Plant Syst Evol (2010) 285:83–101
DOI 10.1007/s00606-009-0259-4

ORIGINAL ARTICLE

Pollen evolution and its taxonomic significance in *Cuscuta* (dodders, Convolvulaceae)

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Received: 12 June 2009 / Accepted: 28 December 2009 / Published online: 27 February 2010

Abstract The pollen morphology of 148 taxa (135 species and 13 varieties) of the parasitic plant genus *Cuscuta* (dodders, Convolvulaceae) was examined using scanning electron microscopy. Six quantitative characters were coded using the gap-weighting method and optimized onto a consensus tree constructed from three large-scale molecular phylogenies of the genus based on nuclear internal transcribed spacer (ITS) and plastid *trn*-LF sequences. The results indicate that 3-zonocolpate pollen is ancestral, while grains with more colpi (up to eight) have evolved only in two major lineages of *Cuscuta* (subg. *Monogynella* and clade O of subg. *Grammica*). Complex morphological intergradations occur between species when their tectum is described using the traditional qualitative types—imperforate, perforate, and microreticulate. This continuous variation is better expressed quantitatively as "percent perforation," namely the proportion of perforated area (puncta or lumina) from the total tectum surface. Tectum imperforatum is likely the ancestral condition, while pollen grains with increasingly larger perforation areas have evolved multiple times. The reticulated tectum, unknown in other Convolvulaceae, has evolved in *Cuscuta* only in two lineages (subg. *Monogynella*, and clade O of subg. *Grammica*). Overall, the morphology of pollen supports *Cuscuta* as a sister to either the "bifid-style" Convolvulaceae clade (Dicranostyloideae) or to one of the members of this clade. Pollen characters alone are insufficient to reconstruct phylogenetic relationships; however, palynological information is useful for the species-level taxonomy of *Cuscuta*.

Keywords Convolvulaceae · *Cuscuta* · Dodders · Evolution · Phylogeny · Pollen morphology · Scanning electron microscopy · Taxonomy

Introduction

The taxonomic significance of pollen morphology in Convolvulaceae has long been recognized. For example, Hallier (1893) assigned the genera within this family to two major groups, "Echinoconieae" and "Psiloconiae," based on their echinate or psilate exine, respectively. Together with other characters, the diversity of pollen morphology in the morning glory family has served for the separation of genera such as *Calystegia* and *Convolvulus* (Lewis and Oliver 1965), *Stylisma* and *Bonamia* (Lewis 1971), *Merremia* and *Operculina* (Ferguson et al. 1977), and *Maripa*, *Dicranostyles*, and *Lysiostyles* (Austin 1973a, 1973b), as well as for the circumscription of species, e.g., *Ipomoea* spp. (Hsiao and Kuoh 1995) and *Convolvulus* spp. (Menemen and Jury 2002). Not surprisingly, pollen has been used to assess the evolutionary relationships in Convolvulaceae. For instance, Sengupta (1972) proposed an evolutionary arrangement of the family with four major pollen types based on the number and distribution of

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Electronic supplementary material The online version of this article (doi:10.1007/s00606-009-0259-4) contains supplementary material, which is available to authorized users.

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apertures. Tellería and Daners (2003) found exine to be more relevant taxonomically than aperture features, and based on the former characters, distinguished three major groups of pollen: tectate, microechinate-perforate; tectate, microechinate-perforate with microspines; and semitectate, microechinate-microreticulate.

Cuscuta (dodders), a genus comprising over 180 species of holoparasitic vines (Stefanović et al. 2007), is nested within Convolvulaceae (Stefanović and Olmstead 2004). It represents the third most economically important group of parasitic plants after *Striga* and *Orobanche*, because infestation by ca. 15 of its species can result in significant yield losses in numerous crops worldwide (Parker and Riches 1993; Dawson et al. 1994; Costea and Tardif 2006). Additionally, numerous *Cuscuta* species are rare and endangered, requiring conservation measures (Costea and Stefanović 2009a).

As a result of their parasitic lifestyle, dodders exhibit extreme reductions of the vegetative structures, limiting the morphological characters available for systematic studies to flowers and fruit (Stefanović et al. 2007). It is therefore imperative to search for and discover new characters useful for the taxonomy of the genus, as well as to create a theoretical basis for character evolution analysis.

Pollen information for *Cuscuta* is relatively scarce. Das and Banerji (1966) described the “rugulate” pollen surface of *C. santapaui* and *C. reflexa*, while Jain and Nanda (1966) compared the pollen morphology of *Cuscuta hyalina* and *Convolvulus pluricaulis* Choisy. Sengupta (1972) studied 21 species of *Cuscuta*, which he divided into two groups according to their tricolpate or penta-hexa-colpate pollen. Liao et al. (2005) analyzed four species from Taiwan, also recognizing two main types of pollen based on exine morphology. Type 1, observed in *C. australis*, *C. campestris*, and *C. chinensis*, is characterized by an ektexine “finely reticulate,” whereas type 2 exhibited a reticulate ektexine, as seen in *C. japonica* (Liao et al. 2005). Despite the limited sampling, these studies concluded that pollen provides important phylogenetic and taxonomic information. Recently, Costea et al. (2006a, 2006b, 2006c, 2006d, 2008a, 2008b) described the pollen of 24 species as a part of taxonomic revisions of major clades that belong to subg. *Grammica*. Therefore, to date, pollen morphology of only about one-quarter of *Cuscuta* species is known. More importantly, pollen diversity in this genus has never been analyzed in a broad-scale evolutionary context, in a firmly established phylogenetic framework.

The precise sister group relationships of *Cuscuta* with other Convolvulaceae members are not clear (Stefanović et al. 2002, 2003; Stefanović and Olmstead 2004). However, well-supported phylogenies based on both plastid and nuclear datasets are available for the genus itself (García and Martin 2007; Stefanović et al. 2007). This newly established phylogenetic framework enables the examination of pollen characters from an evolutionary perspective. Thus, the main goals of this study are to: (1) survey the diversity of pollen morphology across the genus, (2) place this morphological variation into an evolutionary context, and (3) assess the usefulness of pollen exine morphology for the systematics of *Cuscuta*.

Materials and methods

Sampling and scanning electron microscopy

A total of 148 taxa (135 species and 13 varieties) were examined using 372 herbarium specimens (Appendix). Efforts were made to sample multiple accessions, particularly for those species spanning large biogeographical ranges and/or having a diverse morphology. As a result, with the exception of the species known from only one specimen, all of the examined taxa are represented by two or more collections. Mature anthers were fragmented on the stubs without acetolysis to preserve the exine and intine (Harley and Ferguson 1990). Samples were coated with 20 nm of gold using an Emitech K 550 sputter-coater, and examined with a Hitachi S-570, Hitachi SU-1500 or LEO 1530 FE-SEM at 10-15 kV. Photographs illustrating the details of pollen for all the taxa are provided on the Digital Atlas of *Cuscuta* website (Costea 2007 onwards). Pollen measurements were performed on digital SEM images using Carnoy 2.0 for Mac OS X (Schols et al. 2002), and ImageJ (Abramoff et al. 2004) was used for determination of areas.

Pollen characters

We used the terminology of Punt et al. (2007) to preliminarily evaluate the morphological variation of tectum perforations into discrete types, potentially utilizable as qualitative state characters. The corresponding tectum types encountered in *Cuscuta* are: imperforate (no perforations present), perforate (tectum with puncta <1 μm), microreticulate (reticulate ornamentation consisting of muri and lumina <1 μm), and reticulate (similar to the previous, but lumina >1 μm) (Punt et al. 2007). However, the tectum variation in *Cuscuta* could not be consistently separated into these types, because complex morphological intergradations occur, especially among the imperforate, perforate, and microreticulate pollen grains of different species. Therefore, we defined tectum variation quantitatively as “percent perforation,” namely the proportion of the perforation surface (puncta or lumina) from the total surface of the tectum. Comparable quantitative measures, e.g., the "perforation density" (Vezey et al. 1991) and "percent tectum coverage" (Vezey et al. 1992), have previously been used in other groups of plants, yet they have not achieved widespread acceptance despite the fact that they provide a more accurate description of tectum morphology.

Five other quantitative characters—pollen length (polar axis), polar/equatorial (P/E) ratio, average diameter of perforations (puncta or lumina), average surface of perforations, and number of colpi—were also examined. The number of colpi exhibited a discrete variation: 3(–4) or (4–) 5–8 colpi. The remaining characters varied continuously and were coded using Thiele's (1993) gap-weighting method as implemented by MorphoCode (Schols et al. 2004). Gap-weighting was preferred to various gap-coding methods (reviewed by Wiens 2001; Swiderski et al. 1998) because of the better phylogenetic signal recovered (see also García-Cruz and Sosa 2006). The maximum number of resulted character states (*n*) was ten for all the quantitative characters, except for the percent perforation where *n* was eight. Eight character states were sufficient to describe tectum perforation patterns (Table 1).

Using formal outgroup analysis (e.g., Maddison et al. 1984) to determine character polarity in *Cuscuta* is hindered by two factors. First, despite considerable efforts, outgroup relationships of *Cuscuta* are unknown (Stefanović and Olmstead 2004). The position of *Cuscuta* in Convolvulaceae was, however, narrowed down to three possible phylogenetic scenarios (Stefanović et al. 2002; Stefanović and Olmstead 2004): (a) *Cuscuta* as a sister to the "bifid-style" clade (Dicranostyloideae) which comprises the tribes Hildebrandtieae, Cresseae, Dichondreae, and in part Convolvuleae, Poraneae, and Erycibeae; (b) *Cuscuta* as a sister to the "bifid clade" together with "clade 1" (Convolvuloideae) which includes the tribes Ipomoeae, Argyreieae, Merremiae, and some Convolvuleae. Together or individually, these major Convolvulaceae clades account for most of the diversity encountered in the family (e.g., "clade 1" alone has over two-thirds of the species in Convolvulaceae); (c) *Cuscuta* as a sister to one of the members of the "bifid clade," although this possibility was deemed "unlikely" and could not be formally tested because the relationships within this clade were unresolved (Stefanović et al. 2002; Stefanović and Olmstead 2004). Second, not all the Convolvulaceae genera/species from these groups have been studied in regards to their pollen morphology. For these reasons, our interpretation of character polarity in *Cuscuta* also takes into account the ingroup distribution of character states at the level of both *Cuscuta* and Convolvulaceae (reviewed by Stuessy 2008).

Characters were mapped onto a summary consensus tree built in MacClade 4 (Maddison and Maddison 2000), resulting from the combination of two large-scale molecular phylogenies of *Cuscuta* based on plastid *trn*-LF and nuclear ITS sequences (subg. *Cuscuta*, García and Martin 2007; subg. *Grammica* Stefanović et al. 2007), and an unpublished phylogeny of the entire genus (Stefanović and Costea, personal communication). ACCTRAN and DELTRAN were turned off and instances of bootstrap values below 85% were considered unresolved and are indicated in the tree as polytomies.

Results and discussion

Number of apertures

Pollen of *Cuscuta* is heteromorphic (sensu Till-Bottraud et al. 1995) (Fig. 1a–d). Over 95% of the species examined can be characterized as 3-zonocolpate, but this prevalent apertural type may be accompanied in the same anther by a small proportion of 4-, 5- or even 6-zonocolpate grains, and extremely rarely by pantocolpate grains. A similar variation of ±2 apertures can be observed in the species with preponderantly 5- and 7-zonocolpate pollen grains, which in addition may also produce pantocolpate morphs. Apertural heteromorphism is common in numerous angiosperms (Erdtman 1966; Van Campo 1976; Mignot et al.

Table 1 Percent perforation quantitative character states and their corresponding tectum "types" resulted from coding using Thiele's (1993) gap-weighting method

Percent perforation character states determined with MorphoCode (Schols et al. 2004) (%)	Diameter of puncta/lumina (μm)	Corresponding tectum "types"
0–2.1	0.2 (0–0.6)	Tectum imperforatum (TI)
2.6–6.3	0.43 (0.14–1.2)	Tectum perforatum 1 (TP1)
8.3–12.1	0.62 (0.14–1.5)	Tectum perforatum 2 (TP2)
12.5–17.3	0.67 (0.17–1.62)	Microreticulate 1 (MR1)
17.7–21.9	0.7 (0.2–1.77)	Microreticulate 2 (MR2)
30.8–31.3	1.9 (0.85–2.91)	Reticulate 1 (R1)
34.3	2.65 (1.52–3.85)	Reticulate 2 (R2)
43.6–44.6	3.03 (0.8–5.82)	Reticulate 3 (R3)

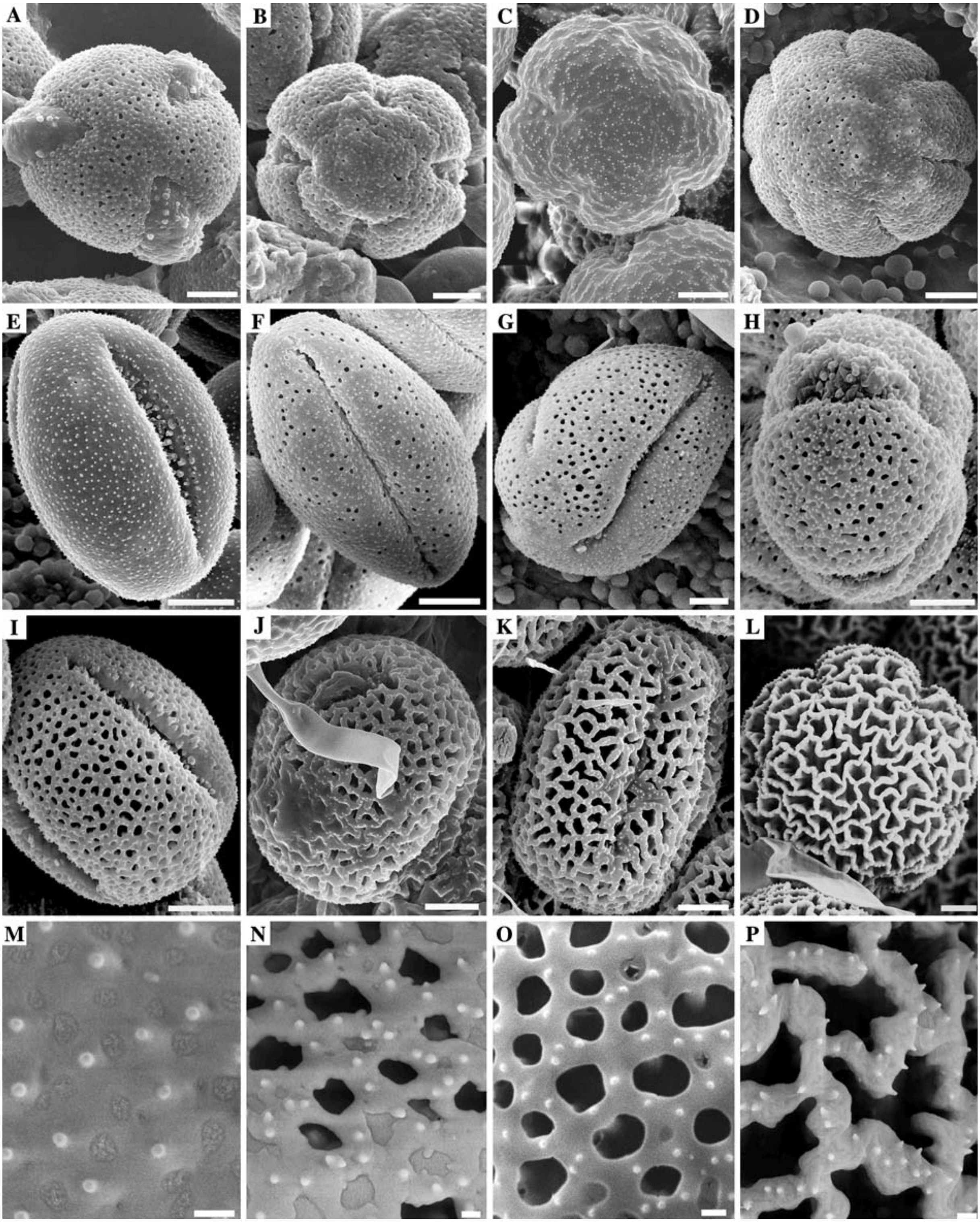

Fig. 1 Variation of colpi number (**a**–**d**). **a** *Cuscuta purpusii*, **b** *C. argentiniana*, **c** *C. parodiana*, **d** *C. boliviana*. Tectum variation (**e**–**l**, respectively); see Table 1 for abbreviations of tectum types. **e** *Cuscuta brachycalyx* (TI), **f** *C. odontolepis* (TP1), **g** *C. polyanthemos* (TP2), **h** *C. cozumeliensis* (MR1), **i** *C. chapalana* (MR2), **j** *C. cassytoides* (R1), **k** *C. japonica* (R2), **l** *C. reflexa* (R3). Tectum, surface detail (Table 1) (**m**–**p**) **m** *C. decipiens* (TI), **n** *C. exaltata* (TP2), **o** *C. mitriformis* (MR2), **p** *C. santapaui* (R3). *Scale bars* (A–L) 5 μm, (M–P) 0.5 μm

1994) and can be linked ontogenetically to the succession of events that take place during meiotic cytokinesis (Blackmore and Crane 1998; Ressayre et al. 2002, 2005). Experimental results from heteromorphic eudicots have shown that 4-apertured grains germinate faster than 3-aperturate ones, but the latter have faster pollenic tube growth and better survival than the former (Dajoz et al. 1991; Till-Bottraud et al. 1999). Together, these different morphotypes and their corresponding pollen strategies maximize the chances of successful fertilization under different conditions.

Although the number of apertures is not perfectly fixed within the species of *Cuscuta*, this character is phylogenetically informative. Sengupta (1972) suggested that the 5–6-colpate grains of *Cuscuta reflexa* are derived compared with the 3-colpate pollen encountered in other dodder species. Our results support this hypothesis, because pollen grains with a higher number of apertures (5–8) have evolved in *Cuscuta* from the ancestral state with three colpi only in two lineages (Fig. 2), in subg. *Monogynella* (*C. reflexa* and *C. japonica*) and in several species of a South American clade that belongs to subg. *Grammica* (clade O, see Stefanović et al. 2007). Sengupta (1972) proposed that an increased number of apertures in *Cuscuta* is associated with polyploidy. While *C. reflexa* is polyploid with several cytotypes ($2n = 28, 30, 32, 42, 48$; Kaul and Bhan 1977), the very scarce cytological information available for the remaining species does not seem to support this hypothesis. The entire genus is a polyploid complex, and some of the species with the highest numbers of chromosomes such as *C. campestris* ($2n =$ ca. 56), *C. cephalanthi*, and *C. gronovii* ($2n = 60$) (Pazy and Plitmann 1995) are tricolpate.

The number of apertures has received considerable attention in Convolvulaceae. Similarly to other eudicots, tricolpate pollen has been regarded as plesiomorphic, while 5-6-zonocolpate, pantocolpate, and pantoporate grains are considered progressively derived in the family (Wodehouse 1936; Vishnu-Mittre 1964; Manitz 1970; Muller 1970; Sengupta 1972; Austin 1973a, 1973b, 1998; Tellería and Daners 2003). This evolutionary sequence, termed “successiformy” by Van Campo (1976), can be encountered in genera from both Convolvuloideae and Dicranostyloideae. For example, in the former clade, zonocolpate pollen grains with five or more apertures are found in *Odonellia*, a genus with two species (Robertson 1982), and several *Meremia* spp. [e.g., *M. umbellata* (L.) Hallier, Sengupta 1972; Tellería and Daners 2003; Leite et al. 2005; *M. vitifolia* (Burm. f.) Hallier f., and *M. sibirica* (L.) Hallier f., Ferguson et al. 1977]. In Dicranostyloideae, *Maripa* and *Jacquemontia* species exhibit complex heteromorphic variation patterns from tricolpate to pantocolpate (Robertson 1971; Austin 1973b).

Exine

Exine in *Cuscuta* is tectate imperforate or semitectate, perforate to reticulate (Fig. 1e–l), with a single layer of unbranched columellae. Supratectal ornamentation typically consists of rounded to acute scabrate processes less than 1 μm long, more or less evenly distributed on the pollen surface (Fig. 1m–p). Larger supratectal conical spines (>1 μm) are present only in subg. *Monogynella* in *C. lehmanniana* and *C. monogyna*. Pollen with reticulate tectum is unknown in other Convolvulaceae (see below) and has evolved in *Cuscuta* only in some species of subg. *Monogynella* and clade O of subg. *Grammica* (Fig. 3; Table 2). Sengupta (1972) characterized the pantoporate pollen grains of *Ipomea* as “complex-reticulate,” namely reticulate with a superimposed hexagonal pattern (the metareticulate pollen of Borsch and Barthlott 1998; Tellería and Daners 2003) and suggested that this type might have originated from the hexacolpate, “simple reticulate” of *C. reflexa*. However, as reported by Tellería and Daners (2003), the metareticulate pollen of many *Ipomoea* spp. in fact has a microreticulate tectum, with a different exine structure and ornamentation.

Pollen grains with smaller perforations (usually ≤1 μm) form a continuous transition from imperforate to microreticulate (Fig. 1e–l) with the former condition prevalent in *Cuscuta* (ca. 60% of species) and encountered in many Convolvulaceae (Sengupta 1972; Austin 1973a, 1973b; Tellería and Daners 2003). Because of the intergradations observed, the types of pollen previously recognized on the basis of perforation size in *Cuscuta* (e.g., Liao et al. 2005) or those derived from the currently accepted tectum categories (Punt et al. 2007) are arbitrary ranges of variation. If a separation of “types” is desirable for description purposes, the template based on the eight quantitative character states (Table 1; Fig. 1e–l) provides a better resolution. Increasingly larger tectum perforation areas have evolved in subg. *Monogynella*, and multiple times in subgenus *Grammica* (Fig. 3), but the advantage of this feature in *Cuscuta* is unclear.

The evolution of tectum in *Cuscuta* parallels that of early angiosperms which were inferred to have had an imperforate or microperforate tectum, with the reticulate condition evolving in the common ancestor of Austrobaileyales and “mesangiosperms” (e.g., all angiosperms other than the ANITA lines; Doyle 2005, 2008). Reticulate exine is common in angiosperms, and it was debated whether if it is associated or not with sporophytic self-incompatibility (Zavada 1984, 1990; Gibbs and Ferguson 1987). Unfortunately, very little is known about the breeding systems in *Cuscuta* (Costea et al. 2006a; Costea and Tardif 2006). Reticulate pollen was functionally linked to entomophily (e.g., Ferguson and Skvarla 1982; Hesse 2000), hydrophily

Fig. 2 Colpi number optimized onto a summary consensus tree resulting from three molecular phylogenies of *Cuscuta* based on nuclear ITS and plastid *trn*-LF sequences (García and Martin 2007; Stefanović et al. 2007; Stefanović and Costea, personal communication). Pollen grains with 5–8 colpi have evolved only in subg. *Monogynella* and in clade O of subg. *Grammica*

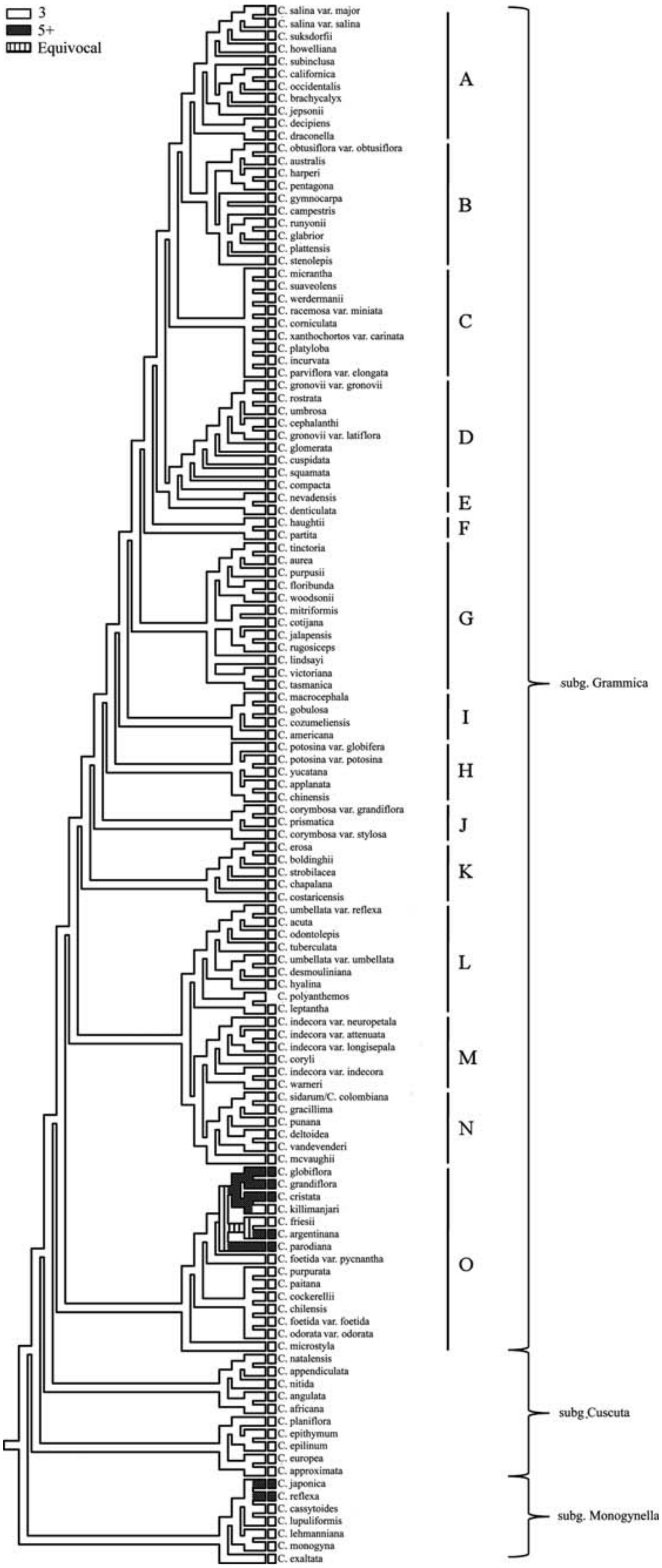

Table 2 Morphology of pollen in *Cuscuta*. Species are grouped into subgenera/major clades (García and Martin 2007; Stefanović et al. 2007; Stefanović and Costea, personal communication). Species for which no molecular data was available (indicated by an asterisk) are also tentatively placed into major infrageneric groups based on their morphology. P = prolate; SP = subprolate; S = spheroidal; SO = suboblate

Infrageneric group	Species	Species details							
		Percent perforation (%)	Avg. perforation area (μm^2)	Perforation diameter (µm)	Length (µm)	Width (µm)	P/E ratio	Shape	Number of colpi
Subg. *Monogynella* (eight species)	*C. cassytoides*	31.3	0.949	1.523–(2.651)–3.885	24.8–(26.4)–28.6	19.5–(22.3)–24.3	1.18	SP	3 (–4)
	C. exaltata	8.3	0.313	0.303–(0.744)–1.366	31.6–(34.5)–37.2	23.1–(25.9)–29.5	1.33	P (–SP)	3 (–4)
	C. japonica	30.8	0.855	1.167–(2.491)–4.518	29.5–(31.5)–33.1	21.6–(23.7)–27.5	1.40	P (–S)	(4–) 5 (–6)
	C. lehmanniana	19	0.63	0.777–(1.285)–1.685	26.0–(29.6)–32.8	24.0–(26.4)–29.3	1.12	(P–) S	3 (–4)
	C. lupuliformis	34.3	0.827	0.855–(1.284)–2.198	27.1–(29.4)–31.4	21.3–(23.5)–26.3	1.25	SP (–S)	3 (–4)
	C. monogyna	0	0	0	31.8–(32.9)–33.7	24.1–(28.2)–30.1	1.17	(P–) SP (–S)	3 (–4)
	C. reflexa	44.6	2.289	3.867–(4.470)–5.827	29.5–(30.6)–31.7	26.2–(27.7)–29.1	1.10	S (–SO)	5–6 (–7)
	*C. santapaui**	38	1.524	1.618–(3.202)–4.541	25.0–(26.3)–27.1	29.5–(30.8)–32.2	0.85	(SP–) SO	(5–6)
Subg. Cuscuta (five species)	*C. approximata*	0.2	0.05	0.286–(0.382)–0.453	22.0–(23.7)–25.1	18.5–(20.6)–24.3	1.15	(P–) SP	3 (–4)
	C. epilinum	0	0.002	0.184–(0.257)–0.322	24.6–(26.0)–27.4	17.1–(18.3)–19.2	1.42	P	3 (–4)
	C. epithymum	0	0	0	18.6–(19.7)–21.8	12.9–(14.2)–15.9	1.39	P (–SP)	3 (–4)
	C. europea	0.4	0.052	0.281–(0.390)–0.584	20.7–(23.1)–25.8	12.4–(13.8)–14.9	1.67	P	3 (–4)
	C. planiflora	0	0	0	22.4–(24.1)–25.3	12.2–(17.0)–19.0	1.42	P (–SP)	3 (–4)
Pachystigma clade (five species)	*C. africana*	3.8	0.257	0.468–(0.564)–0.662	28.0–(29.6)–31.2	17.4–(19.4)–20.6	1.53	P (–S)	3 (–4)
	C. angulata	0.1	0.063	0.415–(0.430)–0.461	26.8–(28.0)–29.5	15.2–(15.8)–17.1	1.77	P	3 (–4)
	C. appendiculata	0	0	0	17.1–(18.9)–20.0	16.0–(17.6)–19.4	1.07	S (–SO)	3 (–4)
	C. natalensis	1.6	0.044	0.168–(0.289)–0.518	17.2–(17.6)–18.3	18.0–(19.2)–19.9	0.92	(P–) S	3 (–4)
	C. nitida	0.1	0.029	0.211–(0.288)–0.393	15.6–(18.2)–22.6	14.9–(19.0)–22.1	0.96	(P–) S	3 (–4)
Subg. *Grammica* (130 taxa) clade A	*C. brachycalyx*	0	0	0	22.9–(24.0)–24.8	15.0–(16.0)–17.0	1.50	P	3 (–4)
	C. californica	0	0	0	19.3–(21.5)–23.0	11.0–(17.5)–20.8	1.23	SP	3 (–4)
	C. decipiens	0.1	0.049	0.243–(0.298)–0.334	19.9–(21.3)–23.0	13.5–(14.3)–14.7	1.49	P (–SP)	3 (–4)
	C. draconella	0	0	0	15.0–(16.2)–17.4	13.2–(14.6)–16.7	1.11	(P–) S	3 (–4)
	C. howelliana	6.3	0.107	0.286–(0.382)–0.542	13.5–(15.6)–16.8	9.2–(11.5)–13.5	1.36	P (–SP)	3 (–4)
	C. jepsonii	0.2	0.048	0.181–(0.237)–0.371	19.2–(21.3)–22.9	13.3–(14.0)–14.9	1.52	P	3 (–4)
	C. occidentalis	0	0	0.161–(0.295)–0.484	19.4–(21.6)–24.6	12.6–(16.2)–18.8	1.33	P (–SP)	3 (–4)
	C. salina var. *salina*	1.2	0.069	0.314–(0.379)–0.547	16.5–(17.2)–21.3	10.6–(11.8)–14.5	1.46	P (–SP)	3 (–4)
	C. salina var. *major*	1	0.022	0.239–(0.367)–0.800	17.1–(17.7)–18.2	14.7–(15.9)–17.2	1.11	S	3 (–4)
	C. subinclusa	0.2	0.04	0.254–(0.327)–0.439	14.7–(15.0)–21.1	15.6–(16.4)–16.9	0.91	(SP–) S	3 (–4)
	C. suksdorfii	0	0	0.287–(0.359)–0.499	24.9–(26.6)–27.6	12.4–(15.7)–17.8	1.69	P	3 (–4)

Table 2 continued

Infrageneric group	Species	Species details							
		Percent perforation (%)	Avg. perforation area (µm^2)	Perforation diameter (µm)	Length (µm)	Width (µm)	P/E ratio	Shape	Number of colpi
Clade B	*C. australis*	0.4	0.045	0.246–(0.317)–0.439	18.9–(20.9)–23.5	11.4–(13.7)–16.9	1.53	P (–SP)	3 (–4)
	C. campestris	0.8	0.056	0.213–(0.282)–0.367	17.6–(23.6)–26.9	18.0–(18.3)–18.7	1.29	(P–) SP	3 (–4)
	C. glabrior	0.1	0.069	0.352–(0.416)–0.485	17.3–(18.0)–21.1	10.8–(12.2)–13.2	1.48	P (–SP)	3 (–4)
	C. gymnocarpa	0.2	0.042	0.222–(0.312)–0.445	20.3–(21.1)–22.3	15.2–(17.4)–19.2	1.21	(P–) SP	3 (–4)
	C. harperi	0.4	0.037	0.197–(0.230)–0.287	16.5–(17.5)–23.4	9.7–(13.3)–16.0	1.32	(P–) SP	3 (–4)
	C. obtusiflora var. *obtusiflora*	4.7	0.03	0.183–(0.260)–0.396	18.9–(20.1)–21.1	12.9–(13.7)–14.9	1.16	SP	3 (–4)
	C. obtusiflora var. *glandulosa*	0.1	0.066	0.222–(0.309)–0.442	17.4–(21.2)–23.6	14.0–(18.3)–20.1	1.47	P (–SP)	3
	C. pentagona	0	0	0	18.6–(19.3)–22.8	12.0–(12.1)–13.5	1.60	P	3 (–4)
	C. plattensis	0	0	0	16.5–(17.5)–22.4	16.6–(17.1)–17.4	1.02	S (–SO)	3 (–4)
	*C. polygonorum**	0	0	0	19.6–(21.8)–23.9	14.2–(16.1)–17.8	1.35	P (–SP)	3 (–4)
	C. runyonii	0.1	0.017	0.166–(0.231)–0.282	13.0–(14.0)–18.6	16.2–(17.3)–18.3	0.81	(SP–) SO	3 (–4)
	C. stenolepis	0	0	0.283–(0.424)–0.643	16.6–(18.2)–20.0	13.7–(14.1)–14.8	1.29	(P–) SP	3 (–4)
Clade C	*C. corniculata*	0.5	0.01	0.153–(0.210)–0.335	20.8–(21.9)–23.2	17.5–(17.8)–18.3	1.23	SP (–SO)	3 (–4)
	C. incurvata	1.3	0.049	0.211–(0.353)–0.500	13.0–(14.0)–14.8	15.1–(16.5)–17.5	0.85	(SP–) SO	3 (–4)
	C. micrantha	0	0	0	13.4–(15.8)–17.9	16.9–(17.5)–18.7	0.90	(SP–) S	3 (–4)
	C. parviflora var. *elongata*	0	0	0.187–(0.199)–0.212	17.5–(18.2)–18.8	16.6–(17.4)–18.8	1.05	S	3 (–4)
	*C. pauciflora**	0.2	0.031	0.207–(0.345)–0.537	14.4–(16.7)–18.5	15.7–(17.5)–19.3	0.95	S	3 (–4)
	C. platyloba	1.2	0.02	0.079–(0.211)–0.383	16.5–(16.9)–17.2	10.8–(12.5)–14.3	1.35	P (–SP)	3 (–4)
	C. racemosa var. *racemosa**	0	0	0	15.2–(16.7)–18.2	12.6–(13.5)–15.1	1.24	SP (–S)	3 (–4)
	C. racemosa var. *miniata*	2.4	0.038	0.145–(0.273)–0.389	14.4–(15.7)–17.8	15.7–(17.4)–19.9	0.90	(SP–) S	3 (–4)
	C. suaveolens	1	0.027	0.195–(0.269)–0.380	14.8–(16.9)–17.6	14.6–(16.1)–17.5	1.05	(P–) S	3 (–4)
	C. werdermanii	0.4	0.048	0.209–(0.268)–0.314	15.1–(17.9)–20.0	14.8–(17.0)–18.0	1.05	(P–) S	3 (–4)
	C. xanthochortos var. *xanthochortos**	0.1	0.023	0.490–(0.521)–0.537	18.9–(19.1)–19.6	21.7–(22.3)–23.3	0.94	(SP–) S	3 (–4)
	C. xanthochortos var. *carinata*	0.2	0.036	0.132–(0.235)–0.420	15.8–(16.9)–19.3	16.6–(18.0)–19.1	0.86	(SP–) SO	3 (–4)
	C. xanthochortos var. *lanceolata**	0	0.009	0.144–(0.177)–0.195	13.6–(14.4)–15.4	16.2–(16.6)–17.2	0.87	(SP–) SO	3 (–4)
Clade D	*C. cephalanthi*	20.1	0.137	0.205–(0.378)–0.707	28.6–(29.3)–30.8	20.7–(22.4)–23.6	1.31	(P–) SP	3 (–4)
	C. compacta	4.3	0.065	0.423–(0.497)–0.561	22.3–(23.4)–24.6	14.3–(17.2)–19.0	1.36	P (–SP)	3
	C. cuspidata	0.2	0.051	0.199–(0.251)–0.331	24.2–(25.2)–25.8	12.2–(14.0)–16.5	1.80	P (–SP)	3 (–4)
	C. glomerata	2.6	0.084	0.227–(0.470)–0.751	21.5–(22.7)–23.7	18.1–(19.0)–19.5	1.19	(P–) SP	3 (–4)
	C. gronovii var. *gronovii*	3.6	0.121	0.279–(0.411)–0.628	19.8–(20.6)–23.6	15.1–(16.9)–19.3	1.22	SP	3 (–4)
	C. gronovii var. *latiflora*	12.1	0.075	0.141–(0.328)–0.685	–	–	–	–	3 (–4)
	C. rostrata	15.2	0.098	0.314–(0.666)–1.246	22.2–(23.9)–25.2	13.8–(15.3)–17.8	1.56	P (–SP)	3 (–4)
	C. squamata	2.1	0.075	0.232–(0.304)–0.431	21.8–(23.1)–23.9	14.7–(15.6)–17.8	1.48	P (–SP)	3 (–4)
	C. umbrosa	1.5	0.029	0.327–(0.508)–0.724	21.9–(23.9)–25.4	14.1–(16.0)–18.3	1.49	P (–SP)	3 (–4)

Table 2 continued

Infrageneric group	Species	Species details							
		Percent perforation (%)	Avg. perforation area (μm^2)	Perforation diameter (μm)	Length (μm)	Width (μm)	P/E ratio	Shape	Number of colpi
Clade E	*C. denticulata*	0	0	0	14.4–(15.2)–16.1	11.4–(11.8)–11.9	1.29	SP	3 (–4)
	C. nevadensis	4.9	0.058	0.150–(0.335)–0.716	20.0–(20.8)–22.2	12.9–(13.7)–14.0	1.52	P	3 (–4)
	C. veatchii	0.4	0.015	0.137–(0.193)–0.311	17.8–(19.4)–20.6	13.9–(14.9)–16.2	1.30	(P–) SP	3 (–4)
Clade F	*C. burrelli*	4	0.044	0.183–(0.319)–0.455	16.7–(17.5)–18.0	13.7–(16.5)–16.6	1.06	(SP–) S	3 (–4)
	C. haughtii	12.5	0.186	0.298–(0.561)–1.053	19.1–(20.8)–21.8	15.4–(18.4)–21.3	1.13	(SP–) S	3 (–4)
	C. longiloba	1.5	0.014	0.210–(0.276)–0.351	15.0–(18.7)–21.8	17.2–(22.6)–25.7	0.83	(S–) SO	3 (–4)
	C. partita	3.2	0.019	0.163–(0.330)–0.562	19.6–(21.5)–23.4	14.3–(15.5)–16.6	1.39	P (–SP)	3 (–4)
	*C. serrata**	0	0	0	18.9–(19.8)–20.7	13.2–(16.8)–19.0	1.18	(P–) SP	3 (–4)
Clade G	*C. aurea*	0.1	0.052	0.302–(0.408)–0.505	17.5–(18.6)–19.6	13.6–(14.9)–15.8	1.25	(P–) SP	3 (–4)
	C. cotijana	20.2	0.405	0.213–(0.306)–1.338	14.3–(18.3)–22.0	13.1–(16.2)–17.4	1.13	(SP–) S	3 (–4)
	C. floribunda	8.6	0.095	0.420–(0.560)–0.693	21.3–(22.7)–24.8	14.6–(17.0)–19.3	1.34	P (–SP)	3 (–4)
	C. jalapensis	14.5	0.28	0.424–(0.670)–0.889	27.8–(28.6)–30.3	18.7–(19.7)–21.2	1.45	P (–SP)	3 (–4)
	C. lindsayi	4.9	0.04	0.305–(0.395)–0.489	22.7–(23.7)–25.6	21.2–(22.0)–23.0	1.08	(SP–) S	3 (–4)
	C. mitriformis	17.7	0.136	0.232–(0.440)–0.781	23.2–(24.7)–25.9	23.9–(25.8)–27.0	0.96	(SP–) S	3 (–4)
	C. purpusii	15	0.103	0.297–(0.573)–1.240	18.7–(20.1)–22.1	20.9–(22.8)–24.7	0.88	S (–SO)	3 (–4)
	C. rugosiceps	9.9	0.166	0.406–(0.773)–1.506	17.7–(18.6)–19.1	15.5–(16.8)–18.2	1.11	(SP–) S	3 (–4)
	C. tasmanica	0.2	0.036	0.228–(0.284)–0.330	21.4–(23.2)–25.0	13.4–(15.0)–16.2	1.55	P (–SP)	3 (–4)
	C. tinctoria	19.7	0.244	0.406–(0.654)–0.905	22.6–(24.3)–26.3	17.3–(18.1)–19.0	1.34	P (–SP)	3 (–4)
	C. victoriana	0	0.005	0.054–(0.117)–0.166	21.7–(25.4)–28.6	16.6–(18.6)–21.0	1.37	P (–SP)	3 (–4)
	C. woodsonii	0.6	0.031	0.254–(0.361)–0.490	17.3–(19.2)–21.3	14.5–(16.3)–17.7	1.17	SP (–S)	3 (–4)
Clade H	*C. applanata*	2	0.066	0.185–(0.311)–0.625	22.2–(23.2)–24.9	14.1–(14.9)–15.9	1.56	P (–S)	3 (–4)
	C. chinensis	0	0	0	18.4–(19.6)–20.6	20.6–(21.8)–22.9	0.90	(SP–) S	3 (–4)
	*C. dentatasquamata**	1	0.076	0.201–(0.324)–0.661	19.3–(20.1)–21.8	13.6–(16.1)–17.9	1.25	SP (–S)	3 (–4)
	C. potosina var. *potosina*	0.2	0.03	0.132–(0.204)–0.318	24.7–(26.6)–29.7	17.2–(18.1)–19.5	1.43	P (–SP)	3 (–4)
	C. potosina var. *globifera*	0.1	0.013	0.229–(0.306)–0.430	24.4–(26.3)–28.9	17.9–(18.4)–18.9	1.47	P (–SP)	3 (–4)
	C. sandwichiana	0	0	0	23.3–(24.9)–26.8	17.4–(19.8)–23.1	1.26	SP	3 (–4)
	C. yucatana	0.1	0.012	0.124–(0.200)–0.287	17.3–(17.9)–18.6	10.5–(12.2)–13.5	1.47	P (–SP)	3 (–4)
Clade I	*C. americana*	2.9	0.036	0.221–(0.328)–0.523	17.1–(19.2)–21.0	14.4–(16.1)–16.6	1.19	SP (–S)	3 (–4)
	C. cozumeliensis	16.4	0.151	0.241–(0.530)–0.924	14.4–(15.3)–16.5	18.1–(20.2)–21.9	0.76	(SP–) SO	3 (–4)
	C. globulosa	1.2	0.05	0.356–(0.454)–0.574	24.2–(25.2)–27.5	18.7–(20.4)–23.1	1.24	SP	3 (–4)
	C. macrocephala	17.7	0.319	0.519–(0.860)–1.398	23.1–(25.0)–26.6	15.5–(16.8)–17.7	1.49	P (–SP)	3 (–4)

Table 2 continued

Infrageneric group	Species	Species details							
		Percent perforation (%)	Avg. perforation area (μm^2)	Perforation diameter (μm)	Length (μm)	Width (μm)	P/E ratio	Shape	Number of colpi
Clade J	*C. corymbosa* var. *corymbosa**	6.4	0.117	0.255–(0.419)–0.579	18.9–(19.5)–20.6	20.5–(21.5)–22.1	0.91	(SP–) S	3 (–4)
	C. corymbosa var. *grandiflora*	0.4	0.043	0.305–(0.491)–0.761	15.5–(16.5)–18.1	19.5–(20.7)–22.1	0.80	(S–) SO	3 (–4)
	C. corymbosa var. *stylosa*	17.7	0.239	0.591–(0.783)–0.983	22.2–(24.5)–27.3	16.1–(17.3)–18.5	1.42	P (–SP)	3 (–4)
	C. prismatica	13.2	0.206	0.565–(0.964)–1.443	20.4–(22.3)–26.1	14.9–(17.5)–19.4	1.27	SP	3 (–4)
Clade K	*C. boldinghii*	0.4	0.006	0.262–(0.571)–0.737	14.9–(15.8)–17.2	17.7–(20.1)–21.3	0.79	(S–) SO	3 (–4)
	C. chapalana	21.2	0.208	0.241–(0.530)–0.822	16.9–(17.6)–18.1	19.4–(21.9)–23.3	0.80	(S–) SO	3 (–4)
	C. costaricensis	0.2	0.06	0.369–(0.386)–0.400	23.0–(25.4)–27.8	16.9–(17.9)–19.3	1.42	P (–SP)	3 (–4)
	C. erosa	0.4	0.055	0.312–(0.357)–0.455	15.6–(16.8)–17.9	16.6–(18.0)–19.6	0.93	(SP–) S	3 (–4)
	*C. ortegana**	4.9	0.087	0.248–(0.470)–0.693	18.8–(19.7)–20.5	11.6–(12.9)–14.4	1.53	P (–SP)	3 (–4)
	C. strobilacea	11.6	0.136	0.237–(0.530)–0.983	21.3–(23.7)–25.5	14.3–(16.4)–18.3	1.44	P (–S)	3 (–4)
Clade L	*C. acuta*	0.2	0.024	0.166–(0.237)–0.299	20.5–(22.3)–25.6	13.5–(15.9)–16.1	1.49	P (–S)	3 (–4)
	C. desmouliniana	0.3	0.055	0.199–(0.357)–0.454	17.9–(19.5)–21.9	10.2–(13.2)–17.2	1.48	P	3 (–4)
	C. hyalina	0	0	0	23.2–(24.9)–26.5	17.6–(18.5)–19.7	1.35	P (–SP)	3 (–4)
	C. leptantha	5.4	0.143	0.282–(0.514)–0.816	21.7–(22.9)–23.6	14.5–(15.1)–15.5	1.52	P (–SP)	3 (–4)
	C. odontolepis	3.5	0.073	0.279–(0.373)–0.488	25.2–(26.7)–29.8	15.0–(16.3)–18.3	1.67	P (–S)	3 (–4)
	C. polyanthemos	15.4	0.114	0.116–(0.368)–0.708	24.6–(27.5)–31.4	18.8–(20.6)–22.9	1.33	P (–SP)	3 (–4)
	C. tuberculata	0	0.02	0.299–(0.358)–0.418	15.5–(16.0)–16.5	19.7–(11.8)–12.6	1.36	P (–SP)	3 (–4)
	C. umbellata var. *umbellata*	0	0	0	16.6–(18.2)–19.0	14.6–(15.9)–17.7	1.14	(P–) SP	3 (–4)
	C. umbellata var. *reflexa*	0	0	0.149–(0.294)–0.431	16.9–(19.1)–21.5	12.4–(14.3)–16.5	1.34	P (–SP)	3 (–4)
Clade M	*C. coryli*	0	0	0.128–(0.173)–0.239	27.9–(29.4)–30.5	19.3–(20.9)–23.2	1.41	P (–SP)	3 (–4)
	C. indecora var. *indecora*	0	0.023	0.366–(0.490)–0.586	26.8–(27.7)–29.8	20.7–(22.5)–24.1	1.23	(P–) SP	3 (–4)
	C. indecora var. *attenuata*	0	0	0.225–(0.269)–0.296	25.9–(27.3)–28.9	17.2–(18.4)–19.0	1.48	P (–SP)	3 (–4)
	C. indecora var. *longisepala*	0.2	0.054	0.226–(0.281)–0.376	26.0–(27.6)–30.3	13.8–(17.6)–21.2	1.57	P (–SP)	3 (–4)
	C. indecora var. *neuropetala*	0	0.011	0.248–(0.322)–0.395	23.7–(27.7)–32.1	16.1–(19.0)–22.2	1.46	P (–SP)	3 (–4)
	C. warneri	0	0	0.152–(0.203)–0.246	23.4–(24.7)–26.5	14.6–(15.1)–15.7	1.64	P (–SP)	3 (–4)
Clade N	*C. choisiana**	0.3	0.073	0.177–(0.346)–0.422	19.7–(20.2)–20.5	16.4–(16.7)–17.0	1.21	SP	3 (–4)
	C. deltoidea	0.6	0.041	0.182–(0.283)–0.460	15.2–(17.5)–19.8	13.8–(15.7)–17.2	1.11	(SP–) S	3 (–4)
	C. gracillima	0	0	0.228–(0.277)–0.360	18.9–(19.8)–20.7	11.1–(13.7)–14.9	1.44	P (–SP)	3 (–4)
	C. mcvaughii	0.1	0.008	0.197–(0.241)–0.298	18.9–(20.6)–22.0	15.8–(18.7)–21.4	1.10	(SP–) S	3 (–4)
	C. punana	0	0	0	20.1–(21.9)–23.9	14.9–(16.2)–17.0	1.35	P (–SP)	3 (–4)
	C. sidarum	0	0	0	16.8–(18.5)–20.0	13.3–(14.1)–15.1	1.31	(P–) SP	3 (–4)
	C. vandevenderi	0	0	0	21.8–(23.9)–25.8	15.3–(16.1)–17.4	1.48	P	3 (–4)

Table 2 continued

Infrageneric group	Species	Species details							
		Percent perforation (%)	Avg. perforation area (μm^2)	Perforation diameter (μm)	Length (μm)	Width (μm)	P/E ratio	Shape	Number of colpi
Clade O	*C. argentiniana*	1.1	0.045	0.307–(0.329)–0.497	17.8–(19.5)–20.7	11.0–(12.4)–13.1	1.57	P (–SP)	4 (–4)
	*C. acutiloba**	0.8	0.037	0.221–(0.292)–0.395	21.1–(21.8)–22.6	15.5–(16.1)–17.1	1.35	P (–SP)	3 (–4)
	*C. bella**	0.7	0.112	0.371–(0.534)–0.784	21.2–(22.4)–24.1	22.6–(24.0)–25.1	0.93	S	3 (–4)
	*C. boliviana**	6.2	0.068	0.204–(0.325)–0.583	18.7–(21.2)–23.5	20.4–(23.2)–26.4	0.91	S	(5–6)–7
	C. chilensis	19.8	0.311	0.443–(0.954)–1.776	21.4–(22.9)–25.9	17.9–(23.3)–26.9	0.98	(SP–) S	3 (–4)
	C. cockerellii	4.9	0.319	0.930–(1.064)–1.224	20.8–(22.6)–24.0	17.0–(18.5)–21.6	1.22	SP	3 (–4)
	C. cristata	6.3	0.086	0.231–(0.421)–0.669	15.9–(16.7)–17.4	14.3–(15.3)–15.9	1.09	S	3–4
	C. flossdorfii var. *pampagrandensis**	26.8	0.47	1.040–(1.469)–2.214	15.8–(19.9)–24.9	17.5–(20.5)–23.4	0.97	(SP–) S	(5–6)–7
	C. foetida var. *foetida*	10.4	0.146	0.485–(0.707)–1.343	21.0–(23.2)–24.6	17.5–(18.4)–19.7	1.26	SP (–S)	3 (–4)
	C. foetida var. *pycnantha*	12.1	0.217	0.478–(0.979)–1.513	21.4–(22.3)–23.6	22.4–(22.9)–23.2	0.97	S (–SO)	3 (–4)
	C. friesii	0.1	0.028	0.158–(0.247)–0.405	17.8–(18.8)–20.1	14.7–(16.0)–17.3	1.17	SP	3 (–4)
	C. globiflora	2.1	0.093	0.302–(0.456)–0.664	21.7–(23.1)–25.6	18.8–(22.0)–25.0	1.05	(SP–) S	6–8
	*C. goyazina**	4.5	0.118	0.294–(0.515)–0.773	15.3–(17.3)–19.0	17.5–(18.2)–20.1	0.95	(SP–) S	3 (–4)
	C. grandiflora	0.3	0.05	0.371–(0.468)–0.564	15.1–(17.3)–18.9	15.2–(16.2)–18.4	1.07	(SP–) S	(4–) 5–6
	C. killimanjari	21.9	0.212	0.203–(0.859)–1.615	17.6–(19.0)–21.0	18.5–(19.9)–21.6	0.95	(SP–) S	3 (–4)
	C. microstyla	21.5	0.189	0.360–(0.702)–1.105	15.3–(17.7)–19.6	10.4–(13.0)–16.8	1.36	P (–SP)	3 (–4)
	C. odorata var. *odorata*	9.6	0.184	0.395–(0.749)–1.190	20.9–(22.8)–25.4	15.3–(15.8)–16.2	1.44	P (–SP)	3 (–4)
	*C. orbiculata**	8.4	0.082	0.242–(0.433)–0.713	18.6–(19.8)–20.4	17.7–(19.4)–22.0	1.02	(SP–) S	3 (–4)
	C. paitana	43.6	0.912	0.749–(1.619)–2.606	16.8–(18.8)–19.9	20.9–(22.7)–23.8	0.83	(S–) SO	3 (–4)
	C. parodiana	34.3	0.903	1.076–(1.933)–2.917	19.0–(20.5)–22.3	21.5–(23.0)–24.0	0.89	(SP–) S	(4–) 5–6
	C. purpurata	0	0	0	19.4–(21.1)–22.7	17.0–(18.3)–20.7	1.15	SP (–SO)	3 (–4)
	*C. rubella**	0.3	0.09	0.468–(0.505)–0.550	17.7–(21.0)–22.9	17.4–(17.9)–19.0	1.17	SP	3 (–4)

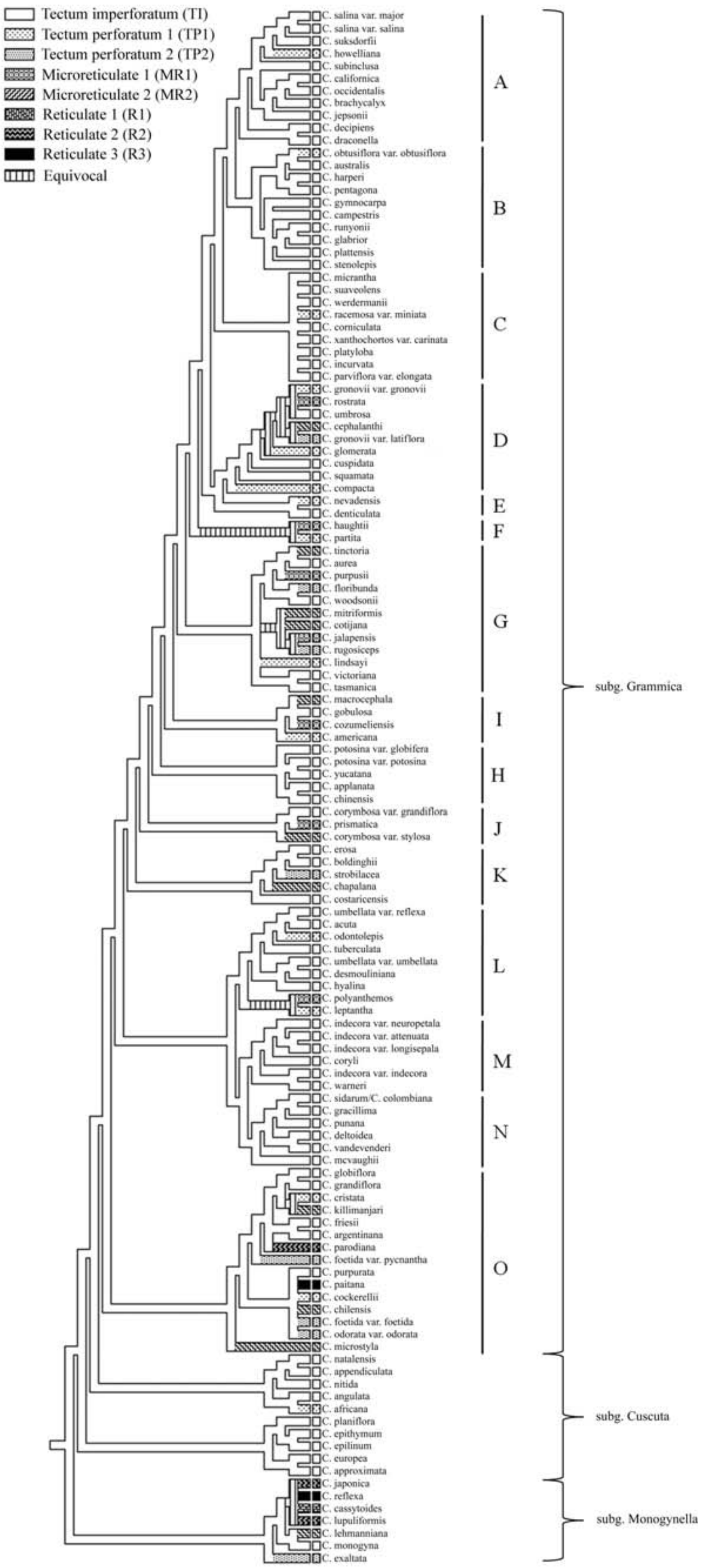

Fig. 3 Evolution of tectum perforation in *Cuscuta*. As a result of implementing the gap-weighting method (Thiele 1993), percent perforation is represented on a continuous scale, with light branches indicating an imperforate tectum and black branches indicating a reticulate tectum. For more information on clades A–O, see Stefanović et al. (2007). Tectum imperforatum is likely the ancestral condition, while pollen grains with increasingly larger perforation areas have evolved multiple times. The reticulated tectum has evolved in *Cuscuta* only in subg. *Monogynella*, and clade O of subg. *Grammica*

(Cox 1988) or anemophily (e.g., Lisci et al. 1994; Tanaka et al. 2004), suggesting that this microarchitectural feature of pollen is not directly correlated with a certain pollination vector.

Pollen size and shape

Pollen size is polymorphic (Table 2; Online resource 1). However, the species of subg. *Monogynella* have the largest pollen grains, 25–37.2 µm long, while in the remaining subgenera the average is 21 µm (Table 2). Convolvulaceae pollen is usually at least twice as large, averaging between 50 and 80 µm (Sengupta 1972; Lewis 1971; Ferguson et al. 1977; Tellería and Daners 2003; Leite et al. 2005; Menemen and Jury 2002; Martin 2001). Humbertieae, which forms a sister lineage to the rest of Convolvulaceae, has also large pollen (50–80 µm) (Lienau et al. 1986). In contrast, small pollen grains were reported from Cardiochlamyeae (e.g., *Cordisepalum*, ca. 12 µm; *Dinetus* 12–18 µm, *Tridynamia*, 12–14 µm, Staples et al. 2009; *Cardiochlamys* and *Poranopsis*, 18–20 µm, Sengupta 1972) and *Erycibe* (28–39.6 µm, Rao and Lee 1970; Sengupta 1972), all inferred to have diverged earlier than *Cuscuta* (Stefanović et al. 2003). Small pollen grains are also known only from the "bifid clade" (Dicranostyloideae), in *Dipteropeltis* (12–14 µm, Staples et al. 2009), *Hildebrandtia* (28–32 µm, Staples et al. 2009), *Dichondra* (22–33 µm), some *Cressa* species (24–30 µm) (Tellería and Daners 2003), *Dicranostyles* (18–21.6 µm), *Lysiostyles* (21.6–25.2 µm) (Austin 1973b), and *Metaporana* (14–16 µm) (Staples et al. 2009). Based on this information, the polarity of this character is equivocal. If the tribe Humbertieae, currently comprising only one genus and species (*Humbertia madagascariensis* Lam.), would be considered a distinct family, Humbertiaceae (Pichon 1947), small pollen grains are likely the ancestral condition in both *Cuscuta* and Convolvulaceae.

Pollen size in *Cuscuta* may be associated with chromosome size, ploidy level, and nuclear genome size. Species with the largest pollen in the subg. *Monogynella* have also the largest chromosomes in the genus (6–23.1 µm in *C. reflexa*; Kaul and Bhan 1977) and among the highest estimates for the nuclear genome (44.93 pg/2C in *C. lupuliformis*; McNeal et al. 2007). Subgenus *Grammica* has typically the smallest chromosomes (typically $\leq$4 µm) and pollen grains, but some of its species with larger chromosomes (8–16 µm in *C. indecora*, Fogelberg 1938) and higher nuclear genome size (65.54 pg/2C McNeal et al. 2007) have also pollen grains approaching 30 µm in length. In this latter size category can also be included some *Grammica* species such as *C. cephalanthi* and *C. campestris* which are characterized by small but numerous chromosomes ($2n = 60$ and $2n =$ ca. 54, respectively; Fogelberg 1938; García and Castroviejo 2003) and higher genome sizes (10.83 pg/2C; McNeal et al. 2007). Similarly, *C. epilinum*, with $2n = 42$ (García and Castroviejo 2003) and 7.74 pg/2C estimated genome size, has larger pollen grains than the other species of subg. *Cuscuta* with $2n = 14$ (genome size is known only in *C. europaea*—2.15 pg/2C). However, a rigorous corroboration of this apparent correlation is not possible because only a few species have their karyotype known (reviewed by García and Castroviejo 2003) and their genome size estimated (McNeal et al. 2007). The presence of 5–8 zonocolpate pollen grains in *Cuscuta* is consistent with all three phylogenetic scenarios mentioned in the "Material and methods" section, but together with the small size, the overall morphology of pollen suggests for *Cuscuta* sisterhood either to the "bifid-style" clade (Dicranostyloideae) or to one of the members of this clade.

Shape of pollen is polymorphic in *Cuscuta* (Table 2; tree not shown). Although over 50% of species have prolate grains, shape varies greatly among species of the same clade, and to a certain extent within the same species in the same flower/anther (Table 2). Nevertheless, because pollen grains with an increased number of apertures (see above) tend to be associated with spheroidal or subspheroidal shapes, the prolate or perprolate shapes (P/E ratios >1.33; Table 2) are likely to be primitive, as suggested by Austin (1998) for Convolvulaceae in general.

Taxonomic significance of pollen characters in *Cuscuta*

Cuscuta is one of the most difficult parasitic groups taxonomically. The last comprehensive treatment of the genus was provided by Yuncker more than 75 years ago (Yuncker, 1932). Following Engelmann (1859), Yuncker (1932) proposed a classification with three subgenera (*Cuscuta*, *Grammica*, and *Monogynella*). While this arrangement has been largely confirmed by phylogenetic studies, the numerous sections and subsections created by Yuncker (especially in subg. *Grammica*) have been shown to be polyphyletic (García and Martin 2007; Stefanović et al. 2007). At the species level, the systematics of *Cuscuta* is currently undergoing major taxonomic revisions through studies aimed at understanding the evolutionary relationships, speciation, and biogeography by using various molecular, morphological, and micromorphological data (Costea et al. 2006a, b, c, d, 2008a; Costea and Stefanović 2009b).

It is clear that pollen characters alone are insufficient to reconstruct phylogenetic relationships within *Cuscuta*, but considering the overall morphological minimalism that characterizes the genus, the variation of pollen (Table 1) is important for future taxonomic revisions at the species level. In general, subg. *Cuscuta* and several of the 15 major clades of subg. *Grammica* exhibit little pollen variation. (e.g., clades A, B, C, E, H, L, and N, see Stefanović et al. 2007; Table 2). Nevertheless, even in groups such as clade A (*C. californica* complex, Costea et al. 2006d), clade B (*C. pentagona* complex, Costea et al. 2006b), and clade L (*C. gracillima* complex, Costea et al. 2008a), basic pollen characters such as size, shape, and diameter of puncta/perforations have already been used together with other characters to separate species. Pollen will play an increasingly significant role in the subg. *Monogynella* and many of the *Grammica* clades (e.g., D, F, G, I, K, and O, see Stefanović et al. 2007) which exhibit significantly more palynological diversity (Table 2). These infrageneric groups are the least known in the genus, and their future species-level taxonomic revisions will benefit enormously from these additional pollen characters. For example, clade O (subg. *Grammica*), comprising over 20 species distributed mostly in South America (but also 1 in Africa), is perhaps the most diverse and challenging in *Cuscuta* (Stefanović et al. 2007). Pollen is equally varied in this group, encompassing practically almost entirely the variation documented in the genus (Table 2). While most species are tricolpate, *C. boliviana*, *C. grandiflora*, and *C. purpurata* are 5-8-colpate. Tectum varies from imperforate in *C. purpurata* to reticulate in *C. parodiniana* and *C. paitana*. Additionally, size and shape can also be used to separate closely related species in this clade.

Conclusions

Placed in the context of the evolutionary history of pollen in Convolvulaceae (3-colpate → 5-6-zonocolpate → pantocolpate → pantoporate), the pollen of *Cuscuta* can be considered one step above the primitive because some species in two major lineages (subg. *Monogynella* and clade O in subg. *Grammica*) have evolved 5–8 zonocolpate pollen, and because pantocolpate grains, although rare, are present. Reticulate pollen has evolved two times in *Cuscuta*: in subg. *Monogynella* and clade O of subg. *Grammica*. The traditional, qualitative tectum “types” represent arbitrary ranges of variation, which in *Cuscuta* are better described quantitatively. Overall, the morphology of pollen supports *Cuscuta* as a sister either to the “bifid-style” clade (Dicranostyloideae) or to one of the members of this clade. Although the pollen characters are insufficient to reconstruct the phylogeny of the genus, pollen morphology is useful for the taxonomy at species level.

Acknowledgments The authors warmly thank the curators and directors of AAU, ALTA, ARIZ, ASU, B, BAB, BOL, BRIT, CANB, CAS, CEN, CHR, CHSC, CIIDIR, CIMI, CTES, DAO, ENCB, F, G, GH, H, HUFU, IAC, IBUG, IEB, IND, J, JEPS, LL, LP, LPB, LPS, K, MEL, MERL, MEXU, MICH, MO, NMC, NU, NY, OKLA, OSU, OXF, PACA, PRE, QCNE, QFA, P, PACA, RB, RSA, SAM, S, SD, SGO, SI, SPF, TEX, TRT, UA, UB, UBC, UCR, UCT, UNB, UNM, UPRRP, UPS, US, USAS, VEN, WTU, and XAL for supplying plant material. We also thank Frédérique Guinel and two anonymous reviewers for their comments on an earlier version of the manuscript. Thierry Deroin provided the information about the pollen of *Humbertia*. This research was supported by Discovery grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada to M. Costea (327013–06) and to S. Stefanović (326439–06).

Appendix

Vouchers used for scanning electron microscopy. Species (arranged in alphabetical order), country, collector, collector number, herbarium acronym from Index Herbariorum.

Cuscuta acuta Engelm. Ecuador, Galapagos Islands, *Anderson 1853* (S); *Fagerlind & Wibom 3401* (S); *Howell 10140* (G). **C. acutiloba** Engelm.: Bolivia, *Mardon 1481* (G); Peru, *Pennell 13242* (USM). **C. africana** Thumb.: South Africa, *Beyers 6968* (NBG); *Oliver 11852* (NBG). **C. americana** L.: USA, Florida, *Small* et al. *11596* (NY); Mexico, *Van Devender and Reina-G 2000-745* (WLU); Bahamas, *Correll 45030* (NY); Cuba, *Britton* et al. *5917* (NY). **C. angulata** Engelm.: South Africa, *Beyers 6968/13* (NBG); *Orchard 460* (NU). **C. appendiculata** Engelm.: South Africa, *Hofmeyr s.n.* (GRA); *Burrows 4666* (J); *Bohnen 7827* (NBG). **C. applanata** Engelm. USA, New Mexico, *Casteller* 7339 (UNM); Mexico, *Van Devender 2001-710* et al. (WLU). **C. approximata** Bab.: USA, California, *Abrams 457* (CAS); Mexico, *Henrickson 13083a* (RSA); Puebla, *McKee 11042* (MEXU)**.** **C. argentiniana** Yunck.: Argentina, *Krapovickas & Schinini 36049, 36757* (CTES); *Brücher s.n.* (S); *Meyer 12467* (UPS). **C. aurea** Liebm.: Mexico, *Palmer 87* (S); Guerrero, *Duran & Garcia 634* (MEXU). **C. australis** R. Br. var. **australis**: New Caledonia, *Bonati 737* (S); Australia, *Conveny 756* (RSA). **C. australis** var. **tinei** (Insenga) Yunck.: Hungary, *Simonkai 2635* (NY); *Karkovány s.n.* (WLU). **C. bella** Yunck.: Peru, *Killip & Smith 21827* (US). **C. boldinghii** Urb.: Mexico, *Van Devender 92-31* et al. (ARIZ); Sinaloa, *Vega 2630* (MEXU). **C. boliviana** Yunck.: Argentina, *Hunzinker 2676* (S); *Ruiz Leal 14817* (MERL), *Burkart 12503* (CTES). **C. brachycalyx** Yunck.: USA, California, *Ahart 9856* (CHICO); *Colwell AC 04-305* (YM/WLU). **C. burrelli** Yunck.: Brazil, *Heringer* et al. *43* (UB); *Alvarenga-Pereira 766* (RB). **C. californica** Hook. & Arn: USA, California, *Sanders 25122* (UCR); *Pinzl 7238a* (NY); *White 5033* (ASU). **C. campestris** Yunck.: USA, Iowa, *Fay 4568* (UC); Oklahoma, *Lipscomb 1894* (SMU); Puerto Rico, *Liogier & Martorell 13908* (UPRRP); Venezuela, *Killip & Tamayo 37010* (GH). **C. cassytoides**

Nees: South Africa, *Balkwill 6968* (NU); *Alexandre 2407* (NBG). **C. cephalanthi** Engelm.: USA, Illinois, *McDonald s.n.* (NMS); Missouri, *Steyermark 79977*(MO); Nebraska, *Churchill 4560* (MO). **C. chapalana** Yunck.: Mexico, *Garcia-Ruiz 7942* (CIMI); Jalisco, *Carrillo-Reyes & al. 468* (CIMI). **C. chilensis** Ker Gawl.: Chile, *Anderson 84-189* (S); *Buchtien s.n.* (S); *Valeutey 94* (S). **C. chinensis** Lam.: Australia, *Carter 628* (CANB); China, *Wang Wen-Tsai 2378* (RSA). **C. choisiana** Yunck.: Mexico, *Véase 490* (MEXU). **C. cockerellii** Yunck.: Peru, *Vargas 2600* (CUS). **C. colombiana** Yunck.: Colombia, *Haught 4535* (NY); Venezuela, *Aristeguieta 4500* (VEN); *Tamayo 4432* (VEN). **C. compacta** Juss.: USA Mississippi, *McDaniel 27291* (MO); Maryland, *Hill 17349* (MO); New Jersey, *Moldenke & Moldenke 25129* (NY). **C. corniculata** Engelm.: Brazil, *Stannard* et al. *51861* (G); Colombia, *Pennell 1453* (GH). **C. coryli** Engelm.: USA, Arkansas, *Demaree 19603* (CAS); Maryland, *Killip 31293* (NY); Michigan, *Hanes 548* (NY); Nebraska, *Reynolds 2727*; Tennessee, *Rydberg 8179* (NY). **C. corymbosa** Ruiz & Pav. var. **grandiflora** Engelm.: Mexico, *Garcia-Ruiz* et al. *7572* (CIMI, WLU); *Iltis & Guzman 29077* (MEXU); *Galván & Galván 4681* (MEXU). **C. corymbosa** var. **stylosa** (Choisy) Engelm.: Mexico, *Rzedowski 28752* (UCR); *Bopp 206* (MEXU); *Pringle s.n.* (MEXU). **C. costaricensis** Yunck.: Mexico, *Reina-G.* et al. *2006-1049* (ARIZ, WLU); *Van Devender 98*-1789 (ARIZ); *Wiens 96-125* et al. (WLU). **C. cotijana** Costea & I. García: Mexico, *García Ruiz 7412* (CIMI,); *García Ruiz* et al. *7557* (CIMI); *Carranza* et al. *7316* (IEB). **C. cozumeliensis** Yunck.: Guatemala, *Kellerman 6580* (F); Mexico, *Calzade & Nivea 9427* (XAL); *Vazquez 176* (MEXU). **C. cristata** Engelm.: *Meyer 10669* (CTES); Argentina, *Cabrera 7144* (NY); *Hunzinker 4927* (S); *Burkart 14000* (SI). **C. cuspidata** Engelm.: USA, Arkansas, *Demaree 15522* (RSA); Texas, *Higgins 12480* (NY); *Runyon 2828* (SMU). **C. decipiens** Yunck.: Mexico, *Henrickson 6362, 13394, 22781* (RSA). **C. deltoidea** Yunck.: Mexico, *Orcutt 4457* (MEXU); *Pringle 5350* (US); *García Ruíz 2516* (CIMI). **C. dentatasquamata** Yunck.: Mexico, *Jones s.n.* (RSA); USA, Arizona, *Lemmon s.n.* (UC). **C. denticulata** Engelm.: USA, Arizona, *Peebles & Parker 14793* (NY); California, *Thomas 8904* (UC); Nevada, *Perish 10299* (CAS); Utah, *Choong s.n.* (NY). **C. desmouliniana** Yunck.: Mexico, *Spellenberg 4943* (NMS); *Van Devender & Reina-G 2002-23* (WLU); *Van Devender 96-360* et al. (WLU). **C. draconella** Costea & Stefanović: USA, New Mexico, *Spellenberg & Mahrt 10497* (NY); *Wagner 3395* (UNM); *Herman 462* (NY). **C. epilinum** Weihe: Canada, Quebec, *Barabe 16914* (DAO); Sweden, *Samuelson 1317* (RSA). **C. epithymum** (L.) L.: Argentina, *Bana 14733* (CTES); USA, New York, *Ahles 67695* (SMU); Belgium, *Meulebrouck s.n.* (WLU). **C. erosa** Yunck.: USA, Arizona, *Jones s.n.* (CAS); Mexico, *Rebman 4275*; *Sánchez NF-172* et al. (WLU). **C. europea** L.: Finland, *Alava* et al. *s.n.* (OSU); Sweden, *Holmgren 19784* (SD). **C. exaltata** Engelm.: USA, Texas, *Snyder 472* (SMU); *Carter 10584* (MO). **C. floribunda** aff. Kunth: Mexico, *Prather & Soule 1221* (TEX). **C. flossdorfii** Hicken var. **pampagrandensis** Yunck.: Bolivia, *Mendoza & Acebo 919* (MO). **C. foetida** Kunth var. ***foetida***: Ecuador, *Holm-Nielsen & Andrade 18480* (AAU); *Holm-Nielsen* et al. *5181* (AAU); Peru, *Smith 1624* (MO). **C. foetida** var. **pycnantha** Yunck: Peru, *Plowman* et al. *14291* (F). **C. friesii** Yunck.: Argentina, *Krapovickas* et al. *21898* (CTES); *Saravia Toledo* et al. *12993* (CTES); *Mulgura 1245* (SI). **C. glabrior** (Engelm.) Yunck.: USA Texas, *Wolff 3270* (NY); Mexico, *Marsh 1115* (SMU); *Henrickson 13676* (RSA). **C. globiflora** Engelm.: Argentina, *Mulgura* et al. *1199* (MO); Bolivia, *Plowman & Davis 5196* (GH); Peru, *King* et al. *247* (USM). **C. globulosa** Benth: Puerto Rico, *Stahl 1064* (S); *Urban 855* (S); *Liogier & Oquendo 180* (UPRRP). **C. glomerata** Choisy: USA, Texas, *Barkley 13886* (RSA); *Wolff 3321* (SMU). **C. goyaziana** Yunck.: Brazil, *Macedo 3731* (NY). **C. gracillima** Engelm.: Mexico, *Pringle 6716* (NML); *Koch and Fryxell 82253* (NY); *García Ruiz 7334* (CIMI, WLU). **C. grandiflora** Kunth: Argentina, *Schinini* et al. *34615* (CTES); Ecuador, *Løjtnant* et al. *11829* (AAU); *Tipaz* et al. *1563* (QCNE); Peru, *Pennell 13613* (GH). **C. gronovii** Willd. ex Roem. & Schult. var. **gronovii:** Canada, Ontario, *Catling 5111* (DAO). USA Alabama, *Kpeooer* et al. *s.n.* (NY); Georgia, *Mellinger s.n.* (SMU). **C. gronovii** var. **latiflora** Engelm.: USA, Missouri, *Brant & Donnell 4813* (MO); Texas, *Lundell 11721* (SMU); New York, *Ferguson 6091* (NY). **C. gymnocarpa** Engelm. Galapagos Islands, *Fagerlind & Wibom 3658* (S); *Werff 2068* (S); *Werff 2136* (NY). **C. harperi** Small: USA, Alabama, *Demaree 46295* (NY); *Harper s.n.* (NY). **C. haughtii** Yunck.: Ecuador, *Asplund 15974* (S); *Holm-Nielsen* et al. *2308* (NY). **C. howelliana** Rubtzoff: USA, California, *True 7407* (DS); *Oswald & Ahart 7978* (JEPS); *Reino & Alava 6809* (JEPS). **C. hyalina** Roth.: India, *Pushpauder s.n.* (CANB); Namibia, *Bosch 25022* (BOL). **C. incurvata** Prog.: Paraguay, *López* et al. *243* (CTES); *Anisits 2395* (S); *Hassler 8170* (S). **C. indecora** Choisy var. **indecora:** USA, California, *Wolf 4392* (UC); Louisiana, *Allen 19239* (BRIT); New Mexico, *Spellenberg* et al. *3427* (NY). **C. indecora** var. **attenuata** (Waterf.) Costea: USA, Oklahoma, *Waterfall 17496* (GH); Texas, *Fisher 4118* (CAS); Mexico, *Palmer 333* (F). **C. indecora** var. **longisepala** Yunck.: Argentina, *Leal 7964* (NY); USA Texas, *Runyon 2819* (NY). **C. jalapensis** Schltdl.: Mexico, *Ton & Lopez 9826* (NY); *Sharp 45380* (NY); *Waterfall & Wallis 14213* (SMU). **C. japonica** Choisy: China, *Bartholomew* et al. *883* (NY); Japan, *Furuse 6890* (RSA). **C. jepsonii** Yunck.: USA, California, *Dudley 1774* (DS);

Heller 5981 (UC); *Tracy 2349* (UC). **C. killimanjari** Oliv.: Malawi, *Lacroix 4559* (MO); Tanzania, *Scheffler 434* (MEL); Zimbabwe, *Eyles 352* (J). **C. lehmanniana** Bunge: Uzbekistan, *Vvedensky s.n* (MEL); *Drobov 3763* (NY). **C. leptantha** Engelm.: Mexico, *Wiggins 17125* (MEXU); *Lindsay 2928* (SD); *Sanders 7523* (UCR); *Van Devender & Reina-G 2000-933* (WLU). **C. lindsayi** Wiggins: Mexico, *Wiggins 13185* (MO). **C. longiloba** Yunck.: Brazil, *Krapovickas & Schinini 31255* (CTES); Paraguay, *Casas & Molero 4384* (MO). **C. lupuliformis** Krock.: Austria, *Barta 2004-302* (NY); Netherlands, *Lennhouts 2514* (CANB). **C. macrocephala** W. Schaffn. ex Yunck.: Mexico, *Rebman 5743* (SD); *Van Devender & Reina-G. 2006-872* (WLU); *Reina-G. & Van Devender 2001-774* (WLU). **C. mcvaughii** Yunck.: Mexico, *Hinton* et al. *12098* (NY). **C. micrantha** Choisy: Chile, *Philippi 489* (G); *Skottsberg 995* (F). **C. microstyla** Engelm. var. **bicolor** Hunz.: Argentina, *Boelcke* et al. *10243* (CTES); *Burkart* et al. *6968* (SI); *Prina* et al. *2362* (CTES). **C. mitriformis** Engelm.: Mexico, *Rzedowski 41379* (IEB); *Bye 2011* (MEXU); *Bye 50488* (UCR). **C. monogyna** Vahl: Grece, *Greuter 11459* (NY); Turkmenistan, *Sintenis 1240* (MO). **C. natalensis** Baker: South Africa, *Rudatis 1319* (NBG); *Rudatis 2412* (NBG). **C. nevadensis** I.M. Johnst.: USA, California, *Raven 12865* (CAS); *Peebles 263* (NY); Nevada, *Brandegee s.n.* (UC), *LaRivers & Hancock 164* (NY). **C. nitida** E. Mey.: South Africa, *Burgers 2649* and *3318* (NBG); *Rogers 17342* (J). **C. obtusiflora** Kunth var. **obtusiflora**: Argentina, *Arbo* et al. *7973* (CTES); *Bordódon s.n.* (CTES). **C. obtusiflora** Kunth var. **glandulosa** Engelm.: Cuba, *Wright 1659* (GH); USA, California, *Parish 2110* (CAS); Delaware, *collector illegible* (“MC”) *s.n.* (CAS); Texas, *Clare 2144* (CAS). **C. occidentalis** Millsp.: USA, California, *Howell 48868* (CAS); *Ertter 7326* (NY); *Schoolcraft* et al. *2220* (NY); Nevada, *Tiehm 12257* (NY); Utah, *Garrett 2170* (NY). **C. odontolepis** Engelm.: Mexico, *White 2730* (GH); *Van Devender & Reina-G 2006-869*; *2006-467* (WLU). **C. odorata** Ruiz & Pav.: Ecuador, *Jaramillo 10372* (AAU); *Sparre 16186* (S); Peru, *Hitchcock 20320* (GH); *Ugent & Ugent 5323* (MO). **C. orbiculata** Yunck.: Brazil, *Alvaregna 93605* (RB); Bolivia, *Krapovickas* et al. *19221* (CTES). **C. ortegana** Yunck.: Mexico, *Hinton* et al. *16294* (MICH); *Van Devender* et al. *2006-74* (WLU). **C. paitana** Yunck.: Ecuador, *Madsen 63940* (QCNE); Peru, *Horton 11575* (GH). **C. parodiana** Yunck.: Argentina, *Eyerdam 22423* (MO); *Novara 7976* (S); *Balegno 447* (SMU). **C. partita** Choisy: Brazil, *Eiten & Eiten 3961* (US); *Krapovickas* et al. *38723* (CTES); *Lindman 3481* (S). **C. parviflora** Engelm. var. **elongata** Engelm.: Brazil, *Filgueiras 1476* (RB); *Oliveira* et al. *745* (US). **C. pauciflora** Phil.: Chile, *Werdermann 1884* (SGO). **C. pentagona** Engelm.: USA, Alabama, *Kral 31225* (SMU); Florida, *Welch 1633* (NY); North Dakota, *Stevens s.n.* (NY); Virginia, *Weatherby 4230* (NY). **C. planiflora** Ten.: Australia, *Howitt & Zaicon-Kunesch s.n.* (PERTH); Palestine, *Musselman 10461* (RSA). **C. plattensis** A. Nelson: USA, Wyoming, *Nelson 2741* (MO); *Nelson 2768* (NY). **C. platyloba** Prog.: Argentina, *Burkart 10554* (CTES); Brazil, *Dusen 10005* (S); Paraguay, *Montes 16599* (CTES). **C. polygonorum** Engelm.: USA Indiana, *Yuncker 10836* (NY); Ohio, *Core & Anderson s.n.* (NY). **C. polyanthemos** Schaffn. ex Yunck.: Mexico, *Wiggins 13153* (SD); *Van Devender & Reina-G 2006-809* (WLU). **C. potosina** W. Schaffn. ex S. Wats. var. **potosina:** Mexico, *Rose* et al. *9650* (NY). **C. potosina** var. **globifera** W. Schaffn.: Mexico, *Perez* et al. *3707* (IEB); *Pringle 6575* (MEXU); *Van Devender 96-451* et al. (WLU); USA, Arizona, *Gooding 290-61* (ASU). **C. prismatica** Pav. ex Choisy: Ecuador, *Mille 112* (F); *Hitchcock 20141* (GH). **C. punana** Costea & Stefanović: Ecuador, *Madsen 63850* (AAU). **C. purpurata** Phil.: Chile, *Dillon & Teillier 5104* (MO); *Johnston 5170* (US); *Morong 1143* (US). **C. purpusii** Yunck.: Mexico, *Martinez 1093*; (MEXU); *Torres Colin 15864* (MEXU); *Hendrickson 6608* (RSA). **C. racemosa** Mart. var. **racemosa**: Brazil, *Pinheiro 55* (SPF). **C. racemosa** Mart. var. **miniata** (Mart.) Engelm.: Brazil, *Menezes* et al. *5100* (CTES); *Name illegible 7835* (S); *Rapini* et al. *491* (WLU). **C. reflexa** Roxb.: India, *Kanta s.n.* (ASU); *Cullelt s.n.* (MEL); *Koelz 21955* (NY). **C. rostrata** Shuttlw. ex Engelm. & A. Gray: USA, North Carolina, *Bozeman* et al. *45268* (OSU); Tennessee, *Jennison 2824* (NY); Texas, *Lundell 11480* (SMU). **C. rubella** Yunck.: Peru, *Macbride & Featherstone 371* (NY). **C. rugosiceps** Yunck.: Mexico, *Carranza & Silva 5997* (IEB); *Taylor 21457* (SMU). **C. runyonii** Yunck. USA, Texas, *Correll & Johnston 14906* (NY); *Lundell 9840* (SMU). **C. salina** Engelm. var. **salina**: USA, Arizona, *Hammond 10349* (NY); California, *Bacigalupi & al. 2667* (DS); Nevada, *Tiehm 5991* (CAS). **C. salina** var. **major** Yunck.: Canada, *Kennedy & Ganders 4947* (UBC); USA, California, *Dudley 267* (CAS); *Moldenke 25731* (NY). **C. sandwichiana** Choisy: USA, Hawaii, *Stern 8416* (CHICO); *Fosberg 9822a* (RSA). **C. santapaui** Banerji & Sitesh Das: Nepal, *Nicolson 2796* (MO). **C. serrata** Yunck.: Brazil, *Acevedo & Lopes 848* (RB); *Smith 15049* (US). **C. sidarum** Liebm.: Guatemala, *Standley 74614* (NY); Mexico, *Ayala 1054* (TEX & LL); *Palmer 51* (S); Nicaragua, *Stevens & Krukoff 20950* (GH). **C. squamata** Engelm.: USA, New Mexico, *Wooton & Standley 3355* (CAS); *Wooton s.n.* (NMC); Texas, *Hutchins 643* (BRIT). **C. stenolepis** Engelm.: Ecuador, *Jaramillo & Carvajal 2307* (AAU); *Nuñez* et al. *2220* (QCNE); *Asplund 6678* (NY). **C. strobilacea** Liebm.: Mexico, *Gentry 5291* (ARIZ); *Jones s.n.* (RSA). **C. suaveolens** Ser.: Australia, *Alcock 10415* (RSA); Chile, *Rusby 2000* (NY); USA, California, *Dudley s.n.* (CAS). **C. subinclusa**

Durand & Hilg.: USA, California, *Dudley 1653* (DS); *Ewan 11049* (NY); *Mason 5766* (NY). **C. suksdorfii** Yunck.: USA, California, *Oswald & Ahart 5874* (CHICO); *Twisselmann 14603* (SD); *Tracy 18430* (UC); *Bailey & Bailey 2672a* (UC). **C. tasmanica** Engelm.: Australia, *Barker s.n.* (CANB); *Lepschi 908* (CAMB); *Watts 1/86* (MEL). **C. tinctoria** Mart. ex Engelm.: Mexico, *Ventura 4248* (IEB); *Rzedowski 34596* (IEB); *Van Devender 94-1008* et al. (WLU). **C. tuberculata** Brandegee: USA, Arizona, *Beauchamp 3112* (SD); Mexico, *Gunn & Felger 19998* (ARIZ); *Rodriguez 1642* (MEXU); *Reina-G. & Van Devender 2001-624* (WLU). **C. umbellata** Kunth var. **umbellata**: Mexico, *Nabhan & Rea 167* (ARIZ); *Moran 24758* (SD). **C. umbellata** var. **reflexa** Yunck.: USA, Arizona, *Felger 92-707* (CAS); Mexico, *Van Devender 93-1123* et al. (UCR); *Van Devender 94-458* et al. (UCR); *Van Devender & Reina-G. 2006-638* (WLU). **C. umbrosa** Beyr. ex Hook: Canada, Alberta, *Allen 150* (DAO); Manitoba, *Criddle s.n.* (DAO); USA Utah, *Jones s.n.* (CAS); Colorado, *Mulford s.n.* (NY). **C. vandevenderi** Costea & Stefanović: Mexico, *Van Devender* et al. *2006-983*; *98-1334* (WLU). **C. veatchii** Brandegee: Mexico, *Porter 198* (MEXU); *Rebman 3189* (SD). **C. victoriana** Yunck.: Australia, *Cowie 9624* (CANB); *Glennon 379* (CANB). **C. werdermanii** Yunck.: Chile, *Werdermann 880* (SGO). **C. woodsonii** Yunck.: Guatemala, *Molina & Molina 30020* (MO); Panama, *Davidson 967* (GH). **C. xanthochortos** Mart. ex Englem. var. **xanthochortos**: Argentina, *Arbo* et al. *6953* (MO); Paraguay, *Zardini & Vera 46124* (MO). **C. xanthochortos** var. **carinata** Yunck.: Paraguay, *Bernardi 18758* (MO), *Billiet & Jadin 3294* (MO). **C. xanthochortos** var. **lanceolata** Yunck.: Argentina, *Schulz 7139* (CTES); Paraguay, *Zardini & Villate 46371* (WLU). **C. yucatana** Yunck.: Mexico, *Alava 1341* (CAS; TEX & LL); *Nee & Taylor 29575* (MO); *Breedlove & Smith 22017* (MO).

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