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Morphology, Structure, Function and Evolution of Infrastaminal Scales in Cuscuta (Convolvulaceae)

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Morphology, Structure, Function and Evolution of Infrastaminal Scales in *Cuscuta* (Convolvulaceae)

by

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Abstract

Cuscuta (dodder, Convolvulaceae) is a genus of about 200 species of obligate stem parasites of cosmopolitan distribution. Infrastaminal scales (IFS) are staminode-like formations that surround the ovary in the flowers of dodders. Their morphological diversity has historically provided some of the most useful taxonomic diagnostic characters at the species-level, however, their function had not been determined. I performed a comparative study of the IFS in 147 taxa using light, scanning and transmission electron microscopy, and results were analyzed in relation to a *Cuscuta* phylogeny obtained from a combined analysis of *rbcL* and *26S rDNA* gene sequences. To test the hypothesis that the role of IFS is related to sexual reproduction, I analyzed the correlations between IFS characters and the production of pollen/ovules in the flower and the number of stomata found in the nectary at the base of the ovary. With a few exceptions, the IFS exhibit numerous fimbriae that contain laticifer cells secreting a complex resin-glycoside latex. In subgenus *Monogynella*, fimbriae are similar to uniseriate glandular hairs. In the derived subgenera *Grammica* and *Cuscuta*, the fimbriae have laticifers that are enclosed and protected by an epidermis which leaves the distal ends of the laticifers exposed. The slightest mechanical contact with the exposed part of the laticifer cells (for example by an insect) causes them to burst open and release the latex. It was found that the relationship between IFS size with both pollen/ovule ratios and nectary stomata number is weak at the level of the entire genus. Subgeneric partitions, however, revealed a strong correlation in subgenus *Monogynella*. These results suggest that scales in *Cuscuta* evolved from nectar holding in the first diverged subgenus *Monogynella*, to ovary protection against herbivorous insects in the derived subgenera *Cuscuta* and *Grammica*. This study further details the morphological diversity of the IFS in *Cuscuta*, confirming their significance for the species-level systematics.

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1. Introduction

Cuscuta (Convolvulaceae), commonly known as the dodders, is a group of parasitic plants (Figure 1.1) that are distributed worldwide and occupy a broad range of habitats (Yuncker, 1932; Costea, 2007-onwards; Stefanović *et al.,* 2007). Some *Cuscuta* species are known as pests that infest many crops globally (Lanini and Kogan, 2005; Costea and Tardif, 2006) and the legislation of most countries places all species on various quarantine lists (Costea and Tardif, 2006; Costea and Stefanović, 2009a). Dodders have also been known to act as strong effectors of ecosystem dynamics (Pennings and Callaway, 1996), and although many species are considered threatened or even extinct, the conservation efforts for *Cuscuta* have remained poor (Costea and Stefanović, 2009a). In addition, some dodders have a long history of usage as medicinal plants, especially in traditional Chinese medicine where they have been used as antibacterial and anti-inflammatory agents, and even for increasing fertility (Bork *et al*., 1996; Srivastava, 2002; Chao *et al*., 2003).

Infrastaminal scales (IFS) are finger-like appendages found within the flower of *Cuscuta*. IFS are historically important for the taxonomy of *Cuscuta* because they are a part of all the species descriptions and identification keys (Choisy, 1841; Engelmann, 1859; Yuncker, 1921, 1932). Despite this, no information is available about the evolution, structure (anatomy), and micromorphological diversity of the IFS, and their function is only at a hypothesis stage (Yuncker, 1921; Tiagi, 1966; Musselman, 1986; Prenner *et al.,* 2002; Wright, 2011). More information on these scales is needed in order to understand their role, and to enhance the classification of the *Cuscuta* species.

Figure 1.1 – Yellowish, slender stems of *Cuscuta gronovii* growing on various hosts near the Grand River in Kaufman Flats, Waterloo, ON

In this study, I took three different approaches to improve current knowledge of the evolution and function of the IFS in *Cuscuta*. First, the morphology, micromorphology, and anatomy of the IFS across the genus were surveyed. Then, the morphological traits were mapped onto a phylogeny of *Cuscuta* and analyzed for their evolutionary significance. Lastly, statistical comparisons were made between IFS data and pollen-ovule ratio data in an attempt to clarify their function in the *Cuscuta* flowers.

2. Background

2.1 Biology and Morphology of *Cuscuta*

Cuscuta is the only parasitic genus in the morning glory family, Convolvulaceae, but its precise positioning within this family is unknown (Stefanović *et al.,* 2003; Stefanović and Olmstead, 2004). Dodders are parasitic plants with ephemeral roots and slender, twining stems that range from yellow to orange or purple in colour. They are categorized as stem parasites because they mainly attach to the stem as opposed to the leaves of various host plants (Figure 2.1). *Cuscuta* are mostly holoparasitic, meaning they depend entirely on the host plants for survival. Most *Cuscuta* species do not contain chlorophyll when they are mature and therefore cannot photosynthesize (Costea and Tardif, 2006). They obtain the host's nutrients through the haustoria, which are specialized organs that penetrate the host tissue and act as a physiological bridge to the host through which water and nutrients are transported from the host to the parasite (Lee and Lee, 1989; Dawson *et al.,* 1994; Vaughn, 2002). *Cuscuta* parasitizes a wide variety of host species, both wild and cultivated (Barath, 2009).

Cuscuta species are seldom subjected to herbivory (Costea and Tardif, 2006). They are attacked only by a few insects, mainly species from the genus *Smicronyx.* Most of them lay their eggs in ovaries or rarely in the stems of *Cuscuta* resulting in the larvae eating the seeds or the internal tissues of the stems (Shimi *et al.,* 1995).

2.1.1 Sexual Reproduction in *Cuscuta*

Cuscuta have reduced scale-like leaves and their small flowers provide the only characteristics to distinguish them among various species (Figure 2.2) (Löffler *et al.,* 1997). Their flower colour ranges from white to pink, and the inflorescences are cymose. They are

Figure 2.1 – *Cuscuta gronovii* at Kaufman Flats, Waterloo, ON. The *Cuscuta* stems are twining around stems of Canada goldenrod (*Solidago canadensis*) host plants.

Figure 2.2 – *Cuscuta draconella* flower (Costea, 2007-onwards).

bisexual and actinomorphic, with 5 calyx lobes, 5 petals, and 5 stamens, as all the members of Convolvulaceae (Yuncker, 1932; Costea and Tardif, 2006). The flower of *Cuscuta* seems to be targeted by generalist pollinators. Field observations suggest that *Cuscuta* species have a wide range of insect visitors including flies, moths, beetles, and larger insects (Wright, 2011). Furthermore, researchers in Spain have demonstrated that the *C. epithymum* pollinators were the same insects that visited its host, *Hormathophylla spinosa* (Gómez, 1994). There is a floral nectary at the base of the ovary of all dodders which consists of a ring of modified stomata (Welsh, 2009; Wright, 2011; Wright *et al*., 2011). The nectary stomata number and volume of secretion vary throughout the genus, with some species being able to secrete a large amount of nectar and others very little (McNeal, 2005; Prenner *et al.,* 2002, Wright *et al.,* 2012).

Despite the above observations suggesting outcrossing, there are reports of self-pollination in *Cuscuta*. Self-pollination is known to occur in most genera of Convolvulaceae (Martin, 1970), and in *Cuscuta* it occurs in many species with small flowers (Beliz, 1986; Prather and Tyrl, 1993; Dawson *et al.,* 1994; Holm *et al*., 1997). The anthers of *C. obtusiflora* have been observed to release their pollen while the flowers are still in buds, and fertilization takes place before outcrossing can occur (i.e. functional cleistogamy, Rodríguez-Pontes, 2009). In contrast, self-incompatibility, a mechanism that helps prevent self-pollinationhas been reported in both *C. chilensis* and *C. rostrata* (McNeal, 2005).

More recently, Wright *et al*. (2012) have demonstrated through a pollen-ovule production study that the *Cuscuta* genus encompasses a broad variation of mating systems. While the majority of *Cuscuta* species exhibit a mixed mating system, some dodders were found to be obligate outcrossers, but none were obligate selfers. Even in species previously thought to self-pollinate before the flowers open (Rodríguez-Pontes, 2009), a small amount of cross-pollination

occurs after anthesis (Wright *et al*., 2012). In general, larger corollas and stigmas were associated with larger pollen-ovule ratio values.

2.2 Systematics and Evolution of *Cuscuta*

Though its taxonomy has been historically controversial, floral characteristics have been important for the systematics of *Cuscuta*, both at the infrageneric and at the species level. The ~200 known species of *Cuscuta* (Costea, 2007 –onwards) have been historically grouped into 3 subgenera, *Grammica*, *Cuscuta*, and *Monogynella,* based on style and stigma morphologies (Engelmann, 1859). Subgenera *Grammica* and *Cuscuta* are characterized by two distinct styles with short, capitate stigmas and linear, elongate stigmas, respectively. Subgenus *Monogynella* is characterized by a single style with a variety of stigma shapes.

More recent phylogenetic studies have largely confirmed the infrageneric classification and broad-scale evolutionary relationships based on plastid and nuclear datasets that have been published for the subgenera *Cuscuta* and *Grammica* (Stefanović *et al.,* 2007; García and Martin, 2007). Within subgenus *Grammica*, the largest and most complicated group, 15 major clades, Clades A – O, have been recently circumscribed (Stefanović *et al*., 2007). Subsequently, more focused studies were initiated to elucidate evolutionary relationships and taxonomy at a species level. To date, 8 of the 15 clades have been extensively studied (Costea *et al*., 2005; Costea *et al.,* 2006a, 2006b, 2006c; Costea *et al.,* 2008; Costea and Stefanović, 2010; Costea *et al.,* 2011a, 2011b).

Studies have recently been conducted on the character evolution for the pollen, gynoecium, and reproductive output of *Cuscuta* (Welsh *et al*., 2010; Welsh, 2009; Wright, 2011). Welsh studied the evolution of pollen and gynoecium characteristics of *Cuscuta*. He concluded that the

pollen characters were marked by convergent evolution, which makes it unsuitable to reconstruct phylogenetic relationships at the scale of the entire genus, but the observed variation is very useful for the species-level systematics of *Cuscuta*. For gynoecium evolution, Welsh (2009) suggested that a single style is plesiomorphic (ancestral), as they characterized the first diverged subgenus *Monogynella*, while the two-styled condition is derived. Wright studied many aspects of sexual reproduction in *Cuscuta* including sex allocation, floral evolution and pollination. He demonstrated that *Cuscuta* have a diverse range of breeding systems, that they possess both pollen and nectar reward strategies, and that the evolution of two styles followed by that of uneven styles was critical for the adaptive radiation of *Cuscuta* (Wright, 2011; Wright *et al*., 2012).

2.3 Infrastaminal Scales

An interesting feature that has only been reported in *Cuscuta* species is the presence of IFS. These are intricate, finger-like structures located at the base of each of the 5 stamens and fused to the corolla tube (Costea, 2007- onwards). They are the last structures to develop in the flower of *Cuscuta*, after the calyx, corolla and reproductive organs (Kuoh and Liao, 1993; Prenner *et al.,* 2002; Clayson, 2012). Flowers of *Cuscuta* are only millimeters long, which means the scales are tiny – the smallest one can be about 100 micrometers (e.g. *C. harperi*). The IFS are diverse in their morphology, ranging from very well-developed to reduced or even absent in some species (Yuncker, 1932). This diversity is what makes the IFS so important for the species-level taxonomy of *Cuscuta*.

In 1932, Yuncker published the most comprehensive monograph of *Cuscuta* species, in which he provided hand-drawn illustrations of the IFS in the 165 species known at that time. More recently, basic morphological information about the scales (e.g. the IFS length vs. corolla

tube length ratio and the shape/size) has been used in the taxonomies of different groups of species mainly from subgenus *Grammica* (García, 1999; Costea *et al*., 2005; Costea *et al.,* 2006a, 2006b, 2006c; Costea and Stefanović, 2009b; Costea *et al.,* 2008; Costea *et al*., 2009; Costea and Stefanović, 2010; Costea *et al.,* 2011a, 2011b). In addition, it was recently discovered that in the *C. umbellata* clade, IFS contain laticifers, which are latex-filled cells that are located at the tips of the scales' fimbriae (Costea and Stefanović, 2010). However, despite the taxonomic significance of the IFS, no study is available about their fine morphology, structure or evolution.

The function of the IFS has only been hypothesized. Prenner *et al*. (2002), relying on observations done in *C. reflexa*, suggested that IFS serve as a secondary nectar receptacle for the nectar secreted at the base of the ovary. Tiagi (1966) suggested that IFS have a secretory role and that they serve to attract pollinators. Yet another hypothesis is that the scales have a protective role because of the way they are positioned around the ovary (Yuncker, 1921; Musselman, 1986). Finally, Wright (2011) suggested that in *C. strobilacea* the scales may play a role in pollination – by shielding the stigmas from self-pollen. None of these hypotheses, however, have been explored at the scale of the entire genus or have been explicitly tested (Costea and Tardif, 2006).

2.4 Development and Function of Laticifers

2.4.1 Laticifers and Latex

In general, laticifers are single cells (non-articulated) or a series of cells (articulated) that have a tube-like growth form and contain fluid latex (Metcalfe, 1967; Evert, 2006). Articulated laticifers are categorized as either anastomosing, which means that the cells are connected to one another and form a network, or non-anastomosing (Pickard, 2008). About 20,000 plant species contain laticifers, which can occur throughout the plant body, including the stems, corolla, calyx,

and gynoecium. Laticifers are under considerable turgor pressure and they exude latex when damaged (Condon and Fineran, 1989a). The latex seems to be a secondary metabolite since it does not serve a function for any primary metabolic processes (Agrawal and Konno, 2009). The amount of latex production has been shown to be phenotypically plastic in the sweet potato (Convolvulaceae), meaning that it can differ from plant to plant (Agrawal and Konno, 2009).

The latex itself can vary in composition in different plant species but is mainly composed of a complex mixture of lipids, phenolics, terpenoids, cardenolides, proteins, and alkaloids (Hagel *et al.,* 2008; Agrawal and Konno, 2009). Phenolics include tannins, lignins and flavonoids and are the most widely distributed class of secondary metabolites in plants (Hättenschwiler and Vitouse, 2000). Terpenoids are carbon-based compounds, which are very common in *Hevea brasiliensis*, also known as the rubber tree (Agrawal and Konno, 2009). Cardenolides are cardiac glycosides which act to inhibit Na^+/K ATPases. Proteins in latex can include cysteine and serine proteases, which are enzymes that catabolize specific proteins. Alkaloids are basic chemical compounds containing nitrogen. Latex differs from other plant residues such as resins or gums because it is more phytochemically diverse (Langenheim, 2003).

Though the function of laticifers is not clear, many studies have linked them with the defense of plants against herbivores (Pickard, 2008; Hagel *et al*., 2008, Ramos *et al*., 2010). Examples of such studies include insects trenching leaves containing latex (Dussourd, 2003), insects halting their feeding because their mouths are smeared with latex from the plant (Dussourd and Eisner, 1987), and the application of latex proteins to crop pests which led to their loss of weight and lower survival rate (Ramos *et al.,* 2007) . Researchers suggest that the formation of latex is an evolutionary response to insect attack as many of the secondary

metabolites found within laticifers are considered toxic (Agrawal and Konno, 2009). Some have also suggested that laticifers may have a wound-healing role in the plant (Evert, 2006).

2.4.2 Laticifers and Latex in Convolvulaceae

Laticifers in the members of the Convolvulaceae which have been studied are articulated and non-anastomosing (Metcalfe, 1967; Dussourd and Denno, 1991; Evert, 2006), and generally have a milky-white latex (Condon and Fineran, 1989a). Articulated laticifers develop from multiple cells and are usually apparent in the mature plant embryo. As the plant begins to develop, meristematic cells are extended, differentiating into laticiferous cells. Articulated laticifers are often associated with external phloem (Condon and Fineran, 1989b; Evert, 2006). Laticifers were found to be primarily abundant in the roots, stems, and petioles of *Calystegia silvatica* and were absent in the epidermis, xylem, and internal phloem of these organs (Condon and Fineran, 1989a).

Studies specifically on the latex of the Convolvulaceae have shown that it commonly contains serine proteases (Agrawal and Konno, 2009). Ether-soluble resin glycosides were isolated from the latex of *Ipomoea batatas*, the sweet potato, and *Ipomoea digitata*, the giant potato, though no function was postulated (Noda and Horiuchi, 2008; Ono *et al.,* 2009). Resin glycosides are a large family of secondary metabolites containing acid derivatives that are unique to Convolvulaceae, and are used in herbal medicine as a laxative (Anaya *et al*., 1990; Pereda-Miranda *et al*., 2010).

2.4.3 Laticifers and Latex in *Cuscuta*

The recent discovery of laticifers in the IFS of *Cuscuta* (Costea and Stefanović, 2010) opens a new direction of research that may shed light not only on their function, but on their evolution

as well. *Cuscuta* have been documented to possess multinucleate and articulated laticifers in the vegetative organs (Lyshede, 1985; Costea and Tardif, 2006), as do other members of the Convolvulaceae (Dussourd and Denno, 1991).

Few studies exist on the chemical composition of dodder extracts from stems and flowers. Researchers studying their phytotoxicity found that they consisted mainly of terpenes, phenols, phenolic acids, fatty acids, and lactone (Khanh *et al.*, 2008). Other constituents of dodder extracts include flavonoids, lignins, quinic acid derivatives, glycosides, sterols, tannins, amino acids, and microelements, with flavonoids being the main metabolite (Ye *et al*., 2005; Mavlonov *et al.,* 2008; Zhusupova, 2009; Ferraz *et al*., 2011). Resin glycoside fractions have been isolated from seeds of *Cuscuta chinensis* (Du *et al*., 1998) and *C. australis* (Du *et al.,* 1999). All of these constituents are classified as part of a large and diverse group of chemical compounds, with none of them having a single confirmed function. Many of these have suspected functions of plant survival and defense. As one can see, the composition of *Cuscuta* is very complex.

There have been a few studies that have recorded a toxic nature of *Cuscuta* extracts. For example, it was reported that *Cuscuta* extracts disrupt the growth of insects (Srivastava *et al.,* 1990). Researchers have shown that *C. hyalina* and *C. reflexa* extracts are highly toxic to mosquitoes (Preeti and Srivastava, 1998; Mehra and Hiradhar, 2002). Studies have also documented that *Cuscuta* extracts can be toxic to nematodes (Haidar *et al*., 1999; Costea and Tardif, 2006).

A researcher from Penn State University has been conducting experiments to test a *Cuscuta* herbivory defense hypothesis. He has been subjecting caterpillars to a diet consisting stems of *Cuscuta*, which also contain laticifers, and so far, they has been proven to be lethal to young caterpillars because they have been avoiding these stems and thus starving to death (Smith and

Costea, personal communication). This project is still ongoing and its results would support that *Cuscuta* extracts could be toxic to insects.

2.5 Staminal/Petal Appendages or Scales

There have been reports of appendages that share a degree of similarity with the IFS in the sense that they are organs related to the androecium and/or corolla of the flower. There are reports of petal appendages in Bromeliaceae, a group of monocots. It was concluded that these appendages are non-vascularized outgrowths of the adaxial petal surface that flank the stamen and that they are the last structures formed in the flower (Brown and Terry, 1992). The authors speculate that they function in intrafloral nectar management such as nectar holding and delivery.

Scale-like structures have also evolved in other Convolvulaceae genera. In *Lepistemon*, "scales" are secretory structures consisting of glandular trichomes which are united by a narrow bridge and are not vascularized. Because of their formation around the ovary, their suggested function is protection (Wilkin, 1999). In a morphological assessment of Convolvulaceae, Govil (1972) states that there are "scales" at the base of the stamen in *Merrmia*, *Evolvulus*, and *Convolvulus*, and that they also do not receive any vascular supply.

3. Objectives

The goal of this study is to gain more knowledge about the IFS in *Cuscuta* species by studying their (a) morphology and structure, (b) evolution, and (c) function. This was done by:

a. Surveying the morphological and structural diversity of the IFS across the genus
b. Placing the diversity of the IFS into an evolutionary context and determining major evolutionary trends
c. Determining the role of the IFS by testing the hypothesis that the scales are associated with sexual reproduction, as well as examining alternative functions

4. Materials and Methods

4.1 Morphology and Micromorphology

The diversity of the IFS was surveyed in 147 taxa (132 species and 15 varieties) using herbarium specimens and field collections from around the world. With the exception of the taxa known only from their type collection or only two collections, a minimum of three specimens collected from different geographical locations were used for each taxon. Ten flowers per specimen were analyzed.

Flowers removed from herbarium specimens were rehydrated by being steeped in gradually warmed 50% ethanol, which was allowed to boil for a few seconds. After removal of the calyces, corollas were dissected and examined with a Nikon SMZ1500 stereomicroscope. Photos were taken to document the IFS length ratio with the corolla tube length (Figure 4.1a) using Pax-it 7.4 software and a PaxCam Arc digital camera (MIS Inc., 2012). Subsequently, the IFS were carefully removed with tweezers from the point of their attachment and imaged separately. For the micromorphological study, rehydrated and dissected corollas were cut in two or three longitudinal strips (Figure 4.1b) to ensure that the IFS remained intact for SEM observation (as opposed to removing the IFS from the corolla). Once the corollas were cut, they were subjected to a hexamethyldisilazane (HMDS) treatment (Wright *et al.,* 2011), which is an alternative to critical-point drying. Corolla strips were passed through an ethanol dehydration series (70%, 85%, 95% and 100%; 1 hour each), and immersed into HMDS overnight (Wright *et al.,* 2011). Samples were dried in the fume-hood and then mounted onto aluminum stubs with the IFS facing up. In each species, some of the IFS were fractured or cut with 3 mm Vannas scissors at the level of fimbriae to allow the observation of their structure under the SEM. Samples were sputter-coated with 30 nanometers of gold using an Emitech K 550 (Emitech, Ltd. Ashfort, UK)

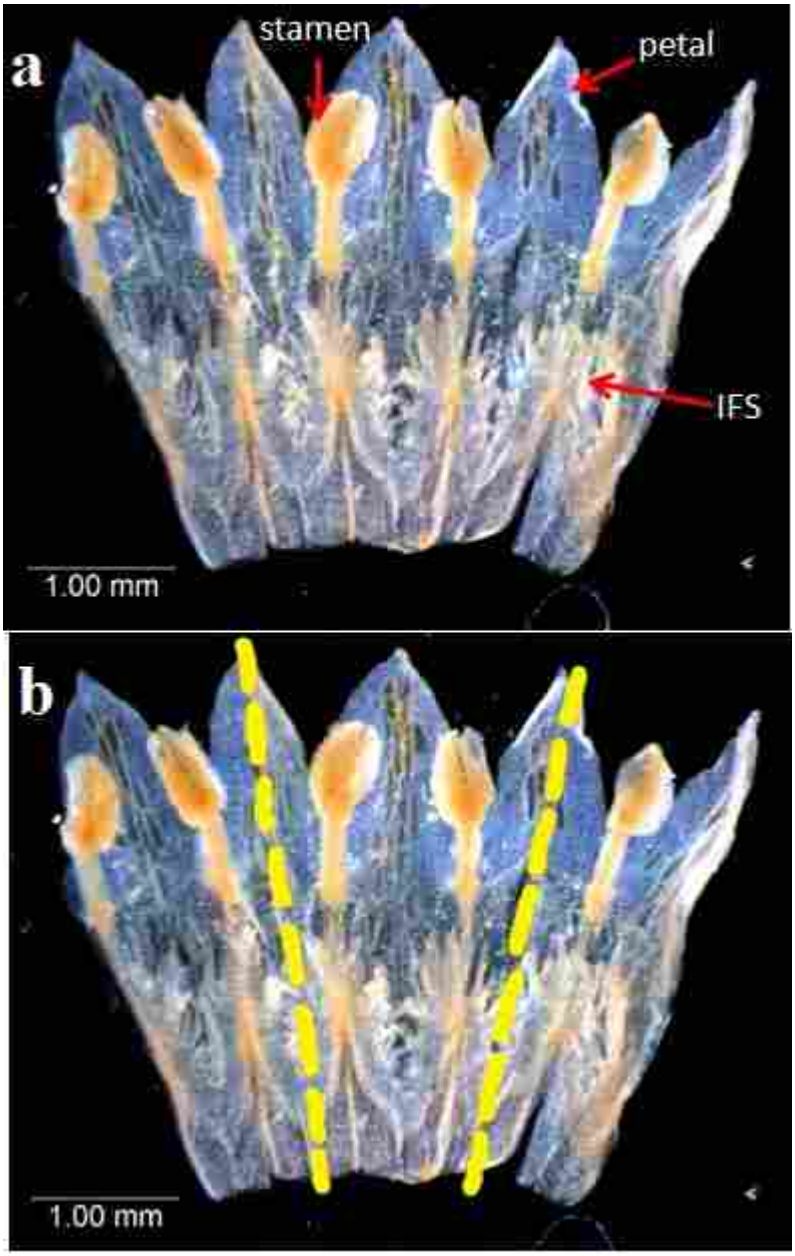

Figure 4.1 – **a**. Dissected corolla of *Cuscuta cuspidata* flower. Note the IFS at the base of the stamens. **b**. Yellow dashed lines indicate where corolla will be cut for specimen pieces for SEM. Photo from Costea(2007-onwards).

sputter coater. Corolla fragments with the attached IFS were then analyzed and photographed under a Hitachi SU1510 variable pressure SEM at 3 kV.

4.2 Structure and Histochemistry

For the anatomical study of the IFS, field collections of *C. chilensis*, *C. costaricensis, C. cotijana*, *C. gracillima, C. gronovii* var. *gronovii*, and *C. strobilacea* (subgenus *Grammica*) were fixed in Formalin-Acetic acid-Alcohol (FAA, Ruzin, 1999). Specimens were dehydrated through an ethanol series (70%: overnight; 70%, 85%, 95%, 100%: 1 hour each; 100%: 2 hours) transferred to xylene, and then infiltrated and embedded in paraffin (Ruzin, 1999). Sections of whole flowers were cut at 5µm and 7µm on an American Optical Co. microtome, and dried for a minimum of 48 hours on glass slides for staining with Sass's safranin-fast green FCF for staining nuclei and differentiating tissue structure (Ruzin, 1999). For *C. approximata, C. epithymum, C. nitida* (subg. *Cuscuta*), *C. japonica* and *C. reflexa* (subg. *Monogynella*), no fresh or fixed material was available, so herbarium specimens were used instead. Observation was conducted on a Nikon Eclipse 50*i* brightfield microscope, and imaging was performed using a Pax-cam Arc digital camera and Pax-it 7.4 software (MIS Inc., 2012).

Specimens embedded in Spurr's resin for transmission electron microscopy (TEM, explained below) were also sectioned for transmitted light microscopy. Specimens were cut 0.5–1.0 µm thick with a diamond histoknife; the sections were mounted on glass slides and stained with Epoxy Tissue Stain (Electron Microscopy Services, Catalog #14960). Slides were then mounted with immersion oil, sealed with nail polish, and imaged.

Laticifer composition was studied via histochemistry in the species mentioned above from all three subgenera for comparatice purposes. IFS were removed after specimens were gradually warmed in 50% ethanol, and submerged into one of five stains (Table 4.1) used for

Table 4.1 - Histochemical stains and target compounds examined. Staining was performed *in situ*.

Stain	*Target Compound(s)*	*Staining Time*	*Mounting Medium*	*Reference(s)*
Nile Blue	Acidic lipids (blue) Neutral lipids (pink)	3 min	7% Acetic acid	Cain, 1947; Passarelli, 1999
Neutral Red	Total lipids (red)	5 min	De-ionized water	Clark, 1981
Oil Red O	Neutral triglycerides, lipoproteins (red)	5-7 min	70% Ethanol	Kirk, 1970
Sudan Black	Total lipids (black)	3-5 min	70% Ethanol	Nemec, 1982
Sudan IV (Scarlet Red)	Neutral lipids (red)	3-5 min	De-ionized water	Cox *et al.,* 1975

the allotted time. Specimens were then viewed on a glass slide under the microscope and imaged with the software mentioned above. Though the IFS have no visible vasculature, phloem staining (Aloni and Sachs, 1973) was conducted in order to confirm that no phloem exists. The corollas of *C. cotijana* specimens fixed in FAA were dissected and in some cases, the IFS were removed with tweezers. Specimens were cleared in 8 M NaOH for 12 hrs at 60ºC (Wright *et al.,* 2011). They were then immersed in lactic acid and brought to a boil. The specimens were then placed in a solution of 0.2% lacmoid in lactic acid for 3 hours. Subsequently, they were rinsed in phosphate buffer at pH 7.5. The specimens were then mounted in 70% sodium lactate and observed on a Nikon Eclipse 50*i* brightfield microscope.

4.3 Ultrastructure

For the ultrastructure study, fresh flowers of *C. gronovii* var. *gronovii* were collected in August, 2011 from a large natural population established in Kaufman flats, near the Grand River, 43°30'11.56"N, 80°29'37.98"W, Ontario, Canada. They were prepared for transmission electron microscopy (TEM) and were subjected to a modified Spurr's Resin protocol (Ma and Peterson, 2000; Fineran, 1982). The specimens were fixed in 3% glutaraldehyde + 2% paraformaldehyde in 0.025 M sodium phosphate buffer at pH 6.8 overnight, and then washed three times in buffer. This was followed by post-fixation in 1% buffered OsO_4 for 1 hour. After three distilled water washes, specimens were subjected to an ethanol dehydration series in 30%, 50%, 70%, 90% and 3x 100%. Specimens were infiltrated and embedded in Spurr's resin (Spurr, 1969) and polymerized at 70ºC. The embedded tissues were cut with a diamond ultraknife at 80 to 100 nm, mounted onto formvar and carbon-coated coppergrids, and then post-stained with 5% uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). Observations were made on a JEOL 2011 Scanning Transmission Electron Microscope at 200 kV, and images were taken with a Gatan Ultrascan digital camera and 'Digital Micrograph' software (Gatan Inc., 2007).

4.4 Characters and Analysis of IFS

Previous descriptions of the scales (Yuncker, 1932; García, 1999; Costea *et al.,* 2005; Costea *et al.*, 2006a, 2006b, 2006c; Costea *et al.*, 2008; Costea *et al.*, 2009; Costea and Stefanović, 2009b; Costea and Stefanović, 2010) were reviewed to choose an initial set of morphological characters to quantify. After the morphological details using different microscopy techniques were observed, eleven quantitative and seven qualitative IFS characters were formulated and scored (Table 4.2; Fugure 4.2). Alternate images documenting the morphology of the IFS and

corolla of many *Cuscuta* species are available in the Digital Atlas of *Cuscuta* online (Costea, 2007 –onwards) and were also used for scoring. Bridge surface was determined by using the formula Surface(mm^2) = base(mm) x width(mm).

Table 4.2 - IFS characters surveyed and their representative codes and states

Character	*Character States*
1. Scales	0 = absent; 1 = present
2. Type of scale	With fimbriae that belong to: 1 = *Monogynella* type; 2 = *Cuscuta* type
3. Shape of scale	1 = *oblong*; 2 = e*lliptic*; 3 = *ovate*; 4 = *ovate-triangular*; 5 = o*bovate*; 6 = *spathulate*; 7 = *bifid*
4. Shape of the scale apex	1 = *rounded*; 2 = *acute*; 3 = *truncate*
5. Total scale length (including bridge and fimbriae)	mm
6. Scale body length (not including bridge)	mm
7. Maximum scale width (including fimbriae)	mm
8. Scale body surface	mm^2
9. Bridge length	mm
10. Bridge surface	mm^2
11. Fimbriae length	mm
12. Fimbriae length on lower half of scale	mm
13. Fimbriae length on upper half of scale	mm
14. Number of fimbriae per scale	Total count of fimbriae
15. Laticifer shape	1 = elongate; 2 = medium elongate (elliptic, ovate, obovate); 3 = short (round, reversed ellipsoid)
16. Papillae on fimbriae	0 = absent; 1 = present
17. Bridge with fimbriae	0 = absent; 1 = present
18. Corolla tube length/ scale length ratio	Ratio

Characters were mapped onto a summary consensus tree of *Cuscuta* built in Mesquite 2.75 (Maddison and Maddison, 2012) resulting from broad-scale phylogenies based on nuclear ITS and plastid *trn*L-F datasets for subgenera *Cuscuta* (Garcia and Martin, 2007) and *Grammica* (Stefanović *et al*., 2007), as well as the entire genus (Costea and Stefanović, personal communication). This phylogeny has been used in three previous studies, the authors of which

studied the diversity and evolution of *Cuscuta* pollen, gynoecium, and breeding systems (Welsh *et al.*, 2010; Wright *et al.,* 2011; Wright *et al.,* 2012). Twenty-seven species studied have been tentatively assigned to various clades of subg. *Grammica* based on their morphology because molecular data were not available for them. The parsimony reconstruction function from the Ancestral States package in Mesquite 2.75 (Maddison and Maddison, 2012) was used with a maximum of ten character states to reconstruct the evolution of infrastaminal scale characters. Assigning character polarities based on an outgroup, however, remains an issue as the position of *Cuscuta* within the Convolvulaceae is unresolved (Stefanović and Olmstead, 2004; Welsh *et al.,* 2010).

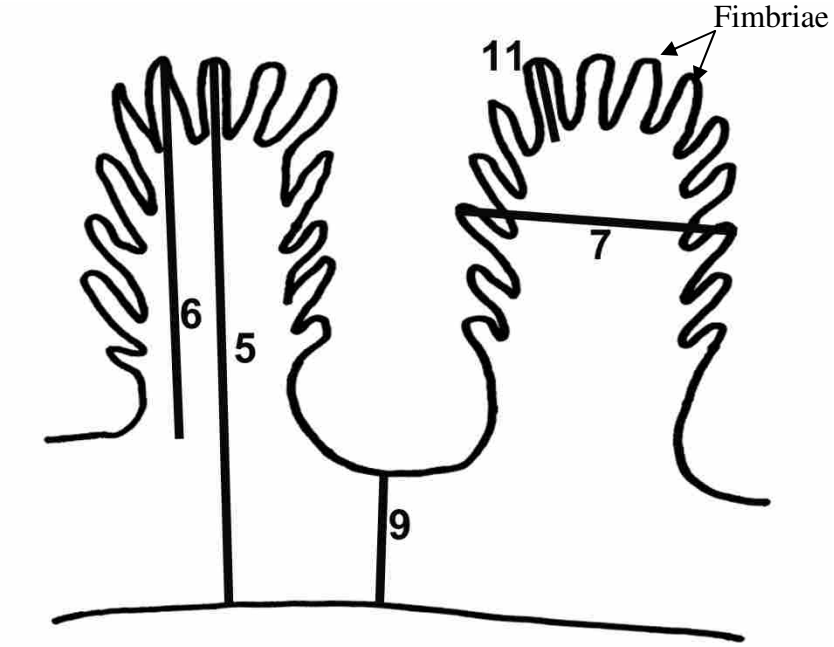

Figure 4.2 – Drawing illustrating the location of the IFS character measurements. 5 = total scale length (including bridge and fimbriae), 6 = scale body length (not including bridge), 7 = maximum scale width (including fimbriae), 9 = bridge length, 11 = fimbriae length. Character

measurement 6 was determined by measuring the highest point of the fimbriae to the lowest portion of the scale that did not include the bridge length (9) measurement.

4.5 Quantitative Analyses

Relationships within IFS characters and between IFS characters and other floral characters were analyzed. To test the hypothesis that scales are associated with sexual reproduction and pollination, the pollen/ovule ratios and number of stomata in the nectary reported by Wright *et al.* (2012) and Wright *et al*. (2011), respectively, were regressed against six IFS quantitative characters (IFS length, IFS width, fimbriae length, fimbriae width, number of fimbriae, and bridge surface). All floral characters reported by Wright *et al*. (2012) were also regressed with the same six IFS quantitative characters. Linear regression and Spearman's rank correlations were conducted using NCSS 2007 (Hintze, 2007). All continuous data were normalized using ln-transformations in order to reduce statistical problems and improve linearity (Niklas, 1994). In addition to the whole genus dataset, subgenus and clade partitions were analyzed as well. Results obtained among scale characters were very similar, and data are shown only for the IFS length, bridge length and surface.

A heirarchial cluster analysis of the IFS characters was performed, and the dendrograms resulted were compared with the current phylogeny used for *Cuscuta*. The statistics software JMP version 10 (SAS Institute Inc., Cary, NC, 1989-2007) was used, and all quantitative IFS characters were inputted to create a hierarchical cluster. With the same software, a principle component analysis (PCA) was also performed using the IFS characters to picture the structure of the data by using as few variables as possible. For all of the above analyses using JMP, the

species without scales (*C. brachycalyx, C. californica, C. occidentalis, C. jepsonii, C. hyalina, C. sandwichiana, C. grandiflora*) were not included in the dataset.

5. Results

5.1 Morphology and Micromorphology

Infrastaminal scales are delicate, translucent, dorsiventral structures that consist of a body that bears lateral and distal fimbriae (Figure 5.1). As Yuncker (1921) indicated, they mostly exhibit a membranous structure with finger-like projections (fimbriae) but, at their simplest, are reduced to a bifid structure (Figure 5.1j) or a few tiny lobes. IFS are the same in number as the stamens, petals, and calyx lobes and are positioned over the staminal vasculature on the ventral surface of the corolla-stamen tube, below the insertion point of the filaments. They are connected to one another at the base of the corolla tube by a "bridge" that varies in size, which may also bear fimbriae in some species (Figure 5.1k). In the flower's natural state the IFS cup the ovary, obscuring its view from the mouth of the corolla tube (Figure 5.2a). The fimbriae may extend beyond the mouth of the corolla tube in some species. When the ovary develops into a capsule, the IFS persist and enclose it. In one species, *C. alata* from subg. *Grammica*, an additional set of scales is present on the exterior of the corolla-stamen tube, opposite to the points of insertion of the filaments (Figure 5.2b). In *C. gigantea* from subg. *Monogynella*, fimbriae are also observed on the adaxial face of the scale body (Figure 5.2c).

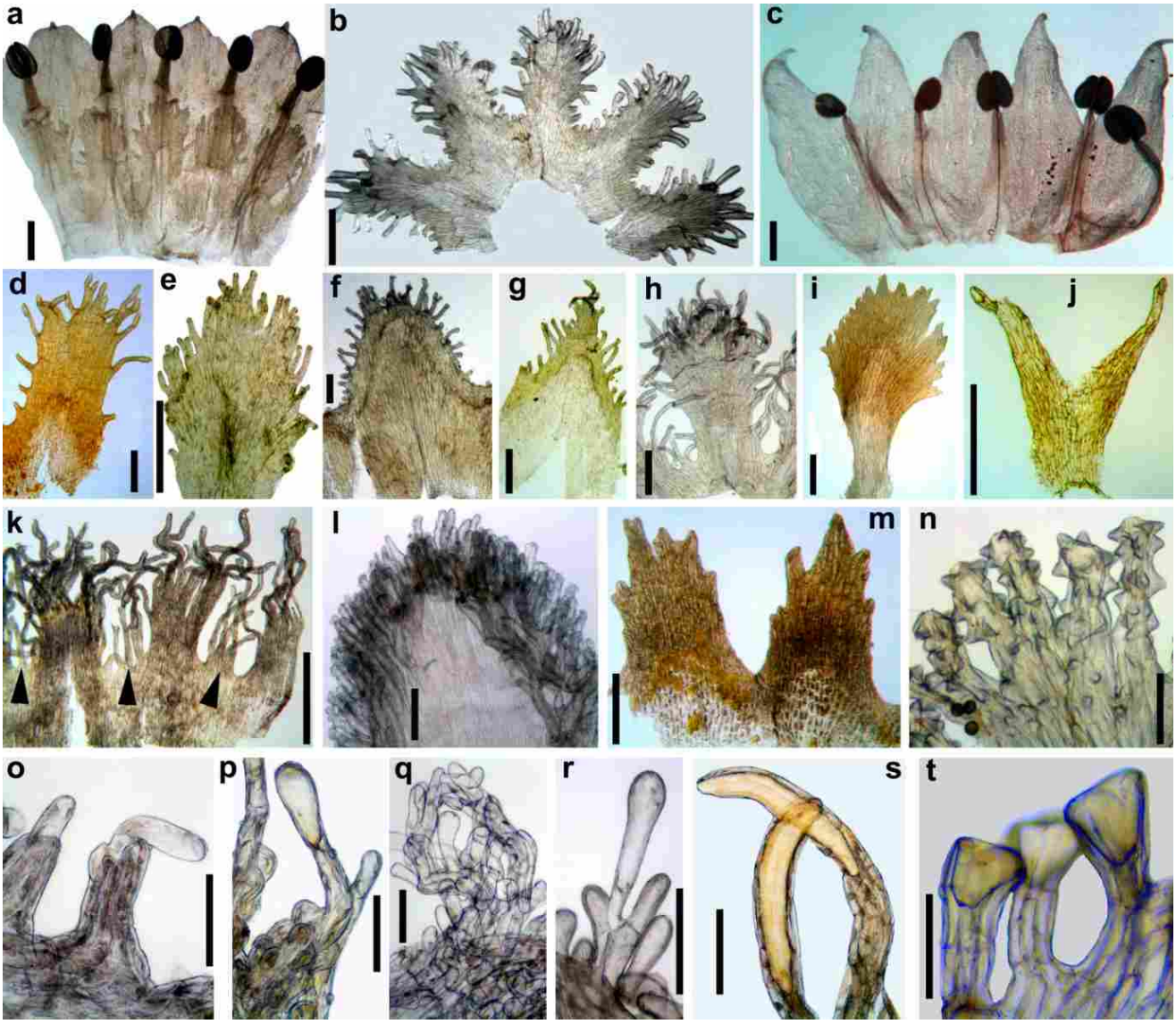

Figure 5.1 – Diversity of infrastaminal scales in *Cuscuta*. **a** Corolla dissected to reveal the IFS (*C. costaricensis*). **b** Detached scales (*C. corniculata*). **c** Absent scales (*C. occidentalis*). **d** Oblong scale (*C. woodsonii*). **e.** Elliptic scale (*C. gracillima*). **f** Ovate scales (*C. kilimanjari*). **g** Ovate-triangular scale (*C. tinctoria*). **h** Obovate scale (*C. glabrior*). **i** Spathulate scale (*C. lindsayi*). **j** Bifid scales (*C. polygonorum*). **k** Fimbriae present on the IFS bridge (arrows, *C. compacta*). **l** Scale with numerous fimbriae (*C. odorata*). **m** Scales with a few fimbriae (*C. dentatasquamata*). **(n-u)** Fimbriae morphology in *Cuscuta*. **n** Papillate (Clade O, *C. cockerellii*). **(o–r)** *Monogynella* type. **o** *C. lupuliformis*. **p** *C. lehmanniana*. **q** *C. reflexa*. **r** *C. japonica*. **(s,t)**

Cuscuta type. **s** *C. gronovii*. **t** *C. umbellata* var. *umbellata*. Scale bars (**a–m**) 0.5 mm, (**n–t**) 100 μm

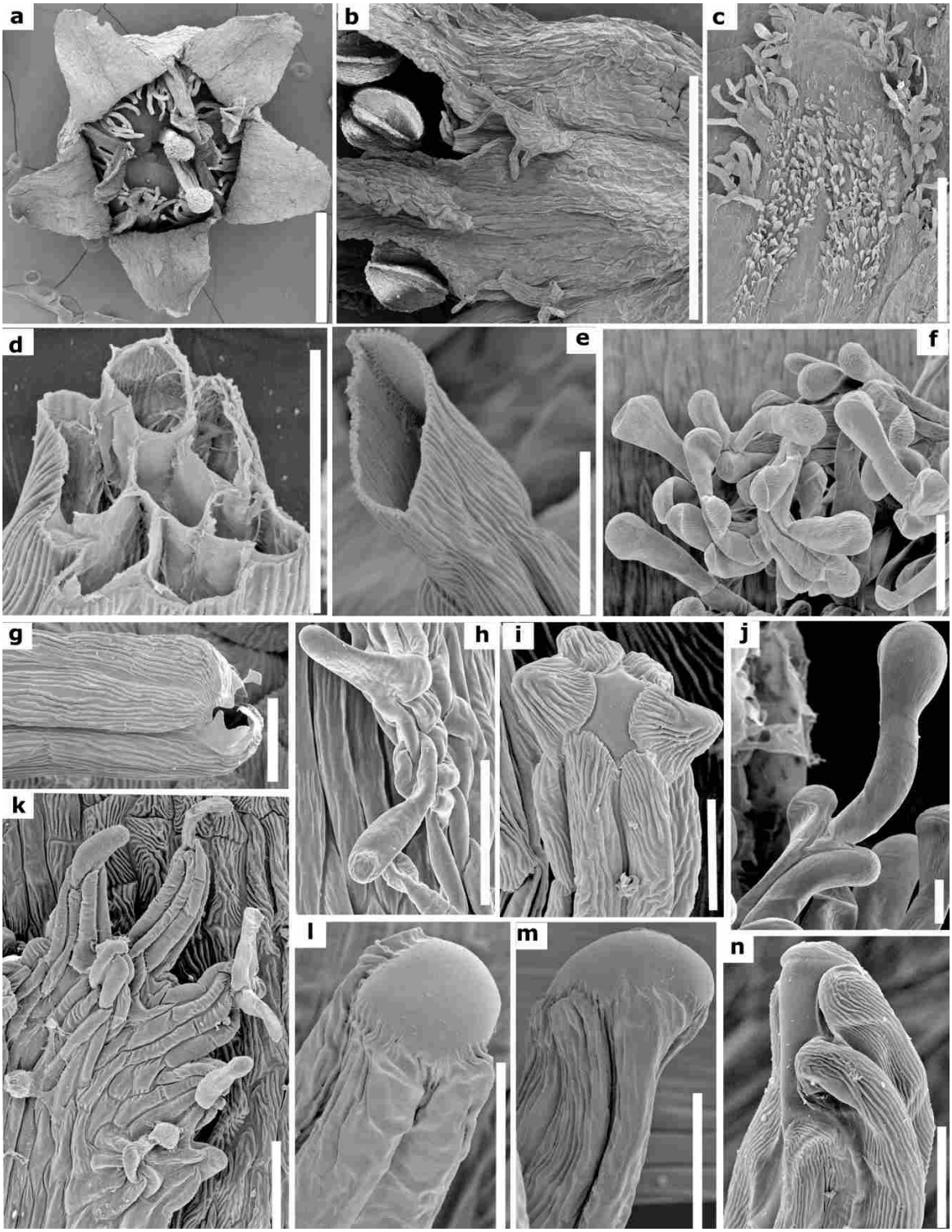

Figure 5.2 – Micromorphology of infrastaminal scales in *Cuscuta*. **a** Top view of flower showing IFS surrounding the ovary (*C. chinensis* var. *applanata*). **b** Scales at the exterior of corolla-stamen tube (*C. alata*) **c** Fimbriae on adaxial surface of the IFS body (*C. gigantea*). (**e,f,h,j,k)** *Monogynella*-type of fimbriae. (**d, g,i,l-n)** *Cuscuta*-type of fimbriae **d** Transversal section (*C. polygonorum*). **e** Transversal section (*C. japonica*). **f** *C. santapaui* fimbriae. **g** Ruptured laticifer (*C. glabrior).* **h** Fimbriae with a multicellular base and one distal secretory cell (*C. lehmanniana*). **i** Fimbria with papillae (*C. purpurata*). **j** *C. japonica* fimbria. **k** Fimbriae with a multicellular base and one distal secretory cell (*C. exaltata*). (**l–n**) Exposed laticifers at distal ends of fimbriae. **l** *C. chinensis* var. *applanata*. **m** *C. umbellata* var. *umbellata*. **n** *C. gronovii*. Ep epidermis of fimbriae, L laticifer cell. Scale bars (**a–c**) 1 mm, (**e–f, h**) 100 µm, (**d, g, i–n**) 30 µm

Most of the IFS characters surveyed are polymorphic in *Cuscuta* (Appendix B). There are several degrees of variation in regards to the IFS shape and size across the genus. The most common IFS shapes encountered are oblong (widest at the middle; margins parallel) and obovate (widest above; margins convex). Other shapes include ovate (widest at the base; margins convex), ovate-triangular, bifid (scales bifurcated distally, with 1–3 fimbriae on both sides of filament), spathulate (spoon-shaped; narrower at the base than at the apex) and elliptic (widest at the middle; margins convex). Elliptic scale shapes are rarely encountered. Species of *Monogynella* seem to possess ovate shapes rather than obovate. Shape of the IFS apex is either rounded, acute, or truncate, with rounded being the most common trend.

IFS length ranges from 1.08 to 2.87 mm, while width ranges from 0.6 to 1.0 mm. Species within Clade G, H, J, K, and O have the largest scale size (Figure 5.3; Appendix B). Species

within Clade O and Clade G have the widest IFS. Fimbriae length ranges from 0.08 to 0.55 mm and is the longest in *C. compacta* of Clade D, with an average length of 0.78 mm. Fimbriae width was constant within the genus, being between 0.03 to 0.06 mm, and has not been included in the character list. Species in 'Clade O' have the most numerous fimbriae, and can have up to 66 fimbriae, with some species from subg. *Monogynella* following close behind, with *C. japonica* having the most fimbriae (69). A common trend seen is longer fimbriae on the upper half of the scale than the lower half. IFS bridge is the longest in subg. *Monogynella*. Clade C and Clade H have the most species with the corolla tube-IFS ratio being over 1, i.e., the fimbriae of the scales extend past the corolla tube in length. The species with scales that have the lowest corolla tube-IFS ratio (0.4 or less) are *C. santapaui* (subg. *Monogynella*), *C. prismatica* (subg. *Grammica*, Clade J), *C. cockerellii* (subg. *Grammica*, Clade O), *C. flossdorfii* var. *pampagrandensis* (Clade O), and *C. paitana* (Clade O).

In subgenus *Grammica*, there are a number of species that have greatly reduced scales (e.g., Clade A: *C. pacifica, C. suksdorfii, C. howelliana, C. salina*) or lack scales entirely (e.g., Clade A: *C.californica, C. brachycalyx, C. jepsonii, C. occidentalis*; Clade J: *C. sandwichiana;* Clade L: *C. hyalina*; Clade O: *C. grandiflora*). Of the species with absent/reduced IFS, eight belong to Clade A.

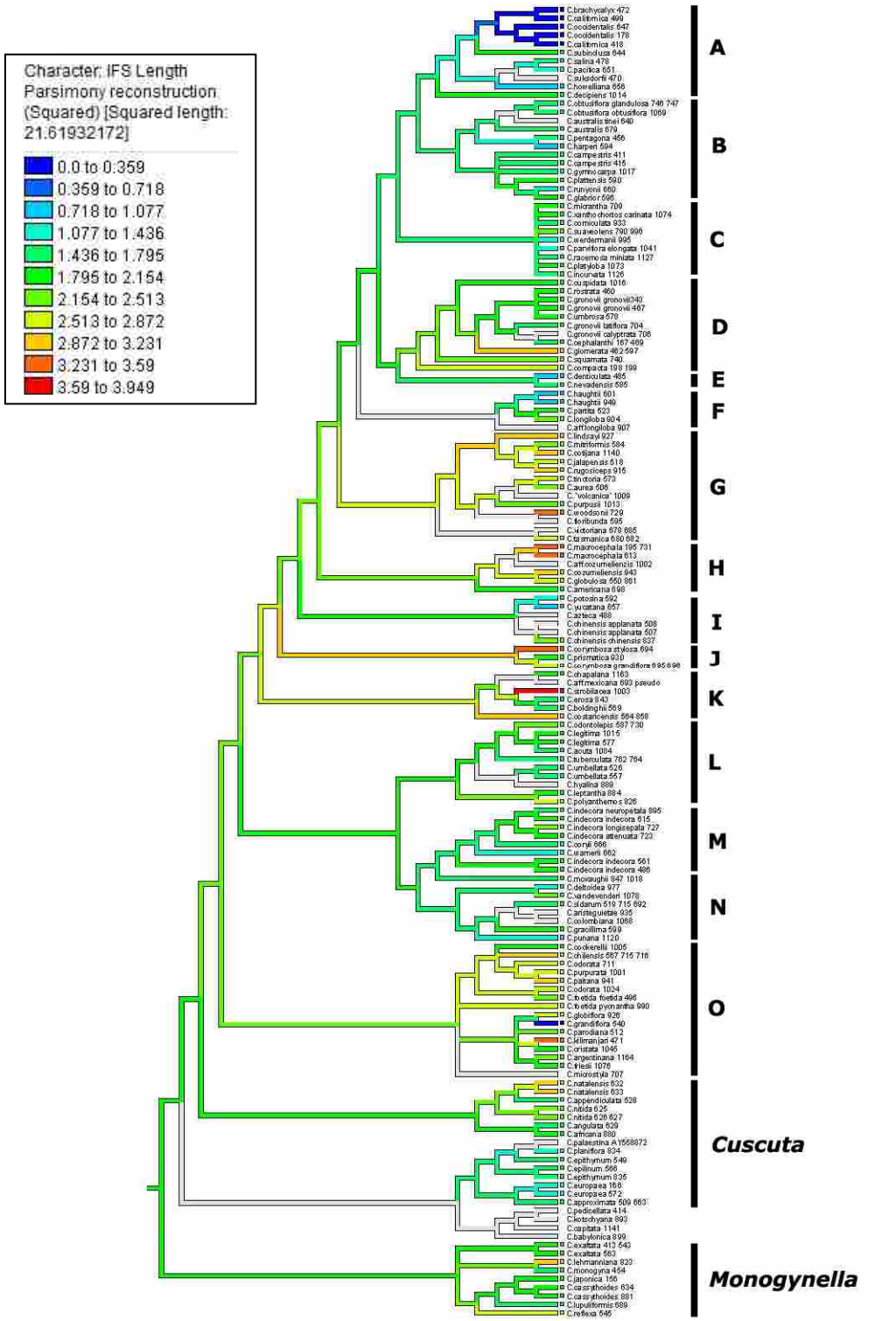

Figure 5.3 – Parsimony reconstruction of the evolution of infrastaminal scale length across the genus *Cuscuta*. Mesquite identified eleven ranges of IFS lengths (mm). Numbers

beside species names refer to the DNA accessions used by Stefanović *et al*. (2007) to produce this phylogeny. Branches with no color belong to species with no data collection. Clades were also inputted manually using the phylogeny used by Stefanovic *et al*. (2007). Clades G, H, J, K and O have the longest IFS lengths.

The fimbriae of the IFS fall into two morphostructural types:

1. *Monogynella type* (glandular trichome type; Figures 5.1 o–r; Figures 5.2e,f, h, j,k). Fimbriae resemble glandular trichomes with a multicellular, multiseriate base, and a distal secretory part that can be either unicellular (*C.* cassytoides, *C. exaltata*, *C. lehmanniana*, *C. monogyna*, and *C. lupuliformis*) or multicellular, uniseriate, and formed from 2–4 cells, and can be sometimes branched (*C. japonica*, *C. reflexa* and *C. santapaui*). This type of fimbriae characterizes subg. *Monogynella*.

2. *Cuscuta type* (laticiferous type; Figures 5.1d–n, s,t; Figures 5.2d,g,i,l–n). This type is exhibited by the majority of *Cuscuta* species. Fimbriae are more complex; they possess one laticifer cell, which is protected by an epidermal sheath, except at its distal end where it remains exposed (Figures 5.2i, l–n). While dissecting fresh or rehydrated flowers it was observed that the cell walls of the exposed portion of the laticifers broke or tore easily (Figure 5.2g) and the latex contained had burst out.

The fimbriae laticifers are most often single elongated cells, but in some species they are round to ovoid, or ellipsoid to broadly obovate. In most species, the epidermal cells of fimbriae have epicuticular waxes with longitudinally reticulated rodlets, while secretory cells or exposed parts of laticifer cells have smooth cell walls. In the South American ‘Clade O’ of subgenus *Grammica* (Stefanović *et al*., 2007), papillae are apparent on the fimbriae in 10 out of the 18

species surveyed. These papillae are bumps or protrusions of the epidermal cell that range from 20 – 40 micrometers in length, with radial ridges of cuticular wax (Figure 5.2i).

5.2 Structure and Ultrastructure

Longitudinal sections through the flower illustrate the position of the scales in between the corolla-stamen tube and the gynoecium (Figure 5.4a). Transversal sections through the basal part of the flower show that the IFS are fused to the corolla-stamen tube (Figure 5.4b). The vasculature of the corolla-stamen tube consists of 5 bundles, and as a result of sympetaly (fusion of petals) bundles are shared by the corolla, scales, and staminal filaments (Figure 5.4d). The free part of the scale body is not vascularized. When observing cross-sections advancing into the distal part of the flower, specific cells that make up the IFS, corolla, and filament become distinguishable (Figures 5.4c–f). The IFS separate first from the corolla, and finally, at about 40% of their length, from the staminal filament (Figures 5.4b–e).

The IFS body is three to six cells wide (Figure 5.4g), and usually does not contain laticifers. Fimbriae are either unicellular in subg. *Monogynella* (Figure 5.2e), or consist of a ring of four to six epidermal cells surrounding elongated central cells, the most distal developing into a laticifer (Figure 5.2d; Figures 5.4h,i). The laticifers appear as clear regions, while the epidermal cells contain excessively-stained amyloplasts. Laticifers are usually isolated and only rarely articulated, in groups or rows of 2 – 3 cells. The epidermal cells exhibit large epicuticular folds or ridges (Figures 5.5b,c). Other organelles were apparent such as mitochondria, vacualoes, and plasmodesmata. Plasmodesmata are apparent between epidermal cells of the fimbriae (Figure 5.5d), and between epidermal cells and laticifer cells of the fimbriae (Figures 5.5e,f).

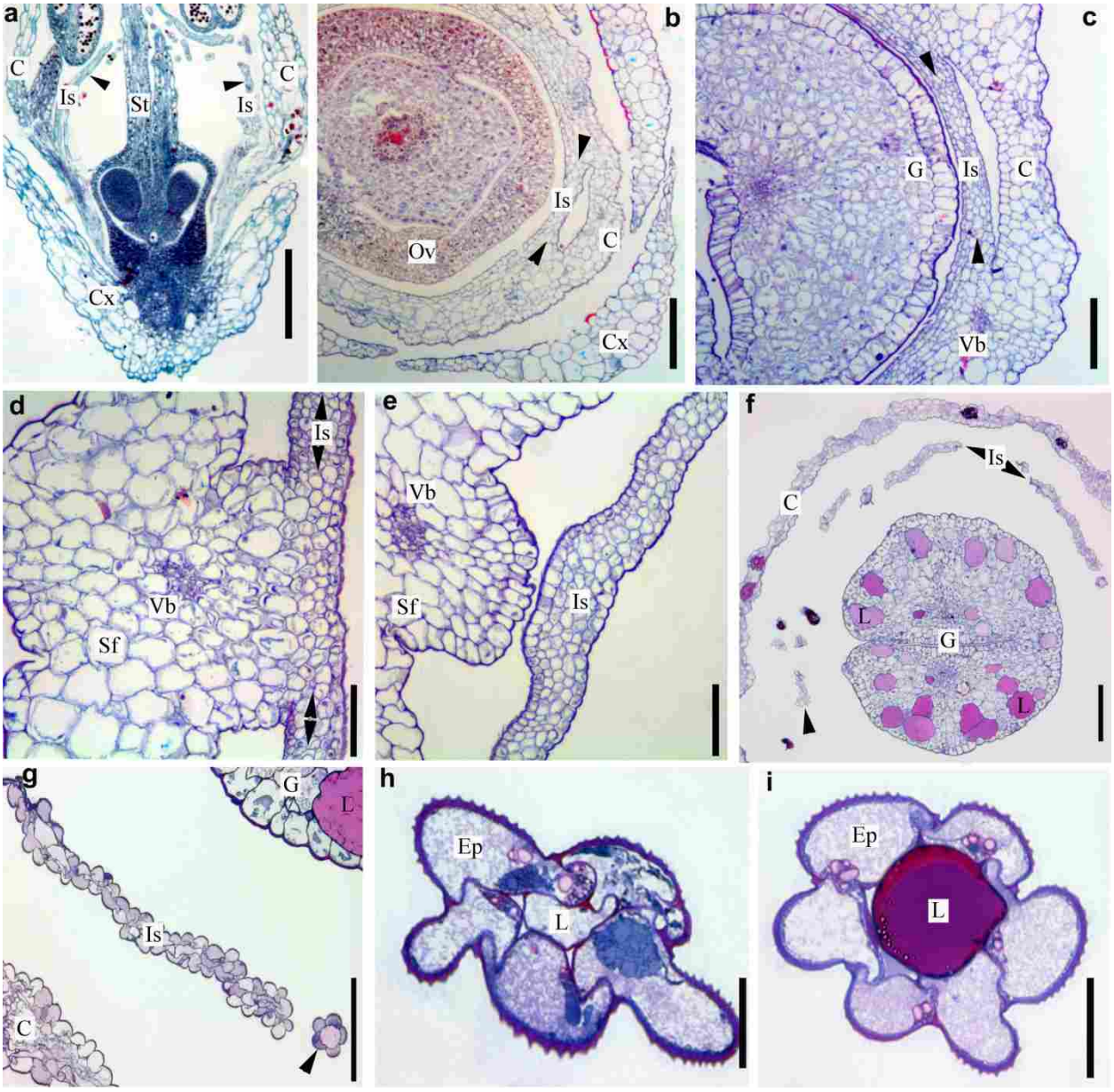

Figure 5.4 – Anatomy of flower and infrastaminal scales. **a** Longitudinal section of *C. gracillima* flower (IFS indicated with arrowheads). **(b–c)** Cross-sections through the base of the flower showing a gradual separation (indicated with arrowheads) of IFS from corolla (*C. gronovii*). **d** Vascular bundle is shared by the corolla-stamen tube and the IFS (*C. strobilacea*). **e** Scales become completely separated from the corolla-stamen tube (*C. strobilacea*). **f** Transversal section showing the position of the scales around the gynoecium. **g** Cross-section through the

body of a scale (one lateral fimbria sectioned transversally indicated with an arrowhead). **h–i** Structure of *Cuscuta* type of fimbriae: laticifer cell is surrounded by five or six epidermal cells. In **h**, the laticifer is not stained, meaning that the laticifer could have been punctured and that there is no fluid latex present. (*C. gronovii*). C corolla, Cx calyx, Ep epidermis of fimbriae, G gynoecium, Is infrastaminal scale, L laticifer, Ov ovary, Sf staminal filament, St style, Vb vascular bundle. Scale bars **a** 500 µm, (**b–c**) 200 µm, (**d–g**) 100 µm, (**h–i**) 20 µm

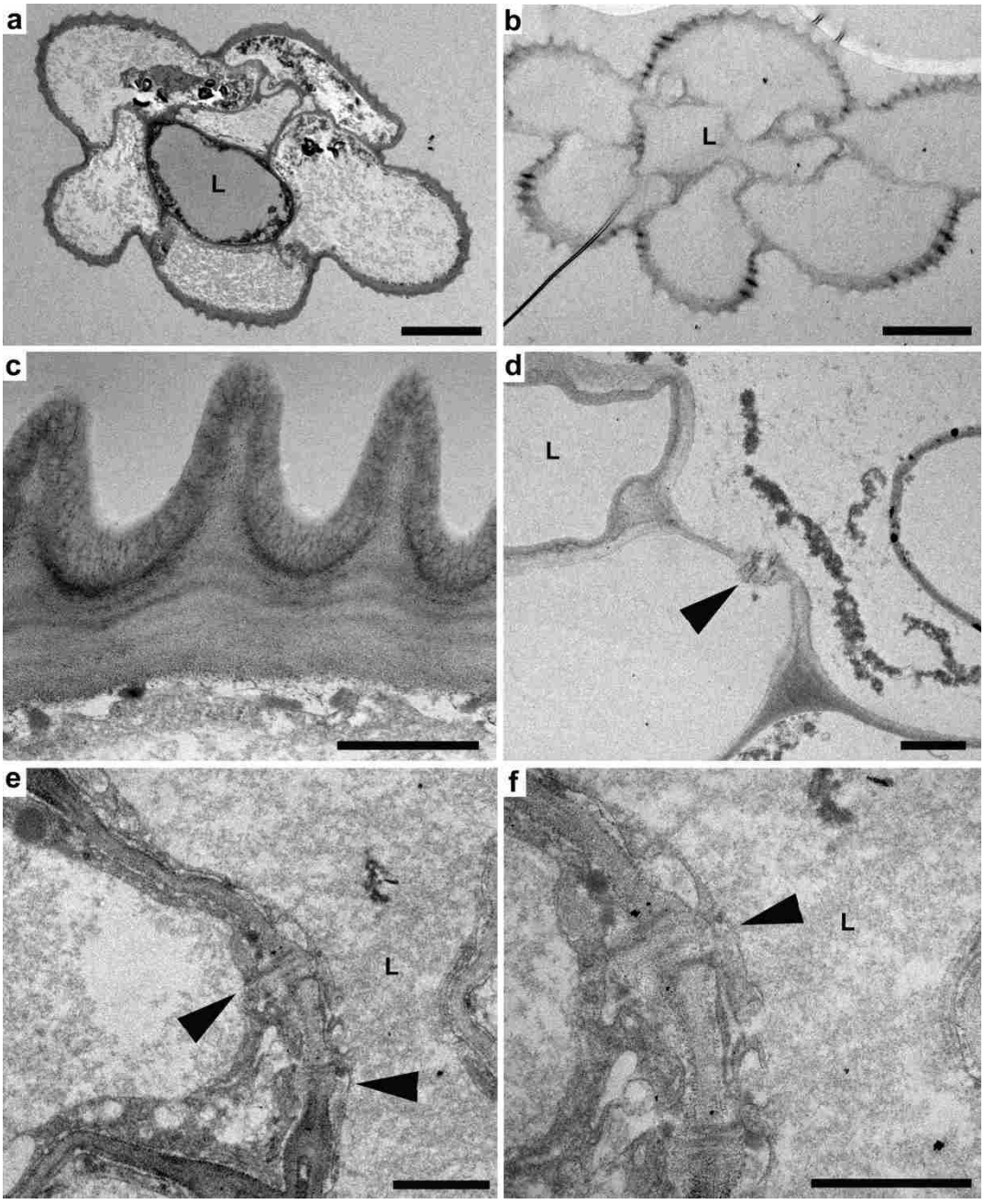

Figure 5.5 – Ultrastructure of IFS fimbriae in *Cuscuta gronovii* (transversal sections). (**a–b)** Six epidermal cells surrounding one laticifer cell. Dark-coloured amyloplasts are seen in some epidermal cells in **a**. **c** External wall of epidermal cells. **d** Plasmodesma (arrowhead) between two epidermal cells. (**e–f)** Plasmodesmata (arrowheads) between laticifer cell and epidermal cell. Ep epidermis of fimbriae, L laticifer cell. Scale bars (**a–b**) 10 µm, (**c–f**) 1 µm

5.3 Histochemistry

The IFS fimbriae and laticifers in the stems of the subg. *Cuscuta* and *Grammica* species and stems of the *Monogynella* species stained blue with Nile Blue, and remained unstained for all other stains used (Table 5.1; Figure 5.6). The fimbriae of the scales of the *Monogynella* species remained unstained with Nile blue, but stained red with Oil Red O. These fimbriae also stained red with Sudan IV. This indicates that in subg. *Cuscuta* and *Grammica*, the latex produced in the laticifers of IFS and stems may share a similar chemical composition. In contrast, in subg. *Monogynella*, the secretion of the IFS frimbriae is different from that of the stem laticifers. This is important because the previous observations on toxicity are based on extracts from the stems.

After the phloem staining protocol was conducted, the specimens (whole flowers) did not have areas that were stained. The controls (corollas with stamens) were not stained blue for where the phloem should be. This will be further discussed in the following discussion section.

5.4 Quantitative Analyses and Relationships

Moderate to strong correlations were found among all IFS characters (Table 5.2). IFS length and width in particular are strongly correlated because they had high r^2 values, as well as the fimbriae length, number of fimbriae, and bridge length and surface. These data show that the length of the scales is reflected in the proportional variation of the other IFS characters and, therefore, it is sufficient to use it for further statistical analyses. There are weak correlations between the IFS length and various floral traits (Table 5.3). For example, the IFS length and

Table 5.1 – Results for five stains used on two species organs. Herbarium and fresh material were used and yielded the same results. + = stained, - = unstained

Taxa	*Organ*	*Stain*				
		Oil Red O	Neutral Red	Nile Blue	Sudan IV	Sudan Black
Cuscuta subg. *Monogynella* (*C. japonica, C. reflexa*)	IFS	+	-	-	+	-
	Stem	-	-	+	-	-
Cuscuta subg. Cuscuta (*C. africana, C. appendiculata*, *C. approximata, C. epithymum, C. nitida*)	IFS	-	-	+	-	-
	Stem	-	-	+	-	-
Cuscuta subg. Grammica (*C. chilensis*, *C. costaricensis, C. cotijana*, *C. gracillima, C. gronovii* var. *gronovii*, *C. strobilacea*)	IFS	-	-	+	-	-
	Stem	-	-	+	-	-

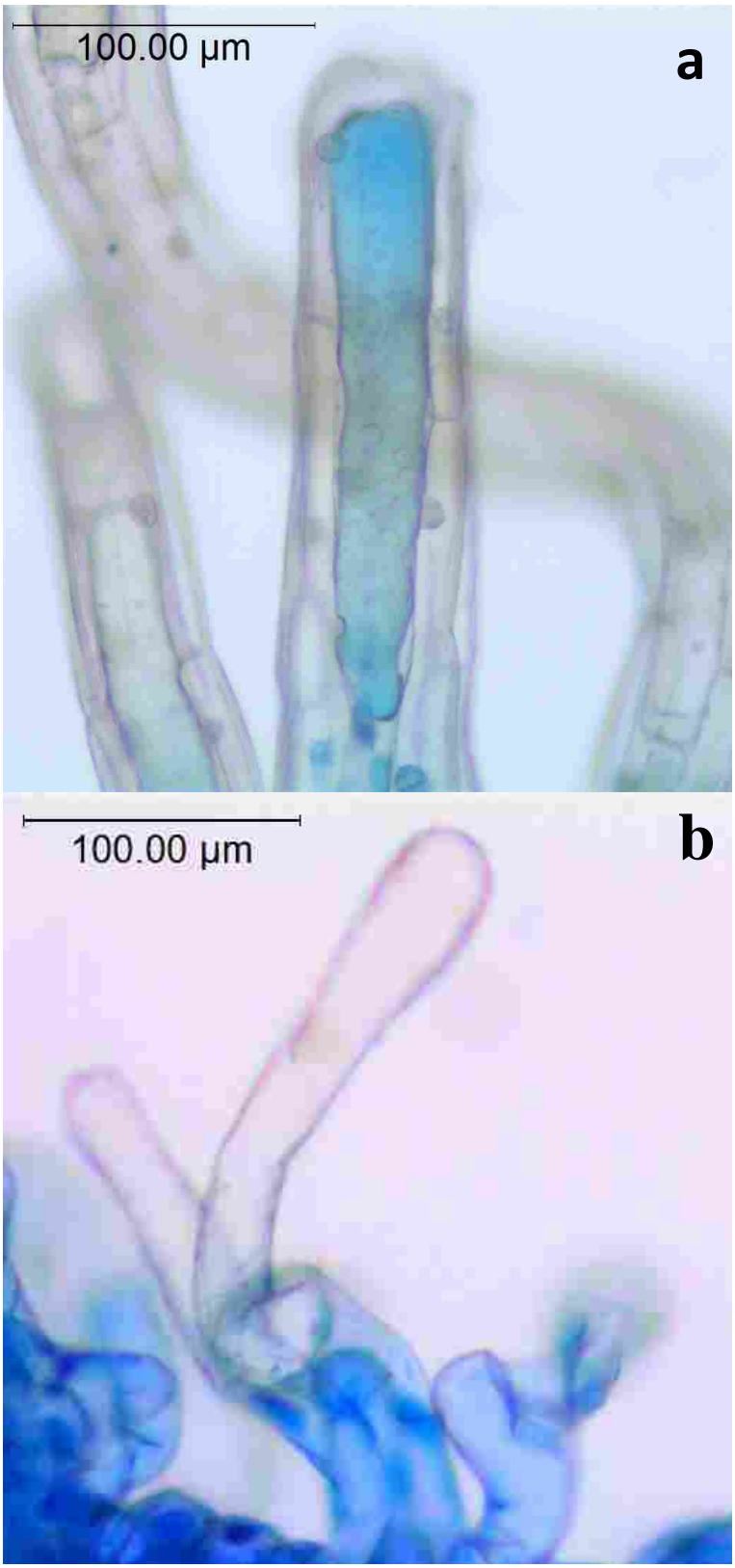
100.00 µm
a
100.00 µm
b

Figure 5.6– Nile Blue staining of *C. gronovii* of subg. *Grammica* (**a**) and *C. santapaui* of subg. *Monogynella* (**b**). **a** cell walls remained unstained and internal laticifer stained blue (acidic lipids). **b** cell wall and fimrbiae did not stain.

Table 5.2 – Regression and correlation coefficients between IFS length and other infrastaminal scale characters

IFS variable		*Regression & Correlation*				*Spearman's Rank*	
		slope	r^2	r	P	r_s	p
IFS length	IFS width	0.5557	0.6859	0.8281	<0.0001	0.7072	<0.0001
	Fimbriae length	0.1752	0.4356	0.66	<0.0001	0.589	<0.0001
	Fimbriae width	0.0423	0.3793	0.6158	<0.0001	0.4623	<0.0001
	Number of fimbriae	2.1044	0.5789	0.7608	<0.0001	0.5446	<0.0001
	Interscale bridge length	0.5115	0.5208	0.7216	<0.0001	0.6642	<0.0001
	Interscale bridge area	1.4621	0.6521	0.8075	<0.0001	0.7979	<0.0001

Table 5.3 – Regression and correlation coefficients for IFS length and various floral characters (floral data taken from Welsh *et al.,* 2010; Wright *et al.,* 2012).

Taxon	*IFS variable*	*Floral variable*	*Regression & Correlation*				*Spearman's Rank*	
			slope	r^2	r	P	r_s	p
Cuscuta (entire genus)	IFS length	Floral tube length	0.4876	0.274	0.5234	<0.0001	0.6594	<0.0001
		Average pollen volume	0.4735	0.0872	0.2952	0.0003	0.4121	<0.0001
		Floral tube mouth width	0.3086	0.1613	0.4016	<0.0001	0.4562	<0.0001
		Corolla flare	0.3038	0.0868	0.2946	0.0006	0.3775	<0.0001
Subgenus *Monogynella*	IFS length	Floral tube length	2.0441	0.6944	0.8333	0.0053	0.8152	0.0074
		Average pollen volume	0	0.0662	0.2572	0.5385	0.0361	0.9323
		Floral tube mouth width	0.6858	0.7036	0.8388	0.0047	0.8488	0.0038
		Corolla flare	0.2755	0.5253	0.7247	0.0272	0.7815	0.0129
Subgenus *Cuscuta*	IFS length	Floral tube length	0.5089	0.5564	0.7459	0.0132	0.8389	0.0024

		Average pollen volume	0	0.0253	0.159	0.6604	0.3091	0.3848
		Floral tube mouth width	0.6452	0.5671	0.7530	0.0119	0.4788	0.1615
		Corolla flare	0.3832	0.7111	0.8432	0.0022	0.7455	0.0133
Subgenus *Grammica*	IFS length	Floral tube length	0.4615	0.259	0.5089	<0.0001	0.6533	<0.0001
		Average pollen volume	0.2144	0.088	0.2966	0.0007	0.442	<0.0001
		Floral tube mouth width	0.5009	0.1396	0.3736	<0.0001	0.4159	<0.0001
		Corolla flare	0.2544	0.0623	0.2495	0.0071	0.3127	0.0007

corolla tube length correlation was the strongest of all floral characters with $r^2 = 0.274$. The analysis of subgeneric partitions revealed, however, that the same relationship was much stronger in subg. *Monogynella* ($r^2 = 0.694$) than in the subgenera *Cuscuta* and *Grammica* ($r^2 = 0.556$ and $r^2 = 0.259$, respectively; Table 5.3).

Pollen-ovule ratios have a weak correlation with the IFS length at the scale of the entire genus ($r^2 = 0.148$; Table 5.4). On the contrary, when species are partitioned into their subgenera/clades, it is seen that there is a strong correlation between the pollen-ovule ratio and IFS length in subg. *Monogynella* ($r^2 = 0.633$; Table 5.4), but not in the subgenera *Cuscuta* and *Grammica* ($r^2 = 0.265$ and $r^2 = 0.148$, respectively). There is also a strong correlation between the IFS bridge length and the nectary stomata count in subg. *Monogynella* ($r^2 = 0.718$; Table 5.4), which, again, is not the case in subgenera *Cuscuta* and *Grammica* ($r^2 = 0.156$ and $r^2 = 0.134$, respectively).

Table 5.4 – Regression and correlation coefficients between pollen/ovule ratios (PO), the number of stomata in the nectary (NS) and several IFS characters. Pollen/ovule data production (PO) and number of stomata (NS) were taken from Wright *et al*. (2012)

			Regression & Correlation				*Spearman's Rank*	
Taxon	*Floral variables*		slope	r^2	r	P	r_s	p
Cuscuta (entire genus)	PO	IFS length	0.9598	0.1489	0.3858	<0.0001	0.5452	<0.0001
		Bridge length	0.9115	0.0606	0.2461	0.004	0.2753	0.0012
		IFS & bridge surface	0.5703	0.1739	0.417	<0.0001	0.4615	<0.0001
	NS	IFS length	0.7129	0.1589	0.3986	<0.0001	0.4573	<0.0001
		Bridge length	0.966	0.1449	0.3806	<0.0001	0.3714	<0.0001
		IFS & bridge surface	0.4534	0.2336	0.4833	<0.0001	0.4741	<0.0001
Subgenus *Monogynella*	PO	IFS length	4.2808	0.6339	0.7961	0.0102	0.8236	0.0064
		Bridge length	1.8329	0.1432	0.3784	0.2809	0.4255	0.2202
		IFS & bridge surface	2.1637	0.5634	0.7505	0.0198	0.85	0.0037
	NS	IFS length	0.0398	0.4484	0.6696	0.0342	0.7333	0.0158
		Bridge length	7.9385	0.7181	0.8474	0.0039	0.636	0.0656
		IFS & bridge surface	6.3561	0.3476	0.5895	0.0947	0.6167	0.0769
Subgenus *Cuscuta*	PO	IFS length	3.6903	0.2651	0.5148	0.1278	0.6364	0.0479
		Bridge length	2.8903	0.0636	0.2521	0.4821	0.4255	0.2202
		IFS & bridge surface	0.3489	0.0095	0.0974	0.7891	0.2727	0.4458
	NS	IFS length	1.4363	0.4616	0.6794	0.0307	0.7333	0.0158
		Bridge length	22.0167	0.1563	0.3953	0.2582	0.5471	0.1017
		IFS & bridge surface	2.6964	0.1775	0.4213	0.2253	0.6606	0.0376
Subgenus *Grammica*	PO	IFS length	0.827	0.1484	0.3852	<0.0001	0.5114	<0.0001
		Bridge length	0.8005	0.0596	0.2441	0.008	0.2693	0.0033
		IFS & bridge surface	0.5425	0.199	0.4460	<0.0001	0.4599	<0.0001
	NS	IFS length	0.2284	0.1549	0.3935	<0.0001	0.441	<0.0001
		Bridge length	20.5668	0.1345	0.3667	0.0005	0.3567	0.0001
		IFS & bridge surface	0.4437	0.2324	0.482	<0.0001	0.4711	<0.0001
Clade A	PO	IFS length	0.0964	0.0046	0.0678	0.8624	-0.007	0.8412
		Bridge length	0.3601	0.0081	0.09	0.8047	0.0063	0.9863
		IFS & bridge surface	0.2792	0.0284	0.1689	0.6419	-0.1534	0.6723

	NS	IFS length	0.4934	0.1741	0.4172	0.4105	0.3947	0.4387
		Bridge length	1.3689	0.2073	0.4553	0.3047	0.3706	0.4131
		IFS & bridge surface	0.6778	0.3339	0.5778	0.1742	0.3706	0.4131

The dendrogram produced from the hierarchical cluster platform report is shown in Figure 5.7. Four different phenotypic clusters have been produced. The PCA analysis revealed the linearily uncorrelated variables variables that best summarize the dataset. The resulting analysis showed that Principle Components One (PC_1), Two (PC_2), and Three (PC_3) jointly explain 80.87% of the total information (57.99%, 13.83%, and 9.05%, respectively; Table 5.5). The factor loadings of each component are summarized in Table 5.6. The loading matrix for PC_1 displayed positive values for all characters. PC_2 displayed positive values for number of fimbriae, IFS width, IFS length, bridge length, bridge surface, and scale surface, and negative values for the remaining five characters (fimbriae length, fimbriae width, fimbriae length on lower half of scale, fimbriae length on upper half of scale, and IFS length not including the bridge). PC_3 displayed positive values for fimbriae length, fimbriae length on lower half of scale, fimbriae length on upper half of scale, number of fimbriae, bridge length, and bridge surface, and negative for the remaining five characters (fimbriae width, IFS width, IFS length, IFS length not including the bridge, and the scale surface). The scatterplot (Figure 5.8) is a summary of the first two principle component values generated for each species. One can see the spatial arrangement of the species in regards to these principle components. *C. compacta* appears as the distinct outlier within the scatterplot.

Table 5.5 - Eigenvalues from PCA analysis of *Cuscuta* IFS characters and resulting cumulative percent of information summarized

Number	*Eigenvalue*	*Percent*	*Cumulative Percent*
1	6.3789	57.990	57.990
2	1.5218	13.835	71.825
3	0.9951	9.046	80.871

Table 5.6 - Loading matrix from PCA analysis of *Cuscuta* IFS characters showing principle component loadings for first three components.

Character	PC_1	PC_2	PC_3
Fimbriae length	0.78823	-0.52924	0.25660
Fimbriae width	0.62025	-0.30527	-0.48560
Fimbriae length on lower half of scale	0.73568	-0.37474	0.30593
Fimbriae length on upper half of scale	0.78872	-0.52443	0.24159
Number of fimbriae	0.56211	0.49272	0.09620
IFS width	0.87466	0.05460	-0.11231
IFS length	0.926558	0.20679	-0.09080
IFS length not including bridge	0.84275	-0.00317	-0.39437
Bridge length	0.63378	0.44096	0.41824
Bridge surface	0.72726	0.44738	0.22630
Scale surface	0.79327	0.22355	-0.36012

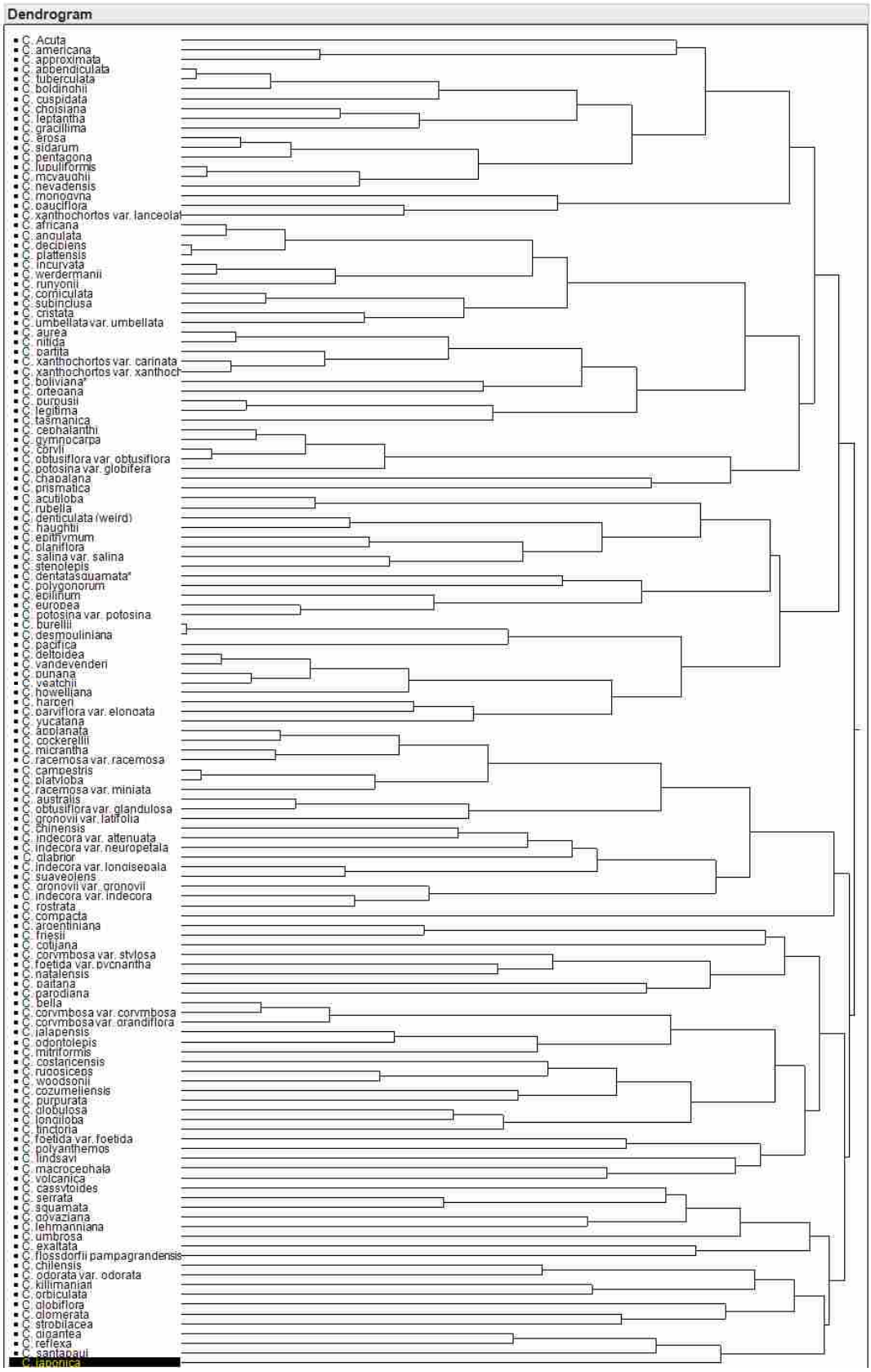
Dendrogram
C. Acuta
C. americana
C. approximata
C. appendiculata
C. tuberculata
C. boldinghii
C. cuspidata
C. choisiana
C. leptantha
C. gracillima
C. erosa
C. sidarum
C. pentagona
C. lupuliformis
C. mcvaughii
C. nevadensis
C. monogyna
C. pauciflora
C. xanthochortos var. lanceolat
C. africana
C. angulata
C. decipiens
C. plattensis
C. incurvata
C. werdermanii
C. runyonii
C. corniculata
C. subinclusa
C. cristata
C. umbellata var. umbellata
C. aurea
C. nitida
C. partita
C. xanthochortos var. carinata
C. xanthochortos var. xanthocr
C. boliviana*
C. orteana
C. purpusii
C. legitima
C. tasmanica
C. cephalanthi
C. gymnocarpa
C. corvii
C. obtusiflora var. obtusiflora
C. potosina var. globifera
C. chapalana
C. prismatica
C. acutiloba
C. rubella
C. denticulata (weird)
C. haughtii
C. epithymum
C. planiflora
C. salina var. salina
C. stenolepis
C. dentatasquamata*
C. polygonorum
C. epilinum
C. europea
C. potosina var. potosina
C. burelli
C. desmouliniana
C. pacifica
C. deltoidea
C. vandevenderi
C. punana
C. veatchii
C. howelliana
C. harperi
C. parviflora var. elongata
C. yucatana
C. applanata
C. cockerellii
C. micrantha
C. racemosa var. racemosa
C. campestris
C. platyloba
C. racemosa var. miniata
C. australis
C. obtusiflora var. glandulosa
C. gronovii var. latifolia
C. chinensis
C. indecora var. attenuata
C. indecora var. neuropetala
C. glabrior
C. indecora var. longisepala
C. suaveolens
C. gronovii var. gronovii
C. indecora var. indecora
C. rostrata
C. compacta
C. argentiniana
C. friesii
C. cotijana
C. corymbosa var. stylosa
C. foetida var. pycnantha
C. natalensis
C. paitana
C. parodiana
C. bella
C. corymbosa var. corymbosa
C. corymbosa var. grandiflora
C. jalapensis
C. odontolepis
C. mitriformis
C. costaricensis
C. rugosiceps
C. woodsonii
C. cozumelensis
C. purpurata
C. globulosa
C. longiloba
C. tinctoria
C. foetida var. foetida
C. polyanthemos
C. lindsayi
C. macrocephala
C. volcanica
C. cassytoides
C. serrata
C. squamata
C. govaziana
C. lehmanniana
C. umbrosa
C. exaltata
C. flossdorfii pampagrandensis
C. chilensis
C. odorata var. odorata
C. killimaniari
C. orbiculata
C. globiflora
C. glomerata
C. strobilacea
C. giganlea
C. reflexa
C. santapaui
C. japonica

Fig 5.7 – Dendogram produced using JMP with IFS quantitative characters using heirarchial clustering. Four distinct clusters have been formed according to phenotypic similarities.

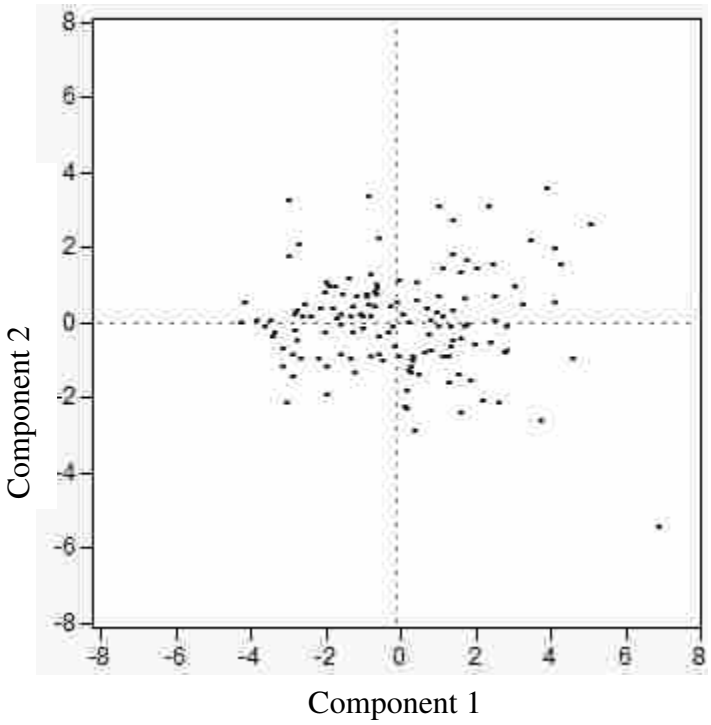

Fig 5.8 – Summary scatterplot from PCA analysis of *Cuscuta* IFS characters. This is a plot of the first two principle components for each species. The outlier to the right is *C. compacta*.

6. Discussion

6.1 Morphology and Micromorphology

This documentation of variation of the IFS is in agreement with Yuncker's (1921) descriptions, but with a great amount of added detail. Dodders exhibit various degrees of variation in regards to their IFS size, shape, and number of fimbriae, however, in general, these variation ranges characterize *Cuscuta* species or varieties. Examples of newly observed qualitative features include the presence of scales at the exterior of the corolla-stamen tube in *C. alata*, the papillate fimbriae of species in clade O, and the fimbriae present on the body of the scale in *C. gigantea*.

The most common scale shapes, oblong and obovate, are widest at the middle, and widest above, respectively. Having more surface area at the upper portion of the scale seems to have been important in this evolutionary trend, except for *Monogynella* species. All of these species except for *C. exaltata* possess oblong- and ovate-shaped scales, which means they tend to have more surface area on the lower half of the scale. This overall shape variation is consistent with the role proposed for the IFS: nectar holding in subg. *Monogynella*, and the protection of the ovary in subg. *Cuscuta* and *Grammica*.

The common round apex of the scales maximizes the surface area of the IFS as well, especially since longer fimbriae are found in general on the upper half of the scales.
The *Monogynella type* fimbriae resemble uniseriate glandular trichomes and are found in subg. *Monogynella*. The second morphostructural fimbriae type, *Cuscuta type*, is more complex with the occurrence of laticifers and is found in the subgenera *Cuscuta* and *Grammica*. The *Monogynella type* fimbriae are believed to be ancestral since it characterizes subg. *Monogynella*,

the first diverged lineage of *Cuscuta*, and because of the increasing complexity of the fimbriae in the derived subgenera *Cuscuta* and *Grammica*.

The presence of laticifers within a plant is ecologically significant; their role is believed to deter herbivory by insects or mammals (Pickard, 2008; Agrawal and Konno, 2009; Ramos *et al.,* 2010). Many *Cuscuta* specimens bore fimbrial laticifers that seemed to have burst at their distal ends, with the presence of a split or tear of the laticifer cell wall (Fig. 5.2g). This may have resulted from the preparation and handling of the IFS but this occurrence suggests that the slightest mechanical contact with the exposed portion of the laticifer cell causes it to burst open and release latex. The easily-breaking fimbriae may influence ovary defense against insect herbivory, for *Cuscuta* is known to be attacked only by few insects (Costea and Tardif, 2006). The low number of insect invaders is perhaps a result of the chemical defense provided by the latex secreted in laticifers found in all the organs of the plant.

The papillae found on the fimbriae of species of Clade O do not occur in any other species of *Cuscuta* and are therefore an apomorphy for this group. Similar epidermal papillae can be seen on the pedicels, perianth, and gynoecium in a number of *Cuscuta* species (e.g. *C. glabrior*, *C. jepsonii, C. pacifica* var. *papillata*; Costea *et al.*, 2006a; Costea and Stefanović, 2009a; Costea *et al,.* 2009; Wright *et al.,* 2011), but papillae are not known to co-occur between these structures and the IFS.

The dendrogram that was produced based on quantitative IFS characters was very different from the phylogeny of *Cuscuta* used in current studies (see phylogeny in Figure 5.3 as an example). By visual observation of the dendrogram, many trends relating to the scale morphology can be drawn. Four distinct clusters were formed. *Cuscuta compacta* was separated on its own because of its unusually long fimbriae. This species is, however, sister to the rest of

the species in its clade in the phylogeny [Clade D; Stefanović *et al.,* 2007]. *Cuscuta exaltata* and *C. flossdorfii var. pampagrandensis* were grouped together based on their similar yet uncommon scale morphology; they have very few, short fimbriae, and large bridge surfaces. Also, *C. gigantea*, *C. reflexa*, *C. santapaui*, and *C. japonica*, all of subg. *Monogynella*, were grouped together because their similar scale morphology. None of the three subgenera, however, were all clustered in one group. The comparison of the phylogeny and the dendrogram illustrates the extent of convergent evolution affecting the morphology of the IFS.

The PCA analysis yielded similar results. The first three principle components have captured 80.87% of the total IFS information. Since all values of the component loadings in PC_1 are positive, PC_1 is considered as a measure of overall size of the IFS. This means that a species with a large PC_1 value would have larger IFS. PC_2 is a contrast between variables associated with fimbriae and variables associated with the rest of the scale. If a species has a large PC_2 value, it would have a large scale body, size, and fimbriae number, and small fimbriae length and width. PC_3 is a contrast between variables associated with width and the scale surface and between variables associated with length, the number of fimbriae and bridge surface. If a species has a large PC_3 value, it would have a large bridge size and long fimbriae length and a short fimbriae and IFS width, and smaller scale surface. This information suggests that there are functional aspects of morphology and tradeoffs being made between the size of the characters. If an IFS has few fimbriae and a smaller length and width, then it will have longer and wider fimbriae to make up for it. If an IFS has a small scale surface, it will have a large bridge surface.

The 3-D scatterplot shows a summary of the principle components of each species. Again, *C.compacta* and the seven species lacking scales were separated from the rest of the genus. These analyses confirm that the IFS characters themselves can be useful taxonomically

within species clades, but have little use in the infrageneric-level systematics because of the extensive convergent evolution observed.

Morphometric analysis of flower characteristics has been shown to reflect evolutionary relationships only when it is used in small groups of closely-related and difficult to separate species. This was done in the study of the *C. salina-pacifica-suksdorfii* complex (Clade A) from western North America (Costea *et al.,* 2009).

6.2 Structure and Ultrastructure

Laticifers in the Convolvulaceae are articulated and non-anastomosing (Dussourd and Denno, 1991; Evert, 2006), and this is true for those found in the stems of *Cuscuta* (Lyshede, 1985). In contrast, the laticifers found in the scales, corolla, and calyx of *Cuscuta* appear often as individual cells, small groups of cells, or as elongated tubes. In the latter case, this may be due to the perforation or resorption of the laticifer cell walls, a process known to occur in other plant species with 'single-cell' laticifers with a tube-like form (Evert, 2006). Articulated laticifers are known to have frequent associations with phloem (Evert, 2006). Again, while this association can be seen in the calyx and corolla of *Cuscuta* (Wright, pers. obs.), no phloem (or xylem) are present in the IFS themselves.

Through TEM observation of the fimbriae, fine details were brought to light. The waxy and highly-folded cell wall of the epidermal cell could have a variety of functions. The petals of some angiosperms also possess cuticular folds, which are hypothesized to make the surface slippery for specific insects by reducing insect adhesion (Prüm *et al.,* 2012). Having a thick waxy layer on the cell wall could also aid in increased moisture retention.

Plasmodesmata were seen between epidermal cells. Though it is impossible to tell by observation of the channel itself, these are most likely primary plasmodesmata since they are

between cells of the same type (Ehlers and Kollmann, 2001). These plasmodesmata can only form during cytokinesis. The plasmodesmata seen between laticifer and epidermal cells are secondary plasmodesmata since they are between different types of cells, i.e. could not have formed during cytokenesis. Secondary plasmodesmata can form into existing cell walls and therefore can be seen between cells of the same type. Condon and Fineran (1989b) observed that the plasmodesmata of laticifer cells in Convolvulaceae are occluded at maturity, suggesting that contents of the laticifer are sealed away. The specimens used in this study were collected at maturity, and plasmodesmata were seen, suggesting that perhaps the laticifers were not fully mature after all, that laticifer contents do not necessarily become sealed off at maturity, or that the plasmodesmata encountered were, in fact, occluded. In future studies, callose staining can be performed to detect plasmodesmata occlusion (Koh *et al.,* 2012). Future studies on the plasmodesmata formation and mechanisms of this plant could lead to insight of transport of secondary latex metabolites in and out of the laticifer cell.

6.3 Phloem Staining

Though I believe there is no phloem present in the IFS because there is no vasculature in the IFS, I still wanted to be able to prove it with phloem stainin. The phloem staining performed did not work effectively. Though the staining gave a negative result (the scales were unstained), this does not prove that there is no phloem present because the positive controls (corolla tube, calyx) were not stained either. There is more than one reason as to why this protocol may not have worked properly for me.

Firstly, it could have been an error in the clearing method I used. The protocol employed by Aloni and Sachs (1973) states phloem staining in cleared material. The authors did not specify how they cleared their plant material, so something that I have done in my clearing

procedure adapted from Wright *et al*. (2011) could have affected my final staining results. It is possible to clear a specimen excessively, which in turn results in poor staining of tissues (Bybd, Jr. *et al.,* 1983).

Secondly, it could have been a problem with the staining procedure itself. What could be done is trying the staining protocol with *Coleus blumei*, the species Aloni and Sachs (1973) used in their study. If this does not stain the phloem, then it is a problem with the way the lacmoid stain was made or used. The next step would be to try using an alternative staining method, such as using aniline blue which stains for callose (Currier, 1957). Callose is a plant polysaccharide that is commonly deposited in sieve elements in phloem when a plant is under stress (Luna *et al.*, 2011). Because the specimens I have been using are not living, it is suspected that there is callose deposition in their phloem from stress resulting from the removing from habitat and drying to create a herbarium specimen.

6.4 Reduction and Absence of Scales in *Cuscuta*

Partially reduced IFS have evolved multiple times in *Cuscuta* (Yuncker, 1932), however absent scales are apparent only in subg. *Grammica* and are associated with seven speciation events (*C. brachycalyx, C. occidentalis, C. californica, C. jepsonii, C. hyalia, C. grandiflora, C. sandwichiana*) in four different clades (Figure 6.1a). Clade A is the only case in *Cuscuta* in which a reduction trend is apparent at the level of the entire clade (Figure 6.1b). Species in this clade are distributed in Western North America ranging from British Columbia to California (Costea and Stefanović, 2009b). *Cuscuta draconella* and *C. decipiens*, which form a sister group to all the species of the clade, have well-developed scales. The next diverging pair of sister clades include on the one hand species with various degrees of partial IFS reductions (*C.*

howelliana, *C. salina*, *C. pacifica*, and *C. suksdorfii*) and on the other, totally reduced, absent scales (*C. californica*, *C. brachycalyx, C. jepsonii*, and *C. occidentalis*; Figure 6.2b)

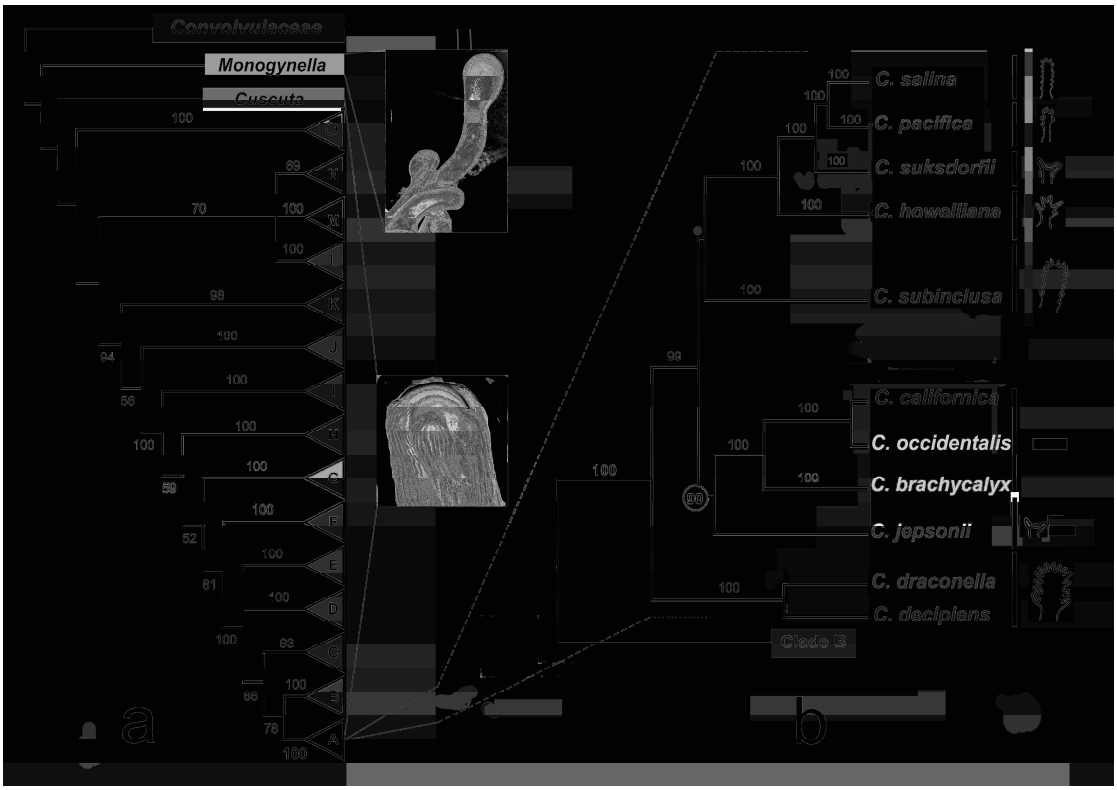

Figure 6.1 - Trends in the evolution of infrastaminal scales in *Cuscuta*. **a** Scales with glandular trichome fimbriae of subg. *Monogynella* are primitive, while scales with laticifers of subgenera *Cuscuta* and *Grammica* are derived. This is a simplified consensus phylogeny of *Cuscuta* based on three molecular phylogenies using nuclear ITS and plastid *trnL-F* sequences (García and Martin, 2007; Stefanović *et al.,* 2007; Stefanović and Costea, personal communication). Fifteen major *Grammica* clades are labeled A–O and their bootstrap support is indicated. Four *Grammica* clades (indicated in white) include speciation events associated with the total reduction of scales. **b** Majority-rule consensus phylogeny of clade A (Costea and Stefanović,

2009b) showing the reduction trend of infrastaminal scales. Horizontal bars indicate the total absence of scales

It appears that outcrossing or selfing can occur independently of the presence/absence of IFS. For example, a visual inspection of the *Cuscuta* breeding system categories reported by Wright *et al.* (2012) shows that the majority of species that lack scales are facultatively xenogamous (only *C. jepsonii* was categorized as facultatively autogamous). On the contrary, species with mating systems that incline towards autogamy possess "normal" scales (e.g., *C. americana, C. approximata, C. australis, C. campestris, C. europaea, C. harperi, C. obtusiflora*; see Wright *et al*., 2012). There are also two varieties of *C. sandwichiana*, var. *sandwichiana* and var. *kailuana*, in which the former has no scales while the latter has scales. Both these varieties have nearly the same pollen-ovule ratio (203.3 and 211, respectively), which supports the suggestions that pollination occurs independently of the presence/absence of IFS.

6.5 Development and Origin of Scales

Due to the fused nature of the corolla-stamen tube, the origins of the IFS as petal or staminal tissue have been historically controversial. The IFS in *Cuscuta* have been referred to by multiple terms, including 'staminodia', 'hypostaminal scales', 'epistamineal scales' and 'corolla appendages' (Babington, 1844; Engelmann, 1859; Yuncker, 1921). There are three general hypotheses of IFS ontogeny. First, Babington (1844) suggested that the IFS should be considered as an alternate whorl of modified stamens since they are opposite of the corolla and alternate with the stamens. However, the IFS do not alternate with the stamens. Furthermore, all Convolvulaceae possess a single whorl of 5 stamens (Stefanović *et al.,* 2003). By contrast, Cunningham (1898) believed that the IFS originate from the corolla because they occur at a

different level than the stamens. This interpretation discounts the existence of the stamina vascular strand beneath the IFS. Endress and Matthews (2006) also calls the IFS "ventral petal scales," and does not mention them possibly being a part of the filamens. Lastly, other researchers have suggested that the IFS are dilatations of the basal part of the staminal filaments (Engelmann, 1859; Yuncker, 1921; Gandhi and Thomas, 1983).

Clayson's (2012) examination of floral development in *Cuscuta gronovii* reveals that it proceeds similarly to that of *C. australis* (Kuoh and Liao, 1993), *C. epithymum* (Erbar, 1991) and *C. reflexa* (Prenner *et al*., 2002). The IFS are the last major floral structures to develop. They are initiated under the staminal filaments after the pollen grains reach maturity and one of the two stigmas become receptive. The bridge that will unite the scales is formed subsequently at the corolla base, as can be seen by the cross-sectional views of the upright flower.

The results of the flower structure in this study shows that the corolla-stamen tube in *Cuscuta* is a structure with a double origin, both the corolla and the androecium (corolla-stamen tube) contributing to its formation. Since the IFS develop last and eventually become fused with the corolla base through their bridges, I am suggesting that the scales are a part of this complex organ that includes both the corolla and androecium. The disagreement regarding the origin of the IFS is a false problem because all these parts are developmentally connected and forming a unique structure: corolla, IFS, and androecium, and this reflects the synorganization of the *Cuscuta* flower.

6.6 Role of Scales in *Cuscuta*

Although Tiagi (1966) believed that the IFS were responsible for the secretion of nectar, it has been demonstrated that *Cuscuta* flowers secrete nectar from a ring of modified stomata within the base of the ovary (Prenner *et al*., 2002, Wright *et al*., 2011). This form of nectary,

either in the ovary wall or elaborated into a nectary disk, is characteristic of all Convolvulaceae (Govil, 1972; Deroin, 1992, 2002). IFS that secreted nectar would merely duplicate this function, and their evolution as a novel structure in the *Cuscuta* flower would be very unlikely.

Pollen-ovule ratios have been suggested as an indicator of breeding systems of angiosperms (Cruden, 1977), and Wright and colleages' (2012) studies show that *Cuscuta* encompasses a mixed mating system. IFS length shows a weak correlation with pollen-ovule ratios when assessing the entire genus. However, the correlation is strong in subgenus *Monogynella*, which declines in subg. *Cuscuta* and it becomes very weak in subg. *Grammica*. This suggests that the role of the IFS could have been related to pollination at first, but this function has been altered in the more derived infrageneric lineages of *Cuscuta*.

In the first diverged subgenus, *Monogynella*, the bridge length correlates with the nectary stomata count. *Monogynella* also tend to have scale shapes that are wider at the lower half of the scale, which makes sense if they were to function in the holding of nectar secreted at the base of the ovary. This could explain why the pollen-ovule ratio and IFS length are correlated in *Monogynella* – they may be associated with sexual reproduction. It is reasonable to assume that the more nectary stomata present, the more nectar is produced, but this is something that needs to be researched further. However, if the above assumption is correct, these results can aid in confirming the idea of Prenner *et al*. (2002) which stated that the scales of *C. reflexa* (subg. *Monogynella*) act as nectar receptacles.Since the nectar holding seems to be only associated with the bridge, the IFS of *Monogynella* can still have an additional role of protection of the ovary because of its fimbriae.

The purpose of exploring the histochemistry of the IFS was to make basic comparisons of composition among the three subgenera of *Cuscuta*,and to determine if the secretions of the

fimbriae are chemically similar to those of the laticifers in the stems. The latter aspect is particularly important because previous *Cuscuta* toxicity reports on insects (Srivastava *et al.,* 1990) involved extracts of the stems in which the latex is an important compoment. The staining results of the fimbriae showed that *Monogynella* species have a different composition than the rest of the genus. The *Monogynella* species' fimbriae stains with Oil Red O and Sudan IV, and not with Nile Blue. This means that the fimbriae contain neutral lipids and lipoproteins, and not acidic lipids. The rest of the genus did have fimbriae which stain blue with Nile blue, meaning that they contain acidic lipids. Also the secretion of fimbriae was different chemically from that of the stems, which stained with Nile Blue. Two different fimbriae types were seen (*Cuscuta*-type and *Monogynella*-type), and these two types also stained differently.

It was also seen that in *Grammica* and *Cuscuta*, the latex in the fimbriae and stems may be similar since they stained the same way – both subgenera stained with Nile Blue and not with the other four stains. Moreover, the latex in the IFS fimbriae stains the same as the latex in the stems. Both the fimbriae and stems stain blue with Nile Blue and do not stain for the other four stains. This means that the latex in the fimbriae may have the same composition based on acidic lipids as the latex in the stems and that the toxicity reported for the latter may also apply to the former. Though studies have shown that latex of *Cuscuta*, along with other Convolvulaceae, contains resin-glycosides which have acid derivatives, the internal composition of the fimbriae latex is unknown, and would need to be analyzed in the future.

In conclusion, based on the current data, the function of IFS is: 1) associated to sexual reproduction/pollination in subgenus *Monogynella*, and 2) to serve most likely as a defense layer against herbivorous insects to protect the ovary/ovules.

6.7 Taxonomic Significance of IFS in *Cuscuta*

Cuscuta's holoparasitic lifestyle has resulted in the reduction of their vegetative structures, leaving their flower as the most important source of characters for systematic and identification purposes. Though IFS characters are not sufficient for reconstructing the *Cuscuta* phylogeny alone, there are some details that can help distinguish particular groups, for example the distinctive glandular trichome-like fimbriae of subg. *Monogynella*, and the presence of papillae on fimbriae of species from Clade O of subg. *Grammica*. The IFS morphology is quite diverse among species (Figure 5.1; Appendix B), and their characters are important for species delimitation and identification as they have been included in both historical and current identification keys (e.g. Choisy, 1841, Engelmann, 1859; Yuncker, 1932; Costea and Stefanović, 2009b, 2010). The diversity documented in this study (Appendix B) will be helpful for improving the taxonomy of *Cuscuta*, especially since this is the first time such detailed quantitative data have been recorded about the IFS. In addition, the morphology of fimbriae, including details about their laticifers, has been explored for the first time in this study.

6.8 Future Directions

There is further study that can be done following the procedures performed and data gathered within this thesis. First of all, concerning the quantitative relationships between the IFS and other floral traits of *Cuscuta*, the floral nectaries can be studied in more detail. Perhaps nectar production can be measured accurately to confirm that the nectary stomata number is positively correlated with the amount of nectar produced.

Concerning the laticifer content, a study can be done on the specific latex components of *Cuscuta* and their variation. Once the components are known, a comparison can be made among

those from the stem, flower, and the IFS. This could help confirm the role of the IFS in *Cuscuta*. This could also have value as a chemotaxonomy study, which may provide new data for species delimitation and identification based on their biochemical composition. Overall, this can help us understand better the evolutionary history of *Cuscuta*.

This study has provided a broad-scale survey and analysis of the IFS, but more in-depth studies are possible. The fact that some species have lost their scales completely is an interesting evolutionary event. More in-depth study can be done that may reveal under what conditions selection against the loss of the IFS dissipates. Ecological and biological factors can be looked at in the future using the handful of species in which scales exhibit degrees of reduction. For example, it would be interesting to study the conditions under which the reduction of IFS takes place by investigating species in which there is a trend of scale reduction (e.g., in *C. sandwichiana* which is found in Hawaii).

Various field experiments can be performed as well. Perhaps careful removal of the IFS in a natural setting can be done and pollination or ovule/seed production can be observed (Liao *et al.,* 2005). One can also try spraying *Cuscuta* latex extracts on other plants/insects and see if they are deterred, as was done in a lettuce latex study (Sethi *et al*., 2008).

7. Summary

The broad survey of infrastaminal scales in *Cuscuta* has shown that the IFS are very diverse morphologically. Although most of the characters surveyed are polymorphic, the ranges of variation observed characterize species or varieties, which makes the scales very important for the taxonomy of the genus. The structure of the IFS is simple. The body of the scales is 3 – 5 cells thick. Fimbriae seen in cross-section are either unicellular in subgenus *Monogynella*, or they consist of a ring of 4 – 6 epidermal cells that surround a laticifer cell. Laticifers are usually isolated and only rarely articulated, in groups or rows of 2 – 3 cells and plasmodesmata are apparent in the cell wall between epidermal cells of the fimbriae and between epidermal and laticifer cells.

The formation of the IFS reflects the synorganisation of the corolla-stamen tube. Although arising at the base of the stamens, the scales become fused to one another through bridges that originate from the corolla base. Thus the scales are neither the product of the stamens nor of the corolla, but rather of the corolla-stamen tube.

Totally reduced (absent) IFS have evolved only in subgenus *Grammica* and are associated with seven speciation events in four different clades. The partial or total reduction of the scales is not accompanied by changes in the breeding system.

The IFS length was moderately to strongly correlated with the other continuous scale characters. Correlations between the IFS length and the pollen/ovule production were very strong in the subgenus *Monogynella* and declined drastically in the subgenera *Cuscuta* and *Grammica*. A similar relationship among subgenera was observed between the length of the IFS bridge and the number of nectary stomata. These results indicate that IFS are associated with pollination/nectar secretion in subgenus *Monogynella*, and they lack this relationship in

subgenera *Cuscuta* and *Grammica*. PCA and cluster analyses show that IFS characters themselves can be useful taxonomically within species clades, but have little use in the infrageneric-level systematics because of the extensive convergent evolution observed.

Scales in *Cuscuta* have likely evolved in connection to a modification of their function in the flower: from nectar protection and holding in the first diverged subgenus *Monogynella*, to ovary/ovule protection against herbivorous insects in the derived subgenera *Cuscuta* and *Grammica*. In subg. *Monogynella*, IFS fimbriae are similar to uniseriate glandular hairs, with the secretory cells entirely exposed, while in the subgenera *Grammica* and *Cuscuta*, the fimbriae become more complex, with an internal laticifer, and a precise, on-contact, mechanism of latex discharge.

There are many future directions that can come forth from this study. There can be additional studies that focus on latex composition of *Cuscuta* and the IFS. There can also be field experiments conducted such as IFS removal that can help determine how much of an effect they have on the flower and its pollination. These examples will help us to better understand the function and evolution of the IFS in *Cuscuta*.

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Appendix A – List of Specimens

Cuscuta acuta Engelm.: Ecuador, Galapagos Islands, *Fagerlind & Wibom 3401* (S); *Howell 110140* (G); *Wheeler et al. 21*(NY); *Howell 10048* (KEW). **C. acutiloba** Engelm.: Bolivia, *Mardon 1481* (G); Peru, *Weberbauer 7443* (F); *Pennell 13242* (S). **C. africana** Thumb.: South Africa, *Beyers 6968* (NBG) [A]; *Muir 156* (GRA); *Oliver 11852* (NBG) [A]; *Durtz 472* (NBG) [A]. **C. americana** L.: U.S.A., Florida,

Small et. al. 11596 (NY); Mexico, *Felger 4087* (SD); Colombia, *Schneider 999* (S); *Billberg 61* (S). **C. angulata** Engelm.: South Africa, *Beyers 12-1985* (NBG); *Orchard 460* (NU); *Williams 2690* (NBG); *Williams 3419* (NBG). **C. appendiculata** Engelm.: South Africa, *Hofmeyr s.n.* (GAA) [A]; *Bohnen 7827* (NBG) [A]. **C. applanata** Engelm.: U.S.A., New Mexico, *Casteller 7339* (UNM); Mexico, *Stewart 1038* (F); *Lyle & Wind 754* (S). **C. approximata** Bab.: U.S.A., California, *Abrams 457* (CAS) [A]; U.S.A., Nevada, *Kennedy s.n.* (CAS); *Kennedy 16422* (CAS); Utah, *Costea & Wright 2009-01* (WLU) [A]. **C. argentiniana** Yunck.: Argentina, *Brücher s.n.*(S); *Krapovickas & Schinini 36049* (CTES). **C. aurea** Liebm.: Mexico, *Palmer 87* (S); *Nesom et al. 5949* (F). **C. australis** R. Br. var. **australis**: New Caledonia, *Bonati 737* (S); China, *Sykes CH99* (CHR). **C. australis** var. **tinei** (Insenga) Yunck.: Hungary, *Simonkai 2635* (NY); *Karkovány s.n.* (WLU). **C. bella** Yunck.: Peru, *Killip & Smith 21827* (US). **C. boldinghii** Urb.: Mexico, *Van Devender 92-31* (ARIZ); *Provance 3403* (UCR); *Breedlove 37373* (NY). **C. boliviana** Yunck.: Argentina, *Hunzinker 2676* (S); *Ruiz Leal 14816* (MERL); *Burkart 12503* (CTES). **C. brachycalyx** Yunck.: U.S.A., California, *Ahart 9856* (CHICO); *Howell 38877* (NY) *Colwell & Coulter AC 04-31* (YM). **C. burrelli** Yunck.: Brazil, *Heringer et. al. 43* (UB); *Alvarenga-Pereira 766* (RB); *Dawson 14278* (NY). **C. californica** Hook. & Arn.: U.S.A., California, *Sanders 25122* (UCR); *Munz 2689* (RSA); *Gregory 1049* (SD). **C. campestris** Yunck: U.S.A., Oklahoma, *Lipscomb 1894* (SMU); Louisiana, *Smith s.n.* (SMU); Mexico, *Pringle 3111* (S). **C. cassytoides** Nees.: South Africa, *Balkwill 6968* (NU); *Alexandre 2407* (NBG); *Garland s.n.* (NY). **C. cephalanthi** Engelm.: U.S.A., Illinois, *McDonald s.n.* (NMS); *Steyermark 79977*(MO); Washington, *Grant s.n.* (RSA). **C. chapalana** Yunck.: Mexico, *García-Ruiz 7942* (CIMI); *Machuca 8981* (IBUG); *García-Ruiz et al. 8064* (WLU) [A]. **C. chilensis** Ker Gawl.: Chile, *Anderson 84-189* (S); *Buchtien 446* (S); *Valeutey 94* (S); *Laudewer 313* (KEW); Muňoz 5169, 5170 (WLU) [A]. **C. chinensis** Lam.: Australia, *Carter 628* (CAN). **C. cockerellii** Yunck: Argentina, *Vargas 2600* (CUS); *Vargas 19383* (CUS); *Nunez 28* (USM). **C. compacta** Juss.: U.S.A., New Jersey, *Moldenke & Moldenke 25129* (AAU); South Carolina, *Godfrey & Taylor 1326* (CAS); Maryland, *Steele 26022* (CAS). **C. corniculata** Engelm.**:** Brazil, *Stannard et. al. 51861*(G); Colombia, *Pennell 1453* (GH). **C. coryli** Engelm.: U.S.A., Arkansas, *Demaree 19603* (CAS); Kansas,

Morley 747 (SMU); 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); *Martinez 3295* (MEXU); *Mendez & de Lopez 9608* (MICH). **C. corymbosa** var. **stylosa** (Choisy) Engelm.: Mexico, *Rzedowski 28752* (UCR); *Borgeau 3353* (S); *Bopp 206* (MEXU); *Pringle s.n.* (MEXU). **C. costaricensis** Yunck.: Mexico, *Van Devender 98-1789* (ARIZ) [A]; *Cházaro et. al. 7527* (MICH); *García-Ruiz et al. 8052* (CIMI, WLU) [A]. **C. cotijana** Costea & I. García: Mexico, *Carranza et al. 7316* (IEB) [A]; *García Ruiz et al. 7557* (CIMI, WLU) [A]. **C. cozumeliensis** Yunck.: Guatemala, *Kellerman 6580* (F); Mexico, *Calzade & Nievea 9427* (XAL); *Vazquez 176* (MEXU). **C. cristata** Engelm.: Argentina, *Burkart 14000* (SI); *Balegna 447* (SMU); *Hunzinker 4927* (S). **C. cuspidata** Engelm.: U.S.A., Arkansas, *Demaree 15522* (RSA); Indiana, *Deam 33011* (IND); Texas, *Higgins 12480* (NY); *Runyon 2828* (SMU); U.S.A., Kansas, *McGregor 15175* (SMU). **C. decipiens** Yunck.: Mexico, *Henrickson 6362*, *13394, 22781* (RSA). **C. deltoidea** Yunck.: Mexico, *Orcutt 4457* (F); *Pringle* 5350 (NMS). **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); *Tiehm 13319* (NY). **C. desmouliniana** Yunck.: Mexico, *Spellenberg et. al. 4943* (NMC); *Rea 1124* (SD); *Spellenberg 4943* (NMS); *Van Devender & Reina-G 2002-23* (WLU). **C. epilinum** Weihe: Sweden, *Samuelson 1317* (RSA); Canada, Quebec, *Barabe 16914* (DAO); *Cayouette s.n.* (QUE). **C. epithymum** (L) L.: Argentina, *Bana 14733* (CTES); Australia, *Clark 107955-212* (RSA) [A]; Belgium, *Meulebrouck s.n.* (WLU) [A]; Mexico, *Pringle 8514* (S); U.S.A., New York, *Ahles 67695* (SMU). **C. erosa** Yunck.: Mexico, Baja California, *Rebman 4275* (UCR); Mexico, *Van Devender 2001-737* (NMS). **C. europea** L.: Finland, *Alava et al. s.n.* (OSU); Sweden, *Holmgren 19784* (SD); Netherlands, *Hekking 635* (NY). **C. exaltata** Engelm.: U.S.A., Texas, *Snyder 472* (SMU); *Carr 12341* (BRIT); *Carter 10584* (MO); *Westlund s.n.* (CAS). **C. flossdorfii** Hicken var. **pampagrandensis** Yunck.: Bolivia, *Mendoza & Acebo 919* (MO) **C. foetida** Kunth. var. **foetida**: Ecuador, *Holm-Neilson & Andrado 18480* (AAU); *Holm-Neilson et. al. 5181* (AAU); *Sparre 16952* (AAU). **C. foetida** var. **pycnantha** Yunck.: Peru, *Plowman et. al. 14291* (F). **C.**

friesii Yunck.: Argentina, *Krapovickas et al. 21898* (CTES); *Mulgura 1245* (SI) *Fulgura 1245* (SI). **C. glabrior** (Engelm.) Yunck.: Mexico, *Marsh 1115* (SMU); *Henrickson 13676* (RSA); U.S.A., Texas, *Palmer 9965* (CAS). **C. globiflora** Engelm.: Argentina, *Mulgura et. al. 1199* (MO); Bolivia, *Plowman & Davis 5196* (GH); *Buchtinen 133* (F). **C. globulosa** Benth.: Puerto Rico, *Stahl 1064* (S); *Urban 855* (S); *Liogier & Oquendo 180* (UPRRP); Cuba, *Ekman 7839* (S). **C. glomerata** Choisy: U.S.A., Texas, *Berkley 13886* (RSA); *Wolff 3321* (SMU); Indiana, *Dean 39229* (NY). **C. goyaziana** Yunck.**:** Brazil, *Macedo 3731* (S); *Duarte & Mattos 8376* (RB). **C. gracillima** Engelm.: Mexico, *Pringle 6716* (NML); *Van Devender 2006-160* (WLU) [A]; *Vazquez 511* (UCR); *García Ruiz 7334* (CIMI, WLU) [A]. **C. grandiflora** Kunth.: Argentina, *Schinini et. al. 34615* (CTES); *Hunzinker 1899* (S); Ecuador, *Løjtnant et al. 11829* (AAU). **C. gronovii** Willd. ex Roem. & Schult. var. **gronovii**: U.S.A., Alabama, *Kpeooer et al. s.n.* (NY) [A]; Georgia, *Mellinger s.n.* (SMU); U.S.A., Massachusetts, *Gates et al. 14841* (SMU); Canada, Ontario, *Wright & Bols 2009-05* (WLU) [A]. **C. gronovii** var. **latifolia** Engelm.: Missouri, *Brant & Donnell 4810* (MO); U.S.A., Texas, *Lundell 11721* (SMU); Connecticut, *Hill 17037* (NY). **C. gymnocarpa** Engelm.: Galapagos Islands, *Fagerling & Wibon 3658* (S); *Werff 2068* (S). **C. harperi** Small: U.S.A., Alabama, *Churchill 861:4* (CAS); *Demaree 46295* (NY); *Harper 6479* (SMU); *Kral 32878* (SMU). **C. haughtii** Yunck.: Ecuador, *Asplund 15974* (S); Venezuela, *Asplund 5618* (F). **C. howelliana** Rubtzoff: U.S.A., California, *True 7407* (DS); *Oswald & Ahart 7645* (CHSC). **C. hyalina** Roth.: India, *Pushpauder s.n.* (CANB); Namibia, *Bosch 25022* (BOL); South Africa, *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., Arizona, *Austin 7599* (RSA); U.S.A., Louisiana, *Allen* 19239 (BRIT); California, *Munz* 12736 (CAS); Arkansas, *Demaree* 18050 (CAS). **C. indecora** var. **attenuata** (Waterf.) Costea**:** U.S.A., Oklahoma, *Waterfall 17496* (GH); Texas, *Whitehouse 16472* (SMU); Mexico, *Palmer 333* (F)]. **C. indecora** var. **longisepala** Yunck.: Argentina, *Leal 7964* (NY); *Burkart s.n.* (KEW); U.S.A., Colorado, *Ewan 15327* (CAS); Texas, *Runyon 2819* (NY). **C. jalapensis** Schltdl.: Mexico, *Waterfall & Wallis 14213* (SMU); *Miller 11561* (MEXU); *García-Ruiz et. al. 7569* (CIMI, WLU). **C. japonica** Choisy: China, *Bartholomew et al. 883* (NY) [A]; *Hill 22616* (MO); Japan, *Furuse 6890* (RSA)

[A]; *Brooks 322* (NY) [A]; U.S.A. South Carolina, *Hill 20079 (BRIT)* [A]. **C. jepsonii** Yunck.: U.S.A., California, *Dudley 1774* (DS); *Tracy 2349* (UC). **C. killimanjari** Oliv.: Malawi, *Lacroix 4559* (MO); Tanzania, *Scheffler 434* (MEL); Zimbabwe, *Eyles 352* (J). **C. legitima** Costea and Stefanović: U.S.A., Arizona, *Felger 92-707* (CAS); *Spellenberg 12966* (NMS); Mexico, *Jones 22633* (UCR), Mexico, *Van Devender & Reina-G. 2006-638* (WLU). **C. lehmanniana** Bunge.: Usbekistan, *Vvedensky s.n* (MEL); *Drobov 3763* (NY); India, *Stewart 21103* (NY); Uzbekistán, *Budogoski 817* (NY). **C. leptantha** Engelm.: Mexico, *Wiggins 17125* (MEXU); *Lindsay 2928* (SD); *Dominguez 3472* (SD); *Moran 8669* (SD); *Van Devender 2000-933* (WLU); *Wiggins 13153* (SD). **C. lindsayi** Wiggins: Mexico, *Wiggins 13185* (MO); *García-Ruiz et al. 7569* (CIMI, WLU). **C. longiloba** Yunck.: Paraguay, *Casas & Molero 4384* (MO); Bolivia, *Krapovickas & Schinini 13255* (F). **C. lucidicarpa** Yunck. Peru, *Pennell 15067* (GH); *Killip & Smith 21858* (US); *Killip & Smith 21909* (NY). **C. lupuliformis** Krock.: Austria, *Barta 2004-302* (NY); Netherlands, *Lennhouts 2514* (CANB); Hungary, *Degen s.n.* (RSA); China, *Bartholomew et al.* 883 (RSA). **C. macrocephala** W. Schaffn. ex Yunck.: Mexico, *Rebman 5743* (SD); *Van Devender & Reina-G 2006-872* (WLU); *Carter et al. 2186* (F); *Moran 18810* (SD). **C. mcvaughii** Yunck.: Mexico, *Hinton et al. 12098* (G). **C. micrantha** Choisy: Chile, *Phillippi 489* (G); *Skottsberg 995* (F). **C. mitriformis** Engelm.: Mexico, *Bye 50488* (UCR); *Bye 2011* (MEXU); *Carranza 5658* (IEB); *Moore & Wood 4329* (MICH). **C. monogyna** Vahl.: Grece, *Greuter 11459* (NY); Turkmenistan, *Sintenis 1240* (MO); Vietnam, *Kung 2024* (NY). **C. natalensis** Baker: South Africa*, Rudatis s.n.* (NBG); *Rudatis 2412* (NBG). **C. nevadensis** I.M. Johnst.: U.S.A., California, *Raven 12865* (CAS); *Peebles 263* (NY); *Twisselmann 16318* (CAS); Nevada, *Brandegee s.n.* (UC), *LaRivers & Hancock 164* (NY). **C. nitida** E. Mey.: South Africa, *Compton 15500* (NBG) [A]; *Edwards 13* (J); *Rogers 17342* (J); *Taylor s.n.* (NBG) [A]. **C. obtusiflora** Kunth var. **obtusiflora:** Argentina, *Arbo et al. 7973* (CTES); *Bordódon s.n.* (CTES). **C. obtusiflora** var. **glandulosa** Engelm.: Mexico, Jalisco, *García-Ruiz 7752* (WLU) [A]; U.S.A., Texas, *Clare 2144* (CAS); *Lundell & Lundell 11717* (NY); U.S.A., Delaware, *collector illegible* ("MC") *s.n* (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); *Palmer 412* (F); *Van Devender 2006-869* (WLU). **C. odorata** Ruiz & Pav.: Ecuador, *Jaramillo 10372* (AAU); *Asplund 7737* (S); Peru, *Hitchcock 20320* (GH); *Ugent & Ugent 5323* (MO). **C. orbiculata** Yunck.: Brazil, *Alvaregna 93605* (RB); *Harley et al. 21452* (AAU). **C. ortegana** Yunck.: Mexico, *Hinton et. al. 16294* (MICH); *Van Devender et al 2006-74* (WLU). **C. pacifica** Costea & Wright: U.S.A., Canada, *Kennedy & Ganders 4947* (UBC); U.S.A., California, *Dudley 267* (CAS); *Eastwood 7971* (CAS); *Moldenke 25731* (NY).**C. paitana** Yunck.: Ecuador, *Madsen 63940* (AAU); Peru, *Horton 11575* (GH). **C. parodiana** Yunck.: Argentina, *Eyerdam 22423* (MO); *Novara 7976* (S); *Balegno 447* (SMU); *Krapovickas 35879* (G); *Novara 7976* (S). **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); *Filgueiras et al. 745* (RB). **C. pentagona** Engelm.: U.S.A., Alabama, *Kral 31225* (SMU); District of Colombia, *Buettcher 122* (CAS); Florida, *Welch 1633* (NY); Virginia, *Herman 10391* (NY). **C. planiflora** Ten.: Australia, *Easkins s.n.* (WLU); *Howitt & Zaicon-Kunesch s.n.* (PERTH); Palestina, *Musselman 10461* (RSA); *Dorn 5420* (NY). **C. plattensis** A. Nelson: U.S.A., Wyoming, *Nelson 2768* (NY); *Nelson 2741* (MO). **C. platyloba** Prog.: Argentina, *Burkart 10554* (CTES); *Karapovickas 2911* (KEW); *Burkart 14250* (SI); Brazil, *Dusen 10005* (S); Paraguay, *Montes 16599* (CTES). **C. polyanthemos** Schaffn. ex Yunck.: Mexico, *Wiggins 13153* (SD); *Van Devender 2006-809 & Reina* (WLU). **C. potosina** Schaffn. ex Yunck.: var. **potosina**: Mexico, *Rose et al. 9650* (GH); *Schaffner 379* (MEXU), *Rzedowski 3894* (MEXU). **C. potosina** var. **globifera** W. Schaffn.: Mexico, *Pringle 6575* (S); *Van Devender et al. 96-451* (WLU); *Pringle 6575* (G); U.S.A., Arizona, *Gooding 290-61* (ASU). **C. prismatica** Pav. ex Choisy: Ecuador, *Mille 112* (F); Peru, *Pilger et al. s.n.* (F). **C. punana** Costea & Stefanović: Ecuador, *Madsen 63850* (AAU). **C. purpurata** Phil.: Chile, *Johnston 5170* (S); *Werdermann 852* (S); *Rechinger 63509* (B). **C. purpusii** Yunck.: Mexico, *Hendrickson 6608* (RSA); *Meyer & Rogers 2878* (UPS). **C. racemosa** Mart. var. **racemosa**: Brazil, *Hatschbach 64867* (KEW); *Pinheiro 55* (SPF); *Smith et al. 14478* (F). **C. racemosa** var. **miniata** (Mart.) Engelm.: Brazil, *Menezes et. al. 5100* (CTES); *Richon 7835* (S); *Arbo et al. 5100* (KEW); *Cordeiro et. al. 8211* (KEW). **C. reflexa** Roxb.: India, *Cullelt s.n.* (MEL) [A]; *Kanta s.n.* (ASU); *Koelz 21955* (NY) [A].

C. rostrata Shuttlw. ex Engelm. & A. Gray: U.S.A., North Carolina, *Bozeman et al. 45268* (OSU); Tennessee, *Churchill 93217* (CAS); *Jennison 2824* (NY); Texas, *Lundell 11480* (SMU). **C. rugosiceps** Yunck.: Mexico, *Taylor 21457* (SMU); *Lindres 4285* (MEXU); Guatemala, *Williams et al. 21950* (NY). **C. runyonii** Yunck.: U.S.A., Texas, *Lundell 9840* (SMU); *Runyon 2622* (BRIT); *Lundell 9827* (SMU). **C. salina** Engelm.: U.S.A., Arizona, *Hammond 10349* (NY); California, *Raven 878* (CAS); Nevada, *Tiehm 5991* (CAS). **C. sandwichiana** Choisy: U.S.A., Hawaii, *Stern 8416* (CHICO); *Fosberg 14019* (RSA). **C. santapaui** Banerji & Sitesh Das: Nepal, *Nicolson 2796* (MO). **C. serrata** Yunck.: Brazil, *Acevedo & Lopes 848* (RB); *Acevedo 757* (RB); *Glaziou 21811* (F). **C. sidarum** Liebm.: Mexico, *Palmer 51* (S); *Standley 12359* (S); *Stevens 20910* (RSA). **C. squamata** Engelm.: U.S.A., New Mexico, *Wooton & Standley 3355* (CAS); *Wooton 1894* (S); Texas, *Gould 7114* (SMU). **C. stenolepis** Engelm.: Ecuador, *Jaramillo & Caravajal 2307* (AAU); *Neilson & Coello 29084* (AAU); *Asplund 6678* (S); *Tipaz 4636* (MO). **C. strobilacea** Liebm.: Mexico, *Jones s.n.* (RSA); *Croat & Hannon 65094* (MEXU); *Jones 27347* (MICH), *Garcìa Ruiz 8071* (WLU) [A]. **C. suaveolens** Ser.: Australia, *Alcock 10415* (RSA); Chile, *Eyerdam 24649* (KEW); U.S.A., California, *Abrams s.n.* (RSA); *Dudley s.n.* (CAS). **C. subinclusa** Durand & Hilg.: U.S.A., California, *Dudley 1653* (DS); *Ewan 11049* (NY); *Mason 5766* (NY); *Rose 39363* (NMS). **C. suksdorfii** Yunck.: U.S.A., California, *Twisselmann 14603* (SD); *Oswald & Ahart 5874* (CHICO); *Tracy 18430* (UC); *Colwell AC05-213* (UC). **C. tasmanica** Engelm.: Australia, *Barker s.n.* (CANB); *Walsh 3045 (*MEL); *Lepschi 909* (MEL). **C. tinctoria** Mart. ex Engelm.: Mexico, *Palmer 87* (S); *García Ruiz et al. 7575* (CIMI, WLU); *Ventura 4248* (IEB); *Rzdowski 34596* (IEB); *Van Devender 94-1008 et al.* (WLU). **C. tuberculata** Brandegee: U.S.A., Arizona, *Beauchamp 3112* (SD); Mexico, *Waterfall 12842* (SMU); *Rebman 7638* (SD); *Reina 2000-465* (WLU). **C. umbellata** Kunth: Mexico, *Moran 24758* (SD); *Nabhan & Rea 167* (ARIZ); U.S.A., Texas, *Bernal 37* (SMU); New Mexico, *Spellenberg 2902* (NMS). **C. umbrosa** Beyr. ex Hook.: Canada, Alberta, *Allen 150* (DAO); Manitoba, *Criddle s.n.* (DAO); U.S.A., Utah, *Jones s.n.* (CAS); Colorado, *Jones 571* (RSA); *Mulford s.n.* (NY). **C. veatchii** Brandegee: Mexico, *Rebman* 3189 (SD); *Porter 198* (MEXU). **C. victoriana** Yunck.: Australia, *Cowie 9624* (CANB); *Glennon 379* (CANB); *Lazarides & Palmer 471* (CANB). **C. warneri** Yunck.:

U.S.A., New Mexico, *Spellenberg 13890* (WLU); Utah, *Warner s.n.* (NY). **C. werdermanii** Yunck.: Chile, *Werdermann 880* (SGO). **C. woodsonii** Yunck.: Guatemala, *Heyde et al. 2912* (KEW); *Brenckle 47-269* (S); Panama, *Davidson 967* (GH). **C. xanthochortos** Mart. ex Engelm. var. **xanthochortos**: Argentina, *Arbo et. al. 6953* (MO); Paraguay, *Zardini & Vera 46124* (MO). **C. xanthochortos** var. **carinata** Yunck.: Paraguay, *Billiet & Jodin 3294* (MO); *Bernardi 18758* (MO). **C. xanthochortos** var. **lanceolata** Yunck.: Argentina, *Schulz 7139* (CTES); Paraguay, *Zardini & Villate 46371* (WLU); *Jorgensen 3478* (S) . **C. yucatana** Yunck.: Mexico, *Nee & Taylor 29575* (MO); *Rzedowski 25728* (IEB); *Steere 1695* (MICH).

Appendix B – Data Matrix

Infrastaminal scales character data in *Cuscuta*. Averages are provided for quantitative characters. 1= scales (0 = absent; 1 = present), 2 = type of scale (1 = *Monogynella* type; 2 = *Cuscuta* type), 3 = shape of scale (1 = *oblong*; 2 = e*lliptic*; 3 = *ovate*; 4 = *ovate-triangular*; 5 = o*bovate*; 6 = *spathulate*; 7 = *bifid*), 4 = shape of scale apex (1 = rounded; 2 = acute; 3 = truncate), 5 = total scale length (including bridge and fimbriae), 6 = scale body length (not including bridge), 7 = maximum scale width (including fimbriae), 8 = scale body surface, 9 = bridge length, 10 = bridge surface, 11 = fimbriae length, 12 = fimbriae length on lower half of scale, 13 = fimbriae length on upper half of scale, 14 = number of fimbriae per scale, 15 = laticifer shape (1 = elongate; 2 = medium elongate; 3 = short), 16 = papillae on fimbriae (0 = absent; 1 = present), 17 = bridge with fimbriae (0 = absent; 1 = present), 18 = corolla tube length/scale length ratio (range of variation). Taxa for which no molecular data is available are indicated with "*", and tentatively placed into major infrageneric groups based on their morphology.

	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Subgenus	*C. cassytoides*	1	1	1, 3, 4	1	1.99	0.83	0.85	0.47	1.12	7.13	0.16	0.11	0.17	25	1	0	0	0.9
Monoynella	*C. exaltata*	1	1	1, 7	3	1.95	1.25	1.55	0.90	1.04	5.67	0.32	0	0.32	6	3	0	0	0.75
(9 taxa)	*C. gigantea*	1	1	1, 3	1	2.11	1.51	1.33	0.98	0.80	7.14	0.22	0.22	0.23	42	1	0	0	0.5
	C. japonica	1	1	1, 3	1	1.95	1.25	0.83	0.80	0.79	3.09	0.13	0.11	0.13	69	2	0	0	0.5
	C. lehmanniana	1	1	1, 3	1	2.95	1.71	0.72	0.85	1.50	7.39	0.18	0.11	0.2	34	1	0	0	1
	C. lupuliformis	1	1	7, 3, 1	1	1.52	0.94	0.87	0.50	0.8	2.56	0.15	0.13	0.15	21	1	0	0	1
	C. monogyna	1	1	1, 3 (7)	3	1.46	0.82	0.65	0.50	0.88	3.30	0	0	0	0	3	0	0	0.75 - 0.9
	C. reflexa	1	1	1, 3	1	2.57	1.76	1.32	1.80	0.78	5.71	0.19	0.16	0.21	58	1	0	0	0.5
	C. santapaui	1	1	1, 3 (5)	1	2.95	2.11	1.04	1.60	1.01	5.79	0.24	0.21	0.26	70	1	0	0	0.4 - 0.5
Subgenus	*C. africana*	1	2	1, 5	1	1.99	1.52	0.90	0.80	0.47	1.70	0.2	0.13	0.22	33	1	0	0	1-1>
Cuscuta	*C. angulata*	1	2	1, 5, 6	1	1.78	1.29	1.06	0.93	0.49	1.14	0.2	0.15	0.23	31	1	0	0	1-1>
(10 taxa)	*C. appendiculata*	1	2	1, 3 (7)	1	1.71	1.15	0.77	0.60	0.60	1.93	0.22	0.16	0.24	24	1	0	0	1-1>
	C. approximata	1	2	1, 3 (7)	1	1.75	1.08	0.76	0.83	0.76	4.24	0.15	0.12	0.16	13	3	0	0	0.9 - 1
	C. epilinum	1	2	1, 3, 5, 7	1	1.58	1.10	0.75	0.90	0.43	1.87	0.16	0	0.16	10	3	0	0	0.9
	C. epithymum	1	2	1, 5	1	1.49	1.05	0.81	0.52	0.43	1.15	0.14	0.11	0.17	18	3	0	0	0.75 - 0.9
	C. europaea	1	2	1, 7	1	1.26	0.90	0.46	0.30	0.34	1.24	0.17	0	0.17	7	2	0	0	0.75 - 0.9
	C. natalensis	1	2	1, 5, 6	1	3.10	2.61	1.13	1.20	0.54	2.64	0.26	0.22	0.27	24	3	0	0	0.8-0.9
	C. nitida	1	2	1, 3	1	2.29	1.65	1.04	1.20	0.69	4.21	0.20	0.16	0.21	36	2	0	0	1
	C. planiflora	1	2	1 (7)	1	1.14	0.93	0.64	0.43	0.17	0.61	0.12	0.09	0.13	12	1	0	0	0.9 - 1
Subgenus	*C. brachycalyx*	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grammica	*C. californica*	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(128 taxa)	*C. decipiens*	1	2	1, 5, 6	1	1.82	1.45	0.97	0.65	0.54	1.26	0.17	0.14	0.19	36	1	0	0	0.75 - 1
	C. howelliana	1	2	1, 3	1	0.96	0.72	0.50	0.43	0.47	0.78	0.14	0.09	0.17	13	0	0	0	0.5
Clade A	*C. jepsonii*	0	1	- (7)	0	0	0	0	0.23	0	0	0	0	0	0	0	0	0	0
	C. occidentalis	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C. pacifica var. pacifica	1	2	1	1	1.20	0.94	0.39	0.25	0.37	0.89	0.08	0.02	0.08	10	3	0	0	0.5
	C. salina	1	2	1, 5	1	1.57	1.31	0.52	0.42	0.43	0.88	0.12	0.10	0.13	24	2	1	0	0.5 - 0.66
	C. subinclusa	1	2	1	1	1.86	1.31	0.77	0.70	0.62	1.65	0.17	0.18	0.17	25	2	1	1	0.5
	C. suksdorfii	1	2	1, 7	1	0	0	0.18	0.07	0.23	0	0.09	0	0	3	3	0	0	0.5 - 0.75
Clade B	*C. australis*	1	2	7, 5	1	1.49	1.18	1.08	0.58	0.38	1.21	0.36	0.22	0.40	15	1	0	1	0.5 - 0.66
	C. campestris	1	2	1, 5	1	1.78	1.40	1.02	1.11	0.47	1.65	0.30	0.26	0.31	21	1	0	1	1-1>
	C. glabrior	1	2	5, 6	1	2.00	1.65	1.28	0.93	0.50	1.48	0.32	0.27	0.34	40	1	0	1	1
	C. gymnocarpa	1	2	1, 5	2	1.62	1.18	0.65	0.36	0.41	1.37	0.25	0.17	0.27	12	2	0	1	1-1>
	C. harperi	1	2	1, 3	1	0.86	0.71	0.86	0.15	0.32	0.83	0.18	0.14	0.20	10	1	0	0	1
	C. obtusiflora var. glandulosa	1	2	1	1	1.45	1.43	1.05	0.90	0.42	1.83	0.33	0.20	0.36	18	2	0	1	1
	C. obtusiflora var. obtusiflora	1	2	1	1	1.66	1.21	0.87	0.50	0.51	2.01	0.25	0.14	0.28	15	2	0	1	0.9-1
	C. pentagona	1	2	1, 5	1	1.47	0.95	0.71	0.55	0.47	1.54	0.16	0.14	0.17	24	1	0	1	1-1>
	C. plattensis	1	2	1, 5	1	1.96	1.50	1.06	1.09	0.46	1.07	0.16	0.14	0.17	35	1	0	0	0.75-0.8
	C. polygonorum	1	2	7	3	0.94	0.76	0.52	0.23	0.10	0.36	0.30	0	0.30	3	3	0	0	0.9 - 1
	C. runyonii	1	2	5, 6	1	1.42	1.21	0.91	0.95	0.25	0.82	0.21	0.18	0.22	35	1	0	1	0.9 - 1
	C. stenolepis	1	2	1	1	1.77	1.23	0.50	0.55	0.52	1.81	0.13	0.08	0.14	12	2	0	1	0.9 - 1
Clade C	*C. corniculata*	1	2	1 (3, 5)	1	1.55	0.96	0.84	0.54	0.61	1.98	0.18	0.16	0.19	26	1	0	1	1
	C. incurvata	1	2	1, 5	1	1.47	1.00	0.77	0.55	0.53	1.13	0.17	0.14	0.18	30	2	0	1	1
	C. micrantha	1	2	1	1	2.00	1.44	1.08	1.20	0.59	2.18	0.28	0.20	0.30	20	2	0	1	1->1
	C. parviflora var. elongata	1	2	1, 5	1	1.19	0.94	0.67	0.44	0.28	0.56	0.18	0.18	0.20	19	1	0	0	1->1
	C. pauciflora	1	2	1	1	1.53	0.95	0.82	0.60	0.62	2.53	0.07	0.07	0.07	17	3	0	1	0.75 - 0.9
	C. platyloba	1	2	1, 5	1	1.87	1.36	0.99	0.50	0.50	1.83	0.29	0.24	0.31	22	1	0	1	1->1

	C. racemosa var. miniata	1	2	1, 5	1	1.66	1.37	0.98	0.78	0.54	1.99	0.28	0.24	0.3	29	1	1	1	1->1
	C. racemosa var. racemosa	1	2	1, 5	1	1.94	1.41	0.92	0.58	0.58	1.80	0.26	0.15	0.30	24	1	0	1	1
	C. suaveolens	1	2	5	1	2.19	1.57	1.16	1.50	0.75	3.70	0.32	0.24	0.35	32	1	0	1	0.9 - 1
	C. werdermanii	1	2	1, 5	1	1.42	1.06	0.85	0.55	0.37	0.95	0.2	0.13	0.22	32	1	0	1	0.75 - 0.8
	C. xanthochortos var. carinata	1	2	1, 5	1	2.06	1.45	0.85	1.06	0.62	3.28	0.22	0.15	0.24	29	1	0	1	1
	C. xanthochortos var. lanceolata	1	2	1	1	1.93	1.06	0.57	0.50	0.79	2.11	0.10	0.07	0.10	24	2	0	0	0.8 - 1
	C. xanthochortos var. xanthochortos	1	2	1, 5	1	2.07	1.37	0.95	0.93	0.72	2.92	0.21	0.17	0.21	30	1	0	1	1->1
Clade D	*C. cephalanthi*	1	2	1	1	1.72	1.35	0.72	0.55	0.62	2.54	0.25	0.18	0.27	14	2	0	0	0.75 - 1
	C. compacta	1	2	1	1	2.64	1.76	1.15	0.70	0.98	4.61	0.78	0.63	0.81	15	1	0	1	0.9 - 1
	C. cuspidata	1	2	1	1	2.01	1.37	0.70	0.51	0.64	2.86	0.29	0.20	0.26	26	1	0	1	0.75
	C. glomerata	1	2	1	1	3.06	1.41	0.80	0.48	1.63	5.19	0.38	0.30	0.41	16	1	0	0	0.8 - 0.9
	C. gronovii var. gronovii	1	2	1, 3	1	2.04	1.48	0.90	0.7	0.59	3.49	0.43	0.24	0.48	13	1	0	1	0.9 - 1
	C. gronovii var. latifolia	1	2	1	1	1.73	1.42	0.73	0.27	0.38	1.85	0.35	0.27	0.38	12	1	0	1	1->1
	C. rostrata	1	2	1, 5	1	2.00	1.53	1.14	0.54	0.66	4.6	0.41	0.21	0.44	20	1	0	1	0.75
	C. squamata	1	2	1	1	2.38	1.37	0.70	0.85	1.12	4.08	0.28	0.23	0.31	19	1	0	0	0.9 - 1
	C. umbrosa	1	2	1 (7)	3	1.84	1.16	1.21	1.2	1.94	3.31	0.26	0.16	0.27	14	2	0	1	0.5 - 0.75
Clade E	*C. denticulata*	1	2	1, 3	1	1.05	0.65	0.46	0.2	0.45	1.36	0.10	0.06	0.10	11	3	0	1	1
	C. nevadensis	1	2	1, 5	1	1.62	0.94	0.62	0.45	0.71	2.45	0.18	0.15	0.19	16	0	0	1	0.9 - 1
	C. veatchii	1	2	1, 5	1	1.28	0.73	0.56	0.4	0.59	2.02	0.13	0.10	0.15	14	2	0	1	1
Clade F	*C. burellii*	1	2	1	1	0.88	0.65	0.52	0.23	0.22	0.67	0.11	0.09	0.12	18	2	0	1	0.75
	C. haughtii	1	2	1	1	0.89	0.46	0.49	0.48	0.40	2.46	0.12	0.12	0.13	11	2	0	1	0.5
	C. longiloba	1	2	1-5	1	2.41	1.50	1.21	0.72	0.94	6.20	0.20	0.17	0.20	33	3	0	0	0.5 - 1
	C. partita	1	2	1, 5	1	2.01	1.18	0.86	0.7	0.87	2.60	0.18	0.14	0.20	27	1	0	1	0.8 - 1
	C. serrata	1	2	1	1	2.29	1.32	0.66	0.5	1.26	4.42	0.21	0.15	0.22	19	1	0	1	0.75 - 0.8
Clade G	*C. aurea*	1	2	1, 3	1	2.31	1.62	1.03	1.56	0.70	4.23	0.22	0.15	0.24	27	1	0	1	0.8 - 1
	C. cotijana	1	2	1, 5	1	3.06	2.31	2.00	2.8	0.76	6.04	0.43	0.17	0.48	26	1	0	1	1
	C. jalapensis	1	2	1, 3	1	2.66	1.70	1.30	0.74	0.98	3.60	0.25	0.19	0.27	23	2	0	1	0.9 - 1
	C. lindsayi	1	2	5	1	3.15	2.69	1.75	2.00	0.38	3.20	0.20	0	0.20	19	0	0	0	0.75 - 0.8
	C. mitriformis	1	2	1	1	2.20	1.41	0.88	0.8	0.85	4.37	0.28	0.15	0.32	12	1	0	0	1
	C. purpusii	1	2	1	1	2.27	1.80	1.12	1.60	0.48	2.29	0.20	0.14	0.23	29	2	0	0	0.75 - 0.9
	C. rugosiceps	1	2	1, 3	1	3.02	1.94	1.14	0.89	1.14	5.95	0.29	0.21	0.31	16	2	0	0	0.9 - 1
	C. tasmanica	1	2	1	1	2.60	1.92	1.18	1.58	0.57	2.31	0.27	0.18	0.3	35	1	0	1	1
	C. tinctoria	1	2	1, 3, 4	1	2.72	1.69	1.50	2.00	1.03	5.98	0.28	0.20	0.30	30	1	0	1	1
	C. 'volcanica'	1	2	1	1	3.61	2.78	1.32	2.30	0.92	7.30	0.24	0.13	0.27	19	2	0	0	0.9
	C. woodsonii	1	2	1	1	3.25	2.24	1.19	2.50	1.06	7.58	0.3	0.22	0.34	22	1	0	0	0.75 - 0.9
Clade H	*C. alata*	1	2	1	1	1.90	1.39	0.92	0.99	0.37	1.69	0.25	0.17	0.29	26	1	0	0	1>
	C. azteca	1	2	1	1	1.40	1.08	0.79	0.53	0.34	1.48	0.23	0.13	0.26	18	1	0	0	0.75 - 1>
	C. chinensis var. applanata	1	2	5	1	1.91	1.56	0.99	0.73	0.36	1.53	0.28	0.17	0.32	26	1	0	1	1>
	C. chinensis var. chinensis	1	2	5	1	2.22	1.86	1.04	1.02	0.37	3.39	0.33	0.17	0.39	27	1	0	0	1
	*C. dentatasquamata**	1	2	1, 3	2	1.37	0.98	0.48	0.35	0.40	1.16	0.27	0.06	0.30	5	3	0	0	0.9 - 1
	C. potosina	1	2	1 (5)	1	1.08	0.86	0.51	0.20	0.25	0.79	0.22	0	0.22	7	1	0	0	0.75 - 1
	*C. sandwichiana var. sandwichiana**	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C. sandwichiana var. kailuana	1	2	1, 7	2	1.10	0.96	0.38	0.34	0.12	0.46	0.19	0	0.19	3	3	0	0	0.5
	C. yucatana	1	2	5	1	0.97	0.86	0.48	0.16	0.11	0.17	0.22	0.15	0.24	13	2	0	0	1 - 1>

Clade I	*C. americana*	1	2	1, 3, 4	1	1.95	1.18	0.68	0.40	0.87	3.34	0.14	0.17	0.14	16	3	0	0	1
	C. cozumeliensis	1	2	1	1	2.99	2.33	0.98	1.70	0.81	4.78	0.24	0.20	0.26	27	1	0	0	1
	C. globulosa	1	2	1, 3	1	2.53	1.31	1.15	0.56	1.32	5.33	0.25	0.22	0.25	33	1	0	0	0.75 - 1
	C. macrocephala	1	2	1	1	3.33	2.64	1.26	2.25	0.75	5.04	0.21	0.18	0.22	43	1	0	0	0.75
Clade J	*C. corymbosa var. corymbosa**	1	2	1	1	2.51	1.85	0.85	1.57	0.82	4.81	0.21	0.18	0.23	20	2	0	1	0.5 - 0.7
	C. corymbosa var. grandiflora	1	2	1	1	2.80	2.13	0.83	1.64	0.92	4.37	0.20	0.18	0.22	19	2	0	1	0.5 - 0.7
	C. corymbosa var. stylosa	1	2	1	1	3.57	3.01	0.86	2.25	0.63	2.62	0.30	0.19	0.36	29	2	0	1	0.5 - 0.7
	C. prismatica	1	2	1,6	1	2.06	1.74	0.71	0.64	0.30	1.16	0.31	0.24	0.33	8	0	0	0	0.25
Clade K	*C. boldinghii*	1	2	1-3	1	1.77	1.15	0.82	0.58	0.77	2.77	0.21	0.15	0.24	25	0	0	1	0.75 - 1
	C. chapalana	1	2	1	1	1.98	1.71	0.49	0.88	0.29	5.17	0.24	0.17	0.25	8	0	0	0	0.5
	C. costaricensis	1	2	1, 5	1	2.89	2.02	1.33	1.45	0.99	3.55	0.34	0.27	0.36	21	1	0	0	0.8 - 1
	C. erosa	1	2	1, 3	1	1.53	0.88	0.74	0.65	0.66	2.84	0.18	0.15	0.19	29	0	0	0	0.5
	C. ortegana	1	2	1, 5	1	1.53	1.17	0.99	1	0.52	3.06	0.20	0.17	0.21	24	1	0	0	0.5
	C. strobilacea	1	2	1, 5	1	3.59	1.84	1.11	1.26	1.88	6.88	0.34	0.28	0.35	39	0	0	0	0.8 - 1
Clade L	*C. acuta*	1	2	1, 5	1	1.49	0.34	0.90	0.80	0.34	1.59	0.15	0.10	0.17	24	2	0	0	0.8 - 1
	C. desmouliniana	1	2	5, 6	1	0.69	0.56	0.48	0.40	0.15	0.49	0.10	0.08	0.11	19	3	0	0	0.75
	C. hyalina var. *hyalina*	0	0	-	0	0	0	0	0	0	0	0	0	0	0		0	0	0
	C. legitima	1	2	5, 6	1	2.08	1.58	1.03	0.77	0.35	1.34	0.22	0.14	0.26	31	3	0	0	0.9 - 1>
	C. leptantha	1	2	1	1	2.08	1.37	0.61	1.50	0.70	2.66	0.14	0.11	0.15	28	0	0	0	0.75-0.85
	C. odontolepis	1	2	1, 5, 6	1	2.27	1.66	1.17	0.79	0.65	3.33	0.24	0.17	0.25	22	0	0	0	0.9
	C. polyanthemos	1	2	1	1	2.69	2.17	0.62	0.68	0.51	3.04	0.13	0.13	0.13	23	2	0	0	0.5
	C. tuberculata	1	2	3	1	1.67	1.10	0.80	1.50	0.67	2.68	0.20	0.18	0.21	22	2	0	0	0.5 - 0.75
	C. umbellata var. umbellata	1	2	5, 6	1	1.50	1.28	0.86	0.67	0.30	2.49	0.16	0.14	0.17	26	2	0	0	1 - 1>
Clade M	*C. coryli*	1	2	7	3	1.60	1.17	0.78	0.52	0.47	1.76	0.21	0.13	0.27	11	2	0	0	1
	C. indecora var. attenuata	1	2	5, 6	1	2.05	1.68	1.34	1.10	0.43	2.07	0.34	0.21	0.38	22	2	0	0	0.8 - 1
	C. indecora var. indecora	1	2	5, 6	1	1.98	1.50	1.20	0.76	0.52	3.49	0.43	0.24	0.48	28	1	0	0	1
	C. indecora var. longisepala	1	2	1, 5, 6	1	2.18	1.54	1.27	1.68	0.74	5.76	0.32	0.20	0.36	28	1	0	0	1
	C. warneri	1	2	1	3	1.37	0	0.44	0.55	0	0.64	0.20	0	0.20	3	3	0	0	0.8 - 1
Clade N	*C. choisiana*	1	2	1	1	2.24	1.42	0.73	0.76	0.81	3.08	0.19	0.15	0.19	25	1	0	0	1
	C. deltoidea	1	2	1, 3	1	1.12	0.60	0.51	0.23	0.50	1.57	0.17	0.14	0.19	15	2	0	0	0.75 - 1
	C. gracillima	1	2	2, 5	1	1.83	1.17	0.91	0.55	0.81	2.94	0.18	0.16	0.18	34	2	0	0	>1
	C. mcvaughii	1	2	1, 5	1	1.69	0.97	0.68	0.45	0.77	2.47	0.14	0.14	0.14	21	2	0	0	0.8 - 1
	C. punana	1	2	1, 3	1	1.14	0.80	0.62	0.32	0.47	2.13	0.14	0.13	0.14	18	2	0	1	0.9 - 1>
	C. sidarum	1	2	1, 3	1	1.59	0.87	0.67	0.45	0.62	1.8	0.15	0.13	0.16	33	1	0	0	1
	C. vandevenderi	1	2	1, 3	1	1.18	0.82	0.54	0.30	0.39	0.95	0.16	0.13	0.18	15	2	0	0	1
Clade O	*C. acutiloba*	1	2	1, 5	2	1.63	1.51	0.55	0.52	0.22	1.24	0.13	0.10	0.17	12	3	0	0	1
	C. argentiniana	1	2	5, 6	1	2.17	1.69	1.49	1.70	0.31	2.21	0.30	0.24	0.32	30	2	1	0	1- 1>
	C. bella	1	2	1	1	2.63	2.04	1.04	1.85	0.69	4.30	0.23	0.20	0.24	19	2	1	0	0.8 - 1
	C. boliviana	1	2	1, 3	1	1.85	1.36	1.25	1.00	0.60	3.78	0.24	0.14	0.27	24	2	0	0	1
	C. chilensis	1	2	1	1	3.11	2.24	1.60	2.00	1.02	8.35	0.30	0.30	0.29	49	1	1	0	0.75
	C. cockerellii	1	2	1, 5, 6	1	2.10	1.66	1.05	1.32	0.26	1.36	0.28	0.21	0.31	23	2	1	0	0.4 - 0.5
	C. cristata	1	2	1, 5, 6	1	1.85	1.30	1.10	0.8	0.56	3.52	0.17	0.13	0.19	26	1	0	0	0.9 - 1
	C. flossdorfii var. pampagrandensis	1	2	5, 7	3	2.32	1.21	0.83	0.6	1.20	8.94	0.33	0	0.33	5	3	0	0	0.25
	C. foetida var. foetida	1	2	1, 5	1	2.28	3.20	1.02	1.6	0.40	2.60	0.20	0.14	0.22	29	2	1	0	0.75
	C. foetida var. pycnantha	1	2	1, 5	1	2.84	2.93	1.13	1.4	0.57	2.13	0.31	0.24	0.34	33	2	1	0	0.75

C. friesii	1	2	5, 6	1	1.95	1.72	1.67	1.35	0.21	1.31	0.24	0.19	0.25	34	2	0	0	1
C. globiflora	1	2	3	1	2.71	1.35	1.47	3.3	1.33	10.56	0.36	0.35	0.35	24	1	1	0	1
C. goyaziana	1	2	1	1	2.61	1.38	0.89	0.82	1.30	6.15	0.28	0.17	0.33	43	1	0	0	0.9 - 1
C. grandiflora	0	1	-	0	0	0	0		0	0	0	0	0	0	0	0	0	0
C. kilimanjari	1	2	3, 4	1	3.45	1.88	1.82	1.8	1.80	10.46	0.21	0.10	0.29	36	2	1	1	1
C. odorata var. odorata	1	2	1, 5	1	2.75	1.86	1.49	2.8	1.03	7.19	0.26	0.23	0.28	66	2	1	0	0.9 - 1
C. orbiculata	1	2	1, 3	1	3.60	2.14	1.47	2.1	1.68	10.66	0.33	0.19	0.37	47	2	0	1	0.9 - 1
C. paitana	1	2	6	1	3.01	2.67	1.62	2.1	0.39	1.66	0.39	0.33	0.39	20	2	0	0	0.4 - 0.5
C. parodiana	1	2	1	1	2.50	3.1	0.93	2.2	0.49	3.08	0.32	0.26	0.36	19	1	0	0	0.5 - 0.75
C. purpurata	1	2	1, 3	1	2.83	1.98	1.11	1.3	1.05	8.37	0.23	0.19	0.24	23	1	1	0	0.8 - 1
C. rubella	1	2	1	2	1.47	1.24	0.51	0.44	0.20	0.64	0.11	0.08	0.11	10	2	0	0	0.8