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Diversity and Evolution of the Multicellular Protuberances in Cuscuta (Convolvulaceae) and the Function of the Stomatiferous Protuberances in Cuscuta subgenus Grammica

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Diversity and Evolution of the Multicellular Protuberances in *Cuscuta* (Convolvulaceae) and the Function of the Stomatiferous Protuberances in *Cuscuta* subgenus *Grammica*

by

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Abstract

Cuscuta (Convolvulaceae), also known as the dodders, is a holoparasitic genus comprised of ca. 200 species grouped into four subgenera: *Monogynella*, *Cuscuta*, *Pachystigma*, and *Grammica*. The presence of unique multicellular structures, referred to as stomatiferous protuberances (SPs), was reported on the stems of subgenus *Grammica* over a century ago and was forgotten until similar SPs were observed on the flowers of several new *Grammica* species. The stems and flowers were examined in 136 *Cuscuta* taxa, and SPs were discovered on all of the haustorial stems of the species in the subgenus *Grammica*, as well as on the perianth of subgenera *Cuscuta* and *Pachystigma*. Other multicellular structures, referred to as extrafloral nectaries (EFNs), found in the subgenus *Monogynella,* differ both morphologically and functionally from the SPs. The diversity and evolution of the multicellular protuberances of *Cuscuta* (including both EFNs and SPs), as well as the function of the SPs are explored throughout this thesis. A morphological survey of both stems and flowers in *Cuscuta* was performed and an examination of the protuberances was completed using light and scanning electron microscopy. Three distinct morphological forms of SPs in *Grammica* were found: dome-like, conical to cylindrical, and crest-like, and in subgenera *Cuscuta* and *Pachystigma* SPs were found with a "diffuse" structure. Each protuberance possesses one or several distal stomata. Species in the subgenus *Grammica* developed two functional types of stems: exploratory stems with no SPs, and haustorial stems with numerous SPs. Using two parasite/host systems in the field, *C. gronovii/Solidago canadensis* in Canada, and *C. costaricensis*/*Tithonia tubiformis* in Mexico, stomatal conductance and water uptake were determined. Stomatal conductance rates were higher in hosts that were not parasitized by *Cuscuta*, suggesting hosts' water preservation when *Cuscuta* is present. Haustorial stems, with SPs, had a higher stomatal conductance rate than

the exploratory stems that are without SPs. This pattern was found in both parasite-host systems. The water uptake of parasitized hosts was significantly higher than non-parasitized hosts and can be explained in part by the water loss at the level of the SPs. Furthermore, *Cuscuta* species within the subgenus *Grammica,* with floral SPs, grow in arid areas (average precipitation less or equal to 90mm) during flowering/fruiting, which suggest that SPs may have evolved to stimulate this water uptake through the host during these stages. Within this thesis, the development of two functional types of stems in the subgenus *Grammica* is discussed for the first time, as well as the evolution of floral SPs across the genus. The flowers of *Cuscuta* are important, as they are the only way to distinguish the ca. 200 species from one another. The documentation of the floral SPs throughout the genus can help with the identification of some of the species. Furthermore, the function of the SPs, which has not been discussed in previous literature, is also studied in this thesis for the first time.

Acknowledgements

First and foremost I would like to thank Dr. Mihai Costea for the opportunity to work under his supervision and for the guidance and support he has given me throughout my time in his lab. I would like to thank my committee members, Dr. Frédérique Guinel and Dr. Kevin Stevens, for their advice and support, which have also contributed to this thesis. I would also like to thank the numerous herbarium curators for providing *Cuscuta* material to work with and dissect (Appendix A).

To my dear friend and past lab-mate, Stephanie Riviere, you have helped me since day one and have provided me with so much guidance and support. I would also like to thank my lab-mates Behrang Behdarvandi, Kristy Dockstader, and Natalie Rey-Caughlin for their help in this thesis as well as support. Susan Belfry of the University of New Brunswick was also a large contributor to the anatomy and ultrastructure section of my thesis.

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Chapter One: Introduction

Parasitic plants have the ability to impact and regulate the structure of the community in which they belong, in some cases causing serious agricultural problems (Irving and Cameron, 2009). One example of a highly complex parasite is *Cuscuta* (dodders), a widely distributed genus of rootless parasitic plants (Figure 1) from the morning glory family (Convolvulaceae). The morning glory family is nearly cosmopolitan in distribution, consisting of 55-60 genera and approximately 1600-1700 species (Stefanović et al., 2003). As the only parasitic lineage in the Convolvulaceae family (Yuncker, 1932; Stefanović et al., 2003; Stefanović and Costea, 2008), *Cuscuta* has been quite successful in its diversification, consisting of approximately 200 species, with 60 varieties, found in a number of different habitats including temperate, riparian, tropical, etc. (Stefanović et al., 2007; Costea et al., 2011a). Several *Cuscuta* species are capable of causing substantial yield losses in numerous vital crops, including tomato, potato, tobacco, soybean, and blueberry (Dawson et al., 1994; Costea and Tardif, 2006) and as a result are commonly placed on quarantine lists by the legislation of many countries. Therefore, many studies continue to focus on eradication methods for *Cuscuta,* even though up to 50% of *Cuscuta* species are threatened, or possibly extinct, and have been neglected in terms of their biology, diversity, and evolution (Costea and Stefanović, 2009). From an ecological point of view, *Cuscuta* have been identified as keystone species and ecosystem engineers due to their ability to increase the richness of plant communities (Pennings and Callaway, 1996). In addition, some species of *Cuscuta* have also been used in traditional Chinese medicine as antibacterial or anti-inflammatory agents, as well as in fertility applications (Bork et al., 1996; Chao et al., 2003). *Cuscuta* has limited morphological characters (Yuncker, 1932) due to drastic reductions of their vegetative organs, and therefore it is important that we develop a more thorough knowledge of their structure, diversity, and evolution

in order to further understand their systematics and natural history. The diversity of the flowers, which are the only way to identify the species from one another, is particularly important.

Two types of multicellular protuberances have been reported in *Cuscuta* in different subgenera. Previously identified extrafloral nectaries are found in subgenus *Monogynella* (Schaffner, 1979), whereas in the remaining three subgenera, *Cuscuta*, *Pachystigma*, and *Grammica*, unique structures are present on the stems and/or perianth of *Cuscuta* species, bearing one or several distal stomata. Initially identified as "proéminences stomatiféres" on the stem epidermis of three species of subgenus *Grammica*, Mirande's (1901) observations fell into oblivion when subsequent authors failed to identify stomata on the stem or flowers (Yuncker, 1943; Patel and Inamdar, 1971; Dawson et al., 1994). More recently, these structures have been reported on the perianth on a number of species that belong to subgenus *Grammica* (Costea et al., 2006c; Costea and Stefanović, 2009; Costea and Stefanović, 2010; Costea et al., 2011a; Costea et al., 2011b; Costea et al., 2013).

The study of these structures is important for taxonomic purposes because *Cuscuta* flowers are vital for the identification of the ca. 200 species, as well as many descriptions of the SPs are present in recent species descriptions and identification keys (Costea et al., 2006c; Costea and Stefanović, 2009; Costea and Stefanović, 2010; Costea et al., 2011a; Costea et al., 2011b; Costea et al., 2013). Despite the more recent descriptions and keys, there is little to no information available on the protuberances diversity, structure, evolution, and function. More information is needed to better classify the *Cuscuta* species and further understand the role of these protuberances in *Cuscuta*.

Figure 1. Yellow stems of *Cuscuta gronovii* growing on vegetation at Kauffman Flats, Grand River, Waterloo, Ontario. This photo highlights the extensive vegetative growth consisting of mostly exploratory stems, before flowering and fruiting.

1.1 Parasitic Plants

Parasitic plants represent approximately 1% of all angiosperms (Nickrent, 2002). They are a diverse, complex group of plants that require a host to provide them with some or all nourishment essential for survival. Parasitism in the angiosperms has evolved approximately 11 times, and occurs in ca. 4000 species, within 270 genera and in over 20 families (Lambers et al., 2008; Irving and Cameron, 2009). Parasitic plants are generally split into two groups of parasitism, facultative and obligate. Facultative parasites are able to complete their life cycle without attachment to their host, whereas obligate parasites depend entirely on their host, and therefore are unable to complete their life cycle without one (Lambers et al., 2008). Parasitic plants can also be divided into hemiparasites and holoparasites. Hemiparasitic plants may be facultative, for example *Rhinanthus major*, (Heide-Jorgensen, 2008) or obligate, for example *Striga gesnerioides* (Kuijt, 1969; Reiss and Bailey, 1998; Heide-Jørgensen, 2008), and only derive some of their resources from their hosts, including water and nutrients. They also contain chlorophyll and have the ability to photosynthesize. In contrast, holoparasites are always obligate because they depend entirely on their host, contain little to no chlorophyll, and therefore usually lack the ability to photosynthesize (Shen et al., 2007; Lambers et al., 2008; Irving and Cameron, 2009). Furthermore, hemiparasites and holoparasites can be root parasites or stem (shoot) parasites. As the names suggest, root parasites attach to the roots of their hosts while stem parasites attach themselves to the stems (shoots) of their hosts (Lambers et al., 2008).

Parasitic plants interact with their hosts through haustoria (Figure 2). The haustorium is a specialized organ that creates a physiological bridge between the parasite and host and functions in the attachment, penetration, and transfer of resources (Kuijt, 1969; Heide-Jørgensen, 2008; Lambers et al., 2008). Haustoria can connect to both the xylem and phloem of the host and the amount of haustorial connections to the host, which can range from 10 to 1000 depending on the

parasite, determines the success of the parasites' ability to take up the required resources, including water and nutrients (Kuijt, 1969; Lambers et al., 2008; Irving and Cameron, 2009).

Most hemiparasitic plants have high stomatal densities and are considered "xylem feeders" that can maximize water uptake (Raven, 1983; Stewart and Press, 1990; Lambers et al., 2008; Irving and Cameron, 2009). In contrast, holoparasitic plants possess low to no water uptake and can be regarded as "phloem feeders" that conserve water (Fer, 1981; Raven, 1983; Stewart and Press, 1990; Seel et al., 1992; Dawson et al., 1994; Ehleringer and Marshall, 1995; Heide-Jørgensen, 2008). The evolution from hemi- to holoparasitism involved drastic reduction of leaves where stomatal densities were low or the stomata were entirely absent (Kuijt, 1969; Heide-Jørgensen, 2008). Although holoparasitic plants are referred to as "phloem feeders" (Fer, 1981; Fer, 1987; Hibberd and Jeschke, 2001) there is sometimes a connection between the hosts' xylem and the parasites' xylem. Due to the low transpiration rates commonly associated with holoparasitic plants, xylem connection is considered to contribute a low, insignificant amount of uptake of water and minerals (Kuijt and Toth, 1976; Hibberd et al., 1998).

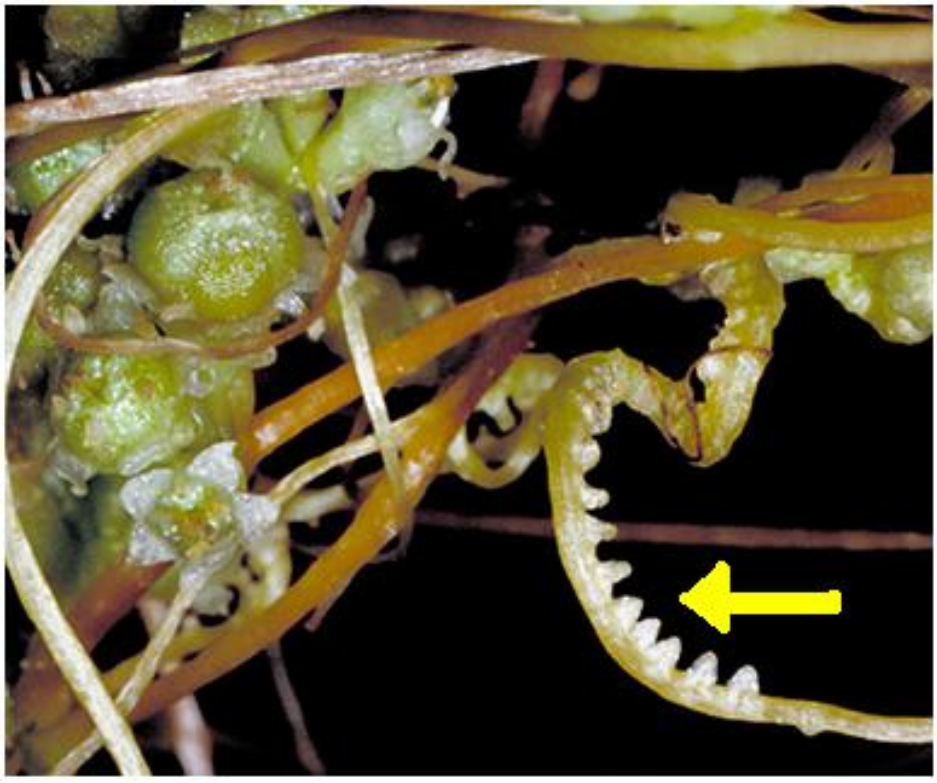

Figure 2. Haustoria developed by *C. sandwichiana* stems, indicated by a yellow arrow [Image from*: http://www.botany.hawaii.edu/faculty/carr/phylo_convolvul.htm*].

1.2. Biology of *Cuscuta*

Cuscuta is considered a holoparasite because it depends entirely on the attachment to its host for survival, contains minimal to no chlorophyll, and in most cases lacks the ability to photosynthesize. Even the most active photosynthetic species that belong to subgenus *Monogynella* derive 99.5% of their carbon from their hosts and therefore are considered functionally holoparasitic (Jeschke et al., 1994b; Hibberd et al., 1998). *Cuscuta* is known to parasitize a wide variety of host species, including both wild and cultivated plants (Lyshede, 1985; Dawson et al., 1994; Barath, 2009).

1.2.1. Stems

Cuscuta have ephemeral roots; in other words, their roots disappear one or two weeks after germination (Lyshede, 1985). The filiform seedling of *Cuscuta* becomes erect and after the initial nutation of the shoot, begins to creep over the ground. *Cuscuta* will continue to grow at the apex and wilt at the base, growing in the direction of higher light intensity until it finds a suitable host (Dawson et al., 1994; Lyshede, 1985). When *Cuscuta* secures contact with its compatible host it will form haustoria (Kuijt, 1969). The haustoria penetrate into the host tissue, connecting the xylem and/or phloem of the host to the *Cuscuta* stem. When the connection is established, the shoot apex of *Cuscuta* continues to develop. The slender stems of *Cuscuta* twine around the host and haustoria develop on the underside of the stem that is connected to the host (Figure 2). These haustorial connections are strong and facilitate the absorption of water, nutrients, and amino acids (Kuijt, 1969).

1.2.2. Flowers

Unlike the scale-like leaves and filiform stems, which provide no useful characters for identification (Yuncker, 1921; Stefanović et al., 2007), the flowers of *Cuscuta* are important from a taxonomic point of view. The flowers provide the only characteristics that can be used to distinguish *Cuscuta* species and varieties from one another. The flowers of *Cuscuta* have a general structure that resembles that of the Convolvulaceae flowers. They are hermaphroditic, actinomorphic, 4-5-merous, more or less fleshy, ranging from white to pink in colour (Costea and Tardif, 2006). More specifically, the calyx is gamosepalous (having sepals united or partly united) and the corolla is gamopetalous (having petals united or partly united), with the lobes overlapping in buds. The stamens alternate with the corolla lobes and are inserted on short filaments near the base of the corolla sinuses. At the base of each of the stamens' filaments are infrastaminal scales, finger-like structures that are fused with the base of the corolla tube. They connect through a bridge that varies in size and surrounds the gynoecium base (Riviere et al., 2013). The gynoecium is located in the middle of the flower with a superior ovary that is 2-locular, each locule with two anatropous (completely inverted) ovules (Costea and Tardif, 2006). There is a floral nectary found at the base of the ovary in all *Cuscuta* flowers; it consists of a ring of modified stomata that vary in number and secretion volume throughout the genus (Prenner et al., 2002; Welsh, 2009; Wright et al., 2011; Wright et al., 2012). *Cuscuta* flowers contain nectar and pollen rewards that are targeted towards generalist pollinators including flies, beetles, moths, and other larger insects (Wright, 2011). Although *Cuscuta* are a target for a variety of insect pollinators, they are seldom subjected to herbivory (Costea and Tardif, 2006). A few insects from the genus *Smicronyx* attack them by laying their eggs on the stems or more commonly, in the ovaries. The larvae then consume and damage the ovules/seeds and the internal tissues of the stems (Shimi et al., 1995).

1.3. Systematics and Evolution of *Cuscuta*

Floral characteristics have been important for the systematics of *Cuscuta*, and continue to be essential for the classification of new species. The ~200 known species of *Cuscuta* are currently classified into four subgenera based on their stigma and style morphology (Engelmann, 1859; Yuncker, 1932; Stefanović et al., 2007; Wright et al., 2011; García et al., 2014). Subgenera *Grammica, Cuscuta*, and *Pachystigma* are characterized by their two distinct styles: *Grammica* display short, capitate stigmas, whereas *Cuscuta* exhibit linear, elongate stigmas, which are as thick as the styles, and *Pachystigma* possess elongate stigmas, thicker than the styles. In contrast, subgenus *Monogynella* is characterized by a single style with varying stigma shape (Engelmann, 1859; Yuncker, 1932; Wright et al., 2011). Recent phylogenetic studies, based on plastid and nuclear datasets have been published for the subgenera *Cuscuta* and *Grammica* (Stefanović *et al.,* 2007; García and Martin, 2007) and for the entire genus (García et al., 2014). *Grammica*, the largest subgenus of *Cuscuta*, accounts for 75% of the genus diversity. It is also the most widespread and complicated subgenus taxonomically (Yuncker, 1932; Stefanović et al., 2007). Yuncker (1932) divided subgenus *Grammica* into two subsections, known as *Cleistogrammica* and *Eugrammica,* based on the capsule (fruit) indehiscence (not splitting open) or dehiscence (splitting open at maturity), respectively. Furthermore, subsections *Cleistogrammica* and *Eugrammica* were initially divided into 12 subsections based on various morphological characters by Yuncker (1932). More recently, within subgenus *Grammica*, fifteen major clades have been recently circumscribed with little resemblance to the taxonomic scheme of Yuncker (1932) (Stefanović et al., 2007; García et al., 2014). Subsequently, to elucidate evolutionary relationships and taxonomy at a species level, more focused studies were initiated. To date, nine of the fifteen clades have been extensively studied at the species level (Costea et al., 2005; Costea

et al., 2006a, 2006b, 2006c; Costea et al., 2008; Costea and Stefanović, 2010; Costea et al., 2011a, 2011b; Costea et al., 2013).

1.4. Stomata

Stomata are small pores that are the gateway between the plant and the atmosphere. Some of the more common structures they serve include: extrafloral nectaries, hydathodes, or pores that allow for transpiration. Stomata may be found on the leaves, stems, or perianth elements of the plant (Keeler, 1980; Hetherington and Woodward, 2003; Irving and Cameron, 2009). The stoma (opening) is surrounded by a pair of guard cells, the site of control for the exchange of gases (CO_2 and water vapour). These guard cells are usually surrounded by subsidiary cells, which are specialized epidermal cells (Hetherington and Woodward, 2003; Hopkins and Hüner, 2009). Stomata respond to a spectrum of signals from the environment as well as the plant and are therefore vital components of a plant.

Stomata are commonly found on the leaves and/or sepals within the family Convolvulaceae in genera such as *Ipomoea*, *Calystegia*, and *Cressa* (Keeler, 1980; Patel and Inamdar, 1971). Within the parasitic genus *Cuscuta*, leaves have been reduced to small, scale-like structures, and do not contain stomata (Yuncker, 1943; Costea and Tardif, 2006). Mirande (1901) made initial reports of stomata on the stems of some species, but his findings were overlooked when subsequent authors reported low stomatal frequencies in other *Cuscuta* species (Yuncker, 1943; Patel and Inamdar, 1971; Dawson et al., 1994). Schaffner (1979) reported stomata on *Cuscuta* species in subgenus *Monogynella* that serve as extrafloral nectaries and are able to produce notable amounts of nectar when parasitizing an appropriate host. More recently, modified stomata have been identified around the base of the ovary in the *Cuscuta* flowers, with the amount of nectar secretion varying across the genus (Prenner et al., 2002; Wright, 2011;

Wright et al., 2011). Structures with stomata were also reported on the perianth of some *Grammica* species but their function remained unknown (Costea et al., 2006c; Costea and Stefanović, 2009; Costea and Stefanović, 2010; Costea et al., 2011a, b; Costea et al., 2013). The function of the SPs will be discussed in this thesis.

1.4.1. Extrafloral Nectaries

Extrafloral nectaries (EFNs) are nectar-secreting structures that are not directly involved with pollination (Keeler, 1980; Falcão et al., 2003). The secretion is composed of an aqueous sugar solution that contains amino acids and can serve to attract/reward insects, such as ants, to protect the plant in return for supplying the insects with food (Keeler, 1980; Falcão et al., 2003). This relationship is viewed as a plant-insect mutualism. Within the Convolvulaceae family, *Ipomoea* have extrafloral nectaries on their leaves as well as sepals, increasing ant visitation and therefore protection from herbivores (Keeler, 1980). Similarly, EFNs are found in *Cuscuta* within the subgenus *Monogynella* (Schaffner, 1979). More specifically, EFNs on the stems of *C. reflexa* and *C. japonica* were found to secrete nectar consisting of sucrose, amino acids, and monosaccharides (Schaffner, 1979). According to Schaffner (1979) the nectar within the two species moves passively through the intercellular spaces that lead up to the stomata. From personal observation, the stem's EFNs can be slightly raised, with a red pigmentationin the epidermal cells surrounding the guard cells (Figure 3).

Unlike the EFNs found in *Monogynella*, the structures with stomata found within the subgenus *Grammica* have a raised morphology, and do not discharge a fluid (e.g., like hydathodes or extrafloral nectaries) during the day or night (Costea et al., 2006c; Costea and Stefanović, 2009; Costea and Stefanović, 2010; Costea et al., 2011a,b; Costea et al., 2013; Costea, unpublished).

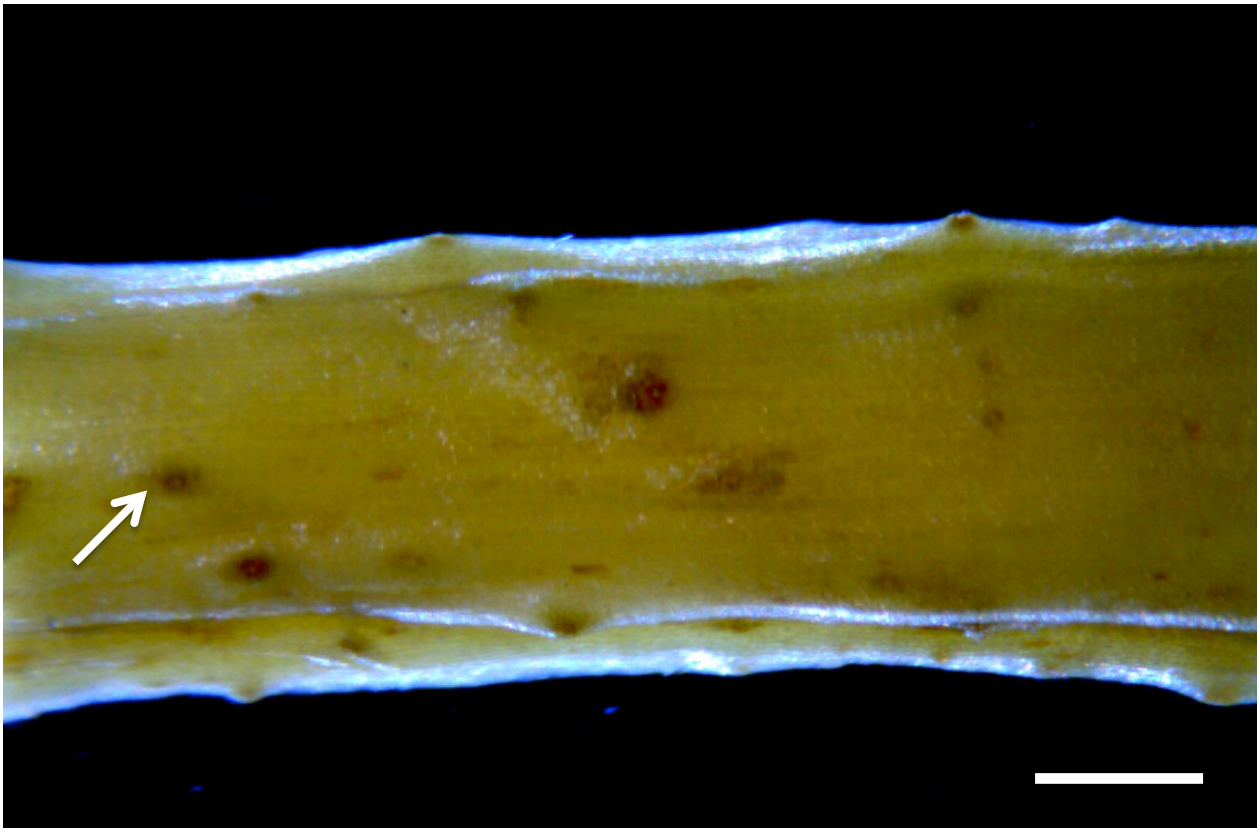

Figure 3. Stem of *Cuscuta japonica* belonging to subgenus *Monogynella*. This image highlights the presence of extrafloral nectaries, which are slightly raised and have a reddened pigment (white arrow).

Scale bar = 1mm.

1.4.2. Stomatiferous Protuberances (SPs)

The presence of unique structures, referred here as the stomatiferous protuberances (SPs), have been observed recently in several species of *Cuscuta* (Costea and Stefanović, 2009, 2010; Costea et al., 2011a; Costea et al., 2011b). They can be found on the stems (Figure 4), pedicels, and even the flowers of *Cuscuta*. On the flowers, SPs can be found on the tips (Figure 5) or dorsal side (Figure 6) of the calyx lobes, the tips of the corolla lobes (Figure 7), as well as on the bracts. These protuberances can be found on multiple areas of the flower (e.g., the tips of the calyx and the tips of the corolla) or are limited to one area depending on the species examined. As the flowers of *Cuscuta* are only millimeters long, the SPs are even smaller, ranging from 0.05 mm-0.7 mm in length (Costea et al., 2006c; Costea and Stefanović, 2009, 2010; Costea et al., 2011a,b; Costea et al., 2013). Floral SPs develop early in the bud, and become fully formed when the reproductive structures are not yet mature (Costea et al., 2011a; Costea et al., 2011b). On each of the SPs are one or several stomata.

Stomatiferous protuberances have evolved multiple times in subgenus *Grammica*, and have so far only been previously documented in 6 clades of subgenus *Grammica*, including clades A, G, H, K, L, and M (Costea et al., 2006c; Costea and Stefanović, 2009, 2010; Costea et al., 2011a,b; Costea et al., 2013). In the subgenus *Monogynella,* structures with stomata on the stems have been documented as extrafloral nectaries in *C. japonica* and *C. reflexa* (Schaffner, 1979), while subgenera *Cuscuta* and *Pachystigma* stems and flowers remain to be examined for the presence of SPs or EFNs. The presence or absence of the SPs in the genus of *Cuscuta*, as well as extrafloral nectaries in other species belonging to *Monogynella* will be documented in this thesis to determine evolutionary trends. It is interesting to note that the SPs on the flowers described thus far in the literature are found in species of subgenus *Grammica* growing in dry

conditions, more specifically in the area of the Southern United States and Mexico (Costea et al., 2006c; Costea and Stefanović, 2009, 2010; Costea et al., 2011a, b; Costea et al., unpublished).

Although Mirande (1901) initially described these structures and termed them "protuberances" or "proéminences stomatifères", his observations fell into oblivion when subsequent authors reported low stem stomatal densities and transpiration rates for *Cuscuta* (Yuncker, 1921; Yuncker, 1943; Patel and Inamdar, 1971; Dawson et al., 1994). Yuncker (1921), in his revision of "North American and West Indian species of *Cuscuta"* did, however, refer to small protuberances found on the flowers in some of his descriptions; for example the calyx and corolla of *C. chapalana* were described as possessing "a prong-like dorsal projection near the apex." Although these structures have been on occasion, briefly documented, their function remains unknown. Our hypothesis is that they serve a role in transpiration and is explored further in the thesis. Currently, there is little information about these structures and this thesis sets out to expand the knowledge of *Cuscuta* SPs for biological, taxonomical, and evolutionary purposes.

Figure 4. *Cuscuta bonafortunae* haustorial stem attached to a host in the field. Note the numerous stomatiferous protuberances present on the surface of the stem.

Photo taken by Mihai Costea.

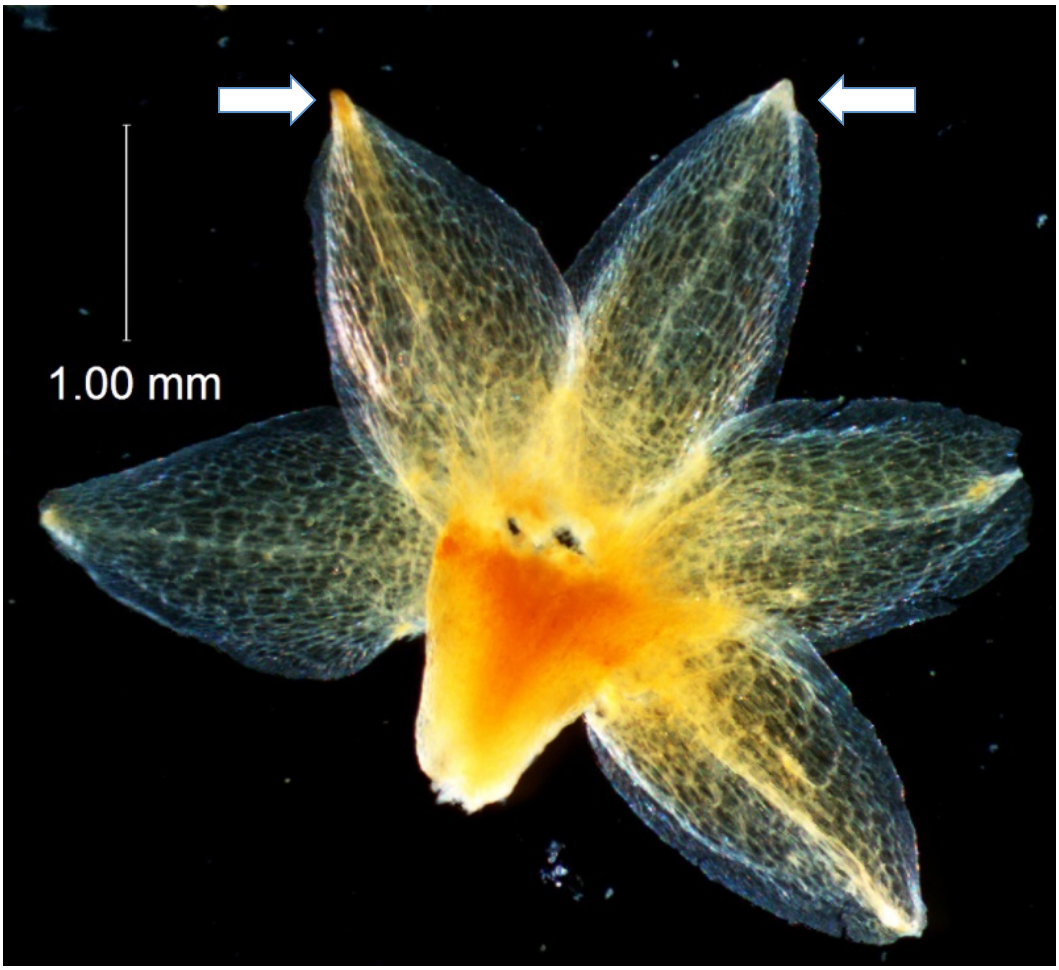

Figure 5. A dissected calyx of *Cuscuta chapalana.* Note the conical stomatiferous protuberances (indicated by white arrows) on the tips of the calyx lobes. Image taken with a SMZ1500 Stereomicroscope by Mihai Costea.

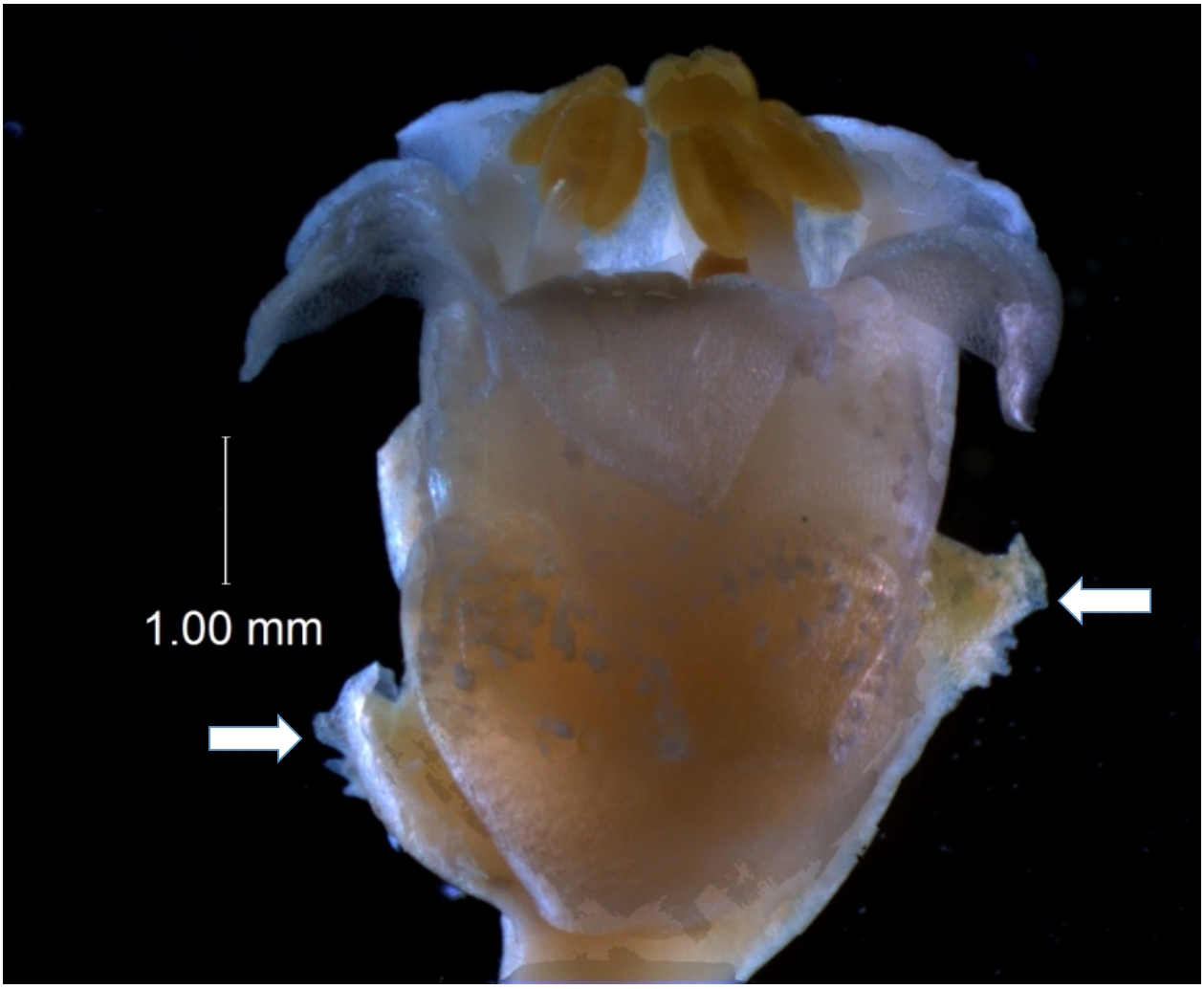

Figure 6. Flower of *Cuscuta cotijana*. Note the presence of crest-like stomatiferous protuberances (identified by white arrows) on the left and right calyx lobes. Image taken with a SMZ1500 Stereomicroscope by Mihai Costea.

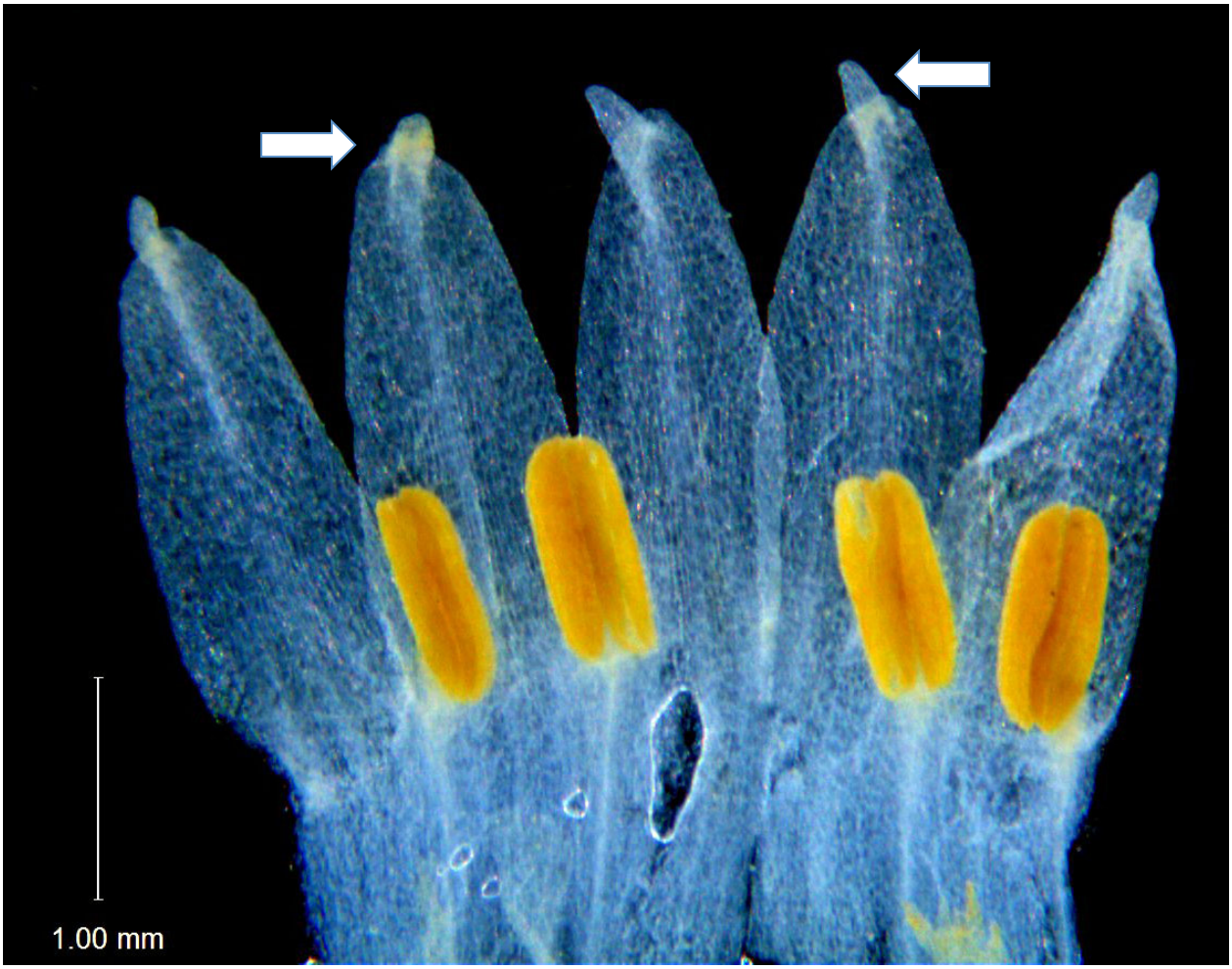

Figure 7. A dissected corolla of *Cuscuta chapalana*. Note the cylindrical stomatiferous protuberances (identified by white arrows) on the tips of the corolla lobes.

Image taken with a SMZ1500 Stereomicroscope.

Objectives

The overall goal of this thesis is to gain more knowledge about the multicellular protuberances (EFNs and SPs) of *Cuscuta* by studying their (a) morphological diversity, (b) evolution, as well as the (c) function of the stomatiferous protuberances. In order to meet this goal, three objectives were devised:

a) To observe and document the morphological diversity of the multicellular protuberances throughout the entire genus.
b) To determine the evolutionary trends of the multicellular protuberances in *Cuscuta*.
c) To determine the function of the unknown stomatiferous protuberances.

Hypotheses

My hypotheses that correspond to the related objectives include:

a) There will be multiple, dissimilar structures of multicellular protuberances found in the species of *Cuscuta* examined.
b) Multicellular protuberances arise through convergent evolution.
c) The primary function of the stomatiferous protuberances is transpiration.

Chapter Two: Materials and Methods

2.1. Taxon Sampling

The diversity of the multicellular protuberances (EFNs and SPs) was surveyed in 136 *Cuscuta* taxa (122 species and 14 varieties; Appendix A; Appendix B) from different herbarium and field specimens. Field specimens were preserved in Formalin-Acetic-Acid (FAA) (Ruzin, 1999). With the exception of the taxa known only for the type specimen, a minimum of three specimens per species was observed for each taxon.

2.2. Morphology

Stem fragments and whole flowers, removed from herbarium and fixed specimens, were rehydrated in 50% ethanol and examined under a Nikon SMZ1500 stereomicroscope. Images of stems, flowers, EFNs, and SPs were taken using the PaxCam Arc digital camera and Pax-it 7.2 software (MIS Inc., 2011) to document observations. Images were also taken at higher magnification under a Nikon Eclipse 50*i* light microscope.

2.3. Micromorphology

Stem fragments, whole flowers, dissected calyces and corolla lobes with and without EFNs or SPs were dehydrated through an ethanol series (50%, 70%, 85%, 95%, 100%; one hour each) and subjected to a hexamethyldisilazane (HMDS) treatment for twenty-four hours as an alternative to critical-point drying (Wright et al., 2011). Samples were then air-dried in the fume-hood, mounted onto aluminum stubs with adhesive, and sputter-coated with 30 nanometers of gold particles using an Emitech K 550 sputter coater (Emitech, Ltd. Ashfort, USA). Images were taken using a Hitachi SU-1510 variable pressure scanning electron microscope (Hitachi Canada

Ltd., Mississauga, Ont.) at 3 kV. Measurements of stomata size on the flowers and stems were taken using Pax-it 7.4 software (MIS Inc., 2011). Stem stomatal densities and the number of stomata per SP were determined using the scanning electron images.

2.4. Anatomy

The structure of SPs was studied in *C. gronovii, C. costaricensis, C. cotijana, and C. bonafortunae.* For each taxon, twenty fragments of stems, whole flowers, and dissected corolla and/or calyx lobes with SPs were dehydrated using an ethanol series (70%, 85%, 95%, 100%; one hour each), transitioned to xylene, and infiltrated in Surgipath Blue Ribbon paraffin (Leica Microsystems; Ruzin, 1999). Samples in paraffin blocks were sectioned both longitudinally and transversally at 8-10µm using an American Optical Corp. microtome. Sections were then stained with Sass's safranin-fast green FCF (Ruzin, 1999), mounted onto slides using Acrytol, and observed under a Nikon Eclipse 50i light microscope. The stained specimens were imaged using a Paxcam digital arc camera and Pax-it 7.4 software (MIS Inc., 2011).

2.5. Ultrastructure

For the ultrastructure study, *C. costaricensis* and *C. cotijana* were collected in the field in Michoacan, Mexico between 11am-12pm. Stem fragments, as well as calyx and corolla lobes with SPs present, were dissected, subjected to a modified Spurr's Resin protocol (Fineran, 1982; Ma and Peterson, 2000) realistic for the field, and prepared for transmission electron microscopy (TEM). Samples were first placed in a solution of 3% glutaraldehyde and 2% paraformaldehyde in a 0.025M sodium phosphate buffer at a pH of 6.8 for twenty-four hours. Specimens were washed three times in the buffer, fifteen minutes for the first two washes and twenty-four hours for the last wash. Samples were brought back to the lab and post-fixed in 1% buffered Osmium

tetroxide for 1 hour. The specimens were washed in buffer three times and treated to an ethanol dehydration series (15%, 30%, 50%, 70%, 90%, 3 x 100%). Specimens were infiltrated and embedded in the Spurr's resin (Spurr, 1969) and polymerized at 70^{o}C. Embedded blocks were cut with a diamond ultra-knife at 80-100 nm, mounted onto formvar and carbon-coated copper grids, and post-stained with 5% uranyl acetate for 10 minutes and Reynold's lead citrate for 5 minutes (Reynolds, 1963). Sections were also stained using Sass's safranin-fast green FCF or Toludine Blue for optical microscopy (Ruzin, 1999). Images were taken with a Gatan Ultrascan digital camera and 'Digital Micrograph' software on a JEOL 2011 Transmission Electron Microscope at 200kV (Gatan Inc. 2007, Pleasanton, CA).

2.6. Field Experiments

Field experiments were conducted on two host-parasite systems: *Cuscuta gronovii* and *Solidago canadensis,* growing naturally in Canada (Waterloo, Ontario, 43°30'10.11"N, 80°29'36.59"W), and *Cuscuta costaricensis* and *Tithonia tubiformis* in Mexico (Abadiano, Michoacan, 19°59'45.18"N, 102°51'41.04"W). *Cuscuta gronovii* is a common riparian species found in Canada and U.S.A, and is without SPs on the flowers (Costea and Tardif, 2006). *Cuscuta costaricensis* is a common Mexican species with SPs both on the haustorial stems and the perianth (Costea et al., 2011a). For both host-parasite systems daytime observations were conducted at 12 pm-2 pm, in full sun, temperature of 25–29°C, and a relative humidity of 45–50%. Nighttime observations were conducted between 10 pm–12 am, temperature of 14–15°C, and a relative humidity of 45–50%.

2.6.1. Water Uptake

Transpiration of *Cuscuta* and host plants was estimated indirectly by measuring water uptake in the field, both during the day as well as the night. Water uptake was determined by measuring host's water uptake without *Cuscuta* but with its leaves, after which the leaves were removed and measurements were taken again. Darwin and Ganong's potometers were tested initially with good results but were abandoned because of the difficulty to set up the experiment for multiple plants in the field. Instead, after a comparison with the potometer results, a much simpler water uptake experiment using 10 mL graduate cylinders was implemented to determine water uptake. Five host plants were selected that were uniform in size for each species. *Solidago canadensis* host plants were cut at approximately 45 cm from the apex, each plant having roughly 50 leaves, whereas *Tithonia tubiformis* host plants were cut at approximately 30 cm from the apex, with 8 leaves on each plant. Additionally, five parasitized host plants with continuous ropes of *Cuscuta* attached were also selected; these were similar in size and number of leaves on the host plants as the previous samples. The number of dodder floral buds/flowers present on the host plants at the time of the readings was ca. 1300 for *C. gronovii* and ca. 1000 for *C. costaricensis*. An internode near the base of the host stems (with and without parasite) was cut with a fresh blade at 45°, placed into the 10 mL graduated cylinders, filled in advance with 8 mL of water, which were then sealed with Parafilm to prevent any evaporation. Therefore, ten plants were running at the same time: 5 host species with leaves without *Cuscuta*, and 5 host species with leaves with *Cuscuta*. This was done during the day and repeated at night. Recordings of the amount of water left in the cylinders were taken after 45 minutes, after which the leaves of the hosts (with and without *Cuscuta*) were removed and the same process was repeated with only the host's stems remaining. The water uptake values were recorded and comparisons within each

host-parasite system were completed by various Mann-Whitney U-test analyses using GraphPad InStat, version 3, San Diego, CA. Comparisons included hosts with *Cuscuta* and with leaves vs. hosts with *Cuscuta* and without leaves, as well as hosts without *Cuscuta* and with leaves vs. hosts without *Cuscuta* and without leaves.

2.6.2. Stomatal Conductance

The stomatal conductance of the host leaves with and without *Cuscuta,* the stomatal conductance of the stems of *Cuscuta* (attached to the host), as well as the flowers/floral buds of *Cuscuta* was determined. This was done on the same host-parasite systems as the water uptake experiment, as well as on the same days. Readings were taken on five different plants, and on five different leaves, stems, or flowers per plant. This was completed using an AP4 Leaf Porometer (Model Ap4, Delta-T Devices, Burwell, Cambridge, UK). Values were recorded in Excel 2010 and comparisons within each host-parasite system as well as between the two hosts were completed by various Mann-Whitney U-test analyses with GraphPad Instat, version 3, San Diego, CA. Comparisons included stems with SPs vs. stems without SPs, *Cuscuta* flowers with SPs vs. *Cuscuta* flowers without SPs, as well as host leaves with *Cuscuta* present vs. host leaves without *Cuscuta* present.

2.7. Character Evolution and Geographical Distribution

There were little to no previous descriptions of the stomatiferous protuberances or extrafloral nectaries of *Cuscuta.* Due to the unresolved position of *Cuscuta* within the Convolvulaceae (Stefanović and Olmstead, 2004), as well as the little information known about EFNs and SPs in other members of the Convolvulaceae, the reconstruction of ancestral character states in *Cuscuta* was analysed by the distribution of character states in-group only (Welsh et al., 2010; Wright et al., 2011; Riviere et al., 2012). A thorough survey and analysis of the multicellular protuberances with stomata, including both the SPs and EFNs, was completed using various microscopy techniques. Eight characters were defined, 6 qualitative and 2 quantitative, and scored in the 122 species and 14 varities (Appendix B). Many of the images of the SPs were taken from the Digital Atlas of *Cuscuta* online (Costea, 2007 – onwards; online). The characters were mapped onto the recent genus phylogeny based on *rbcL* and nrLSU sequences (García et al., 2014). Using Mesquite 2.75 (Maddison and Maddison, 2011), scenarios of character evolution were analyzed using the parsimony reconstruction method, treating the character state changes as unordered. The Markov k-state 1 parameter model (MK1) of evolution was used (Maddison and Maddison, 2011). Two qualitative characters, types of MPs present on stems, and types of MPs present on the flowers, were also analyzed with the likelihood reconstruction method provided by the same software.

Correlation between the presence/absence of SPs on the calyx and corolla was determined using Pagel's method (Pagel, 1994) in Mesquite. Two average precipitation values (average annual precipitations and average precipitation during the three months of maximum flowering and beginning of fruiting) were determined for all 136 *Cuscuta* taxa to test for a possible association between a dry climate and the presence of floral SPs. The geographical data and

flowering/fruiting time was obtained from herbarium specimen labels. The two precipitation values for each species were collected using the 30 seconds resolution precipitation database from WorldClim – Global Climate Data (Hijmans et al., 2005) that was imported in DIVA GIS version 7.5 (Hijmans et al., 2001). Ten herbarium specimens per taxon were selected to represent the scale of the geographical distribution of each species, with the exception of species known only from the type collection. The annual average precipitations and average for the maximum flowering/beginning of fruiting precipitations were treated as continuous characters and were analyzed using the parsimony reconstruction and correlation methods of the PDAP package (Midford et al., 2002) implemented by Mesquite 2.75 (Maddison and Maddison, 2011).

Chapter Three: Results

3.1. Stomatiferous Protuberances on the Stems

3.1.1. Morphology and Diversity

Species of subgenus *Grammica* develop two functional types of stems during their life cycle: exploratory stems and haustorial stems. The exploratory (vegetative) stems have a smooth appearance, lacking SPs (Figure 8; Figure 9). Exploratory stems also have very low stomatal densities (< 1 per mm^2) (Figure 9). Haustorial stems are attached to the host by haustoria and have numerous SPs (20-30 per mm^2) on their surface (Figure 10). The SPs on the stems of the subgenus *Grammica* are more or less conical and 0.1-0.3 mm in length. Each SP usually has one distal stoma (Figure 11; Table 1) with stomata guard cells that range from 16-29 μm in length. During flowering and fruiting the exploratory stems disappear, leaving only haustorial stems with SPs as well as flowers/fruits to remain on the host (Figure 12).

Whereas *Grammica* has two functional types of stems (Figure 8; Figure 9; Figure 10), species of *Monogynella* develop only one functional type of stem, with extrafloral nectaries (EFNs) (Figure 3; Table 2; Appendix B). EFNs have guard cells ranging from 50–70 μm in length, and are only slightly raised. In some species, there are red-pigmented epidermal cells surround the EFN stomata (e.g., *C. japonica, C. lehmanniana,* and *C. lupuliformis*) (Figure 3). Similar to subgenus *Monogynella*, subgenera *Cuscuta* and *Pachystigma* have only one type of stem, but they are smooth and lack both SPs and EFNs (Table 2; Appendix B).

3.1.2. Stem evolution

The diversity of the stems among the *Cuscuta* subgenera has a phylogenetic significance. Parsimony reconstruction indicates that either subgenera *Monogynella* or *Cuscuta* stem types are the ancestral states. The likelihood reconstruction test favours the *Monogynella* type of stem as an ancestral character state for the entire genus (proportional likelihoods include: *Monogynella* type = 0.6332; *Cuscuta* type = 0.3558; *Grammica* type = 0.0109; Figure 13). *Cuscuta* is favoured as the common ancestor of subgenera *Cuscuta* and *Pachystigma* (proportional likelihood = 0.9582; Figure 13). Subgenus *Grammica* stem type is inferred as derived regardless of the ancestral reconstruction method used (Figure 13).

3.1.3. Stem anatomy

The anatomy of the two types of stems in subgenus *Grammica* is different. The exploratory stems, without SPs, have a smaller number of vascular bundles (Figure 14A) than the haustorial stems (Figure 14B). The stems also differ in the amount of xylem that is present within the bundles. More specifically, the vascular bundles in the exploratory stems having less xylem present (Figure 14A) than those in the haustorial stems (Figure 14B). There is also more starch grains present within the haustorial stems compared to the exploratory stems (Figure 14A-B), as well as more intercellular spaces within the stem cortex of the haustorial stems that connect to the SPs (Figure 14A-C). Both stems have a thick waxy cuticle present, but in the case of the SPs on the haustorial stems, the cuticle stops at the stomatiferous part of the SP (Figure 14C-D). The stem SPs consists of a lacuna (or substomatal chamber) at the base of the stoma (Figure 14C-D) that connects to the intercellular spaces in the stem cortex (Figure 14C-D).

Figure 8. The haustorial stems (with SPs) and exploratory stems (without SPs) of *Cuscuta cotijana* in the field in Mexico. Note that the haustorial stems (blue arrow) are twined around the host and have numerous SPs present, whereas the exploratory stems (white arrow) have a smooth appearance and no SPs. Flowers with crest-like SPs are also present. Photo taken by Mihai Costea.

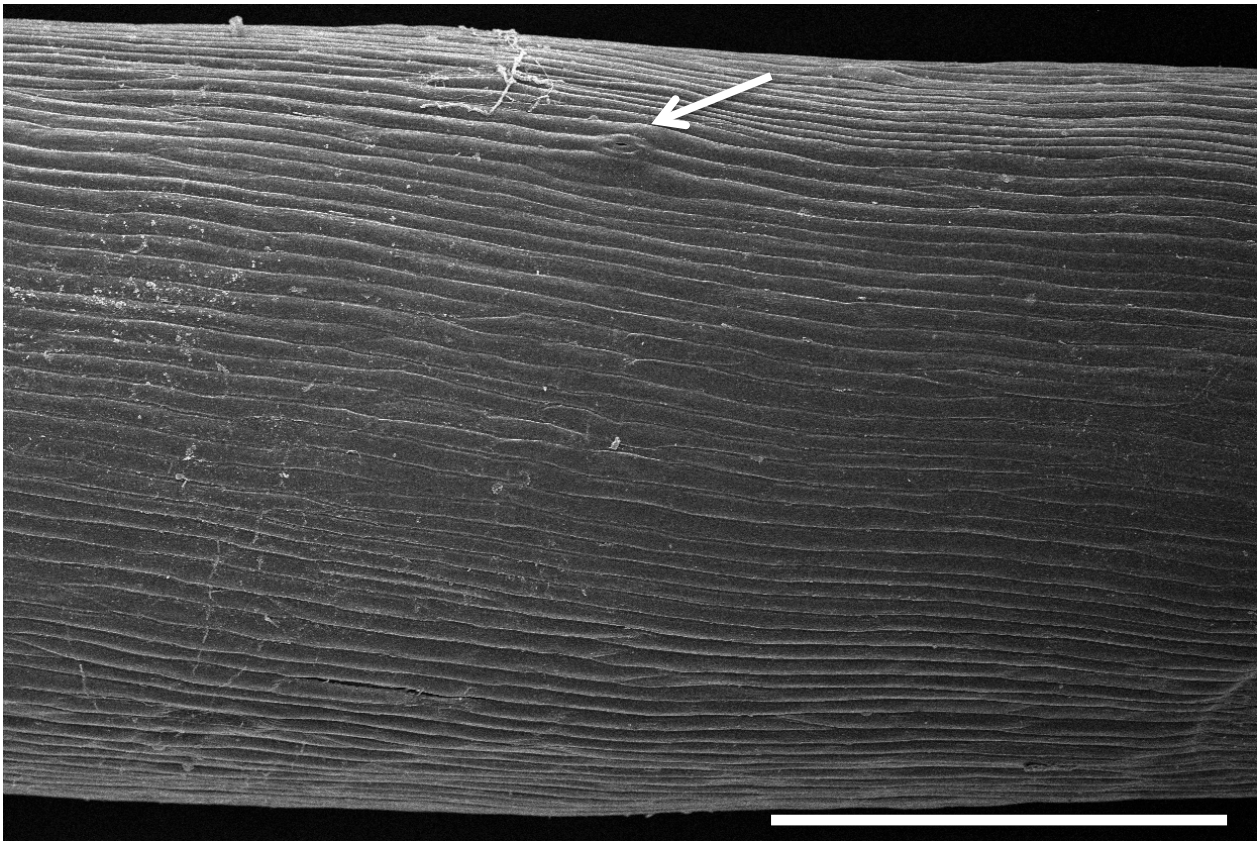

Figure 9. Exploratory stem segment of *Cuscuta cotijana*. This stem has a more or less smooth appearance because no stomatiferous protuberances present. There is sometimes a flattened stoma (identified by white arrow) present (typically < $1/mm^{-2}$). Image taken with the Hitachi SU-1510 variable pressure Scanning Electron Microscope at 3kV. Scale = 1mm

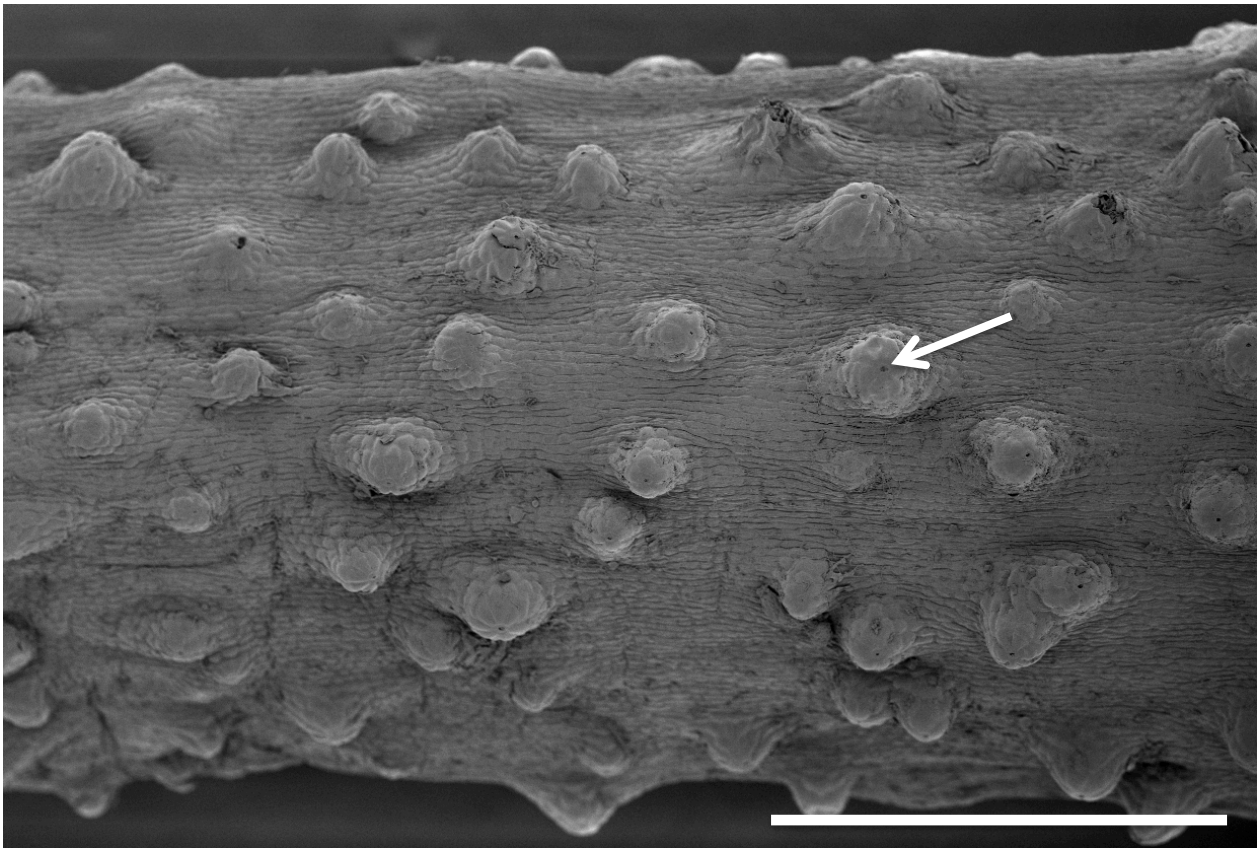

Figure 10. Haustorial stem segment of *Cuscuta cotijana*. Note the presence of multiple stomatiferous protuberances on the surface of the stem. Each protuberance has a distal stoma present (identified by a white arrow). Image taken with the Hitachi SU-1510 variable pressure Scanning Electron Microscope at 3kV. Scale bar = 1mm

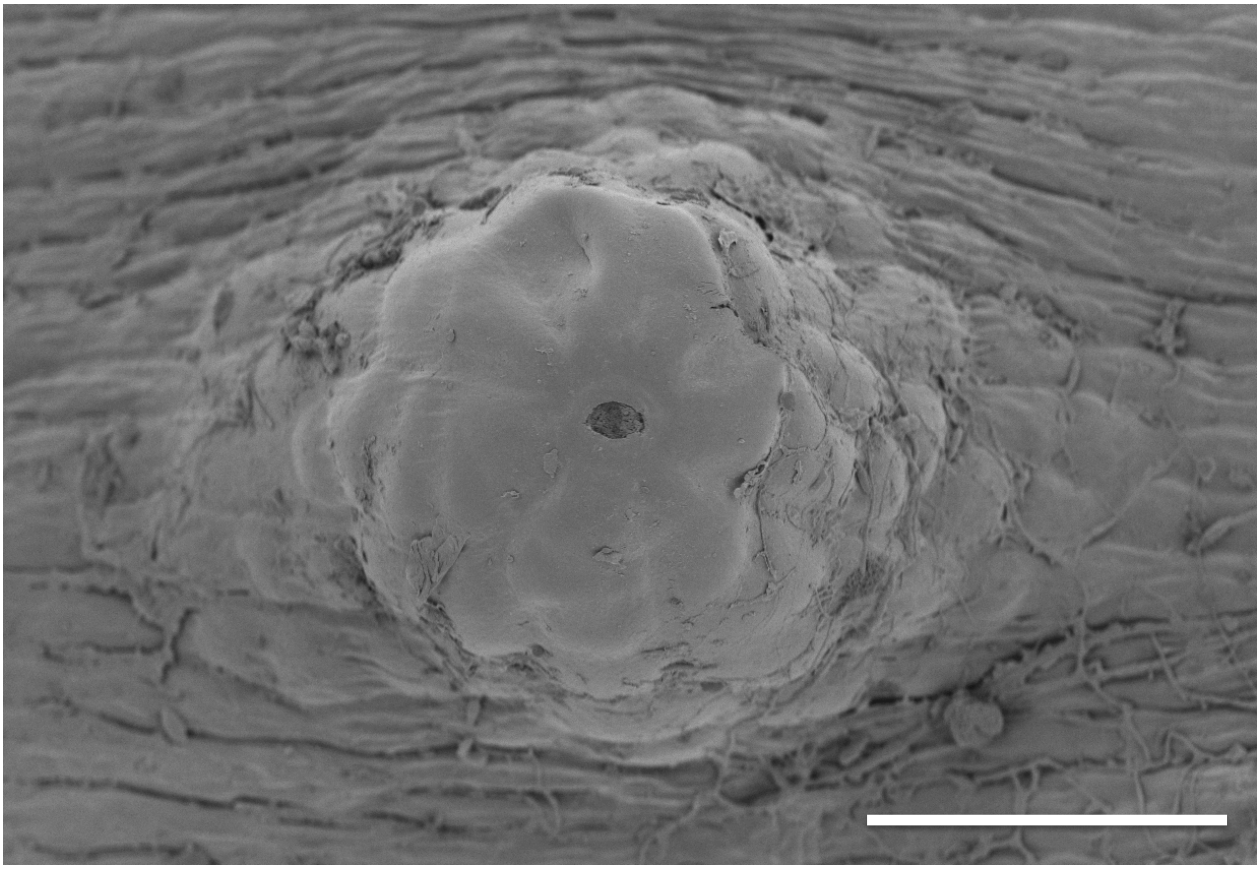

Figure 11. Top view of a stomatiferous protuberance on the haustorial stem of *Cuscuta cotijana*. Image taken with the Hitachi SU-1510 variable pressure Scanning Electron Microscope at 3kV. Note the presence of a unique stoma at the tip of the protuberance. Scale = 0.50mm

Figure 12. Inflorescences of *Cuscuta costaricensis* develop from haustorial stems and form a "rope" around the host's stem. Note that the exploratory stems (arrowheads) are going to disappear as flowering and fruiting progress.

Photo taken by Mihai Costea.

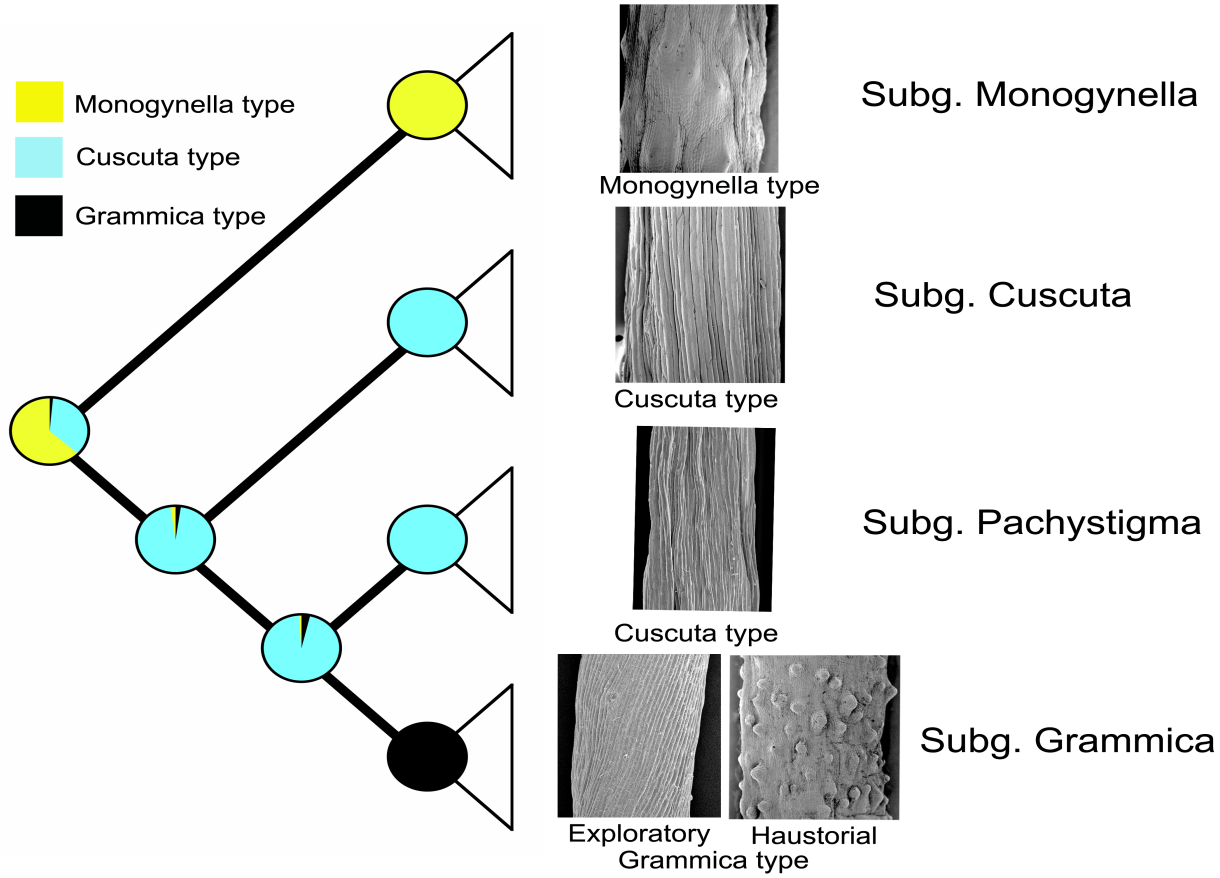

Figure 13. Likelihood ancestral reconstruction of the stem types in *Cuscuta* made in Mesquite. The stems of *Monogynella* have only one type of stem with extrafloral nectaries. Subgenera *Cuscuta* and *Pachystigma* stems have one type of stem with neither extrafloral nectaries nor stomatiferous protuberances (SPs). The stems of *Grammica*, inferred as derived, have two functional types of stems, exploratory (vegetative), with low stomatal densities but no SPs, and haustorial (reproductive), with numerous SPs.

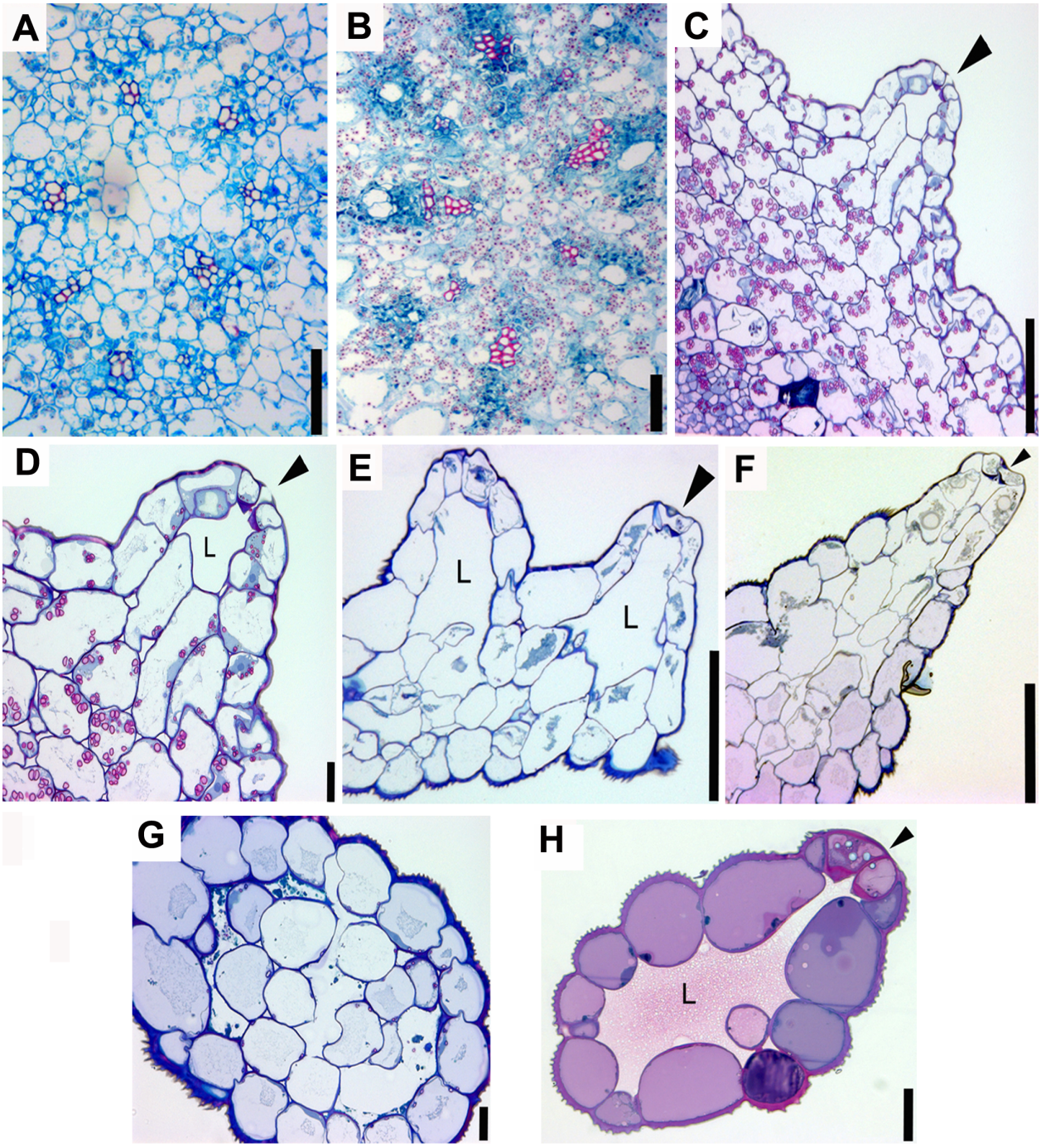
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Figure 14. Anatomy of the stem and flower SPs in *Cuscuta*. A-B Transversal stem sections stained with Safranin O and Fast Green FCF. (A) Part of a cross-section through the exploratory stem of *C. cotijana*; note the lignin stained red. (B) Part of a cross-section through the haustorial stem of *C. cotijana;* note the more extensive xylem development. C-D Stem SPs stained with safranin O and fast green FCF. (C) SP on a haustorial stem of *C. costaricensis* with presence of starch grains in the cortex parenchyma. (D) Higher magnification of a haustorial stem SP of *C. costaricensis*; note lacuna/substomatal chamber (L) under the stoma. E-I Flower SPs stained with safranin O and fast green FCF. (E) Longitudinal section through crest-like SPs on the calyx of *C. cotijana*. (F) Longitudinal section through the cylindrical SP on the corolla of *C. costaricensis*. (G) Tranversal section through cylindrical SP of the corolla lobe of *C. costaricensis*. (H) Transversal section through the tip of the corolla of *C. costaricensis* but a longitudinal section through the stoma; note the large lacuna/substomatal chamber under the stoma.

Arrowheads pointing to distal stomata; L = Lacuna.

Scale bar = 100 μm (A-F); 20 μm (G-I).

Photos C-H taken by Susan Belfry.

Table 1. Number of stomatiferous protuberances (SP) and the corresponding number of stomata on haustorial stems and flowers/floral buds taken from ten samples. SPs are always absent from exploratory stems.

Species	Haustorial stems		Flowers		
	Number of SPs (mm^{-2})	Number of stomata/SP	Number of SPs/flower	Number of stomata/SP	Number of stomata/flower
C. costaricensis	20 ± 3	1.1	5	6 ± 2	30 ± 4
C. bonafortunae	30 ± 4	1.2	10	5 ± 2	50 ± 5
C. cotijana	22 ± 3	1.2	5 ± 3	10 ± 2	55 ± 5
C. gronovii	18 ± 2	1.1	0	0	0

Table 2. Multicellular protuberance characters surveyed and their representative codes and states.

Character	Character states
Stems	0 = All stems have ENs (*Monogynella* type). 1 = All stems without SPs
1. Types of stems	or ENs (*Cuscuta* type). 2 = Exploratory stems without SPs and reproductive stems with SPs (*Grammica* type; see text and Fig. 1).
Flowers	
2. Types of multicellular protuberances present on flowers (calyx or calyx and corolla)	0 = SPs or ENs absent; 1 = ENs present; 2 = SPs present
3. Shape of SPs on calyx lobes	0 = Both SPs and ENs absent; 1 = ENs present; 2 = SPs with stomata found on fleshy area of bract/calyx lobe ("diffuse" SPs); 3 = SPS dome-like; 4 = SPs conical to cylindrical; 5 = SPs crest-like.
4. SP position on bracts and calyx lobes	0 = ENs or SPs absent; 1 = ENs present; 2 =SPs subterminal (dorsal, under the tip of calyx or bract lobes) or terminal (continuing tips of calyx or corolla lobes); 3 = SPs several along the midvein; 4 = SPs basal
5. Average number of stomata per calyx lobe SP	
6. Shape of SPs on corolla lobes	0 = ENs or SPs absent; 1 = SPs with stomata on fleshy area of corolla lobe (“diffuse”); 2 = SPs dome-like; 3 = SPs conical to cylindrical; 4 = SPs crest-like
7. SP position on corolla lobes	0 = ENs or SPs absent; 1 = SPs subterminal (dorsal, under the tip of corolla lobes) or terminal (continuing tips of corolla lobes); 2 = SPs several along the midvein
8. Average number of stomata per corolla lobe SP	

3.2. Stomatiferous Protuberances on the Flowers

3.2.1. Morphology and Diversity

Floral SPs are found on all the species of subgenera *Cuscuta* and *Pachystigma* examined, and on 24 species of subgenus *Grammica*. Floral SPs range from 0.2 mm – 1 mm in length, and bear up to 10 stomata on their distal part (Table 1), ranging from 15-25 μm in length. Floral SPs differentiate before the androecium and gynoecium and vary in shape and size among the different species and clades. Floral SPs may develop only on the bracts and calyx lobes in some species in subgenus *Grammica* (e.g., *C. cotijana* (Figure 15D, 17E-F), *C. iguanella* (Figure 15E), *C. alata* (Figure 15F, Figure 17G), and *C. draconella* (Figure 15H)), and on all of the species of subgenus *Pachystigma* (Figure 16H-I, Figure 18H; Appendix B). Floral SPs may develop on the bracts, calyx, as well as the corolla lobes of some species in subgenus *Grammica* (e.g., *C. boldinghii* (Figure 15A-B), *C. chapalana* (Figure 15C), and *C. costaricensis* (Figure 17A-B)) and on all of the species in subgenus *Cuscuta* (Figure 16F-G, Figure 18E-F; Appendix B). The position of the floral SPs can also vary depending on the species. These positions include: subterminal SPs on the calyx and/or corolla lobe (e.g., *C. boldinghii* (Figure 15A-B), *C. costaricensis* (Figure 17A-B), and *C. warneri* (Figure 16B, Figure 17J)), basal SPs on the calyx and/or corolla lobe (e.g., *C. desmouliniana* (Figure 15I), *C. werdermannii* (Figure 16A, Figure 17I), and *C. tuberculata* (Figure 16C)) or multiple SPs along the midvein of the calyx lobe (e.g. *C. cotijana* (Figure 15D, 17E-F), *C. iguanella* (Figure 15E), and *C. alata* (Figure 15F, Figure 17G)).

The shapes of the floral SPs also differ across the subgenera. Within *Cuscuta* and *Pachystigma*, the SPs have a “diffuse” shape (Figure 16F-I; Figure 18E-F & H) because they are not as distinct morphologically on the calyces and corollas as the floral SPs of *Grammica*. They

do however have distal stomata, similar to those of subgenus *Grammica*, on the SPs (Figure 18G-H). The shapes of the floral SPs in subgenus *Grammica* vary, including dome-like (Figure 15H-I), with 1 or 2 distal stomata (Figure 17D), conical to cylindrical (Figure 15A-C & G, Figure 17A-B & I), with 2-5 distal stomata (Figure 17C), and crest-like (Figure 15D-F, Figure 17E-G), with 5-10 stomata on the entire SP, but a single stoma on each of the tips of the crest (Figure 17F, H). Floral SPs differ from the EFNs that are found on the flowers of *Monogynella*. There are no EFNs on the corolla lobes (Figure 16D), but they are present on the calyx lobes (Figure 16E). The stomata are more or less flat with a smaller stomatal pore (Figure 18A-B). More specifically, on the corolla lobes stomata are present but they are not associated with EFNs or SPs (Figure 18C-D).

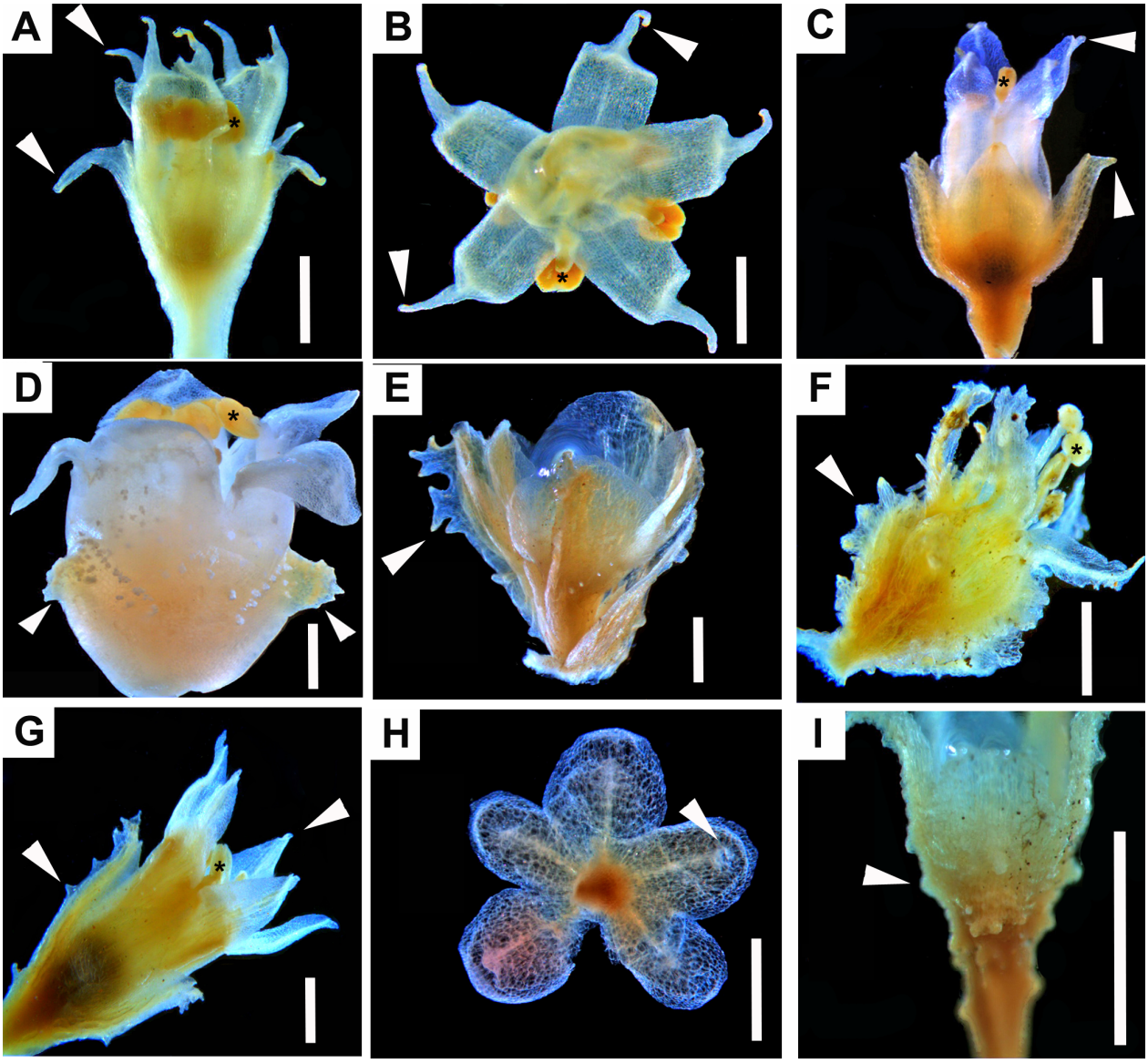
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Figure 15. Diversity of the stomatiferous protuberances (SPs) in *Cuscuta,* subgenus *Grammica,* as seen with the SMZ1500 Stereomicroscope. (A) Flower with cylindrical, subterminal SPs on the tips of the corolla lobes and calyx lobes (*C. boldinghii*). (B) Dissected calyx with cylindrical, subterminal SPs on the tips its calyx lobes (*C. boldinghii*). (C) Flower with conical to cylindrical, subterminal SPs on the tips of corolla and calyx lobes (*C. chapalana*). (D) Flower with crested SPs along the middle of the calyx lobes (*C. cotijana*). (E) Dissected calyx with more defined, crested SPs along the midvein of the calyx lobes (*C. iguanella*). (F) Flower with crested SPs along the midvein of the calyx lobes (*C. alata*). (G) Flower with conical SPs on the calyx and corolla lobes (*C. insolita*). (H) External view of a dissected calyx with domed SPs along the midvein of the calyx (*C. draconella*). (I) External view of a dissected calyx with domed SPs on the base of the calyx and on the calyx tube (*C. desmoulinana*).

White arrowheads point to the SPs on the flowers; Black asterisk (*) indicates the orange stamens of the flowers. Scale Bar = 1mm.

Images 15D-I taken by Mihai Costea.

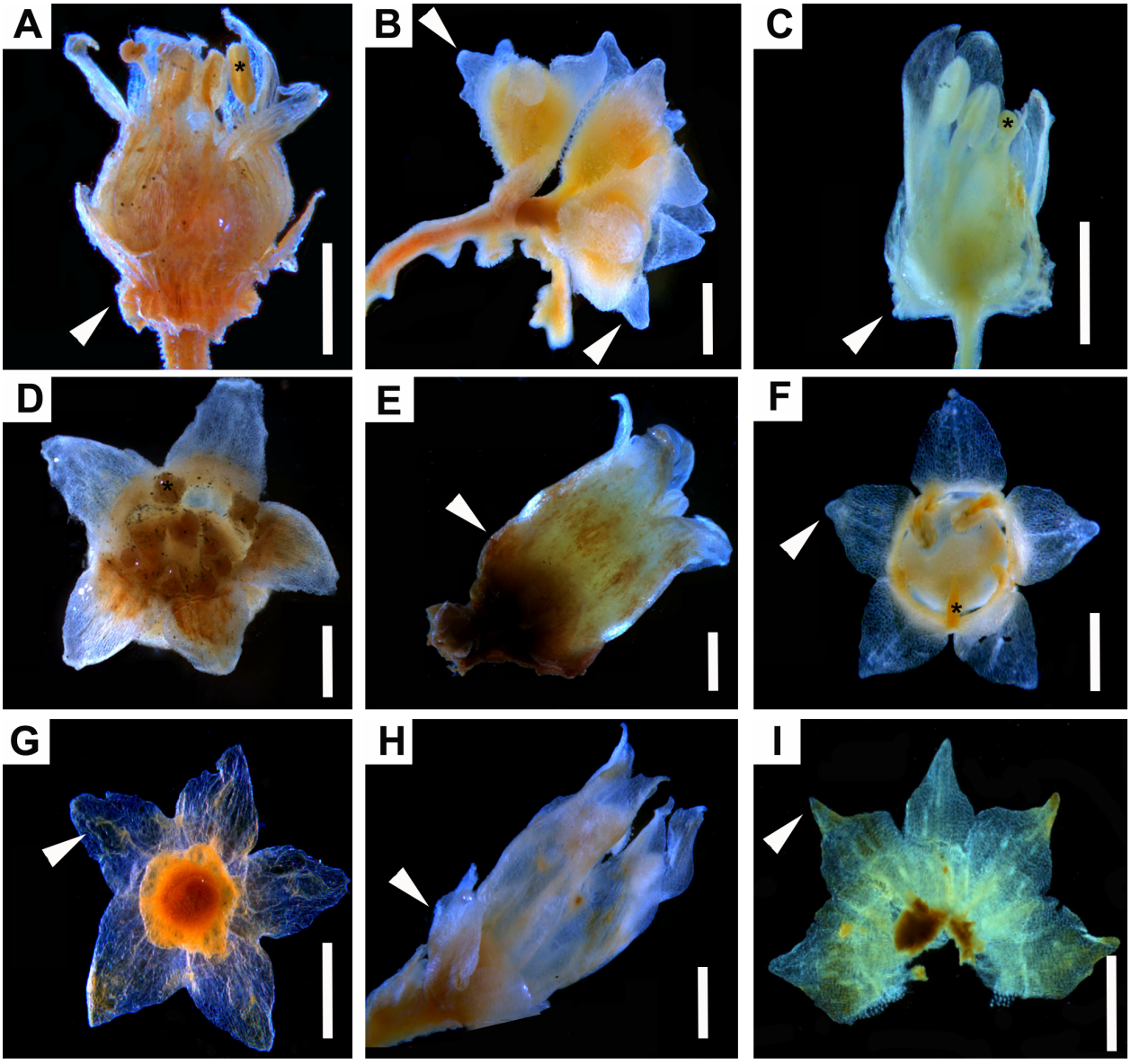
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Figure 16. Diversity of the multicellular protuberances (SPs and EFNs) in *Cuscuta* as seen under the SMZ1500 Stereomicroscope. A-C. Subgenus *Grammica* SPs. (A) Flower with conical to cylindrical SPs on the base of the calyx (*C. werdermanii*). (B) Flowers with conical, subterminal SPs on the calyx lobes (*C. warneri*). (C) Flower with crested SPs on the base of the calyx lobes (*C. tuberculata*). D-E. Subgenus *Monogynella*. (D) Top view of flower with no EFNs present on the corolla (*C. cassythoides*). (E) Flower with flattened EFNs along the calyx lobes (*C. japonica*). F-G. Subgenus *Cuscuta* SPs. (F) Top view of flower with diffused SPs along the corolla lobes (*C. epithymum*). (G) External view of a dissected calyx with diffused SPs along the calyx lobes (*C. apppendiculata*). H-I. Subgenus *Pachystigma* SPs. (H) Flower with diffused SPs along the calyx lobes (*C. natalensis*). (I) Dissected calyx with diffused SPs along the lobes (*C. nititida*).
White arrowheads pointing to the MPs on the flowers; Black asterisk (*) indicates the orange stamens of the flowers. Scale Bar = 1mm.
Images 16B, D, F-I taken by Mihai Costea.

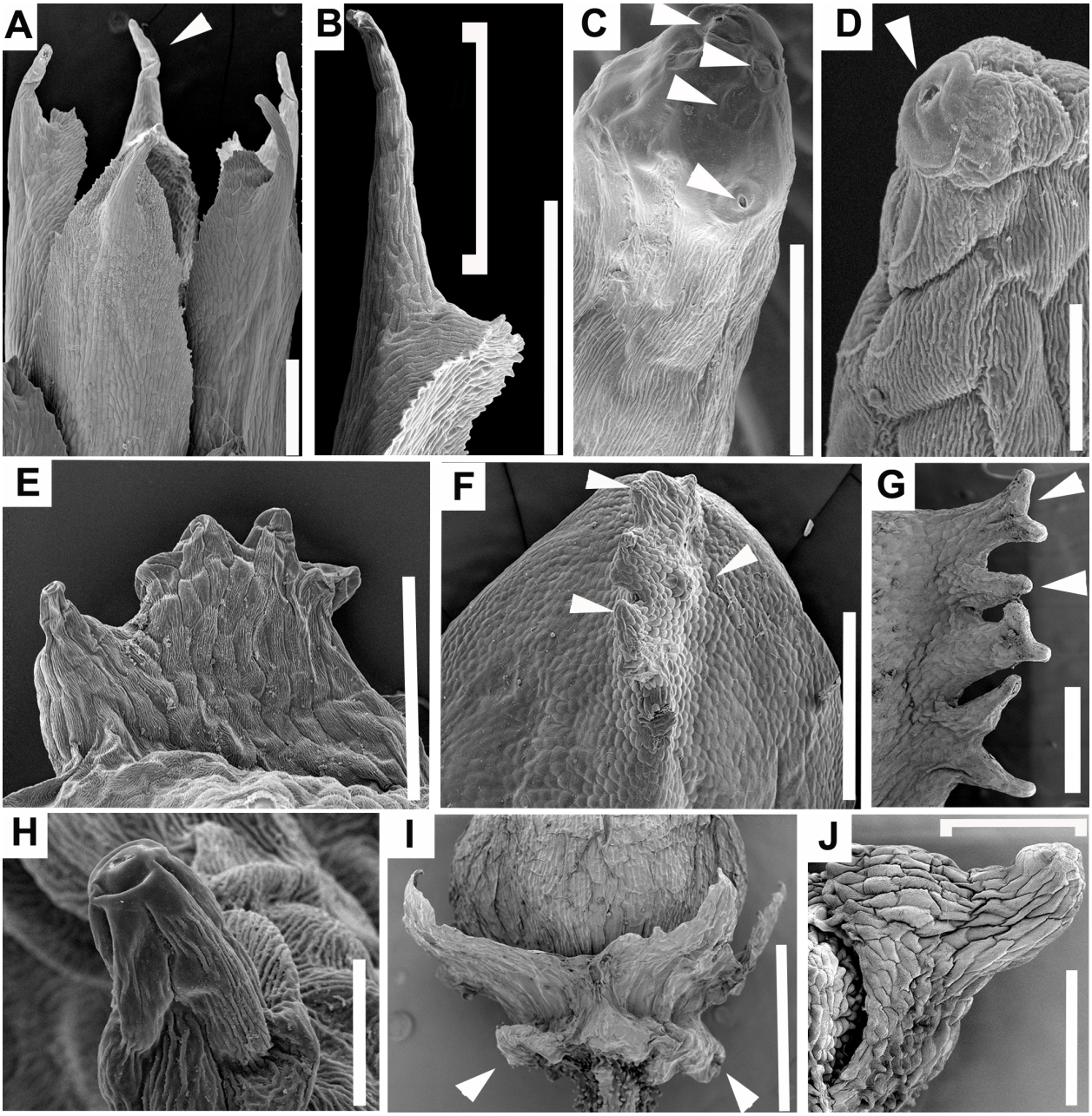
A
B
C
D
E
F
G
H
I
J

Figure 17. Micromorphology of the stomatiferous protuberances in *Cuscuta,* subgenus *Grammica,* examined under a Hitachi SU-1510 variable pressure Scanning Electron Microscope at 3kV. A-C. SPs on corolla lobes. (A) Cylindrical, subterminal SPs (arrowhead) on the tips of the lobes (*C. costaricensis*). (B) Higher magnification of a cylindrical SP (bracket) (*C. costaricensis*). Note the length of the SP and its connection to the corolla lobe. (C) Stomata on the tip of the cylindrical SP (arrowheads) (*C. costaricensis*). D-J. SPs on calyx lobes. (D) Stoma on the tip of a conical, subterminal SP (*C. chapalana*). Note the presence of one single stoma compared to the multiple stomata that can be seen in other species. (E) Dissected calyx lobe with a crested SP present (*C. cotijana*). (F) Top view of a crested SP with multiple stomata (arrowheads) (*C. cotijana*). (G) Crested SP on dissected calyx lobe (*C. alata*). Note the more defined branching of the SP. (H) Stoma on the tip of a crested SP (*C. cotijana*). (I) Conical SPs located on the base of the calyx (arrowheads) (*C. werdermanii*). (J) Conical, subterminal SP on dissected calyx lobe (bracket) (*C. warneri*).

Scale bars = 1mm (A, B, I); 0.5mm (C, E, F, G, J); 50 μm (D, H).

Images 17A-C & F-G taken by Mihai Costea.

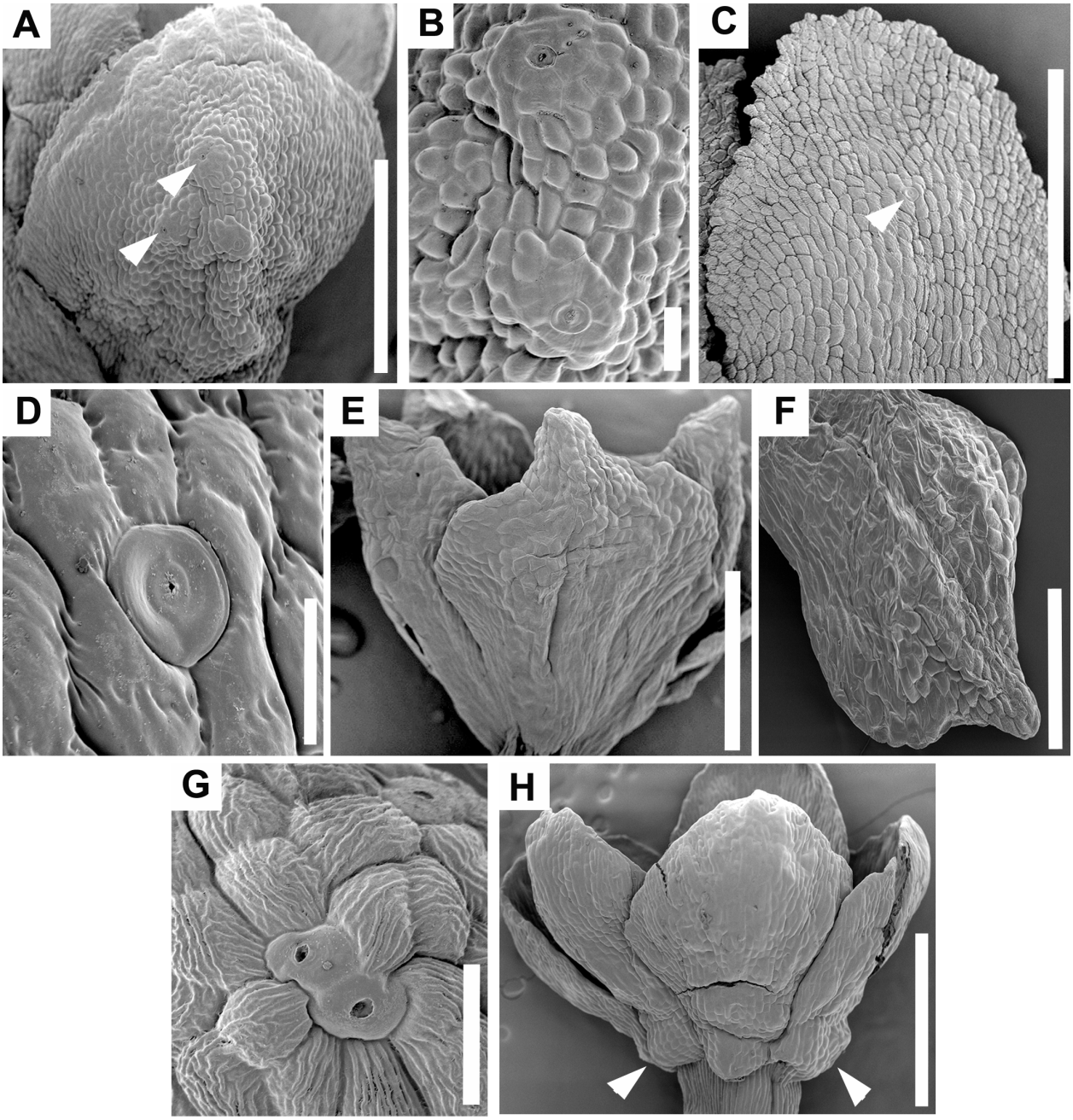
A
B
C
D
E
F
G
H

Figure 18. Micromorphology of the multicellular protuberances in *Cuscuta* examined under a Hitachi SU-1510 variable pressure Scanning Electron Microscope at 3kV. A-D Subgenus *Monogynella*. (A) Dissected calyx lobe with flattened EFNs (arrowheads) (*C. japonica*); (B) Higher magnification of stomata of EFNs on calyx lobe (*C. japonica*); (C) Corolla lobe with normal stoma (arrowhead) (*C. japonica*); (D) Higher magnification of normal stoma on corolla lobe (*C. japonica*); E-G Subgenus *Cuscuta*. (E) Dissected calyx with "diffuse" SPs along the lobes (*C. approximata*); (F) Diffuse SP along one calyx lobe (*C. approximata*); (G) Higher magnification of stomata on the tip of a diffused SP (*C. approximata*); H. Subgenus *Pachystigma*. (H) Dissected calyx with diffused SPs at the base (arrowheads) (*C. africana*). Scale bars = 1mm (A, C, E, H); 0.5mm (F); 100μm (B, I); 50μm (D, G).

Images 16A-E, G-H taken by Mihai Costea.

3.2.2. Floral evolution

Floral EFNs that are found in subgenus *Monogynella* are strongly supported as the ancestral state by the likelihood reconstruction (absent ENs or SPs: 0.0809, present ENs: 0.6763, present SPs: 0.2426). Floral SPs have evolved in nine of the fifteen major clades, and more specifically on 24 of the 122 species examined in the subgenus *Grammica* (Figure 19; Appendix B). Due to the amount of species with SPs versus the ones without SPs, the most parsimonious character state is absent SPs. Two clades of subgenus *Grammica* are entirely characterized by the presence of SPs (Sections *Grammica* (Clade H) and *Ceratophorae* (Clade K)), while the other seven clades contain species with SPs on either a single species (e.g. Section *Californicae* (Clade A)) or on a few species (e.g. Section *Lobostigmae* (Clade G)) (Figure 19).

Within the three subgenera that have floral SPs, *Grammica*, *Cuscuta*, and *Pachystigma*, the "diffuse" structures of SPs are inferred as the common ancestor (Figure 19). The shape of the floral SPs, including dome-like, conical/cylindrical, and crest-like, have evolved multiple times, with crest-like being the least common structure of floral SPs (Figure 19). Within subgenus *Grammica*, there is also the presence of polymorphic character states, where some species have more than one type of floral SP shape and/or position. For example, *Cuscuta draconella* (Clade A) may have dome-like SPs and conical SPs on the same plant, depending on the individual flower examined. This species can also have floral SPs along the calyx midvein, located basally or subterminal (Appendix B).

The presence/absence of the SPs on the flowers of *Cuscuta* are not independent according to Pagel's (1994) test of correlated discrete character evolution (difference log likelihood = 9.7268; p-value from 1000 simulations = 0.0). Species with corolla SPs always have SPs on their calyces as well, but only a few species with calyx SPs have corolla SPs (Figure 19; Appendix B). In subgenus *Cuscuta* there are corolla SPs on all the species, whereas in subgenus *Pachystigma*

there is none (Figure 19; Appendix B). In subgenus *Grammica* every species in Section *Ceratophorae* (Clade K) (Figure 19; Appendix B), one species in Section *Grammica* (Clade H), *C. alata* (Figure 19; Appendix B), and three species in Section *Lobostigmae* (Clade G), *C. cotijana, C. iguanella, C. insolita* (Figure 19; Appendix B) have SPs on both the calyx and corolla lobes.

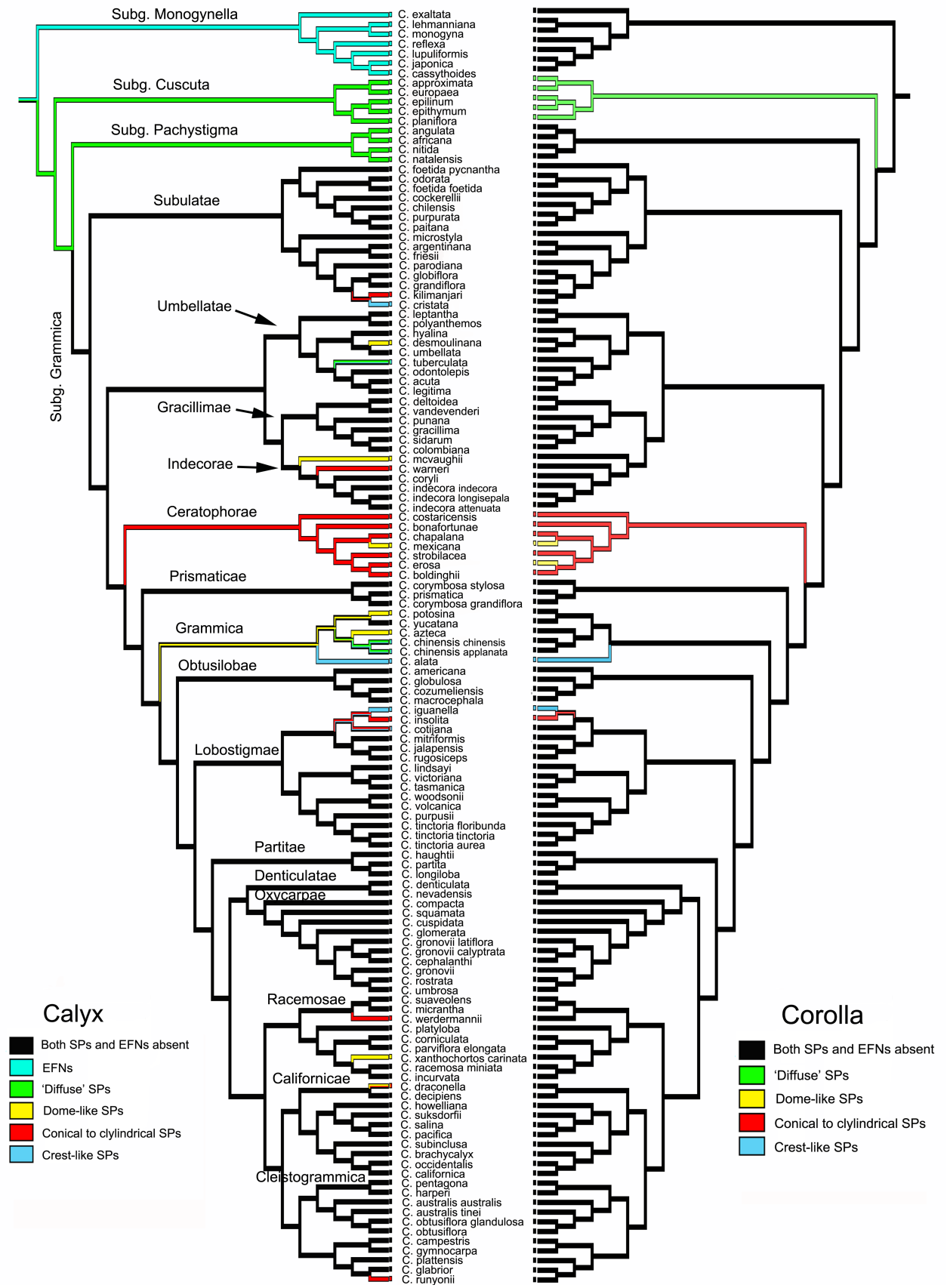
Subg. Monogynella
Subg. Cuscuta
Subg. Pachystigma
Subg. Grammica
Subulatae
Umbellatae
Gracillimae
Indecorae
Ceratophorae
Prismaticae
Grammica
Obtusilobae
Lobostigmae
Partitae
Denticulatae
Oxycarpae
Racemosae
Californicae
Cleistogrammica
C. exaltata
C. lehmanniana
C. monogyna
C. reflexa
C. lupuliformis
C. japonica
C. cassythoides
C. approximata
C. europaea
C. epilinum
C. epithymum
C. planiflora
C. angulata
C. africana
C. nitida
C. natalensis
C. foetida pycnantha
C. odorata
C. foetida foetida
C. cockerellii
C. chilensis
C. purpurata
C. paitana
C. microstyla
C. argentiniana
C. friesii
C. parodiana
C. globiflora
C. grandiflora
C. kilimanjari
C. cristata
C. leptantha
C. polyanthemos
C. hyalina
C. desmoulinana
C. umbellata
C. tuberculata
C. odontolepis
C. acuta
C. legitima
C. deltoidea
C. vandevenderi
C. punana
C. gracillima
C. sidarum
C. colombiana
C. mcvaughii
C. warneri
C. coryli
C. indecora indecora
C. indecora longisepala
C. indecora attenuata
C. costaricensis
C. bonafortunae
C. chapalana
C. mexicana
C. strobilacea
C. erosa
C. boldinghii
C. corymbosa stylosa
C. prismatica
C. corymbosa grandiflora
C. potosina
C. yucatana
C. azteca
C. chinensis chinensis
C. chinensis applanata
C. alata
C. americana
C. globulosa
C. cozumeliensis
C. macrocephala
C. iguanella
C. insolita
C. cotijana
C. mitriformis
C. jalapensis
C. rugosiceps
C. lindsayi
C. victoriana
C. tasmanica
C. woodsonii
C. volcanica
C. purpusii
C. tinctoria floribunda
C. tinctoria tinctoria
C. tinctoria aurea
C. haughtii
C. partita
C. longiloba
C. denticulata
C. nevadensis
C. compacta
C. squamata
C. cuspidata
C. glomerata
C. gronovii latiflora
C. gronovii calyptrata
C. cephalanthi
C. gronovii
C. rostrata
C. umbrosa
C. suaveolens
C. micrantha
C. werdermannii
C. platyloba
C. corniculata
C. parviflora elongata
C. xanthochortos carinata
C. racemosa miniata
C. incurvata
C. draconella
C. decipiens
C. howelliana
C. suksdorfii
C. salina
C. pacifica
C. subinclusa
C. brachycalyx
C. occidentalis
C. californica
C. pentagona
C. harperi
C. australis australis
C. australis tinei
C. obtusiflora glandulosa
C. obtusiflora
C. campestris
C. gymnocarpa
C. plattensis
C. glabrior
C. runyonii
Calyx
Both SPs and EFNs absent
EFNs
'Diffuse' SPs
Dome-like SPs
Conical to clylindrical SPs
Crest-like SPs
Corolla
Both SPs and EFNs absent
'Diffuse' SPs
Dome-like SPs
Conical to clylindrical SPs
Crest-like SPs

Figure 19. Character Evolution of MPs on the *Cuscuta* flowers. The left tree is the parsimony reconstruction of extrafloral nectaries (EFNs) and shape of stomatiferous protuberances (SPs) on the calyx and the right tree is the parsimony reconstruction of EFNs and shape of SPs on the corolla.

3.2.3. Anatomy and ultrastructure

The floral SP anatomy is similar to that of the stem SPs, consisting of a lacuna or substomatal chamber at the base of the stomata (Figure 14E-F, H) with intercellular spaces through the calyx and/or corolla lobes (Figure 14F-G). Similar to the stem SPs, the flowers have a waxy cuticle present except at the distal part of the SP where the stomata are present (Figure 14E-F, H). This is the only area on the parasite's body where there is no thick cuticle present. Another similarity to the stem SPs is the presence of the lacuna or sub-stomatal chamber. In the case of the floral SPs there is a larger lacuna (Figure 14E-F, H). Due to the similar, raised morphology, as well as the presence of stomata, the stem and floral SPs most likely serve the same role.

The ultrastructure of the guard cells and epidermal cells of the floral SP of *C. costaricensis* illustrated that there are multiple organelles present. More specifically, the guard cells of the stomata contain numerous amyloplasts, mitochondria, plastids, and endoplasmic reticulum (Figure 20A-D). The presence of these organelles in the guard cells of the SP stomata suggests that these cells are alive and metabolically active.

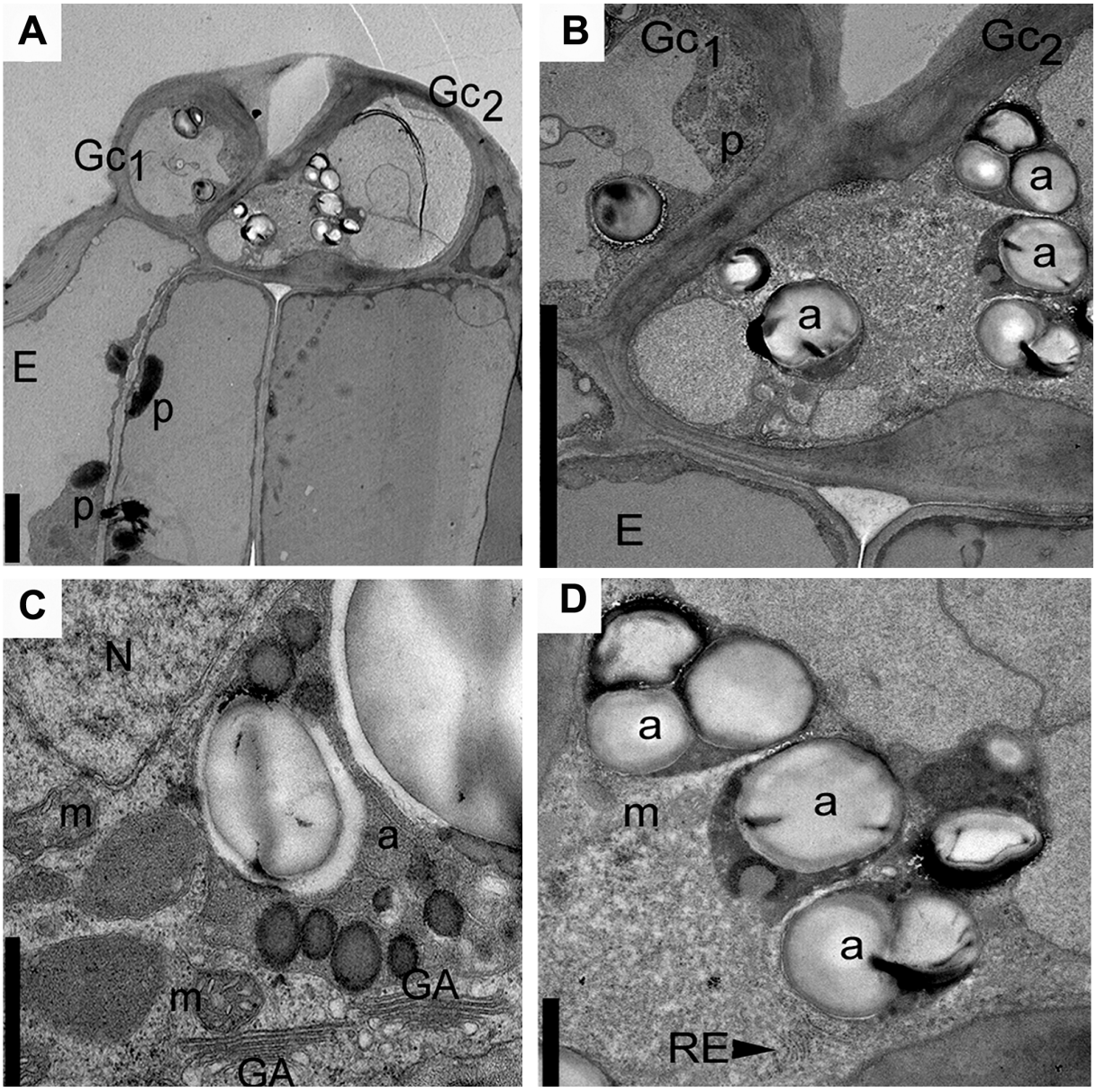
A
Gc1
Gc2
E
p
p
B
Gc1
Gc2
p
a
a
a
E
C
N
m
a
m
GA
GA
D
a
a
m
a
RE

Figure 20. Transmission Electron Microscopy (TEM) of a cylindrical floral SP of *Cuscuta costaricensis*. (A) Oblique section of guard cells and surrounding epidermal cells with plastids present; note that due to the plane of sectioning, the substomatal chamber is not present. (B) Higher magnification of guard cells with amyloplasts and plastids present. (C) Higher magnification of guard cell illustrating the organelles present, including mitrochondria, nucleus, and Golgi Apparatus. (D) Higher magnification of the other guard cell with multiple amyloplasts present, as well as mitochondria and endoplasmic reticulum.

Gc_1, Gc_2, = guard cells; E = epidermal cell; p = plastid; a = amyloplast; N = nucleus; m = mitochondria; GA = Golgi apparatus; RE = Endoplasmic reticulum.

Scale bars: A–B, 10 μm; C–D, 1 μm.

Images taken by Susan Belfry.

3.3. Field Experiments

3.3.1. Water Uptake

The water uptake experiment revealed that hosts with *Cuscuta* (and SPs present) had a higher water uptake than the hosts without *Cuscuta* (and SPs). The stomata on the SPs were able to create a greater pull of water through the host to *Cuscuta* in both parasite-host systems. When *Tithonia tubiformis* plants (with leaves) were infested by *C. costaricensis*, the species with SPs on both the haustoria stems and the flowers, there was a significantly higher water uptake than for the host plant without *Cuscuta* (Figure 21A; Table 3). When the leaves of *Tithonia tubiformis* plants were removed and all that was remaining was the host stem and the parasite, there was also a significantly higher water uptake than in *Tithonia tubiformis* plants without leaves and without *Cuscuta* (Figure 21B; Table 3).

Similar results were found with *Solidago canadensis* and *C. gronovii*, the species with SPs only on the haustorial stems. When *Solidago canadensis* plants (with leaves) were infested by *C. gronovii* there was only a slightly, however non-significant, higher water uptake than in the host without *Cuscuta* (Figure 21D; Table 3). When the leaves of *Solidago canadensis* plants were removed and all that remained was the host stem and *Cuscuta gronovii*, there was a significantly higher water uptake than in the *Solidago canadensis* plants without leaves and without *Cuscuta* (Figure 21E; Table 3).

Similar results were found when the experiment was repeated at night. During the night, the water uptake of the parasitized hosts (with leaves) was higher than that of the non-parasitized hosts (Figure 21C&F; Table 3), even though it was much more reduced than in the day (Figure 21A&D; Table 3). The non-parasitized host plants without leaves at night had a negligible/zero water uptake (Table 3) and therefore stats were not performed. Most of the water uptake observed

in the case of the parasitized hosts without leaves can be attributed to *Cuscuta* with SPs present on the stems (*C. gronovii* and *C. costaricensis)* and flowers (*C. costaricensis).*

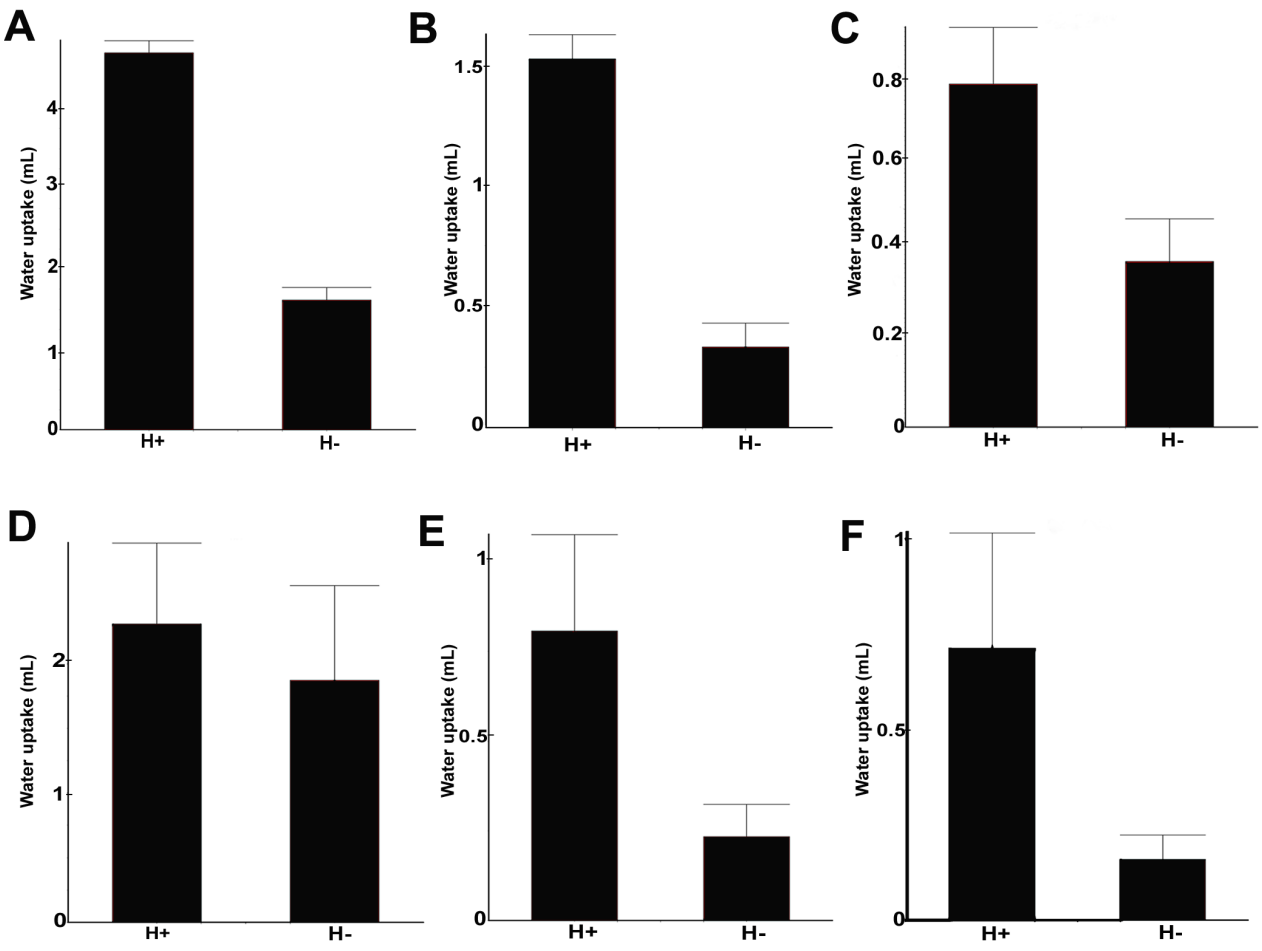
A
Water uptake (mL)
0
1
2
3
4
H+
H-
B
Water uptake (mL)
0
0.5
1
1.5
H+
H-
C
Water uptake (mL)
0
0.2
0.4
0.6
0.8
H+
H-
D
Water uptake (mL)
0
1
2
H+
H-
E
Water uptake (mL)
0
0.5
1
H+
H-
F
Water uptake (mL)
0
0.5
1
H+
H-

Figure 21. Comparison of the water uptake of host plants with and without parasite with leaves (A, D) as well as without their leaves (B, E) during the day. Water uptake of host plants with and without parasite with leaves was also compared during the night (C, F). Water uptake was assessed in the field using a modified potometer. Statistical testing was performed using a Mann Whitney U-test. (A) *Tithonia tubiformis* with leaves, with *C. costaricensis* attached (H+) or without *C. costaricensis* attached (H-); (B) *Tithonia tubiformis* without leaves, with *C. costaricensis* attached (H+) or without *C. costaricensis* attached (H-); (C) *Tithonia tubiformis* with leaves, with *C. costaricensis* attached (H+) or without *C. costaricensis* attached (H-); (D) *Solidago canadensis* with leaves, with *C. gronovii* attached (H+) or without *C. gronovii* attached (H-); (E) *Solidago canadensis* without leaves, with *C. gronovii* attached (H+) or without *C. gronovii* attached (H-); (F) *Solidago canadensis* with leaves, with *C. gronovii* attached (H+) or without *C. gronovii* attached (H-). Note the standard deviation bars.

A, B, C, E & F are significantly different (<0.0001).

Table 3. Water uptake of parasitized and non-parasitized host plants during the day/night and Mann–Whitney U test comparisons within the two parasitic systems.

(H+) = parasitized host; (H-) non-parasitized host.

* = Differences not significant.

Parasitic system	Time	Variables	Water Uptake (ml)	p
Tithonia tubiformis +/- *Cuscuta costaricensis*	Day	(H+) with leaves	4.69 ± 0.15	p<0.0001
		(H-) with leaves	1.61 ± 0.16	
		(H+) without leaves	1.52 ± 0.33	p<0.0001
		(H-) without leaves	0.33 ± 0.098	
	Night	(H+) with leaves	0.77 ± 0.13	p<0.0001
		(H-) with leaves	0.37 ± 0.096	
		(H+) without leaves	0.34 ± 0.097	N/A
		(H-) without leaves	0	
Solidago canadensis +/- *Cuscuta gronovii*	Day	(H+) with leaves	2.31 ± 1.87	0.1220*
		(H-) with leaves	0.63 ± 0.73	
		(H+) without leaves	0.81 ± 0.27	p<0.0001
		(H-) without leaves	0.23 ± 0.089	
	Night	(H+) with leaves	0.71 ± 0.30	p<0.0001
		(H-) with leaves	0.16 ± 0.063	
		(H+) without leaves	0.39 ± 0.22	N/A
		(H-) without leaves	0	

3.3.2. Stomatal Conductance

The stomatal conductance of the non-parasitized and parasitized host plant leaves showed surprising results. Both host plants, *Solidago canadensis* and *Tithonia tubiformis*, had a higher stomatal conductance when they were not parasitized by *Cuscuta gronovii* and *Cuscuta costaricensis*, respectively, than when they were fully parasitized by them (Figure 22C-D; Table 4). This suggests that when the host plants are parasitized, they try to minimize their water uptake and therefore transpiration. The haustorial stems (with SPs) of both *Cuscuta gronovii* and *Cuscuta costaricensis* had a very high stomatal conductance rate that was significantly different from that of exploratory stems (without SPs) which had a close to zero rate (Figure 22A-B; Table 4). Similarly, the stomatal conductance of the flowers of *Cuscuta costaricensis*, with SPs, was very high and it was significantly higher than that of the flowers of *Cuscuta gronovii* that are without SPs (Table 4).

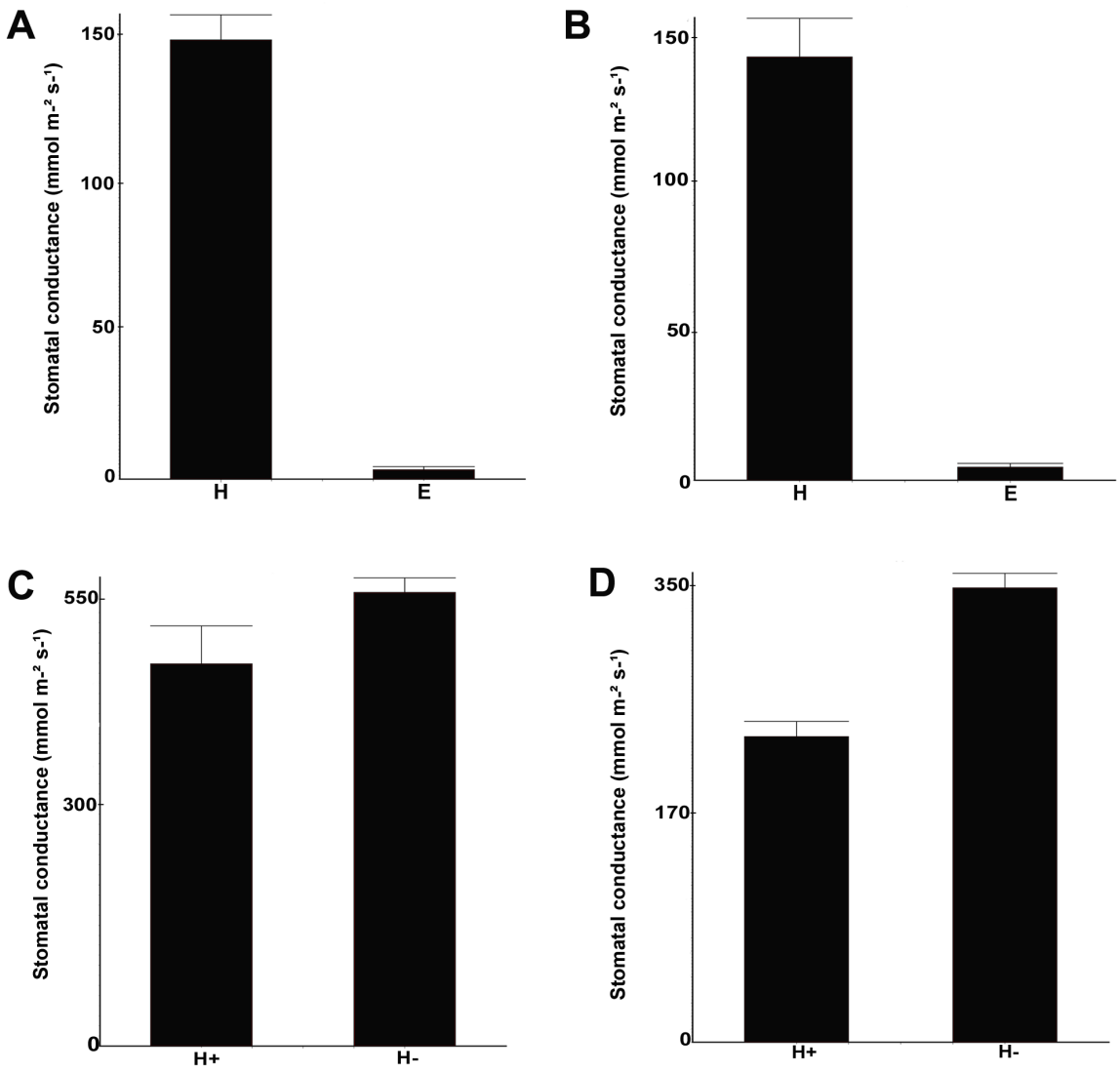
A
Stomatal conductance (mmol m-2 s-1)
150
100
50
0
H
E
B
Stomatal conductance (mmol m-2 s-1)
150
100
50
0
H
E
C
Stomatal conductance (mmol m-2 s-1)
550
300
0
H+
H-
D
Stomatal conductance (mmol m-2 s-1)
350
170
0
H+
H-

Figure 22. Comparison of stomatal conductance ($mmol/m^{-2}/s^{-1}$) of the haustorial (H) and exploratory (E) stems of Cuscuta (A, B) as well as the host leaves with (H+) and without (H-) *Cuscuta* present (C, D). The conductance was assessed in the field using an AP4 leaf porometer. Statistical testing was performed using a Mann-Whitney U-test. (A) *C. costaricensis*; (B) *C. gronovii;* (C) *Tithonia tubiformis* leaves with (H+) or without (H-) *C. costaricensis* present; (D) *Solidago canadensis* leaves with (H+) or without (H-) *C. gronovii* present. Note the standard deviation bars.

A, B, C & D = significantly different (<0.0001).

Table 4. Stomatal conductance and Mann–Whitney U test comparisons within two parasitic systems: *Tithonia tubiformis*/*Cuscuta costaricensis* and *Solidago canadensis*/*Cuscuta gronovii.*

Parasitic system	Variables	Stomatal conductance [mmol/m^{-2}/s^{-1}]	p
Tithonia tubiformis +/- *C. costaricensis*	Leaves of parasitized host (H+)	473.79 ± 47.49	p <0.0001
	Leaves of non-parasitized host (H-)	562.33 ± 18.40	
	Haustorial stems of *Cuscuta*	148.40 ± 8.52	p<0.0001
	Exploratory stems of *Cuscuta*	3.2400 ± 0.99	
	Flowers/floral buds of *Cuscuta*	118.62 ± 8.29	N/A
Solidago canadensis +/- *C. gronovii*	Leaves of parasitized host (H+)	233.99 ± 11.65	p<0.0001
	Leaves of non-parasitized host (H-)	348.28 ± 10.97	
	Haustorial stems of *Cuscuta*	138.43 ± 12.70	p<0.0001
	Exploratory stems of *Cuscuta*	4.2000 ± 1.36	
	Flowers/floral buds of *Cuscuta*	1.1330 ± 1.42	N/A

3.4. Geographical Distribution and Precipitation

When considering species with floral SPs, we examined their geographic distribution and found the corresponding precipitation values for the areas in which these species were located. We then determined if there were any patterns between the precipitation values of the areas where species with floral SPs were located. Most species with floral SPs grow in arid and/or semiarid areas (<250mm and 250-500mm/year), although species without SPs also grow in these areas. The average precipitation values, during the time of flowering and fruiting of each of the species, were determined and recorded. All the species with SPs in subgenus *Grammica* grow in areas with 90mm precipitations or less during the time of flowering/fruiting (Figure 23; Appendix B). For example, *Cuscuta cotijana*, a species that has SPs on the flowers, occurs in an area with an average annual precipitation of 1082 mm/year. However, when this species flowers and begins to fruit, during December-February, the average precipitation in this area is only 40mm (Figure 23; Appendix B). This pattern suggests that species with floral SPs have evolved in areas that are characterized by a dry season as a way to uptake more water from their hosts. *Cuscuta* utilizes the host as a "straw," and although the host may try to preserve water in the dry climates, can increase the amount of water that is being taken up by the host. It is important to note that there are some cases where a species, that does not have floral SPs, occupy areas with similar dry conditions during flowering/fruiting (Figure 22; Appendix B).

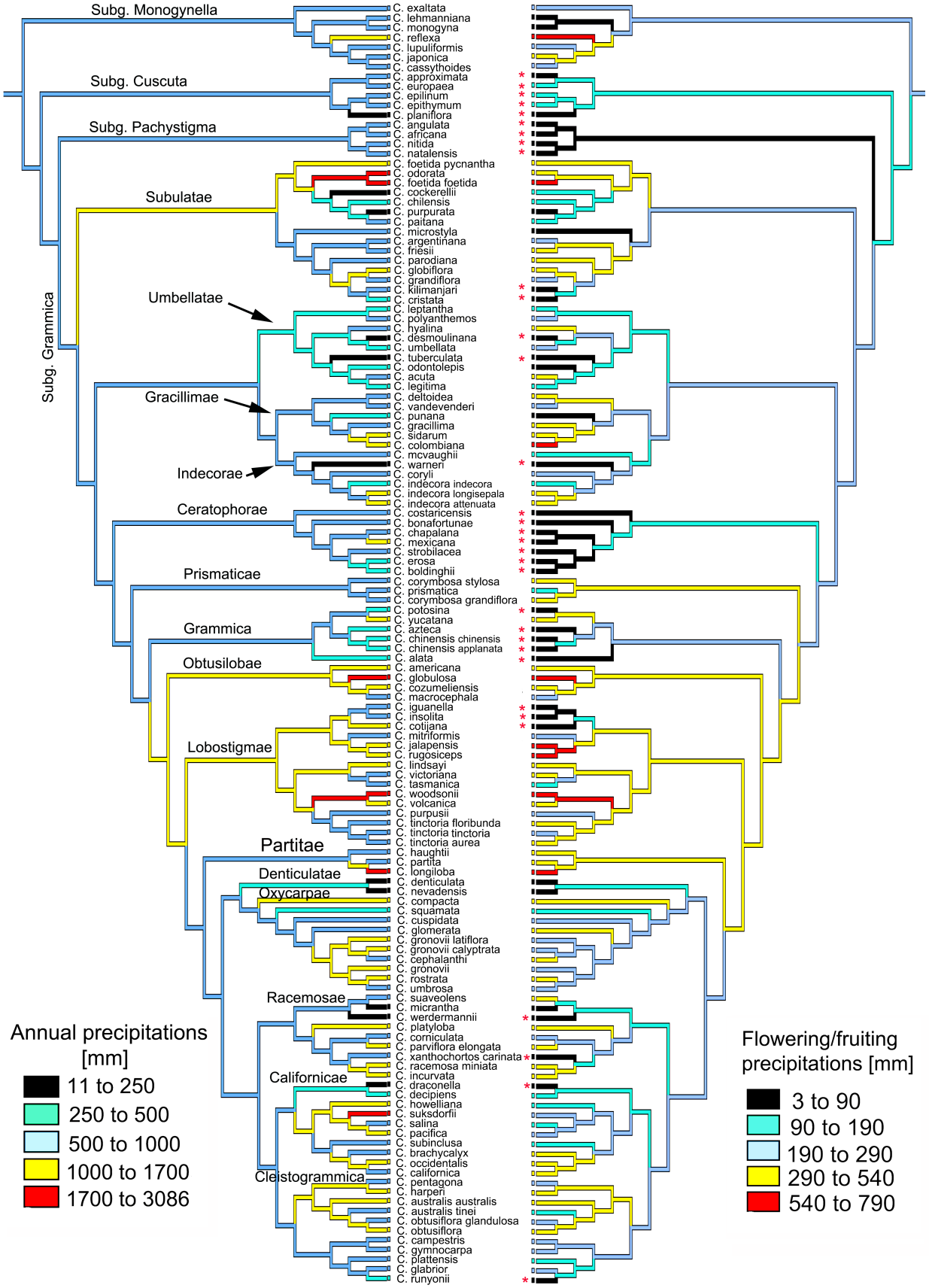
Subg. Monogynella
Subg. Cuscuta
Subg. Pachystigma
Subg. Grammica
Subulatae
Umbellatae
Gracillimae
Indecorae
Ceratophorae
Prismaticae
Grammica
Obtusilobae
Lobostigmae
Partitae
Denticulatae
Oxycarpae
Racemosae
Californicae
Cleistogrammica
C. exaltata
C. lehmanniana
C. monogyna
C. reflexa
C. lupuliformis
C. japonica
C. cassythoides
C. approximata
C. europaea
C. epilinum
C. epithymum
C. planiflora
C. angulata
C. africana
C. nitida
C. natalensis
C. foetida pycnantha
C. odorata
C. foetida foetida
C. cockerellii
C. chilensis
C. purpurata
C. paitana
C. microstyla
C. argentinana
C. friesii
C. parodiana
C. globiflora
C. grandiflora
C. kilimanjari
C. cristata
C. leptantha
C. polyanthemos
C. hyalina
C. desmoulinana
C. umbellata
C. tuberculata
C. odontolepis
C. acuta
C. legitima
C. deltoidea
C. vandevenderi
C. punana
C. gracillima
C. sidarum
C. colombiana
C. mcvaughii
C. warneri
C. coryli
C. indecora indecora
C. indecora longisepala
C. indecora attenuata
C. costaricensis
C. bonafortunae
C. chapalana
C. mexicana
C. strobilacea
C. erosa
C. boldinghii
C. corymbosa stylosa
C. prismatica
C. corymbosa grandiflora
C. potosina
C. yucatana
C. azteca
C. chinensis chinensis
C. chinensis applanata
C. alata
C. americana
C. globulosa
C. cozumeliensis
C. macrocephala
C. iguanella
C. insolita
C. cotijana
C. mitriformis
C. jalapensis
C. rugosiceps
C. lindsayi
C. victoriana
C. tasmanica
C. woodsonii
C. volcanica
C. purpusii
C. tinctoria floribunda
C. tinctoria tinctoria
C. tinctoria aurea
C. haughtii
C. partita
C. longiloba
C. denticulata
C. nevadensis
C. compacta
C. squamata
C. cuspidata
C. glomerata
C. gronovii latiflora
C. gronovii calyptrata
C. cephalanthi
C. gronovii
C. rostrata
C. umbrosa
C. suaveolens
C. micrantha
C. werdermannii
C. platyloba
C. corniculata
C. parviflora elongata
C. xanthochortos carinata
C. racemosa miniata
C. incurvata
C. draconella
C. decipiens
C. howelliana
C. suksdorfii
C. salina
C. pacifica
C. subinclusa
C. brachycalyx
C. occidentalis
C. californica
C. pentagona
C. harperi
C. australis australis
C. australis tinei
C. obtusiflora glandulosa
C. obtusiflora
C. campestris
C. gymnocarpa
C. plattensis
C. glabrior
C. runyonii
Annual precipitations [mm]
11 to 250
250 to 500
500 to 1000
1000 to 1700
1700 to 3086
Flowering/fruiting precipitations [mm]
3 to 90
90 to 190
190 to 290
290 to 540
540 to 790

Figure 23. Parsimony reconstruction of precipitation values in the areas of geographical distribution of various *Cuscuta* species. Left tree is the annual average precipitation values and the right tree is the flowering/fruiting average precipitation. Note that all of the species of subgenus *Grammica* that have floral SPs grow in areas with less or equal to 90mL during flowering and fruiting.

* = Species with floral SPs

Chapter Four: Discussion

4.1 Morphology and Evolution of the stems in *Cuscuta*

Stomatiferous protuberances (SPs) were first reported on the stems of *Cuscuta* by Mirande (1901) and were termed "protubérances" or "proéminences stomatifères." This initial observation was soon forgotten due to subsequent authors studying stems that were devoid of SPs, including: vegetative/exploratory stems of *Grammica* species that have low stoma densities (Figure 9) and thereby low transpiration rates, the stems of subgenus *Cuscuta* that are without SPs and also have low stomatal densities (Yuncker, 1943; Dawson et al., 1994), and/or the stems of subgenus *Monogynella* that have slightly raised stomata that serve as EFNs (Yuncker 1943; Schaffner, 1979). Stomatiferous protuberances with a transpiration role are unknown in other Convolvulaceae, which suggests that these structures are unique and have evolved only in *Cuscuta*. Similar to the extrafloral nectaries that are found in *Cuscuta* (subgenus *Monogynella*), EFNs can be found on the stems and sepals of other Convolvulaceae species, such as *Ipomoea* (Keeler, 1980) and *Merremia* (Blüthgen and Reifenrath, 2003). Nectar secreted by the EFNs in *Ipomoea* and *Merremia* serve as a reward for insects, more specifically ants, which provide protection to the plants (Bentley, 1977; Keeler, 1980). An explanation as to why species of subgenus *Monogynella* have extrafloral nectaries has not been clearly identified but has been thought to play a similar role in plant-insect mutualism, or as a role in removal of excess carbon from the plant (Jeschke et al., 1994b). The stems of *Cuscuta* have received minimal attention in terms of their morphology in the literature. The general concept that *Cuscuta* possess stems with low stomatal densities is common (Yuncker, 1932; Dawson et al., 1994; Jeschke et al., 1994a,b) and has contributed to the lack of awareness of the functionally different types of stems within the four subgenera.

Although we grouped stomatiferous protuberances (SPs) and extrafloral nectaries (EFNs) together as multicellular protuberances (MPs) in *Cuscuta*, they are quite different both morphologically and functionally. SPs have lost the ability to secrete nectar, as well as the red pigmentation in the epidermal cells found in the EFNs. SPs have a more raised morphology and are found on the haustorial stems (Figure 10), and on some flowers (Figure 5, 6, 7, 15 and 17; Appendix B). More specifically, all *Grammica* species have SPs on their haustorial stems and only on some flowers, whereas species in subgenera *Cuscuta* and *Pachystigma* do not have SPs on the stems and are found on all of the flowers instead (Figure 16F-I, 18E-H; Appendix B). The likelihood ancestral reconstruction for the stems in *Cuscuta* showed that the most ancestral state is likely the *Monogynella* type with the EFNs, followed by the stems of *Cuscuta* and *Pachystigma* with no EFNs/SPs present (Figure 13). The subgenus *Grammica* type, with two functional types of stems (haustorial and exploratory; Figure 13), was inferred as the most derived state. This is similar to that of the gynoecia in *Cuscuta,* where subgenus *Monogynella* had the most likely ancestral gynoecia with one-style, and subgenera *Cuscuta* and *Grammica* were more derived with two-styles (Wright et al., 2011). This pattern suggests that the species in the subgenera *Cuscuta, Pachystigma,* and *Grammica* develop more complex structures both in gynoecia and MPs that support the development of the species.

More recently, Hong et al. (2011) described the development of multicellular protuberances with "pores" on the stems (haustorial) of *Cuscuta campestris*, a species of subgenus *Grammica* that does not develop SPs on the flowers. The authors proposed that these structures are "pseudo-haustoria" that are non-functional, vascularized, and develop on the inside of the haustorial stem coils. This idea does not coincide with our findings and is not supported. SPs are not found on the inside of the haustorial stem coils, and they are not vascularized. There is no mention of the two functional types of stems, which is clearly observed in all of the species

of subgenus *Grammica*. The authors believe that because these protuberances form on the stems with haustoria, and are similar in shape to the haustoria, they are infact 'false' haustoria that are unable to attach to a host and therefore do not serve a purpose. Hong et al. (2011) provide a plate with some unclear, transversal sections of the stem with a protuberance present. The section is not in the correct plane and does not show the stoma. It is clear from our morphological and anatomical studies that these structures are not the same as the haustoria. In the subgenus *Grammica,* the two types of stems are distinct, with the SPs on the haustorial stems resembling those of the SPs on the flowers. The ability to produce two functional types of stems in the subgenus *Grammica* is a unique system that combines water conservation, during the vegetative stage of *Cuscuta*, with water loss, during the flowering and fruiting stage when the SPs develop on the stems.

4.2 Floral stomatiferous protuberances in *Cuscuta*

As previously mentioned, SPs are found on all of the haustorial stems of subgenus *Grammica*. Differing in shape and location are the floral SPs, which have been found on the calyx and/or corolla on species in the subgenus *Cuscuta*, on the calyx on species in the subgenus *Pachystigma* and found on the calyx, corolla and/or bracts on twenty-four species in the subgenus *Grammica* (Appendix B). Similar to the stems, and the gynoecia (Wright et al., 2012), the most ancestral state of the multicellular protuberances (MPs) on the flowers are the EFNs in subgenus *Monogynella* (Figure 19). The floral SPs in subgenus *Grammica* are inferred as the most derived, evolving in nine major clades (Figure 19; Appendix B). In these nine clades all the species with SPs grow in areas that receive average precipitations of 90 mm or less during the flowering and beginning of fruiting periods (Figure 23). As we predicted, species with SPs on the corolla lobes

also had SPs present on the calyx lobes, whereas some species had floral SPs only on the calyx lobes (Figure 19). Also as expected, the least common shape of the floral SPs was crest-like. Crest-like SPs are the most diverse and are found on a limited number of species in the subgenus *Grammica* (Figure 15D-F; Figure 17E-G) compared to dome-like and/or conical to cylindrical floral SPs that are more common (Figure 15A-C, G-I; Figure 17A-B, I-J). Floral SPs are not discussed in detail in the literature, as most studies focused on species within subgenus *Monogynella* with the previously documented EFNs (Schaffner, 1979; Jeschke et al., 1994a,b).

The presence of SPs on the flowers, as well as the haustorial stems, in areas with low precipitations during the flowering/fruiting period, suggests a possible, important link between the evolution of these structures and *Cuscuta* speciation. These data also aid in the idea that *Cuscuta* species with SPs are able to increase the amount of water uptake from their hosts. This adaptation to increase the amount of water uptake and overall transpiration rates in dry, arid weather conditions is unusual and thus deserves further research. Species such as *Cuscuta costaricensis*, *C. chapalana,* and *C. bonafortunae* grow vegetatively during the rainy season in June-August and start to flower at the beginning of the dry season (end of August/beginning of September). When flowering and fruiting begins, exploratory stems disappear completely and the bulk of the parasite consists of haustorial stems and inflorescences that remain on the hosts (Figure 12). The observations made in the field found that the *Cuscuta* species with floral SPs in dry, arid climates were able to flower and fruit during until the end of the dry season when the hosts were dried and had no leaves or flowers (Figure 24). This suggests that the parasite may be capable of using the host xylem in extreme conditions as a "straw" to absorb the water and/or minerals necessary to complete its life cycle. Further research to confirm this observation should be completed.

Although all species with floral SPs are found in dry, arid climates, there are also species without floral SPs that are found in the dry climates with low average precipitation values during flowering and fruiting, for example: *C. denticulata, C. micrantha, C. microstyla, C. nevadensis, C. odontolepis, C. punana,* and *C. purpurata*. Assuming that the SPs play a transpiration role to stimulate water uptake of the host, it is unknown how the species, without floral SPs, compensate for the uptake of water in these dry, arid conditions. It would be interesting to observe the amount of water uptake with only the haustorial stems with SPs in the species mentioned above and compare them to the haustorial stems with SPs as well as the floral SPs (e.g. *C. costaricensis)* in these dry, arid conditions. It would also be interesting to determine the water uptake of species from subgenera *Cuscuta* or *Pachystigma*, without SPs on their stems but only on their flowers. This was not a possibility due to the location of the species as well as the time and budget restraints of this project.

Figure 24. Flowers and fruits of *Cuscuta bonafortunae* on a dried perennial host (*Salvia tiliifolia)* with no leaves in Mexico. Note that *C. bonafortunae,* a species with SPs on both the haustorial stems and flowers, is still able to complete its life cycle in such extreme conditions. The parasite may be capable of using the host xylem, from the roots and stems, to absorb water and presumably minerals. Photo taken by Mihai Costea.

4.3. Water regime

The water uptake experiment proved successful, and allowed us to determine indirectly the water loss at the level of the SPs. In each scenario, water uptake was always higher when *Cuscuta* was present on the host. The only water uptake difference that was not found significant was in the case of *Solidago canadensis* with leaves with and without *Cuscuta gronovii* (Table 3). This exception could be explained in part by the lower stomatal conductance of the parasitized plant leaves in general (Table 4), as well as the possibility of a lack of uniformity among the host leaves or dodders, as suggested by the high standard deviations (Table 3). More specifically, when the host species with *Cuscuta* were collected we made sure the amount of *Cuscuta* present on the host stem was as uniform as possible among the five samples. This approximation could have affected the results. Although there was not a significant difference of the water uptake of *Solidago canadensis,* with leaves, and with or without *C. gronovii,* when the leaves were removed, and all that remained was a host stem with or without *C. gronovii,* there was a significantly higher water uptake (Table 3). The host stems, without leaves, and without the host present had minimal water uptake, whereas the host stem with just *Cuscuta* tightly wound around the stem and SPs present on the stems and/or flowers were able to take up significantly more water (Table 3). These results suggest that the presence of the SPs on the surface of the haustorial stems, and/or flowers, allow for a higher water uptake. It would be interesting to test the amount of water uptake between host stems with only the vegetative stems of *Cuscuta* present, when the SPs have not yet formed, and compare them to the host stems with the haustorial stems and SPs present. The stomatal conductance of both host-parasite systems were found significantly different but with the host plants without *Cuscuta* present having a higher stomatal conductance rate than when *Cuscuta* was present (Table 4). It has been reported in the past that host plants

have lower transpiration rates when a parasite is present to conserve as much water as possible (e.g. Shen et al., 2007). In these two host-parasite systems this is evident and would suggest that these parasitized hosts lower their transpiration rate to compensate for the water loss at the level of the SPs.

The stem and floral SPs have a raised morphology with a lacuna present underneath the stoma. More specifically, the floral SPs have a diverse structure and have also evolved in multiple clades in the subgenus *Grammica*. Although *Cuscuta* is functionally holoparasitic, and is believed to have a low transpiration rate (Kuijt and Toth, 1976; Hibberd et al., 1998), the results from the field experiments, including the porometer and potometer, support our hypothesis that these structures are involved in transpiration. Three of the four subgenera (*Cuscuta, Pachystigma,* and *Grammica*) have evolved SPs to enhance their transpiration, especially during flowering and fruiting (Figure 21A-E; Table 3). These elevated transpiration rates, similar to those in hemiparasitic plants (Ehrelinger and Marshall, 1995), play an important role in the plant and can drive the uptake of water, as well as the solute flow through the xylem continuum that is formed between the host and the parasite by contributing to the higher negative water potentials. Whereas hemiparasitic plants are known to have strong xylem connections from host to parasite (Ehrelinger and Marshall, 1995), the holoparasitic dodder in previous literature is referred to as "phloem feeders" since the phloem supplies a majority of the nutrients to the parasite (Fer, 1981; Fer, 1987; Hibberd and Jeschke, 2001). *Cuscuta* not only has a strong connection with the phloem, but the haustoria form an extensive xylem-to-xylem connection with the host (Fer, 1981; Fer et al., 1987; Jeschke et al., 1994a). It is important to note that although one study stated that the xylem transfers were "non-negligible" (Penot, 1986), no further research has been done since. Also, most detailed nutrient flow studies on *Cuscuta* are done on the vegetative dodders, without SPs, most commonly with *Cuscuta reflexa* in subgenus *Monogynella* (Jeschke et al., 1994a, b).

More research needs to be done on the nutrient flow between host and parasites on flowering species of *Cuscuta* in subgenus *Grammica* that have SPs.

4.4. Systematic significance

Cuscuta consists of ~200 species, within four subgenera, and until now the only way to distinguish morphologically the subgenera from one another was through the gynoecia (Wright et al., 2011; García et al., 2014; Costea et al., 2014). Stem morphology was not considered relevant for the systematics of *Cuscuta* since most authors observed the vegetative stems, which are all similar morphologically (Yuncker, 1932, 1943). Subgenera can now be characterized and identified by the different stem types, including: the number of functional stems, one versus two, as well as the presence/absence of SPs or EFNs (Figure 13).

The presence as well as the diversity of the floral SPs of *Cuscuta* is also a great way to identify the species from one another. The "diffuse" floral SPs of subgenera *Cuscuta* and *Pachystigma* are more discrete than those of subgenus *Grammica* (Figure 16F-I, 18E-H; Appendix B). More specifically, *C. approximata* and *C. planiflora* were previously described by Yuncker (1932) as having appendages or fleshy apices of the calyx lobes and contributed to the identification of these species. Within subgenus *Grammica*, species such as *C. cotijana* (Figure 15D, 17E-F) or *C. iguanella* (Figure 15E) have distinct SPs that would allow for an easy identification. Other species can be identified by observations under the scanning electron microscope (Figure 17A-I) of stereomicroscope (Figure 15A-I; Figure 16A-C). There is a limited amount of floral characters available for *Cuscuta* and therefore the diversity of the floral SPs is important within each clade they are present in. These structures are also useful in species

delimitation as proven by the more recent taxonomic revisions (e.g., Costea and Stefanović, 2009; Costea et al., 2011a, b; Costea et al., 2013).

Concluding Remarks

Cuscuta develop unique structures, with one or more stomata present, referred to as stomatiferous protuberances (SPs) at flowering/fruiting. These structures develop on the haustorial stems of all the species within subgenus *Grammica* and also on the flowers of twenty-four species in *Grammica.* SPs are also found on the flowers of all the species in subgenera *Cuscuta* and *Pachystigma*. In the subgenus *Grammica,* species produce two functional types of stems during their life cycle: haustorial stems (with SPs) as well as exploratory stems (without SPs). The remaining three subgenera only develop one functional stem that lack SPs. These structures differ from the extrafloral nectaries (EFNs) that were previously studied in the subgenus *Monogynella*. Experiments in the field using a potometer revealed that the water uptake of the parasitized hosts was higher compared to the non-parasitized hosts, which can be explained at least in part by the water loss at the level of the SPs, because they are the only structures of the parasite that are adapted to lose water. Species with SPs on their flowers develop in areas with average precipitations of 90mm or less during flowering and fruiting.

This thesis incorporated multiple components of biology allowing for an integrative study. More specifically, integrating the different types of microscopy, including stereomicroscopy, scanning electron microscopy, and transmission electron microscopy, allowed for an extensive study of the SPs. Other components of biology, including the evolutionary analysis study, as well as experiments completed in the field with statistical analyses were also used and allowed for a more in-depth study of these structures. All of the objectives were

completed in this thesis and a better understanding of the multicellular protuberances in *Cuscuta,* and more specifically the function of the SPs, was determined.

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Appendices

Appendix A – List of Species

***Cuscuta* acuta** Englm.: 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. azteca** Costea & Stefanvoic. **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. bonafortunae** Costea and I. Garcia. **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. draconella** Costea & Stefanovic. **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. iguanella** Costea & I. Garcia. **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. insolita** Costea and I. Garcia. **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** 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. volcánica** Costea and I. Garcia. **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** var. **carinata** Yunck.: Paraguay, *Billiet & Jodin 3294* (MO); *Bernardi 18758* (MO). **C. yucatana** Yunck.: Mexico, *Nee & Taylor 29575* (MO); *Rzedowski 25728* (IEB); *Steere 1695* (MICH).

Appendix B – Data Matrix

Table 1 Multicellular protuberances character data in *Cuscuta*. 1-8 see characters from Table 2; 9: Annual average precipitations (mL) from the locations listed on the herbaria curators; 10: Average precipitations during flowering and beginning of fruiting (mL).

Subgenus/ Section	Species/variety	1	2	3	4	5	6	7	8	9	10
Subg. **Monogynella**	*C. cassythoides* Nees	0	1	1	1	-	0	0	-	869	197
(7 sp.)	*C. exaltata* Engelm.	0	1	1	1	-	0	0	-	794	240
	C. japonica Choisy	0	1	1	1	-	0	0	-	660	342
	C. lehmanniana Bunge	0	1	1	1	-	0	0	-	520	72
	C. lupuliformis Krock.	0	1	1	1	-	0	0	-	851	207
	C. monogyna Vahl.	0	1	1	1	-	0	0	-	704	59
	C. reflexa Roxb.	0	1	1	1	-	0	0	-	1445	773
Subg. **Cuscuta**	*C. approximata* Bab.	1	2	2	2	3±1	1	1	2±1	532	90
(5 sp.)	*C. epilinum* Weihe	1	2	2	2	1	1	1	1	722	152
	C. epithymum L.	1	2	2	2	2±1	1	1	1	778	152
	C. europaea L.	1	2	2	3	3±1	1	1	1	677	126
	C. planiflora Ten.	1	2	2	2	3±1	1	1	1	134	40
Subg. **Pachystigma**	*C. africana* Thunb.	1	2	2	4	1	0	0	0	626	83
(4 sp.)	*C. angulata* Engelm.	1	2	2	2	1	0	0	0	589	40
	C. natalensis Baker	1	2	2	4	1	0	0	0	848	85
	C. nitida E. Mey.	1	2	2	4	1	0	0	0	602	39
Subg. **Grammica**	*C. brachycalyx* Yunck.	2	0	0	0	0	0	0	0	922	404
(106 sp, 14 vars.)	*C. californica* Hook. & Arn.	2	0	0	0	0	0	0	0	882	318
Sect. *Californicae*	*C. decipiens* Yunck.	2	0	0	0	0	0	0	0	380	92
(10 sp.)	*C. draconella* Costea & Stefanović	2	2	3,4	2,3	3±1	0	0	0	206	66
	C. howelliana Rubtzoff	2	0	0	0	0	0	0	0	1118	122
	C. occidentalis Millsp.	2	0	0	0	0	0	0	0	1560	492
	C. pacifica Costea & Wright	2	0	0	0	0	0	0	0	1526	260
	C. salina Engelm.	2	0	0	0	0	0	0	0	563	156
	C. subinclusa Durand & Hilg.	2	0	0	0	0	0	0	0	682	101
	C. suksdorfii Yunck.	2	0	0	0	0	0	0	0	1897	252
Sect. *Cleistogrammica*	*C. australis* R. Br. var. *australis*	2	0	0	0	0	0	0	0	1192	418
(9 sp., 2 vars.)	*C. australis* var. *tinei* (Insenga) Yunck.	2	0	0	0	0	0	0	0	650	165
	C. campestris Yunck.	2	0	0	0	0	0	0	0	931	235
	C. glabrior (Engelm.) Yunck.	2	0	0	0	0	0	0	0	596	216
	C. gymnocarpa Engelm.	2	0	0	0	0	0	0	0	696	260

	C. harperi Small	2	0	0	0	0	0	0	0	1431	310
	C. obtusiflora Kunth var. *obtusiflora*	2	0	0	0	0	0	0	0	1110	354
	C. obtusiflora var. *glandulosa* Engelm.	2	0	0	0	0	0	0	0	722	211
	C. pentagona Engelm.	2	0	0	0	0	0	0	0	965	279
	C. plattensis A. Nelson	2	0	0	0	0	0	0	0	524	145
	C. runyonii Yunck.	2	2	4	4	5±2	0	0	0	445	53
Sect. *Racemosae*	*C. corniculata* Engelm.	2	0	0	0	0	0	0	0	743	265
(9 sp., 3 vars.)	*C. incurvata* Prog.	2	0	0	0	0	0	0	0	1187	372
	C. micrantha Choisy	2	0	0	0	0	0	0	0	80	37
	C. parviflora Englem. *var. elongata* Engelm.	2	0	0	0	0	0	0	0	1144	454
	C. platyloba Prog.	2	0	0	0	0	0	0	0	1187	391
	C. racemosa Mart. var. *miniata* (Mart.) Engelm.	2	0	0	0	0	0	0	0	1152	462
	C. suaveolens Ser.	2	0	0	0	0	0	0	0	822	378
	C. werdermannii Yunck.	2	2	4	4	3±1	0	0	0	87	10
	C. xanthochortos Mart. ex Engelm. var. *carinata* Yunck.	2	2	3	3	2±1	0	0	0	518	27
Sect. *Oxycarpae*	*C. cephalanthi* Engelm.	2	0	0	0	0	0	0	0	814	321
(8 sp., 2 vars.)	*C. compacta* Juss.	2	0	0	0	0	0	0	0	1064	331
	C. cuspidata Engelm.	2	0	0	0	0	0	0	0	612	237
	C. glomerata Choisy	2	0	0	0	0	0	0	0	802	298
	C. gronovii Willd. ex Roem. & Schult var. *gronovii*	2	0	0	0	0	0	0	0	1111	215
	C. gronovii var. *calyptrata* Engelm.	2	0	0	0	0	0	0	0	1160	269
	C. gronovii var. *latiflora* Engelm.	2	0	0	0	0	0	0	0	1201	260
	C. rostrata Shuttlew. ex Engelm.	2	0	0	0	0	0	0	0	1437	296
	C. squamata Engelm.	2	0	0	0	0	0	0	0	302	158
	C. umbrosa Beyr. ex Hook.	2	0	0	0	0	0	0	0	690	280
Sect. *Denticulatae*	*C. denticulata* Engelm.	2	0	0	0	0	0	0	0	150	56
(2 sp.)	*C. nevadensis* I. M. Johnst.	2	0	0	0	0	0	0	0	132	51
Sect. *Partitae*	*C. haughtii* Yunck.	2	0	0	0	0	0	0	0	802	377
(3 sp.)	*C. longiloba* Yunck.	2	0	0	0	0	0	0	0	1784	693
	C. partita Choisy	2	0	0	0	0	0	0	0	779	300
Sect. *Lobostigmae*	*C. cotijana* Costea & I. García	2	2	4,5	2,3	10±2	0	0	0	1082	40
(13 sp., 2 vars.)	*C. iguanella* Costea & I. García	2	2	5	4	10±2	4	3	5±2	711	70
	C. insolita Costea & I. García	2	2	4	2	5±2	3	1	2±1	756	78
	C. jalapensis Schltdl.	2	0	0	0	0	0	0	0	1481	663
	C. lindsayi Wiggins	2	0	0	0	0	0	0	0	1123	303
	C. mitriformis Engelm.	2	0	0	0	0	0	0	0	799	251
	C. purpusii Yunck.	2	0	0	0	0	0	0	0	655	286
	C. rugosiceps Yunck.	2	0	0	0	0	0	0	0	1121	625

	C. tasmanica Engelm.	2	0	0	0	0	0	0	0	701	185
	C. tinctoria Mart. ex. Engelm var. *tinctoria*	2	0	0	0	0	0	0	0	816	248
	C. tinctoria var. *aurea* (Liebm.) Costea	2	0	0	0	0	0	0	0	801	332
	C. tinctoria var. *floribunda* (Kunth) Costea	2	0	0	0	0	0	0	0	887	309
	C. victoriana Yunck.	2	0	0	0	0	0	0	0	619	304
	C. volcanica Costea & I. García	2	0	0	0	0	0	0	0	1356	474
	C. woodsonii Yunck.	2	0	0	0	0	0	0	0	3086	790
Sect. *Grammica*	*C. alata* Brandegee	2	2	5	3	5±2	4	1	4±1	406	78
(5 sp., 1 var.)	*C. azteca* Costea & Stefanović	2	2	3	3	4±2	0	0	0	380	90
	C. chinensis Lam. var. *chinensis*	2	2	2,5	3	3±1	0	0	0	422	89
	C. chinensis Lam. var. *applanata* (Engelm.) Costea & Stefanović	2	2	2,5	3	6±2	0	0	0	346	71
	C. potosina Schaffn. ex. Yunck.	2	2	3	3	4±2	0	0	0	375	88
	C. yucatana Yunck.	2	0	0	0	0	0	0	0	1210	489
Sect. *Obtusilobae*	*C. americana* L.	2	0	0	0	0	0	0	0	1528	522
(4 sp.)	*C. cozumeliensis* Yunck.	2	0	0	0	0	0	0	0	1210	445
	C. globulosa Benth.	2	0	0	0	0	0	0	0	1830	568
	C. macrocephala W. Schaffn. ex Yunck.	2	0	0	0	0	0	0	0	701	279
Sect. *Prismaticae*	*C. corymbosa* Ruiz & Pav. var. *grandiflora* Engelm.	2	0	0	0	0	0	0	0	981	411
(3 sp., 2 vars.)	*C. corymbosa* var. *stylosa* (Choisy) Engelm.	2	0	0	0	0	0	0	0	922	442
	C. prismatica Pav. ex Choisy	2	0	0	0	0	0	0	0	802	188
Sect. *Ceratophorae*	*C. boldinghii* Urb.	2	2	4	2	4±1	3	1	4±1	433	89
(7 sp.)	*C. bonafortunae* Costea & I. García	2	2	4	2	5±1	3	1	5±1	792	49
	C. chapalana Yunck.	2	2	4	2	3±1	3	1	3±1	765	42
	C. costaricensis Yunck.	2	2	4	2	6±2	3	1	6±2	821	86
	C. erosa Yunck.	2	2	4	2	3±1	2	1	3±1	466	82
	C. mexicana Yunck.	2	2	3	2	3±1	2	1	3±1	1421	77
	C. strobilacea Liebm.	2	2	4	2	6±2	3	1	6±2	652	46
Sect. *Umbellatae*	*C. acuta* Engelm.	2	0	0	0	0	0	0	0	670	291
(9 sp.)	*C. desmouliniana* Yunck.	2	2	3	3	3±1	0	0	0	122	36
	C. hyalina Roth. var. *hyalina*	2	0	0	0	0	0	0	0	772.8	341
	C. legitima Costea & Stefanović	2	0	0	0	0	0	0	0	495	155
	C. leptantha Engelm.	2	0	0	0	0	0	0	0	265	116
	C. odontolepsis Engelm.	2	0	0	0	0	0	0	0	402	67
	C. polyanthemos Schaffn. ex Yunck.	2	0	0	0	0	0	0	0	522	201
	C. tuberculata Brandegee	2	2	2,5	3	5±1	0	0	0	102	35

	C. umbellata Kunth var. *umbellata*	2	0	0	0	0	0	0	0	422	204
Sect. *Indecorae*	*C. coryli* Engelm.	2	0	0	0	0	0	0	0	995	284
(3 sp., 2 vars.)	*C. indecora* Choisy var. *indecora*	2	0	0	0	0	0	0	0	489	181
	C. indecora var. *attenuata* (Waterf.) Costea	2	0	0	0	0	0	0	0	1115	299
	C. indecora var. *longisepala* Yunck.	2	0	0	0	0	0	0	0	1123	464
	C. warneri Yunck.	2	2	4	2	4±2	0	0	0	210	54
Sect. *Gracillimae*	*C. colombiana* Yunck.	2	0	0	0	0	0	0	0	1556	614
(7 sp.)	*C. deltoidea* Yunck.	2	0	0	0	0	0	0	0	945	489
	C. gracillima Engelm.	2	0	0	0	0	0	0	0	945	363
	C. mcvaughii Yunck.	2	2	3	3	2±1	0	0	0	688	98
	C. punana Costea & Stefanović	2	0	0	0	0	0	0	0	451	87
	C. sidarum Liebm.	2	0	0	0	0	0	0	0	1095	338
	C. vandevenderi Costea & Stefanović	2	0	0	0	0	0	0	0	695	281
Sect. *Subulatae*	*C. argentinana* Yunck.	2	0	0	0	0	0	0	0	650	240
(14 sp., 1 vars.)	*C. cristata* Engelm.	2	2	5	3	3±1	0	0	0	278	77
	C. chilensis Ker Gawl.	2	0	0	0	0	0	0	0	420	178
	C. cockerellii Yunck.	2	0	0	0	0	0	0	0	220	110
	C. foetida Kunth var. *foetida*	2	0	0	0	0	0	0	0	2321	586
	C. foetida var. *pycnantha* Yunck.	2	0	0	0	0	0	0	0	1221	322
	C. friesii Yunck.	2	0	0	0	0	0	0	0	723	328
	C. globiflora Engelm.	2	0	0	0	0	0	0	0	1624	445
	C. grandiflora Kunth.	2	0	0	0	0	0	0	0	934	196
	C. kilimanjari Oliv.	2	2	4	3	3±1	0	0	0	993	44
	C. microstyla Engelm.	2	0	0	0	0	0	0	0	632	36
	C. odorata Ruiz & Pav. var. *odorata*	2	0	0	0	0	0	0	0	2221	420
	C. paitana Yunck.	2	0	0	0	0	0	0	0	697	173
	C. parodiana Yunck.	2	0	0	0	0	0	0	0	824	328
	C. purpurata Phil.	2	0	0	0	0	0	0	0	11	3