2009

# Evolution and Systematic Significance of Reproductive Structures in the Genus *Cuscuta* (dodders, Convolvulaceae): Pollen and Gynoecium

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## Recommended Citation

Welsh, Mark, "Evolution and Systematic Significance of Reproductive Structures in the Genus *Cuscuta* (dodders, Convolvulaceae): Pollen and Gynoecium" (2009). *Theses and Dissertations (Comprehensive)*. 957.
https://scholars.wlu.ca/etd/957

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395, rue Wellington
Ottawa ON K1A 0N4
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*Your file Votre référence*
*ISBN:* 978-0-494-64347-1
*Our file Notre référence*
*ISBN:* 978-0-494-64347-1

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# Evolution and systematic significance of reproductive structures in the genus *Cuscuta* (dodders, Convolvulaceae): pollen and gynoecium

by

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(B.A. Biology, Wilfrid Laurier University, 2007)

THESIS
Submitted to the Department of Biology
Faculty of Science
in partial fulfillment of the requirements for the
Master of Science in Integrative Biology
Wilfrid Laurier University

Waterloo, Ontario, Canada, 2009

# Abstract

The genus *Cuscuta* (dodders, Convolvulaceae) is one of the most significant lineages of parasitic plants from economic, conservation, and anthropogenic perspectives. Members of the genus are twining stem parasites with no roots, lacking almost completely chlorophyll or its function, and gather required nutrition from their host via specialized haustorial connections. While there are almost 200 described species, problems with species identification exist because many diagnostic characters are restricted to their tiny flowers. Probably contributing to this identification difficulty is the fact that the group has not received taxonomic attention for over 75 years; even knowledge of its basic reproductive biology is sparse. Together these conditions have had negative consequences for applied research on this group. More recently, *Cuscuta* have witnessed a scientific rejuvenation. Molecular phylogenies have been published for 2 of the 3 major infrageneric lineages, 5 of the 15 clades of subg. *Grammica* have received taxonomic revisions, and work on their basic reproductive ecology has gained momentum. Still lacking, however, are reliable morphological characters that are able to support molecular phylogenies, aid in the description of new sections or species, or provide a solid morphological framework within which ecological variables can be compared. This work presents a thorough survey of the structural diversity of two of the most important, and systematically relevant, reproductive structures in *Cuscuta*: pollen and the gynoecium. While a number of characters were initially considered, a total of 15 qualitative and 14 quantitative characters were included in analysis. A reclassification of pollen and stigma morphology was required to better account for the variation that is present in these structures. Character states were coded using Thiele's gap-weighting, and many of the characters were optimized onto a phylogenetic supertree that resulted from the combination of three large-scale phylogenies based on plastid *trnL*-F and nuclear ITS sequences. Key results are discussed in terms of their ecologic, systematic, taxonomic, cytological or developmental significance.

# Acknowledgements

Professional acknowledgements are included at the end of each major chapter in this thesis; therefore, these acknowledgements are more of personal thanks than business. My supervisor, Mihai Costea, is not included in those acknowledgements as he is listed as a co-author, so it is only timely that I thank him here. Mihai was incredibly supportive throughout my M.Sc. and he continually challenged me to push my capabilities both as a researcher and an individual. While the opportunity to work in the laboratory is greatly appreciated, my fondest memories will stem from our time in the field, for which I consider myself very fortunate. My committee, Dr Scott Ramsay and Dr. Frédérique Guinel, whom I must also thank for their humble guidance throughout my undergraduate years, provided me with their expert advice on everything from methodologies to deadlines. I would also like to thank Michael Wright for his help in the lab, in the field, and for lending an ear to bounce ideas off of. Thanks to Dr. David Pearson for graciously donating a bottle of acrylic resin and his sectioning advice. I want to thank my family for all they have done for me throughout my academic career; without them I would never have made it this far. Lastly, Jess, thank you for your love and support, and being with me every step of the way.

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# Chapter 1

# General Introduction

## 1.1 The study of character evolution

An emerging trend in evolutionary biology is the practice of mapping characters (morphological, anatomical, behavioral, etc.) onto molecular phylogenies in an effort to determine ancestral character states or discover important synapomorphies that define groups of monophyletic taxa (and support phylogenetic hypotheses). To date, this practice has been largely led by paleontologists and biologists interested in studying vertebrates (for example see Brochu 1997; Shaffer et al. 1997; O'Leary 1999; Asher et al. 2005; Geisler & Uhen 2005; Ksepka et al. 2006), and to a lesser extent invertebrates (see Smith et al. 1995; Arango & Wheeler 2007; Cardinal & Packer 2007). Regardless, although such studies are comparatively fewer, a dizzying amount of work on phylogenetics and character evolution has also been accomplished in plants (Frye & Kron 2003, Friedman et al. 2004, Barrett 2008).

Our knowledge of relatedness between organisms, coupled with an understanding of their evolutionary history, has tremendously increased in recent decades (ex. Friedman et al. 2004; Yokoyama 2005; Judd et al. 2007). Theoretical progress in phylogenetic tree reconstruction, combined with increased computer-power and molecular tools, has made it possible to reconstruct phylogenies of groups of closely-related species, or of whole domains, kingdoms, phyla and orders, from which evolutionary trees can be constructed (Soltis et al. 2005; Yokoyama 2005). It is therefore of little surprise that numerous studies have utilized these hypotheses as a backbone to study evolution of the traits that have shaped the current vascular flora of the world. Among plant life-history traits, reproductive characters are of particular importance because they affect both microevolutionary processes and macroevolutionary patterns (Barrett 2008). Reproductive characters acquire these fundamental roles because they not only influence genetic transmission and population genetic structure, but also selection response and patterns of evolutionary diversification (Harder & Barrett 2007).

## 1.2 Pollen development and the fertilization pathway

A particularly intriguing area of sexual plant reproduction involves the series of events that surround pollen production, its successful germination, and the factors that affect fertilization of the ovules. Two of the major floral parts involved in this pathway, the pollen and the gynoecium, are the structures of interest in my thesis. Pollen is the male gametophyte, a unique multicellular (2 or 3 celled) 'organism' that most higher plants (though not all) utilize as part of their sexual reproductive system. Pollen production occurs in the anther where a complex suite of developmental factors account for the enormous morphological variation present in the pollen of angiosperms (D'Arcy & Keating 1996). One of the most distinctive features of pollen grains is the pollen wall. This outermost wall, termed the exine (or tectum), shows a great deal of variation and is usually ornamented in a species-characteristic fashion (Hesse 2000; Edlund et al. 2004). Advances in molecular genetics, especially using *Arabidopsis thaliana*, have propelled our understanding of the complex events that lead to this differential patterning (reviewed in Lersten 2004).

The path a pollen grain takes once released from the anther is largely a subject of pollination ecology. For the purposes of this study, it is noteworthy that it has long been thought that the shape and surface features of the grain are correlated with the particular pollination mechanism of the plant (e.g. Wodehouse 1935; 1936); direct evidence for this, however, was slow to accumulate. Although some studies have revealed positive correlations between pollen exine and pollination mechanisms (e.g. Hesse 1981; Ferguson and Skvarla 1982; Grayum 1986; Bolick 1990; Hesse 2000; Salter et al. 2002; Schwendemann et al. 2007) or pollen-ovule ratio differences (e.g. Niklas 1992), others show no correlation (Taylor and Levin 1975; Lee 1978; Kress 1986).

The stigma is the receptive part of the female reproductive system, which in conjunction with the styles and ovary/ovule(s) comprise the gynoecium. Functionally, the main purposes of the stigma are to capture the pollen, support its rehydration, stimulate its germination, and guide the pollen tubes towards the ovules; however, recognition of intraspecific pollen is also a task generally accomplished by the stigma (Edlund et al. 2004; Lersten 2004). Aside from sporophytic self-incompatibility, pollen recognition occurs via a number of regulatory pathways at the stigma surface, including ‘lock and key’ mechanisms, hydrostatic forces, or spatial (herkogamy) and temporal maturation (dichogamy) differences between stigma and anther (Lersten 2004). Once the germinating pollen tube enters the stigma, it is soon led by the transmitting tissue down the style towards the ovules (Kim et al. 2004). Depending on the type of style, the transmitting tissue may either be a strand of elongate, thick-walled cells (closed style), or an interior tube lined with secretory cells (open style) (Edlund et al. 2004). Transmitting tissue of either open or closed styles has two certain functions, to provide physical guidance and nutrients for pollen tubes (Cheung et al. 1995; Lersten 2004). The precision with which pollen tubes advance indicates that a complex system is in place, yet whether the mode is anatomical or chemotrophic in nature still awaits elucidation (Lennon et al. 1998; Kim et al. 2004).

## 1.3 The genus *Cuscuta*

### 1.3.1 Biology

This thesis is mainly concerned with reproductive character evolution in the genus *Cuscuta* (commonly known as dodders). *Cuscuta* is the only parasitic lineage within the family Convolvulaceae; it consists of ca. 200 holoparasitic species, and it has an almost worldwide distribution. The flowers of *Cuscuta* offer more taxonomic information than all other parts of the plant combined (Engelmann 1859; Yuncker 1932, 1965). They are small (generally less than 5 mm), hermaphroditic, (3-) 4-5-merous, typically white-cream and fleshy; the calyx is gamosepalous generally persistent in fruit; the corolla is gamopetalous, variously shaped. The stamens alternate with the corolla lobes, and anthers are sessile or on short filaments. Infrastaminal scales, a character unique to *Cuscuta*, are present in most species (Yuncker 1932). These are scale-like appendages dentate or fringed, bridged and fused with the corolla tube base, forming a corona alternating with the corolla lobes. Their function is unknown, however, it has been hypothesized that they have a protective role (Yuncker 1932; Musselman 1986), a secretory function (Tiagi 1966), or that they may act as secondary nectar receptacles (Prenner et al. 2002). The ovary is two-locular, each locule containing two anatropous ovules; styles are one or two, sometimes fused to varying degrees, equal or unequal in length. Stigmas are variable morphologically (Yuncker 1932).

*Cuscuta* has been subject to hundreds of molecular, anatomical, physiological, developmental and ecological studies (for a review see Costea and Tardif 2006), yet still lacking are data pertaining to their fundamental biology and ecology. Literature on the reproductive biology of *Cuscuta* species is generally sparse (reviewed for weed species by Dawson et al. 1994; Costea et al 2006b; Costea and Tardif 2006). Only one study reports experimental crosses and it was determined that *C. attenuata* is mainly autogamous, but with some potential for allogamy because intra- and inter-population crosses were successful (Prather and Tyrl 1993). Other data that support autogamy includes the presence of cleistogamous (or functionally cleistogamous) flowers observed in some species (e.g. Verdcourt 1948; Dawson et al. 1994; Costea and Tardif 2006; Rodriguez-Pontes 2008), and the low pollen/ovule ratios reported in others (Beliz 1986). However, some indirect evidence suggests at least the capacity for allogamy: for example, nectary pores found at the ovary base in *C. reflexa* (Prenner et al. 2002), the discovery of several ancient hybridization events (Stefanović and Costea 2008), and insect visitation observed in the flowers of some species (Pierce 1939; Anderson 1962; Musselman 1986; Holm et al. 1997; Costea and Tardif 2006). In addition to sexual reproduction, vegetative multiplication from tissues overwintering inside the host has been observed in several species (Dean 1937; Strivastava and Dwivedi 2001; Meulebrouck et al. 2009).

### 1.3.2 Importance of the genus

As weeds *Cuscuta* are economically one of the most important groups of parasitic plants, and infestation by their seeds can cause major yield loss (see Dawson et al. 1994). For example, *C. campestris*, likely the most widespread and common species, is known to infest at least 25 important crop species in 55 countries (Holm et al. 1997). Globally, ~15 *Cuscuta* species are considered weeds (Parker and Riches 1993; Dawson et al. 1994), but the detrimental nature of these species (combined with the negative "stigma" associated with parasitic organisms in general) has resulted in major conservation issues for the entire genus (Costea and Stefanović 2009b). Legislation in Canada, for example, places "*Cuscuta* spp." as a whole on "noxious weeds" lists in Quebec, Ontario, Manitoba, and British Columbia, and as "restricted weeds" in Alberta (Costea & Tardif 2006; Rice 2009; Costea & Stefanović 2009b). Despite this hurdle it is important to remember that the vast majority of *Cuscuta* are indeed beneficial. Similar to other parasitic plants (Bardgett et al. 2006), *Cuscuta* spp. play key roles in natural ecosystems. Dodders are able to modify the plant community structure and dynamics, impact multiple trophic levels, and they can even alter abiotic factors (Pennings and Callaway 1996, 2002; Press and Phoenix 2005). In a more anthropogenic sense, some species (e.g. *C. chinensis* and *C. japonica*) have been used for thousands of years in ancient Chinese medicine (known as 'Tu Si Zi') (Chao et al. 2003). Herbal uses include a decoction of the seeds or stems to treat ailments of the kidney (Anis et al. 2000) and liver, and for reproductive problems in both males and females (Quin et al. 2000).

### 1.3.3 Taxonomy and systematics

Precise phylogenetic relationships of parasitic angiosperms relative to non-parasitic members are typically difficult to infer (Kuijt 1969; Nickrent et al. 1998). Similarly, the exact "position" of *Cuscuta* in Convolvulaceae is currently unknown, even after analysis of more than 6800 DNA bp (Stefanović 2002; Stefanović and Olmsted 2004). It is now understood, however, that *Cuscuta* is nested in Convolvulaceae, and many alternative hypotheses were rejected (Stefanović and Olmsted 2004). Although evolutionary affinities at the family level are still unclear, the infrageneric relationships within *Cuscuta* have recently begun to be unraveled. Molecular phylogenies have been published for two of the three major infrageneric lineages. Using ITS *r*DNA and *trn*L intron sequences, Garcia and Martin (2007) studied phylogenetic relationships within the subgenus *Cuscuta*. Subgenus *Grammica*, by far the largest and most diverse of the subgenera, has also been subjected to a molecular study (Stefanović et al. 2007). The ca. 130 species sampled in the study clustered into 15 major clades with little correspondence to the previous taxonomic scheme of Yuncker (1932). At the genus level, a combined analysis of *rbc*L and 26S *r*DNA gene sequences has been used to infer an unpublished phylogeny (Stefanović and Costea, in preparation); the phylogeny includes the three subgenera, plus one new one, '*Pachystigma*' from South Africa (Costea and Stefanović, in preparation). This is the most complete phylogeny to date and will serve as a basis for the current study. At the species level, taxonomic revisions have been published for only five North American clades in subgenus *Grammica* (Costea et al. 2005; Costea et al. 2006 a,b,c,d; Costea et al. 2008b; Costea and Stefanović 2009 a,b).

## 1.4 Rationale behind this study

- As a result of its parasitic lifestyle, a major problem studying the genus *Cuscuta* (compared to other angiosperms) is the reduced number of morphological characters that are available for taxonomic purposes. A clear indication of the taxonomic difficulty of this genus is the fact that it has not been studied for more than 75 years since Yuncker's monograph (1932). This has not only negatively affected our understanding of evolutionary relationships within *Cuscuta*, but it has also had negative repercussions on applied research, both biological (e.g. biodiversity, floristics, ecology) and agronomical (e.g. control methods), because researchers simply do not have the tools to distinguish among *Cuscuta* species. The field of molecular systematics has recently generated an unprecedented progress in the phylogenetics of *Cuscuta*, but these molecular phylogenies must be supplemented with morphological characters necessary for the description of the new sections (e.g. clades A-O from Stefanović et al. 2007) or species. Costea et al. (2008a) indicated that up to 20 more species must be described in the future to better account for the diversity in this genus. This emphasizes even more the necessity to discover new characters useful for the systematics of the genus.
- There is also a need to accumulate a theoretical basis for character evolution comparison, not only within *Cuscuta* and Convolvulaceae, but with other parasitic groups as well.

- Lastly, a prerequisite for understanding the reproductive biology of *Cuscuta* is sound knowledge of the structural variation of floral structures across the genus, and inferring their connection with potential reproductive functions.

## 1.5 Objectives

1. To survey variation of pollen morphology across the entire genus and propose evolutionary scenarios for pollen characters.

2. To survey the variation of gynoecium morphology, micromorphology, and anatomy (ovary, stigma, style) across the genus, and analyze patterns of evolution of their characters.

3. Assess the taxonomic and systematic significance of pollen and style/stigma characters in *Cuscuta* and their usefulness to provide differences between various infrageneric taxa and species.

## 1.6 Thesis overview

The first chapter of this thesis was intended to provide a brief introduction to the practice of character evolution, the floral structures of interest to this study, as well as a general literature review of the group of organisms under study. From this point, the second chapter has been formatted and submitted to Plant Systematics and Evolution. Authorship of this manuscript is shared between myself, Sasa Stefanović and Mihai Costea. My role in this project was fivefold: I sampled many of the herbarium specimens that were used; prepared and photographed much of the material; designed and performed the analysis; prepared the initial draft; and actively participated in revisions. The manuscript involves a comprehensive survey of morphological variations of pollen in *Cuscuta*, information that is unavailable for the vast majority of species in this genus. Making extensive use of the scanning electron microscope, both qualitative and quantitative characters were scored based on the micromorphology of pollen and this information was analyzed in an evolutionary context for the first time. As one of the most important palynological characters, the tectum 'types' were analyzed using a novel method after it was determined that a morphological intergradation occurs among pollen grains of *Cuscuta* species. Therefore, in an attempt to reduce coding bias, this traditionally qualitative character was converted to a quantitative character (i.e. 'percent perforation') while we ensured that the coded states were also expressed for descriptive purposes in relationship to the traditional qualitative types. Ecological context is then given to the most informative characters with an attempt at determining the cause of variation between character states. To finish, the taxonomic significance of pollen in *Cuscuta* is discussed.

The third chapter in this thesis is also formatted for submission to Plant Systematics and Evolution. The decision to submit the second manuscript to the same journal as the first manuscript was made because the character coding and optimization techniques are the same as those used in the pollen study. Thus, to avoid method repetition, both manuscripts will be submitted to the same journal. I am, together with my supervisor, a co-author on this manuscript. Again I was assigned the task of sampling material, preparing and photographing said material, planning and performing analysis, writing initial drafts, and participating in revisions. In essence, this study involved a treatment of the gynoecia of *Cuscuta* similar to that which was given to the pollen, though a more extensive character list was developed to accommodate the high morphological diversity found in this structure. The gynoecium study involved a comprehensive survey of 136 taxa using anatomical, morphological, and micromorphological techniques. Similar to the pollen study, data generated was optimized onto a phylogenetic supertree. In addition to highlighting a number of characters important for both the taxonomy and systematics of *Cuscuta*, a novel technique for embedding dried herbarium material into acrylic plastic for sectioning was developed for this thesis. Although it was difficult to relate trends in many of the observed characters directly to the reproductive biology of *Cuscuta* (simply because so little knowledge of it exists), an attempt was made to link observations with their possible ecological significances. To end, a brief statistical analysis was conducted to determine any correlations between pollen and stigma/style characteristics.

The forth and final chapter of this thesis contains a general discussion on the impacts this work will have on related biological fields based on a brief synthesis of the results from Chapter 2 and 3. It also comprises a brief description of the integrative nature of this project, potential future work, and a condensed summary.

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# Chapter 2

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

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## 2.1 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. Five quantitative characters were coded using the gap weighting method and optimized onto a supertree constructed from three large-scale molecular phylogenies of the genus based on nuclear ITS and plastid *trnLF* sequences. The results indicate that 3-zonocolpate pollen is the ancestral character state, while grains with more colpi (up to 8) 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. Imperforate tectum is likely the ancestral condition, while pollen grains with increasingly larger perforation areas have evolved multiple times. The reticulated tectum, unknown in other Convolvulaceae, probably represents an apomorphy which is present in *Cuscuta* only in two lineages (subg. *Monogynella*, and Clade O of subg. *Grammica*). Overall, the morphology of pollen supports either *Cuscuta* as a sister to 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*.

**Key Words** Convolvulaceae - *Cuscuta* - dodders - evolution - phylogeny - pollen morphology - scanning electron microscopy - taxonomy

## 2.2 Introduction

The taxonomic significance of pollen morphology in Convolvulaceae has been long recognized. For example, Hallier (1893) assigned the genera within this family into 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), *Maripa, Dicranostyles* and *Lysiostyles* (Austin 1973a,b), 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 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 the parasitic lifestyle, dodders exhibit extreme reductions of the vegetative structures limiting the number of morphological characters available for systematic studies to flowers and fruit (Stefanović et al. 2007). It is, therefore, imperative to search 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 in 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 the 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. (2006 a,b,c,d; 2008 a,b) 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; Stefanović et al. 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*.

## 2.3 Materials and methods

### 2.3.1 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 a LEO 1530 FE-SEM at 10-15KV. 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), while ImageJ (Abramoff et al. 2004) was used for the determination of areas.

### 2.3.2 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 (a reticulate ornamentation consisting of muri and lumina < than 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 the 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 a widespread acceptance despite the fact that they provide a more accurate description of tectum morphology.

Five other quantitative characters, the pollen length [polar axis], polar/equatorial [P/E] ratio, the average diameter of perforations (puncta or lumina), the average surface of perforations, and the 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 1991; 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 10 for all the quantitative characters except for the percent perforation where n was 8. Eight character states were sufficient to describe tectum perforation patterns (Table 1).

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 these 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., only "clade 1" has over 2/3 of the species in Convolvulaceae); c) *Cuscuta* as a sister to one of the members of the "bifid clade"—but 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* is also taking into account the ingroup distribution of character states both at the level *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 *trnL*-F 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). Both ACCTRAN and DELTRAN were turned off, and instances of bootstrap values below 85% were considered unresolved and are indicated in the tree as polytomies.

## 2.4 Results and discussion

### 2.4.1 Number of apertures

Pollen of *Cuscuta* is heteromorphic (sensu Till-Bottraud et al. 1995) (Fig. 1, A–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 ± two 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. 1994), and can be linked ontogenetically to the succession of events that take place during the 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 a faster pollenic tube growth and a 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 to 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 3 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 a 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,b, 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 5 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., *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).

### 2.4.2 Exine

Exine in *Cuscuta* is tectate imperforate or semitectate, perforate to reticulate (Fig. 1, E–L) with a single layer of unbranched columellae. Supratectal ornamentation typically consists of rounded to acute scabrate processes less than 1 μm long, ± evenly distributed on the pollen surface (Fig. 1, M–P). Larger supratectal conical spines (> 1 μm) are present only in subg. *Monogynella* in *C. lehmanniana* and *C. monogyna*. Pollen with a reticulate tectum is unknown in other Convolvulaceae (see below) and has evolved in Cuscuta only in some species of subg. *Monogynella* and the 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. has in fact a microreticulate tectum, with a different exine structure and ornamentation.

Pollen grains with smaller perforations (usually ≤ 1μm) form a continuous transition from imperforate to microreticulate (Fig. 1, E–L) with the former condition prevalent in *Cuscuta* (ca. 60% of species), and encountered in many Convolvulaceae (Sengupta 1972; Austin 1973a,b; 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. 1, E–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 a tectate 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 Skavarla 1982; Hesse 2000), hydrophily (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.

### 2.4.3 Pollen size and shape

Pollen size is only relatively homogenous within major clades of *Cuscuta* (Table 2; tree not shown). Generally, 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 50–80 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). However, small pollen grains were reported from Cardiochlamydeae (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 both in *Cuscuta* and Convolvulaceae.

Pollen size in *Cuscuta* may be associated with the 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 (e.g. 6–23.1 µm in *C. reflexa*; Kaul and Bhan 1977), and among the highest estimates for the nuclear genome (e.g. 44.93 pg/2C in *C. lupuliformis*; McNeal et. al. 2007). Subgenus *Grammica* has typically the smallest chromosomes (typically ≤ 4 µm) and pollen grains, but some of its species with larger chromosomes (e.g. 8–16 µm in *C. indecora*, Fogelberg 1938) and a 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 at 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 at al. 2007). The presence of 5–8 zonocolpate pollen grains in *Cuscuta* is consistent with all the three phylogenetic scenarios mentioned in the "Materials and methods" section, but together with the small size, the overall morphology of pollen suggests for *Cuscuta* either a sisterhood 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) are associated with spheroidal or subspheroidal shapes, the prolate or perprolate shapes (P/E ratios > 1.33) are likely to be primitive as suggested by Austin (1998) for Convolvulaceae in general.

### 2.5 Taxonomic significance of pollen characters in *Cuscuta*

*Cuscuta* is one of the most difficult taxonomically parasitic groups. 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 such groups, e.g. 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, 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 one 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.

## 2.6 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, albeit 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 either *Cuscuta* as a sister 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.

## 2.7 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, 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, WTU and XAL for supplying plant material. We also thank 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 Natural Sciences and Engineering Research Council (NSERC) of Canada to M. Costea (327013–06) and to S. Stefanović (326439–06).

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**Table 2.1** 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 (μm2) | 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 perforatuml (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) |

**Table 2.2** 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). The species for which no molecular data was available (indicated with "*") are also tentatively placed into major infrageneric groups based on their morphology. P = prolate; SP = subprolate; S = spheroidal; SO = suboblate.

| | | Species Details | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Infrageneric group | Species | Percent perforation (%) | Avg. perforation area (µm2) | Perforation diameter (µm) | Length (µm) | Width (µm) | P/E ratio | Shape | Number of colpi |
| Subg. *Monogynella* (8 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* (5 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 |
|  | *C. europea* | 0.4 | 0.052 | 0.281–(0.390)–0.584 |
|  | *C. planiflora* | 0 | 0 | 0 |
| Pachystigma clade (5 species) | *C. africana* | 3.8 | 0.257 | 0.468–(0.564)–0.662 |
|  | *C. angulata* | 0.1 | 0.063 | 0.415–(0.430)–0.461 |
|  | *C. appendiculata* | 0 | 0 | 0 |
|  | *C. natalensis* | 1.6 | 0.044 | 0.168–(0.289)–0.518 |
|  | *C. nitida* | 0.1 | 0.029 | 0.211–(0.288)–0.393 |
| Subg. *Grammica* (130 taxa) Clade A | *C. brachycalyx* | 0 | 0 | 0 |
|  | *C. californica* | 0 | 0 | 0 |
|  | *C. decipiens* | 0.1 | 0.049 | 0.243–(0.298)–0.334 |
|  | *C. draconella* | 0 | 0 | 0 |
|  | *C. howelliana* | 6.3 | 0.107 | 0.286–(0.382)–0.542 |
|  | *C. jepsonii* | 0.2 | 0.048 | 0.181–(0.237)–0.371 |
|  | *C. occidentalis* | 0 | 0 | 0.161–(0.295)–0.484 |
|  | *C. salina* var. *salina* | 1.2 | 0.069 | 0.314–(0.379)–0.547 |
|  | *C. salina* var. *major* | 1 | 0.022 | 0.239–(0.367)–0.800 |
|  | *C. subinclusa* | 0.2 | 0.04 | 0.254–(0.327)–0.439 |
|  | *C. suksdorfii* | 0 | 0 | 0.287–(0.359)–0.499 |

| | | | | |
|---|---|---|---|---|
| 18.6–(19.7)–21.8 | 12.9–(14.2)–15.9 | 1.39 | P (–SP) | 3 (–4) |
| 20.7–(23.1)–25.8 | 12.4–(13.8)–14.9 | 1.67 | P | 3 (–4) |
| 22.4–(24.1)–25.3 | 12.2–(17.0)–19.0 | 1.42 | P (–SP) | 3 (–4) |
| 28.0–(29.6)–31.2 | 17.4–(19.4)–20.6 | 1.53 | P (–S) | 3 (–4) |
| 26.8–(28.0)–29.5 | 15.2–(15.8)–17.1 | 1.77 | P | 3 (–4) |
| 17.1–(18.9)–20.0 | 16.0–(17.6)–19.4 | 1.07 | S (–SO) | 3 (–4) |
| 17.2–(17.6)–18.3 | 18.0–(19.2)–19.9 | 0.92 | (P–) S | 3 (–4) |
| 15.6–(18.2)–22.6 | 14.9–(19.0)–22.1 | 0.96 | (P–) S | 3 (–4) |
| 22.9–(24.0)–24.8 | 15.0–(16.0)–17.0 | 1.50 | P | 3 (–4) |
| 19.3–(21.5)–23.0 | 11.0–(17.5)–20.8 | 1.23 | SP | 3 (–4) |
| 19.9–(21.3)–23.0 | 13.5–(14.3)–14.7 | 1.49 | P (–SP) | 3 (–4) |
| 15.0–(16.2)–17.4 | 13.2–(14.6)–16.7 | 1.11 | (P–) S | 3 (–4) |
| 13.5–(15.6)–16.8 | 9.2–(11.5)–13.5 | 1.36 | P (–SP) | 3 (–4) |
| 19.2–(21.3)–22.9 | 13.3–(14.0)–14.9 | 1.52 | P | 3 (–4) |
| 19.4–(21.6)–24.6 | 12.6–(16.2)–18.8 | 1.33 | P (–SP) | 3 (–4) |
| 16.5–(17.2)–21.3 | 10.6–(11.8)–14.5 | 1.46 | P (–SP) | 3 (–4) |
| 17.1–(17.7)–18.2 | 14.7–(15.9)–17.2 | 1.11 | S | 3 (–4) |
| 14.7–(15.0)–21.1 | 15.6–(16.4)–16.9 | 0.91 | (SP–) S | 3 (–4) |
| 24.9–(26.6)–27.6 | 12.4–(15.7)– | 1.69 | P | 3 (–4) |

|  |  |  |  |  |
|---|---|---|---|---|
| Clade B | *C. australis* | 0.4 | 0.045 | 0.246–(0.317)–0.439 |
|  | *C. campestris* | 0.8 | 0.056 | 0.213–(0.282)–0.367 |
|  | *C. glabrior* | 0.1 | 0.069 | 0.352–(0.416)–0.485 |
|  | *C. gymnocarpa* | 0.2 | 0.042 | 0.222–(0.312)–0.445 |
|  | *C. harperi* | 0.4 | 0.037 | 0.197–(0.230)–0.287 |
|  | *C. obtusiflora var. obtusiflora* | 4.7 | 0.03 | 0.183–(0.260)–0.396 |
|  | *C. obtusiflora var. glandulosa** | 0.1 | 0.066 | 0.222–(0.309)–0.442 |
|  | *C. pentagona* | 0 | 0 | 0 |
|  | *C. plattensis* | 0 | 0 | 0 |
|  | *C. polygonorum** | 0 | 0 | 0 |
|  | *C. runyonii* | 0.1 | 0.017 | 0.166–(0.231)–0.282 |
|  | *C. stenolepis* | 0 | 0 | 0.283–(0.424)–0.643 |
| Clade C | *C. corniculata* | 0.5 | 0.01 | 0.153–(0.210)–0.335 |
|  | *C. incurvata* | 1.3 | 0.049 | 0.211–(0.353)–0.500 |
|  | *C. micrantha* | 0 | 0 | 0 |
|  | *C. parviflora* var. *elongata* | 0 | 0 | 0.187–(0.199)–0.212 |
|  | *C. pauciflora** | 0.2 | 0.031 | 0.207–(0.345)–0.537 |
|  | *C. platyloba* | 1.2 | 0.02 | 0.079–(0.211)–0.383 |

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|---|---|---|---|---|
| | 17.8 | | | |
| 18.9–(20.9)–23.5 | 11.4–(13.7)–16.9 | 1.53 | P (–SP) | 3 (–4) |
| 17.6–(23.6)–26.9 | 18.0–(18.3)–18.7 | 1.29 | (P–) SP | 3 (–4) |
| 17.3–(18.0)–21.1 | 10.8–(12.2)–13.2 | 1.48 | P (–SP) | 3 (–4) |
| 20.3–(21.1)–22.3 | 15.2–(17.4)–19.2 | 1.21 | (P–) SP | 3 (–4) |
| 16.5–(17.5)–23.4 | 9.7–(13.3)–16.0 | 1.32 | (P–) SP | 3 (–4) |
| 18.9–(20.1)–21.1 | 12.9–(13.7)–14.9 | 1.16 | SP | 3 (–4) |
| 17.4–(21.2)–23.6 | 14.0–(18.3)–20.1 | 1.47 | P (–SP) | 3 |
| 18.6–(19.3)–22.8 | 12.0–(12.1)–13.5 | 1.60 | P | 3 (–4) |
| 16.5–(17.5)–22.4 | 16.6–(17.1)–17.4 | 1.02 | S (–SO) | 3 (–4) |
| 19.6–(21.8)–23.9 | 14.2–(16.1)–17.8 | 1.35 | P (–SP) | 3 (–4) |
| 13.0–(14.0)–18.6 | 16.2–(17.3)–18.3 | 0.81 | (SP–) SO | 3 (–4) |
| 16.6–(18.2)–20.0 | 13.7–(14.1)–14.8 | 1.29 | (P–) SP | 3 (–4) |
| 20.8–(21.9)–23.2 | 17.5–(17.8)–18.3 | 1.23 | SP (–SO) | 3 (–4) |
| 13.0–(14.0)–14.8 | 15.1–(16.5)–17.5 | 0.85 | (SP–) SO | 3 (–4) |
| 13.4–(15.8)–17.9 | 16.9–(17.5)–18.7 | 0.90 | (SP–) S | 3 (–4) |
| 17.5–(18.2)–18.8 | 16.6–(17.4)–18.8 | 1.05 | S | 3 (–4) |
| 14.4–(16.7)–18.5 | 15.7–(17.5)–19.3 | 0.95 | S | 3 (–4) |
| 16.5–(16.9)–17.2 | 10.8–(12.5)–14.3 | 1.35 | P (–SP) | 3 (–4) |

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|  | *C. racemosa* var. *racemosa** | 0 | 0 | 0 |
|  | *C. racemosa* var. *miniata* | 2.4 | 0.038 | 0.145–(0.273)–0.389 |
|  | *C. suaveolens* | 1 | 0.027 | 0.195–(0.269)–0.380 |
|  | *C. werdermanii* | 0.4 | 0.048 | 0.209–(0.268)–0.314 |
|  | *C. xanthochortos* var. *xanthochortos** | 0.1 | 0.023 | 0.490–(0.521)–0.537 |
|  | *C. xanthochortos* var. *carinata* | 0.2 | 0.036 | 0.132–(0.235)–0.420 |
|  | *C. xanthochortos* var. *lanceolata** | 0 | 0.009 | 0.144–(0.177)–0.195 |
| Clade D | *C. cephalanthi* | 20.1 | 0.137 | 0.205–(0.378)–0.707 |
|  | *C. compacta* | 4.3 | 0.065 | 0.423–(0.497)–0.561 |
|  | *C. cuspidata* | 0.2 | 0.051 | 0.199–(0.251)–0.331 |
|  | *C. glomerata* | 2.6 | 0.084 | 0.227–(0.470)–0.751 |
|  | *C. gronovii* var. *gronovii* | 3.6 | 0.121 | 0.279–(0.411)–0.628 |
|  | *C. gronovii* var. *latiflora* | 12.1 | 0.075 | 0.141–(0.328)–0.685 |
|  | *C. rostrata* | 15.2 | 0.098 | 0.314–(0.666)–1.246 |
|  | *C. squamata* | 2.1 | 0.075 | 0.232–(0.304)–0.431 |
|  | *C. umbrosa* | 1.5 | 0.029 | 0.327–(0.508)–0.724 |
| Clade E | *C. denticulata* | 0 | 0 | 0 |
|  | *C. nevadensis* | 4.9 | 0.058 | 0.150–(0.335)–0.716 |

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| 15.2–(16.7)–18.2 | 12.6–(13.5)–15.1 | 1.24 | SP (–S) | 3 (–4) |
| 14.4–(15.7)–17.8 | 15.7–(17.4)–19.9 | 0.90 | (SP–) S | 3 (–4) |
| 14.8–(16.9)–17.6 | 14.6–(16.1)–17.5 | 1.05 | (P–) S | 3 (–4) |
| 15.1–(17.9)–20.0 | 14.8–(17.0)–18.0 | 1.05 | (P–) S | 3 (–4) |
| 18.9–(19.1)–19.6 | 21.7–(22.3)–23.3 | 0.94 | (SP–) S | 3 (–4) |
| 15.8–(16.9)–19.3 | 16.6–(18.0)–19.1 | 0.86 | (SP–) SO | 3 (–4) |
| 13.6–(14.4)–15.4 | 16.2–(16.6)–17.2 | 0.87 | (SP–) SO | 3 (–4) |
| 28.6–(29.3)–30.8 | 20.7–(22.4)–23.6 | 1.31 | (P–) SP | 3 (–4) |
| 22.3–(23.4)–24.6 | 14.3–(17.2)–19.0 | 1.36 | P (–SP) | 3 |
| 24.2–(25.2)–25.8 | 12.2–(14.0)–16.5 | 1.80 | P (–SP) | 3 (–4) |
| 21.5–(22.7)–23.7 | 18.1–(19.0)–19.5 | 1.19 | (P–) SP | 3 (–4) |
| 19.8–(20.6)–23.6 | 15.1–(16.9)–19.3 | 1.22 | SP | 3 (–4) |
| – | – | – | – | 3 (–4) |
| 22.2–(23.9)–25.2 | 13.8–(15.3)–17.8 | 1.56 | P (–SP) | 3 (–4) |
| 21.8–(23.1)–23.9 | 14.7–(15.6)–17.8 | 1.48 | P (–SP) | 3 (–4) |
| 21.9–(23.9)–25.4 | 14.1–(16.0)–18.3 | 1.49 | P (–SP) | 3 (–4) |
| 14.4–(15.2)–16.1 | 11.4–(11.8)–11.9 | 1.29 | SP | 3 (–4) |
| 20.0–(20.8)–22.2 | 12.9–(13.7)–14.0 | 1.52 | P | 3 (–4) |

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| | *C. veatchii* | 0.4 | 0.015 | 0.137–(0.193)–0.311 |
| Clade F | *C. burrelli** | 4 | 0.044 | 0.183–(0.319)–0.455 |
| | *C. haughtii* | 12.5 | 0.186 | 0.298–(0.561)–1.053 |
| | *C. longiloba** | 1.5 | 0.014 | 0.210–(0.276)–0.351 |
| | *C. partita* | 3.2 | 0.019 | 0.163–(0.330)–0.562 |
| | *C. serrata** | 0 | 0 | 0 |
| Clade G | *C. aurea* | 0.1 | 0.052 | 0.302–(0.408)–0.505 |
| | *C. cotijana* | 20.2 | 0.405 | 0.213–(0.306)–1.338 |
| | *C. floribunda* | 8.6 | 0.095 | 0.420–(0.560)–0.693 |
| | *C. jalapensis* | 14.5 | 0.28 | 0.424–(0.670)–0.889 |
| | *C. lindsayi* | 4.9 | 0.04 | 0.305–(0.395)–0.489 |
| | *C. mitriformis* | 17.7 | 0.136 | 0.232–(0.440)–0.781 |
| | *C. purpusii* | 15 | 0.103 | 0.297–(0.573)–1.240 |
| | *C. rugosiceps* | 9.9 | 0.166 | 0.406–(0.773)–1.506 |
| | *C. tasmanica* | 0.2 | 0.036 | 0.228–(0.284)–0.330 |
| | *C. tinctoria* | 19.7 | 0.244 | 0.406–(0.654)–0.905 |
| | *C. victoriana* | 0 | 0.005 | 0.054–(0.117)–0.166 |
| | *C. woodsonii* | 0.6 | 0.031 | 0.254–(0.361)–0.490 |
| Clade H | *C. applanata* | 2 | 0.066 | 0.185–(0.311)–0.625 |

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| 17.8–(19.4)–20.6 | 13.9–(14.9)–16.2 | 1.30 | (P–) SP | 3 (–4) |
| 16.7–(17.5)–18.0 | 13.7–(16.5)–16.6 | 1.06 | (SP–) S | 3 (–4) |
| 19.1–(20.8)–21.8 | 15.4–(18.4)–21.3 | 1.13 | (SP–) S | 3 (–4) |
| 15.0–(18.7)–21.8 | 17.2–(22.6)–25.7 | 0.83 | (S–) SO | 3 (–4) |
| 19.6–(21.5)–23.4 | 14.3–(15.5)–16.6 | 1.39 | P (–SP) | 3 (–4) |
| 18.9–(19.8)–20.7 | 13.2–(16.8)–19.0 | 1.18 | (P–) SP | 3 (–4) |
| 17.5–(18.6)–19.6 | 13.6–(14.9)–15.8 | 1.25 | (P–) SP | 3 (–4) |
| 14.3–(18.3)–22.0 | 13.1–(16.2)–17.4 | 1.13 | (SP–) S | 3 (–4) |
| 21.3–(22.7)–24.8 | 14.6–(17.0)–19.3 | 1.34 | P (–SP) | 3 (–4) |
| 27.8–(28.6)–30.3 | 18.7–(19.7)–21.2 | 1.45 | P (–SP) | 3 (–4) |
| 22.7–(23.7)–25.6 | 21.2–(22.0)–23.0 | 1.08 | (SP–) S | 3 (–4) |
| 23.2–(24.7)–25.9 | 23.9–(25.8)–27.0 | 0.96 | (SP–) S | 3 (–4) |
| 18.7–(20.1)–22.1 | 20.9–(22.8)–24.7 | 0.88 | S (–SO) | 3 (–4) |
| 17.7–(18.6)–19.1 | 15.5–(16.8)–18.2 | 1.11 | (SP–) S | 3 (–4) |
| 21.4–(23.2)–25.0 | 13.4–(15.0)–16.2 | 1.55 | P (–SP) | 3 (–4) |
| 22.6–(24.3)–26.3 | 17.3–(18.1)–19.0 | 1.34 | P (–SP) | 3 (–4) |
| 21.7–(25.4)–28.6 | 16.6–(18.6)–21.0 | 1.37 | P (–SP) | 3 (–4) |
| 17.3–(19.2)–21.3 | 14.5–(16.3)–17.7 | 1.17 | SP (–S) | 3 (–4) |
| 22.2–(23.2)–24.9 | 14.1–(14.9)– | 1.56 | P (–S) | 3 (–4) |

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| | *C. chinensis* | 0 | 0 | 0 |
| | *C. dentatasquamata** | 1 | 0.076 | 0.201–(0.324)–0.661 |
| | *C. potosina* var. *potosina* | 0.2 | 0.03 | 0.132–(0.204)–0.318 |
| | *C. potosina* var. *globifera* | 0.1 | 0.013 | 0.229–(0.306)–0.430 |
| | *C. sandwichiana* | 0 | 0 | 0 |
| | *C. yucatana* | 0.1 | 0.012 | 0.124–(0.200)–0.287 |
| Clade I | *C. americana* | 2.9 | 0.036 | 0.221–(0.328)–0.523 |
| | *C. cozumeliensis* | 16.4 | 0.151 | 0.241–(0.530)–0.924 |
| | *C. globulosa* | 1.2 | 0.05 | 0.356–(0.454)–0.574 |
| | *C. macrocephala* | 17.7 | 0.319 | 0.519–(0.860)–1.398 |
| Clade J | *C. corymbosa* var. *corymbosa** | 6.4 | 0.117 | 0.255–(0.419)–0.579 |
| | *C. corymbosa* var. *grandiflora* | 0.4 | 0.043 | 0.305–(0.491)–0.761 |
| | *C. corymbosa* var. *stylosa* | 17.7 | 0.239 | 0.591–(0.783)–0.983 |
| | *C. prismatica* | 13.2 | 0.206 | 0.565–(0.964)–1.443 |
| Clade K | *C. boldinghii* | 0.4 | 0.006 | 0.262–(0.571)–0.737 |
| | *C. chapalana* | 21.2 | 0.208 | 0.241–(0.530)–0.822 |
| | *C. costaricensis* | 0.2 | 0.06 | 0.369–(0.386)–0.400 |
| | *C. erosa* | 0.4 | 0.055 | 0.312–(0.357)–0.455 |

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| | 15.9 | | | |
| 18.4–(19.6)–20.6 | 20.6–(21.8)–22.9 | 0.90 | (SP–) S | 3 (–4) |
| 19.3–(20.1)–21.8 | 13.6–(16.1)–17.9 | 1.25 | SP (–S) | 3 (–4) |
| 24.7–(26.6)–29.7 | 17.2–(18.1)–19.5 | 1.43 | P (–SP) | 3 (–4) |
| 24.4–(26.3)–28.9 | 17.9–(18.4)–18.9 | 1.47 | P (–SP) | 3 (–4) |
| 23.3–(24.9)–26.8 | 17.4–(19.8)–23.1 | 1.26 | SP | 3 (–4) |
| 17.3–(17.9)–18.6 | 10.5–(12.2)–13.5 | 1.47 | P (–SP) | 3 (–4) |
| 17.1–(19.2)–21.0 | 14.4–(16.1)–16.6 | 1.19 | SP (–S) | 3 (–4) |
| 14.4–(15.3)–16.5 | 18.1–(20.2)–21.9 | 0.76 | (SP–) SO | 3 (–4) |
| 24.2–(25.2)–27.5 | 18.7–(20.4)–23.1 | 1.24 | SP | 3 (–4) |
| 23.1–(25.0)–26.6 | 15.5–(16.8)–17.7 | 1.49 | P (–SP) | 3 (–4) |
| 18.9–(19.5)–20.6 | 20.5–(21.5)–22.1 | 0.91 | (SP–) S | 3 (–4) |
| 15.5–(16.5)–18.1 | 19.5–(20.7)–22.1 | 0.80 | (S–) SO | 3 (–4) |
| 22.2–(24.5)–27.3 | 16.1–(17.3)–18.5 | 1.42 | P (–SP) | 3 (–4) |
| 20.4–(22.3)–26.1 | 14.9–(17.5)–19.4 | 1.27 | SP | 3 (–4) |
| 14.9–(15.8)–17.2 | 17.7–(20.1)–21.3 | 0.79 | (S–) SO | 3 (–4) |
| 16.9–(17.6)–18.1 | 19.4–(21.9)–23.3 | 0.80 | (S–) SO | 3 (–4) |
| 23.0–(25.4)–27.8 | 16.9–(17.9)–19.3 | 1.42 | P (–SP) | 3 (–4) |
| 15.6–(16.8)–17.9 | 16.6–(18.0)–19.6 | 0.93 | (SP–) S | 3 (–4) |

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| | *C. ortegana** | 4.9 | 0.087 | 0.248–(0.470)–0.693 |
| | *C. strobilacea* | 11.6 | 0.136 | 0.237–(0.530)–0.983 |
| Clade L | *C. acuta* | 0.2 | 0.024 | 0.166–(0.237)–0.299 |
| | *C. desmouliniana* | 0.3 | 0.055 | 0.199–(0.357)–0.454 |
| | *C. hyalina* | 0 | 0 | 0 |
| | *C. leptantha* | 5.4 | 0.143 | 0.282–(0.514)–0.816 |
| | *C. odontolepis* | 3.5 | 0.073 | 0.279–(0.373)–0.488 |
| | *C. polyanthemos* | 15.4 | 0.114 | 0.116–(0.368)–0.708 |
| | *C. tuberculata* | 0 | 0.02 | 0.299–(0.358)–0.418 |
| | *C. umbellata* var. *umbellata* | 0 | 0 | 0 |
| | *C. umbellata* var. *reflexa* | 0 | 0 | 0.149–(0.294)–0.431 |
| Clade M | *C. coryli* | 0 | 0 | 0.128–(0.173)–0.239 |
| | *C. indecora* var. *indecora* | 0 | 0.023 | 0.366–(0.490)–0.586 |
| | *C. indecora* var. *attenuata* | 0 | 0 | 0.225–(0.269)–0.296 |
| | *C. indecora* var. *longisepala* | 0.2 | 0.054 | 0.226–(0.281)–0.376 |
| | *C. indecora* var. *neuropetala* | 0 | 0.011 | 0.248–(0.322)–0.395 |
| | *C. warneri* | 0 | 0 | 0.152–(0.203)–0.246 |
| Clade N | *C. choisiana** | 0.3 | 0.073 | 0.177–(0.346)–0.422 |
| | *C. deltoidea* | 0.6 | 0.041 | 0.182–(0.283)–0.460 |

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| 18.8–(19.7)–20.5 | 11.6–(12.9)–14.4 | 1.53 | P (–SP) | 3 (–4) |
| 21.3–(23.7)–25.5 | 14.3–(16.4)–18.3 | 1.44 | P (–S) | 3 (–4) |
| 20.5–(22.3)–25.6 | 13.5–(15.9)–16.1 | 1.49 | P (–S) | 3 (–4) |
| 17.9–(19.5)–21.9 | 10.2–(13.2)–17.2 | 1.48 | P | 3 (–4) |
| 23.2–(24.9)–26.5 | 17.6–(18.5)–19.7 | 1.35 | P (–SP) | 3 (–4) |
| 21.7–(22.9)–23.6 | 14.5–(15.1)–15.5 | 1.52 | P (–SP) | 3 (–4) |
| 25.2–(26.7)–29.8 | 15.0–(16.3)–18.3 | 1.67 | P (–S) | 3 (–4) |
| 24.6–(27.5)–31.4 | 18.8–(20.6)–22.9 | 1.33 | P (–SP) | 3 (–4) |
| 15.5–(16.0)–16.5 | 19.7–(11.8)–12.6 | 1.36 | P (–SP) | 3 (–4) |
| 16.6–(18.2)–19.0 | 14.6–(15.9)–17.7 | 1.14 | (P–) SP | 3 (–4) |
| 16.9–(19.1)–21.5 | 12.4–(14.3)–16.5 | 1.34 | P (–SP) | 3 (–4) |
| 27.9–(29.4)–30.5 | 19.3–(20.9)–23.2 | 1.41 | P (–SP) | 3 (–4) |
| 26.8–(27.7)–29.8 | 20.7–(22.5)–24.1 | 1.23 | (P–) SP | 3 (–4) |
| 25.9–(27.3)–28.9 | 17.2–(18.4)–19.0 | 1.48 | P (–SP) | 3 (–4) |
| 26.0–(27.6)–30.3 | 13.8–(17.6)–21.2 | 1.57 | P (–SP) | 3 (–4) |
| 23.7–(27.7)–32.1 | 16.1–(19.0)–22.2 | 1.46 | P (–SP) | 3 (–4) |
| 23.4–(24.7)–26.5 | 14.6–(15.1)–15.7 | 1.64 | P (–SP) | 3 (–4) |
| 19.7–(20.2)–20.5 | 16.4–(16.7)–17.0 | 1.21 | SP | 3 (–4) |
| 15.2–(17.5)–19.8 | 13.8–(15.7)– | 1.11 | (SP–) S | 3 (–4) |

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|---|---|---|---|---|
|  | *C. gracillima* | 0 | 0 | 0.228–(0.277)–0.360 |
|  | *C. mcvaughii* | 0.1 | 0.008 | 0.197–(0.241)–0.298 |
|  | *C. punana* | 0 | 0 | 0 |
|  | *C. sidarum* | 0 | 0 | 0 |
|  | *C. vandevenderi* | 0 | 0 | 0 |
| Clade O | *C. argentiniana* | 1.1 | 0.045 | 0.307–(0.329)–0.497 |
|  | *C. acutiloba** | 0.8 | 0.037 | 0.221–(0.292)–0.395 |
|  | *C. bella** | 0.7 | 0.112 | 0.371–(0.534)–0.784 |
|  | *C. boliviana** | 6.2 | 0.068 | 0.204–(0.325)–0.583 |
|  | *C. chilensis* | 19.8 | 0.311 | 0.443–(0.954)–1.776 |
|  | *C. cockerellii* | 4.9 | 0.319 | 0.930–(1.064)–1.224 |
|  | *C. cristata* | 6.3 | 0.086 | 0.231–(0.421)–0.669 |
|  | *C. flossdorfii* var. *pampagrandensis** | 26.8 | 0.47 | 1.040–(1.469)–2.214 |
|  | *C. foetida* var. *foetida* | 10.4 | 0.146 | 0.485–(0.707)–1.343 |
|  | *C. foetida* var. *pycnantha* | 12.1 | 0.217 | 0.478–(0.979)–1.513 |
|  | *C. friesii* | 0.1 | 0.028 | 0.158–(0.247)–0.405 |
|  | *C. globiflora* | 2.1 | 0.093 | 0.302–(0.456)–0.664 |
|  | *C. goyazina** | 4.5 | 0.118 | 0.294–(0.515)–0.773 |

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| | 17.2 | | | |
| 18.9–(19.8)–20.7 | 11.1–(13.7)–14.9 | 1.44 | P (–SP) | 3 (–4) |
| 18.9–(20.6)–22.0 | 15.8–(18.7)–21.4 | 1.10 | (SP–) S | 3 (–4) |
| 20.1–(21.9)–23.9 | 14.9–(16.2)–17.0 | 1.35 | P (–SP) | 3 (–4) |
| 16.8–(18.5)–20.0 | 13.3–(14.1)–15.1 | 1.31 | (P–) SP | 3 (–4) |
| 21.8–(23.9)–25.8 | 15.3–(16.1)–17.4 | 1.48 | P | 3 (–4) |
| 17.8–(19.5)–20.7 | 11.0–(12.4)–13.1 | 1.57 | P (–SP) | 4 (–4) |
| 21.1–(21.8)–22.6 | 15.5–(16.1)–17.1 | 1.35 | P (–SP) | 3 (–4) |
| 21.2–(22.4)–24.1 | 22.6–(24.0)–25.1 | 0.93 | S | 3 (–4) |
| 18.7–(21.2)–23.5 | 20.4–(23.2)–26.4 | 0.91 | S | (5–6)–7 |
| 21.4–(22.9)–25.9 | 17.9–(23.3)–26.9 | 0.98 | (SP–) S | 3 (–4) |
| 20.8–(22.6)–24.0 | 17.0–(18.5)–21.6 | 1.22 | SP | 3 (–4) |
| 15.9–(16.7)–17.4 | 14.3–(15.3)–15.9 | 1.09 | S | 3–4 |
| 15.8–(19.9)–24.9 | 17.5–(20.5)–23.4 | 0.97 | (SP–) S | ? |
| 21.0–(23.2)–24.6 | 17.5–(18.4)–19.7 | 1.26 | SP (–S) | 3 (–4) |
| 21.4–(22.3)–23.6 | 22.4–(22.9)–23.2 | 0.97 | S (–SO) | 3 (–4) |
| 17.8–(18.8)–20.1 | 14.7–(16.0)–17.3 | 1.17 | SP | 3 (–4) |
| 21.7–(23.1)–25.6 | 18.8–(22.0)–25.0 | 1.05 | (SP–) S | 6–8 |
| 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 |
| *C. killimanjari* | 21.9 | 0.212 | 0.203–(0.859)–1.615 |
| *C. microstyla* | 21.5 | 0.189 | 0.360–(0.702)–1.105 |
| *C. odorata var. odorata* | 9.6 | 0.184 | 0.395–(0.749)–1.190 |
| *C. orbiculata** | 8.4 | 0.082 | 0.242–(0.433)–0.713 |
| *C. paitana* | 43.6 | 0.912 | 0.749–(1.619)–2.606 |
| *C. parodiana* | 34.3 | 0.903 | 1.076–(1.933)–2.917 |
| *C. purpurata* | 0 | 0 | 0 |
| *C. rubella** | 0.3 | 0.09 | 0.468–(0.505)–0.550 |

| | | | | |
|---|---|---|---|---|
| 15.1–(17.3)–18.9 | 15.2–(16.2)–18.4 | 1.07 | (SP–) S | (4–) 5–6 |
| 17.6–(19.0)–21.0 | 18.5–(19.9)–21.6 | 0.95 | (SP–) S | 3 (–4) |
| 15.3–(17.7)–19.6 | 10.4–(13.0)–16.8 | 1.36 | P (–SP) | 3 (–4) |
| 20.9–(22.8)–25.4 | 15.3–(15.8)–16.2 | 1.44 | P (–SP) | 3 (–4) |
| 18.6–(19.8)–20.4 | 17.7–(19.4)–22.0 | 1.02 | (SP–) S | 3 (–4) |
| 16.8–(18.8)–19.9 | 20.9–(22.7)–23.8 | 0.83 | (S–) SO | 3 (–4) |
| 19.0–(20.5)–22.3 | 21.5–(23.0)–24.0 | 0.89 | (SP–) S | (4–) 5–6 |
| 19.4–(21.1)–22.7 | 17.0–(18.3)–20.7 | 1.15 | SP (–SO) | 3 (–4) |
| 17.7–(21.0)–22.9 | 17.4–(17.9)–19.0 | 1.17 | SP | 3 (–4) |

**Fig. 2.1**

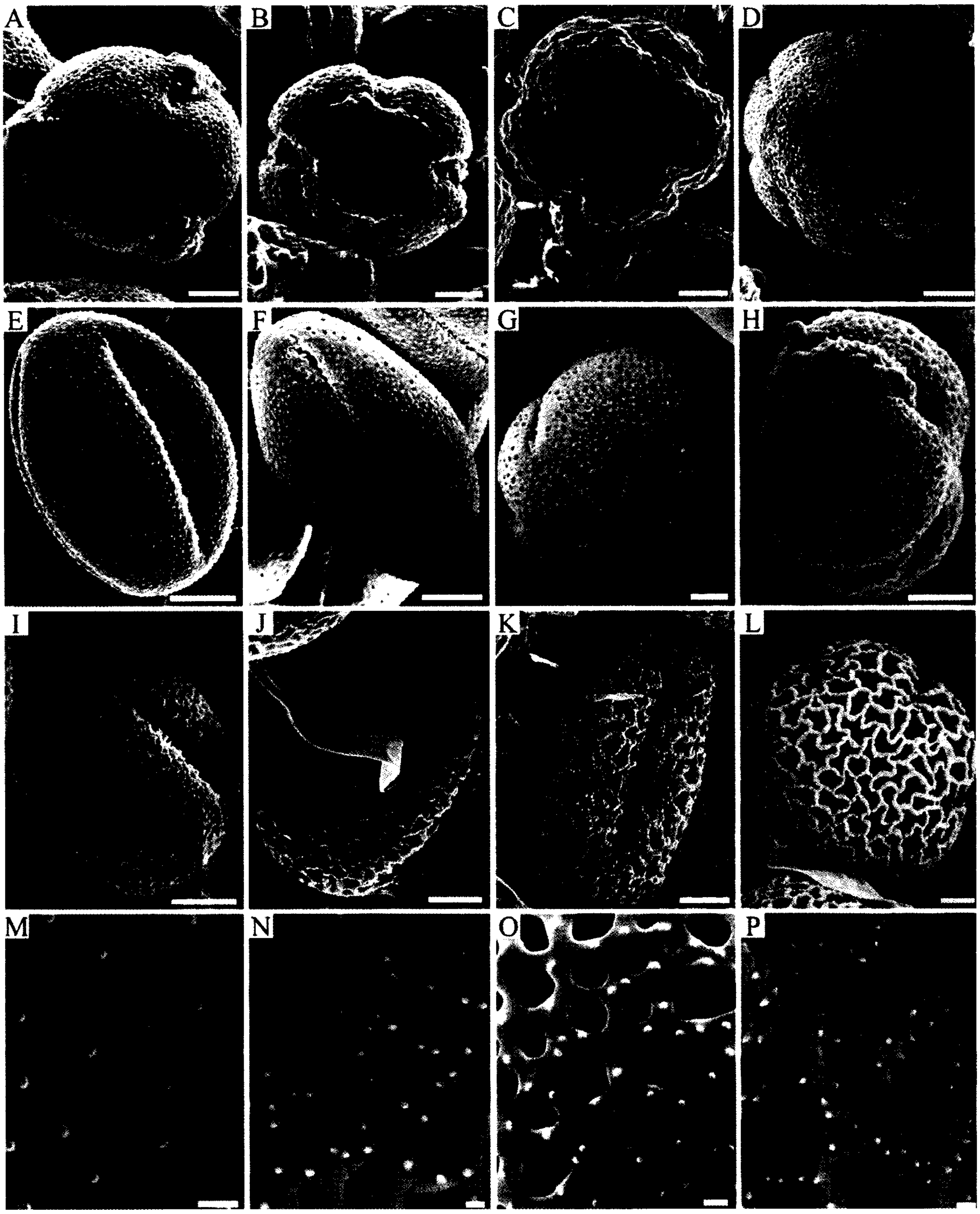

**Fig. 2.1** Variation of colpi number (A–D). A. *Cuscuta purpusii*, B. *C. argentiniana*, C. *C. parodiana*, D. *C. boliviana.* Tectum variation (E–L respectively). E. *Cuscuta brachycalyx* (TI), F. *C. odontolepis* (TP1), G. *C. polyanthemos* (TP2), H. *C. cozumeliensis* (MR1), I. *C. chapalana* (MR2), 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) 5μm, (M–P) 0.5μm.

**Fig. 2.2**

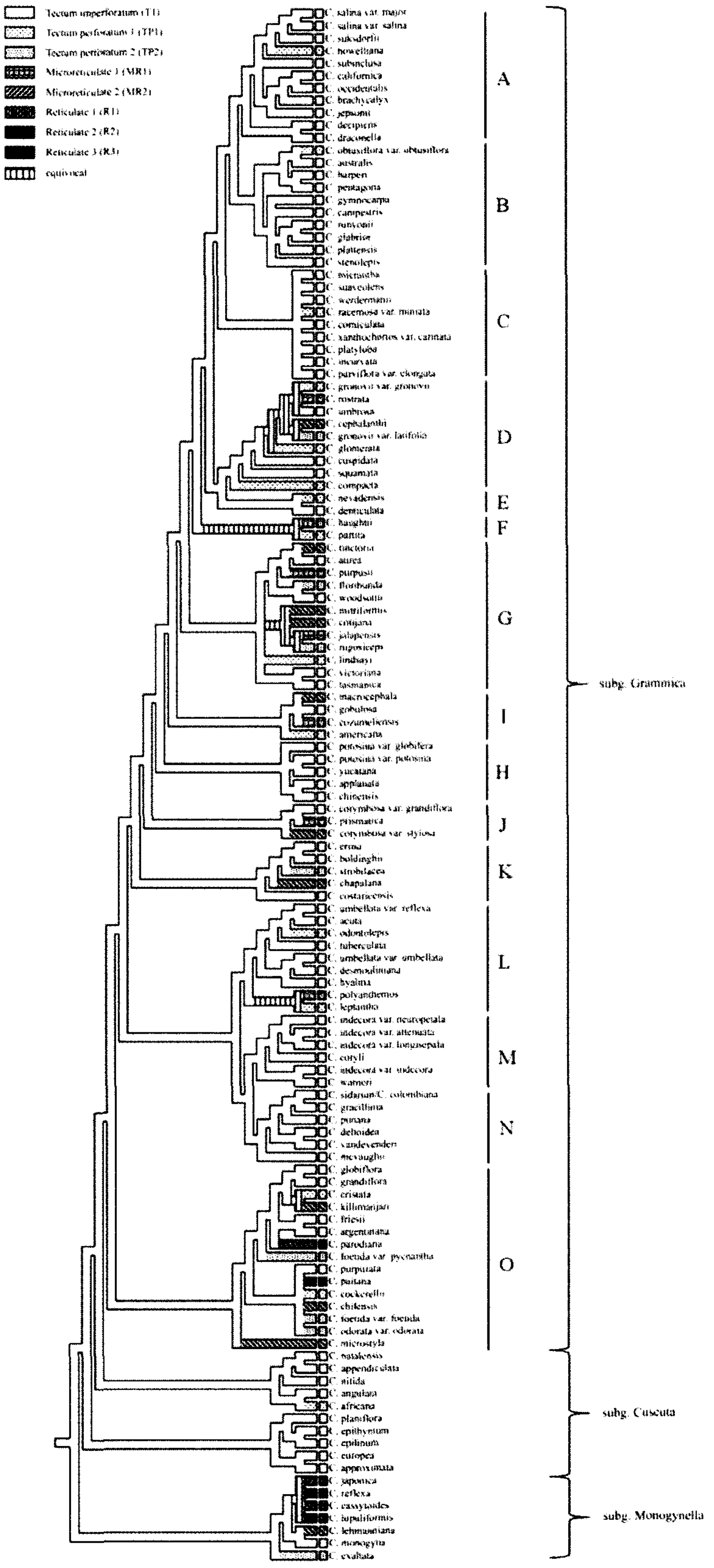
Tectum imperforatum (TI)
Tectum perforatum 1 (TP1)
Tectum perforatum 2 (TP2)
Microreticulate 1 (MR1)
Microreticulate 2 (MR2)
Reticulate 1 (R1)
Reticulate 2 (R2)
Reticulate 3 (R3)
equivocal
C. salina var. major
C. salina var. salina
C. suksdorfii
C. howelliana
C. subinclusa
C. californica
C. occidentalis
C. brachycalyx
C. jepsonii
C. decipiens
C. draconella
C. obtusiflora var. obtusiflora
C. australis
C. harperi
C. pentagona
C. gymnocarpa
C. campestris
C. runyonii
C. glabrior
C. plattensis
C. stenolepis
C. micrantha
C. suaveolens
C. werdermannii
C. racemosa var. miniata
C. corniculata
C. xanthochortos var. carinata
C. platyloba
C. incurvata
C. parviflora var. elongata
C. gronovii var. gronovii
C. rostrata
C. umbrosa
C. cephalanthi
C. gronovii var. latiflora
C. glomerata
C. cuspidata
C. squamata
C. compacta
C. nevadensis
C. denticulata
C. haughtii
C. partita
C. tinctoria
C. aurea
C. purpusii
C. floribunda
C. woodsonii
C. mitriformis
C. cotijana
C. jalapensis
C. rugosiceps
C. lindsayi
C. victoriana
C. tasmanica
C. macrocephala
C. globulosa
C. cozumeliensis
C. americana
C. potosina var. globifera
C. potosina var. potosina
C. yucatana
C. applanata
C. chinensis
C. corymbosa var. grandiflora
C. prismatica
C. corymbosa var. stylosa
C. erosa
C. boldinghii
C. strobilacea
C. chapalana
C. costaricensis
C. umbellata var. reflexa
C. acuta
C. odontolepis
C. tuberculata
C. umbellata var. umbellata
C. desmouliniana
C. hyalina
C. polyanthemos
C. leptantha
C. indecora var. neuropetala
C. indecora var. attenuata
C. indecora var. longisepala
C. coryli
C. indecora var. indecora
C. warneri
C. sidarum/C. colombiana
C. gracillima
C. punana
C. deltoidea
C. vandevenderi
C. mcvaughii
C. globiflora
C. grandiflora
C. cristata
C. killimanjari
C. friesii
C. argentiniana
C. parodiana
C. foetida var. pycnantha
C. purpurata
C. paitana
C. cockerellii
C. chilensis
C. foetida var. foetida
C. odorata var. odorata
C. microstyla
C. natalensis
C. appendiculata
C. nitida
C. angulata
C. africana
C. planiflora
C. epithymum
C. epilinum
C. europaea
C. approximata
C. japonica
C. reflexa
C. cassytoides
C. lupuliformis
C. lehmanniana
C. monogyna
C. exaltata
A
B
C
D
E
F
G
I
H
J
K
L
M
N
O
subg. Grammica
subg. Cuscuta
subg. Monogynella

**Fig. 2.2** 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 depicting a reticulate tectum. For more information on clades A to O, see Stefanović et al. (2007).

**Fig. 2.3**

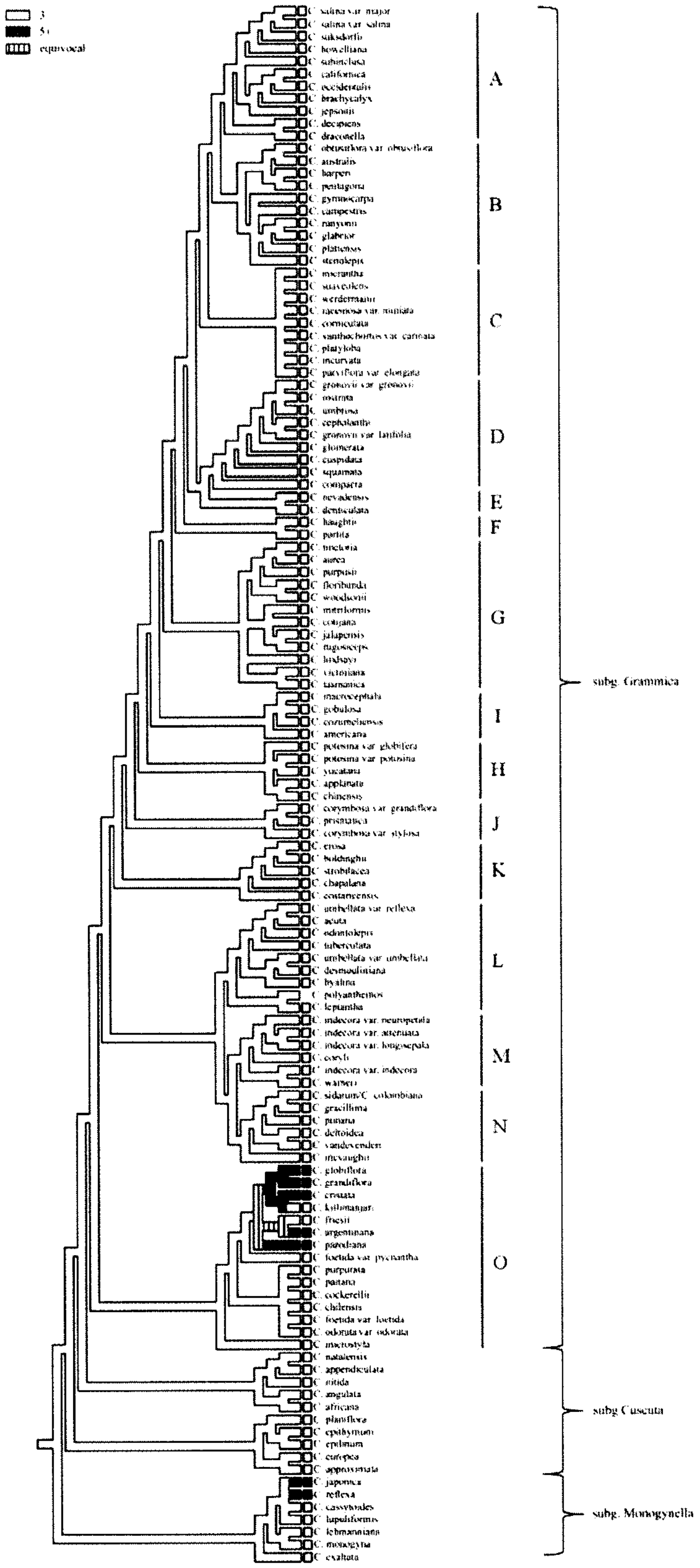
3
5+
equivocal
C. salina var. major
C. salina var. salina
C. suksdorfii
C. howelliana
C. subinclusa
C. californica
C. occidentalis
C. brachycalyx
C. jepsonii
C. decipiens
C. dracontella
C. obtusiflora var. obtusiflora
C. australis
C. harperi
C. pentagona
C. gymnocarpa
C. campestris
C. runyonii
C. glabrior
C. plattensis
C. stenolepis
C. micrantha
C. suaveolens
C. werdermannii
C. racemosa var. miniata
C. corniculata
C. xanthochortos var. carinata
C. platyloba
C. incurvata
C. parviflora var. elongata
C. gronovii var. gronovii
C. rostrata
C. umbrosa
C. cephalanthi
C. gronovii var. latiflora
C. glomerata
C. cuspidata
C. squamata
C. compacta
C. nevadensis
C. denticulata
C. haughtii
C. partita
C. tinctoria
C. aurea
C. purpusii
C. floribunda
C. woodsonii
C. mitriformis
C. cotijana
C. jalapensis
C. rugosiceps
C. lindsayi
C. victoriana
C. tasmanica
C. macrocephala
C. globulosa
C. cozumeliensis
C. americana
C. potosina var. globifera
C. potosina var. potosina
C. yucatana
C. applanata
C. chinensis
C. corymbosa var. grandiflora
C. prismatica
C. corymbosa var. stylosa
C. erosa
C. boldinghii
C. strobilacea
C. chapalana
C. costaricensis
C. umbellata var. reflexa
C. acuta
C. odontolepis
C. tuberculata
C. umbellata var. umbellata
C. desmouliniana
C. hyalina
C. polyanthemos
C. leptantha
C. indecora var. neuropetala
C. indecora var. attenuata
C. indecora var. longisepala
C. coryli
C. indecora var. indecora
C. warneri
C. sidarum/C. colombiana
C. gracillima
C. punana
C. deltoidea
C. vandevenderi
C. mcvaughii
C. globiflora
C. grandiflora
C. cristata
C. kilimanjari
C. friesii
C. argentiniana
C. parodiana
C. foetida var. pycnantha
C. purpurata
C. paitana
C. cockerellii
C. chilensis
C. foetida var. foetida
C. odorata var. odorata
C. macrostyla
C. natalensis
C. appendiculata
C. nitida
C. angulata
C. africana
C. planiflora
C. epithymum
C. epilinum
C. europaea
C. approximata
C. japonica
C. reflexa
C. cassytoides
C. lupuliformis
C. lehmanniana
C. monogyna
C. exaltata
A
B
C
D
E
F
G
I
H
J
K
L
M
N
O
subg. Grammica
subg. Cuscuta
subg. Monogynella

**Fig. 2.3** Colpi number optimized onto a supertree resulted from three molecular phylogenies of *Cuscuta* based on nuclear ITS and plastid *trnLF* sequences (García and Martin 2007; Stefanović et al. 2007; Stefanović and Costea, personal communication).

## 2.9 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 110140* (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.: U.S.A., Florida, *Small et al. 11596* (NY); Mexico, V*an 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.* (GAA); *Burrows 4666* (J); *Bohnen 7827* (NBG). **C. applanata** Engelm. U.S.A, New Mexico, *Casteller* 7339 (UNM); Mexico, *Van Devender 2001-710 et al.* (WLU). **C. approximata** Bab.: U.S.A., 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, *VanDevender 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.: U.S.A, 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: U.S.A., California, *Sanders 25122* (UCR); *Pinzl 7238a* (NY); *White 5033* (ASU). **C. campestris** Yunck.: U.S.A., 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.: U.S.A, 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 446* (S); *Valeutey 94* (S). **C. chinensis** Lam.: Australia, *Carter 628* (CAN); 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.: U.S.A. 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.: U.S.A, 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) Englm.: Mexico, *Rzedowski 28752* (UCR); *Bopp 206* (MEXU); *Pringle s.n.* (MEXU). **C. costaricensis** Yunck.: Mexico, *Reina-G. et al. 2006-1049* (ARIZ, WLU); *VanDevender 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.: U.S.A., 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 Ruiz 2516* (CIMI). **C. dentatasquamata** Yunck.: Mexico, *Jones s.n.* (RSA); U.S.A., Arizona, *Lemmon s.n.* (UC). **C. denticulata** Engelm.: U.S.A., 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: U.S.A., 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); U.S.A, New York, *Ahles 67695* (SMU); Belgium, *Meulebrouck s.n.* (WLU). **C. erosa** Yunck.: U.S.A., 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.: U.S.A, Texas, *Snyder 472* (SMU); *Carter 10584* (MO). **C. floribunda** Kunth aff.: Mexico, *Prather & Soule 1221* (TEX). **C. flossdorfii** Hicken var. **pampagrandensis** Yunck.: Bolivia, *Mendoza & Acebo 919* (MO). **C. foetida** Kunth var. ***foetida***: Ecuador, *Holm-Nielson &*

*Andrado 18480* (AAU); *Holm-Nielson 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.: U.S.A. 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: U.S.A., Texas, *Berkley 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). U.S.A. Alabama, *Kpeooer et al. s.n.* (NY); Georgia, *Mellinger s.n.* (SMU). **C. gronovii** var. **latiflora** Engelm.: U.S.A., 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: U.S.A., Alabama, *Demaree 46295* (NY); *Harper s.n.* (NY). **C. haughtii** Yunck.: Ecuador, *Asplund 15974* (S); *Holm-Nielsen et al. 2308* (NY). **C. howelliana** Rubtzoff: U.S.A., 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:**

U.S.A., California, *Wolf 4392*; Louisiana, *Allen 19239* (BRIT); New Mexico, *Spellenberg et al. 3427* (NY). **C. indecora** var. **attenuata** (Waterf.) Costea: U.S.A., Oklahoma, *Waterfall 17496* (GH); Texas, *Fisher 4118* (CAS); Mexico, *Palmer 333* (F). **C. indecora** var. **longisepala** Yunck.: Argentina, *Leal 7964* (NY)*; 208* (NY); U.S.A. 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.: U.S.A., 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: Usbekistan, *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.: U.S.A, 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); U.S.A., California, *Parish 2110* (CAS); Delaware, *collector illegible* ("MC") *s.n.* (CAS); Texas, *Clare 2144* (CAS). **C. occidentalis** Millsp.: U.S.A., 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.: U.S.A, 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: U.S.A, 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.: U.S.A. 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); U.S.A., 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**: Brasil, *Pinheiro 55* (SPF). **C. racemosa** Mart. var. **miniata** (Mart.) Engelm.: Brazil, *Menezes et al. 5100* (CTES); *Richon 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: U.S.A, 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. U.S.A., Texas, *Correll & Johnston 14906* (NY); *Lundell 9840* (SMU). **C. salina** Engelm. var. **salina:** U.S.A., Arizona, *Hammond 10349* (NY); California, *Bacigalupi & al. 2667* (DS); Nevada, *Tiehm 5991* (CAS). **C. salina** var. **major** Yunck.: Canada, *Kennedy & Ganders 4947* (UBC); U.S.A., California, *Dudley 267* (CAS);

*Moldenke 25731* (NY). **C. sandwichiana** Choisy: U.S.A., 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.: U.S.A., New Mexico, *Wooton & Standley 3355* (CAS); *Wooton s.n.* (NMC) Texas, *Hutchins 643* (BRIT). **C. stenolepis** Engelm.: Ecuador, *Jaramillo & Caravajal 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); U.S.A., California, *Dudley s.n.* (CAS). **C. subinclusa** Durand & Hilg.: U.S.A., California, *Dudley 1653* (DS); *Ewan 11049* (NY); *Mason 5766* (NY). **C. suksdorfii** Yunck.: U.S.A., 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); *Rzdowski 34596* (IEB); *Van Devender 94-1008 et al.* (WLU). **C. tuberculata** Brandegee: U.S.A, 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.**:** U.S.A., 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); U.S.A. Utah, *Jones s.n.* (CAS); Colorado, *Mulford s.n.*

(NY). **C. vandevenderi** Costea & Stefanović: Mexico, *VanDevender 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).

# Chapter 3

# Evolution and taxonomic significance of the gynoecium in *Cuscuta* (dodders, Convolvulaceae) with emphasis on style and stigma characters

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## 3.1 Abstract

The gynoecia of 136 taxa (121 species and 15 varieties) of the holoparasitic plant genus *Cuscuta* (dodders, Convolvulaceae) were examined for their morphological, micromorphological and anatomical diversity using light and scanning electron microscopy. Thirteen qualitative and eight quantitative characters were analyzed and the latter characters were coded with Thiele's gap-weighting method. Characters were optimized onto a phylogenetic supertree resulting from the combination of three broad-scale phylogenies based on plastid *trnL*-F and nuclear ITS sequences. The high morphological diversity found in gynoecium features of *Cuscuta* essentially "summarizes" the diversity and evolution of style and stigma structures in Convolvulaceae. Contrary to earlier hypothesis, our results suggest that a single style (restricted to subg. *Monogynella*) is plesiomorphic, while the two-style condition is derived. Similarly two unequal styles are probably derived, while equal styles are primitive. Stigma morphology is best described by eleven morphological types based on the overall shape. Subg. *Monogynella*, the smallest infrageneric lineage, exhibits more stigma variation than either of the remaining subgenera (*Cuscuta* and *Grammica*). Stigma surface is generally small in *Cuscuta*, but stigmas with larger areas have evolved multiple times, uncorrelated with particular stigma shapes. Pollen size is moderately correlated with stigma papillae size, however, there is no association between pollen size and style or stigma length. A ring of basal stomata was found for the first time at the ovary base in most *Cuscuta* species. This stomata ring is the reduced equivalent of the hypogynous nectary disk of many Convolvulaceae. Overall, while gynoecium characters do not usually provide resolution at the species level, they are the only morphological characters bearing a phylogenetic signal at the subgenus level. Additionally gynoecium characters are useful for the taxonomy of the genus. The evidence based on stigma/style morphology suggests *Cuscuta* as a sister group either to the "bifid style" Convolvulaceae clade (Dicranostyloideae), or to one of the members of this clade.

**Key Words**: *Cuscuta* – dodders – evolution – gynoecium morphology – anatomy – scanning electron microscopy

## 3.2 Introduction

*Cuscuta* (dodders) is the only parasitic lineage that has evolved within Convolvulaceae (Stefanović et al. 2002; Stefanović et al. 2003; Stefanović and Olmstead 2004). The genus includes ca. 200 holoparasitic species (Yuncker 1932; Stefanović et al. 2007; Costea 2007 onwards) that are important from economic (Dawson et al 1994), ecological (Pennings and Callaway 1996, 2002; Press and Phoenix 2005) and conservation points of view (Costea and Stefanović 2009b). As in other parasitic plants (Kuijt 1969; Mauseth et al. 1992; Hsiao et al. 1993), the complete reliance on the host has rendered many of the vegetative structures obsolete, and *Cuscuta* spp. exhibit profound morphological and to a lesser extent genomic reductions (Krause et al. 2003; McNeal et al. 2007).

Recent molecular phylogenetic studies in *Cuscuta* (Stefanović et al. 2007; Garcia and Martin 2007; Costea et al. 2008a; Costea and Stefanović 2009ab; Costea et al. 2009) have spurred an impetus to find new morphological characters that support evolutionary hypotheses and improve the taxonomy at the species level; yet the morphology of *Cuscuta* limits the number of informative characters available for these purposes (Garcia and Martin 2007; Stefanović et al. 2007). Due to its significance for Convolvulaceae systematics, pollen has been suggested as a useful character for *Cuscuta* as well; however, results have indicated that although helpful for taxonomic purposes (e.g., for morphologically separating species), palynological characters bear relatively little phylogenetic signal in *Cuscuta* (Welsh et al. submitted). The gynoecium is similarly a structure of great interest in Convolvulaceae because of the morphological diversity encountered in this family (Govil 1972; Austin 1973; Deroin 1992, 2002, 2004;

Stefanović et al. 2003). Style morphology ranges from a single style (sometimes with two branches) to two distinct styles, while extremely short, practically absent styles also exist in some genera (e.g., *Erycibe*) (Austin 1973). Stigmas of Convolvulaceae are dry, with the receptive cells consisting of unicellular, non-secretory papillae (Heslop-Harrison and Shivanna 1977). Stigma morphology is variable, but in general they are elongate/linear, clavate, conical, or globose (Stefanović et al. 2003). Not surprising, this variation has been of great systematic importance for the family. Some of the earliest and most influential classifications of the Convolvulaceae (Hallier 1893; Peter 1897) are based on pollen exine types, style/stigma morphology and fruit characters.

Historically the taxonomy of *Cuscuta* has been controversial because dodders have been understood either as a family (Cuscutaceae) or as a genus (*Cuscuta*, part of Convolvulaceae). Yet regardless of the taxonomic scheme, the morphology of the style/stigmas has always provided the most important taxonomic characters. For example, Pfeiffer (1845) treated *Cuscuta* as a family in which he distinguished three genera based on stigma characters: *Cuscuta* with linear stigmas, *Epilinella* with clavate stigmas, and *Engelmannia* with capitate stigmas. Similarly, Des Moulins (1853) divided *Cuscuta* (Cuscutaceae) into four genera using stigma and style characteristics. When circumscribed as a genus, stigma and style features have been used to separate the major infrageneric taxa of *Cuscuta*. Choisy (1842) divided the genus into two sections based on stigma shape; one section with acute to clavate stigmas, and the second having globose stigmas. Engelmann (1859) separated *Cuscuta* into three 'groups'. The first group, *Monogynella*, included species with one style and variously shaped stigmas. Members of the second group, *Cuscuta*, had two distinct styles and linear stigmas. *Grammica*, the third and most diverse group, similarly comprised species with two styles, but exhibited capitate stigmas. More recently, Yuncker (1932) formally recognized Engelmann's groups as subgenera, *Monogynella*, *Cuscuta* and *Grammica*, thus proposing the most widely accepted infrageneric classification of *Cuscuta*. In addition, Yuncker (1932) used several morphological gynoecium characters at a species level (e.g., the style length in relation to the ovary length; uniformly filiform styles versus subulate styles; the "convoluted" stigmas of Subsect. *Subulatae*), and proposed some "evolutionary tendencies" for the styles/stigmas of *Cuscuta* (Yuncker 1932, pp. 115). Despite their proven taxonomic significance, these reproductive structures have not been the subject of further investigations in *Cuscuta*. Such information would be useful not only for the systematics of the genus, but also for much needed research into the reproductive biology and ecology of dodders. Therefore, the objectives of the present study are to 1) survey the morphological, micromorphological, and anatomical diversity of gynoecium; 2) place this information into evolutionary and ecological contexts; and 3) determine the usefulness of gynoecium characters for the systematics and taxonomy of *Cuscuta*. The anatomy of the ovary/pericarp and the evolution of dehiscence/indehiscence will be dealt with elsewhere.

## 3.3 Material and methods

### 3.3.1 Plant material

Morphology and micromorphology were studied in 136 taxa (121 species and 15 varieties) using over 360 herbarium specimens (Appendix 1). The anatomy of the style/stigma was documented in eight species chosen to represent major infrageneric clades of *Cuscuta*: *C. reflexa*, *C. japonica* (subg. *Monogynella*); *C. epithymum*, *C. approximata*, *C. nitida*, *C. africana* (subg. *Cuscuta*); *C. cotijana*, and *C. gronovii* (subg. *Grammica*). Herbarium vouchers used for anatomy are marked with "A" in Appendix 1.

### 3.3.2 Microscopy

Dried flowers from the herbarium specimens were rehydrated in a warm solution of 50% EtOH for two minutes. Ten mature flowers and receptive gynoecia per specimen were dissected to determine basic morphology. Gynoecia were examined with a Nikon SMZ1500 stereo microscope and imaged using a PaxCam Arc digital camera with Pax-it 7.0 image fusion software (MIS Inc., Villa Park, IL). Hundreds of images documenting the morphology of the gynoecium in *Cuscuta* are available in the Digital Atlas of *Cuscuta* (Costea 2007-onward).

The vasculature of the gynoecium was studied using a technique modified from Gardner (1975). Dried flowers were placed directly into a solution of 10% NaOH and 1% basic fuchsin and maintained at 60˚C for 6-8 hours. Once becoming transparent, the flowers were passed through an increasing EtOH series. At 70% EtOH, the solution was acidified with a few drops of concentrated HCl, and the overall vasculature of flowers examined. Gynoecia were then dissected from the flowers, mounted and analyzed with a Nikon SMZ 1500 stereomicroscope, a Jenalumar fluorescent microscope, and an Olympus FV1000 confocal microscope (365-520nm).

Previously published methods of softening, embedding and sectioning rehydrated herbarium specimens (Ayensu 1967; Peterson et al. 1978, Deroin 2004; De Smet et al. 2004) resulted in unsatisfactory results for *Cuscuta*. Instead, we discovered that excellent results are achieved if clarified flowers from the vasculature study are dehydrated to 100% EtOH and embedded directly in LR White (London Resin Company, U.K.) (modified from O'Brien and McCully 1981). Semi-thin transverse and longitudinal sections (ca. 2 µm) through the stigma and style were cut with a Sorvall ultra-microtome, stained with toluidine blue O (pH 4.4) for 2 minutes, and examined under a Nikon Eclipse 50i equipped with the above-mentioned camera and imaging software.

For scanning electron microscopy (SEM), herbarium material was analyzed both uncoated and coated with 30 nm of gold using an Emitech K 550 sputter coater. Digital micrographs were taken using either a Hitachi SU 1550 VP SEM or a Hitachi S-570 at 10-15KV.

### 3.3.3 Characters and analysis

In total 8 quantitative and 12 qualitative characters were analyzed in this study (Table 3.1). Other characters were initially considered as well (e.g. the style divergence angle, stigma papillae shape, etc.) but because no meaningful variation was encountered, they were discarded. Measurements for both SEM and light microscopy were taken using ImageJ 1.40g (Abramoff et al. 2004). Quantitative character states were coded using Thiele's (1993) gap weighting as implemented by Morphocode (Schols et al. 2004), with n=10 as the maximum number of allowed character states. Measurements and character states for all the species can be found in Appendix 2. Stigma surface area was calculated using general surface area formulae [ie. sphere $SA = 4\pi r^2$, depressed sphere $SA = 3\pi r^2$, hemisphere $SA = 2\pi r^2$, cylinder $SA = (\pi \cdot r^2) + (2\pi \cdot r)*h$, or cone $SA = \pi \cdot r\ (r+s)$] and approximating these formulae to the prevalent stigma shape of each taxon. Characters with little variation [e.g., when a certain character state was present in only a few taxa] are presented directly in the text. Optimization of character state methodology follows Welsh et al. (submitted).

Relationships between pollen and stigma/style were analyzed on 128 taxa. Three pollen quantitative characters [percent perforation, pollen size, and P/E ratio; taken from Welsh et al. (submitted)] and four style/stigma variables (papillae diameter, style length, stigma length, and stigma surface area) were analyzed with nonparametric Spearman's rank correlation coefficients using NCSS 2007 (Hintze 2007).

## 3.4 Results

### 3.4.1 Structure, morphology and micromorphology of the gynoecium

The gynoecium is syncarpous, superior and 2(-3) carpelar; the ovary is 2-locular but the septum separating the two locules is incomplete at the center of the ovary where it is replaced by placental tissue originating at the base of the ovary. Placentation is axial and each locule has two anatropous ovules. The vasculature entering the gynoecium is scanty and directed mostly towards the placental area. Two vascular bundles corresponding to the carpelar dorsals pass from the receptacle directly in the ovary wall where they remain unbranched. Vascular bundles consist mostly of phloem; xylem is reduced and represented by helical tracheids. Laticifers are usually present in the ovary wall and styles. Starch grains can be observed in all the parts of the gynoecium.

Ovary—In general, the characters of the ovary bear little phylogenetic significance (Appendix 2; trees not shown). In species with dehiscent fruit, the dehiscence line is usually visible from the ovary stage. A ring of actinocytic stomata was observed in most *Cuscuta* species for the first time at the base of the ovary (Appendix 1). This ring is located under the future dehiscence line in the species with dehiscent fruit, and immediately above the receptacle in the species with indehiscent fruit (Fig. 3.1, A-C). While they appear to be sporadic in some species (e.g., *C. cassytoides*, *C. nitida*, *C. pentagona*), in most others they form a well-defined band at the base of the ovary. The stomata ring is present in all three subgenera.

The morphology of the distal part of the ovary is variable and useful to distinguish among species (Table 3.1; Appendix 2). Distal ovary thickenings are more common in subg. *Grammica*. The interstylar aperture is normally visible in the ovary of all species with indehiscent fruit and large interstylar apertures at the capsule stage. With the exception of papillae and epicuticular wax present on the surface, the morphology of the ovary gradually changes after fertilization as the capsules develop. As the overall size of the ovary increases, the distal thickenings become more accentuated; the interstylar aperture enlarges, and a transparent pericarp may replace the opaque ovary wall. At the mature capsule stage, the ring of stomata may be difficult to observe in many species.

Papillae on the ovary and style have evolved only in species of four clades within subg. *Grammica* (data not shown in Appendix 2): *C. salina* var. *papillata*, *C. californica* var. *papillata*, *C. jepsonii* (Clade A), *C. glabrior*, *C. runyonii* (clade B), *C. desmouliniana*

(Clade L), *C. argentinana*, *C. bella*, *C. lucidicarpa* (Clade O). Subulate styles have similarly evolved only in subg. *Grammica*, in species from the clades G (*C. cotijana*, *C. jalepensis*, *C. mitriformis*, *C. rugosiceps* and *C. woodsonii*), Clade K (*C. boldinghii*, *C. chapalana* and *C. erosa*), and clade O (*C. chilensis*, *C. foetida*, *C. globiflora*, *C. grandiflora*, *C. parodiana* and *C. purpurata*) (not shown in Appendix 2).

Styles—The number of styles is one of the most informative phylogenetic characters in *Cuscuta* (Appendix 2). Subg. *Monogynella* has gynoecia with one style while subgenera *Cuscuta* and *Grammica* have two (rarely three) styles. In most *Monogynella* species, the style forms a column up to the level of the stigmas, however, in *C. cassythoides* and *C. exaltata* the style is divided in the distal 1/4 part into two branches (Fig. 3.2 K). In Clade O of subg. *Grammica* (e.g., *C. parodiana*), we observed a number of abnormal gynoecia in which the two styles and stigmas were fused, resembling species of subg. *Monogynella* (Fig 3.2 L).

Gynoecia with two unequal style lengths characterize only subg. *Grammica* (visible in Fig. 3.2 A, B, C, D). The overall style length is polymorphic in *Cuscuta* (Appendix 2). Very short (e.g., *C. gigantea*, *C. microstyla*, *C. victoriana*), absent (e.g., *C. reflexa*, *C. friesii*) or long styles (> 2mm; *C. gracillima*, *C. glomerata*, *C. polyanthemos*) have evolved multiple times (tree not shown; Appendix 2).

The style is "solid"; the transmitting tissue connects the stigma with the centre of the ovary, where the placental tissue and septum form a glandular tissue that acts as an obturator. Species of subg. *Monogynella* have a single transmitting tissue and compitum which may become separated distally only when the style has two diverging branches. In the subgenera *Cuscuta* and *Grammica*, each style has its own transmitting tissue, and a fusion occurs only in the apical portion of the ovary. The ground (cortical) tissue of the style is parenchymatous and often includes laticifers. The style is vascularised only in subg. *Monogynella* by the two carpelar dorsals that enter at the base of the style but do not reach the stigmas (Fig. 3.1, D, F, G).

Stigma—The stigmas are dry. Their receptive surface consists of unicellular (subgenera *Monogynella* and *Cuscuta*; Fig. 3.1 H, I) or 2-4 celled, uniseriate (subg. *Grammica*, Fig. 3.1 J) nonsecretory papillae. The diameter of papillae tend to be the largest in subg. *Monogynella* (and a few members of subg. *Grammica*; ex. *C. parodiana*, 12.668 μm; *C. compacta*, 12.569 μm).

In subgenus *Monogynella*, the two stigmas can be entirely fused and forming a unique 2-lobed stigma (*C. lehmaniana*); they can be fused only at their base and connivent in the rest (the majority of species), or they may be entirely distinct, separated on their own style branch (*C. cassytoides* and *C. exaltata*, not shown in Appendix 2). Species of subgenera *Cuscuta* and *Grammica* always have two distinct stigmas (except teratologic, "one style" gynoecia). The stigma shape is particularly diverse and important both from phylogenetic and taxonomic points of view; we have documented a total of 11 stigma shapes which can be grouped into two major types: "head-like" and "elongated" (Table 3.2; Fig. 3.2). The base of the "head-like" stigmas (at the junction with the styles) is rolled inward. For this reason, often the larger "head-like" stigmas in subgenus *Grammica* have a "mushroom-like" appearance (Fig. 3.2 A, B, C, F). Pollen grains are often trapped in the groove created by the overhanging stigma base. The "elongated" stigmas lack such an area; the receptive surface ends abruptly at the junction with the styles. Although it is the smallest infrageneric taxon, *Monogynella* exhibits the greatest diversity of stigma shapes (Fig. 3.3). *Cuscuta japonica*, an extremely variable species, may have globose, ellipsoid, ovoid, obovoid, rectangle and conical shaped stigmas (Appendix 2). Nevertheless, the most common stigma shapes in subg. *Monogynella* are ellipsoid, ovoid and conical (Appendix 2;). Subgenus *Cuscuta* has species with "elongate stigmas" (cylindrical, terete or clavate) (Fig. 3.3). Species of Sect. *Pachystigma* display stigmas that are thicker than the styles, while in the rest of the subg. *Cuscuta*, the stigmas are ± as thick as the styles. The largest and most diverse subgenus, *Grammica*, has mostly globose or depressed-globose stigmas with rare exceptions of flattened (e.g. *C. friesii*, *C. argentiniana*, *C. parodiniana*; Appendix 2) or ellipsoid stigmas (*C. globiflora* and *C. purpurata*) (Fig. 3.3). The stigma surface is smooth in subgenera *Monogynella* and *Cuscuta*, while in subg. *Grammica* the stigmas of some species can have superficial lobes or wrinkles (Fig. 3.2 A, C; Appendix 2). The majority of species have a relatively small stigmatic surface area (avg. = 0.232 mm$^2$; Fig. 3.4), however, this character is quite variable (Table 3.2; Appendix 2) and ranges from 0.026 mm$^2$ (*C. harperi*) to 1.105 mm$^2$ (*C. lindsayi*). Interestingly, large surface area is apparently not associated with a particular stigma shape (Appendix 2; also see Discussion).

### 3.4.2 Quantitative relationships with pollen

Pollen size was moderately correlated with stigma papillae diameter ($r_s = 0.4656$, $p = 0.0010$; Fig. 3.5 B). Large pollen grains (>30μm) are mainly restricted to subgenus *Monogynella* and a few members of Clade O of subg. *Grammica* (Welsh et al.

submitted). Members of these groups also have species with larger stigma papillae diameter on average. Conversely, members of subg. *Cuscuta* and the remainder of subg. *Grammica* tend to have small (<10μm) to medium (10-20μm) pollen, and smaller stigma papillae. Additionally, there was a moderate positive correlation between pollen percent perforation and stigma surface area ($r_s = 0.4325, p = 0$; Table 3.3). Pollen size and style length ($r_s = -0.0126, p = 0.8884$; Fig. 3.5 A,), and pollen size and stigma height ($r_s = 0.1701, p = 0.0559$) were not correlated (Table 3.3). No other significant correlations were found between the variables analyzed.

## 3.5 Discussion

The gynoecium is so variable among different infrageneric *Cuscuta* lineages (and even species) that it effectively "summarizes" most of the morphological diversity encountered in Convolvulaceae. As indicated by Welsh et al. (submitted), evaluating character polarity in *Cuscuta* is hindered by the unknown sister group relationships of the genus with other Convolvulaceae (Stefanović et al. 2002; Stefanović et al. 2003; Stefanović and Olmstead 2004), and by the lack of style and stigma information at the level of the entire family. Likewise, assessing the biological and ecological advantages of various reproductive character states is complicated by the poor knowledge of *Cuscuta* reproductive biology in general (Costea et al. 2006b). While selfing apparently plays an important role in some species (e.g., *C. indecora*, Prather and Tyrl 1993; *C. salina*, Beliz 1986; *C. campestris*, Costea and Tardif 2006), in other species the flowers are visited by insects suggesting that cross-pollination is also taking place (*C. epithymum*, Meulebrouck et al. 2009; *C. rostrata*, Costea and Tardif 2006; *C. chilensis*, McNeal et al. 2007). Large differences exist among various species in regards to their Pollen/Ovule ratios (Wright and Costea, unpublished). These differences, combined with the extremely variable floral morphologies, indicate that *Cuscuta* has evolved different reproductive strategies which are—regrettably—unknown at the moment.

### 3.5.1 Ovary

Fahn (1953) found that in the more advanced angiosperm families there is an acrocentripetal migration of nectariferous tissue towards the centre of the flower. In this respect, a discoidal nectary ring surrounds the base of the ovary in many Convolvulaceae (Fahn 1979; Cronquist 1981); however, its absence has also been reported in some members of the family (e.g., *Evolvulus alsinodes*, *Cressa cretica* and *Porana paniculata*; Govil 1972). The nectary structures in Convolvulaceae have been studied in some detail in connection to nectar composition, floral development and pollinator guild (ex. Govil 1972; Real 1981; Stucky and Beckmann 1982; Galetto and Bernardello 2004), but in *Cuscuta*, the stomatal nectaries have received little attention. Cronquist (1981) indicated that the bases of "at least some" Cuscutaceae gynoecia are nectiferous. Govil and Lavania (1980) studied the floral anatomy of select *Cuscuta* and found that the stomata ring of *C. europaea* is supplied with phloem elements. More recently, Prenner et al. (2002) reported nectar-secreting stomata at the ovary base of *C. reflexa*. We expanded the number of reported species with gynobasal stomata to the size of almost the entire genus (Appendix 2). However, it is unclear if these stomata serve as a nectar outlet in all the species, or if they are just “regular” stomata such as those present in the ovary walls of *C. australis* (Kuoh and Liao 1993). What is known is that even if fulfilling a nectary role, the gynobasal nectary region in *Cuscuta* is much diminished compared to that of other Convolvulaceae, which is in line with the reductive morphological trend exhibited by *Cuscuta* as a result of its parasitic lifestyle.

### 3.5.2 Style(s)

The one-style gynoecium of subg. *Monogynella* is essentially the only morphological character that separates this lineage from the more derived subgenera, *Cuscuta* and *Grammica*, which have two distinct styles. Yuncker (1932) hypothesized that the “evolutionary trend” for this character in *Cuscuta* has been from distinct to fused styles. Although over 95% of the *Cuscuta* species have two styles, and this would be the “most parsimonious” in-group interpretation (see Stuessy 2009), our results contradict this hypothesis. First, although the sister group of dodders in Convolvulaceae is unknown, lineages that are recognized to have diverged earlier than *Cuscuta* (e.g. *Humbertieae* and *Cardiochlamydeae*) have 1-style gynoecia. Similarly, members of Solanaceae, the sister family to Convolvulaceae, exhibit one style (Bell and Hicks 1976; Gane et al. 1994). Second, a few species of subg. *Monogynella* are characterized by a distally branched style (e.g. *C. cassytoides* and *C. exaltata*, Fig. 3.2 K). *Cuscuta japonica* var. *fissistyla*, also in *Monogynella* (not examined in this study), was described as having deeply divided style (ca. 1/2 the length of the style) (Yuncker 1932). In contrast, in the subgenera where the presence of two styles is the rule (*Cuscuta* and *Grammica*), the one-style condition appears only rarely in some flowers as a developmental abnormality (e.g. in subg. *Grammica*, *C. parodiana*, Fig. 3.2 L). Therefore, it is most likely that the one-style gynoecium is the ancestral character state in *Cuscuta*.

Two unequal styles are present only in subg. *Grammica*, and this character state (versus equal styles) is most probably derived. A similar spatial condition exists in other "bifid" Convolvulaceae genera where either the distal branches of the partially-fused style are unequal, the two distinct styles are unequal (*Cressa*, Austin 2000; *Bonamia*, Lejoly and Lisowski 1982, Austin 1994), or both of these possibilities are present within the genus (*Stylisma*, Myint 1966). In the latter case, Myint (1966) similarly suggested that the unequal nature of stylar branches is the derived condition in *Sylisma*.

The reproductive significance of one vs. two styles may be associated to a certain extent with the selective advantages of apocarpy vs. syncarpy in general. Thus, one style (versus two styles) decreases the necessity for multiple pollination events, imposes pollen competition and selection to occur in a single structure (see also style anatomy, below), and provides a better spatial interaction with animal visitors (e.g., Endress 1982; Raven and Weyers 2001; Armbruster et al. 2002; Erbar 2003). The evolution of two styles and its two morphotypes—equal in length in subg. *Cuscuta*, and unequal in subg. *Grammica*—provides a spatial separation of the two stigmas in the flower, and creates the conditions for a differential development/receptivity of the two stigmas (see below).

The structure of styles in subg. *Monogynella* also differs from that of the remaining subgeneric lineages. The most significant difference is the presence of a single compitum (see Carr and Carr 1961) in the latter (Fig. 3.1 D), whereas in the subgenera *Cuscuta* and *Grammica*, an amalgamation of the transmitting tissues of the two styles occurs only in the distal portion of the ovary. The presence of a compitum within the style is not uncommon for Convolvulaceae with a single style, and it is found in both the ancestral *Humbertia madagascariensis* (Deroin 1992), and in more derived *Ipomoea* spp. (Martin and Ortiz 1967). Similarly, *Nicotiana tabacum* (Bell and Hicks 1976) and *Capsicum eximium* (Onus 2000), both members of the sister family Solonaceae, also exhibit this condition. The number of transmitting tracts has important ecological consequences from the perspective of germinating pollen tubes. Pollen tube competition has been shown to significantly affect offspring fitness (Winsor et al. 1987; Snow and Spira 1996; Johannsson and Stephenson 1997), and as the arena in which this occurs is expanded, sexual selection will play an enhanced role in fused-style species of *Cuscuta.* Also distinguishing subg. *Monogynella* is the presence of xylem elements within the style, a situation also found in many related species with a single fused style (Martin and Ortiz 1967; Govil 1972; Bell and Hicks 1976).

### 3.5.3 Stigma(s)

Variation in stigma morphology is considerable within the *Cuscuta.* Despite being the smallest infrageneric lineage, subgenus *Monogynella* exhibits more stigma morphologies than any of the other subgenera (Fig. 3.3; Table 3.2), including globose, depressed-globose, flattened, ellipsoid, ovoid, obovoid, rectangular and conical stigmas. Similarly, a high amount of variation was reported for *Monogynella* in the pollen exine morphology, the subgenus exhibiting 6 of the 9 tectum "types" (Welsh et al. submitted). However, stigma shape and tectum morphology are not correlated.

Linear stigmas are restricted to subg. *Cuscuta* and traditionally they have been used as a defining feature for this infrageneric lineage (Yuncker 1932; Garcia and Martin 2007). With the addition of *C. appendiculata* (depressed-globose stigmas) to subg. *Cuscuta* sect. *Pachystigma* (Stefanović et al. 2007), however, linear stigmas are no longer a unanimous feature for this group, though still valid for sect. *Cuscuta*. The reproductive role that linear stigmas play has been of interest to ecologists and evolutionary biologists. In *Arachis*, increased stigma length has been associated with an annual lifecycle and self-pollination (Mayer and Pickersgill 1990). Yu and Huang (2006) found that the linear stigmas of *Aquilegia yabeana* progressively mature from the tip and subsequently recurve in order to expose themselves to pollinators if previous pollination has not occurred. In *Spinacea oleracea*, Miglia and Freeman (1996) have shown that stigmas exhibit indeterminate growth and continue to elongate if pollen outcrossing is delayed.

Globose, depressed-globose and flattened stigmas are characteristic of subgenus *Grammica*. Because in this subgenus the two styles are unequal in length, the stigmas reach different relative positions within the flower. This spatial separation of the two stigmas in flower may be associated with different degrees of stigma receptivity before and at anthesis (Wright and Costea, personal communication). However, the functional significance of these various morphological types of stigmas will remain unknown until further studies give a better understanding of the reproductive biology and ecology in *Cuscuta*.

The majority of *Cuscuta* species have a relatively small stigma surface area (Fig 3.3; Appendix 2), which is especially true for many clades in subg. *Grammica* (A- F, H, K, M, N). The distribution of this character in the trees indicates that stigmas with large surface area have evolved multiple times throughout the genus, but is not associated with particular stigma shapes (Appendix 2). For instance, the moderately large (0.491 $mm^2$) conical stigmas of *C. reflexa* (subgenus *Monogynella*) are more or less matched by the capitate stigmas of *C. paitana* (subgenus *Grammica*) (0.443 $mm^2$), or the depressed capitate stigmas of *C. mitriformis* (subgenus *Grammica*) (0.531 $mm^2$). Conversely, examples of each stigma shape may possess a surface area that is below the average (Appendix 2). While increased stigma surface area permits an increased pollen load, it may also promote pollen competition (Armbruster 1996; Mulcahy 1983; Purrington 1993) and possibly reduce the "cost of inbreeding depression" (Armbruster and Rogers 2004).

### 3.5.4 Functional aspects of pollen-stigma/style relationships

The quantitative pollen-stigma/style relationships found in this study can be interpreted in view of three early stages of interaction of these reproductive structures: capture, lodging, and hydration of pollen (Dumas and Gaude 1981). The primary interaction between pollen and stigma is largely concerned with the relative size of both the grain and papillae, although electrostatic forces may be involved (Chaloner 1986). The positive correlation between pollen and papillae in *Cuscuta* is consistent with results published in other plants (e.g., Heslop-Harrison 1981; Cruden and Lyon 1985; Bigazzi and Selvi 1999), suggesting that a great deal of morphological reciprocity and co-adaptation exist between these two structures to ensure the favorable contact for capture and rehydration of pollen grains (Lee 1978; Heslop-Harrison 1981).

This study does not support the hypothesis that the size of the pollen grains is correlated with style length (Fig 3.4 A) as described in other plants (Lee 1978; Baker and Baker 1979; Plitman and Levin 1983). In addition, only a weak correlation was found between pollen size and stigma height in *Cuscuta* (Table 3.3) indicating that these two variables are unrelated (Bigazzi and Selvi 1999).

### 3.5.5 Taxonomic and systematic significance of gynoecium characters in *Cuscuta*

Throughout the past decade *Cuscuta* has experienced a great deal of both taxonomic and systematic reorganization, building upon its last major revision which dates back more than 75 years (Yuncker 1932). Although the relationships that Yuncker (1932) proposed at the subgeneric level have in general received support, his sections and subsections are not monophyletic and require both taxonomic and systematic revision.

To date, 5 of the 15 major clades of subg. *Grammica* (Stefanović et al. 2007) (Costea et al. 2005; Costea et al. 2006 a,b,c,d; Costea et al. 2008a; Costea et al. 2009) and subg. Cuscuta (Garcia and Martin 2007) have been subject to taxonomic revisions; however, the remaining clades (A-C, G-L,O, see Stefanović et al. 2007 ) and subgenus *Monogynella* include the most unfamiliar and morphologically diverse species and are groups that still await treatment. Given the morphologically reductive nature of the genus, the style, and especially stigma morphology, will play an increasingly significant role in species delimitation and description. As has already been suggested for pollen (Welsh et al. unpublished data), Clade O of subg. *Grammica*, for example, will benefit enormously from enhanced stigma characters that highlight species boundaries. In addition, although subg. *Monogynella* is the smallest of the subgenera, it exhibits more stigma variation than either of the remaining subgenera. While most species here have conical or ovoid stigmas (*C. japonica*, *C. monogyna*, *C. reflexa*), ellipsoid (*C. lehmanniana*) and even depressed-globose (*C. cassytoides*, *C. exaltata*) stigmas are found.

From a systematic perspective it is clear that stigma or style characters alone are insufficient to reconstruct phylogenetic relationships at the species level, but distinct vs. fused styles together with the stigma shape are the only morphologic characters that provide resolution at the subgeneric level. Additionally, characters such as the presence of a basal stomata ring, location of the compitum, number of styles, and/or stigma shape may prove useful in determining a more accurate phylogenetic position of *Cuscuta* within Convolvulaceae. Based on stigma/style evidence, it is likely that *Cuscuta* is either sister to the "bifid clade" or to one of its members, as already suggested based on pollen morphology (Welsh et al. submitted). This phylogenetic hypothesis is supported by two observations: first, despite a single style being probably the plesiomorphic state for the genus, over 95% of *Cuscuta* species have two styles. Second, based on recent molecular evidence (Stefanović and Olmstead 2004), it is apparent that style number is not perfectly fixed within the "bifid clade" of Convolvulaceae. While most members have distally branched or distinct styles, genera with a single style (eg. *Jacquemontia*, *Dicranostyles*) are also nested within this clade (Stefanović et al. 2002, Stefanović and Olmstead 2004).

## 3.6 Conclusions

The high morphological diversity found in gynoecium features of *Cuscuta* essentially "summarize" the diversity and evolution of style and stigma structures in Convolvulaceae. Contrary to previous evolutionary hypotheses it is likely that a single style is plesiomorphic in *Cuscuta* while the two-style condition is derived. The classification of stigma characters in *Cuscuta* required revision to accurately describe the morphological variation that exists within this genus. As a result, recognition of eleven stigma types better accounts for this variation. Subgenus *Monogynella*, the smallest infrageneric lineage, contains the greatest variation in stigma morphology and includes globose, sub-globose, ellipsoid, ovoid, obovoid, rectangle or conical stigmas. Species of subg. *Cuscuta* exhibit cylindrical, terete or clavate (except *C. appendiculata* with sub-globose) stigmas. The largest and most diverse subgenus, *Grammica*, contains relatively little variation with globose, sub-globose, flattened, and rarely ellipsoid stigmas. A band of modified stomata were observed for the first time in many *Cuscuta* species. This ring of stomata is probably equivalent to the hypogynous nectary disc present in many Convolvulaceae, however, it has undergone a morphological reduction in *Cuscuta*. A moderate positive correlation exists between pollen length and stigma papillae width, as well as between pollen percent perforation and stigma surface area; pollen length is not correlated with either style or stigma length. Based on stigma/style evidence, it is likely that *Cuscuta* is sister to the "bifid clade" of Convolvulaceae, or to one of its members. Qualitative and quantitative gynoecium characters will be beneficial for taxonomic revisions at the species level.

### 3.7 Acknowledgements

The authors would like to thank the curators and directors of the following herbaria: AAU, ARIZ, ASU, BOL, BRIT, CAN, CANB, CAS, CHICO, CHR, CHSC, CIMI, CTES, DS, ENCB, F, G, GAA, GH, GRA, IBUG, IEB, KEW, MERL, MEXU, MICH, MO, NBG, NML, NMS, NU, NY, QFA, QUE, RB, RSA, S, SD, SI, SMU, UB, UC, UCR, UN, UNM, US, WLU, WTL, and XAL for supplying plant material. Thanks also to Michael Wright for supplying a fresh specimen of *C. gronovii*. This research was supported by a Discovery grant from Natural Sciences and Engineering Research Council (NSERC) of Canada to M. Costea (327013-06).

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**Table 3.1** Characters used in this study and their representative codes and states

| Character | Character States |
|---|---|
| 1. Ovary length | (mm) |
| 2. Ovary width | (mm) |
| 3. Basal stomata ring | 0 = absent; 1 = present |
| 4. Thickenings in the distal part of ovary | 0 = absent; 1 = interstylar aperture margins (IAM) thickened, but not raised; 2 = (IAM) raised in a collar around the styles; 3 = tip of ovary forms a broad depression from which styles emerge; 4 = distal part of the ovary forms a rostrum under the styles; 5 = irregular distal ovary protuberances (2-5) present |
| 5. Interstylar aperture | 0 = not visible; 1= visible |
| 6. Translucence of ovary wall | 0 = transparent; 1 = opaque |
| 7. Style length | (mm) Longest |
| 8. Style width | (mm) Measured at the middle |
| 9. Number of styles | 0 = one style (unbranched); 1 = one style with 2 distal branches holding the stigmas (< than 1/2 the length of the style); 2 = two styles |
| 10. Unequal styles on the same gynoecium (only for subgenera *Cuscuta* and *Grammica*) | 0 = absent; 1 = present |

| | |
|---|---|
| 11. Style shape | 0 = filiform (± uniform in thickness); 1 = subulate (enlarged at the base, gradually tapering toward stigma). |
| 12. Ovary and style epidermis papillae | 0 = absent; 1= present (not shown in Appendix 2, examples provided directly the results) |
| 13. Ovary and style epicuticular wax | 0 = absent; 1= present (not shown in Appendix 2, examples provided directly the results) |
| 14. Stigma length | (mm) |
| 15. Stigma width | (mm) |
| 16. Stigma papillae diameter | (μm) |
| 17. Stigma lobes [applies only for subg. *Monogynella*] | 0 = connivent; 1 = distinct; 2 = fused |
| 18. Stigma surface | 0 = smooth; 1= irregular: superficially lobed or convoluted (wrinkled) |
| 19. Stigma type | 1 = globose; 2 = depressed-globose; 3 = flattened; 4 = ellipsoid; 5 = ovoid; 6 = obovoid; 7 = rectangle; 8 = conical; 9 = cylindrical; 10 = terete; 11 = clavate |
| 20. Stigma surface area | ($mm^2$) |

**Table 3.2** Classification of stigma in *Cuscuta* based on their shape.

| |
|---|
| **A.** “Head-like”; ratio l/w ≤ 1 : 3 |
| A.1. Axes are equal or stigmas are dorsiventrally compressed with a longer axis that is perpendicular to the style. Possible shapes:<br>1) **globose**: ± spherical; l/w ratio = ~ 1:1.<br>2) **depressed-globose:** dorsiventrally compressed; l/w ratio = 1:1.2-1.6);<br>3) **flattened**: even more compressed; l/w = 1:2-3) |
| A.2. Stigmas are compressed laterally; the longer axis of stigmas is in continuous with the style. Shape types:<br>4) **ellipsoid** (widest at the middle); 5) **ovoid** (widest at the base); 6) **obovoid** (widest at the top); 7) **rectangle** shaped; 8) **conical** |
| **B.** “Elongated”; ratio l/w > 4 : 1<br>9) **cylindrical** (margins parallel, ends are blunt); 10) **terete** (gradually attenuate from base to apex); 11) **clavate** (club shaped) |

**Table 3.3** Spearman's rank correlation coefficients ($r_s$) between pollen and stigma/style variables. Moderate positive correlations were found between pollen percent perforation and stigma surface area, and between pollen size and stigma papillae diameter. Number of taxa = 128; ns = not significant ($p > 0.05$); † see Fig. 3.5.

| Pollen Variable | Stigma/Style Variable | $r_s$ | t (N-2) | p |
|---|---|---|---|---|
| Percent | Style length | 0.1683 | 1.9315 | ns |
| Perforation | Papillae diameter | 0.1427 | 1.6054 | ns |
| | Stigma height | 0.2768 | 3.2588 | 0.0014 |
| | Stigma surface area | 0.4325 | 5.4477 | 0 |
| Pollen Size | Style length† | -0.0126 | 0.1406 | ns |
| | Papillae diameter† | 0.4656 | 5.7874 | 0.0010 |
| | Stigma height | 0.1701 | 1.9297 | ns |
| | Stigma surface area | 0.0786 | 0.886 | ns |
| P/E Ratio | Style length | -0.1984 | 2.2721 | 0.0248 |
| | Papillae diameter | 0.0462 | 0.5107 | ns |
| | Stigma height | -0.2776 | 3.2432 | 0.0015 |
| | Stigma surface area | -0.3137 | 3.7236 | 0.0003 |

Fig. 3.1

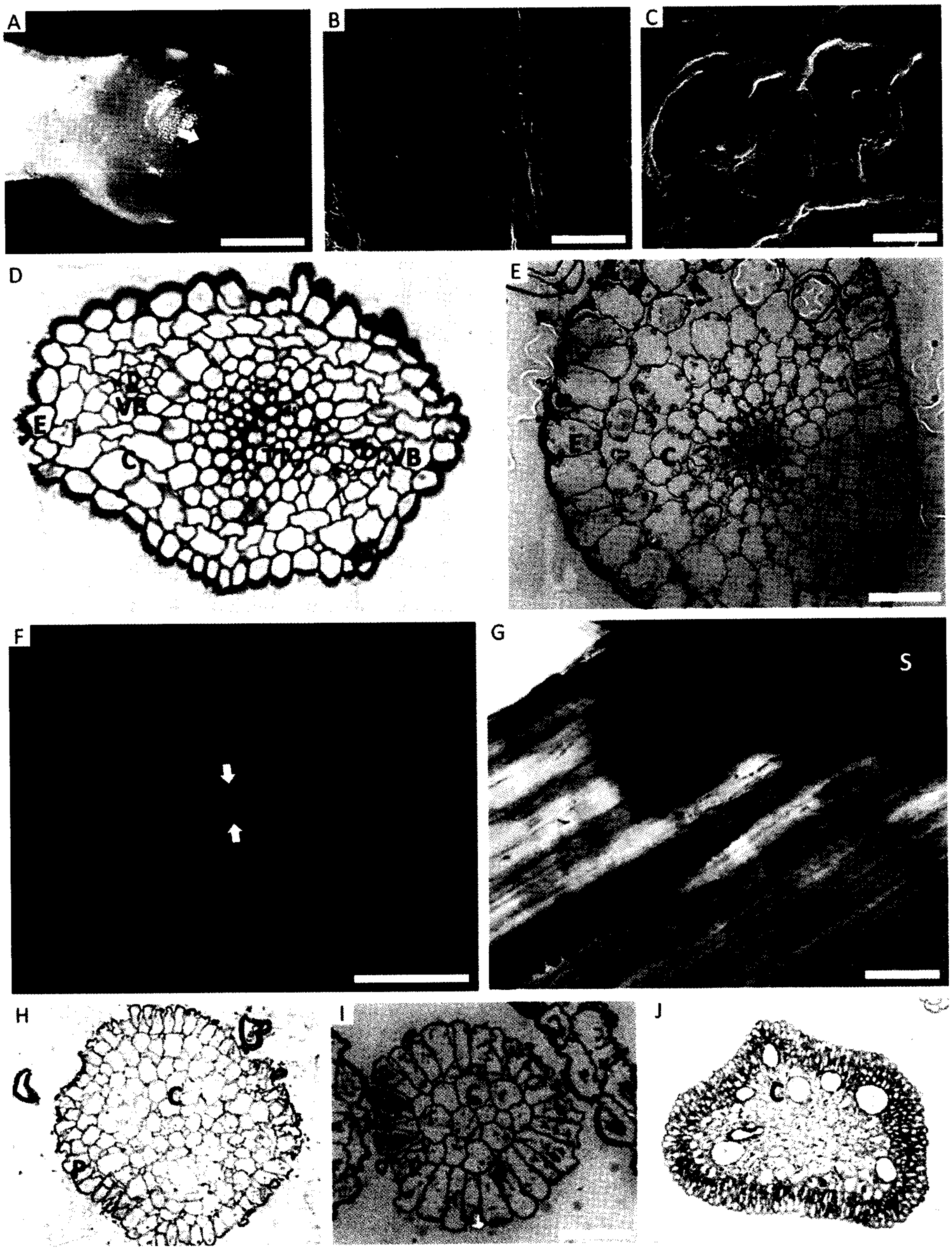

**Fig. 3.1** Ovary morphology and stigma/style anatomy in *Cuscuta*. Stomata at the ovary base (A-C). A. Stereomicroscope image of the ovary base of *Cuscuta rostrata* stained with Toluidine blue O (arrow indicates the band of stomata); B. SEM micrograph showing linear arrangement stomata at the ovary base of *C. monogyna*; C. Detailed view of stomatal structure in *C. purpurata*. Style anatomy. Style anatomy in *Cuscuta* (D-G): D. Transverse section of *C. japonica* style stained with Toluidine Blue O (notice the presence of two vascular strands and fusion of the transmitting tissue [compitum] in the style); E. Transverse style section of *C. cotijana* stained with Toluidine Blue O; F. Stereomicroscope image of the gynoecium of *C. japonica* (arrows indicate vascular strands), tissues were cleared with NaOH to better illustrate the vascular supplies; G. Compound microscope image of the helical trachery elements as the approach the stigma. Transverse sections stained with Toluidine Blue O of representative stigmas of the three major infrageneric lineages of *Cuscuta* (H-J) H. *C. japonica* I. *C. nitida* J. *C. cotijana*. Notice that papillae are single celled in subg. *Monogynella* and *Cuscuta*, but 2-4 celled in subg. *Grammica*. (C) cortex, (E) epidermis, (P) papillae, (S) stigma, (TT) transmitting tissue, (VB) vascular bundle. Scale bar: A, F = 0.5mm; B, D, E, H, J = 50μm; G, I = 25μm.

Fig. 3.2

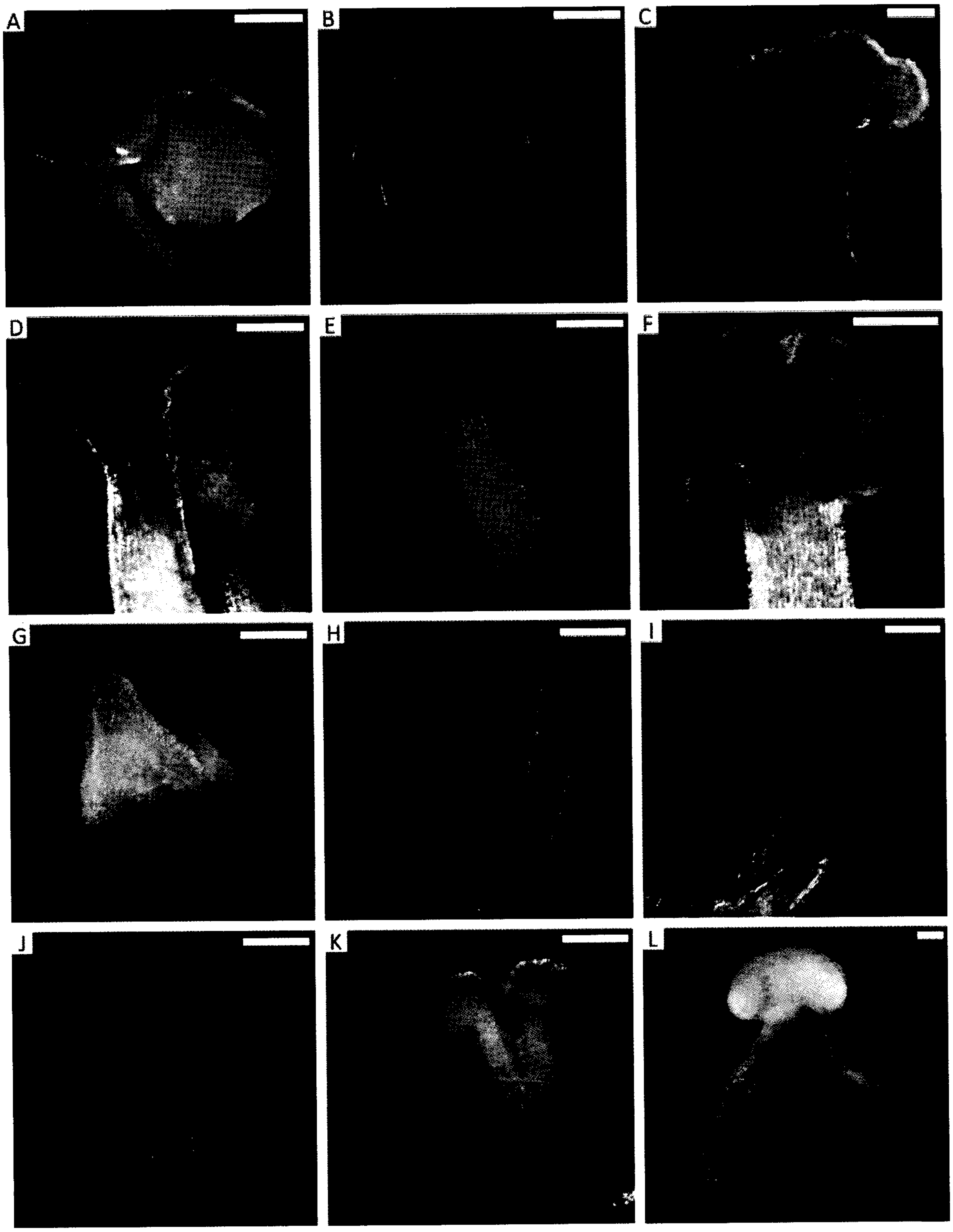

**Fig. 3.2** Variation of style and stigma morphology in *Cuscuta*. Illustration of the eleven stigma types of *Cuscuta* using stereomicroscopy without staining (A-J). A. *Cuscuta paitana* (globose); B. *C. umbellata* (depressed-globose); C. *C. parodiana* (flattened); D. *C. globiflora* (ellipsoid); E. *C. japonica* (ovoid); F. *C. japonica* (rectangle); G. *C. reflexa* (conical); H. *C. natalensis* (cylindrical); I. *C. approximata* (terete); J. *C. europea* (clavate). Please note an example of an obovoid stigma is not presented as it is essentially a rotation of the ovoid stigma. K. Stigma and style of *C. exaltata* showing partial style separation. L. An abnormal style in *C. parodiana*. Scale bars = 0.25 mm.

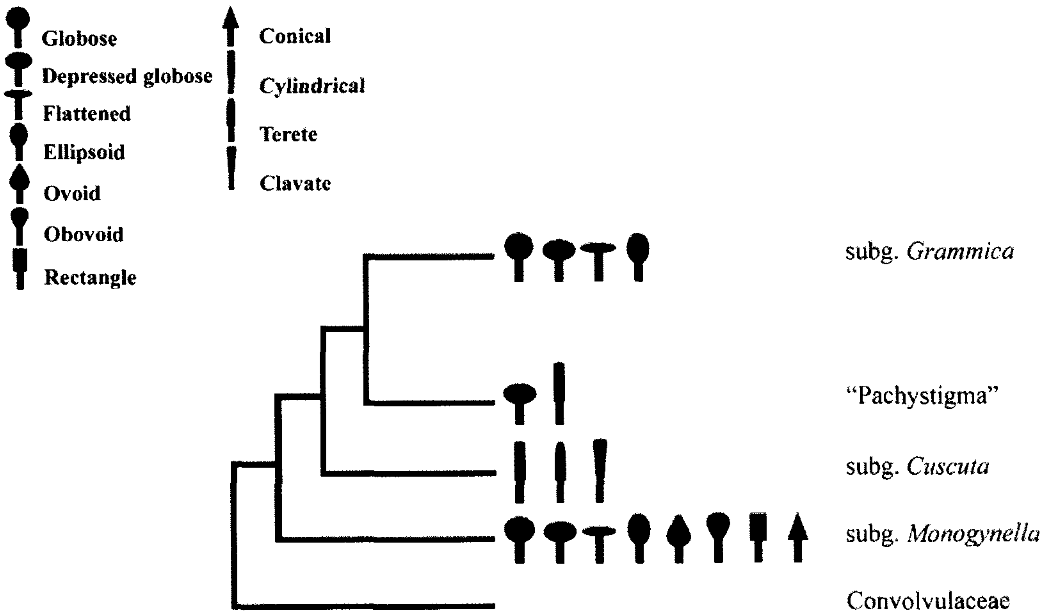

**Fig. 3.3** Distribution of stigma shape types among major infrageneric lineages of *Cuscuta*. Although subg. *Monogynella* is the smalled subgeneric lineage it comprises more stigma variation than the others combined.

**Fig. 3.4**

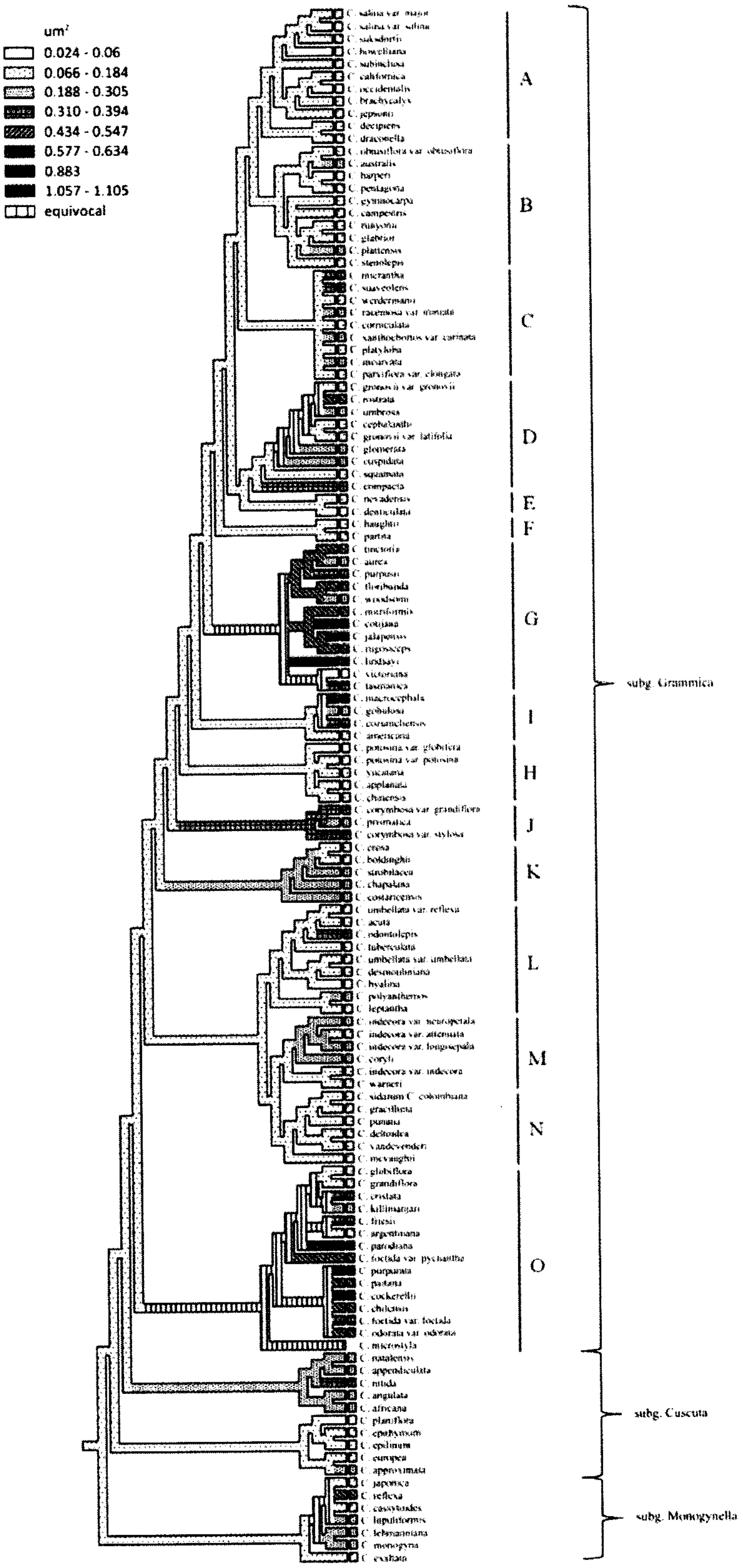
um²
0.024 - 0.06
0.066 - 0.184
0.188 - 0.305
0.310 - 0.394
0.434 - 0.547
0.577 - 0.634
0.883
1.057 - 1.105
equivocal
C. salina var. major
C. salina var. salina
C. suksdorfii
C. howelliana
C. subinclusa
C. californica
C. occidentalis
C. brachycalyx
C. jepsonii
C. decipiens
C. draconella
C. obtusiflora var. obtusiflora
C. australis
C. harperi
C. pentagona
C. gymnocarpa
C. campestris
C. runyonii
C. glabrior
C. plattensis
C. stenolepis
C. micrantha
C. suaveolens
C. werdermannii
C. racemosa var. miniata
C. corniculata
C. xanthochortos var. carinata
C. platyloba
C. insquamata
C. parviflora var. elongata
C. gronovii var. gronovii
C. rostrata
C. umbrosa
C. cephalanthi
C. gronovii var. latiflora
C. glomerata
C. cuspidata
C. squamata
C. compacta
C. nevadensis
C. denticulata
C. haughtii
C. partita
C. tinctoria
C. aurea
C. purpusii
C. floribunda
C. woodsonii
C. mitriformis
C. cotijana
C. jalapensis
C. rugosiceps
C. lindsayi
C. victoriana
C. tasmanica
C. macrocephala
C. globulosa
C. cozumeliensis
C. americana
C. potosina var. globifera
C. potosina var. potosina
C. yucatana
C. applanata
C. chinensis
C. corymbosa var. grandiflora
C. prismatica
C. corymbosa var. stylosa
C. erosa
C. boldinghii
C. strobilacea
C. chapalana
C. costaricensis
C. umbellata var. reflexa
C. acuta
C. odontolepis
C. tuberculata
C. umbellata var. umbellata
C. desmouliniana
C. hyalina
C. polyanthemos
C. leptantha
C. indecora var. neuropetala
C. indecora var. attenuata
C. indecora var. longisepala
C. coryli
C. indecora var. indecora
C. warneri
C. sidarum/C. colombiana
C. gracillima
C. punana
C. deltoidea
C. vandevenderi
C. mcvaughii
C. globiflora
C. grandiflora
C. cristata
C. kilimanjari
C. friesii
C. argentiniana
C. parodiana
C. foetida var. pycnantha
C. purpurata
C. paitana
C. cockerellii
C. chilensis
C. foetida var. foetida
C. odorata var. odorata
C. microstyla
C. natalensis
C. appendiculata
C. nitida
C. angulata
C. africana
C. planiflora
C. epithymum
C. epilinum
C. europaea
C. approximata
C. japonica
C. reflexa
C. cassytoides
C. lupuliformis
C. lehmanniana
C. monogyna
C. exaltata
A
B
C
D
E
F
G
I
H
J
K
L
M
N
O
subg. Grammica
subg. Cuscuta
subg. Monogynella

**Fig. 3.4** Evolution of stigma surface area in *Cuscuta*. Surface area coded using the Thiele's gap-weighting method and reconstructed onto a phylogenetic supertree. A large surface area has evolved independently multiple times throughout the genus (see Clade G and O), yet this trend is not associated with a particular stigma shape.

Fig. 3.5

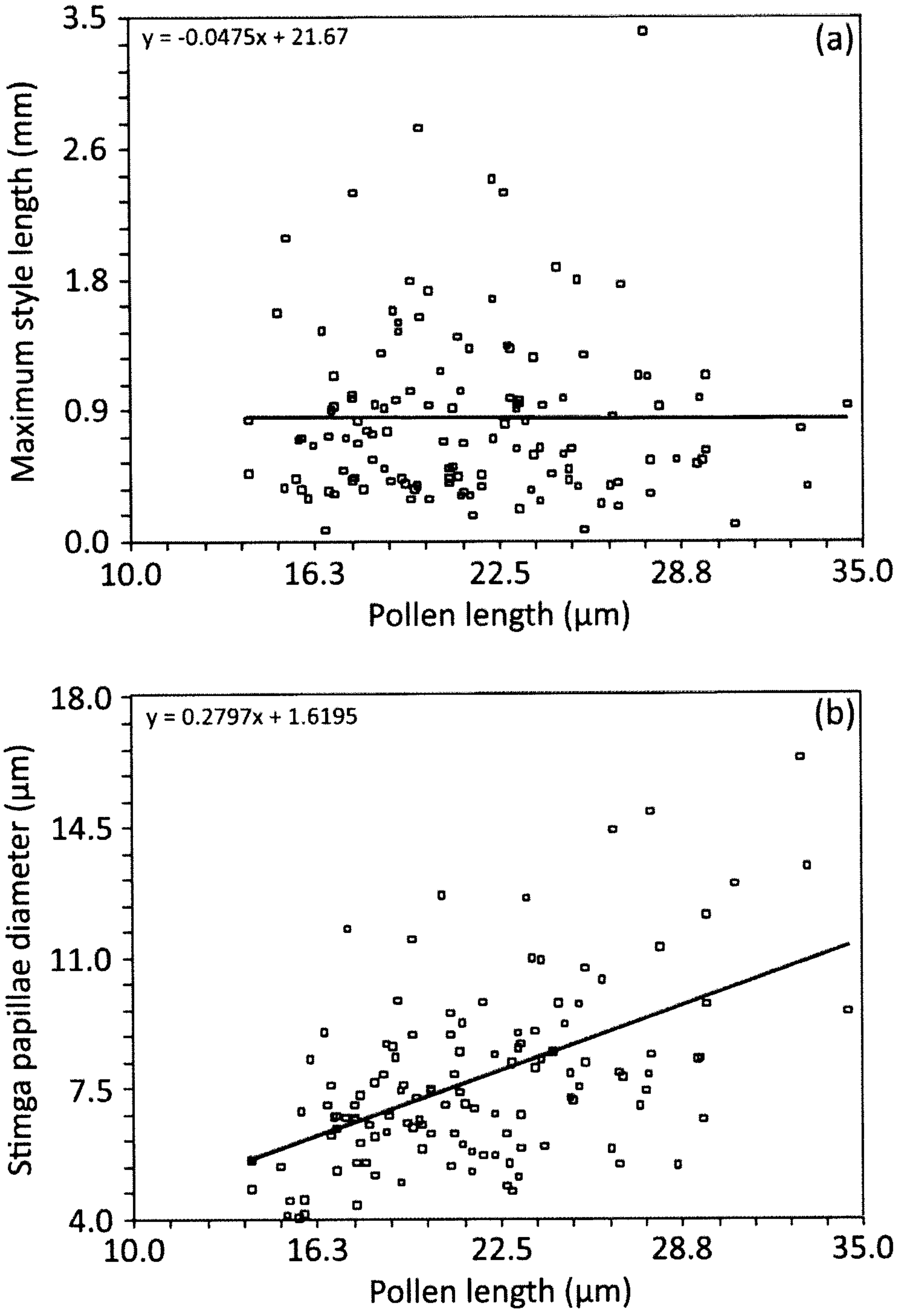
y = -0.0475x + 21.67
(a)
Maximum style length (mm)
3.5
2.6
1.8
0.9
0.0
10.0
16.3
22.5
28.8
35.0
Pollen length (µm)
y = 0.2797x + 1.6195
(b)
Stimga papillae diameter (µm)
18.0
14.5
11.0
7.5
4.0
10.0
16.3
22.5
28.8
35.0
Pollen length (µm)

**Fig. 3.5** Quantitative correlations between pollen and stigma characters in *Cuscuta*. Correlation plots based on Spearman's rank correlation coefficient ($r_s$) between pollen length and style length (a), pollen length and stigma papillae size (b).

## 3.9 Appendix 1

List of vouchers used for scanning electron microscopy [SE], stereomicroscopy [ST], and stigma/style anatomy [A]. Species (arranged in alphabetical order), country, collector, collector number, herbarium acronym from Index Herbariorum.

**Cuscuta acuta** Englm.: Ecuador, Galapagos Islands, *Fagerlini & Wilson 3401* (S) [SE]; *Howell 110140* (G) [SE]; *Wheeler et al. 21*(NY) [SE]; *Howell 10048* (KEW) [ST]. **C. acutiloba** Engelm.: Bolivia, *Mardon 1481* (G) [SE]; Peru, *Weberbauer 7443* (F) [SE]; *Pennell 13242* (S) [ST]. **C. africana** Thumb.: South Africa, *Beyers 6968* (NBG) [SE]; *Muir 156* (GRA) [SE]; *EGH & Oliver 11852* (NBG) [ST, A]; *Durtz 472* (NBG) [A]. **C. americana** L.: U.S.A., Florida, *Small et. al. 11596* (NY) [SE]; Mexico, *Felger 4087* (SD) [SE]; Colombia, *Schneider 999* (S) [ST]; *Billberg 61* (S). **C. angulata** Engelm.: South Africa, *Beyers 12-1985* (NBG) [SE]; *Orchard 460* (UN) [SE]; *Williams 2690* (NBG) [SE]; *Williams 3419* (NBG) [ST]. **C. appendiculata** Engelm.: South Africa, *Hofmeyr s.n.* (GAA) [SE, ST]; *Bohnen 7827* (NBG) [A]. **C. applanata** Engelm.: U.S.A., New Mexico, *Casteller 7339* (UNM) [SE]; Mexico, *Stewart 1038* (F) [SE]; *Lyle & Wind 754* (S) [ST]. **C. approximata** Bab.: U.S.A., California, *Ahams 457* (CAS) [SE]; U.S.A., Nevada, *Kennedy s.n.* (CAS) [SE]; *Kennedy 16422* (CAS) [ST]; Utah, *Costea & Wright 2009-01* (WLU) [A]. **C. argentiniana**Yunck.: Argentina, *Bruchner s.n.* (S) [SE]; *Krapovickas & Sohinini 36757* (CTES) [ST]. **C. aurea** Liebm.: Mexico, *Palmer 87* (S) [SE, ST]; *Nesom et al. 5949* (F) [ST]. **C. australis** R. Br. var. **australis**: New Caledonia, *Bonati 737* (S) [SE, ST]; China, *Sykes CH99* (CHR) [SE, ST]. **C. australis** var. **tinei** (Insenga) Yunck.: Hungary, *Tibiseum et. al. 2635* (NY) [SE, ST]. **C. bella** Yunck.: Peru, *Smith 21827* (US) [SE, ST]. **C. boldinghii** Urb.: Mexico, *Van Devender 92-31* (ARIZ) [SE]; *Provance 3403*

(UCR) [SE, ST]; *Breedlove 37373* (NY) [ST]. **C. boliviana** Yunck.: Argentina, *Hunzinker 2676* (S) [SE, ST]; *Ruiz 14816* (MERL) [SE, ST]. **C. brachycalyx** Yunck.: U.S.A., California, *Ahart 9856* (CHICO) [SE]; *Howell 38877* (NY) [SE, ST]; *Colwell & Coulter AC 04-31* (YM) [ST]. **C. burrelli** Yunck.: Brazil, *Heringer et. al. 43* (UB) [SE]; *Alvarenga Pereira 766* (RB) [SE, ST]; *Dawson 14278* (NY) [ST]. **C. californica** Hook. & Arn.: U.S.A., California, *Sanders 25122* (UCR) [SE]; *Munz 2689* (RSA) [ST]; *Gregory 1049* (SD) [SE, ST]. **C. campestris** Yunck: U.S.A., Oklahoma, *Lipscomb 1894* (SMU) [SE, ST]; U.S.A., Louisiana, *Smith s.n.* (SMU) [SE]; Mexico, *Pringle 3111* (S) [SE, ST]. **C. cassytoides** Nees.: South Africa, *Balkwill 6968* (NU) [SE]; *Hlokozi 2407* (NBG) [SE]; *Garland s.n.* (NY) [ST]. **C. cephalanthi** Engelm.: U.S.A., Illinois, *McDonald s.n.* (NMS) [SE]; *Hill 34308* (BRIT) [SE]; U.S.A., Washington, *Grant s.n.* (RSA) [ST]. **C. chapalana** Yunck.: Mexico, *Garcia-Ruiz 7942* (CIMI) [SE]; *Machuca 8981* (IBUG) [SE]; *Garcia-Ruiz et al. 8064* (CIMI) [ST, A]. **C. chilensis** Ker Gawl.: Chile, *Anderson 84* (S); *Buchtien 446* (S) [SE]; *Valeutey 94* (S) [SE, ST]; *Laudewer 313* (KEW) [ST]. **C. chinensis** Lam.: Australia, *Carter 628* (CAN) [SE, ST]. **C. cockerellii** Yunck: Argentina, *Vorgos 2600* (CUS) [SE]; Unknown lovation, *Vorgos 19383* (CUS) [SE, ST]. **C. colombiana** Yunck.: Colombia, *Haught 4535* (KEW) [SE, ST]. **C. compacta** Juss.: U.S.A., New Jersey, *Moldenke & Moldenke 25129* (AAU) [SE]; U.S.A., South Carolina, *Godfrey & Taylor 1326* (CAS) [SE]; U.S.A., Maryland, *Steele 26022* (CAS) [ST]. **C. corniculata** Engelm.**:** Brazil, *Stannard et. al. 51861*(G) [SE, ST]; Colombia, *Dawe 270* (KEW) [SE, ST]. **C. coryli** Engelm.: U.S.A., Arkansas, *Demaree 19603* (CAS) [SE]; U.S.A., Kansas, *Morley 747* (SMU) [SE, ST]; Unknown location, *Atwood s.n.* (RSA) [ST]. **C. corymbosa** Ruiz & pav. var. **corymbosa**: Mexico, *Garcia-*

*Ruiz et al. 7572* (CIMI) [SE. ST]. **C. corymbosa** var. **grandiflora** Engelm.: *Mexico, Iltis & Guzman 29077* (MEXU) [SE]; *Martinez 3295* (MEXU) [SE]; *Mendez & de Loyez 9608* (MICH) [ST]. **C. corymbosa** var. **stylosa** (Choisy) Engelm.: Mexico, *Rzedowski 28752* (UCR) [SE]; *Borgeau 3353* (S) [SE, ST]. **C. costaricensis** Yunck.: Mexico, *Van Devender 98-1789* (ARIZ) [SE]; *Chazaro et. al. 7527* (MICH) [SE]; *Garcia-Ruiz et al. 8052* (CIMI) [ST]. **C. cotijana** Costea & I. Garcia: Mexico, *Carranza et al. 7316* (IEB) [SE]; *Garcia Ruiz et al. 7557* (CIMI) [SE, ST, A]. **C. cozumeliensis** Yunck.: Guatemala, *Kellerman 6580* (F) [SE]; Mexico, *Calzade & Nievea 9427* (XAL) [SE]; *Vazquez 176* (MEXU) [ST]. **C. cristata** Engelm.: Argentina, *Burkart 14000* (SI) [SE]; *Balegna 447* (SMU) [SE]; *Hunzinker 4927* (S) [SE, ST]. **C. cuspidata** Engelm.: U.S.A., Texas, *Higgins 12480* (NY) [SE]; *Runyen 2828* (SMU) [SE]; U.S.A., Kansas, *McGregor 15175* (SMU) [ST]. **C. decipiens** Yunck.: Mexico, *Purjus 4973* (GH) [SE, ST]. **C. deltoidea** Yunck.: Mexico, *Pringle* 5350 (NMS) [SE, ST]. **C. dentatasquamata** Yunck.: Mexico, *Jones s.n.* (RSA) [SE, ST]; U.S.A., Arizona, *Lemmon s.n.* (UC) [SE, ST]. **C. denticulata** Engelm.: U.S.A., Nevada, *Perish 10299* (CAS) [SE]; *Tiehm 13319* (NY) [SE]; U.S.A., California, *Munz 11731* (SD) [ST], *Thomas 8904* (SD) [ST]. **C. desmouliniana** Yunck.: Mexico, *Spellenberg et. al. 4943* (NMC) [SE]; *Rea 1124* (SD) [SE]; *Christian & Matsamura 14130* (SMU) [ST]. **C. epilinum** Weihe: Sweden, *Samuelson 1317* (RSA) [SE]; Canada, Quebec, *Cayounette s.n.* (QUE) [SE, ST]; *Cartier s.n.* (QFA) [ST]. **C. epithymum** (L) L.: U.S.A., New York, *Ahles 67695* (SMU) [SE,A]; Mexico, *Pringle 8514* (S) [SE, ST]. **C. erosa** Yunck.: Mexico, Baja California, *Rebman 4275* (UCR) [SE]; Mexico, *Van Devender 2001-737* (NMS) [SE, ST]. **C. europea** L.: Sweden, *Holmgren 19784* (SD) [SE]; Netherlands, *Hekking 635* (NY) [SE, ST]. **C. exaltata** Engelm.:

U.S.A., Texas, *Snyder 472* (SMU) [SE]; *Carr 12341* (BRIT) [SE]; *Cam 12341* (BRIT) [ST]; *Westlund s.n.* (CAS) [ST]. C. **floribunda** Kunth aff.: Mexico, *Fisher 37022* (S) [SE]; *Ugent & Flores 2440* (SMU) [SE, ST]; **C. flossdorfii** Hicken var. **pompagrandensis** Yunck.: Argentina, *Accbo 919* (MO) [SE, ST]. **C. foetida** Kunth. var. **foetida**: Ecuador, *Holm-Neilson & Andrado 18480* (AAU) [SE]; *Holm-Neilson et. al.5181* (AAU) [SE]; *Sparre 16952* (AAU) [ST]. **C. foetida** var. **pycnantha** Yunck.: Peru, *Plowman et. al. 14291* (F) [SE]. **C. friesii** Yunck..: Argentina, *Krapovickas et al. 21898* (CTES) [SE]; *Mulgura 1245* (SI) [SE, ST]; *Fulgura 1245* (SI) [ST]. **C. glabrior** (Engelm.) Yunck.: Mexico, *Marsh 1115* (SMU) [SE]; *Heudrickson 13676* (RSA) [ST]; U.S.A., Texas, *Palmer 9965* (CAS) [SE]. **C. globiflora** Engelm.: Argentina, *Mulgura et. al. 1199* (MO) [SE]; Catauaeca?, *Jorgusen 1613* (SMU) [SE]; Bolivia, *Buchtinen 133* (F) [ST]. **C. globulosa** Benth.: Puerto Rico, *Stahl 1064* (S) [SE]; *Urban 855* (S) [SE, ST]; Cuba, *Ekman 7839* (S) [ST]; Mexico, *Morgensen 1073* (AAU) [SE]. **C. glomerata** Choisy: U.S.A., Texas, *Berkley 13886* (RSA) [SE]; *Wolff 3321* (SMU) [SE]; U.S.A., Indiana, *Dean 39229* (NY) [ST]. **C. goyaziana** Yunck.: Brazil, *Macedo 3731* (S) [SE]; *Duarte & Mattos 8376* (RB) [SE, ST]. **C. gracillima** Engelm.: Mexico, *Pringle 6716* (NML) [SE]; *Vazquez 511* (UCR) [SE, ST]. **C. grandiflora** Kunth.: Argentina, *Schinini et. al. 34615* (CTES) [SE]; *Hunzinker 1899* (S) [ST]; Ecuador, *Løjtnant et al. 11829* (AAU) [SE, ST]. **C. gronovii** Willd. ex Roem. & Schult. Var. **gronovii**: U.S.A., Georgia, *Mellinger s.n.* (SMU) [SE, ST]; U.S.A., Massachusetts, *Gates et al. 14841* (SMU) [SE, ST]; Canada, Ontario. *Wright & Bols 2009-05* (WLU) [A]; unknown location, *Keaswhy s.n.* (S) [SE]. **C. gronovii** var. **latifolia** Engelm.: Unknown location, *Brant & Donnell 4810* (MO) [SE]; U.S.A., Texas, *Lundell 11721* (SMU) [SE]; Connecticut, *Hill 17037*

(NY) [ST]. **C. gymnocarpa** Engelm.: Galapagos Islands, *Fagerling & Wibon 3658* (S) [SE]; *Werff 2068* (S) [SE, ST]. **C. harperi** Small: U.S.A., Alabama, *Demaree 46295* (NY) [SE, ST]; *Harper 6479* (SMU) [SE]; *Kral 32878* (SMU) [ST]. **C. haughtii** Yunck.: Ecuador, *Asplund 15974* (S) [SE, ST]; Venezuela, *Asplund 5618* (F) [SE, ST]. **C. howelliana** Rubtzoff: U.S.A., California, *True 7407* (DS) [SE, ST]; *Oswald & Ahart 7645* (CHSC) [SE, ST]. **C. hyalina** Roth.: South Africa, *Bosch 25022* (BOL) [SE, ST]. **C. incurvata** Prog.: Unknown location, *Anisits 2395* (S) [SE, ST]; *Anisits 2555* (S) [ST]; Paraguay, *Lopez Vanni et. al. 243* (CTES) [SE]. **C. indecora** Choisy var. **indecora** U.S.A., Louisiana, *Allen* 19239 (BRIT) [SE]; U.S.A., California, *Munz* 12736 (CAS) [SE]; U.S.A., Arkansas, *Demaree* 18050 (CAS) [ST]. **C. indecora** var. **attenuata** (Waterf.) Costea**:** U.S.A., Oklahoma, *Waterfall 17496* (GH) [SE]; Mexico, *Palmer 333* (F) [SE]; U.S.A., Texas, *Whitehouse 16472* (SMU) [ST]. **C. indecora** var. **longisepala** Yunck.: Argentina, *Leal 7964* (NY) [SE]; *Burkart s.n.* (KEW) [SE]; U.S.A., Colorado, *Ewan 15327* (CAS) [ST]; **C. indecora** var. **neurpetala** (Engelm.) Hitchc.: U.S.A., New Mexico, *Spellenberg et. al. 3427* (NY) [SE, ST]. **C. jalapensis** Schltdl.: Mexico, *Waterfall & Wallis 14213* (SMU) [SE, ST]; *Miller 11561* (MEXU) [SE]; *Garcia-Ruiz et. al. 7569* (CIMI) [ST]. **C. japonica** Choisy: Japan, *Brooks 322* (NY) [A]; China, *Bartholomew et al. 883* (NY) [SE]; Unknown location, *Hill 22616* (MO) [SE, ST]. **C. jepsonii** Yunck.: U.S.A., California, *Dudley 1774* (DS) [SE]; *Tracy 2349* (UC) [SE, ST]. **C. killimanjari** Oliv.: Zimbabwe, *Eyles* 352 (J) [SE, ST]. **C. lehmanniana** Bunge.: Unknown location, *Vvedensky & Syr-Darja s.n.* (MEL) [SE]; India, *Stewart 21103* (NY) [SE]; Uzbekistán, *Budogoski 817* (NY) [ST]. **C. leptantha** Engelm.: Mexico, *Lindsay 2928* (SD) [SE]; *Dominguez 3472* (SD) [SE]; *Moran 8669* (SD) [ST]; *Wiggins 13153*

(SD) [ST]. **C. lindsayi** Wiggins: Mexico, *Wiggins 13185* (MO) [SE]; *Garcia-Ruiz et al. 7569* (CIMI) [SE, ST]. **C. longiloba** Yunck.: Paraguay, *Casas & Molow 4384* (MO) [SE]; Bolivia, *Krapovickas & Schinini 13255* (F) [SE, ST]. **C. lucidicarpa** Yunck. Peru, *Pennell 15067* (GH) [ST, SE]; *Killip & Smith 21858* (US) [ST]; *Killip & Smith 21909* (NY) [SE, ST]. **C. lupuliformis** Krock.: Netherlands, *Lennhouts 2514* (CANB) [SE, ST]; Hungary, *Greknari s.n.* (NY) [SE, ST]; China, *Bartholomew et al.* 883 (RSA) [SE]. **C. macrocephala** W. Schaffn. ex Yunck.: Mexico, *Rebman 5743* (SD) [SE]; *Carter et al. 2186* (F) [SE]; *Moran 18810* (SD) [ST]. **C. mcvaughii** Yunck.: Mexico, *Hinton et al. 12098*(G) [SE, ST]. **C. micrantha** Choisy: Chile, *Phillippi 489* (G) [SE]; *Skottsberg 995* (F) [SE, ST]; Unknown collector and number (WTU) [SE]. **C. microstyla** Engelm. var. **bicolor** Hunz.: Argentina, *Boelcke et. al. 26-1-1969* (CTES) [SE]; *Burkart et. al. 6968* (SI) [SE, ST]. **C. mitriformis** Engelm.: Mexico, *Bye 50488* (UCR) [SE]; *Moore & Wood 4329* (MICH) [SE, ST]. **C. monogyna** Vahl.: Turkmenistan, *Sintenis 1240* (MO) [SE]; Vietnam, *Kung 2024* (NY) [SE]; Austria, *Kovats 203* (NY) [ST]. **C. natalensis** Baker: South Africa, *Rudatis s.n.* (NBG) [SE]; *Rudatis 2412* (NBG) [SE, ST]. **C. nevadensis** I.M. Johnst.: U.S.A., California, *Raven 12865* (CAS) [SE]; *Angel 111* (SD) [SE, ST]; *Twisselmann 16318* (CAS) [ST]. **C. nítida** E. Mey.: South Africa, *Compton 15500* (NBG) [A]; *Rogers 17342* (J) [SE, ST]; *Taylor s.n.* (NBG) [SE]. **C. obtusiflora** Kunth var. **obtusiflora** U.S.A., California, *Parish 2110* (CAS) [SE]; Unknown location, *Holm-Nielson et al. 7240* (AAU) [SE]; Mexico, *Chase 7563* (F) [ST]. **C. obtusiflora** var. **glandulosa** Engelm.: U.S.A., Texas, *Clare 2144* (CAS) [SE]; *Lundell & Lundell 11717* (NY) [SE]; U.S.A., Delaware, *Canlez 1872* (CAS) [ST]. **C. occidentalis** Millsp.: U.S.A., California, *Howell 48868* (CAS) [SE]; *Keil 19626* (UCR) [SE]; *Taylor 4964* (CHICO)

[ST]. **C. odontolepis** Engelm.: Mexico, *White 2730* (GH) [SE]; *Hartman 52* (KEW) [SE]; *Palmer 412* (F) [ST]. **C. odorata** Ruiz & Pav. var. **odorata**: Ecuador, *Jaramillo 10372* (AAU) [SE]; *Asplund 7737* (S) [SE]; Peru, *Moebird & Featherstone 756* (F) [SE, ST]. **C. orbiculata** Yunck.: Brazil, *Alvaregna 93605* (RB) [SE]; *Harley et al. 21452* (AAU) [SE. ST]. **C. ortegana** Yunck.: Mexico, *Hinton et. al. 16294* (MICH) [SE]; *Van Devender et al 2006-74* (WLU) [SE, ST]. **C. paitana** Yunck.: Ecuador, *Madsen 63940* (AAU) [SE, ST]; Peru, *Haught s.n.* (F) [SE, ST]; **C. parodiana** Yunck.: Argentina *Eyerdam 22423* (KEW) [SE]; *Novara 7976* (S) [SE, ST]; *Balegno 447* (SMU) [ST]. **C. partita** Choisy: Brazil *Lindman 3481* (S) [SE]; *Arrias 6737* (KEW) [SE, ST]. **C. parviflora** Engelm. var. **elongata** Engelm.: Brazil, *Filgueiras 1476* (RB) [SE]; *Filgueiras et al. 745* (US) [SE, ST]. **C. pentagona** Engelm.: U.S.A., Alabama, *Kral 31225* (SMU) [SE]; U.S.A., District of Colombia, *Buettcher 122* (CAS) [SE]; U.S.A., Virginia, *Herman 10391* (NY) [ST]. **C. planiflora** Ten.: Australia, *Howitt & Zaicon-Kunesch s.n.* (PERTH) [SE]; Unknown location, *Priva 82* (S) [SE]; Palestina, *Musselman 10461* (RSA) [ST], *Dorn 5420* (NY) [ST]. **C. plattensis** A. Nelson: U.S.A., Wyoming, *Nelson 2768* (NY) [SE]; *Nelson 2741* (MO) [SE, ST]; *Segeuer & Peiler 16242* [SE]. **C. platyloba** Prog.: Brazil, *Dusen 1000* (S) [SE, ST]; *Gillosin 2538* (S) [SE, ST]; Argentina, *Karapovickas 2911* (KEW) [SE]. **C. polyanthemos** Schaffn. ex Yunck.: Mexico, *Wiggins 13153* (SD) [SE]; *Van Devender 2006-809 & Reina* (WLU) [SE, ST]. **C. potosina** Schaffn. ex Yunck.: var. **potosina**: Mexico, *Pringle 6575* (S) [SE]; *Rose et al. 9650* (GH) [SE]; *Rzedowski 3894* (ENCB) [ST]. **C. potosina** var. **globifera** W. Schaffn.: U.S.A., Arizona, *Gooding 290-61* (ASU) [SE]; Mexico, *Van Devender et al. 96-451* (WLU) SE, ST]; *Pringle 6575* (G) [SE]. **C. prismatica** Pav. ex Choisy: Ecuador, *Mille*

*112* (F) [SE, ST]; Peru, *Pilger et al. s.n.* (F) [SE]. **C. punana** Costea & Stefanović: Ecuador, *Madsen 63850* (AAU) [SE, ST]. **C. purpurata** Phil.: Chile, *Johnston 5170* (S) [SE]; *Werdermann 852* (S) [SE]; *Rechinger 63509* (B) [ST]. **C. purpusii** Yunck.: Mexico, *Hendrickson 6608* (RSA) [SE]; *Meyer & Rogers 2878* (UPS) [SE, ST]. **C. racemosa** Mart. var. **racemosa**: Brazil, *Hatschbach 64867* (KEW) [SE]; *Dechioni 192* (RB) [SE, ST]. C. **racemosa** var. **miniata** (Mart.) Engelm.: Brazil, *Menezes et. al. 5100* (CTES) [SE]; *Richon 7835* (S) [SE]; *Arbo et al. 5100* (KEW) [SE, ST]; *Cordeiro et. al. 8211* (KEW) [SE, ST]. **C. reflexa** Roxb.: India, *Cullelt s.n.* (MEL) [SE]; *Kanta s.n.* (ASU) [SE, ST]; *JDH s.n.* (MO) [SE]; *Koelz 21955* (NY) [SE, ST]; *J.J. s.n.* (NY) [A]. **C. rostrata** Shuttlw. ex Engelm. & A. Gray: U.S.A., Texas, *Lundell 11480* (SMU) [SE]; U.S.A., Alleghany Mountains, *Rydberg 9386* (CAS) [SE]; U.S.A., Tennessee, *Churchill 93217* (CAS) [ST]. **C. rugosiceps** Yunck.: Mexico, *Taylor 21457* (SMU) [SE, ST]; *Lindres 4285* (MEXU) [SE]; Guatemala, *Williams et al. 21950* (NY) [SE, ST]. **C. runyonii** Yunck.: U.S.A., Texas, *Lundell 9840* (SMU) [SE, ST]; *Runyon 2622* (BRIT) [SE]; *Lundell 9827* (SMU) [ST]. **C. salina** Engelm. var. **salina**: U.S.A., Nevada, *Tiehm 5991* (CAS) [SE, ST]; California, *Raven 878* (CAS) [SE, ST]. **C. salina** Engelm. var. **major**: U.S.A., California, *Eastwood 7971* (CAS) [SE, ST]. **C. sandwichiana** Choisy: U.S.A., Hawaii, *Stern 8416* (CHICO) [SE]; *Fosberg 14019* (RSA) [SE, ST]. **C. santapaui** Banerji & Sitesh Das: Nepal, *Nicolson 2796* (MO) [SE, ST]. **C. serrata** Yunck.: Brazil, *Asevedo & Lopes 848* (RB) [SE]; *Azevedo 757* (RB) [SE]; *Glaziou 21811* (F) [ST]. **C. sidarum** Liebm.: Mexico, *Palmer 51* (S) [SE, ST]; *Standley 12359* (S) [SE, ST]. **C. squamata** Engelm.: U.S.A., New Mexico, *Wooton & Standley 3355* (CAS) [SE, ST]; *Wooton 1894* (S) [SE, ST]; Texas, *Gould 7114* (SMU) [SE]. **C. stenolepis** Engelm.:

Ecuador, *Jaramillo & Caravagal 2307* (AAU) [SE]; *Neilson & Coello 29084* (AAU) [SE]; *Asplund 6678* (S) [ST]. **C. strobilaceae** Liebm.: Mexico, *Jones s.n.* (RSA) [SE]; *Croat & Hannon 65094* (MEXU) [SE]; *Jones 27347* (MICH) [ST]. **C. suaveolens** Ser.: U.S.A., California, *Dudley s.n.* (CAS) [SE]; *Dudley 1653* (DS), *Smith s.n.* (CAS) [SE]; Chile, *Eyerdam 24649* (KEW) [ST]. **C. subinclusa** Durand & Hilg.: U.S.A., California, *Dudley 1653* (DS) [SE, ST]; *Rose 39363* (NMS) [SE]; *Rose 69091* (AAU) [ST]. **C. suksdorfii** Yunck.: U.S.A., California, *Twisselmann 14603* (SD) [SE]; *Oswald & Ahart 5874* (CHICO) [SE]; *Colwell & Sanders AC 04-159* (YM) [ST], Colwell AC05-213 (UC) [SE]. **C. tasmanica** Engelm.: Australia, *Barker s.n.* (CANB) [SE]; *Walsh 3045* (MEL) [SE]; *Lepschi 909* (MEL) [ST]. **C. tinctoria** Mart. ex Engelm.: Mexico, *Palmer 87* (S) [SE]; *King & Soderstrom 4838* (SMU) [SE]; *Garcia Ruiz et al. 7575* (CIMI) [SE]; *Mexia 8995* (S) [ST]. **C. tuberculata** Brandegee: U.S.A., Arizona, *Beauchamp 3112* (SD) [SE]; Mexico, *Waterfall 12842* (SMU) [SE]; *Rebman 7638* (SD) [ST]. **C. umbellata** Kunth var. **umbellata**: Mexico, *Moran 24758* (SD) [SE]; U.S.A.,Texas, *Bernal 37* (SMU) [SE]; U.S.A., New Mexico, *Spellenberg 2902* (NMS) [ST]. **C. umbellata** var. **reflexa** Yunck.: U.S.A., Arizona, *Felger 92-707* (CAS) [SE]; *Spellenberg 12966* (NMS) [ST]; Mexico, *Jones 22633* (UCR) [SE]. **C. umbrosa** Beyr. ex Hook.: U.S.A., Utah, *Jones s.n.* (CAS) [SE]; *Mejones 6889* (CAS) [SE]; U.S.A., Colorado, *Jones 571* (RSA) [ST], *Bacigalupl 870* (CAS) [ST]. **C. veatchii** Brandegee: Mexico, *Rebman* 3189 (SD) [SE]; *Harbison* 6901 (SD) [SE]. **C. victoriana** Yunck.: Australia *Cowie 9624* (DNA) [SE]; *Glennon 379* (CANB); *Lazarides & Palmer 471* (CANB) [ST]. **C. warneri** Yunck.: U.S.A., New Mexico, *Spellenberg 13890* (WLU) [ST]; U.S.A., Utah, *Warner s.n.* (NY) [SE, ST]. **C. werdermanii** Yunck.: Chile, *Werdermann 880* (G) [SE, ST]. **C. woodsonii** Yunck.:

Guatemala, *Heyde et al. 2912* (KEW) [SE]; *Brenckle 47-269* (S) [SE, ST]. **C. xanthochortos** Mart. ex Engelm. var. **xanthochortos**: Argentina, *Arbo et. al. 6953* (MO) [SE, ST]; *Balegmo 1107* (S) [SE]. **C. xanthochortos** var. **carinata** Yunck.: Paraguay, *Billiet & Jodin 3294* (MO) [SE]; *Bernardi 18758* (MO) [SE, ST]. **C. xanthochortos** var. **lanceolata** Yunck.: Paraguay, *Zardini & Villate 46371* (WLU) [SE]; *Jorgensen 3478* (S) [SE]. **C. yucatana** Yunck.: Mexico, *Nee & Taylor 29575* (MO) [SE, ST]; Mexico, *Rzedowski 25728* (G) [SE, ST].

## 3.10 Appendix 2

Data matrix of ovary, style, and stigma characters. 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 is available are indicated with "*", and tentatively placed into major infrageneric groups based on their morphology. Characters correspond to those provided in Table 3.1; characters missing from this table are presented directly in the text.

| Infrageneric Group | Species | Character | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 14 | 15 | 16 | 18 | 19 | 20 |
| Monogynella (7 taxa) | C. cassytoides | 0.767-(0.887)-1.087 | 0.906-(1.296)-1.546 | 1 | 0 | n/a | 1 | 0.621-(0.836)-1.026 | 0.174-(0.252)-0.320 | 0 | n/a | 0.161-(0.170)-0.182 | 0.270-(0.269)-0.345 | 11.283-(14.438)-16.646 | 0 | 2, 3 | 0.113 |
| | C. exaltata | 0.941-(1.106)-1.215 | 1.076-(1.234)-1.408 | 1 | 0 | n/a | 1 | 0.795-(0.908)-0.993 | 0.303-(0.340)-0.387 | 0 | n/a | 0.149-(0.180)-0.207 | 0.214-(0.257)-0.311 | 9.097-(9.554)-10.160 | 0 | 2 | 0.155 |
| | C. japonica | 0.408-(0.417)-0.443 | 0.396-(0.419)-0.453 | 1 | 0 | n/a | 1 | 0.223-(0.368)-0.438 | 0.120-(0.129)-0.140 | 0 | n/a | 0.289-(0.304)-0.326 | 0.166-(0.205)-0.258 | 10.053-(13.448)-18.512 | 0 | 1, 4, 5, 6, 7, 8 | 0.146 |
| | C. lehmanniana | 0.751-(0.921)-0.988 | 1.074-(1.192)-1.291 | 1 | 0 | n/a | 1 | 0.563-(0.609)-0.641 | 0.353-(0.384)-0.417 | 0 | n/a | 0.453-(0.509)-0.574 | 0.483-(0.498)-0.511 | 9.839-(12.133)-16.926 | 0 | 4, 5 | 0.584 |
| | C. lupuliformis | 0.959-(1.115)-1.279 | 0.991-(1.042)-1.099 | 1 | 0 | n/a | 1 | 0.880-(0.955)-1.0 | 0.266-(0.310)-0.357 | 0 | n/a | 0.316-(0.344)-0.373 | 0.273-(0.355)-0.417 | 7.287-(8.304)-10.784 | 0 | 4, 5 | 0.305 |
| | C. monogyna | 0.949-(1.083)-1.293 | 0.811-(0.905)-0.994 | 1 | 0 | n/a | 1 | 0.667-(0.753)-0.938 | 0.234-(0.275)-0.312 | 0 | n/a | 0.273-(0.315)-0.361 | 0.200-(0.259)-0.318 | 15.083-(16.345)-19.023 | 0 | 5, 8 | 0.194 |
| | C. reflexa | 0.932- | 0.905- | 1 | 0 | n/a | 1 | 0.102- | 0.331- | 0 | n/a | 0.456- | 0.336- | 8.642- | 0 | 8 | 0.491 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (1.150)-1.389 | (1.085)-1.195 | | | | | (0.115)-0.131 | (0.337)-0.344 | | | (0.586)-0.767 | (0.389)-0.479 | (12.986)-16.272 | | | |
| Cuscuta (5 taxa) | C. approximata | 1.007-(1.054)-1.112 | 0.934-(0.991)-1.096 | 1 | 0 | 0 | 0 | 0.431-(0.575)-0.709 | 0.080-(0.108)-0.132 | 1 | 0 | 0.783-(0.868)-0.986 | 0.100-(0.139)-0.162 | 6.885-(8.038)-9.203 | 0 | 9, 10 | 0.241 |
| | C. epilinum | 0.956-(1.254)-1.519 | 0.882-(0.900)-0.921 | 1 | 1 | 0 | 1 | 0.132-(0.251)-0.441 | 0.100-(0.156)-0.235 | 1 | 0 | 0.301-(0.447)-0.582 | 0.127-(0.170)-0.237 | 9.264-(10.405)-12.268 | 0 | 9 | 0.151 |
| | C. epithymum | 0.603-(0.645)-0.708 | 0.489-(0.612)-0.734 | 1 | 0 | 0 | 0 | 0.347-(0.371)-0.394 | 0.099-(0.107)-0.111 | 1 | 0 | 0.798-(0.826)-0.865 | 0.081-(0.093)-0.103 | 4.898-(6.639)-9.989 | 0 | 10 | 0.154 |
| | C. europea | 0.950-(1.268)-1.500 | 1.415-(1.507)-1.623 | 1 | 1 | 0 | 1 | 0.513-(0.619)-0.698 | 0.091-(0.114)-0.149 | 1 | 0 | 0.267-(0.327)-0.384 | 0.113-(0.124)-0.134 | 7.220-(8.566)-9.509 | 0 | 9, 11 | 0.081 |
| | C. planiflora | 0.486-(0.568)-0.610 | 0.554-(0.719)-0.803 | 1 | 0 | 0 | 0 | 0.229-(0.248)-0.270 | 0.061-(0.075)-0.090 | 1 | 0 | 0.248-(0.277)-0.298 | 0.080-(0.085)-0.090 | 4.526-(5.105)-6.153 | 0 | 9 | 0.047 |
| "Pachystigma" (5 taxa) | C. africana | 0.567-(0.590)-0.607 | 0.815-(0.881)-0.939 | 1 | 0 | 0 | 1 | 0.986-(1.106)-1.213 | 0.090-(0.111)-0.129 | 1 | 0 | 0.742-(0.806)-0.861 | 0.148-(0.154)-0.167 | 7.850-(9.754)-12.837 | 0 | 9 | 0.284 |
| | C. angulata | 0.543-(0.578)-0.628 | 0.692-(0.748)-0.795 | 1 | 1 | 0 | 1 | 0.763-(0.902)-1.072 | 0.081-(0.103)-0.124 | 1 | 0 | 0.627-(0.789)-0.925 | 0.158-(0.178)-0.193 | 8.334-(11.276)-13.867 | 0 | 9 | 0.281 |
| | C. appendiculata | 0.685-(0.771)-0.869 | 0.866-(0.897)-0.924 | 1 | 2.5 | 0 | 1 | 1.367-(1.541)-1.724 | 0.105-(0.119)-0.130 | 1 | 0 | 0.209-(0.220)-0.235 | 0.277-(0.313)-0.346 | 6.825-(8.331)-9.817 | 0 | 2 | 0.231 |
| | C. natalensis | 0.705-(0.746)-0.813 | 0.721-(0.797)-0.892 | 1 | 0 | 0 | 1 | 0.301-(0.424)-0.618 | 0.132-(0.148)-0.160 | 1 | 0 | 0.614-(0.740)-0.897 | 0.134-(0.156)-0.179 | 4.637-(5.514)-6.701 | 0 | 9 | 0.230 |
| | C. nitida | 0.653- | 0.778- | 1 | 0 | 0 | 1 | 0.544- | 0.114- | 1 | 0 | 0.981- | 0.129- | 4.866- | 0 | 9 | 0.321 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (0.705)- 0.781 | (0.821) -0.868 | | | | | (0.716) -0.880 | (0.121) -0.135 | | | (1.098) -1.339 | (0.146) -0.170 | (6.199)- 8.398 | | | |
| Grammica (119 taxa) Clade A | C. brachycalyx | 0.627- (0.670)- 0.718 | 0.608- (0.689) -0.755 | 0 | 3 | 0 | 0 | 0.847- (0.910) -1.038 | 0.096- (0.11)- 0.128 | 1 | 1 | 0.132- (0.154) -0.171 | 0.188- (0.202) -0.229 | 5.138- (5.927)- 7.076 | 0 | 1, 2 | 0.096 |
| | C. californica var. californica | 0.805- (0.936)- 1.069 | 0.959- (0.999) -1.049 | 0 | 3 | 0 | 1 | 1.000- (1.288) -1.653 | 0.111- (0.122) -0.132 | 1 | 1 | 0.144- (0.149) -0.159 | 0.198- (0.219) -0.239 | 4.634- (5.797)- 7.200 | 0 | 1, 2 | 0.113 |
| | C. decipiens | 1.015- (1.136)- 1.230 | 0.948- (1.065) -1.196 | 1 | 2 | 0 | 1 | 0.589- (0.654) -0.718 | 0.105- (0.114) -0.123 | 1 | 1 | 0.151- (0.170) -0.185 | 0.223- (0.226) -0.229 | 4.943- (7.082)- 9.406 | 0 | 1, 2 | 0.120 |
| | C. draconella | 0.583- (0.783)- 0.940 | 0.711- (0.841) -0.998 | 0 | 2 | 0 | 1 | 0.466- (0.644) -0.851 | 0.063- (0.102) -0.141 | 1 | 1 | 0.131- (0.167) -0.198 | 0.179- (0.215) -0.267 | - | 0 | 1, 2 | 0.108 |
| | C. howelliana | 0.434- (0.560)- 0.650 | 0.311- (0.428) -0.547 | 1 | 1 | 0 | 0 | 0.277- (0.417) -0.683 | 0.059- (0.075) -0.084 | 1 | 1 | 0.094- (0.112) -0.143 | 0.070- (0.111) -0.171 | 3.323- (4.032)- 4.991 | 0 | 1, 2 | 0.039 |
| | C. jepsonii | 0.725- (0.809)- 0.862 | 0.769- (0.812) -0.871 | 1 | 3 | 0 | 0 | 0.267- (0.325) -0.407 | 0.089- (0.100) -0.112 | 1 | 1 | 0.106- (0.132) -0.168 | 0.129- (0.156) -0.170 | - | 0 | 1, 3 | 0.076 |
| | C. occidentalis | 0.717- (0.867)- 0.995 | 0.555- (0.756) -0.884 | 1 | 2 | 1 | 0 | 0.078- (0.172) -0.408 | 0.076- (0.092) -0.103 | 1 | 1 | 0.075- (0.096) -0.130 | 0.086- (0.112) -0.155 | 5.405- (6.942)- 9.075 | 0 | 1, 2 | 0.029 |
| | C. salina var. salina | 1.121- (1.175)- 1.268 | 0.813- (0.822) -0.826 | 1 | 2 | 0 | 0 | 0.371- (0.476) -0.547 | 0.105- (0.115) -0.127 | 0 | 1 | 0.103- (0.133) -0.160 | 0.133- (0.158) -0.178 | 4.433- (6.701)- 7.702 | 0 | 1, 2 | 0.058 |
| | C. salina var major | 0.909- (1.250)- 1.389 | 0.666- (0.897) -0.994 | 1 | 1, 2 | 0 | 1 | 0.522- (0.652) -0.815 | 0.096- (0.162) -0.166 | 1 | 1 | 0.134- (0.170) -0.202 | 0.174- (0.205) -0.236 | 5.742- (7.309)- 8.795 | 0 | 1, 2 | 0.098 |
| | C. subinclusa | 0.922- | 0.888- | 0 | 2 | 0 | 1 | 1.174- | 0.131- | 1 | 1 | 0.182- | 0.200- | 4.196- | 0 | 1, 2 | 0.145 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (0.981)-1.012 | (0.996)-1.101 | | | | | (1.526)-1.853 | (0.145)-0.157 | | | (0.206)-0.221 | (0.215)-0.221 | (5.398)-8.372 | | | |
| | C. suksdorfii | 0.945-(1.049)-1.104 | 0.727-(0.889)-0.978 | 0 | 1 | 1 | 0 | 0.327-(0.390)-0.429 | 0.092-(0.104)-0.115 | 1 | 1 | 0.109-(0.128)-0.152 | 0.148-(0.167)-0.207 | 3.958-(5.472)-7.098 | 0 | 1, 2 | 0.066 |
| Clade B | C. australis | 0.879-(0.980)-1.057 | 1.027-(1.215)-1.379 | 1 | 1 | 1 | 1 | 0.369-(0.494)-0.597 | 0.135-(0.167)-0.210 | 1 | 1 | 0.133-(0.161)-0.178 | 0.237-(0.293)-0.366 | 5.125-(6.276)-7.936 | 1 | 2 (3) | 0.202 |
| | C. campestris | 0.711-(0.800)-0.891 | 0.853-(0.912)-1.022 | 0 | 1 | 1 | 0 | 0.268-(0.338)-0.390 | 0.107-(0.129)-0.148 | 1 | 1 | 0.103-(0.136)-0.157 | 0.183-(0.210)-0.229 | 8.835-(10.977)-13.816 | 0 | 2 | 0.104 |
| | C. glabrior | 0.750-(0.939)-1.226 | 1.134-(1.255)-1.420 | 1 | 1 | 1 | 1 | 0.419-(0.741)-1.047 | 0.119-(0.147)-0.167 | 1 | 1 | 0.164-(0.195)-0.265 | 0.257-(0.277)-0.303 | 4.584-(6.522)-7.626 | 1 | 2 | 0.181 |
| | C. gymnocarpa | 1.032-(1.222)-1.442 | 1.041-(1.174)-1.298 | 1 | 1 | 1 | 0 | 0.363-(0.429)-0.477 | 0.106-(0.127)-0.136 | 1 | 1 | 0.162-(0.195)-0.223 | 0.189-(0.198)-0.212 | 6.988-(8.482)-9.524 | 0 | 2 | 0.092 |
| | C. harperi | 0.817-(0.881)-0.931 | 0.879-(0.944)-0.987 | 1 | 1 | 1 | 1 | 0.239-(0.409)-0.614 | 0.073-(0.086)-0.101 | 1 | 1 | 0.058-(0.081)-0.122 | 0.066-(0.105)-0.146 | 5.672-(6.716)-8.630 | 0 | 1, 2 | 0.026 |
| | C. obtusiflora var. obtusiflora | 0.967-(1.118)-1.277 | 0.985-(1.107)-1.289 | 1 | 3 | 1 | 1 | 0.238-(0.277)-0.350 | 0.141-(0.150)-0.166 | 1 | 1 | 0.130-(0.155)-0.180 | 0.230-(0.251)-0.291 | 4.032-(6.290)-7.332 | 0 | 2 (3) | 0.148 |
| | C. obtusiflora var. glandulosa | 0.786-(0.814)-0.847 | 0.827-(0.983)-1.112 | 1 | 3 | 1 | 1 | 0.191-(0.303)-0.434 | 0.136-(0.164)-0.193 | 1 | 1 | 0.150-(0.175)-0.200 | 0.252-(0.283)-0.329 | 7.799-(9.252)-11.270 | 0 | 2(3) | 0.188 |
| | C. pentagona | 0.623-(0.674)-0.743 | 0.624-(0.663)-0.695 | 1 | 3 | 1 | 0 | 0.289-(0.382)-0.484 | 0.061-(0.080)-0.091 | 1 | 1 | 0.093-(0.125)-0.156 | 0.127-(0.158)-0.187 | 4.071-(6.569)-8.960 | 0 | 1, 2 | 0.078 |
| | C. plattensis | 1.217- | 1.274- | 1 | 0 | 1 | 1 | 0.798- | 0.117- | 1 | 1 | 0.194- | 0.221- | 4.400- | 0 | 1, 2 | 0.231 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (1.587)-1.957 | (1.636)-1.998 | | | | | (0.976)-1.145 | (0.150)-0.188 | | | (0.268)-0.355 | (0.313)-0.392 | (6.663)-10.509 | | | |
| | C. runyonii | 0.587-(0.660)-0.732 | 0.896-(0.976)-1.047 | 0 | 1 | 1 | 1 | 0.306-(0.453)-0.626 | 0.120-(0.137)-0.150 | 1 | 1 | 0.161-(0.182)-0.215 | 0.256-(0.278)-0.312 | 3.935-(4.813)-5.481 | 0 | 1, 2 | 0.182 |
| | C. stenolepis | 0.748-(0.940)-1.087 | 0.681-(0.800)-0.875 | 1 | 1,2 | 0 | 1 | 0.414-(0.543)-0.730 | 0.090-(0.121)-0.164 | 1 | 1 | 0.092-(0.124)-0.167 | 0.154-(0.187)-0.216 | 2.852-(5.197)-7.440 | 0 | 1, 2 | 0.082 |
| Clade C | C. corniculata | 0.714-(0.757)-0.825 | 0.690-(0.768)-0.903 | 1 | 5 | 0 | 0 | 0.257-(0.365)-0.507 | 0.083-(0.091)-0.100 | 1 | 1 | 0.143-(0.149)-0.155 | 0.175-(0.193)-0.213 | 4.822-(5.693)-6.392 | 0 | 1, 2 | 0.088 |
| | C. incurvata | 0.872-(0.936)-0.976 | 0.828-(0.904)-0.979 | 1 | 3 | 0 | 1 | 0.502-(0.811)-1.039 | 0.147-(0.167)-0.188 | 1 | 1 | 0.175-(0.201)-0.214 | 0.204-(0.286)-0.326 | 4.603-(5.584)-6.970 | 0 | 1, 2 | 0.193 |
| | C. micrantha | 1.248-(1.347)-1.456 | 1.285-(1.678)-2.046 | 1 | 0 | 0 | 1 | 0.607-(0.687)-0.874 | 0.194-(0.207)-0.229 | 1 | 1 | 0.181-(0.239)-0.290 | 0.287-(0.340)-0.423 | 3.524-(4.140)-4.909 | 0 | 1 | 0.363 |
| | C. parviflora var. elongata | 0.708-(0.773)-0.851 | 0.561-(0.654)-0.761 | 1 | 3 | 0 | 0 | 0.605-(0.717)-0.889 | 0.057-(0.070)-0.086 | 1 | 1 | 0.108-(0.153)-0.208 | 0.190-(0.210)-0.254 | 6.117-(7.644)-9.473 | 0 | 1, 2 | 0.104 |
| | C. platyloba | 0.592-(0.688)-0.730 | 0.535-(0.735)-0.915 | 1 | 5 | 0 | 1 | 1.535-(0.902)-1.519 | 0.083-(0.112)-0.130 | 1 | 1 | 0.176-(0.198)-0.230 | 0.180-(0.234)-0.268 | 5.184-(6.774)-7.595 | 0 | 1, 2 | 0.172 |
| | C. racemosa var. racemosa | 0.692-(0.815)-0.934 | 0.692-(0.650)-0.669 | 1 | 1 | 0 | 1 | 0.562-(0.764)-1.023 | 0.122-(0.142)-0.154 | 1 | 1 | 0.187-(0.195)-0.210 | 0.220-(0.258)-0.286 | - | 0 | 1, 2 | |
| | C. racemosa var. miniata | 0.566-(0.583)-0.598 | 0.574-(0.616)-0.661 | 1 | | 0 | 1 | 0.546-(0.680)-0.827 | 0.084-(0.103)-0.113 | 1 | 1 | 0.161-(0.195)-0.228 | 0.263-(0.294)-0.319 | 5.397-(6.881)-10.074 | 0 | 1, 2 | 0.203 |
| | C. suaveolens | 1.269- | 1.087- | 1 | 5 | 1 | 1 | 0.745- | 0.195- | 1 | 1 | 0.178- | 0.314- | 5.338- | 1 | 1, 2 | 0.310 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (1.419)-1.606 | (1.233)-1.359 | | | | | (1.106)-1.534 | (0.218)-0.251 | | | (0.227)-0.312 | (0.363)-0.398 | (6.412)-8.361 | | | |
| | C. werdermanii | 0.505-(0.648)-0.740 | 0.507-(0.671)-0.768 | 1 | 1 | 0 | 1 | 0.446-(0.568)-0.785 | 0.098-(0.111)-0.136 | 1 | 1 | 0.119-(0.158)-0.193 | 0.138-(0.188)-0.226 | 5.651-(6.780)-8.832 | 0 | 1 | 0.111 |
| | C. xanthochortos var. xanthochortos* | 0.644-(0.887)-1.038 | 0.582-(0.740)-0.862 | 1 | 1, 5 | 0 | 1 | 1.002-(1.462)-1.961 | 0.131-(0.165)-0.207 | 1 | 1 | 0.163-(0.222)-0.260 | 0.308-(0.330)-0.351 | 3.581-(4.975)-6.684 | 0 | 1, 2 | 0.256 |
| | C. xanthochortos var. carinata | 0.573-(0.589)-0.604 | 0.642-(0.649)-0.657 | 1 | 1, 5 | 0 | 1 | 0.237-(0.314)-0.504 | 0.129-(0.149)-0.164 | 1 | 1 | 0.249-(0.270)-0.287 | 0.257-(0.291)-0.316 | 4.102-(5.284)-6.426 | 0 | 1, 2 | 0.199 |
| Clade D | C. cephalanthi | 0.790-(0.877)-0.984 | 0.749-(0.796)-0.882 | 1 | 0, 5 | 0 | 1 | 0.445-(0.516)-0.662 | 0.100-(0.121)-0.163 | 1 | 1 | 0.122-(0.146)-0.172 | 0.198-(0.225)-0.270 | 7.015-(8.284)-9.153 | 0 | 1, 2 | 0.119 |
| | C. compacta | 1.069-(1.098)-1.139 | 0.841-(0.919)-1.013 | 1 | 2 | 0 | 1 | 0.704-(0.807)-0.889 | 0.134-(0.160)-0.209 | 1 | 1 | 0.209-(0.221)-0.239 | 0.330-(0.368)-0.398 | 9.103-(12.596)-17.216 | 1 | 1, 2, 3 | 0.319 |
| | C. cuspidata | 0.217-(0.238)-0.260 | 0.199-(0.230)-0.266 | 1 | 2 | 0 | 1 | 0.215-(0.368)-0.503 | 0.025-(0.030)-0.037 | 1 | 1 | 0.182-(0.215)-0.261 | 0.281-(0.300)-0.326 | 6.173-(7.546)-9.159 | 0 | 1, 2 | 0.212 |
| | C. glomerata | 1.328-(1.444)-1.621 | 0.564-(0.631)-0.726 | 1 | 2 | 0 | 1 | 1.627-(2.327)-3.217 | 0.120-(0.132)-0.156 | 1 | 1 | 0.164-(0.214)-0.266 | 0.236-(0.253)-0.283 | 2.800-(4.894)-6.332 | 0 | 1, 2 | 0.201 |
| | C. gronovii var. gronovii | 1.288-(1.389)-1.520 | 0.898-(1.028)-1.092 | 1 | 1, 2 | 0 | 1 | 0.577-(0.668)-0.806 | 0.106-(0.138)-0.158 | 1 | 1 | 0.133-(0.163)-0.202 | 0.189-(0.221)-0.269 | 5.945-(7.044)-9.650 | 0 | 1, 2 | 0.115 |
| | C. gronovii var. latifolia | 0.896-(1.016)-1.132 | 0.821-(0.911)-1.031 | 1 | 1 | 1 | 0 | 0.313-(0.493)-0.613 | 0.081-(0.098)-0.121 | 1 | 1 | 0.095-(0.129)-0.156 | 0.143-(0.184)-0.225 | 6.242-(8.672)-11.027 | 1 | 1, 2 | 0.080 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. rostrata | 1.323-(1.478)-1.614 | 0.801-(0.901)-0.993 | 1 | 4 | 0 | 1 | 0.432-(0.625)-0.746 | 0.129-(0.141)-0.159 | 1 | 1 | 0.245-(0.346)-0.427 | 0.334-(0.372)-0.426 | 5.336-(8.288)-9.917 | 0 | 1, 2 | 0.434 |
| | C. squamata | 0.732-(0.990)-1.342 | 0.522-(0.626)-0.712 | 1 | 2 | 0 | 1 | 0.372-(0.884)-1.933 | 0.083-(0.127)-0.170 | 1 | 1 | 0.165-(0.202)-0.245 | 0.185-(0.258)-0.315 | 3.831-(5.132)-6.818 | 0 | 1, 2 | 0.157 |
| | C. umbrosa | 1.521-(1.643)-1.806 | 0.914-(1.239)-1.414 | 1 | 2, 5 | 0 | 0 | 0.167-(0.265)-0.369 | 0.144-(0.157)-0.188 | 1 | 1 | 0.196-(0.205)-0.221 | 0.213-(0.316)-0.392 | 8.392-(10.924)-14.038 | 0 | 2 | 0.235 |
| Clade E | C. denticulata | 0.700-(0.962)-1.271 | 0.603-(0.767)-0.930 | 0 | 2, 5 | 0 | 0 | 0.287-(0.359)-0.420 | 0.079-(0.113)-0.138 | 1 | 1 | 0.102-(0.112)-0.117 | 0.128-(0.145)-0.176 | 4.107-(5.440)-7.741 | 0 | 1, 2 | 0.049 |
| | C. nevadensis | 0.628-(0.760)-0.938 | 0.442-(0.572)-0.749 | 0 | 2 | 0 | 1 | 0.302-(0.489)-0.662 | 0.092-(0.108)-0.125 | 1 | 1 | 0.135-(0.158)-0.181 | 0.147-(0.182)-0.213 | 4.104-(5.417)-7.385 | 0 | 1, 2 | 0.078 |
| Clade F | C. burellii* | 0.639-(0.680)-0.722 | 0.675-(0.686)-0.697 | 1 | 0 | 0 | 1 | 0.375-(0.551)-0.770 | 0.122-(0.128)-0.135 | 1 | 1 | 0.199-(0.227)-0.247 | 0.195-(0.250)-0.293 | - | 0 | 1, 2 | 0.147 |
| | C. haughtii | 0.832-(0.917)-1.028 | 0.779-(0.895)-1.072 | 1 | 1, 2 | 0 | 1 | 0.223-(0.389)-0.494 | 0.087-(0.097)-0.113 | 1 | 1 | 0.144-(0.159)-0.174 | 0.165-(0.188)-0.219 | 5.613-(8.938)-12.279 | 1 | 2 | 0.083 |
| | C. longiloba* | 0.702-(0.828)-0.997 | 0.610-(0.713)-0.806 | 1 | 0, 5 | 0 | 1 | 0.341-(0.732)-1.225 | 0.123-(0.142)-0.173 | 1 | 1 | 0.180-(0.219)-0.247 | 0.211-(0.258)-0.291 | 6.943-(6.756)-7.639 | 1 | 1, 2 | 0.157 |
| | C. partita | 0.556-(0.676)-0.798 | 0.521-(0.623)-0.797 | 1 | 0, 5 | 0 | 1 | 0.159-(0.304)-0.610 | 0.062-(0.087)-0.110 | 1 | 1 | 0.124-(0.146)-0.187 | 0.153-(0.184)-0.227 | 3.952-(5.273)-7.353 | 0 | 1, 2 | 0.080 |
| | C. serrata* | 0.742-(0.838)-0.927 | 0.709-(0.760)-0.812 | 1 | 0 | 0 | 1 | 1.321-(1.501)-1.667 | 0.110-(0.133)-0.149 | 1 | 1 | 0.154-(0.227)-0.298 | 0.190-(0.256)-0.313 | 4.925-(5.868)-6.940 | 0 | 1 | 0.221 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clade G | C. aurea | 0.883-(0.928)-1.005 | 0.868-(0.954)-1.096 | 1 | 0 | 0 | 1 | 0.635-(0.891)-1.122 | 0.135-(0.175)-0.230 | 1 | 1 | 0.238-(0.254)-0.268 | 0.325-(0.359)-0.393 | 6.843-(8.682)-11.048 | 1 | 2 | 0.304 |
| | C. cotijana | 1.365-(1.707)-2.024 | 1.497-(1.809)-2.091 | 1 | 0 | 0 | 1 | 0.680-(0.912)-1.179 | 0.210-(0.272)-0.332 | 1 | 1 | 0.282-(0.317)-0.351 | 0.427-(0.519)-0.615 | - | 0 | 2 | 0.634 |
| | C. floribunda | 0.924-(1.105)-1.198 | 0.788-(1.109)-1.366 | 1 | 4 | 0 | 1 | 0.555-(0.780)-1.068 | 0.190-(0.230)-0.263 | 1 | 1 | 0.331-(0.351)-0.383 | 0.445-(0.459)-0.483 | 3.443-(6.276)-8.396 | 1 | 2 | 0.469 |
| | C. jalapensis | 1.708-(1.776)-1.867 | 1.848-(1.986)-2.120 | 1 | 2, 4 | 0 | 1 | 0.331-(0.544)-1 | 0.233-(0.293)-0.369 | 1 | 1 | 0.296-(0.387)-0.458 | 0.555-(0.670)-0.793 | 4.229-(5.441)-6.283 | 0 | 2 | 1.057 |
| | C. lindsayi | 0.934-(2.059)-2.184 | 1.982-(2.069)-2.156 | 1 | 2 | 0 | 1 | 0.833-(1.226)-1.583 | 0.272-(0.471)-0.711 | 1 | 1 | 0.315-(0.353)-0.381 | 0.584-(0.685)-0.781 | 7.186-(9.013)-11.521 | 1 | 2(3) | 1.105 |
| | C. mitriformis | 1.026-(1.261)-1.536 | 1.143-(1.497)-1.939 | 1 | 0 | 0 | 1 | 0.796-(0.952)-1.103 | 0.237-(0.334)-0.387 | 1 | 1 | 0.247-(0.304)-0.407 | 0.375-(0.475)-0.553 | 7.736-(9.226)-11.486 | 1 | 2 | 0.531 |
| | C. purpusii | 0.845-(0.985)-1.123 | 0.739-(1.062)-1.323 | 1 | 2 | 0 | 1 | 1.067-(1.671)-2.212 | 0.128-(0.171)-0.212 | 1 | 1 | 0.252-(0.282)-0.332 | 0.381-(0.394)-0.409 | 5.841-(7.497)-9.703 | 0 | 1, 2 | 0.366 |
| | C. rugosiceps | 1.435-(1.757)-1.991 | 1.390-(1.680)-1.827 | 1 | 0 | 0 | 1 | 0.374-(0.486)-0.594 | 0.178-(0.260)-0.319 | 1 | 1 | 0.281-(0.324)-0.380 | 0.435-(0.479)-0.524 | 5.026-(6.335)-9.044 | 1 | 2(3) | 0.540 |
| | C. tasmanica | 0.815-(0.974)-1.053 | 0.931-(1.026)-1.130 | 1 | 2 | 0 | 0 | 0.610-(0.935)-1.072 | 0.094-(0.114)-0.135 | 1 | 1 | 0.139-(0.165)-0.191 | 0.316-(0.373)-0.450 | 5.667-(6.778)-7.480 | 1 | 2 | 0.327 |
| | C. tinctoria | 1.110-(1.166)-1.221 | 1.188-(1.264)-1.315 | 1 | 2 | 0 | 1 | 0.298-(0.447)-0.561 | 0.182-(0.208)-0.232 | 1 | 1 | 0.249-(0.294)-0.334 | 0.389-(0.440)-0.556 | 7.091-(8.480)-10.685 | 0 | 2 | 0.456 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. victoriana | 0.584-(0.680)-0.785 | 0.552-(0.691)-0.818 | 1 | 5 | 0 | 1 | 0.041-(0.074)-0.106 | 0.073-(0.092)-0.110 | 1 | 1 | 0.069-(0.081)-0.097 | 0.357-(0.125)-0.159 | 7.730-(10.706)-13.348 | 0 | 3 | 0.024 |
| | C. woodsonii | 1.124-(1.169)-1.216 | 1.030-(1.249)-1.431 | 1 | 0 | 0 | 1 | 0.711-(0.891)-1.018 | 0.216-(0.262)-0.294 | 1 | 1 | 0.319-(0.336)-0.348 | 0.328-(0.349)-0.477 | 6.170-(7.860)-10.589 | 0 | 2 | 0.286 |
| Clade H | C. applanata | 0.391-(0.425)-0.458 | 0.403-(0.489)-0.549 | 1 | 5 | 1 | 0 | 0.166-(0.215)-0.268 | 0.068-(0.086)-0.101 | 1 | 1 | 0.098-(0.139)-0.178 | 0.111-(0.174)-0.215 | 4.555-(5.899)-7.705 | 0 | 2 | 0.071 |
| | C. chinensis | 0.580-(0.735)-0.821 | 0.571-(0.748)-0.910 | 1 | 5 | 1 | 1 | 0.246-(0.347)-0.419 | 0.114-(0.120)-0.127 | 1 | 1 | 0.170-(0.191)-0.219 | 0.229-(0.255)-0.268 | 6.636-(7.221)-8.693 | 0 | 2 | 0.153 |
| | C. dentatasquamata* | 0.814-(0.930)-1.096 | 1.060-(1.205)-1.313 | 1 | 3 | 0 | 1 | 0.871-(0.909)-0.927 | 0.135-(0.153)-0.165 | 1 | 1 | 0.165-(0.187)-0.205 | 0.222-(0.249)-0.271 | 6.588-(7.429)-8.058 | 0 | 2 | 0.203 |
| | C. potosina var. potosina | 0.537-(0.656)-0.773 | 0.658-(0.739)-0.811 | 1 | 1 | 1 | 0 | 0.157-(0.235)-0.390 | 0.094-(0.109)-0.126 | 1 | 1 | 0.095-(0.119)-0.116) | 0.146-(0.160)-0.179 | 6.543-(7.922)-9.389 | 0 | 1, 2 | 0.060 |
| | C. potosina var. globifera | 0.668-(0.803)-0.893 | 0.702-(0.892)-0.986 | 1 | 1 | 1 | 0 | 0.275-(0.372)-0.504 | 0.074-(0.106)-0.134 | 1 | 1 | 0.114-(0.134)-0.153 | 0.152-(0.170)-0.210 | 4.662-(5.868)-7.966 | 0 | 1, 2 | 0.068 |
| | C. sandwichiana* | 1.276-(1.395)-1.506 | 1.417-(1.575)-1.710 | 1 | 1 | 1 | 1 | 0.422-(0.481)-0.587 | 0.115-(0.145)-0.169 | 1 | 1 | 0.122-(0.155)-0.176 | 0.138-(0.188)-0.242 | 6.528-(7.890)-9.517 | 0 | 1, 2 | 0.083 |
| | C. yucatana | 0.494-(0.641)-0.806 | 0.511-(0.703)-0.907 | 1 | 5 | 1 | 0 | 0.285-(0.349)-0.412 | 0.095-(0.124)-0.170 | 1 | 1 | 0.126-(0.149)-0.165 | 0.161-(0.175)-0.189 | 4.628-(5.492)-7.391 | 0 | 1, 2 | 0.072 |
| Clade I | C. americana | 0.528-(0.611)-0.656 | 0.582-(0.639)-0.674 | 1 | 2 | 0 | 1 | 0.314-(0.419)-0.635 | 0.094-(0.113)-0.133 | 1 | 1 | 0.118-(0.149)-0.168 | 0.198-(0.238)-0.262 | 5.656-(7.583)-10.257 | 0 | 1, 2 | 0.133 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. cozumeliensis | 1.096-(1.296)-1.421 | 0.906-(0.994)-1.082 | 1 | 5 | 0 | 0 | 1.194-(2.030)-2.584 | 0.123-(0.153)-0.180 | 1 | 1 | 0.209-(0.239)-0.260 | 0.284-(0.375)-0.443 | 4.504-(6.231)-7.789 | 0 | 2 | 0.331 |
| | C. globulosa | 1.298-(1.447)-1.658 | 1.111-(1.227)-1.341 | 1 | 2 | 0 | 1 | 1.592-(1.744)-1.979 | 0.130-(0.148)-0.160 | 1 | 1 | 0.205-(0.234)-0.257 | 0.285-(0.312)-0.362 | 7.907-(9.755)-11.515 | 0 | 1, 2 | 0.229 |
| | C. macrocephala | 1.106-(1.349)-1.685 | 1.013-(1.289)-1.544 | 1 | 2, 5 | 0 | 1 | 0.379-(0.620)-0.966 | 0.226-(0.281)-0.367 | 1 | 1 | 0.313-(0.355)-0.430 | 0.370-(0.503)-0.589 | 5.282-(7.178)-9.430 | 1 | 2 | 0.595 |
| Clade J | C. corymbosa var. corymbosa* | 1.321-(1.369)-1.440 | 1.013-(1.093)-1.177 | 1 | 1, 2 | 0 | 0 | 1.657-(1.735)-1.789 | 0.108-(0.123)-0.145 | 1 | 1 | 0.155-(0.180)-0.209 | 0.295-(0.328)-0.373 | 7.299-(8.924)-10.510 | 0 | 2 | 0.253 |
| | C. corymbosa var. grandiflora | 1.356-(1.476)-1.563 | 1.151-(1.250)-1.313 | 1 | 0 | 0 | 1 | 1.306-(1.406)-1.662 | 0.104-(0.128)-0.164 | 1 | 1 | 0.188-(0.236)-0.266 | 0.348-(0.373)-0.388 | 7.688-(9.001)-10.206 | 0 | 2 | 0.327 |
| | C. corymbosa var. stylosa | 0.904-(1.034)-1.103 | 0.816-(0.853)-0.936 | 1 | 1, 2 | 0 | 1 | 1.146-(1.829)-2.451 | 0.138-(0.150)-0.178 | 1 | 1 | 0.261-(0.291)-0.312 | 0.352-(0.388)-0.433 | 7.911-(9.779)-12.334 | 1 | 2 | 0.354 |
| | C. prismatica | 1.209-(1.265)-1.339 | 1.136-(1.223)-1.272 | 1 | 0 | 0 | 1 | 1.953-(2.421)-2.951 | 0.127-(0.138)-0.153 | 1 | 1 | 0.237-(0.266)-0.294 | 0.212-(0.338)-0.415 | 5.336-(6.798)-7.681 | 0 | 1, 2 | 0.269 |
| Clade K | C. boldinghii | 0.426-(0.450)-0.474 | 0.545-(0.593)-0.657 | 1 | 0 | 0 | 1 | 0.265-(0.345)-0.409 | 0.117-(0.140)-0.178 | 1 | 1 | 0.105-(0.148)-0.182 | 0.184-(0.225)-0.292 | 3.929-(4.525)-5.138 | 0 | 1, 2 | 0.119 |
| | C. chapalana | 0.830-(1.083)-1.221 | 0.718-(0.941)-1.228 | 1 | 0 | 0 | 1 | 1.406-(2.328)-3.317 | 0.241-(0.262)-0.281 | 1 | 1 | 0.189-(0.254)-0.343 | 0.283-(0.356)-0.443 | 2.676-(4.372)-5.489 | 1 | 1, 2 | 0.298 |
| | C. costaricensis | 0.796-(0.813)-0.839 | 0.647-(0.842)-1.010 | 1 | 0 | 0 | 1 | 0.978-(1.248)-1.603 | 0.115-(0.126)-0.136 | 1 | 1 | 0.181-(0.219)-0.252 | 0.275-(0.308)-0.366 | 6.989-(8.187)-10.365 | 0 | 1, 2 | 0.223 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | C. erosa | 0.468-(0.616)-0.744 | 0.605-(0.687)-0.739 | 1 | 0 | 0 | 1 | 0.577-(0.877)-1.100 | 0.121-(0.131)-0.155 | 1 | 1 | 0.169-(0.182)-0.241 | 0.198-(0.244)-0.303 | 5.102-(6.741)-9.170 | 0 | 1, 2 | 0.140 |
|  | C. strobilacea | 1.068-(1.239)-1.331 | 0.905-(1.067)-1.220 | 1 | 0 | 0 | 1 | 0.569-(0.705)-0.940 | 0.167-(0.192)-0.231 | 1 | 1 | 0.240-(0.291)-0.369 | 0.288-(0.335)-0.372 | 4.221-(6.219)-7.609 | 0 | 1, 2 | 0.264 |
| Clade L | C. acuta | 0.879-(0.991)-1.083 | 1.505-(1.562)-1.624 | 1 | 1, 5 | 1 | 1 | 0.495-(0.686)-0.893 | 0.136-(0.149)-0.170 | 1 | 1 | 0.104-(0.141)-0.173 | 0.173-(0.208)-0.245 | 4.498-(5.714)-6.532 | 0 | 1, 2 | 0.101 |
|  | C. desmouliniana | 0.569-(0.613)-0.670 | 0.552-(0.667)-0.793 | 1 | 5 | 0 | 0 | 0.713-(1.005)-1.294 | 0.066-(0.081)-0.099 | 1 | 1 | 0.106-(0.120)-0.146 | 0.154-(0.165)-0.182 | 5.199-(6.448)-7.755 | 0 | 1, 2 | 0.085 |
|  | C. hyalina | 0.761-(0.853)-0.944 | 0.513-(0.593)-0.674 | 1 | 5 | 0 | 1 | 0.284-(0.407)-0.552 | 0.055-(0.072)-0.091 | 1 | 1 | 0.132-(0.142)-0.150 | 0.127-(0.140)-0.151 | 5.642-(7.235)-8.561 | 0 | 1, 2 | 0.046 |
|  | C. leptantha | 0.560-(0.630)-0.768 | 0.411-(0.473)-0.510 | 0 | 5 | 0 | 0 | 0.729-(0.952)-1.234 | 0.079-(0.082)-0.085 | 1 | 1 | 0.147-(0.178)-0.195 | 0.181-(0.218)-0.254 | 4.028-(4.727)-5.490 | 0 | 1, 2 | 0.111 |
|  | C. odontolepis | 1.238-(1.304)-1.417 | 1.280-(1.439)-1.568 | 1 | 3 | 0 | 0 | 1.290-(1.713)-2.045 | 0.173-(0.195)-0.218 | 1 | 1 | 0.224-(0.241)-0.251 | 0.373-(0.387)-0.408 | 6.265-(7.794)-9.135 | 0 | 1, 2 | 0.352 |
|  | C. polyanthemos | 0.891-(1.044)-1.147 | 0.577-(0.698)-0.807 | 1 | 2 | 0 | 1 | 2.057-(3.407)-4.204 | 0.096-(0.110)-0.122 | 1 | 1 | 0.193-(0.245)-0.293 | 0.305-(0.358)-0.427 | 5.641-(7.433)-11.144 | 0 | 1, 2 | 0.301 |
|  | C. tuberculata | 0.407-(0.476)-0.519 | 0.459-(0.590)-0.644 | 1 | 2 | 0 | 1 | 0.137-(0.287)-0.445 | 0.088-(0.100)-0.111 | 1 | 1 | 0.135-(0.170)-0.193 | 0.179-(0.237)-0.276 | 6.919-(8.288)-9.543 | 0 | 1, 2 | 0.132 |
|  | C. umbellata var. umbellata | 0.555-(0.649)-0.736 | 0.424-(0.585)-0.719 | 1 | 2, 5 | 0 | 0 | 0.323-(0.805)-1.564 | 0.069-(0.097)-0.115 | 1 | 1 | 0.122-(0.161)-0.191 | 0.170-(0.224)-0.289 | 4.761-(6.025)-7.144 | 0 | 1, 2 | 0.118 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. umbellata var. reflexa | 0.906-(1.021)-1.114 | 0.903-(1.043)-1.150 | 1 | 2, 5 | 0 | 1 | 1.030-(1.404)-1.905 | 0.229-(0.249)-0.271 | 1 | 1 | 0.186-(0.201)-0.227 | 0.234-(0.260)-0.287 | 6.117-(7.433)-8.648 | 0 | 1, 2 | 0.159 |
| Clade M | C. coryli | 1.202-(1.221)-1.252 | 1.004-(1.092)-1.151 | 1 | 3 | 0 | 1 | 0.425-(0.541)-0.657 | 0.141-(0.158)-0.175 | 1 | 1 | 0.164-(0.179)-0.202 | 0.260-(0.283)-0.299 | 4.581-(6.661)-8.003 | 0 | 1, 2 | 0.188 |
| | C. indecora var. indecora | 0.714-(0.813)-0.879 | 0.696-(0.807)-0.913 | 1 | 2 | 0 | 1 | 0.309-(0.540)-0.831 | 0.101-(0.127)-0.157 | 1 | 1 | 0.170-(0.218)-0.254 | 0.241-(0.261)-0.280 | 6.463-(8.401)-10.110 | 0 | 1, 2 | 0.160 |
| | C. indecora var. attenuata | 0.933-(1.227)-1.420 | 0.904-(1.009)-1.079 | 1 | 2, 3 | 0 | 1 | 0.435-(1.107)-1.503 | 0.091-(0.119)-0.141 | 1 | 1 | 0.182-(0.205)-0.244 | 0.189-(0.239)-0.286 | 5.505-(7.031)-9.072 | 0 | 1, 2 | 0.134 |
| | C. indecora var. longisepala | 1.412-(1.552)-1.670 | 1.415-(1.531)-1.686 | 1 | 2 | 0 | 1 | 0.633-(1.103)-1.917 | 0.174-(0.221)-0.265 | 1 | 1 | 0.235-(0.276)-0.314 | 0.292-(0.335)-0.375 | 6.340-(7.861)-10.614 | 1 | 1, 2 | 0.264 |
| | C. indecora var. neuropetala | 0.792-(0.877)-0.948 | 0.704-(0.860)-0.976 | 1 | 2 | 0 | 1 | 0.293-(0.315)-0.351 | 0.104-(0.106)-0.111 | 1 | 1 | 0.213-(0.236)-0.268 | 0.269-(0.330)-0.381 | 13.721-(14.902)-15.649 | 0 | 1, 2 | 0.256 |
| | C. warneri | 1.019-(1.085)-1.152 | 1.103-(1.144)-1.193 | - | 5 | 0 | 0 | 0.327-(0.578)-0.733 | 0.098-(0.108)-0.115 | 1 | 1 | 0.185-(0.207)-0.245 | 0.219-(0.258)-0.290 | - | 0 | 1, 2 | 0.156 |
| Clade N | C. deltoidea | 0.588-(0.649)-0.749 | 0.705-(0.809)-0.941 | 1 | 3 | 0 | 1 | 0.813-(0.957) 1.144 | 0.066-(0.097)-0.134 | 1 | 1 | 0.112-(0.132)-0.148 | 0.159-(0.185)-0.207 | 5.645-(7.041)-8.833 | 0 | 1, 2 | 0.080 |
| | C. gracillima | 0.701-(0.779)-0.862 | 0.826-(0.912)-0.996 | 1 | 2,5 | 0 | 1 | 2.450-(2.766)-2.989 | 0.116-(0.124)-0.147 | 1 | 1 | 0.161-(0.191)-0.221 | 0.213-(0.236)-0.250 | 4.641-(6.517)-8.311 | 0 | 1, 2 | 0.174 |
| | C. mcvaughii | 0.445-(0.560)-0.632 | 0.379-(0.515)-0.595 | 1 | 0 | 0 | 1 | 0.254-(0.419)-0.632 | 0.090-(0.094)-0.100 | 1 | 1 | 0.139-(0.197)-0.254 | 0.163-(0.211)-0.254 | 7.919-(9.504)-10.855 | 0 | 1, 2 | 0.139 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. punana | 0.662-(0.733)-0.823 | 0.596-(0.750)-0.852 | - | 2 | 0 | 0 | 0.202-(0.442)-0.681 | 0.090-(0.099)-0.106 | 1 | 1 | 0.173-(0.190)-0.229 | 0.177-(0.219)-0.277 | 8.297-(9.803)-11.520 | 0 | 1, 2 | 0.113 |
| | C. sidarum | 0.586-(0.672)-0.739 | 0.598-(0.696)-0.767 | 1 | 2, 5 | 0 | 0 | 0.920-(1.255)-1.608 | 0.090-(0.098)-0.114 | 1 | 1 | 0.174-(0.192)-0.212 | 0.183-(0.233)-0.264 | 4.436-(7.874)11.065 | 0 | 1, 2 | 0.127 |
| | C. vandevenderi | 0.998-(1.027)-1.055 | 0.808-(0.861)-0.913 | - | 1 | 1 | 0 | 0.938-(1.096)-1.229 | 0.100-(0.106)-0.116 | 1 | 1 | 0.181-(0.198)-0.225 | 0.221-(0.238)-0.253 | 7.816-(8.638)-9.552 | 0 | 1, 2 | 0.133 |
| Clade O | C. argentiniana | 0.814-(0.933)-1.004 | 1.113-(1.192)-1.236 | 1 | 2, 5 | 0 | 0 | 0.268-(0.283)-0.302 | 0.127-(0.143)-0.152 | 1 | 1 | 0.124-(0.134)-0.148 | 0.288-(0.311)-0.333 | 7.882-(11.475)-14.464 | 1 | 3 | 0.151 |
| | C. bella* | 1.213-(1.361)-1.473 | 1.406-(1.546)-1.673 | 1 | 0 | 0 | 1 | 0.569-(0.612)-0.646 | 0.220-(0.257)-0.268 | 1 | 1 | 0.225-(0.259)-0.292 | 0.331-(0.337)-0.343 | - | 0 | 1, 2 | 0.267 |
| | C. boliviana* | 0.884-(1.061)-1.161 | 0.915-(1.092)-1.354 | 1 | 1, 5 | 1 | 1 | 0.753-(1.006)-1.156 | 0.151-(0.184)-0.243 | 1 | 1 | 0.261-(0.294)-0.373 | 0.229-(0.333)-0.462 | 4.687-(5.978)-8.643 | 0 | 2 | 0.261 |
| | C. chilensis | 1.514-(1.751)-2.110 | 1.528-(1.765)-2.059 | 1 | 0 | 0 | 1 | 1.090-(1.285)-1.439 | 0.198-(0.269)-0.333 | 1 | 1 | 0.343-(0.441)-0.578 | 0.442-(0.514)-0.576 | 6.632-(8.182)-10.285 | 0 | 1, 2 | 0.622 |
| | C. cristata | 0.771-(0.913)-1.112 | 0.757-(1.003)-1.208 | 1 | 2 | 0 | 0 | 0.280-(0.335)-0.440 | 0.246-(0.301)-0.382 | 1 | 1 | 0.126-(0.148)-0.187 | 0.288-(0.372)-0.420 | 6.108-(7.575)-8.4493 | 1 | 2, 3 | 0.325 |
| | C. foetida var. foetida | 1.569-(1.727)-1.835 | 1.553-(1.679)-1.759 | 1 | 0 | 0 | 0 | 0.760-(0.917)-1.211 | 0.172-(0.234)-0.292 | 1 | 1 | 0.283-(0.357)-0.388 | 0.433-(0.482)-0.588 | 5.692-(8.678)-10.721 | 0 | 2 | 0.547 |
| | C. foetida var. pycnantha | 1.831-(1.960)-2.110 | 1.633-(1.660)-1.679 | 1 | 0 | 0 | 1 | 1.465-(1.619)-1.781 | 0.315-(0.345)-0.403 | 1 | 1 | 0.322-(0.399)-0.435 | 0.493-(0.542)-0.607 | 6.493-(8.398)-10.183 | 0 | 2 | 0.461 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. friesii | 1.090-(1.228)-1.342 | 1.242-(1.375)-1.542 | 1 | 5 | 0 | 1 | 0.062-(0.078)-0.094 | n/a | n/a | 1 | 0.170-(0.192)-0.207 | 0.339-(0.409)-0.455 | 4.884-(7.051)-10.891 | 1 | 3 | 0.394 |
| | C. globiflora | 1.061-(1.146)-1.260 | 1.545-(1.649)-1.804 | 1 | 3 | 0 | 1 | 0.798-(0.907)-1.125 | 0.173-(0.198)-0.216 | 1 | 1 | 0.172-(0.233)-0.274 | 0.216-(0.280)-0.322 | 8.239-(8.965)-10.497 | 0 | 1, 4 | 0.184 |
| | C. grandiflora | 1.226-(1.480)-1.593 | 1.483-(1.929)-2.135 | 1 | 2,5 | 0 | 1 | 0.489-(0.687)-0.910 | 0.208-(0.246)-0.284 | 1 | 1 | 0.197-(0.240)-0.291 | 0.195-(0.260)-0.317 | 8.619-(11.778)-14.039 | 0 | 2 | 0.159 |
| | C. killimanjari | 2.149-(2.368)-2.587 | 2.556-(2.645)-2.733 | 1 | 0 | 0 | 1 | 0.898-(0.943)-0.988 | 0.178-(0.215)-0.252 | 1 | 1 | 0.191-(0.195)-0.198 | 0.306-(0.332)-0.357 | 8.087-(9.862)-12.952 | 0 | 1, 2 | 0.259 |
| | C. microstyla var. bicolor | 0.592-(0.620)-0.648 | 0.997-(1.013)-1.030 | 1 | 0 | 0 | 1 | 0.167-(0.196)-0.224 | 0.164-(0.171)-0.179 | 1 | 1 | 0.162-(0.179)-0.192 | 0.336-(0.356)-0.379 | - | 0 | 2 | 0.298 |
| | C. lucidicarpa | 0.844-(0.879)-0.915 | 0.989-(1.215)-1.441 | 1 | 0 | 0 | 1 | 0.589-(0.674)-0.760 | 0.157-(0.168)-0.179 | 1 | 1 | 0.109-(0.111)-0.114 | 0.176-(0.191)-0.207 | - | 0 | 2 | 0.085 |
| | C. odorata var. odorata | 1.186-(1.486)-1.851 | 1.241-(1.640)-2.110 | 1 | 0 | 0 | 1 | 0.598-(1.307)-1.962 | 0.173-(0.271)-0.377 | 1 | 1 | 0.233-(0.291)-0.335 | 0.349-(0.464)-0.567 | 4.408-(5.482)-7.522 | 0 | 2 | 0.507 |
| | C. paitana | 0.767-(0.875)-0.958 | 0.793-(0.799)-0.809 | 1 | 0 | 0 | 1 | 0.299-(0.401)-0.472 | 0.139-(0.154)-0.168 | 1 | 1 | 0.256-(0.325)-0.412 | 0.378-(0.434)-0.488 | 6.853-(8.615)-9.724 | 1 | 2 | 0.443 |
| | C. parodiana | 1.759-(1.878)-2.017 | 1.688-(1.771)-1.934 | 1 | 0 | 0 | 1 | 0.867-(1.134)-1.404 | 0.339-(0.373)-0.404 | 1 | 1 | 0.252-(0.306)-0.355 | 0.563-(0.750)-1.000 | 10.493-(12.668)-15.731 | 1 | 3 | 0.883 |
| | C. purpurata | 1.390-(1.589)-1.727 | 1.808-(2.021)-2.265 | 1 | 0 | 0 | 1 | 1.094-(1.362)-1.513 | 0.239-(0.305)-0.370 | 1 | 1 | 0.319-(0.390)-0.506 | 0.327-(0.429)-0.516 | 5.844-(7.379)-8.622 | 1 | 1, 4 | 0.577 |

# Chapter 4
# General Discussion

## 4.1 Contributions

These studies have surveyed the morphological diversity of the two most important reproductive structures, in terms of taxonomic and systematic significance, in the genus *Cuscuta*. In itself, knowledge of this diversity will be the most important contribution this thesis makes to the taxonomic understanding of this group. As previously mentioned, it has been over 75 years (Yuncker 1932) since the last comprehensive taxonomic treatment on *Cuscuta*, and a revised monograph, based on molecular phylogenetic work and more accurate descriptions of species, is now in preparation (Costea, personal communication). This study is extremely-information rich (ie. Pollen, Table 2.2; Gynoecium, Appendix 2) and both pollen and gynoecium data will be included in this revised monograph where it will prove valuable for the delimitation of *Cuscuta* species, especially in challenging groups such as Clade O of subg. *Grammica* (Stefanović et al. 2007). Qualitative and quantitative characters for the pollen and gynoecium will also be useful for future students studying the reproductive biology of *Cuscuta*. The characters provides a morphological framework that will assist in comparing pollination mechanisms and reproductive strategies to the reproductive morphological attributes associated with a particular taxon. For example, a current graduate student in the lab is examining male investment and pollination strategies in *Cuscuta*, and it is likely that knowledge of the morphological diversity present in the associated structures developed in this study will be of tremendous help to his work. Studies such as his resonate one of the primary elements of biology: the relationship between form and function.

In addition, this work may aid in determining a more precise phylogenetic position of *Cuscuta* within the Convolvulaceae, and therefore provide characters that are of use to systematists. For palynologists, the new method of quantifying tectum types that was proposed may be of value. In addition, hundreds of pollen micrographs of *Cuscuta* are now online as part of the Digital Atlas of *Cuscuta* (Costea 2007-onward), and this page has been linked to a larger Convolvulaceae Pollen Atlas (Austin et al. 2008-onward) which is set to include photos for each species in the family.

## 4.2 The integrative nature of this project

The term "integrative biology" has different meanings depending on the user (Wake 2001). It is also clear that the scientific results of integrative biology should be incorporated into much wider scientific disciplines such as chemistry, physics and engineering, to name a few (Wake 2003). While production and linkage of scientific information in this fashion is desirable, it is difficult to achieve this level of complexity over the course of a Master's degree. However, I believe the work presented here is truly integrative on its own level, or at least as much as it can be in such short duration, and this "integrativeness" is best observed at multiple "scales".

From a fine scale it is apparent that the methods that were used, and the resulting types of data, are very diverse in nature. The umbrella of this project required me to complete both field and lab work. Field work was performed in Mexico, primarily from Puerto Vallarta in the north to Salina Cruz in the south. Fresh samples were collected and preserved in various ways, while some of the basic ecology of *Cuscuta* was studied in order to gain a better understanding of population dynamics and the general reproductive biology of Mexican species. In the lab, I used a variety of microscopy techniques to study multiple levels of cellular organization (ie. anatomical, micromorphological, morphological) that could be compiled in order to address the central issue of character evolution in *Cuscuta.*

Part of the core concept behind integrative biology involves the use of innovative or novel methods that enhance the capability to gain new insight into important biological questions (Wake 2003; also a mandate of the Journal of Integrative Biology). This work involved a number of such techniques. For instance, because a complete phylogeny that includes all *Cuscuta* species has not yet been generated, multiple phylogenies were combined to create a phylogenetic supertree, using not the original data matrices, but the resultant phylogenies themselves. This technique allows for the inclusion of a large number of taxa into an analysis, and although it is relatively new and controversial (see Sanderson et al 1998; Bininda-Emonds 2004), it has been applied to a wide variety of organisms (ex. bats, Jones et al. 2002; bacteria, Daubin et al. 2001). Additionally, for analysis of the pollen tectum I used the combination of digital image analysis software and coding strategies that resulted in a quantitative character that had not previously been used for the study of pollen grain surfaces. Lastly, because of my reliance on herbarium material and the necessity to understand variation at the anatomical level, a reliable method for embedding this type of material into acrylic resin had to be derived.

At a broader scale, the long-term goal of increasing knowledge of reproductive biology in *Cuscuta* was partially fulfilled in this project by examining two very important, but associated, structures of the sexual reproduction pathway. It is well known that pollen and the gynoecium play integral roles in this pathway, but less well understood are the relationships between these structures at the morphologic and mechanistic levels, especially in *Cuscuta*. Therefore, both the pollen and gynoecium were selected to assess their diversity and determine any correlated relationships. Consequently, although pollen and gynoecia are very distinct structures, both morphologically and physiologically, they are ecologically linked and can be considered an integrated system.

At its widest reach this study represents a source of new scientific data in combination with the collation of old, multi-source information (i.e., a very broad range of journal articles were consulted) in an attempt to integrate and make sense of floral reproductive structures in *Cuscuta*. Lastly, many of the major results of these two studies were discussed in relation to their developmental, cytological, or ecological significance. To conclude, this thesis truly conforms to the ideals of integrative biology entirely on its own levels; the methods and results (also see Contributions above) are expandable and formatted in such a way that they can easily be incorporated into ongoing studies of other biological disciplines.

## 4.3 Future work

While an attempt was made to produce the most complete survey of pollen and gynoecia in *Cuscuta* to date, many species are very rare or known only from their type collection. As a result, at least 60 taxa were not included in this thesis, but if material does become available it would be beneficial to examine missing taxa in a similar fashion to that presented here. This would ensure that their variation is in-line with the morphological types that are described, and that such types are a true representation of the genus as a whole.

Still unclear is the role of the stomata at the base of the ovary in *Cuscuta*. While they are suspected of secreting nectar in some species, it is uncertain whether this is consistent with the remainder of the genus. Much of my work involved staining the ovary with toluidine blue and locating the stomata under a stereomicroscope. However, when it was not possible to see stomata under this magnification, ovaries where cut in half and observed under the compound microscope where the stomata became more apparent. It is the difference in staining ability that is interesting, and it remains unclear why stomata of some species stain while those of others do not. It is possible that they play a secretory role in many species, while in others the stomata are vestigial, and while still present, do not perform this function. Nectar secretion is one of the most powerful tools to infer pollination mechanisms in angiosperms, and it would be beneficial to know which species secrete nectar and which do not. This could be performed a number of ways including quantification of nectar production in living plants, specific staining protocols for herbarium material, or examination for structural changes in the stomata under the scanning electron microscope. The latter of these methods would require special preparation of the ovary using critical point drying and the use of material at various stages of development.

Another possible extension of this work involves the mode of seed dispersal in *Cuscuta*. Dispersal is regulated by the capsule, and functionally, this structure is either dehiscent or indehiscent. A third type of dehiscence, known as 'irregularly dehiscent' is found in a number of species, yet the exact mechanism leading to this irregularity is unknown. The ability to embed and section dried herbarium material in acrylic resin can be very useful, especially for small samples like those typical of *Cuscuta*, and an anatomical comparison between these three capsule types would be particularly well suited to this technique.

As mentioned previously, the evolution of the stigma and style of the Convolvulaceae represents an interesting topic in its own right. As such, recent systematic revisions of the family permit both the gynoecium and pollen work presented in this thesis to be expanded to include the entire family. This is particularly true for the gynoecium as the variation found in *Cuscuta* is essentially a "mirror image" of that found in the remainder of Convolvulacae, and many of the characters and their states would be directly transferrable.

Lastly, one of the major aims of this study was to optimize systematically informative characters onto a well-supported molecular phylogeny. In theory, however, phylogenetic trees originating from combined morphological and molecular data sets may provide increased resolution over molecular data alone (Wortley and Scotland 2006).

This is a possible avenue for the phylogeny of *Cuscuta* which currently has a number of polytomies due to a lack of support at some of its nodes (ex. Clade C, Clade O).

## 4.4 Summary

1. Evolution of pollen in *Cuscuta*
    a. The trend in colpi number is from tricolpate to pentahexa-colpate; shape is polymorphic; pollen size may be associated with chromosome size, ploidy level, and nuclear genome size.
    b. A morphological intergradation exists between the traditional tectum "types", and this character is better represented quantitatively as "percent perforation".
    c. Evidence based on pollen morphology suggests *Cuscuta* as sister to the "bifid clade" of Convolvulaceae, or to one of the members of this clade.
    d. In terms of systematics, pollen is relatively uninformative for the genus, yet many of the characters will be extremely valuable for taxonomic delimitation.
2. Evolution of the gynoecium in *Cuscuta*
    a. A basal stomata ring is found in nearly every species, and is probably homologous with the discoidal nectary of Convolvulaceae.

   b. A single style is plesiomorphic, while the two-styled condition is derived.
   c. Stigma morphology is best categorized into 11 shapes: globose, depressed-globose, flattened, ellipsoid, ovoid, obovoid, rectangle, conical, cylindrical, terete, clavate.
   d. Stigma/style evidence suggests *Cuscuta* as sister to the "bifid clade" of Convolvulaceae, or to one of the members of this clade.

3. <u>Overall</u>
   a. In general, there is a great deal of morphological variation within the pollen and gynoecium characters, however, many states are consistent with those found in the remainder of Convolvulaceae.
   b. Alone pollen is of little systematic significance in *Cuscuta*, while style/stigma morphology is important for subgeneric delimitation in the genus. However, in combination with molecular phylogenetics, the morphology of both structures suggests that *Cuscuta* is either sister to the "bifid clade" (Dicranostyloideae) or nested within this clade.
   c. Pollen and stigma characters are uncorrelated in *Cuscuta* with the exceptions of pollen length and stigma papillae size, and percent perforation and stigma surface area, which both showed moderate, positive correlations.

d. Qualitative and quantitative data from both studies will be valuable for the taxonomy of this difficult group

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# Appendix A

## Glossary

Allogamy: the fertilization of an ovum from one individual with the spermatozoa of another; cross-fertilization

Anemophily: a form of pollination whereby pollen is distributed by wind

Apocarpous: carpels separated by individual pistils; a gynoecium consisting of one or more carpels that are free, simple, and distinct from one another; having two or more simple pistils[2]

Apomorphy: designation or relating to a novel derived character which distinguishes the organisms or taxa that possess it from others descended from the same ancestor[1]

Autogamy: self-fertilization in plants[2]

Bifid: divided into two parts by a deep cleft or notch[2]

Carpel: a more or less abstract unit of floral structure conceptually equivalent to a simple pistil or its putative evolutionary precursor (megasporophyll) or derivative (constituent of compound pistil); often regarded as the basic evolutionary unit of the gynoecium[2]

Clavate: elongate and basically round in transverse section, the diameter greatest at or near a blunt apex, thence attenuate toward the base, the degree of taper greatest between the middle and the upper quarter[1]

Cleistogamous: having the perianth remaining closed through anthesis, preventing deposit of pollen from other flowers, thus only self-pollination possible[2]

Compitum: a fusion of the carpelar transmitting tissue prior to reaching the ovules

Dehiscence: mode of opening (splitting or forming apertures)[2]

Echinate: bearing spines[2]

Entomophily: a form of pollination whereby pollen is distributed by insect

Eudicots: a group of flowering plants; “true eudicots”

Filiform: elongate and very slender, basically round in transverse section and of more or less uniform diameter, variously curved over its length or not[2]

Gamopetalous: having two or more petals that are partially or wholly connate[2]

Gamosepalous: having two or more sepals that are partially or wholly connate[2]

Globose: uniformly convex, circular in any median section and in outline when viewed from any angle; like a globe or sphere[2]

Gynobasic: apparently arising from the ovary base[2]

Heteromorphic: occurring in two or more distinctly different forms within the taxon in point, at either the same or different times, in either the same or different plants[2]

Holoparasite: an obligate parasite, unable to exist except in association with its host[1]

Hydrophily: a form of pollination whereby pollen is distributed by water

Hypogynous: perianth and stamens are attached to the receptacle below the gynoecium

Indehiscent: not splitting or forming an aperture at maturity, the contents being released for dispersal only after decay, digestion, or erosion of the structure[2]

Infrageneric: lineages below the rank of genus

Laticifiers: producing and exuding latex[2]

Palynology: the branch of science that deals with the structure and dispersal of pollen grains and other spores, considered as indicators of plant taxonomy and distribution, as fossils for dating geological formations or archaeological remains, and as causative agents of allergic reactions[1]

Pantocolpate: describing a pollen grain having many colpi with no distributional pattern[3]

Pantoporate: describing a pollen grain with pores spread over the surface, sometimes forming a regular pattern[3]

Parenchyma: the fundamental or ground tissue of plants, typically consisting of living, thin-walled, often polyhedral cells, as in the pulp of fruits, the softer parts of leaves, the pith of stems, etc[1]

Pericarp: the portion of a fruit wall that is derived from the ovary wall; consisting of three more or less distinct tissue layers (exocarp or epicarp, mesocarp, and endocarp) that may or may not differ greatly in structure and/or function; the wall of a fruit, excluding any tissues of extra-ovarian (accessory) origin[2]

Perprolate: describing the shape of a pollen grain or spore in which the ratio between the polar axis and the equatorial diameter is more than 2[3]

Plesiomorphic: ancestral or primitive condition as compared to a later derived state[1]

Polytomy: a section of a phylogeny in which the evolutionary relationships cannot be fully resolved

Prolate: describing the shape of a pollen grain or spore in which the polar axis is larger than the quitorial diameter[3]

Psilate: without spines or superficial ornamentation[3]

Sporophytic self-incompatability: a general name for several genetic mechanisms in angiosperms which prevents self-fertilization

Subulate: slender, essentially terete, and straight, gradually attenuate from a relatively narrow base to a very narrow, blunt to angular apex[2]

Synapomorphy: a shared, derived character state

Syncarpy: having one or more compound pistils[2]

Teratologic: study of abnormalities of physiological development

Tracheids: elongated cells in the xylem of vascular plants that serve in the transport of water and mineral salts[1]

Zonocolpate: arrangement of colpi around the equatorial axis of a pollen grain[3]

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