Entangled evolutionary history of *Cuscuta pentagona* clade: A story involving hybridization and Darwin in the Galapagos

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Abstract The distribution of *Cuscuta* subg. *Grammica* sect. *Cleistogrammica* (*Cuscuta pentagona* clade) is centered in North America (*C. campestris*, *C. glabrior*, *C. harperi*, *C. pentagona*, *C. obtusiflora*, *C. plattensis*, *C. polygonorum*, *C. runyonii*); however, long-distance dispersal was documented to Hawaii (*C. sandwichiana*), South America (*C. gymnocarpa*, *C. stenolepis*, and in part *C. obtusiflora*), Africa (*C. bifurcata*, *C. schlechteri*), Eurasia, and Australia (C. *australis*). Hybrid speciation has already been documented for some members of sect. *Cleistogrammica* (*C. sandwichiana*, *C. bifurcata*) but previous studies strongly suggested that the extent of reticulate evolution is underestimated in *Cuscuta* generally, and in this section in particular. Sequence data from the nuclear internal transcribed spacer (ITS) and the plastid *trnL-F* region were used to reconstruct the phylogeny and gain a better understanding of the evolutionary history within the clade. Additionally, a morphometric analysis was conducted to test the phenetic distinctiveness of a select number of species with taxonomic problems: *C. campestris*, *C. glabrior*, *C. gymnocarpa*, and *C. pentagona*. Discordances between phylogenies derived from plastid and nuclear data showed that *C. campestris* is a hybrid, likely involving the *C. runyonii*/*glabrior* lineage as a maternal progenitor and an undiscovered species as a paternal progenitor. This latter species, an extinct or unsampled lineage, was itself inferred to be a hybrid between *C. pentagona*/*harperi* and *C. australis*/*obtusiflora*/*polygonorum* lineages. Both the evolutionary and morphometric results clearly showed that *C. campestris* is a distinct species and the negative consequences of its amalgamation with *C. pentagona* during the last decades are discussed. *Cuscuta gymnocarpa*, an enigmatic species described from specimens collected by Darwin from the Galapagos, was inferred as conspecific with *C. campestris* and proposed as a variety of the latter. Because *C. gymnocarpa* is only a form of *C. campestris*, the possible means of dispersal of the latter species to the Galapagos are discussed. *Cuscuta modesta*, a new species discovered while studying the systematics of the clade, is described and illustrated.

Keywords Convolvulaceae; dispersal; field dodder; Galapagos Islands; ITS; molecular phylogeny; morphometrics; reticulate evolution; *trnL-F*

Supplementary Material The Electronic Supplement (Figs. S1–S2) is available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/content/iapt/tax; alignments have been submitted to TreeBase with study reference number S18533 (http://purl.org/phylo/treebase/phylows/study/TB2:S18533)

■ INTRODUCTION

Cuscuta sect. *Cleistogrammica* Engelm., informally also referred to as the *C. pentagona* clade, is one of the largest infrageneric groups of *C.* subg. *Grammica* (Lour.) Peter & al. (15 spp.; García & al., 2014; Costea & al., 2015a). This clade has a complex biogeography and evolutionary history. Biogeographically, it is centered in North America, where most of its species occur (*C. campestris* Yunck., *C. glabrior* Yunck., *C. harperi* Small, *C. obtusiflora* Kunth, *C. pentagona* Engelm., *C. plattensis* A.Nelson, *C. polygonorum* Engelm., and *C. runyonii* Yunck.; Stefanović & al., 2007; García & al., 2014; Costea & al., 2015a). However, long-distance dispersal was documented to Hawaii (*C. sandwichiana* Choisy) and all the other continents: South America (*C. gymnocarpa* Engelm., *C. stenolepis* Engelm., and in part *C. obtusiflora*), Africa (*C. bifurcata* Yunck., *C. schlechteri* Yunck.), Asia, Australia, and Europe (*C. australis* R.Br.). In addition, during the last century, despite strict quarantine legislation measures adopted by most countries, *C. campestris* has been dispersed worldwide as a seed contaminant of forage legume crops (Dawson & al., 1994; Costea & Tardif, 2006). Reticulate evolution was reported for *C. sandwichiana* and *C. bifurcata* involving most likely maternal progenitors from sect. *Cleistogrammica* and paternal progenitors from sect. *Grammica* and sect. *Racemosae* (Yunck.) Costea & Stefanović, respectively, two other clades of subg. *Grammica* (Lour.) Peter & al. (Stefanović & Costea, 2008; García & al., 2014). The same studies strongly suggested that the extent of reticulate evolution in subg. *Grammica* is largely underestimated and that more extensive taxonomic sampling, cloning, and analysis of additional genes are likely to reveal more cases of reticulate evolution (see also Costea & Stefanović, 2010).

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The complicated evolutionary history and biogeography of sect. *Cleistogrammica* have generated some systematics problems. One of these problems involves the circumscription and taxonomic status of *C. pentagona* (Engelmann, 1859) and one of its segregate species, *C. campestris* (field dodder, common dodder; Yuncker, 1932). The latter is perhaps the most common dodder pest species worldwide (Dawson & al., 1994; Costea & Tardif, 2006) and one of the worst parasitic invasive weeds in general (Holm & al., 1997). In contrast, *C. pentagona* appears to be limited only to North America, primarily in central and eastern parts of the U.S.A. (Costea & al., 2006). A superficial Google Scholar (2015, https://scholar.google.com) literature search of "Cuscuta pentagona" from 2006 to 2015 retrieved over 700 references linked to this binomial; however, as we are going to show, many of these references may refer in fact to *C. campestris*. This confusion persists especially in North America despite the fact that *C. campestris* was repeatedly reported as morphologically distinct from *C. pentagona* (Yuncker, 1965; Austin, 1986; Musselman, 1986; Costea & al., 2006), and both species were included in several molecular phylogenetic studies over the last decade (e.g., Stefanović & al., 2007; Stefanović & Costea, 2008; García & al., 2014).

Another interesting taxonomic conundrum, linked to the previous, is in relation to *C. gymnocarpa*, a charismatic species described from Galapagos by Engelmann (1859) from specimens collected by Charles R. Darwin in 1835 from Santiago (James) Island during the (second) Beagle voyage (Darwin, 1839). The species has been considered endemic to the Galapagos (Robinson, 1902; Wiggins & Porter, 1971; Austin, 1982). Both Engelmann (1859) and Yuncker (1932) mentioned that *C. gymnocarpa* resembles morphologically *C. pentagona* and *C. campestris*, respectively. Also, a sample of *C. gymnocarpa* was found to be nearly identical from a molecular point of view to *C. campestris* in a phylogeny of subg. *Grammica* (Stefanović & al., 2007). If *C. gymnocarpa* is indeed conspecific with *C. campestris*, this would raise the question how was *C. campestris* introduced to the Galapagos before 1835, when Darwin collected it?

The two taxonomic problems mentioned above and the possibility of more extensive reticulate evolution within this clade, have prompted us to investigate in more detail the evolutionary history of this species complex. Thus, the specific objectives of this study are to: (1) unravel the molecular evolutionary history of sect. *Cleistogrammica* based on nuclear ITS and plastid *trnL-F* sequences; (2) test the morphological distinctiveness of *C. pentagona*, *C. campestris*, *C. gymnocarpa*, and *C. glabrior* using morphometric approaches; (3) describe a new species, *C. modesta* sp. nov., discovered while studying the systematics of this clade.

■ MATERIALS AND METHODS

Taxon sampling for molecular analyses. — A set of 68 accessions, representing 13 ingroup species of sect. *Cleistogrammica*, was used for the molecular phylogenetic analyses (Appendix 1). Efforts were made to sample multiple accessions, particularly for those species spanning large biogeographical ranges or worldwide anthropogenic dispersal (e.g., *C. australis*, 8 individuals; *C. campestris*, 21 individuals). As a result, 1 to 21 individuals are included in the molecular analyses for all but one species, *C. schlechteri*, which is known only from its type locality in Africa. Based on our previous, more inclusive phylogenetic analyses of subg. *Grammica* (Stefanović & al., 2007; Stefanović & Costea, 2008), as well as preliminary analyses conducted in this study, we selected *C. stenolepis* as functional outgroup.

Molecular techniques and alignments. — Sequences for the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) as well as *trnL-F* intron/spacer region from the plastid genome (ptDNA) were obtained to infer phylogenetic relationships among species of sect. *Cleistogrammica*. In addition to the DNA samples used in previous studies (Stefanović & al., 2007; Stefanović & Costea, 2008; García & al., 2014), total genomic DNA was isolated from newly obtained specimens as well (Appendix 1). DNA extractions, polymerase chain reaction (PCR) reagents and conditions and amplicon purifications followed the protocols detailed in Stefanović & al. (2007). Cleaned products were sequenced at the McGill University and Génome Québec Innovation Centre (Canada). By direct sequencing of ITS amplicons, significant amounts of additive polymorphic sites were detected primarily in individuals belonging to *C. campestris* and *C. gymnocarpa*. Other species also showed polymorphic sites, albeit to a much lesser extent. Purified PCR products were cloned for all the species using the pGEM-T Easy Vector II cloning kit (Promega, Madison, Wisconsin, U.S.A.) and multiple clones per individual were sequenced. A total of 282 ITS and 70 *trnL-F* sequences were analyzed; new sequences generated for this work were deposited in GenBank (accession numbers KT371706–KT371747 for *trnL-F* and KT383062–KT383307 for ITS; see Appendix 1). Sequences were aligned manually using Se-Al v.2.0a11 (Rambaut, 2002). Alignments of the sequences analyzed have been submitted to TreeBase with study reference number S18533 (http://purl.org/phylo/treebase/phylows/study/TB2:S18533).

We tested for evidence of recombination in the unreduced ITS dataset using the Phi test (Bruen & al., 2006) as implemented in SplitsTree v.4.6 (Huson & Bryant, 2006). Additionally, to check for intra-individual recombination or PCR-derived chimeric sequences the programs Recombination Analysis Tool (RAT; Etherington & al., 2005) and Bellerophon (Huber & al., 2004) were run. Finally, to detect potential pseudogenes in the ITS dataset, the ITS2 secondary structure of the most divergent clones was predicted using the hidden Markov model-based method (Keller & al., 2009) and the web interface ITS2 prediction tool (Koetschan & al., 2012), as well as compared for putatively functional sequence motifs (Mai & Coleman, 1997).

The original matrix prevented the production of convergence in the Bayesian analyses even after 6 million generations. To reduce computational burden and uninformative repetition in the ITS clones matrix, sequences identical to each other for any given accession were included as a single ribotype, resulting in a matrix with 253 sequences. A preliminary

phylogenetic analysis of this ITS matrix (see Electr. Suppl.: Fig. S1) produced trees with low support for some backbone relationships, primarily owing to the phylogenetically unfavorable ratio of operational taxonomic units (253) compared to the number of parsimony-informative (180) and variable (224) characters, especially for *C. campestris*/*gymnocarpa* accessions. To reduce the negative impact the of this high taxon-to-character ratio on the phylogenetic analyses, a safe deletion rule, first introduced as the Safe Taxonomic Reduction (STR) strategy by Wilkinson (1995) and modified by Zarrei & al. (2014), was used to reduce the size of the original dataset. The only sequences retained for a given individual were those found in different clades in the consensus tree shown in Fig. S1 (Electr. Suppl.). Therefore, for a particular individual of *C. campestris*/*gymnocarpa*, we retained one clone resolved with *C. pentagona* and another clone resolved in the clade with *C. australis*, *C. obtusiflora*, and *C. polygonorum*. For *C. pentagona*, all the clones were resolved in the basal polytomy and only one of them from each individual was included in the reduced matrix. For all the other species we kept two clones per individual. Additionally, incomplete sequences lacking more than 100 bp and some of the most divergent clones were also removed. The matrix reduced in this fashion consists of 106 ITS ribotypes (Table 1). In both ITS and *trnL-F* datasets, ribotypes and haplotypes from putative hybrid species, including *C. sandwichiana* and *C. bifurcata*, two species with paternal progenitors from other sections in subg. *Grammica* (Stefanović & Costea, 2008; García & al., 2014), were removed and analyzed separately. The ITS and *trnL-F* matrices from which hybrid taxa were excluded contained 63 and 43 sequences, respectively. Finally, a 41 accessions matrix containing concatenated ITS and *trnL-F* sequences was produced for the "total evidence" analyses (Table 1); for this matrix only one ITS sequence per accession was used.

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

Under parsimony criterion, nucleotide characters were treated as unordered and all changes were equally weighted. Searches for most parsimonious (MP) trees for all the matrices were performed using a two-stage strategy using PAUP* v.4.0b10 (Swofford, 2002). First, the analyses involved 10,000 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) branch swapping saving no more than 10 trees per replicate, and MULTREES off. The second round of analyses was performed on all trees in memory with the same settings except with MULTREES on. Both stages were conducted to completion or until 1 million trees were found. 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 (DeBry & Olmstead, 2000). Nodes receiving bootstrap (BS) values <60%, 60%–75%, and >75% were considered weakly, moderately, and strongly supported, respectively.

Bayesian phylogenetic inferences were performed using MrBayes v.3.2.2 (Ronquist & al., 2012) run on the CIPRES Science Gateway (Miller & al., 2010). The program MrModeltest v.2.3 (Nylander, 2004) was used to determine the model of sequence evolution for each dataset by the Akaike information criterion (AIC). For all matrices, the general time reversible (GTR; Tavaré, 1986) model of DNA substitution was selected as the best-fit. In some cases this base model was further modified either by addition of rate variation among nucleotides

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

	Plastid (*trnL-F*)		Nuclear (ITS)		Combined
	All species	Hybrids excluded	All species	Hybrids excluded	Hybrids excluded
Number of OTUs included	70	43	106	63	41
Sequence characteristics:					
Aligned length	508	508	664	664	1172
Variable sites	59	47	256	196	162
Parsimony-informative sites	47	41	108	91	121
Most parsimonious tree characteristics:					
Length	75	54	349	232	191
Consistency/Retention index	0.807/0.968	0.907/0.975	0.834/0.957	0.940/0.982	0.916/0.976
Bayesian analyses:					
Model of DNA evolution	GTR+G	GTR+I	GTR+G	GTR	GTR+G
Number of generations	750,000	275,000	920,000	225,000	2,000,000
Number of trees retained	11,252	4127	13,802	3378	30,002
Mean $-lnL$	1435.38	1098.77	3703.26	2598.50	2995.143

GTR, general time reversible (Tavaré, 1986); G, discrete gamma distribution rate variation among nucleotides; I, proportion of invariable sites; OTU, operational taxonomic unit

following a discrete gamma distribution (GTR+G) or with a proportion of invariable sites (GTR+I); see Table 1 for details. Each Bayesian analysis consisted initially of two runs, each with two million generations, starting from a random tree using the default priors, and four Markov chains sampled every 100 generations. However, the analyses were run until the average standard deviation of split frequencies between two runs was <0.01 (see Table 1 for details on MrBayes settings and number of generations used for each of the analyses). Of the trees obtained from the two runs, the first 25% were discarded as burn-in. The 50% majority-rule consensus trees and the Bayesian posterior probabilities (PP) were obtained in MrBayes from the remaining trees. Only the nodes receiving ≥0.95 PP were considered statistically significantly supported (Rannala & Yang, 1996).

Alternative hypothesis testing. — Several alternative phylogenetic hypotheses for *C. campestris*/*gymnocarpa* were tested on both nuclear and plastid datasets (listed in Table 2). Constrained topologies were constructed using MacClade v.4.06 (Maddison & Maddison, 2003) and their cost in parsimony was assessed using PAUP* (Swofford, 2002). To evaluate the significance among these alternative phylogenetic hypotheses, we implemented the one-tailed Shimodaira-Hasegawa tests (SH tests; Shimodaira & Hasegawa, 1999; Goldman & al., 2000) in PAUP*, using the same models of DNA evolution as implemented in corresponding Bayesian analyses (GTR+G; see Table 1). The test distributions were obtained using the re-estimated log likelihoods (RELL; Kishino & Hasegawa, 1989) with 10,000 bootstrap replicates.

Morphometric analyses and herbarium survey. — Herbarium specimens were used for the morphometric analyses (Appendix 2). Four operational taxonomic units (OTUs), corresponding to *C. pentagona*, *C. campestris*, *C. gymnocarpa*, and *C. glabrior* were included in the morphometric analyses, to test their morphological distinctiveness (Appendix 2). *Cuscuta glabrior* was selected for comparison because this species is also a segregate of *C. pentagona* (Engelmann, 1859; Yuncker, 1932). However, unlike *C. campestris*, *C. glabrior* is currently considered a "good" species (exception, Gandhi & al., 1987, regarded it as a variety of *C. pentagona*). Efforts were made to ensure a sampling size that reflects the scale of the geographical distribution of each species (Costea & al., 2006). Thus, morphometric analyses included 131 specimens (*C. campestris* 59; *C. pentagona* 33; *C. glabrior* 28; *C. gymnocarpa* 11; Appendix 2). A previous morphometric study of species within sect. *Californicae* (Yunck.) Costea & Stevanovic (Costea & al., 2009), which is sister to sect. *Cleistogrammica* (Stefanović & al., 2007; García & al., 2014), provided the list of useful characters. These characters were further refined using some recent character evolution studies (gynoecium and perianth; Wright & al., 2011, 2012; infrastaminal scales; Riviere & al., 2013). In total, 32 characters, 25 continuous and 7 binary were formulated (Appendix 3). The majority of herbarium specimens had both flowers and fruits/seeds (exception: three collections of *C. glabrior* did not have mature seeds). Flowers and fruits removed from herbarium specimens were steeped in gradually warmed 50% ethanol, which was then allowed to boil for a few seconds to rehydrate tissues. For basic morphology, flowers were dissected under a Nikon SMZ1500 stereomicroscope and imaged with PaxCam Arc digital camera (MIS, Villa Park, Illinois, U.S.A.) equipped with a Pax-it 7.6 imaging software. For scanning electron microscopy (SEM), we used hexamethydisilazane (HMDS) as an alternative for critical dry point (Costea & al., 2011a, b), and the examination was done at 10 kV using a Hitachi SU1510 variable pressure scanning electron microscope. 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 v.1.89 (Hammer & 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. We also run a principal component analysis (PCA) analysis using correlation (normalized var-covar) and the iterative imputation algorithm for missing data (Ilin & Raiko, 2010) but since groups were similar to those obtained from PCoA, they are not shown.

We also wanted to verify the known geographical distributions of *C. gymnocarpa* (Wiggins & Porter, 1971), *C. campestris*, and especially that of *C. pentagona* (Costea & al., 2006). However, in the process, all the species of sect. *Cleistogrammica* from the following herbaria were studied and annotated (herbaria in italics are examined here for the first time): *AAU*, *ABH*, ALTA, *ARAN*, ARIZ, ASU, *B*, *BAB*, *BC*, *BCN*, *BM*, *BOL*, *BORD*, *BR*, BRIT, *CAL*, *CANB*, *CAS*, *CEN*, *CHR*, *CHSC*, *CIIDIR*, *CIMI*, *COI*, *CTES*, DAO, *E*, F, *FT*, *G*, GH, *H*, HAM, *HUFU*, *HUJ*, *IAC*, *IEB*, *IND*, *J*, *JACA*, *JE*, *JEPS*, *K*, *L*, *LAU*,

Table 2. Results of the Shimodaira-Hasegawa (SH) tests for alternative hypothesis testing in *Cuscuta* sect. *Cleistogrammica*. Probabilities below 0.05 (i.e., tree topology rejected as significantly worse) indicated in bold.

Dataset	Constrained topology	Length	Length difference	SH test
Nuclear (ITS)	Optimal tree (Fig. 1)	349	Best	1.000
	All *C. campestris* Yunck./*gymnocarpa* Engelm. ribotypes together (anywhere on the tree)	380	31	**<0.001**
	All *C. campestris*/*gymnocarpa* ribotypes with *C. glabrior* Yunck./*runyonii* Yunck./*plattensis* A.Nelson	374	25	**<0.001**
Plastid (*trnL-F*)	Optimal tree (Fig. 2)	75	Best	1.000
	All *C. campestris*/*gymnocarpa* haplotypes with *C. pentagona* Engelm./*harperi* Small	81	6	**0.037**
	All *C. campestris*/*gymnocarpa* haplotypes with *C. australis* R.Br./*obtusiflora* Kunth/*polygonorum* Engelm.	82	7	**0.019**

LD, *LE*, *LL*, *LP*, *LPB*, *LPS*, *M*, *MA*, *MACB*, *MAF*, *MEL*, *MERL*, MEXU, MGC, *MICH*, *MO*, MT, MTMG, *MPU*, *MSTR*, *NAP*, *NBG*, NFLD, *NMC*, NSPM, NY, OAC, *OKLA*, *OSC*, *OXF*, *P*, *PACA*, *PRE*, *QCNE*, QFA, QUE, *RB*, RBG, *RNG*, *RSA*, *S*, *SALA*, *SAM*, SASK, *SD*, *SEV*, SFS, *SGO*, *SI*, *SPF*, TEX, *TRT*, *TRTE*, *UA*, *UB*, UBC, *UCR*, UC, *UCT*, *UNB*, *UNM*, *UPRRP*, *UPS*, *US*, UWO, *VAL*, *W*, WAT, WIN, WIS, WTU and XAL.

■ RESULTS

Unconstrained analyses and overall levels of support. — Summary descriptions for sequences obtained from ITS and *trnL-F* regions are presented in Table 1.

There was no evidence for recombination or chimeric sequences within the ITS dataset. All the clones showed apparently intact four-helix secondary structure, a U-U mismatch on helix II, and a UGGA motif near the end of helix III (results not shown).

All the species showed some intra-individual ITS sequence variation, but the divergence was substantially higher within individuals of *C. campestris* and *C. gymnocarpa*. In these two species, the number of variable sites between pairs of sequences from a single individual ranged from 0 to 33 (up to 4.9%); only in one accession of *C. campestris* (1260) the number of variable sites was 9 or less. The rest of the species showed intra-individual variation that ranged from 0 and 13 sites (up to 1.9%), with the exception of accession 639 of *C. australis* var. *tinei* (Insenga) Yunck. in which one of the clones differed in 22–27 sites from the other clones obtained from the same individual, and one clone from accession 747 of *C. obtusiflora* var. *glandulosa* Engelm. (17–20 sites). The analyses of the matrix containing all 253 ITS clones resulted in trees with low support for some of the backbone relationships (see Electr. Suppl.: Fig. S1). The analysis of the reduced ITS matrix, containing 106 terminal units, resulted in trees with well supported backbone relationships (Fig. 1; solid lines). With *C. stenolepis* as functional outgroup, two strongly supported clades were resolved. The first major clade (90% BS; ≥0.95 PP) contains all the clones belonging to *C. australis*, *C. obtusiflora*, *C. polygonorum*, and part of the clones of *C. campestris* and *C. gymnocarpa*. Within this clade, very little internal resolution was recovered. The second major clade (82% BS; ≥0.95 PP) is resolved in two well supported subclades: one (80% BS; ≥0.95 PP) that includes the clones of *C. harperi*, *C. pentagona* plus all but one of the remainder of the clones obtained from *C. campestris* and *C. gymnocarpa*, and the other (95% BS; ≥0.95 PP) that groups together accessions from *C. plattensis*, *C. modesta* sp. nov., *C. runyonii* and *C. glabrior*. Only the clones of *C. modesta* sp. nov., were resolved as reciprocally monophyletic, with high support (99% BS; ≥0.95 PP). A clade containing sequences from *C. glabrior* and *C. runyonii* together, received strong support as well (98% BS; ≥0.95 PP), but without internal resolution to segregate these two species. Finally, the sequences derived from *C. plattensis* were resolved as monophyletic, but with mixed, moderate to strong, support (63% BS; ≥0.95 PP). One divergent clone sequenced from a South African individual of *C. campestris* (527, cl. 4) and another clone from a North American specimen (1264, cl. 6) were unexpectedly resolved in the *C. plattensis* clade (Fig. 1; Electr. Suppl.: Fig. S1). We interpret these results as an artifact (e.g., chimeric sequence, contamination, etc.), because if this phylogenetic relationship was real and the result of a natural process, some of the clones from other specimens of *C. campestris* should also be resolved in this clade.

When all the clones derived from *C. campestris* and *C. gymnocarpa*, two putative hybrids, were removed from the ITS matrix and the reduced matrix reanalyzed (63 terminal units), the same underlining tree topology was recovered, but with clades generally receiving higher support across the board (Fig. 1; dotted lines).

Analyses of plastid *trnL-F* matrix recovered trees (Fig. 2) with different topologies to those obtained with ITS sequences. Similar to the ITS trees, the split between *C. stenolepis* as the functional outgroup and the rest of the species of sect. *Cleistogrammica* sampled have received a strong support (100% BS; ≥0.95 PP). However, the backbone relationships, albeit resolved on the consensus trees, received very weak support. Instead, five terminal clades were recovered with strong support, all of them with ≥80% BS and ≥0.95 PP. The *trnL-F* sequences derived from *C. campestris* and *C. gymnocarpa* samples were almost exclusively found in one strongly supported clade (98% BS; 1.00 PP), containing the individuals of *C. runyonii*, *C. glabrior*, and *C. plattensis*, unlike either of the placements obtained with nuclear data. Sister to this clade is the group containing individuals of *C. modesta* sp. nov., but this sister relationship was only weakly supported. Three additional accessions identified morphologically as *C. campestris* (468, 1263, 1272) were resolved in a strongly supported clade (83% BS; 1.00 PP) composed of the individuals of *C. pentagona* and *C. harperi*. The fourth clade (89% BS; 1.00 PP) contained sequences from all individuals of *C. australis*, *C. obtusiflora*, *C. polygonorum*, and *C. bifurcata*, whereas the accessions belonging to *C. sandwichiana* were resolved in another clade (100% BS; 1.00 PP).

When all the sequences derived from putative hybrids species (*C. campestris*, *C. gymnocarpa*, *C. sandwichiana*, *C. bifurcata*) were removed from the *trnL-F* matrix and the reduced matrix reanalyzed (43 terminal units), the same underlining tree topology was recovered, with clades generally receiving similar or higher support across the board and backbone relationships weakly supported (Fig. 2; dotted lines).

The combined ("total evidence") analyses were conducted on a dataset in which nuclear and plastid sequences were concatenated but excluding the accessions with strongly supported conflicting position (*C. campestris*, *C. gymnocarpa*) and the species of hybrid origin with paternal progenitors from other sections of subg. *Grammica* (*C. bifurcata*, *C. sandwichiana*). After this exclusion, no significantly supported conflict existed between the two datasets. The resulting consensus trees (Fig. 3) recovered the same two major clades obtained with ITS sequences and the internal topology was overall similar to the ITS trees, but with generally much improved internal resolution and better support.

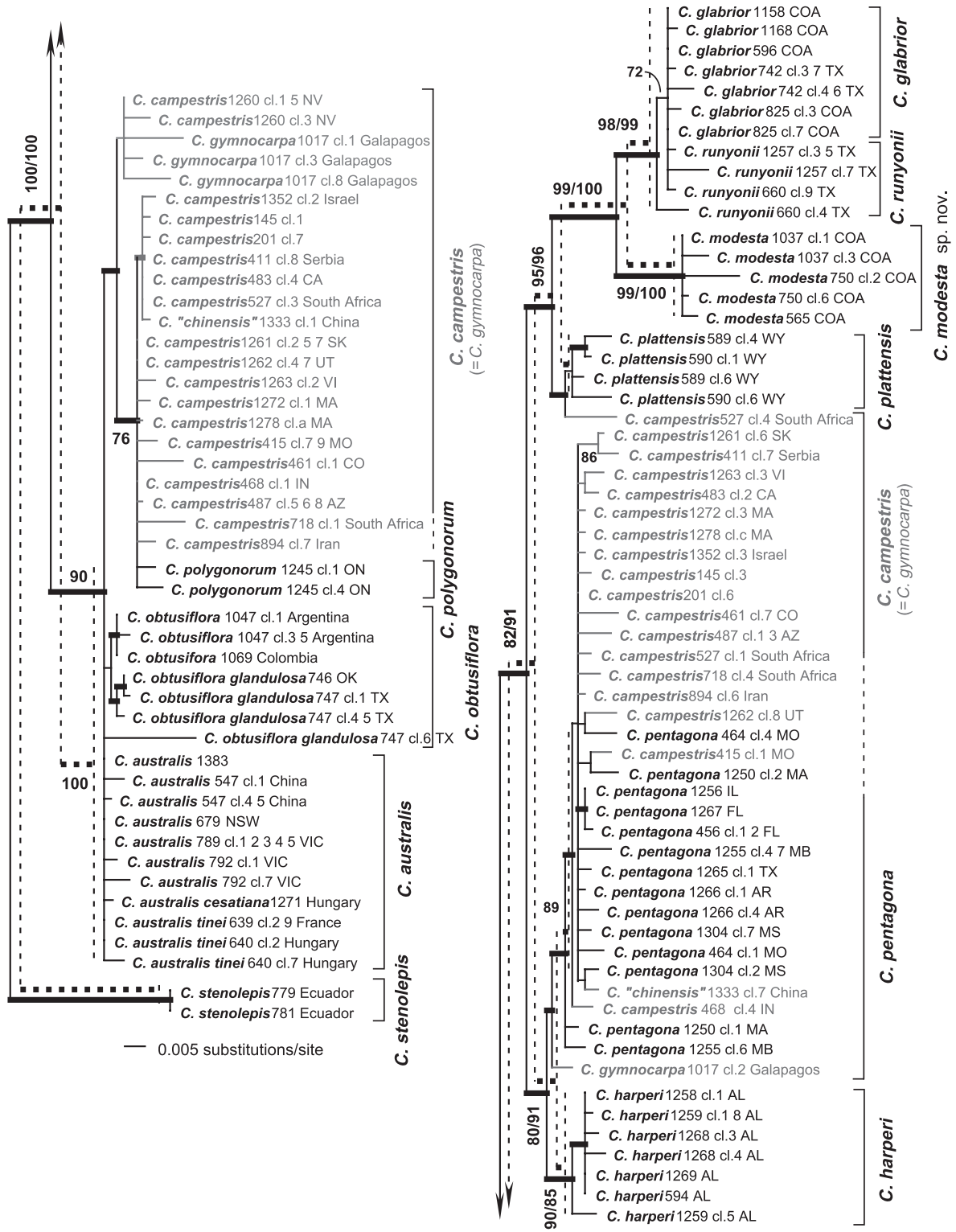

Fig. 1. Phylogenetic relationships among all sampled species (solid lines) of *Cuscuta* sect. *Cleistogrammica* resulting from the Bayesian analysis of the nuclear (ITS) sequence data. Superimposed (dotted lines) is the phylogeny obtained after the exclusion of putative hybrid species (OTUs shaded in grey). Thick lines (solid or dotted) indicate Bayesian posterior probabilities ≥0.95. The MP searches resulted in strict consensus trees with nearly identical topologies. Parsimony bootstrap values are indicated for nodes supported ≥65%; when two values are provided they refer to full sampling (solid line)/reduced sampling (dotted line). The trees are rooted using individuals of *C. stenolepis* as functional outgroup. Species names are followed by their respective DNA accession numbers (Appendix 1) and geographic locations where the specimens were collected (countries, or abbreviations of states/provinces for the U.S.A., Mexico, Australia, and Canada, are provided). In addition, different ITS clones are labeled, when applicable.

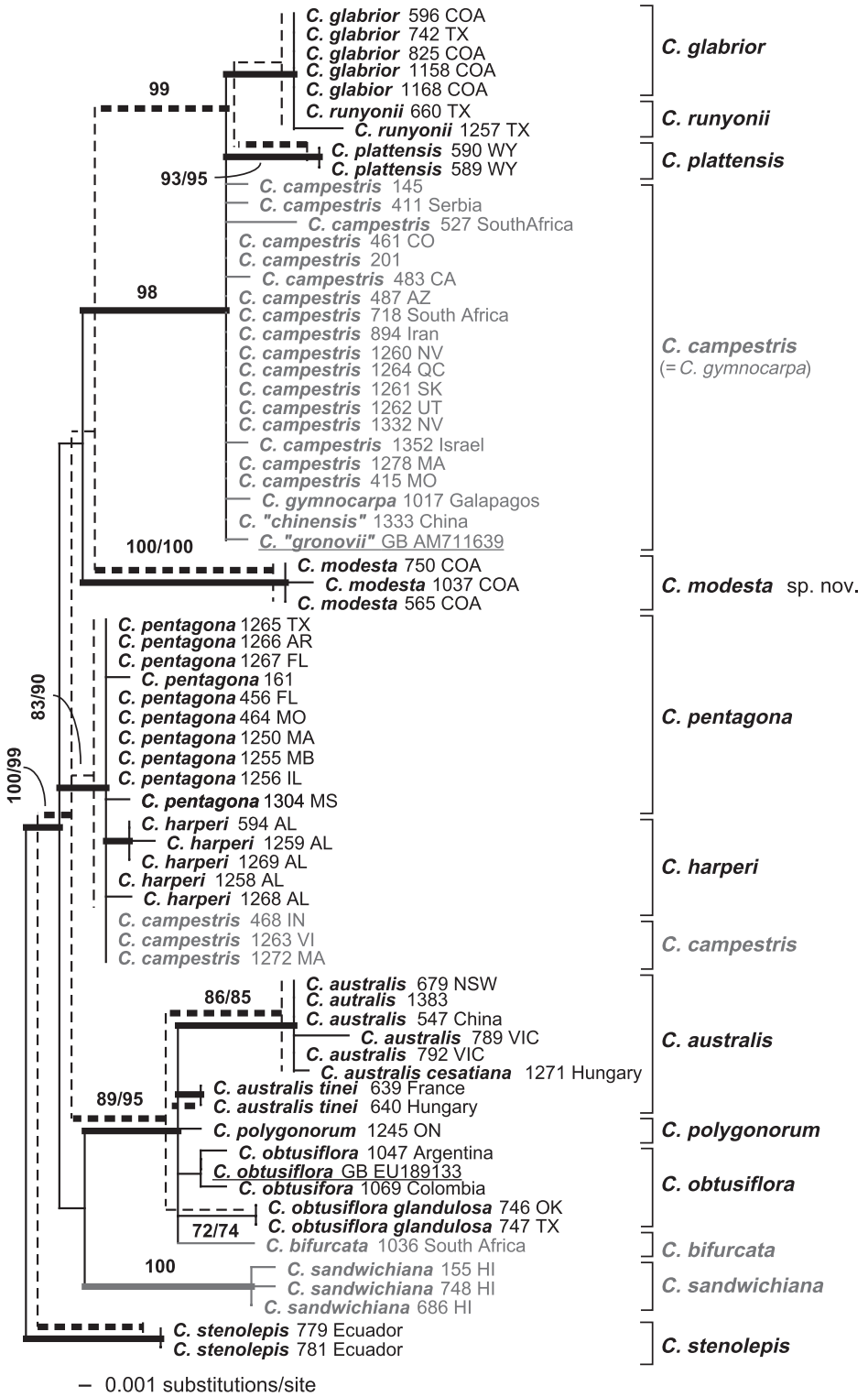

Fig. 2. Phylogenetic relationships among all sampled species (solid lines) of *Cuscuta* sect. *Cleistogrammica* resulting from the Bayesian analysis of the plastid (*trnL-F*) sequence data. Superimposed (dotted lines) is the phylogeny obtained after the exclusion of putative hybrid species (OTUs shaded in grey). Thick lines (solid or dotted) indicate Bayesian posterior probabilities ≥0.95. The MP searches resulted in strict consensus trees with nearly identical topologies. Parsimony bootstrap values are indicated for nodes supported ≥65%; when two values are provided they refer to full sampling (solid line)/reduced sampling (dotted line). The trees are rooted using individuals of *C. stenolepis* as functional outgroup. Species names are followed by their respective DNA accession numbers (Appendix 1) and geographic locations where the specimens were collected (countries, or abbreviations of states/provinces for the U.S.A., Mexico, Australia, and Canada, are provided). Two individuals whose sequences are obtained from online databases (GenBank accession numbers indicated) are underlined.

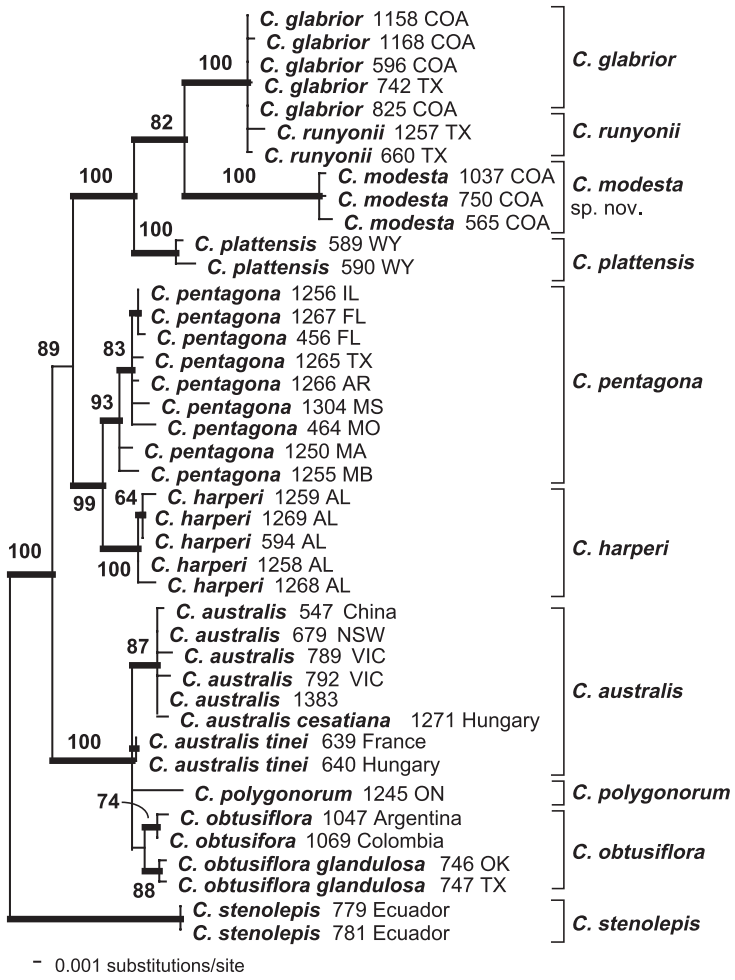

Fig. 3. Majority-rule consensus tree with mean branch lengths from the Bayesian analysis of combined plastid (*trnL-F*) and nuclear (ITS) data showing the backbone relationships among species of *Cuscuta* sect. *Cleistogrammica*. The MP searches resulted in strict consensus trees with nearly identical topologies. Thick lines indicate Bayesian posterior probabilities ≥0.95; parsimony bootstrap values are indicated for nodes supported ≥65%. Trees are rooted using *C. stenolepis* as functional outgroup. Species names are followed by their respective DNA accession numbers (Appendix 1) and geographic locations where the specimens were collected (countries, or abbreviations of states/provinces for the U.S.A., Mexico, Australia, and Canada, are provided).

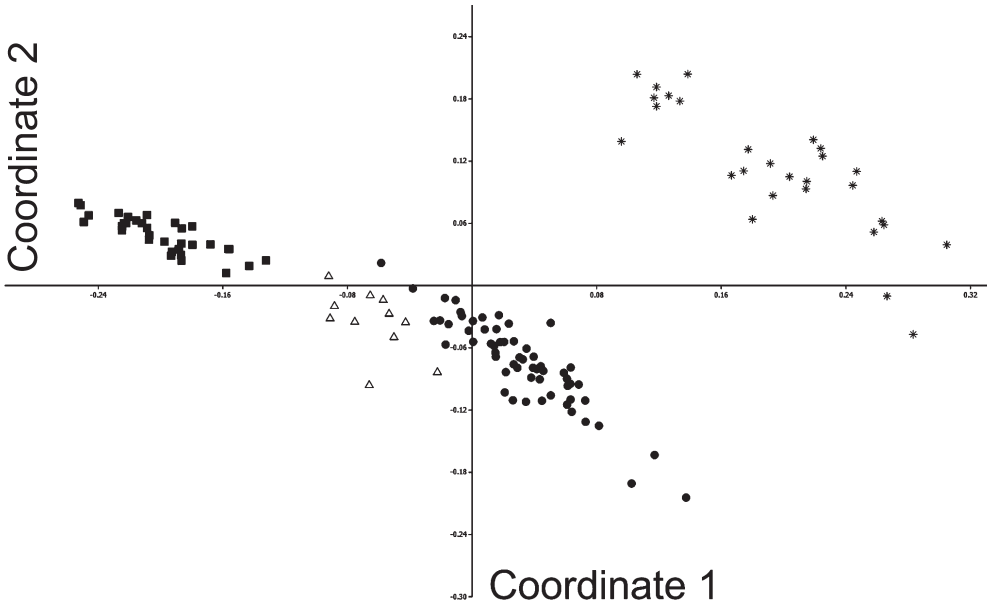

Fig. 4. Principal coordinates analysis (PCoA) ordinated specimens in three groups corresponding to three species. The first coordinate axis (43.12% of the variance) separated *C. glabrior*, *C. campestris*, and *C. pentagona*. The second coordinate axis (15.89% of the variance) separated *C. pentagona* from *C. campestris*. *Cuscuta gymnocarpa* specimens were grouped together with *C. campestris*. Squares, *C. pentagona*; triangles, *C. gymnocarpa*; Circles, *C. campestris*; stars, *C. glabrior.*

Tests of alternative tree topologies. — The conflicting topological positions of *C. campestris*/*gymnocarpa* were further investigated by enforcing the topological results obtained from ITS sequences on *trnL-F* data and vice-versa. When the *C. campestris*/*gymnocarpa* haplotypes were forced in a clade with *C. pentagona*/*harperi* and with *C. australis*/*obtusiflora*/*polygonorum* (following the ITS results) the MP trees were 6 and 7 steps longer respectively than the optimal trees. When all the ribotypes were forced to be together anywhere on the tree or in the clade of *C. glabrior*/*runyonii*/*plattensis* (following *trnL-F* results), the MP trees were 31 and 25 steps longer, respectively, than the optimal tree. All these length differences were deemed strongly significant, and were rejected as worse solutions compared to the optimal, based on the SH tests (Table 2).

Morphometric analyses. — Principal coordinates analysis (PCoA) produced three distinct groups of specimens: one that corresponded to *C. pentagona*, one for *C. campestris* (including *C. gymnocarpa*), and one for *C. glabrior* (Fig. 4). The first coordinate axis accounted for 43.12% of the variance and separated the three species: *C. campestris* (including *C. gymnocarpa*), *C. pentagona*, and *C. glabrior*. The second coordinate axis accounted for 15.89% of the variance and separated *C. pentagona* from *C. campestris* (including *C. gymnocarpa*). The dendrogram obtained from the UPGMA cluster analysis revealed also three distinct backbone clusters that had a similar composition to the major groups obtained through PCoA analysis: a cluster that included *C. pentagona*, one that comprised both *C. campestris* and *C. gymnocarpa*, and one for *C. glabrior* (Electr. Suppl.: Fig. S2). The 11 specimens of *C. gymnocarpa* formed a distinct cluster within *C. campestris*). Within each species, in general, samples from the same geographical areas did not cluster together. Only in the case of *C. glabrior*, the samples from Mexico formed three different sub-clusters. The cophenetic correlation coefficient was 0.8438. In conclusion, both ordination and clustering methods produced essentially the same results: *C. pentagona* and *C. campestris* formed separated morphological groups/clusters, while the samples of *C. gymnocarpa* grouped together within *C. campestris*.

Geographic distribution and ecology of *C. pentagona*, *C. campestris*, and *C. gymnocarpa*. —The most comprehensive herbarium survey undertaken to date confirms that *C. pentagona* is limited to the territory of the U.S.A., and can be found in the following states: Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kansas, Illinois, Indiana, Iowa, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Montana, New Jersey, New York, North Carolina, North Dakota, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, and Virginia. One specimen of *C. pentagona* was collected by Macoun from Manitoba in 1872 (*Macoun s.n.*, MTMG), but the species was never collected subsequently in Canada. There is not even a single occurrence of *C. pentagona* outside North America. Most specimens of *C. pentagona* are relatively old (1950s and earlier), and specimens collected after 1990 exist only from Arkansas, Florida, Missouri, and Texas. This suggests potentially a degree of rarity in some U.S.A. states. Habitats recorded in the herbarium labels of *C. pentagona* specimens include flats, meadows, prairies, shores, and open areas in forests, generally at elevations lower than 300 m, on sandy, gravel, limestone or dolomite substrates. Although some specimens of *C. pentagona* were collected from ruderal habitats (e.g., margins or roads, abandoned fields), there are no occurrences of this species as an agricultural weed. *Cuscuta campestris* is in many respects the exact opposite. The species has currently a subcosmopolitan distribution between the latitudes of 60° North (Europe) and 30° South (South Africa), at elevations up to 3000 m. In North America, it is commonly misidentified as *C. pentagona*; in Asia, as *C. australis* or *C. chinensis* (e.g., accessions no. 1383 and 1333, respectively, included in this study); and in South America it is sometimes mistaken with *C. obtusiflora* (var. *obtusiflora*). The species prefers habitats with anthropomorphic disturbance, both ruderal and agricultural, but it is especially the latter that set apart *C. campestris* from *C. pentagona*. *Cuscuta gymnocarpa* is currently present at elevations lower than 300 m on all the Galapagos Islands.

■ DISCUSSION

Evidence for hybridization in *Cuscuta* sect. *Cleistogrammica*. — When the plastid DNA tree is compared with an independently derived phylogenetic tree (from morphology or other molecular data), conflicting position of a taxon between phylogenies may be taken as evidence for the hybrid origin of this taxon (Sang & Zhong, 2000). Although alternative biological phenomena, such as horizontal gene transfer, paralogy, gene duplication followed by differential deletion, and lineage sorting could result in similar incongruences, Stefanović & Costea (2008) showed that in *Cuscuta* these alternative hypotheses are more complex than the possibility of hybridization or introgression and the hybrid origin of *C. sandwichiana* and *C. bifurcata*, with maternal progenitors in sect. *Cleistogrammica*, and paternal progenitors in sect. *Grammica* and sect. *Racemosae* respectively (Fig. 5). Stefanović & Costea (2008) suggested *C. obtusiflora* as the maternal progenitor for *C. bifurcata*. Bayesian analyses resolve a clade that includes *C. sandwichiana* as sister to the clade of *C. australis*/*obtusiflora*/*polygonorum*/*bifurcata*, suggesting that the maternal progenitor of the Hawaiian endemic could be an extinct or unsampled species in this lineage. However, this clade received very low support (<50% BS; 0.87 PP) and the maternal progenitor of *C. sandwichiana* remains unknown.

Stefanović & Costea (2008) found several examples of reticulation between and within sections of subg. *Grammica*. All these examples were confirmed subsequently in a broader phylogenetic context, including representatives of the entire genus (García & al., 2014), and additional new cases were detected (e.g., Costea & Stefanovic, 2010). The case of *C. campestris*/*gymnocarpa* described here appears to be another example of a species originated by more recent events of hybridization within the same section (*Cleistogrammica*). Our results show the presence of two divergent groups of ITS ribotypes in almost all the individuals of *C. campestris*/*gymnocarpa*

sampled. This finding is independent of the geographical origin of the samples, which spanned the entire North America (native range), and it also included representatives from Europe, Middle East, South Africa, and Asia (recent anthropogenic dispersal). The presence of divergent ITS ribotypes within the same individual is likely the result of their reunion in a single genome following a hybridization event. In the absence of inter-genic homogenization through concerted evolution the divergent copies (i.e., two nuclear ribosomal arrays, maternal and paternal) are maintained, and serve as evidence of the reticulation event (Álvarez & Wendel, 2003). Our results indicate the presence of two paternal lineages in *C. campestris*/*gymnocarpa*, one derived from the *C. pentagona*/*harperi* clade and the other one from the *C. australis*/*obtusiflora*/*polygonorum* clade (Fig. 5). However, the origin of the maternally inherited plastid genome is strongly supported to be in the lineage of *C. runyonii*/*glabrior*. Our results are consistent with reticulation involving a maternal progenitor from the *C. runyonii*/*glabrior* lineage and a paternal progenitor from an undiscovered species originated by an older hybridization event between species from the *C. pentagona*/*harperi* and *C. australis*/*obtusiflora*/*polygonorum* lineages. This paternal progenitor is at present unknown but it most likely involved a hybridization between *C. pentagona* and *C. polygonorum*, two species with overlapping distribution, albeit the support for this particular set of species is weak to moderate. For three individuals identified morphologically as *C. campestris* (accessions 468, 1263, 1272), *trnL-F* sequences were resolved in the *C. pentagona*/*harperi* clade, not in the *C. runyonii*/*plattensis* clade, as was the case for the remaining 19 accessions (Fig. 2). This is one of the two possible topologies expected from the undiscovered paternal progenitor of *C. campestris* (Fig. 5), in this case with the maternal progenitor in the *C. pentagona*/*harperi* lineage and the paternal progenitor in the *C. australis*/*obtusiflora*/*polygonorum* lineage. We have identified specimens 468, 1263 and 1272 as *C. campestris*, but the undiscovered hybrid might be similar morphologically to the latter species. Alternatively, these individuals may not belong to the undiscovered species that was the paternal progenitor of *C. campestris*, but their existence indicates that the hybridization event between species of *C. harperi*/*pentagona* and *C. australis*/*obtusiflora*/*polygonorum* lineages may have happened multiple times. Also, a chloroplast capture through backcrossing hybridization might explain the presence of *C. pentagona*/*harperi* haplotypes in these specimens.

From one individual of *C. campestris* originated in Nevada (accession 1260), all the ITS clones sequenced belonged exclusively to the *C. australis*/*obtusiflora*/*polygonorum* lineage, despite the eight clones sequenced from this specimen (see Fig. 2 and Electr. Suppl.: Fig. S1). Insufficient sampling is a possible but unlikely explanation because in all the other cases a similar cloning effort was enough to obtain sequences from both lineages. Another explanation is that the repeats derived from the *C. pentagona*/*harperi* have been reduced or eliminated from the genome as a consequence of concerted evolution mechanisms that may occur in some populations.

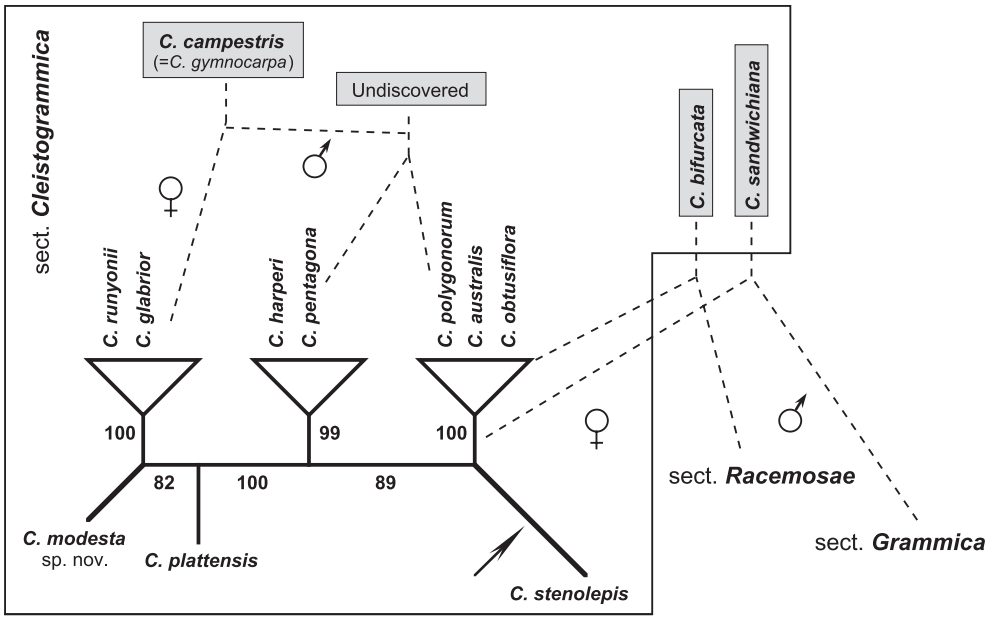

Fig. 5. Schematic overview of phylogenetic relationships among species of *Cuscuta* sect. *Cleistogrammica*. Unrooted topology (solid lines) and support for the backbone relationships is based on analyses of combined plastid (*trnL-F*) and nuclear (ITS) data (compare with Fig. 3). Arrow indicates the placement of the root. Intra- and inter-sectional reticulation events, inferred from conflicting phylogenetic position between plastid- and nuclear-based phylogenies (Figs. 1 and 2; see also Stefanović & Costea, 2008; García & al., 2014), are indicated with dashed lines. Species of putative hybrid origin are highlighted in grey. An undiscovered lineage is inferred to be paternal progenitor of *C. campestris* s.l. This extinct or unsampled species is itself deduced to be a hybrid between *C. pentagona*/*harperi* and *C. australis*/*obtusiflora*/*polygonorum* clades (most likely between *C. pentagona* and *C. polygonorum*).

García & Martín (2007) found that in *C. approximata* Bab., a species of possible allopolyploid origin in subg. *Cuscuta*, most of the individuals showed polymorphic ITS sequences and two pairs of longer chromosomes associated to nucleolar organizer regions (NOR). However, one individual lacked ITS polymorphisms and presented a single 45S locus located only on one of the longer pairs of chromosomes. There is no evidence on the number and location on the NORs in this complex but the maintenance of divergent ribotypes in *C. campestris*/*gymnocarpa* suggests that they are located in more than one chromosome pair.

The same chromosome number, $2n = 56$ ($n = 28$), was reported for both *C. campestris* and *C. pentagona* (Fogelberg, 1938), and these data are trustworthy because Yuncker had provided the seeds of *C. pentagona* and verified the herbarium specimen of *C. campestris* that was used as a seed source in the study. With the exception of *C. australis* ($2n$ = ca. 28; García & Castroviejo, 2003), unfortunately, chromosome numbers are currently not available for the other putative species involved in this reticulation event. In general, more karyotype data is necessary for subg. *Grammica* because this is the least known infrageneric group from a cytological point of view, and the only one in which hybridization has been documented.

***Cuscuta campestris* is a good species. —** The morphological results and evolutionary history strongly confirm that *C. campestris* is a distinct species, also with a different ecology and biogeography compared to *C. pentagona*. From a systematic point of view, not accepting *C. campestris* would be as arbitrary as ignoring any of the other related and accepted species: *C. australis*, *C. glabrior*, *C. harperi*, *C. obtusiflora*, *C. plattensis*, *C. polygonorum*, and *C. runyonii* Yunck.

In addition to the typical form, Engelmann (1859) distinguished three varieties of *C. pentagona*: var. *calycina* Engelm., var. *verrucosa* Engelm., and var. *microcalyx* Engelm. Yuncker (1932) accepted var. *calycina* and var. *verrucosa* at specific rank, as *C. campestris* and *C. glabrior*, respectively. He also treated var. *microcalyx* as a synonym of *C. pentagona*. Ironically, Yuncker had anticipated that *C. campestris* would be received with reluctance. In a letter addressed to W.C. Ferguson in 1929 (four years before the publication of *C. campestris*; see herbarium specimen *Ferguson 7795*, NY), he wrote: "I suspect I will be accused of various bad tendencies in the way of species making […]". Although subsequent studies have in general recognized *C. glabrior* as a species (except Gandhi & al., 1987), the situation of *C. campestris* was different. While some excellent floristic studies (e.g., Austin, 1986; Musselman, 1986) accepted *C. campestris*, other authors considered it a nomenclatural synonym of *C. pentagona* (e.g., Beliz, 1986; Gandhi & al., 1987). Most importantly, major North American biodiversity overviews since the 1980s have also followed the merged concept of *C. pentagona* (e.g., NatureServe, 2015; Tropicos, 2015; USDA NRCS, 2015) regardless of the increasing evidence supporting *C. campestris* as a distinct species over the last decade (Costea & Tardif, 2006; Costea & al., 2006; Stefanović & al., 2007). Non-systematists commonly assume that taxonomic problems are solved in biodiversity overviews. Often, plant biology researchers receive the seeds necessary for their study from local weed scientists or agricultural extension specialists (e.g., Neelima Sinha, pers. comm.), who also follow the same biodiversity overviews. Many of these studies have been conducted in the context of dodder as an agricultural pest, which is only the case of *C. campestris*. As a result, many of the over 700 articles published since 2006 (Google Scholar, 2015, https://scholar.google.com) that refer directly or indirectly to *C. pentagona* likely used in their studies *C. campestris* instead. We can confirm this because sometimes authors included in their articles flower images that allowed us to unambiguously identify *C. campestris* (e.g., Alakonya & al., 2012; Ranjan & al., 2014); cited a seed source that we know uses this species (e.g., Jiang & al., 2013); published DNA sequences that we could verify bioinformatically (e.g., Chen & al., 2014), or subsequently sent us plant material for identification (e.g., Runyon & al., 2006). *Cuscuta campestris* accessions offered by some important germplasm collections may also be misidentified as *C. pentagona* (e.g., USDA ARS; accession 1332 in this study). Thus, some of the most exciting studies done in *Cuscuta* and parasitic plants in general, were done on *C. campestris*, not *C. pentagona* as it has been assumed. For example, those include, among many others, host location using volatile compounds (Runyon & al., 2006); cross-specific transport of mRNA through haustoria (Roney & al., 2007; David-Schwartz & al., 2008); transcriptome characterization (Ranjan & al., 2014).

Because *C. campestris* is so common and widespread worldwide, misidentification is frequent not only with the closely related *C. pentagona*, but also with *Cuscuta* species that belong to entirely different and more distantly related sections. For example, Funk & al. (2007) published the entire plastid genome of supposedly *C. gronovii* Willd. ex Roem. & Schult., another North American species but belonging to sect. *Oxycarpae* (Engelm. ex Yunck.) Costea & Stefanović (Costea & al., 2015a). Although we could not sequence its ITS, the phylogenetic analysis indicates that the haplotype matches that of *C. campestris* (Fig. 2). *Cuscuta chinensis* Lam. var. *chinensis* is native to Asia and has evolved in sect. *Grammica* (Costea & al., 2015a); its seeds, widely used and commercialized as traditional Asian herbal medicine (reviewed by Donnapee & al., 2014) often belong in fact to *C. campestris* (Costea & al., 2011b; accession no. 1333 in this study) or *C. australis* (e.g., accession no. 1383).

***Cuscuta gymnocarpa* is a form of *C. campestris* that has evolved after the introduction of the latter in the Galapagos Islands. —** The new morphological and molecular results that place *C. gymnocarpa* together with *C. campestris* did not come entirely as a surprise because they resonate with those of previous molecular studies (Stefanović & al., 2007; García & al., 2014). Before Engelmann, Hooker (1847) had already examined the Darwin specimens and described this plant as "*Cuscuta sandvicensis* Choisy" var. *mimosae* Hook.f. Hooker (1847) noted that he could not distinguish this plant "from the Sandwich Island plant described by Choisy", leaving no doubt that he was referring to *C. sandwichiana* from Hawaii (Choisy, 1841). After studying the Darwin collections, Engelmann (1859) described this variety as a new species, *C. gymnocarpa*, but noted that it was "much closer to *C. arvensis* [= *C. pentagona* s.l.] than

to *C. sandwichiana*". Subsequently, Yuncker (1932) also noticed the close morphological similarity between *C. gymnocarpa* and *C. campestris* because he felt compelled to provide some characteristics that separate the two taxa. However, we found that none of these characters, "more upright corolla lobes, shorter filaments and more globose capsules and with the calyx lobes not overlapping" (Yuncker, 1932), can distinguish *C. gymnocarpa* from *C. campestris*. *Cuscuta gymnocarpa* has on average slightly smaller flowers (flower length 2.36 mm versus 2.59 mm in *C. campestris*) and therefore, in general, shorter flower parts (data not shown), but these quantitative characters (Appendix 3) overlap and its separation within *C. campestris* is possible only through a morphometric analysis. It was the mystique of the captivating "Enchanted Islands" and its famous collector, Charles R. Darwin, that have probably contributed to the recognition of this plant as a species. Although similar to *C. campestris* both from a morphological and molecular points of view, we proposed a varietal rank for *C. gymnocarpa* (see Taxonomic treatment) because this Galapagos form of *C. campestris* has value as a long-distance dispersal event and incipient case of allopatric speciation in *Cuscuta*.

The introduction of *C. campestris* to the Galapagos Islands had to take place earlier than 1835 when found by Darwin on Santiago Island where, as he indicated in the herbarium label, the plant was already growing "in immense abundance among *Mimosa* bushes". The question emerging is whether *C. campestris* was introduced to the Galapagos and subsequently dispersed among the islands by humans, or naturally from the mainland. Either possibility is interesting. In the first case, such an early human introduction to the Galapagos would suggest that possibly *C. campestris* had been introduced even earlier from North America to Europe because it was mostly Spanish and British ships that visited the Galapagos Islands since 1532.

However, the introduction of *C. campestris* to Europe appears to be much more recent, at the beginning of the 20th century (Feinbrun, 1972). Furthermore, although Santiago Island was subject to anthropomorphic disturbance (e.g., it was visited by pirates, whalers, and turtle hunters; Hickman, 1985), it was not colonized and farmed like Floreana Island since 1830 (Slevin, 1959), from where *C. gymnocarpa* was collected only in the 20th century (e.g., in 1932, *Howell 8835*, NY). Aside from Santiago Island, earlier than 1900 herbarium specimens of *C. gymnocarpa* were gathered from the eastern part of Isabela Island (Aug 1891, *Baur 205*, GH), which was colonized in its southern part in 1893 (Slevin, 1959). It was also collected from Marchena and Ferdinanda Islands in 1899 (*Snodgrass & Heller 769* and *318*, respectively, mounted on the same sheet at GH), which were never colonized and farmed. In the 20th century, *C. gymnocarpa* was documented from all the islands, and recent biodiversity surveys indicate that it is not threatened (Tye, 2007; León-Yánez & al., 2011). The lack of agriculture on the four islands where *C. gymnocarpa* was collected initially, together with its current presence on the uninhabited islands, makes human introduction unlikely because the main avenue of dispersal of weedy dodders, including *C. campestris*, has been through contaminated seed crops, particularly alfalfa and other legumes (Dawson & al., 1994; Costea & Tardif, 2006).

The second possibility, natural introduction, is more probable despite the fact that *Cuscuta* seeds lack obvious morphological dispersal features (e.g., Dawson & al., 1994; Costea & Tardif, 2006). Vargas & al. (2014) reported that 55.6% of the endemic Galapagos plant species possess "unspecialized" diaspores, but the authors did not find evidence for an evolutionary loss of dispersability from non-endemic to endemic species in the islands. Indeed, the seeds of *C. gymnocarpa* are "unspecialized", identical morphologically to those of *C. campestris*. Nevertheless, the lack of a clear dispersal syndrome for many of the Galapagos species does not preclude the possibility of "mud dispersal", various forms of endozoochory, or other overlooked dispersal mechanisms (e.g., Porter, 1976; Heleno & al., 2011; Nogales & al., 2012; Vargas & al., 2012). Recently, Andrew Green and collaborators (pers. comm.) retrieved seeds of *Cuscuta* sp. from the end of the digestive tract of the migratory northern pintail duck in coastal marshes of northern California, and these seeds germinated easily on filter paper. Another species of dodder, *C. acuta* Engelm. (sect. *Umbellatae* (Yunck.) Costea & Stefanović; Costea & al., 2015a) was also considered initially endemic to the Galapagos (Engelmann, 1859; Yuncker, 1932; Wiggins & Porter, 1971) only to be discovered more recently on the Pacific coast of South America (Austin, 1982; Costea & Stefanović, 2010). This latter species is not weedy and human introduction to the Galapagos can be ruled out with even more confidence. Therefore, more research is necessary to clarify the natural dispersal means of *Cuscuta* but this direction of investigation may provide the necessary biological clue to understand other cases of long-distance dispersal both in sect. *Cleistogrammica* and other clades of *Cuscuta* (García & al., 2014). Thus, even as a variety of *C. campestris*, *C. gymnocarpa* offers an opportunity to study long distance-dispersal and incipient stages of allopatric speciation in *Cuscuta*.

■ TAXONOMIC TREATMENT

In view of the fact that *C. campestris* is currently the most common worldwide species of dodder, merging *C. gymnocarpa* to it would generate a significant amount of nomenclatural confusion because the latter binomial has priority. For this reason, to preserve nomenclatural stability, we proposed separately (Costea & al., 2015b) to conserve the name *C. campestris* against *C. gymnocarpa*.

Cuscuta modesta Costea & Stefanović, **sp. nov.** – Holotype: MEXICO. Coahuila. Paila, between Torreon and Saltillo, 14 Oct 1958, *Jones 22545* (MO!; isotype NY!). — For images of the holotype, see Fig. 6.

Cuscuta modesta resembles morphologically *Cuscuta glabrior* but differs from it in the fleshier flowers, especially in the receptacle and calyx base area, the absence of a saccate corolla, and much larger seeds.

Stems medium or slender, orange. Inflorescences few-flowered but usually confluent; pedicels 0.2–0.6 mm long; bracts 1 at the base of clusters, absent at the base of flowers, 0.8–1.2 × 1–1.5 mm, broadly ovate, obtuse, margins entire.

Flowers (4)5-merous, 3–4 mm long, fleshy, white when fresh, reddish-brownish when dried; papillae absent but cells of perianth epidermis and ovary large with external periclinal walls convex; laticifers visible in the calyx and corolla, isolated, ovoid; calyx 1.3–1.6 mm long, red-brownish, not reticulate or glossy, fleshy at the base, cupulate, shorter or equaling corolla tube, divided ca. ½–⅔ the length, tube 0.5–0.8 mm long, lobes 0.8–1.2 mm long, overlapping at the base, broadly ovate to 1.5–2 times wider than long, not carinate or with multicellular protuberances on the midveins, margins entire, apex rounded; corolla 2.8–3.6 mm long, tube 1.6–2 mm long, cupulate, not saccate between the lines of stamen attachments, lobes 1.2–1.6 mm

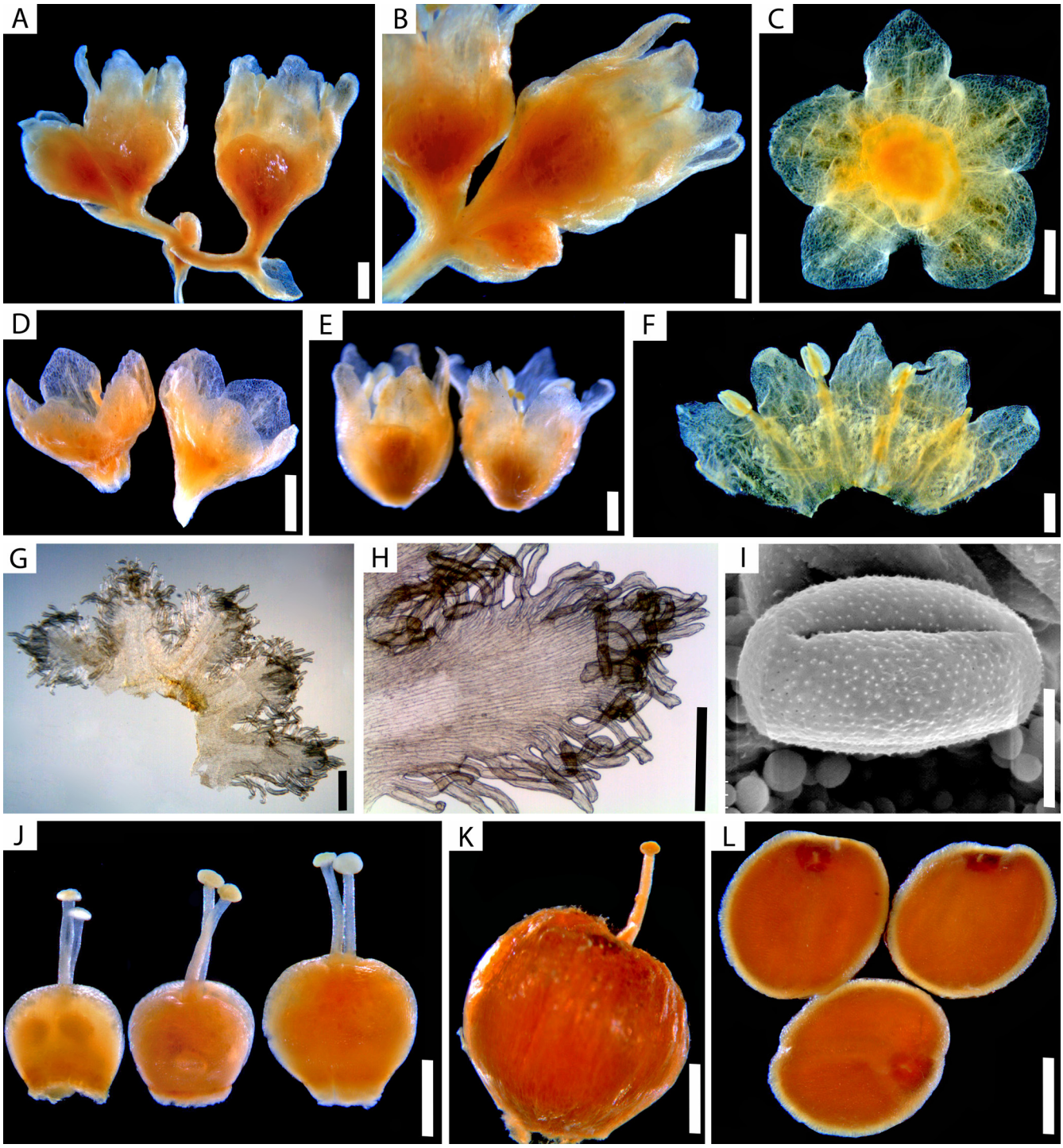

Fig. 6. Morphology of *Cuscuta modesta* (holotype; *Jones 22545*, MO): **A,** inflorescence; **B,** flowers; **C,** calyx, dissected; **D,** calyx (not dissected); **E,** corolla; **F,** corolla, dissected; **G–H,** infrastaminal scales (removed from the flower; **G,** general view; **H,** detail of fimbriae); **I,** pollen; **J,** gynoecium at different stages of flower maturation; **K,** capsules (one style is broken); **L,** seeds removed from one capsule. — Scale bars: A–F, J–L = 1 mm; G–H = 0.5 mm; I = 10 µm.

long, initially erect, later slightly spreading, shorter or nearly equaling the tube, ovate-triangular, not overlapping, margin entire or irregular, apex acute to subobtuse, usually ± inflexed; stamens not exerted, shorter than corolla lobes, anthers 0.5–0.7 × 0.4–0.5 mm, broadly oblong, filaments 0.4–0.7 mm long; pollen tricolpate, 21–25 µm long, prolate to subprolate, tectum imperforate or with a few isolated puncta, 0.1–0.2 µm in diameter; infrastaminal scales 1.6–2 mm long, equaling corolla tube, obovate to oblong, bridged at 0.5–0.7 mm, fimbriae numerous, 0.3–0.5 mm long; styles 0.8–1.3 mm long, shorter to equaling the ovary, thin, cylindrical. Capsules indehiscent, 2.5–4 × 2.5–5 mm, globose, not thickened and/or risen around the relatively large interstylar aperture, not translucent, surrounded by the withered corolla. Seeds 3–4 per capsule, 2.1–2.6 × 2–2.2 mm, angled, broadly-elliptic to subround, seed coat cells alveolate/papillate, hilum area 0.4–0.6 mm in diameter, scar 0.14–0.2 mm long.

Note. – The few specimens available, including the type, were identified by Yuncker as *Cuscuta decipiens* Yunck. or *C. aurea* Liebm., two species that belong to two different subg. *Grammica* clades (sect. *Californicae* (Yunck.) Costea & Stefanovic and sect. *Lobostigmae* Engelm., respectively; Costea & Stefanovic, 2009; Costea & al., 2013; Costea & al., 2015a). It resembles only superficially these two species in the fleshy flowers which become reddish-brown upon drying.

Etymology. – From the Latin "modestus" alluding to the unassuming and discreet nature of this species that managed to pass unnoticed by Yuncker, the genus monographer.

Distribution and ecology. – Mexico, Chihuahuan Desert in Coahuila. It grows in arid flats, parasitizing on *Flourensia* (Asteraceae). Flowering takes place between July and October.

Specimens examined. – Mexico. Coahuila. [Mpio. Metamoros], Filipinas, Oct 1910, *Purpus 4973* (GH, NY, MO, US); Hwy 40, 1.5 mi W of junction to Parras (W of Saltillo), 25°38′ N 102°11′ W, flat, many cacti, 22 Jul 1977, *Lehto & al. L21709* (ASU).

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

Cuscuta australis R.Br.: **547,** *Sykes 99* (CHR), China, EF194457, KT383063*/KT383064*/KT383065*/KT383066*/KT383067*/KT383068*/KT383069*; **679,** *Hosking 938* (CANB), Australia, NSW, EF194458, EF194668; **789,** *Beaughlehole 83203* (MEL), Australia, VIC, KT371708*, EF194669; **792,** *Curtis 124* (MEL), Australia, VIC, KT371709*, KT383070*/KT383071*/KT383072*/KT383073*/KT383074*/KT383075*; **1383,** Seed extraction (unknown origin; commercial herbal product sold as "*C. chinensis*"), KT371707*, KT383062*. ***C. australis*** var. ***cesatiana*** (Bertol.) Yunck.: **1271,** *Barath s.n.* (TRTE), Hungary, KT371710*, KT383076*. ***C. australis*** var. ***tinei*** (Insenga) Yunck.: **639,** *Thiebaut 3098* (NY), France, EF194460, KT383077*/KT383078*/KT383079*/KT383080*/KT383081*/KT383082*/KT383083*/KT383084*; **640,** *Simonkai 2635* (NY), Hungary, EF194459, KT383085*/KT383086*/KT383087*/KT383088*/KT383089*/KT383090*/KT383091*. ***C. bifurcata*** Yunck.: **1036,** *Paterson 578* (PRE), South Africa, EF194461, –. ***C. campestris*** Yunck.: **145,** *Stefanović SS-98-85* (no voucher), grown from seeds of unknown origin, KT371720*, KT383143*/KT383144*/KT383145*/KT383146*/KT383147*/KT383148*/KT383149*; **201,** *Rose 46281* (WTU), CA, KT371721*, KT383150*/KT383151*/KT383152*; **411,** *Stefanović SS-03-103* (TRTE), Serbia, EF194450, KT383153*/KT383154*/KT383155*/KT383156*; **415,** *Solomon 17192* (IND), U.S.A., MO, EF194455, KT383157*/KT383158*/KT383159*; **461,** *Weber 7446* (IND), U.S.A., CO, KT371722*, KT383160*/KT383161*/KT383162*/KT383163*; **468,** *Deam 63612* (IND), U.S.A., IN, KT371723*, KT383164*/KT383165*/KT383166*; **483,** *Pitzer 3765* (ASU), U.S.A., CA, EF194453, KT383167*/KT383168*/KT383169*/KT383170*/KT383171*/KT383172*/KT383173*; **487,** *Baker & Wright 11575-1* (ASU), U.S.A., AZ, EF194452, KT383174*/KT383175*/KT383176*/KT383177*; **527,** *Reddy & al. 1049* (J), South Africa, KT371724*, KT383178*/KT383179*/KT383180*/KT383181*/KT383182*/KT383183*/KT383184*; **718,** *Linder 3399* (BOL), South Africa, KT371725*, KT383185*/KT383186*/KT383187*/KT383188*; **894,** *Alava 11039* (RSA), Iran, EF194454, KT383189*/KT383190*/KT383191*/KT383192*/KT383193*/KT383194*; **1260,** *Tiehm 13390* (NY), U.S.A., NV, KT371711*, KT383092*/KT383093*/KT383094*/KT383095*/KT383096*/KT383097*/KT383098*; **1261,** *Diedrichsen V2000-10* (NY), Canada, SK, KT371712*, KT383099*/KT383100*/KT383101*/KT383102*/KT383103*/KT383104*; **1262,** *Welsh & al. 27834* (NY), U.S.A., UT, KT371713*, KT383105*/KT383106*/KT383107*/KT383108*/KT383109*; **1263,** *Henderson 1527* (USAS), U.S.A., VI, KT371714*, KT383110*/KT383111*/KT383112*/KT383113*/KT383114*/KT383115*/ KT383116*; **1264,** *Néron 01-02* (QUE), Canada, QE, KT371715*, KT383117*/KT383118*; **1272,** *Stefanović SS-11-23* (TRTE), U.S.A., MA, KT371743*, KT383119*/KT383120*/KT383121*/KT383122*/KT383123*/KT383124*/KT383125*; **1278,** Cranberry Research Station, no voucher, U.S.A., MA, KT371716*, KT383126*/KT383127*/KT383128*/KT383129*/KT383130*/KT383131*/KT383132*; **1332** (no voucher), grown from seeds obtained from USDA NPGS, ARS-WRPIS (accession W6 30332, Donor accession identifier NV030-037), misidentified as *C. pentagona* var.

Appendix 1. Continued.

pentagona, NV, KT371717*, –; **1333** (no voucher), China, extraction from seeds sold as "*C. chinensis*" herbal supplement, KT371718*, KT383133*/KT383134*/KT383135*/KT383136*/KT383137*; **1352,** Extraction from seed (no voucher), Israel, KT371719*, KT383138*/KT383139*/KT383140*/KT383141*/KT383142*. ***C. glabrior*** (Engelm.) Yunck.: **596,** *Palmer 723* (GH), Mexico, COA, EF194470, KT383195*; **742,** *Cory 42164* (NY), U.S.A., TX, EF194471, KT383197*/KT383198* /KT383199*/KT383200*/KT383201*/KT383202*; **825,** *Villarreal & Vásquez 6154* (XAL), Mexico, COA, EF194472, KT383203*/KT383204*/KT383205*/KT383206*/KT383207*; **1158,** *Villarreal & Valdés 5676* (ARIZ), Mexico, COA, KT371706*, KT383195*; **1168,** *Villarreal & Valdés 5676* (BRIT), Mexico, COA, KT371726*, –. ***C. gymnocarpa*** Engelm.: **1017,** *Mears & Andersen 5288* (TEX), Ecuador, Galapagos, EF194456, KT383208*/KT383209*/KT383210*/KT383211*/KT383212*/KT383213*. ***C. harperi*** Small: **594,** *Demaree 46295* (NY), U.S.A., AL, EF194464, EF194681; **1258,** *Harper 147* (ARIZ), U.S.A., AL, KT371727*, KT383214*; **1259**, *Harper 3997* (NY), U.S.A., AL, KT371728*, KT383215*/KT383216*/KT383217*/KT383218*/KT383219*; **1268,** *Harper 6479* (CAS), U.S.A., AL, KT371729*, KT383220*/KT383221*/KT383222*/KT383223*/KT383224*; **1269,** *Churchill 86124* (CAS), U.S.A., AL, KT371730*, KT383225*. ***C. modesta*** Costea & Stefanović: **565,** *Purpus 4973* (GH), Mexico, COA, KT371731*, KT383231*; **750,** *Jones 22545* (NY), Mexico, COA, KT371732*, KT383232*/KT383233*/KT383234*/KT383235*/KT383236*/KT383237*; **1037,** *Lehto & al. L21709* (ASU), Mexico, COA, KT371733*, KT383226*/KT383227*/KT383228*/KT383229*/KT383230*. ***C. obtusiflora*** Kunth: **1047,** *Pedersen 3688* (US), Argentina, CR, KT371734*, KT383238*/KT383239*/KT383240*/KT383241*; **1069,** *Skolnik & Barkley 19ANL23* (US), Colombia, Antioquía, EF194463, EF194674. ***C. obtusiflora*** var. ***glandulosa*** Engelm.: **746**, *Mitchell 3387* (NY), U.S.A., OK, EF194462, EF194675; **747**, *Lundell & Lundell 11717* (NY), U.S.A., TX, KT371736*, KT383242*/KT383243*/KT383244*/KT383245*/KT383246*/KT383247*. ***C. pentagona*** Engelm.: **161**, *Taylor 5765* (WTU), U.S.A., MO KT371745*, –; **456,** *Lakela 26019* (IND), U.S.A., FL, EF194465, EF194664/EF194678/KT383267*; **464,** *Taylor 5765* (IND), U.S.A., MO, EF194467, KT383268*/KT383269*/KT383270*/KT383271*/KT383272*/KT383273*; **1250,** Cranberry Research Station, no voucher, U.S.A., MA, KT371737*, KT383248*/KT383249*, **1255**, *Marshal M1874* (DAO), Canada, MB, KT371738*, KT383250*/KT383251*/KT383252*/KT383253*; **1256**, *Chase 1128* (DAO), U.S.A., IL, KT371739*, KT383254*; **1265,** *Shimmers 15030* (BRIT), U.S.A., TX, KT371740*, KT383255*/KT383256*/KT383257*/KT383258*; **1266,** *Smith 1692* (SMU), U.S.A., AR, KT371741*, KT383259*/KT383260*; **1267,** *Curtis 5881* (CAS), U.S.A., FL, KT371742*, KT383261*; **1304,** *Taylor & Taylor 13414* (BRIT), U.S.A., MS, KT371744*, KT383262*/KT383263*/KT383264*/KT383265*/KT383266*. ***C. plattensis*** A. Nelson: **589,** *Degener & Peiler 16242* (NY), U.S.A., WYO KT371746*, KT383274*/KT383275*/KT383276*/KT383277*/KT383278*/KT383279*; **590,** *Dorn 5470* (NY), U.S.A., WY, EF194468, KT383280*/KT383281*/KT383282*/KT383283*/KT383284*/KT383285*/KT383286*. ***C. polygonorum*** Engelm.: **1245,** *Gregory s.n.* (TRTE), Canada, ON, KT371735*, KT383287*/KT383288*/KT383289*/KT383290*/KT383291*/KT383292*. ***C. runyonii*** Yunck.: **660,** *Flyr 368* (TEX/LL), U.S.A., TX, EF194469, KT383299*/KT383300*/KT383301*/KT383302*/KT383303*/KT383304*/KT383305*/KT383306*; **1257,** *Runyon 2622* (DAO), U.S.A., TX, KT371747*, KT383293*/KT383294*/KT383295*/KT383296*/KT383297*/KT383298*. ***C. sandwichiana*** Choisy: **155,** *Degener & Degener 36596* (WTU), U.S.A., HI, EU288333, –; **686,** *Sylva & Rumel s.n.* (NY), U.S.A., HI, EU288335, –; **748,** *Degener & Degener 35248A* (CANB), U.S.A., HI, EU288334, –. ***C. stenolepis*** Engelm.: **779,** *Ollgaard 99142* (QCNE), Ecuador, Pichincha, EF194473, EF194687; **781,** *Núñez & al. 034* (QCNE), Ecuador, Pichincha, EF194474, KT383307*.

Appendix 2. Taxa sampled for the morphometric studies and herbarium vouchers.

Cuscuta campestris Yunck. (cam). **ARGENTINA** (Arg1) Corrientes, 17 Nov 2010, *González & Medina 317* (WLU); (Arg2) Valle del Rio Chubut, 25–30 Jan 1944, *Hunziker 4632* (US). **AUSTRALIA** (Aus1) Laidley, 27 Jan 1944, *Clemens s.n.* (OSC); (Aus2) Iron Pot Creek, 30 Dec 1981, *Wilson 4253* (RSA). **BAHAMA ISLANDS** (BI), Grand Bahama, 20 Aug 1974, *Correll & Kral 43076* (GH). **BRITISH HONDURAS** (BH), Stann Creek Distr., 13 Apr 1953, *Gentle 7914* (GH). **CANADA.** BC, Osoyos, 28 Jun 1992, *Lomer 92-98* (UBC). ON(1), Essex Co., East Sister Island, 31 Aug 1988, *Oldham & al. 8621* (DAO); ON(2) Kenora Co., shoreline E of Grassy Narrow Lodge, 18 Sep 2003, *Oldham & Foster 29902a* (WLU); ON(3) Aulneau Peninsula, 15 Sep 2003, *Oldham & Bakowsky 29774* (WLU); ON(4) Assabaska Ojibway Park, *Oldham & Bakowsky 29758* (WLU). QUE, Ile-de-Montreal, Aug 2001, *Neron 01-12* (QUE). **CHILE** (Chi), Valle de Teno, 18 Jan 2011, *Muñoz 5174* (WLU). **CHINA** (Chi), Xinjiang Uygur Zizhiqu, N margin of Tien Shan, 16 Jun 1989, *Liston 823-21* (RSA). **ENGLAND** (Eng), Kew, 14 Aug 1958, *Halliday & Uchlem 182* (ASU). **FRANCE** (Fra), Drôme, *Convolvulus arvensis*, summer 1987, *Labbe s.n.* (OSC). **HAITI** (Ha), Navassa Island, 20–23 Jun 1956, *Proctor 15489* (GH). **LESSER ANTILLES** (LA1) Marie Galante, 4 Dec 1959, *Proctor 20280* (GH); (LA2) Marie Galante, 27 May 1960, *Proctor 21056* (GH). **MEXICO** (Mex1), Guanajuato, Salvatierra, 2006, *Carranza 7193* (WLU). (Mex2) Jalisco, Mpio. Autlan de Navarro, 17 Jul 2012, *Robles s.n.* (WLU). (Mex3) Sonora, Mpio Etchojoa, Los Tejabanes, Etchojoa, 12 Jun 2010, *Sainz s.n.* (WLU). **PARAGUAY** (Par1) Asunción, 24 Dec 1972, *Schinini 5691* (CTES); (Par2) 10 km SW of Nueva Italia, 7 Dec 1990, *Zardini & Velázquez 24883* (MO). **SLOVAKIA** (Slo) close to Leopoldov, 29 Aug 1948, *Stanek 1355* (ASU). **THAILAND** (Tha), Northern Chiang Mai Province, Chiang Dao, 17 Nov 2010, *Staples & al. 1381* (WLU). **U.S.A.** AZ, Maricopa Co., Salt/Gila River confluence, 17 Aug 1985, *Amadeo 845b* (UCR). CA(1), Imperial Co., Brawley, 16 Aug 1966, *Wright s.n.* (UCR); CA(2) Riverside Co., Riverside, 23 Oct 1978, *Clarke 17381* (UCR); CA(3) Riverside, 7 Jul 1995, *White 3452* (UCR); CA(4) 4 Jul 2000, *Provance 2105* (UCR); CA(5) Temecula Valley, 21 Oct 1994, *White 2505* (UCR); CA(6) Santa Ana River, 28 Jul 1994, *Sanders 15174* (UCR); CA(7) San Jacinto Valley, 26 Sep 1999, *Sanders & Provance 23127* (UCR); CA(8) 15 Oct 1999, *Sanders 23171* (UCR); CA(9) San Bernardino Co., Mojave Desert, 26 Sep 1991, *Myers & White s.n.* (UCR); CA(10) San Bernardino Mts., 6 Oct 1997, *Pitzer 3284* (UCR); CA(11) 17 Oct 1998, *Pitzer 4210* (UCR); CA(12) Colton, 25 Jul 2000, *Provance 2161* (UCR); CA(13) Bloomington/Crestmore, 1 Sep 2000, *Provance 2227B* (UCR). FL, Dade Co., Miami, 12 Nov 1974, *Correll 43759* (NY). ID, Ada Co., NE side of Boise, 13 Aug 1980, *Ertter & Strachan 3951* (NY). IL, Kendall Co., 1.5 mi NE of Yorkville, 17 Aug 2005, *Hill 36581* (NY). KS, Anderson Co., S Edge of Garnett, 18 Jun 2002, *Morse & Loring 8193* (UCR). KY, Fayette Co., Lexington, 2 Sep 1944, *McFarland 70* (NY). MD, Wicomico Co., Willards, 4 Sep 1942, *Moldenke 13847* (OSC). NC, Madison Co., 23 Jul 1966, *Pence 45040* (OSC). NV(1), Lander Co., Smoky Valley, 17 Sep 1968, *Howell & True 45430* (NY); NV(2) Humbolt Co., Humboldt River, 31 Aug 2000, *Tiehm 13390* (OSC). OK(1), Payne Co., Stillwater, 14 Jul 1938, *Whitehead 56* (OSC); OK(2) 1984, *Lipscomb s.n.* (SMU). OR, Truax Island, 2 Oct 2007, *Halse 7419* (OSC). PA, cultivated at PEN State University, *Smith s.n.* (WLU). TX, Travis Co., 2 mi of Bull Creek, 5 Jul 1944, *Barkley & Ripperton s.n.* (OSC). UT(1), Salt Lake Co., Wasatch Range, 1 Sep 1975, *Arnow 4694* (NY); UT(2) Sanpete Co., 4 mi NW of Fountain Green, *Neese & White 3682* (NY). WV, Gilmer Co., Glenville, 10 Sep 1949, *Davis & Davis 9021* (OSC). **VENEZUELA** (Ven1) Federal District, 7 Jan 1924, *Pittier 11359* (GH); (Ven2) Miranda, 6–8 Mar 1943, *Killip & Tamayo 37010* (US).

Cuscuta glabrior Yunck. (Gla). **MEXICO.** (Mex1) Coahuila, Sierra de Parras, 21 Aug 1982, *Cowan 3644* (MEXU); (Mex2) Sierra de Parras, 9 May 1987, *Villarreal 3623* (MEXU); (Mex3) Muzquiz, Apr 1938, *Marsh 1115* (SMU); (Mex4) Sierra de Los Alamitos, 29 Sep 1973, *Henrickson 13676c* (RSA); (Mex5) Nova Rosita, 13 Aug 1948, *Kenoyer & Crum 4143* (GH); (Mex6) 50 Km S of Saltillo, 9 Jun 1990, *Villarreal & Valdés 5676* (ARIZ); (Mex7) Chojo Grande, 16 Jul 1905, *Palmer 723* (GH); (Mex8) Coahuila, Saltillo, 10–20 Nov 1902, *Palmer 307* (MO); (Mex9) Coahuila, 18 mi NE of Saltillo, 6 Aug 1957, *Waterfall & Wallis 13240* (BRIT); (Mex10) Nuevo León, 8 Km E of Saltillo, 18 Dec 1991, *Prather & Soule 900* (CAS). **U.S.A.** OK, Love Co., 8 mi W of Marietta, 28 Sep 1973, *Taylor & Taylor 15121* (SD). TX(1), *Drummond 247* (Type of *C. pentagona* var. *verrucosa*; GH). TX(2) Angelina Co., E of Wells, 8 Sep 1942, *Lundell & Geiser 11792* (NY). TX(3) Bell Co., Near Little River, 11 Jun 1930, *Wolff 2274* (NY); TX(4) near Killen, 14 Aug 1931, *Wolff 3270* (NY). TX(5) Cameron Co., San Benito, 2 May 1941, *Runyon 2624* (NY). TX(6) Coleman Co., 7 mi E of Santa Anna, 29 Jun 1959, *Correll & Johnston 19019* (NY). TX(7) Dallas Co., Highland Park, 21 Jun 1940, *Lundell & Lundell 9595* (NY); TX(8), E side of White Rock Lake, 21 Aug 1942, *Lundell 11596* (NY); TX(9) along White Rock Creek, 26 Aug 1942, *Lundell 11674* (MO); TX(10) Deaf Smith Co., 15 mi N and 15 mi W of Hareford, 23 Jul 1966, *Waller 962* (TEX/LL). TX(11) Del Rio, 10 Apr 1930, *Jones 26237* (CAS). TX(12) Kerr Co., 31 May 1916, *Palmer 9965* (CAS). TX(13) Neuces Co., Corpus Christi, 9–12 Apr 1894, *Heller 549* (NY). TX(14) Randall

Appendix 2. Continued.

Co., Buffalo Lake, 3 Aug 1975, *Higgins 9567* (NY). TX(15) Val Verde Co., Seminole Canyon State Park, 8 Aug 1975, *Snyder 395* (BRIT); TX(16) along Devil's River, 26 Sep 1953, *Warnock 11655* (SD); TX(17) W side of Devil's River, 8 May 1947, *Whitehouse 18582* (BRIT).

Cuscuta gymnocarpa Engelm. (Gym). **ECUADOR,** Galapagos (Ga1) **Ferdinanda Isl.,** 3–5 Feb 1964, *Fosberg 45043* (US). (Ga2) **Isabela Isl.,** Tagus Cove, 24 May 1932, *Howell 9493* (US); (Ga3) Tagus Cove, Mar 1927, *Stewart 3092* (F); (Ga4) 19 Jun 1974, *Van der Werff 1254* (CAS); (Ga5**) Española Isl.,** [no date], *Fagenlind & Wibom 3641* (S). (Ga6) **Floreana Isl.,** 6 May 1967, *Eliasson 2079* (S); (Ga7) May 1975, *Van der Werff 2068* (CAS); (Ga8) Post Office Bay, 23 Oct 1932, *Howell 8825* (CAS). (Ga9) 8 Feb 1964, *Hendrickson H-68* (CAS); (Ga10) **Santa Cruz Isl.,** summit of El Chato, 31 Jul 1966, *Wiggins 458* (CAS); (Ga11) 8 mi W of Academy Bay, 11 Apr 1930, *Svenson 242* (F).

Cuscuta pentagona Engelm. (Pen). **U.S.A.** AK, Stone Co., Optimus, 12 Jul 1942, *Demaree 23483* (NY). AL, Marengo Co., 2.3 mi S of Demopolis, 6 Jun 1968, *Kral 31225* (SMU). DE, Dover, Aug 1863, *Canby s.n.* (NY). DC(1) Eckington, 15 Jul 1893, *Buettcher 122* (CAS); DC(2) Washington, Jul 1893, *Holm s.n.* (AAU). FL(1) Levy Co., Cedar Key, 10 May 1958, *Goodfrey* 56580 (RSA); FL(2) Gulf Co., Wawahitchaka, 17 Jun 1964, *Demaree 50393* (SMU). GA, Kalb Co., Little Stone Mt, 25 Jul 1893, *Small s.n.* (F). IN(1) near Lake Maxinkuckee, 14 Oct 1900, *Scovell & Clark* 1095 (CAS); IN(2) Posey Co., 12 mi SE of Mt. Vernon, 5 Jan 1920, *Deam 25430* (IND); IN(3) probably 1910–1012, *Grimes s.n.* (NY); IN(4) Starke Co., 2.5 mi SE of North Judson, 18 Jul 1930, *Deam 49139* (IND); IN(5) Cass Co., 1.5 mi NW of Lake Cicott, 1 Oct 1940, *Deam 60219* (IND). KS, Trego Co., 19 mi W of Collier, *David & Horr 4136* (NY). MA(1) Tonset, 27 Aug 1901, *Elmondson* 2777 (NY); MA(2) Middlesex Co., 15 Sep 1906, *Bartlett 691* (IND); MA(3) Middlesex Co., Winchester, 22 Sep 1908, *Fernald & Weatherby 259* (RSA). MD, Calvert Co., Scientists' Cliffs, 13 Aug 1957, *Seymour 17466* (MO). MI, Kalamazoo Co., Fort Custer, 12 Aug 1945, *Hanes 4541* (NY). MO(1) St. Louis Co., Allenton, 13 Aug 1933, *Lodewyks 38* (MO); MO(2) Cockerell, 3 Jul 1898, *Bush 10* (MO). MS, Jackson Co., Petit Bois Island, 27 May 1973, *Taylor & Taylor 13414* (BRIT). NJ(1) Bay Head, 31 Jul 1910, *MacKenzie 4742* (NY); NJ(2) 24 Aug 1900, *Stockton s.n.* (NY). NY, Long Island, 8 Aug 1909, *Bicknell s.n.* (NY). TN(1) Jun 1894, *Ruth 315* (NY); TN(2) vicinity of Nashville, [no date], *Gattinger s.n.* (CAS). TX(1) Brazos Co., College Station, 8 Jul 1946, *Parks s.n.* (RSA); TX(2) Wise Co., near West Cross Timbers, 21 Jun 2003, *O'Kennon & McLemore 18605* (TEX/LL). VA(1), Norfolk 1849, *Rugel s.n.* (MO; type); VA(2) Arlington Co., Hatfield, 9 Jul 1939, *Herman 10391* (NY); VA(3) W of Williamsburg, 15 Aug 1921, *Weatherby 4230* (NY); VA(4) Bedford Co., Aug 1872, *Curtiss s.n* (NY).

Appendix 3. Characters scored for the morphometric study.

Continuous characters. **1.** Flower length (mm; measured from base of receptacle to the tip of corolla lobes). **2.** Calyx lobe length (mm). **3.** Calyx lobe width (mm). **4.** Calyx tube length (mm). **5.** Calyx surface (mm²). **6.** Corolla lobe length (mm). **7.** Corolla lobe width (mm). **8.** Calyx tube length (mm). **9.** Corolla tube circumference (mm). **10.** Corolla surface (mm²). **11.** Stamen filament length (mm). **12.** Anther length (mm). **13.** Anther width (mm). **14.** Infrastaminal scale (IFS) length (mm). **15.** Width of IFS at the base (mm). **16.** Width of unfringed part of IFS (mm). **17.** Interscale bridge length (mm). **18.** Longest fimbria length (mm). **19.** Number of fimbriae per IFS (nr). **20.** Capsule width (mm). **21.** Capsule length. **22.** Seed length (mm). **23.** Seed width (mm). **24.** Hilum area diameter (mm). **25.** Vascular scar length (mm).

Qualitative characters. **26.** Calyx lobes with auricles at the base: present (1), absent (0). **27.** Calyx lobes overlapping: present (1), absent (0). **28.** Calyx papillae: present (1), absent (0). **29.** Saccate corolla tube: present (1), absent (0). **30.** Corolla papillae: present (1), absent (0). **31.** Ovary papillae: present (1), absent (0). **32.** Corolla persistence on the capsule: enveloping 1/2–2/3 capsule (1) or found at the base of the capsule (0).