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Diversity and Evolution of Seeds in Cuscuta (dodders, Convolvulaceae): Morphology and structure

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DIVERSITY AND EVOLUTION OF SEEDS IN *CUSCUTA* (DODDERS, CONVOLVULACEAE): MORPHOLOGY AND STRUCTURE

by

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ABSTRACT

Cuscuta is a genus of nearly 200 obligate stem parasites with a nearly cosmopolitan distribution and considerable agricultural and ecological significance. Dodder seeds are considered “unspecialized”, with no morphological adaptations towards particular dispersal vectors; however, the seed coat anatomy has recently suggested an adaptation to endozoochory. This is the first attempt to provide a genus-wide overview of the diversity in morphology and anatomy of *Cuscuta* seeds*,* together with an assessment of the water gap and exploration of various form-function relationships. I surveyed 104 species belonging to all four *Cuscuta* subgenera. Seeds of the species of the first infrageneric dodder lineage diverged, subg. *Monogynella*, exhibit epidermal cells that are elongated and puzzle-like, morphologically uninfluenced by dryness/wetness (Type I) and possess a seed coat with an incomplete outer palisade layer. In contrast, epidermal cells of the other subgenera, *Cuscuta*, *Pachystigma* and *Grammica*, are more or less isodiametric and have evolved the ability to alternate their morphology and physiology between two states: deeply pitted when dry, and papillose through hydration (Type II). With the exception of four species, taxa of these three subgenera *have* a complete outer palisade layer throughout the entire seed. An embryo with a globose radicular end has evolved in sect. *Denticulatae* and sect. *Subulatae* of subg. *Grammica,* likely in connection with a storage function. In this thesis, I also examined the route of water entry — the water gap, in seven species and suggested two possible mechanisms for initial physical dormancy break. I investigated the relationship between seed size and geographical distribution as well as between the average number of seeds per capsule and the breeding systems. Finally, using other characters, such as the seed size, hilar region morphology, seed coat micromorphology and the number of palisade layers, I prepared an identification key for 16 species present and of concern in Canada.

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1. INTRODUCTION

1.1 GENERAL OVERVIEW OF *CUSCUTA*

Cuscuta (Convolvulaceae), otherwise known as dodders, is a group of obligate parasitic plants consisting of nearly 200 species that is sub-cosmopolitan in distribution and thriving in a great variety of habitats (Yuncker 1932; Good 1947; García et al. 2014). Parasitic plants, in general, have an immense influence on their natural ecosystems as well as in agricultural environments (Dawson et al. 1994; Pennings and Callaway 2002; Costea and Tardif 2006). Once successfully attached to the host via haustoria, *Cuscuta* becomes a powerful sink for water and assimilates, dramatically reducing growth and fitness of the host (e.g., Dawson et al. 1994; Kim and Westwood 2015). When it attacks crops, it decreases both the quantity and the quality of the yield (e.g., amount of sugars and other nutrients in edible organs), thus causing significant economic losses (Knepper et al. 1990; Sandler et al. 1997). In an effort to eliminate the potential threat of dodders, legislation has been enforced worldwide, often with the entire genus being placed on quarantine lists (Costea and Tardif 2006). Commercial seed lots are screened at the border and quarantined if containing seeds of *Cuscuta* (McDonald and Copeland 2012; Canadian Food Inspection Agency 2018). However, within the genus, only a relatively small number of *Cuscuta* species (~15) have been documented as noxious weeds of crops globally (Dawson et al. 1994; Knepper et al. 1990; Costea and Tardif 2006). Furthermore, numerous species require conservation efforts and there is evidence that some species may have become recently extinct (Costea and Stefanović 2009).

In natural ecosystems, dodders and other parasitic plants are said to be “keystone species” because through their host preferences they affect the structure and dynamics of their plant communities (Pennings and Callaway 1996). The presence of parasitic plants increases the diversity of species in natural plant communities, as

parasitism can hinder a hosts, ability to compete, thus allowing other present species to thrive (Shen et al. 2005; Fisher et al. 2013; Kim and Westwood 2015; Cruz Neto et al. 2017).

The geographical range of infestation for invasive *Cuscuta* species is vast, with an artificial dispersal mechanism well studied and widely accepted, through contaminated seed shipments of common legume crops (e.g. alfalfa, trefoil; Knepper et al. 1990; Costea and Tardif 2006). Understanding non-human vectors of dispersal has been more challenging, but zoochory was suspected (Kuhn 1868; Kuijt 1969) and endozoochory through waterfowl has been recently confirmed in North America (Costea et al. 2016). Examining seed and fruit attributes, like number of seeds per capsule, capsule properties, seed size and weight alone or in combination may further the understanding of the apparent lack of morphological dispersal syndromes (Howe and Smallwood 1982; Van der Pijil 1982; Haig and Westoby 1988; Poorter et al. 1996; Ho and Costea 2018).

The worldwide prevalence of dodders must factor in the ability of the seeds to disperse, remain viable, germinate, of the seedlings to find and successfully attach to suitable hosts. In many natural or artificial settings, *Cuscuta* species are represented only by their seeds (e.g., soil seed banks or contaminated commercial seed shipments), so in the interest of both conservation and control, it is imperative to have a good understanding of their seeds. *Cuscuta* seed morphology, anatomy, embryology, and dispersal have all been investigated, but these studies detailed only a few species at a time (Gaertner 1950; Knepper et al. 1990; Abdel Khalik et al. 2006).

1.2 NATURAL HISTORY AND REPRODUCTIVE BIOLOGY OF *CUSCUTA*

Cuscuta is the only parasitic genus evolved in the morning glory family Convolvulaceae (Stefanović et al. 2002; Stefanović and Olmstead 2004; Stefanović et al. 2007). Seedlings possess a reduced ephemeral root capable of absorbing water and interacting with AMF fungi (Behdarvandi et al. 2015). Seedlings are short-lived, capable of surviving alone a few days to several weeks depending on the species (Lyshede 1985; Dawson et al. 1994; Sandler et al. 1997; Marambe et al. 2002; Costea and Tardif 2006). Immediately after germination, they begin their search for a suitable host, with nutation of the growing shoot and "creeping" towards an appropriate host (Lyshede 1985; Marambe et al. 2002; Costea and Stefanović 2009; Meulebrouck et al. 2009a). *Cuscuta* stems vary in color from yellow to orange or red-purple; they are slender and twining, possessing rudimentary, scale-like, alternate leaves (Yuncker 1921,1932; Kuijt 1969; Dawson et al. 1994). After twining around the host stems, they produce haustoria for attachment and vascular connection with their host plants. All *Cuscuta* species are functionally holoparasitic, i.e. they rely entirely on their host plants in order to survive as most species lack chlorophyll at maturity and do not photosynthesize (Pierce 1984; Lyshede 1985; Machado and Zetsche 1990; Costea and Tardif 2006). The haustorium is a physiological bridge through which water and nutrients from the host's vascular tissues are extracted by the parasite. Haustoria are also a conduit for various macromolecules, mRNA, and hormones, which the parasite uses to "communicate" with the host (Knuston 1979; Pennings and Callaway 2002; Westwood et al. 2010).

Flowers of *Cuscuta* are crucial for taxonomical purposes as they provide enough variation in their characteristics for humans to distinguish species and they are currently the only reliable morphological identification means (Engelmann 1859; Yuncker 1932; Costea et al. 2015). The inflorescences are cymose, usually compound. Flowers range

from white to purple; they are bisexual, actinomorphic, and four to five-merous (Yuncker 1932; Johri and Tiagi 1952; Wright et al. 2011, 2012). The fruit, generally considered a capsule, can be either circumscissile dehiscent, opening at the base to release its seeds, or indehiscent, where it remains closed (Engelmann 1859; Yuncker 1932; Ho and Costea 2018). Previous studies on their reproductive biology suggested that dodder species use a wide range of sexual reproductive strategies (Wright et al. 2011, 2012). A mixed mating system is present in most *Cuscuta* species but to what extent selfing vs. outcrossing is relied upon in different individuals, populations and species, shows much variation (Rodriguez-Pontes 2009; Wright et al. 2011, 2012). The floral morphology seems to be targeting generalist pollinators (i.e. flies, moths, beetles), which come in contact with the stigmas and anthers (Wright et al. 2012). Arthropod visitors in many cases are attracted by the nectar ring at the base of the *Cuscuta* ovary, which secretes copious amounts of nectar in some species (Gómez 1994; McNeal 2005; Meulebrouck et al. 2009a; Wright et al. 2011, 2012; Riviere et al. 2013). Dodders can also spread vegetatively through stem fragments (Parker and Riches 1993).

The number of seeds per capsule, upon further investigation, can be linked with the type of breeding system of species (Haig and Westoby 1988). Studies in Convolvulaceae (*Calystegia*) and other angiosperm families (Amaranthaceae - *Alternanthera*; Asteraceae - *Adenostemma*; Fabaceae - *Lupinus*) indicated that selfing species produce more seeds than outcrossing species; also the autogamous species exhibited other higher measures of reproductive success (e.g. fruit/flower ratio, pollen count, seed/ovule ratio, brood size) than xenogamous ones (Wiens 1984; McMullen 1987; Lyons and Antonovics 1991; Ushimaru and Kikuzawa 1999). *Cuscuta* species possess a wide range of mixed-mating systems, therefore making dodders ideally suited for an exploration of possible functional relationships between breeding system and reproductive success.

1.3 SYSTEMATICS AND EVOLUTION OF *CUSCUTA*

Since its description as a genus in 1700 by Tournefort, *Cuscuta* has received considerable systematic investigation. Choisy (1841), Engelmann (1859) and Yuncker (1932) agreed on the inclusion of the genus in Convolvulaceae; however, other authors, based on the parasitic nature of *Cuscuta*, supported its classification within its own distinct family: Cuscutaceae (Dumortier 1829; Pfeiffer 1845, 1846; Des Moulins 1853). Traditionally, the genus was informally divided into three ”groups” (Engelmann 1859) which were subsequently accepted as subgenera: *Grammica, Cuscuta* and *Monogynella* based on the number of styles and the morphology of the stigmas (Yuncker 1921,1932).

Cuscuta and *Grammica* have two distinct styles, the former with equal styles and linear stigmas and the latter with unequal styles and capitate stigmas. *Monogynella* is characterized by a single style, sometimes partially divided, with a variety of stigma shapes (Engelmann 1859; Wright et al. 2011; García et al. 2014). Recent molecular phylogenetic results (Stefanović et al. 2007; García and Martin 2007; García et al. 2014) confirmed the validity of this morphological classification with one exception. Subgenus *Cuscuta* was found to be paraphyletic as another major infrageneric lineage was revealed (reviewed by García et al. 2014). This lineage was formally accepted as subgenus *Pachystigma* in the first phylogenetic classification of *Cuscuta* (Costea et al. 2015). *Pachystigma* is characterized by two elongated stigmas, broader than the styles, and has a very restricted geographical distribution (García et al. 2014; Costea et al. 2015).

Historically, the sections and subsections of *Cuscuta* subgenera have varied considerably, especially in subgenus *Grammica* (Engelmann 1859; Yuncker 1921,1932; reviewed by Costea et al. 2015), but in more recent studies our understanding has been enhanced substantially through molecular phylogenetic investigations (Stefanović et al.

2007; García and Martin 2007; García et al. 2014). Currently, the phylogenetic classification groups the 194 accepted species and varieties into four subgenera and 18 sections (15 sections for subg. *Grammica* and three for subgenus *Cuscuta*) (Costea et al. 2015). These sections have strong morphological and biogeographical predictive values and include from one to 31 species (Costea et al. 2015).

Subgenus *Grammica* is the largest and most diverse subgenus containing 154 species distributed throughout the New World, but centered in Mexico and the southern U.S.A. The classification of the 15 well-supported clades (Stefanović et al. 2007; García et al. 2014) currently accepted as sections (Costea et al. 2015) have little resemblance to the original taxonomic scheme proposed by Yuncker (1932). Subgenus *Cuscuta* is the second largest with three sections, *Cuscuta, Epistigma* and *Babylonicae* consisting of 21 species having a mostly Eurasian distribution. Subgenus *Monogynella* is mostly distributed throughout Eurasia, it has 15 species, and is the most "basal" group having the closest relation to the remainder of the morning glory family (García and Martin 2007; Wright et al. 2011, 2012; Clayson et al. 2014; Ho and Costea 2018). Finally, the newly resolved (Costea et al. 2015) subgenus *Pachystigma* is endemic to South Africa and consists of five species.

Recent character evolution studies revealed that the majority of morphological characters in *Cuscuta* are affected greatly by convergent evolution. Such characters include: pollen morphology (Welsh et al. 2010), gynoecium characteristics (Wright et al. 2011), perianth features (Wright et al. 2012); infrastaminal scales (Riviere et al. 2013); multicellular protuberances with stomata (Clayson et al. 2014) and fruit features (Ho et and Costea 2018). These morphological characters are insufficient to reconstruct the infrageneric phylogeny of *Cuscuta,* but they provide useful taxonomic data at species level within various clades (reviewed by Costea et al. 2015). The same is likely true for

the seeds, however, a comprehensive comparative study is necessary to reveal the variation of their traits.

1.4 SIGNIFICANCE OF *CUSCUTA*

Historically, the genus *Cuscuta* received attention for its parasitic nature and, at times, misunderstood oddities (Costea and Tardif 2004). Dodders are amongst the earliest referenced parasitic plants. The “father of botany” Theophrastus (ca. 371 BC – ca 287 BC), colleague and mentor of Aristotle, was acquainted with them and mentioned their peculiar nature (Theophrastus, De Causis Plantarum 2:17.5 1976). Their “curiosities” (having no apparent roots or seemingly springing forth from their host plants) (Kuijt 1969; Parsons and Cuthbertson 2001; Costea and Tardif 2004) inspired not only folklore but also superstitions about the origin of the species around the world (Grigson 1955; Silverthorne 2003; Watts 2007). Additionally, *Cuscuta* was mentioned in ancient medical texts of the Arabs, Persians, Indians and Chinese as remedy for a multitude of ailments (Ruellius 1529; Gerald 1597; Parkinson 1640; Culpeper 1652; Ghahreman and Okhovvat 2004; Ramawat and Goyal 2008).

The initial interest in the peculiarities of these parasitic plants stimulated the early study of their taxonomy, physiology and anatomy by early botanists (Choisy 1841; Engelmann 1842,1859; Mirande 1909). Subsequent studies have been motivated by *Cuscuta* being amongst the most destructive and economically detrimental parasites on important crop plants including species from Brassicaceae, Fabaceae and Solanaceae (Knepper et al. 1990; Cudney et al. 1992; Costea and Tardif 2006). Studies have indicated yield reductions upwards of 60 - 80%. *Cuscuta campestris,* for example, one of the most common species world-wide, has been reported to be a weed of 25 crop plants in 55 countries and to reduce yields up to 100% (Holme et al. 1997; Nickrent 2002; Mishra et al. 2005). Nonetheless, *C. campestris* is among only 15-20 species that are of

detrimental economic importance, but due to identification difficulties, all the species of *Cuscuta* are treated equally as noxious/and or invasive weeds (Dawson et al. 1994; Costea and Tardif 2006).

As previously mentioned, *Cuscuta* species, similar to other parasitic plants, are keystone species in their natural environments and thus extremely important ecologically (e.g., Pennings and Callaway 2002). In addition, many dodders though largely detrimental to their hosts may impart some advantages. A few recent studies on signaling between *Cuscuta* and its hosts showed that dodders stimulated the activation of the hosts' defenses against pathogens and herbivores (Hettenhausen et al. 2017; Saric-Krsmanovic et al. 2018).

2. BACKGROUND

2.1 DEVELOPMENT (EMBRYOLOGY)

The ovary of *Cuscuta* species is bilocular with two ovules per locule (Yuncker 1932; Vásquez-Santana et al. 1992). The ovary wall consists of six to eight cell layers depending on the species, with an external cuticularized epidermis and well differentiated inner epidermis and hypodermis (Johri and Tiagi 1952; Tiagi 1966; Govil and Lavania 1980; Wright et al. 2011). The vasculature consists of four to five collateral bundles that diverge into four terminal branches to supply the placenta and the ovules (Govil and Lavania 1980; Wright et al. 2011).

Each ovule grows as a "protuberance" at the base of the ovary (basal placentation), it begins to invert by the time the archesporial cell has differentiated and it is completely inverted by the time the megaspore mother cell (MMC) begins to differentiate (Tiagi 1966; Govil and Lavania 1980). In *Cuscuta*, the archesporial cell functions as the MMC (Tiagi 1966; Govil and Lavania 1980; Vásquez-Santana et al.

1992). By the time megasporogenesis commences, the single integument of the ovule completely covers the nucellus, with the exception of the narrow micropyle.

The MMC undergoes successive meiotic and then mitotic divisions that give rise to a seven-celled, eight-nucleate embryo sac. In dodder species, two types of embryo sacs can be encountered depending on the second meiotic division and whether or not two nuclei (bisporic) or one nucleus (monosporic) proceed to divide. In the *Polygonum* type, there are four megaspores with one "functional" megaspore (as the other three undergo programmed cell death) which divides mitotically three times giving rise to the mature embryo sac. In the *Allium* type, two megaspores are produced at the end of megasporogenesis, each containing two haploid nuclei, and the nearest to the micropyle undergoes cell death leaving a single functional megaspore. This megaspore will then divide mitotically three times and the result is also an eight-nucleate embryo sac (Davis 1966; Corner 1976; Govil and Lavania 1980; Rodriguez-Pontes 2009; Taiz et al. 2014).

Monosporic *Polygonum* type is most common in *Cuscuta* species (Davis 1966); however, the bisporic *Allium* type was reported to coexist together with *Polygonum* type in *C. reflexa, C. chinensis, C. lupuliformis, C. hyalina,* and *C. campestris* (Johri and Nand 1934; Johri and Tiagi 1952; Govil and Lavania 1980; Johri et al. 1992). The embryo sac consists of the egg apparatus that is formed by two synergids and one large egg cell, three ephemeral antipodals and the polar nuclei that will give rise to the endosperm (Corner 1976; Govil and Lavania 1980; Vásquez-Santana et al. 1992). The fusion of the polar nuclei is said to be delayed until the arrival and discharge of the pollen tube (Corner 1976), after which the endosperm development is initiated by rapid divisions (Johri 1934). The mature ovule is unitegmic, tenuinucellate and anatropous with a multilayered integument. Pollen of *Cuscuta* species is generally 3-zonocolpate or 4-6-zonocolpate, and in some rare cases pantocolpate (Hamed 2005; Liao et al. 2005). It is

polymorphic, with different numbers of colpi within the same anther (Costea et al. 2006a, 2006b, 2006c, 2006d, 2008a, 2008b; Welsh et al. 2010).

The seed coat develops from the single ovule integument (Johri et al. 1992; Corner 1976; Rodriguez-Pontes 2009). The mature embryo is either filiform and curved or coiled up to five times in the endosperm, which is completely consumed during germination (Yuncker 1921; Johri 1951; Johri and Tiagi 1952; Lyshede 1992; Behdarvandi et al. 2015). As an exception, the embryo of species from section *Denticulatae* (Clade E) and that of *C. microstyla* of section *Subulatae* possess an "abruptly enlarged ball-like end" (Hunzinker 1949; Costea et al. 2005). The cotyledons are absent, and the reduced radicle-like organ does not have a meristem (MacPherson 1921; Johri 1987; Johri et al. 1992; Behdarvandi et al. 2015).

2.2 MORPHOLOGY AND ANATOMY

Seeds of the genus *Cuscuta* are considered "simple" with similar morphological features that make species identification very difficult or impossible by seed characters alone (Kuijt 1969; Lyshede 1984, 1990; Knepper et al. 1990). Each capsule contains one to four seeds with color ranging from light grey to yellow-brown (Gaertner 1950; Lyshede 1984; Knepper et al. 1990; Ho 2017). The seeds of the subgenera *Cuscuta*, *Pachystigma* and *Grammica* resemble one another in both epidermal cell morphology and seed size, while *Monogynella* seeds are larger with an interlocking pattern of linear epidermal cells (Knepper et al. 1990; Abdel Khalik 2006; Costea et al. 2015). When the seeds are dry, the epidermal cells of studied species in subgenera *Cuscuta*, *Pachystigma,* and *Grammica* are pitted and when they are hydrated, epidermal cells show an "inversion" of the pits and become dome-like or papillose (Lyshede 1984; Jayasuriya et al. 2008). The hilum is located in a small circular depression and it is longest in *Monogynella* and shortest in *Cuscuta* (Johri and Tiagi 1952; Lyshede 1992;

Costea et al. 2015). No remnants of the micropyle can be observed in mature seeds (Jayasuriya et al. 2008).

The seed coat consists of an epidermal layer with a cuticle, two palisade layers, followed by one to four parenchyma layers. The size of the palisade cells is dependent on their location within the seed, generally the inner palisade layer thickens underneath the hilar area (Haenlein 1879; Breymann 1914; Gaertner 1956; Corner 1976). The impermeable layer or region responsible for the physical dormancy of seeds has been reported to be located at the junction between the two palisade layers or just above the linea lucida (or light line) of the inner palisade layer (*C. campestris*; Hutchison and Ashton 1979; *C. pedicellata* and *C. campestris*; Lyshede 1992). Seeds become dormant once they have desiccated (Gaertner 1950) or upon the developmental completion of the inner palisade layer (Hutchison and Ashton 1979).

The endosperm has been variously described, from appearing “scanty” (*C. gronovii* - MacPherson 1921) to having a peripheral aleurone layer containing both proteins and lipids (Lyshede 1984). Authors generally described the endosperm in different species and at different moments of seed development, therefore making descriptions difficult to compare. However, in all species, upon germination the endosperm is usually partially to completely absorbed.

2.3 SEED DISPERSAL AND GEOGRAPHY

Capsules of *Cuscuta* are characterized by different modes of indehiscence/dehiscence, resulting in seeds that are either dispersed enclosed within the fruit, or individually (Costea et al. 2015; Ho and Costea 2018). An important factor in the geographical distribution of plant species is the effectiveness of their dispersal syndromes (Lloyd et al. 2003; Lowry and Lester 2006; Gaston 2003; Hanski 1993;

Birand et al. 2011). Many *Cuscuta* species are sub-cosmopolitan in their distribution and occur in a great variety of habitats, but their dispersal vectors are little known (Costea et al. 2016). The artificial means of dispersal, however, has been well documented: through agricultural activities and seed contamination of common commercial seed crops (Musil 1944; Kujit 1969; Musselman 1986; Knepper et al. 1990). Seeds of *Cuscuta* are considered “unspecialized”, without any clear morphological adaptations that match known dispersal syndromes (Kuijt 1969; Musselman 1986; Lyshede 1992). Lyshede (1984) suggested that wind may play a role in dispersal due to the pitted surface of dry seeds; however, this was argued to be an adaptation for seed imbibition rather than an example of classic dispersal mechanism (Costea and Tardif 2006). Kuijt (1969) indicated that seeds remained viable after passing through the digestive tract of sheep. Kuhn (1868) also explored the germination of *Cuscuta* seeds that had passed through digestive tracts of common farm animals, including sheep, to determine the percentage of viable seeds. More recently, endozoochory was examined by Costea et al. (2016); their findings confirmed that dodder seeds can be spread along the migratory pathways of waterfowl and noted that seeds passed through the guts of the birds resembled those scarified with sulfuric acid. Hydrochory, dispersal of seeds or fruit by water, is another possibility of natural dispersal, as capsules of some species with indehiscent fruit such as *C. gronovii* can float for more than three weeks, and some species grow in wetlands or along waterways (Verdecourt 1948; Ho and Costea 2018).

Seed size is a relevant trait when considering the dispersal potential, competitive ability, seed bank behavior, distribution and abundance of plants (Westoby et al. 1990,1992; Hanski 1993; Thompson et al. 1993; reviewed in Gaston 1996; Poorter et al. 1996; Kidson and Westoby 2000). Larger seeds are assumed to have more reserves for seedling survival when faced with seedling hazards (e.g. deep shade, drought, physical damage etc.; Baker 1972; Venable and Brown 1988; Bekker et al. 1998), while smaller

seeds are viewed as having greater dispersal capacity because they are usually produced in larger numbers and they have longer seed bank persistence (Harper et al. 1970; Grime et al. 1988). Seed size and the geographical distribution have been analyzed in various plant families and correlations usually indicate a negative relationship (Venable and Brown 1988; Oakwood et al. 1993; Reese 1995; Guo et al. 1998,1999, 2000). The aforementioned literature generally reviewed multiple genera and species from different families with varying seed sizes, intricate dispersal syndromes and variable distribution. However, investigations specifically within the same family and genus (e.g. Fabaceae, Tribe Acacieae - *Acacia,* Brassicaceae*,* Myrtaceae, Tribe Eucalypteae - *Eucalyptus,* Fagaceae - *Quercus*) contradicted the negative relationship and found a positive association between seed size and geographical range (Aizen and Paterson 1990; Edwards and Westoby 1996; Willis et al. 2014a). The relationship between seed size and distribution has yet to be studied and examined for any genus of Convolvulaceae, including *Cuscuta*.

2.4 GERMINATION BIOLOGY

Many members of the Convolvulaceae family have species with seeds capable of physical dormancy caused by water-impermeable mechanical layers within their seed coat (reviewed by Baskin and Baskin 2004). *Cuscuta* is apparently the only holoparasitic plant capable of this type of dormancy (Baskin and Baskin 2000, 2006; Willis et al. 2014b). Species exhibiting physical dormancy cannot germinate without having their seed coat structurally altered, and practical methods for this have been reported for at least 21 *Cuscuta* species (e.g. Subg. *Cuscuta*: *C. epithymum* subsp. *epithymum* and subsp. *trifolii, C. europea, C. planiflora*; *Grammica*: *C. gronovii, C. umbrosa, C. campestris, C. chinensis, C. epilinum*; *Monogynella*: *C. monogyna, C. lupuliformis*) using

different methods, from physical treatments to chemical scarification (Gaertner 1950,1956; Baskin and Baskin 2000, 2005; Benvenuti et al. 2005; Jayasuriya et al. 2009).

Physiological dormancy, where seeds require varying lengths of moist, cold stratification periods before germinating, has also been documented in this genus (Meulebrouck et al. 2009a; Jayasuriya et al. 2008; Willis et al. 2014), along with a combined physical and physiological dormancy (Jayasuriya et al. 2008; Baskin and Baskin 2004). Physical and physiological dormancy have been confirmed only in a few *Cuscuta* species but can be inferred in others when physically or chemically scarified seeds do not germinate unless they are cold-stratified for different periods of time (Gaertner 1950; Tingey and Allred 1961, Allred and Tingey 1964; Meulebrouck et al. 2008).

Physiological dormancy is regulated by cycling of “sensitivity” and “insensitivity”. If a seed is in the sensitive stage, once it has responded to physical dormancy breaking treatments and becomes permeable to water, it can *then* respond to moist, cold stratification and eventually start the germination process. The route of water entry has not yet been studied in full in this genus, but based on work with *C. australis,* Jayasuriya et al. (2009) determined it occurs through the hilar fissure, unlike the micropyle passage documented in other tribes of Convolvulaceae (Jayasuriya et al. 2007, 2008). By possessing physical or physical + physiological dormancy some of the seeds of *Cuscuta* species can avoid germination and form a persistent seed bank which allows a bet-hedging strategy (Ho and Costea 2018). Bet-hedging occurs when a small portion of the seeds in the seed bank germinate in seasonal peaks, with some delay during each vegetative season and throughout the years (Cohen 1968; Ho 2017).

3. OBJECTIVES AND HYPOTHESES

Morphology and anatomy of Cuscuta seeds: diversity and evolution

1) To survey the diversity of morphology, micromorphology and anatomy of *Cuscuta* seeds.
2) To reconstruct ancestral states for seed characters and discuss their systematic/taxonomic significance. Although, seed characters may not hold systematic significance, I hypothesize they will have some taxonomic importance and will be useful to the creation of identification keys.
3) To investigate possible relationships among seed coat characters.
4) To analyze and investigate the water-gap anatomy in multiple species. I hypothesize that the initial route of water entry is through the hilar fissure.

Explore functional relationships

5) To examine a possible relationship between the geographical distribution of North American *Grammica* species and the size of their seeds.
6) To analyze possible correlations between the number of seeds per capsule and breeding systems in *Cuscuta*. I hypothesize that the number of seeds per capsule will be positively correlated with the species breeding system, i.e. factitively autogamous species will have on average more seeds per capsule than fully xenogamous and facultatively xenogamous species.

4. MATERIALS AND METHODS

The morphology and anatomy of seeds was examined in 104 taxa (99 species and five varieties) using ca. 300 dried herbarium specimens (Appendix A, based on abundance and availability of material).

4.1 MORPHOLOGY AND MICROMORPHOLOGY

Five seeds were collected from each herbarium specimen and a minimum of three specimens were used per taxon (15 seeds/taxon). Seeds were rehydrated in warm (not boiling) 50% ethanol and 50% deionized water solution until they were softened (an indicator of successful imbibition) and were preliminarily examined under a SMZ1500 stereomicroscope. Seeds were then dried with a Tousimis Autosamdri-931 critical point dry instrument. Seeds of each herbarium specimen were mounted onto an aluminum stub and sputter-coated with 30 nm of gold using Emitech K550 sputter coater. A Hitachi SU1510 scanning electron microscope (SEM) was used to examine and image the overall morphology and micromorphology of seeds. Measurements were taken with Quartz PCI version 5.1. (Quartz Imaging Corp).

4.2 ANATOMY

Five to seven seeds were collected per herbarium specimen for anatomical investigation, for a total of approximately 15-21 seeds per taxon. Seeds were rehydrated using the same method as for morphology and micromorphology (see above). At least five seeds were sectioned by hand with a razor, longitudinally along the hilum to analyze whole seed anatomy. Examining sectioned seeds under the SEM has been shown to be a valid methodology to study the seed coat anatomy in *Cuscuta* (e.g. Costea et al. 2016), as this approach eliminates the need for embedding, sectioning and optical

microscopy. Nonetheless, 26 species, **Subg. *Grammica***: *C. californica, C. campestris, C. argentiniana, C. cephalanthi, C. acutiloba, C. chapalana, C. chilensis, C. compacta, C. corymbosa, C. cristata, C. cuspidata, C. denticulata, C. desmouliniana, C. erosa, C. foetida, C. grandiflora, C. iguanella, C. indecora, C. mitriformis*; **subg. *Cuscuta:*** *C. epilinum, C. epithymum, C. europea*; **subg. *Monogynella***: *C. exaltata C. gigantea, C. monogyna,* and *C. reflexa* were also hand-sectioned for optical microscopy. Sections were stained using the polychromatic Toluidine Blue O dye (TBO), with a pH of 4.4. TBO absorbs different colors depending on the nature of its chemical binding with the components of the cell walls, with pH being an important factor affecting binding and coloration. As the pH decreases and becomes more acidic, TBO only gives a blue or green colour as there are no carboxylated carbohydrates carrying negative charges to react with. Whereas at a higher and more basic pH any carboxylated groups will be negatively charged, and if rich in polysaccharides there will be a dark pink reaction (O'Brien et al. 1964). Observations and imaging of these sections were conducted using a Nikon Eclipse 50i Brightfield, Nikon SMZ1500 stereo-microscope and imaged with a PaxCam Arc digital camera equipped with Pax-it! 2 Version 1.5 software (MIS Inc, Villa Park, IL).

4.3 WATER GAP ANATOMY

The protocol has been adapted from Jayasuiraya et al. (2008). Seven species from three of the four subgenera (*Grammica*: *C. gronovii, C. veatchii, C. volcanica, C. tasmanica, C. sandwichiana; Cuscuta*: *C. epithymum* and *Monogynella*: *C. monogyna*) were examined, and 20 seeds were used per species. Physical dormancy was removed in 14 seeds by using the rehydration protocol mentioned in sect. 4.1. Non-dormant seeds exhibited an open hilar fissure (an indicator of physical dormancy break; Jayasuiraya et al. 2008). The hilum region of seven of the seeds was painted with

petroleum gel to block the hilar fissure, while the remaining seven seeds were left with no obstruction at the hilar pad. Six seeds were left dormant and were not prepared in any way to serve as a control. Both dormant or non-dormant seeds were placed in an aqueous solution of 25% Aniline Blue, in glass trays with one seed/basin. Seeds were removed at 15 min intervals from the solution beginning at time 0. After 15 min to 1h and 30 min, seeds were sectioned longitudinally by hand through the hilar pad along the hilar fissure, to observe the penetration of the dye. Observation was conducted using a Nikon Eclipse 50i brightfield and Nikon SMZ1500 stereo-microscope. Images were then takren with a PaxCam Arc digital camera equipped with Pax-it! 2 Version 1.5 software (MIS Inc, Villa Park, IL).

4.4 CHARACTERS AND ANALYSES

A list of seed characters, both morphological and anatomical, was created based on the available literature (Haenlein 1879; Gaertner 1950; Lyshede 1984; Abdel Kahlik 2006; Costea et al. 2006a, 2006b, 2006c, 2006d, 2008a, 2008b, 2011, 2013; Costea and Stefanović 2010). Ten categorical and 12 continuous characters are summarized in **Table 1** and illustrated in **Figure 1**. Additionally, three characters were subsequently added upon the completion of initial character survey and scoring for a more detailed comparison of characters between taxa.

Table 1: Seed characters and their representative codes and states used for surveying morphology and anatomy of 104 *Cuscuta* taxa. Subsequently added characters are bolded.

Character	Character states
Categorical characters	
1. Compression of seed or the number of ± flat faces that a seed has.	1 = dorsoventrally compressed = seeds with one flat face and one convex face; 2 = “angled” seeds with 2 flat faces and one

	convex face; 3 = no compressions, spherical
2. Seed shape (seeds are positioned in such a way the hilum is at the base of the seed	1 = elliptic; 2 = obovate; 3 = circular; 4 = ovate, 5 = oblong
3. Radicular end of embryo	1 = spherically enlarged, 2 = filiform
4. Hilum location	1 = terminal; 2 = subterminal
5. Hilum compression	1= flat; 2 = concave
6. Dry seed epidermal cells	1 = pitted; 2 = non-pitted
7. Hydrated seed epidermal cells	1 = papillose; 2 = non-papillose
8. Seed epidermis cell shape (as seen in surface SEM images)	1 = elongated ("puzzle-like"); 2 = isodiametric
9. Presence of outer palisade layer	0 = absent; 1 = present
10. Presence of inner palisade layer	0 = absent; 1 = present
Continuous characters	
11. Seed length	µm
12. Seed width	µm
13. Seed thickness	µm
14. Hilum area length	µm
15. Hilum area width	µm
16. Epidermal cell diameter	µm
17. Length of funicular “line” of the hilum	µm
18. Thickness of epidermal cell (anatomy)	µm
19. Width of epidermal cell (anatomy)	µm
20. Thickness of outer palisade layer	µm
21. Thickness of inner palisade layer	µm
22. Number of seeds per capsule	N/A
23. Ratio of epidermal cell diameter and seed length (“seed size”)	Epidermal cell diameter (µm)/ seed size (µm)
24. Ratio of epidermal cell thickness and seed size	Epidermal cell thickness (µm)/ seed size (µm)

25. Ratio of inner + outer palisade thickness and seed size	Inner + outer palisade thickness (µm)/ seed size (µm)

In order to trace character history, character states obtained (Table 1) were analyzed with Mesquite (3.40; Maddison and Maddison 2018). Broad-scale phylogenies based on ITS and plastid-trn LF datasets are available separately for subgenera *Cuscuta* and *Grammica* (García and Martin 2007; Stefanović et al. 2007) and now for the entire genus (García et al. 2014). Analysis of character polarity in *Cuscuta* using outgroup analysis is hindered by the still unresolved position of *Cuscuta* within Convolvulaceae (Stefanović and Olmstead 2004). Therefore, distribution of characters was analyzed only in-group, as previously done in other character evolution studies conducted for other *Cuscuta* traits (e.g. pollen, gynoecium, infrastaminal scales, protuberances with stomata and fruit capsules) (Welsh 2010; Wright et al. 2011; Riviere et al. 2013; Clayson et al. 2014; Ho and Costea 2018).

Scenarios of character evolution were analyzed using the parsimony reconstruction method provided by Mesquite 3.40 (Maddison and Maddison 2011). Markov k-state 1 parameter model (MK1) of evolution was used; in the parsimony reconstruction, character-state changes were treated as unordered. Three qualitative, non-polymorphic characters (outer palisade layer, epidermal cell type and type of embryo) (see Table C1 in Appendix B) were also analyzed with the likelihood reconstruction method.

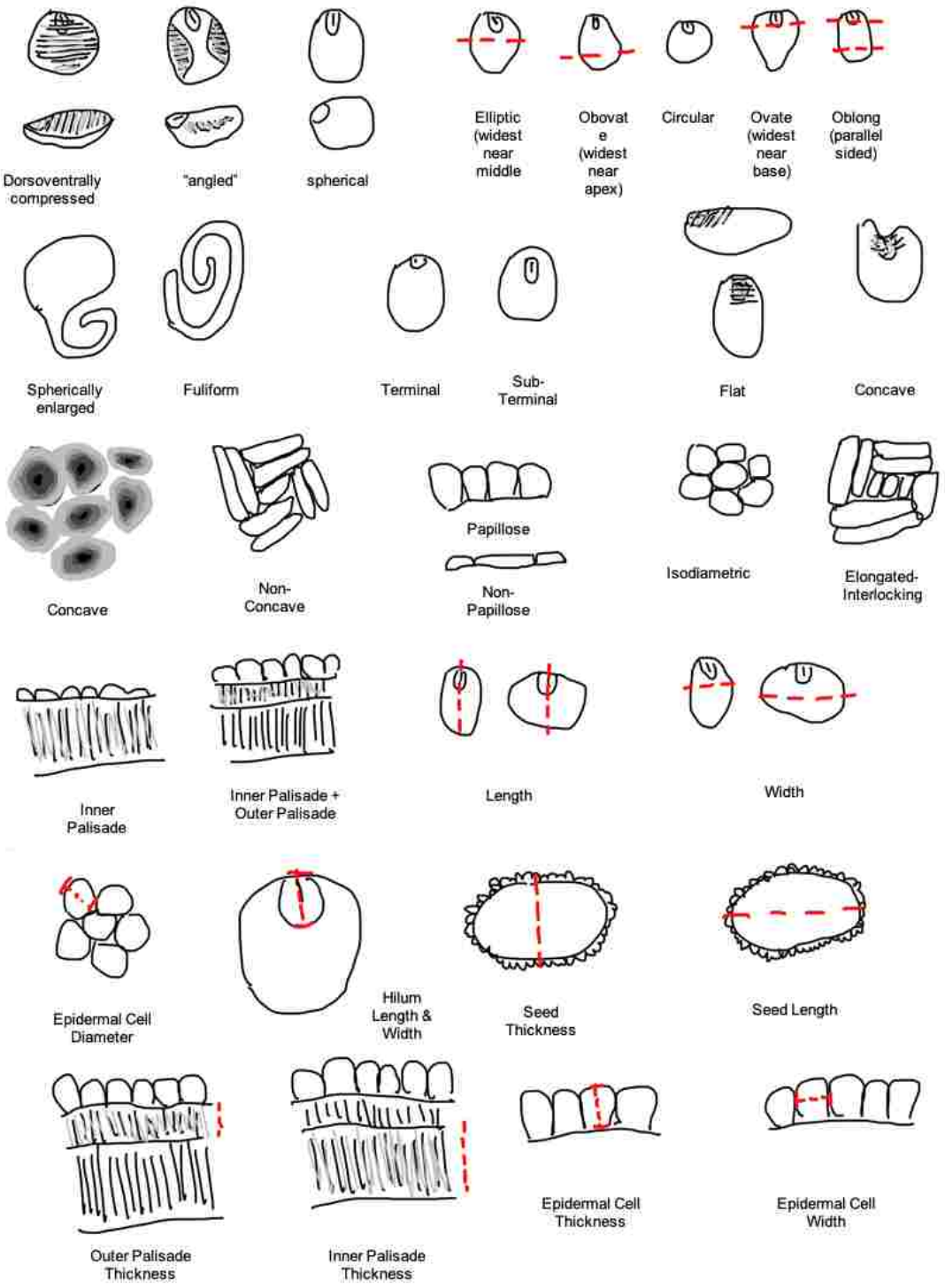

FIGURE 1. Illustration of characters scored (Table 1).

4.5 STATISTICAL ANALYSES

All seed characters (Table 1) were first checked for deviations from a normal distribution (Table 5), using PAST version 3.16 (Hammer et al. 2001), and plotted on an X-Y graph to determine if any linear relationships were present. In addition to the entire genus dataset, subgenus and section clade partitions were also examined. In order to visualize the similarity and structure of data among all of the characters (see Table B1 in Appendix B), a multivariate analysis of all taxa was conducted. Principal Coordinate Analysis (PCoA) was run using Gower's coefficient (Gower 1971) available from the statistical software program PAST version 3.16 (Hammer et al. 2001). A PCoA was used as it can handle (dis)similarity matrices calculated from quantitative, qualitative and mixed variables as well as data gaps. Then, using the transposed matrix, an additional PCoA was run to visualize the grouping of seed characters on the basis of their similarity/dissimilarity.

The relationships among quantitative characters were analyzed with a Pearson Product-Moment Correlation (Pearson's Correlation) and an Ordinary Least Squares Regression using the same software. Characters were first verified to meet the five assumptions of the Pearson's Correlation (interval or ratio measurements, normal distribution, linearity, minimum outliers and homoscedasticity of the data) and the four assumptions of the Ordinary Least Squares Regression (linearity, independence, normal distribution, equality of variance).

Additionally, the seed size was of interest for character comparisons and required for statistical investigations into functional relationships. A "seed size" index involving the length, width and thickness of seeds was considered initially, but because similar values were obtained from different combinations of the three size variables, this metric was abandoned. Instead, an Ordinary Least Squares Regression and Pearson's Correlation

were conducted to evaluate the relationship between these three variables to determine if one of the characters could be a suitable representative for “seed size” on its own. Seed length had a very strong positive correlation to both width and thickness and therefore became the proxy for seed size (see Results; Table 4).

4.6 FUNCTIONAL RELATIONSHIPS

Breeding systems and number of seeds per capsules in Cuscuta

A putative relationship between the (average) number of seeds per capsule (S/C), as a proxy for the reproductive output, and the breeding systems categories was investigated. Wright et al. (2012) determined the total (average) number of pollen grains per flower in 138 species. This number was then divided by the ovule number, which is four per ovary in all *Cuscuta* species, to generate the pollen/ovule (P/O) ratio (Wright et al. 2012). Based on Cruden’s P/O ranges (Cruden 1977; Cruden et al. 1990), the 138 taxa were assigned to different breeding system categories: six dodder taxa were inferred to be fully xenogamous, 108 taxa were regarded as facultatively xenogamous and at least 23 taxa facultatively autogamous (Wright et al. 2012).

In this study, a bipartite network graph was initially constructed to visually represent the relationship between the number of seeds per capsule and the breeding system categories (based on the P/O ratios published by Wright et al. 2012). Then a Kendall’s Tau Correlation was performed, using the PAST software version 3.20 (Hammer et al. 2001) to determine if and to what extent a correlation was present between the two variables. Kendall’s Tau was suitable as it is a non-parametric rank correlation that is not dependent on the assumptions of the underlying distributions. Differences among the breeding system categories were analyzed using an Analysis of Variance (ANOVA). Additionally, a general regression tree was constructed, (R package

"r.part" – McCullagh and Nelder 1989; Appendix C) with the response variable being the P/O ratios, and the explanatory variables being the breeding system categories and the number of seeds per capsule as a prediction model. Regression trees iteratively divide data into two homogenous groups along the values of one of the explanatory variables in such a way that they have mutually exclusive memberships and minimize the variation (sum of squares) of the response variable(s) within the two groups (Breiman et al. 1984; De'ath and Fabricius 2000; De'ath 2002). A regression tree was suitable for the dataset as it can be constructed using continuous (average S/C) and/or categorical predictor variables (breeding system categories).

Distribution of North American subg. Grammica species and seed size

A Spearman's Rank Correlation between the total geographical distribution range of 50 North American *Grammica* species (km^2) from Ho and Costea (2018) and their seed size (indicated by the seed length, Table B1 in Appendix B) was conducted to explore a possible relationship. Analysis was run using PAST version 3.16. This non-parametric rank correlation does not depend upon the assumptions of various underlying distributions.

5. RESULTS

5.1 MORPHOLOGY AND MICROMORPHOLOGY

The seeds of *Cuscuta* develop from a two-locular ovary, with a constant two ovules per locule, meaning there can be a maximum of four seeds set per fruit. Average number of seeds per fruit varies from 1 to 3.8 (Table B2 in Appendix B) with no apparent systematic trend amongst subgenera and clades, other than subgenus *Cuscuta* the taxa of which generally average highest S/C (3.1-3.8 S/C; Table B2 in Appendix B).

Many of the seed characters were polymorphic (Table B1 in Appendix B). For example, the majority of taxa exhibit more than one character state for both seed shape and compression. As the ovules/seeds develop in close proximity to one another within the same ovary/capsule, they will be variously compressed depending on the final number of seeds per capsule. Just under half of the seeds examined display an "angled" compression (two flat faces on either side of the hilum and one convex face), while the remaining majority of seeds were found to be dorsoventrally compressed (one flat face, usually the face with the hilum, and one convex face) (Fig. 2; A-D). A small minority of seeds did not display any obvious compressions and were categorized as spherical. The most commonly encountered seed shapes were elliptic (widest near the middle; margins convex) and ovate (widest at the base). Other seeds exhibited oblong (widest at the middle; margins parallel) and circular (approximately the same distance to the centre at every point) shapes. The least common shape was obovate (widest near the apex; margins convex).

In general, there are several degrees of variation among taxa in regard to quantitative characters like seed size, hilar pad size, and epidermal cell diameter across the genus. However, the variation within each taxon is relatively constant. Seed length among species ranges from 704.55 µm to 3158.30 µm, while seed width ranges from 668.28 µm to 2910.05 µm. Seed size within species had a standard deviation of as little as 16.87 µm (*C. membranacea)* to as much as 196.2 µm (*C. monogyna*), which indicates that seed size is a reliable character within species (Table B1 in Appendix B). Seeds of subg. *Monogynella* are the largest, whereas those of subg. *Cuscuta* have, on average, the smallest (Table B1 in Appendix B). Subgenus *Grammica* has the largest variation in seed size, for example species within Clade G (sect. *Lobostigmae*) have an average seed length of 1658 µm and width of 1420 µm while taxa of Clade L (sect.

Umbellatae) have an average seed length of 972 µm and width of 846 µm, exemplifying the convergent evolution of seed size (Figure B4 in Appendix B).

Based on their morphology, two types of epidermal cells ("Type I" and "Type II") were observed (Fig. 2; G-J). Type I includes epidermal cells that are elongated and puzzle like. This type of seed coat epidermis is morphologically unaffected by dryness and wetness and characterizes subg. *Monogynella*, the first infrageneric dodder lineage to diverge. Type II exhibits more or less isodiametric epidermal cells (Fig. 2; G-H), which can alternate their morphology and physiology between two states: pitted (or concave) when dry or bulging, papillose when hydrated. This type is present in the subgenera *Cuscuta*, *Pachystigma* and *Grammica*. Parsimony and Maximum Likelihood reconstruction analyses indicated that the Type I is likely the ancestral state and Type II is derived (Proportional Likelihood, Type I: 0.5622; Type II: 0.4378). No reversals to Type I were found in subg. *Cuscuta, Pachystigma* and *Grammica* (see Appendix C3).

The variation of the three character ratios (Table 1) was found useful to separate *Cuscuta* subgenera (Appendix C2). Thus, the ratio between epidermal cell diameter in relation to the seed size (Table 2) was largest on average in subg. Cus*cuta* (0.0407) and smallest in subg. *Grammica* (0.0282). Subgenus *Grammica* displayed the most variation, with Clade J (sect. *Prismaticae*; 0.0187) showing the smallest values and Clade A (sect. *Californicae*; 0.0318) the highest. *Pachystigma* exhibited intermediate values between the other subgenera (0.0310). Parsimony reconstruction displayed an inverse trend for an increase in epidermal cell diameter in relation to seed size (Tree not shown).

The hilum area is more or less round in shape and includes the funicular scar or hilar fissure which is morphologically distinct from the remainder of the epidermis regardless of the type of seed coat. In this area, cells are substantially smaller, elongated and rectangular with thin cell walls, elongated and rectangular, and appear concentrically arranged around the hilar fissure. The hilar pad varies in size amongst the

subgenera, as does the length of the hilar fissure. Quantitative characters associated with the hilar pad (length, width, size of hilar fissure) were the largest in subg. *Monogynella*, in some cases three to four times larger than in the remaining taxa. Within subg. *Grammica*, Clades D (sect. *Oxycarpae*) and G (sect. *Lobostigmae*) also had relatively large hilar pads and fissures.

The hilar fissure is thought to be the initial route of water entry upon the breaking of physical dormancy in *Cuscuta*, thus preparation of samples in warm water broke the physical dormancy of the seeds observed. In all four subgenera, whole seeds were consistently imaged with their hilar fissures open. The micropyle or its remnants were not observed.

TABLE 2. Ratio of epidermal cell diameter to seed size in the three subgenera of *Cuscuta* that have isodiametric cells. Excludes subg. *Monogynella* taxa as cell diameter cannot be measured in their seeds.

Subgenus	Ratio of epidermal cell diameter to seed size (average)
Grammica	0.0282
Pachystigma	0.0310
Cuscuta	0.0407

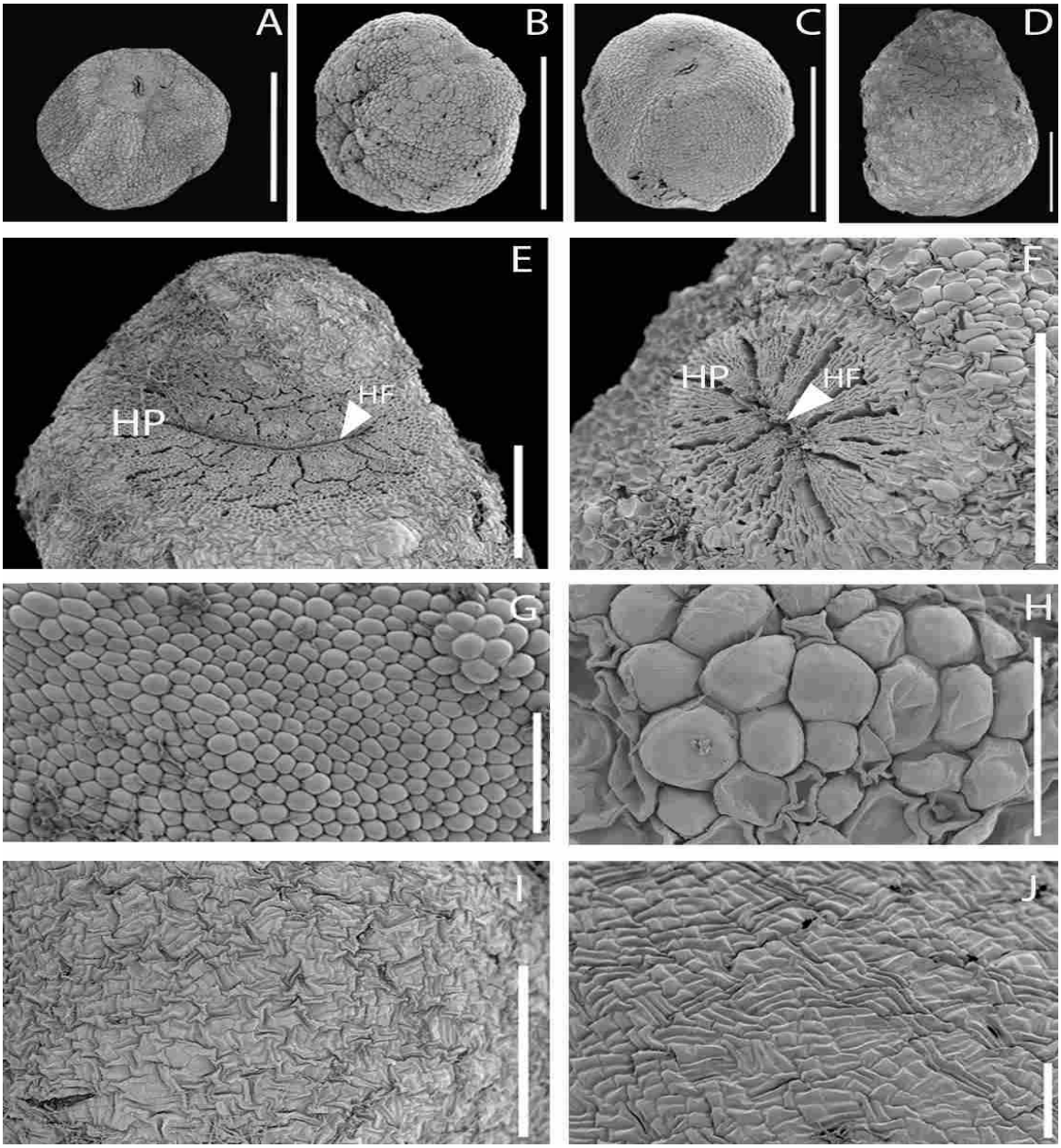

FIGURE 2. Morphology of seeds. A. Circular seed of *C. cristata* (subg. *Grammica*) dorsoventrally compressed with a subterminal, flat hilum and isodiametric papillose epidermal cells. **B.** Circular seed of *C. babylonica* (subg. *Cuscuta*) spherical with relatively no compression, terminal concave hilum and papillose epidermal cells. **C.** Circular seed of *C. campestris,* "angled" with 2 flat faces and one convex face; a subterminal flat hilum and papillose epidermal cells. **D.** Dorsal view of ovate seed of *C. lupuliformis* (subg. *Monogynella*), dorsoventrally compressed with terminal, concave hilum and elongated interlocking epidermal cells. **E.** Hilar region of *C. lupuliformis* (subg. *Monogynella*) showing large hilar pad (HP) and hilar fissure (HF); cells surrounding the fissure are substantially smaller, with thin cell walls, appear isodiametric along the perimeter of the hilar pad but begin to elongate surrounding the fissure. **F-H.** *C. gronovii* (subg. *Grammica*). F. Flat hilar pad, with central, raised hilar fissure. Cells of the hilar pad are elongated and rectangular and appear concentrically arranged around the hilar fissure. G. Isodiametric epidermal cells of rehydrated seed. H. Isodiametric epidermal cells displaying an intermediate stage prior to complete rehydration, concave cells mixed with papillose cells. **I-J.** Elongated, interlocking cells of *C. lupuliformis* (subg. *Monogynella*) are not affected by hydration. Scale bars: **A-D** = 1.00 mm; **E-G** = 500 µm; **H** = 200 µm; **I** = 1.00 mm; **J** = 200 µm.

5.2 ANATOMY

As seen in longitudinal sections, the epidermal cells of subg. *Monogynella* species are more or less rectangular (22.96 - 47.43 μm thick; Fig. 4; G) and covered by a cuticle. In hand sections, taxa of subg. Monogynella, stain a blue/green color with TBO indicating no carboxylated carbohydrates and an acidic pH (Fig. 3; D-F). Epidermal cells of *Grammica, Cuscuta* and *Pachystigma* species appear radially elongated (thickness; 25.04 - 116.56 μm) and are often tapered at the base and rounded at the apex (Fig. 3; A-C, Fig. 4; E). When stained, species of these subgenera exhibit both blue/green and purple/pink reactions which indicate cells are rich in polysaccharides and a more basic pH (Fig. 3; A-C, J-K) The ratio between the epidermal cell thickness and the seed size is largest on average in subg. *Cuscuta* (0.0523) and smallest in subg. *Monogynella* (0.0119) (Appendix C2). Within subg. *Grammica,* Clade A (sect. *Californicae*) displays the largest (0.0367) and Clade D (sect. *Oxycarpae*) the smallest (0.0275) ratio. Parsimony reconstruction indicated that larger epidermal cell thickness: seed size ratio is likely ancestral in species with Type II epidermal cells (pitted-papillose), though in *Grammica* species there are at least four cases where that ratio evolved to become larger (Fig. 5). Immature seeds of *Grammica, Pachystigma* and *Cuscuta* exhibit abundant starch granules within the epidermal cell (Fig. 3; G), whereas at seed maturity, starch is no longer present in the epidermal cells (Fig. 3; A). In subg. *Monogynella,* abundant starch and tannins persist in the epidermal cells at seed maturity.

The hilar pad is visible in the sections as the morphologically distinct epidermal cells in this area appear much smaller and compressed than the remainder of the seed coat. The outer palisade layer increases slightly in size in this region, whereas the inner palisade layer can be more than twice as thick as that layer is to the remainder of the seed coat. In the majority of species, both the inner and outer palisade layers are

"complete" and found directly below the epidermal cells, continuous throughout the entire seed coat.In subgenus *Monogynella*, the outer palisade layer is "incomplete", present only under and around the hilar pad and is absent in the rest of the seed. Likelihood reconstruction determined a proportional likelihood of 0.5727 for the incomplete outer palisade layer which is thus more likely to be the ancestral state. Reversals to this ancestral character have occurred in four species of two clades within subg. *Grammica*: the species of sect. *Denticulatae* (Clade E; *C. denticulata, C. nevadensis* and *C. veatchii*) and *C. microstyla* in sect. *Subulatae* (Clade O) (Tree not shown). The inner palisade layer is consistently three to four times thicker than the outer palisade in subgenera *Grammica, Pachystigma* and *Cuscuta* with the exception of sect. *Denticulatae* (Clade E) in *Grammica*, the inner palisade layer of which is only about twice the size of the outer palisade layer (Table B1 in Appendix B).

Upon rehydration, the endosperm of mature seeds becomes gelatinized. Gelatinization is a consistent indicator of seed maturity, as immature seeds, when sectioned, exhibit copious amounts of starch and readily release the embryo. The endosperm is gradually consumed during embryo development. A peripheral cell layer with large nuclei is present around the endosperm which demarcates it from any remaining parenchymal layers of the seed coat. This "membranous" layer surrounds the gelatinous content of the endosperm and varies in thickness. Ten species in both subg. *Grammica* and *Pachystigma (Grammica: C. sandwichiana* - Clade B (sect. *Racemosae)*; *C. nevadensis, C. denticulata, C. veatchii* - Clade E (sect. *Denticulatae*); *C. haughtii* – Clade F (sect. *Partitae*); *C. tinctoria* – Clade G (sect. *Lobostigmae*); *C. strobilacea* – Clade K (sect. *Ceratophorae*); *C. acuta* – Clade L (sect. *Umbellatae*); *C. microstyla* – Clade O (sect. *Subulatae*); *Pachystigma: C. nitida)* displayed a substantially thicker outer membrane that could easily be removed from the remainder of the endosperm.

A spherically enlarged embryo has evolved in the subg. *Grammica* in four species: *C. denticulata, C. veatchii, C. nevadensis* (Clade E – sect. *Denticulatae*) and in *C. microstyla* (Clade O – sect. *Subulatae*). These taxa have a swelling at the distal portion of the embryo, similar morphological forms, deviating only in the size of swelling and proximal end coiling (between zero and two coils). This type has evolved from the ancestral filiform embryo typical of the remainder of the genus (tree not shown but see Fig. 6). The filiform embryos vary in the number of coils in subg. *Monogynella;* some taxa will appear to curve but will not form a full coil, whereas the remaining species and subgenera display one to five coils. The number of coils varies considerably amongst subgenera but remains somewhat consistent within individual species.

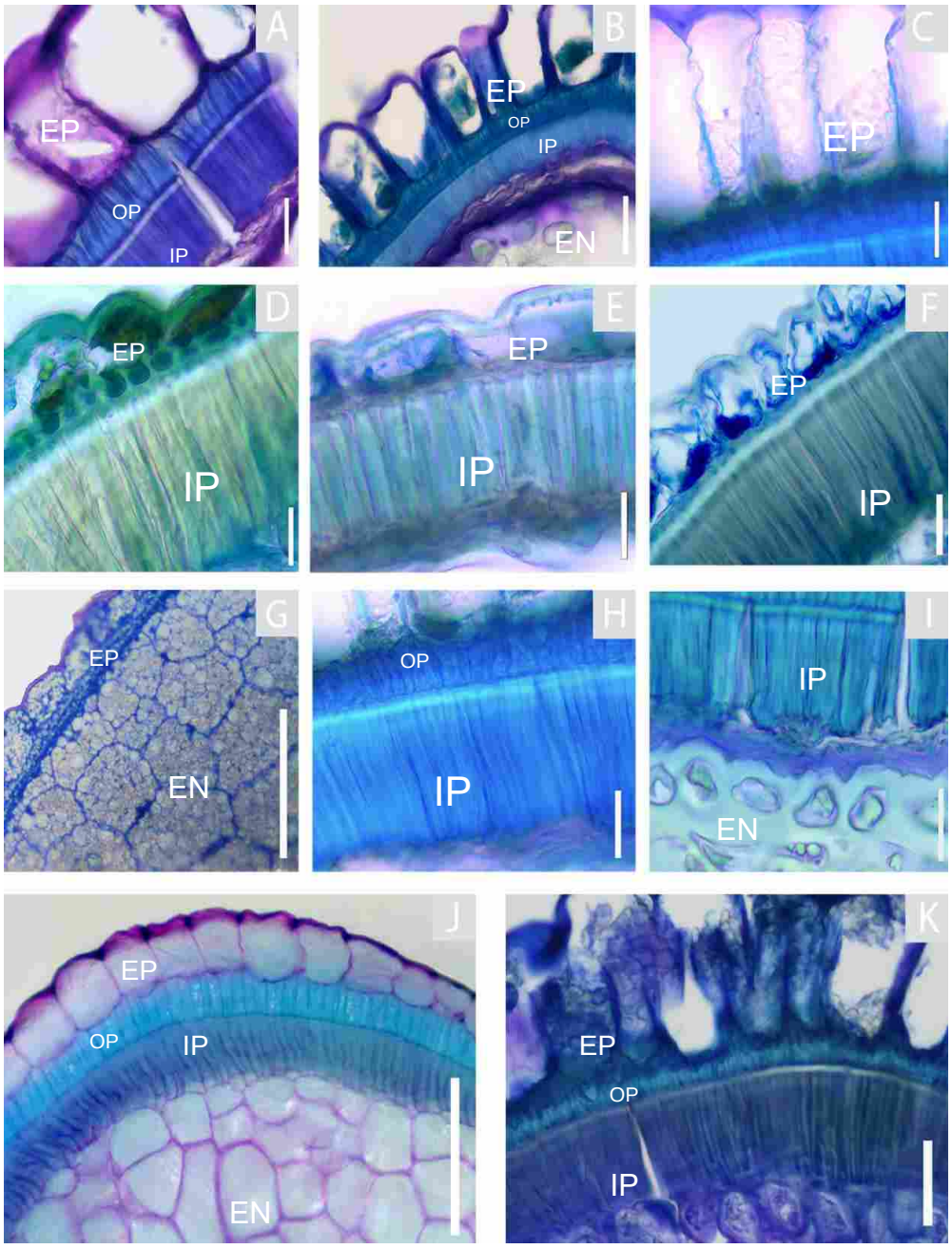

FIGURE 3. Light microscopy anatomy of seeds. A. Seed coat of *C. cristata* (subg. *Grammica*) with papillose epidermis (EP). **B.** Seed coat of *C. epithymum* (subg. *Cuscuta*); epidermal cells in subg. *Cuscuta* are the largest if their ratio with seed size is considered. **C.** Epidermis of *C. mitriformis* (subg. *Grammica*) fully hydrated. **D.** Seed coat of *C. reflexa* (subg. *Monogynella*) with Type I epidermal cells and exhibiting only the inner palisade (IP) layer as the image is not taken in the vicinity of the hilum. **E-F.** Seed coat of subg. *Monogynella* species showing Type I epidermal cells, evident linea lucida. E. *C. monogyna*; F. *C. gigantea*. **G.** Immature seed coat of *C. argentiniana* (subg. *Grammica*); epidermis and parenchyma of endosperm (EN) peripheral layer are filled with starch; the palisade layers are not fully developed. **H.** Palisade layers of *C. mitriformis* (subg. *Grammica*); outer (OP) is less lignified than the inner palisade layer (OP). **I.** Peripheral membrane of the EN in *C. cristata* (subg. *Grammica*) just below the IP layer. **J.** Seed coat of *C. campestris* (subg. *Grammica*) with the papillose EP followed by the OP, IP and the endosperm. **K.** Seed coat of *C. foetida* (subg. *Grammica*), epidermal cells contain starch granules indicating the seed is immature. **EP** = Epidermal cell layer; **OP** = Outer palisade layer; **IP** = Inner palisade layer; **EN** = Endosperm. Scale bars: **A** = 25 µm; **B** = 50 µm; **C**= 25 µm; **D-F** = 25 µm; **G**= 100 µm; **H-I**= 25 µm; **J** = 100 µm; **K** = 25 µm.

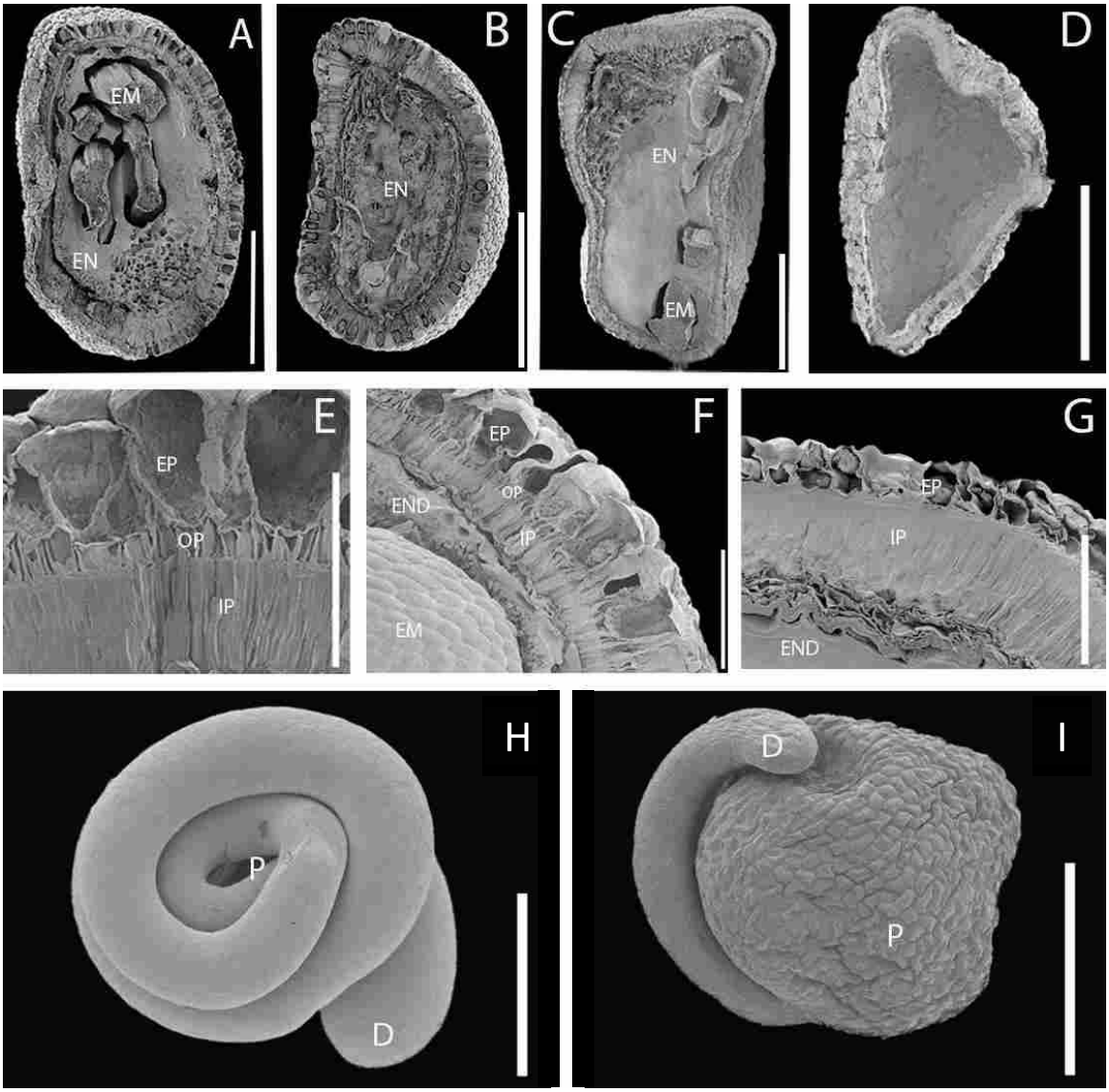

FIGURE 4. Anatomy of seed of *Cuscuta.* A-D. Longitudinal sections of various species. A. *C. cuspidata* (subg. *Grammica*); embryo (EM) is coiled 2.5 times and embedded in the gelatinized endosperm (EN). B. *C. epithymum* (subg. *Cuscuta*); EM removed. C. *C. reflexa* (subg. *Monogynella*); EM coiled two times in EN. D. *C.* denticulate; EN and EM were removed. **E.** Seed coat anatomy (longitudinal section) of *C. gronovii* (subg. *Grammica*) displaying large, papillose epidermal cells (EP), outer and inner palisade layers (OP; IP). **F.** Same in *C. americana* (subg. *Grammica*) showing also the EM embedded within EN. **G.** *C. reflexa* (subg. *Monogynella*) seed coat away from the hilum showing only the IP and the Type I epidermal cells. **H.** Type I EM of *C. pacifica* (subg. *Grammica*), coiled 3 times. **I.** Type II EM of *C. nevadensis* (subg. *Grammica*) with proximal "bulbous" structure. **EP** = Epidermal cell layer; **OP** = Outer palisade layer; **IP** = Inner palisade layer; **EN** = Endosperm; **P** = proximal; **D** = Distal. Scale bar: **A-B** = 500 µm; **C**= 1.00 mm; **D** = 500µm; **E-F** =100 µm; **G**= 500 µm; **H-I**= 500 µm.

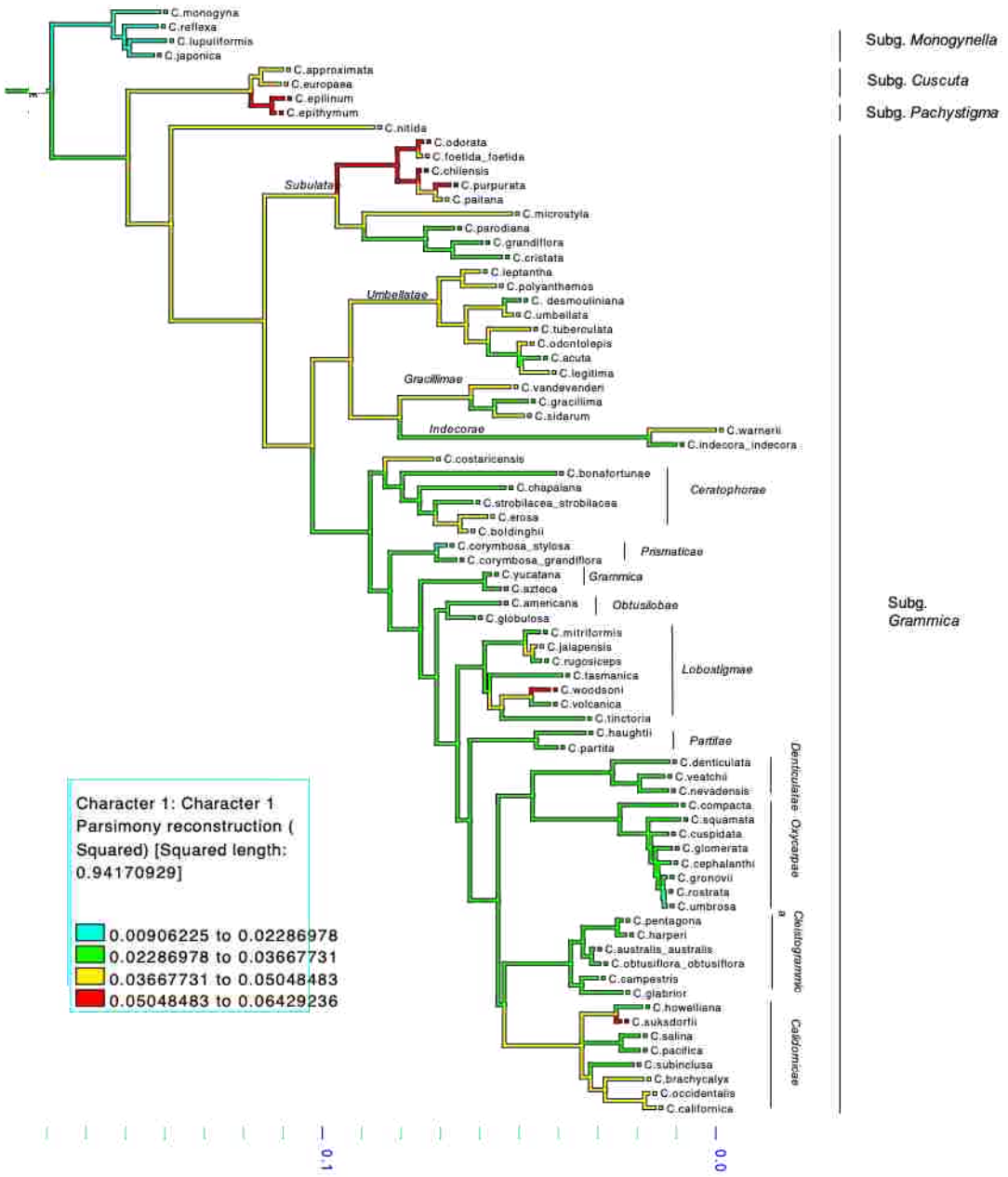

FIGURE 5. Ancestral character state reconstruction of the epidermal cell thickness: seed size ratio in *Cuscuta* mapped onto the recent genus phylogeny based on rbcL and nrLSU sequences (García et al. 2014). Parsimony reconstruction using 4 size bins identified larger epidermal cell thickness: seed size ratio to be likely ancestral in *Monogynella* species with Type II epidermal cells, though in *Grammica* species, this pattern of evolution is not as clear and there are at least 4 cases where the cell/seed ratio evolved to become larger.

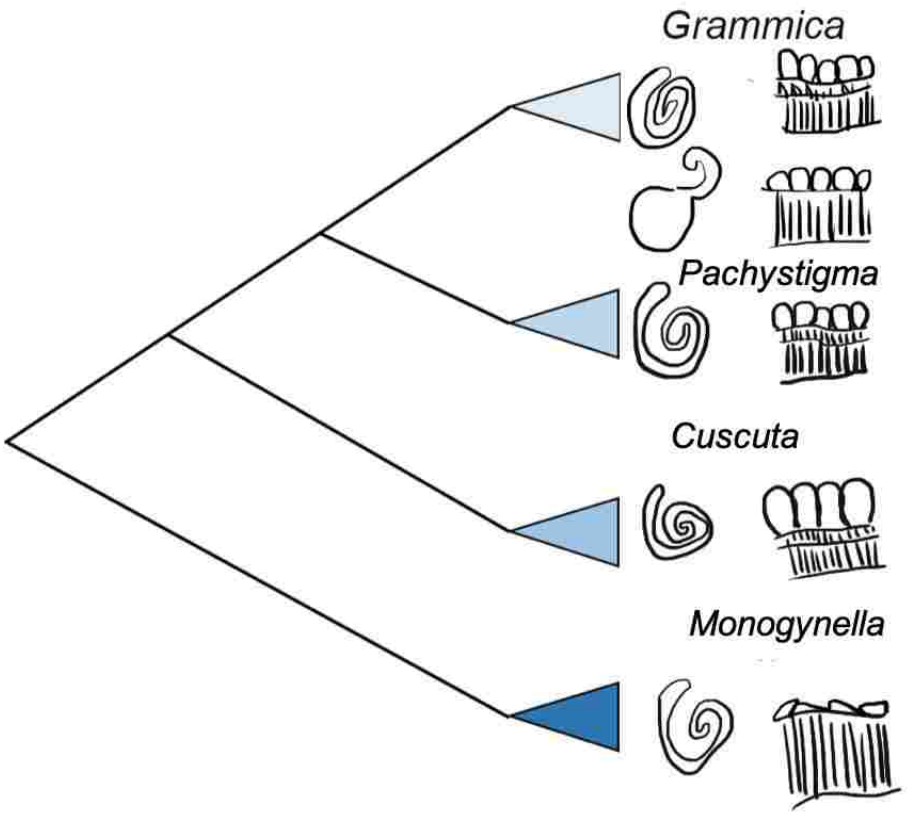

FIGURE 6. Overview *Cuscuta* phylogeny illustrating key characters for all four subgenera of *Cuscuta*. Ancestral subgenus *Monogynella* with long, interlocking epidermal cells (Type I), incomplete outer palisade layer, and a filiform embryo. Subg. *Cuscuta* with large papillose epidermal cells (Type II), complete inner and outer palisade layers, and a filiform embryo. Majority of species of subg. *Grammica* have a complete outer and inner palisade layer and a filiform embryo. Four species have evolved to only have a complete inner palisade layer and a "bulbous" embryo. All taxa in subg. *Grammica* have pitted-papillose epidermal cells (Type II).

5.3 SYSTEMATIC AND TAXONOMIC SIGNIFICANCE

As shown in the previous section (see also Appendix C1), most characters are polymorphic and exhibit extensive convergent evolution. Such characters include: seed length, width, thickness; inner and outer palisade thickness; hilar position and compression; and epidermal cell thickness and diameter. For example, subg. *Grammica* taxa of Clade D (sect. *Oxycarpae*) and Clade G (sect. *Lobostigmae*) have evolved similar seed lengths, epidermal cell thickness, inner and outer palisade thicknesses, whereas, taxa of Clade A (sect. *Calif*ornicae) and Clade L (sect. *Umbellatae*) have similar hilar pad length and width and similar palisade layer thickness in common. Clade E (sect. *Denticulate*) and an isolated species from Clade O (sect. *Subulatae*) share strong morphological and anatomical affinities (see Sect. 6.1). Unfortunately, in most of these cases, little is known about the ecology, dispersal and germination of the species to attempt a biological explanation of their convergent evolution. Seed traits are therefore insufficient to reconstruct the infrageneric lineages within the *Cuscuta* phylogeny. Only the epidermal cell type carries a phylogenetic signal (see previous section and Appendix C3) as the Type I epidermal cells are exclusive to subg. *Monogynella*.

Although not systematically significant at an infrageneric level, the large variation in characters and the consistency within species provide enough taxonomic information for identification purposes. The morphological and anatomical characters of seeds, in combination, are practical in the formation of an identification key (Table 3) for 16 species that are present in Canada and/or of interest to the Canadian Food Inspection Agency (Brouillet et al. 2016; Canadian Food Inspection Agency 2019).

TABLE 3. Key to Canadian *Cuscuta* species based on Weed Risk Analysis documents by the Canadian Food Inspection Agency and Database of Vascular Plants of Canada (Brouillet et al. 2016). It includes species that pose risk to Canadian economy and environment and are present, not yet present or present but not widely distributed and under official control.

Note: A minimum of 10 seeds should be examined to increase chances of identification; seeds should be rehydrated until they have softened to observe morphological characters; sectioned longitudinally parallel to the hilar fissure for observation of anatomical characters; seed length should be measured parallel to the hilar fissure, width perpendicular; inner and outer palisade layers should be measured at their thickest points.

Seeds length: ≥2000 µm; interlocking linear epidermal cells unaffected by dryness or wetness; hilum terminal, ≥400 µm long; outer palisade layer is incomplete, inner palisade layer is complete ...…….....**Subgenus *Monogynella***

Seed length: <2000 µm; isodiametric epidermal cells, concave when dry and papillose upon hydration; hilum subterminal to terminal; hilum <400 µm long; inner and outer palisade layer are complete……**Subgenera *Cuscuta, Grammica***

Subgenus Monogynella

1A. Seed length ≥2900 µm x seed width ≥2700 µm x seed thickness ≥1800 µm; hilar pad length ≥750 µm, length of funicular scar < 530 µm; inner palisade layer thickness >160 µm……***C. reflexa***

1B. Seed length <2900 µm x seed width < 2700 x seed thickness < 1800 µm; hilar pad length < 750 µm, length of funicular scar ≥ 530 µm; inner palisade layer thickness <160 µm.

2A. Seed length ≥ 2500 µm x seed width ≥2100 µm x seed thickness <1500 µm; hilum length <500 µm x hilum width <400 µm; length of funicular scar ≥ 550 µm; inner palisade thickness <120 µm……. ***C. monogyna***

2B. Seed length <2500 µm x seed width <2100 µm x seed thickness ≥1600 µm; hilum length ≥600 µm x hilum width ≥400 µm; length of funicular scar < 550 µm; inner palisade thickness ≥120 µm …….. ***C. japonica***

Subgenera *Cuscuta, Grammica*

1A. Ratio of epidermal cell diameter and seed length ≥ 0.030; seed width <1100; ratio of epidermal cell thickness and seed length ≥ 0.040……... ***Subgenus Cuscuta***

1B. Ratio of epidermal cell diameter and seed length <0.030; seed width ≥1100; ratio of epidermal cell thickness and seed length <0.040…… ***Subgenus Grammica***

Subgenus *Cuscuta*

1A. Seed length ≥1200 µm x seed width ≥1000 µm x seed thickness ≥ 800 µm; hilum length ≥250 µm x hilum width ≥ 200 µm…… **2.**

1B. Seed length <1200 µm x seed width < 1000 µm x seed thickness <850 µm; hilum length < 250 µm x hilum width <200 µm…… **3.**

2A. Seed length ≥1270 µm x seed thickness ≥850 µm; ratio between epidermal cell diameter and seed length <0.035; length of funicular scar ≥80 µm……...***C. europaea***

2B. Seed length <1270 µm x seed thickness <850 µm; ratio between epidermal cell diameter and seed length ≥0.035; length of funicular scar <80 µm…… ***C. epilinum***

3A. Seed length ≥900 µm x seed width ≥800 µm x seed thickness ≥700; ratio between epidermal cell diameter and seed length <0.040; outer palisade thickness ≥20 µm, inner palisade thickness ≥60 µm……***C. approximata***

3B. Seed length <900 µm x seed width <800 µm x seed thickness <700; ratio between epidermal cell diameter and seed length ≥0.040; outer palisade thickness < 20 µm, inner palisade thickness <60 µm……..***C. epithymum***

Subgenus Grammica

1A. Seed length <1200 µm x seed thickness <700 µm ……. ***C. pentagona***

1B. Seed length ≥1200 x seed thickness ≥700 µm……..…… **2.**

2A. Seed length <1400 x seed width <1400 µm……... **3.**

2B. Seed length ≥1400 x seed width ≥1400 µm…….…….**5.**

3A. Seed length ≥1300 µm x seed thickness ≥ 900 µm; hilum length <200 µm x hilum width ≥900 µm; inner palisade thickness <80 µm; length of funicular scar <70µm…….... ***C. pacifica***

3B. Seed length <1300 µm x seed thickness <900 µm; hilum length ≥200 µm x hilum width <900 µm; inner palisade thickness ≥80 µm; length of funicular ≥70 µm……. **4.**

4A. Seed thickness <800µm; hilum length <250 µm x hilum width <220 µm; epidermal cell diameter <30 µm; length of funicular scar ≥80 µm……..... ***C. australis***

4B. Seed thickness ≥800 µm; hilum length ≥250 µm x hilum width ≥220 µm; epidermal cell diameter ≥ 30 µm; length of funicular scar <80µm…… ***C. campestris***

5A. Seed length <1600 µm x seed width <1550µm x seed thickness <900 µm; ratio of epidermal cell thickness and seed length ≥ 0.025; ratio between total palisade thickness and seed length ≥0.185……. ***C. indecora***

5B. Seed length ≥1600 µm x seed width ≥1550 µm x seed thickness ≥900 µm; ratio of epidermal cell thickness and seed length ≥0.025; ratio between total palisade thickness and seed length <0.185……... **6.**

6A. Seed length <1850 x seed width <1600 x seed thickness ≥1200 µm; inner palisade thickness <100 µm; length of funicular scar <160 µm……..……. ***C. gronovii***

6B. Seed length ≥1850 x seed width ≥1600 µm x seed thickness <1200 µm; inner palisade thickness ≥ 110 µm; length of funicular scar ≥ 160 µm……. ***C. umbrosa***

5.4 QUANTITATIVE ANALYSES AND RELATIONSHIPS

A PCoA (Principal Coordinate Analysis) of the entire dataset of seed characters, (Table 1, Appendix C) resulted in a clear separation of only subg. *Monogynella* and Clade E (sect. *Denticulatae*) of subg. *Grammica,* while the remaining taxa grouped closely together (Fig. 7). The PCoA of the transposed matrix grouped characters in four major clusters; the characters closest amongst themselves were 1. Hilum length and width 2. Outer and inner palisade thickness and 3. Epidermal cell shape, epidermal cell width, epidermal cell thickness and epidermal cell diameter (Fig. 8); seed thickness remained isolated from the other characters. Once the similarities between characters were revealed, selected characters meeting the assumptions for Pearson's Correlation (see Sect. 4.5) were analyzed.

Moderate to strong Pearson correlations were found among multiple seed characters (Table 4). Particularly, seed length and width (r =0.95136 r^2 =0.90905) and seed length and thickness (r =0.91537 r^2 =0.8379) are very strongly correlated. These strongly correlated characters identified seed length as a sufficient character to represent seed

size in further statistical analyses. An Ordinary Least Squares Regression led to the same conclusion (Table not shown).

Strong correlations were also revealed between the size of the inner and outer palisade layers (r = 0.79283, r^2 = 0.62858) and length and width of the hilar pad (r = 0.96906, r^2 = 0.93907). A moderate correlation was obtained between seed size and epidermal cell diameter (r =0.58388 r^2 =0.34091). Additional correlations can be found in Table 4.

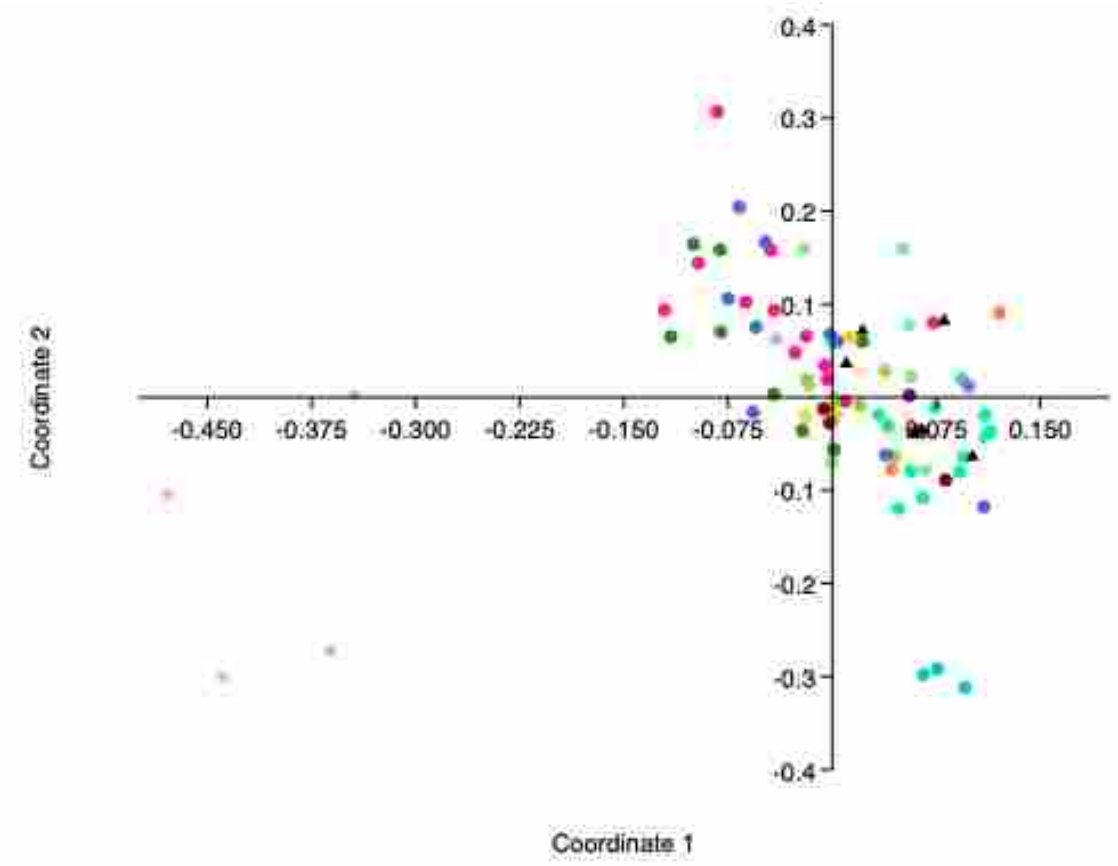

FIGURE 7: Principal Coordinate Analysis (PCoA) plot based on all the seed characters. Subg. *Grammica* (**dots**), species of each section (clade) are represented by a different color; clades generally clustered together with the exception of Clade E – Sect. *Denticulatae* (pale blue). Subg. *Pachystigma* (**plus**) clustered together with subg. *Grammica*, but it was represented only by one species in this study. Subg. *Cuscuta* (**triangles**), grouped together but within subg. *Grammica.* Subg. *Monogynella* (asteriska) species formed a separate group from the remainder of the genus. The leading eigenvalue represents 26.703 % of the variation while the second 15.336 %.

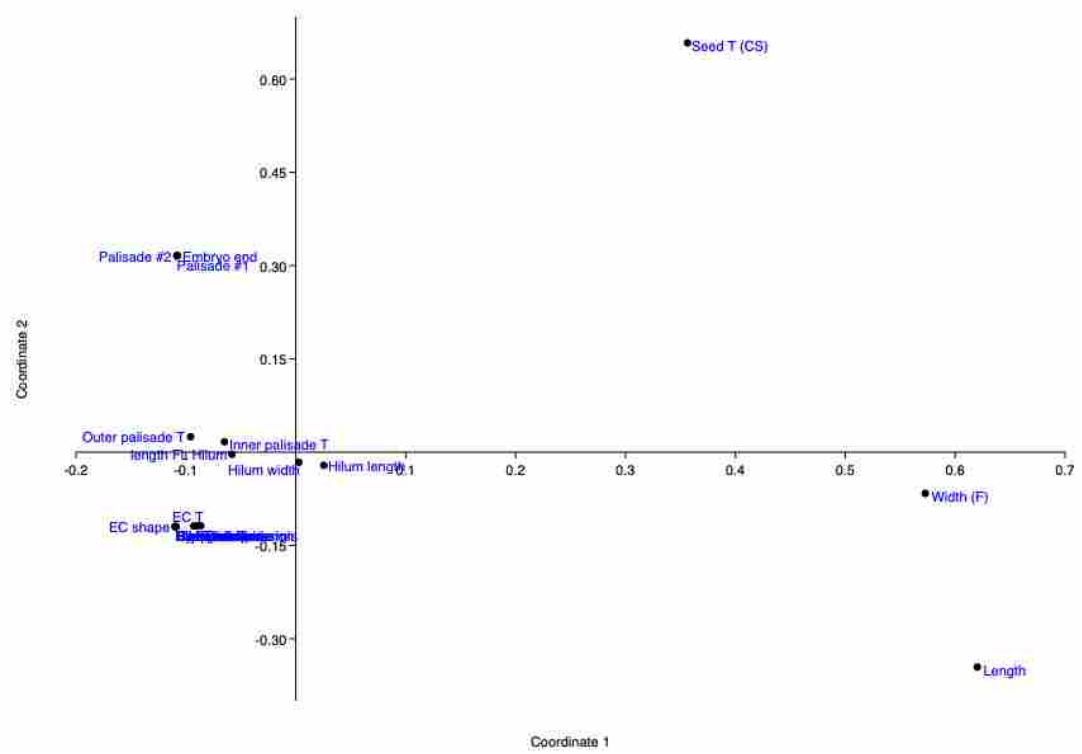

FIGURE 8. PrincipalCoordinate Analysis (PCoA) of transposed data matrix. Seed T. = seed thickness. Inner palisade T. = inner palisade thickness. EC shape = epidermal cell shape. EC T. = epidermal cell thickness. Length FL Hilum = length of funicular scar on hilar pad. Palisade #1 = Presence of outer palisade layer. Palisade #2 = Presence of inner palisade layer. Embryo end = Type of embryo. Characters grouped in four clusters: hilum length and width, outer and inner palisade thickness and epidermal cell shape, epidermal cell width, epidermal cell thickness and epidermal cell diameter. Length and width are grouped in relatively the same area whereas seed thickness appears on its own. The leading eigenvalue represents 98.648 % of the variation while the second 0.66851%.

TABLE 4. Pearson's r correlation statistic between various seed characters of *Cuscuta.*

Variables	r	r^2	Strength of Correlation
Seed length x seed width	0.95136	0.90905	Very strong
Seed length x seed thickness	0.91537	0.8379	Very strong
Inner palisade layer x outer palisade layer	0.79283	0.62858	Strong
Length of hilar pad x width of hilar pad	0.96906	0.93907	Very strong
Length of hilar pad x length of funicular scar	0.93074	0.86627	Very strong
Seed size x epidermal cell diameter	0.58388	0.34091	Modest
Seed Size x epidermal cell thickness	0.30262	0.091576	Weak
Outer palisade layer x epidermal cell thickness	0.53551	0.28677	Modest
Distribution of subg. *Grammica* x seed size	0.094412	0.0089136	No relationship

TABLE 5. Summary statistics for quantitative characters of *Cuscuta*

Seed trait (µm)	Mean	Median	Std. Error	Min	Max	n	p-value (Shapiro)
Seed length	1353.5	1265.9	45.1	704.55	3158.3	100	8.877E-08
Seed width	1235.3	1156.2	40.9	668.28	2910.05	98	2.178E-06
Seed thickness	842.3	806.7	29.2	388.4	2133.2	98	7.212E-07
Epidermal cell thickness	45.7	43.4	1.7	18.0	116.6	91	2.374E-06
Epidermal cell width	32.4	30.9	0.92	13.6	56.2	91	0.006912
Outer palisade thickness	25.6	24.4	0.8	10.4	60.5	94	7.586E-06
Inner palisade thickness	81.6	77.9	2.5	42.3	163	95	0.003854
Hilum length	256.5	228.3	13.2	94.3	811.3	95	3.323E-11
Hilum width	215.4	192.7	10.2	97.97	659.9	95	1.464E-10
Epidermal cell diameter	36.9	36.7	0.9	17.5	58.5	89	0.9864
Length of funicular scar	99	77.7	9.7	32.1	575.4	87	5.46E-16

5.5. WATER GAP ANATOMY AND DYE TRACKING

Water gap anatomy was examined in seven species of *Cuscuta*. Though the number of palisade layers may vary amongst the subgenera outside the hilar area (see section 5.2. and Appendix B), at the hilar pad the seed coat is similar across the entire genus; it is composed of an epidermal layer overlaying two palisade layers. In this area, both inner and outer palisade layers increase in thickness until a suture-type discontinuity at the centre of the hilar pad where the linear-shaped fissure can be observed (Fig. 10).

The aniline blue stain was unable to penetrate the seed coat of dormant seeds even after 60 min of soaking (Fig. 9; G). However, in non-dormant seeds, the stain began to penetrate the hilar fissure after 15 min. After 120 min, the stain was observed around the endosperm and embryo. Interestingly, epidermal cells of both dormant and non-dormant petroleum gel painted seeds stained randomly with varying degrees of

saturation until the entire epidermis was saturated. When sectioned, it was observed that even if the epidermal layer was completely saturated, the stain was unable to penetrate the palisade layers of the hilum-painted non-dormant seeds. Tracheid-like structures, that are most likely remnants of the vasculature from the funiculus, were observed at the hilar fissure.

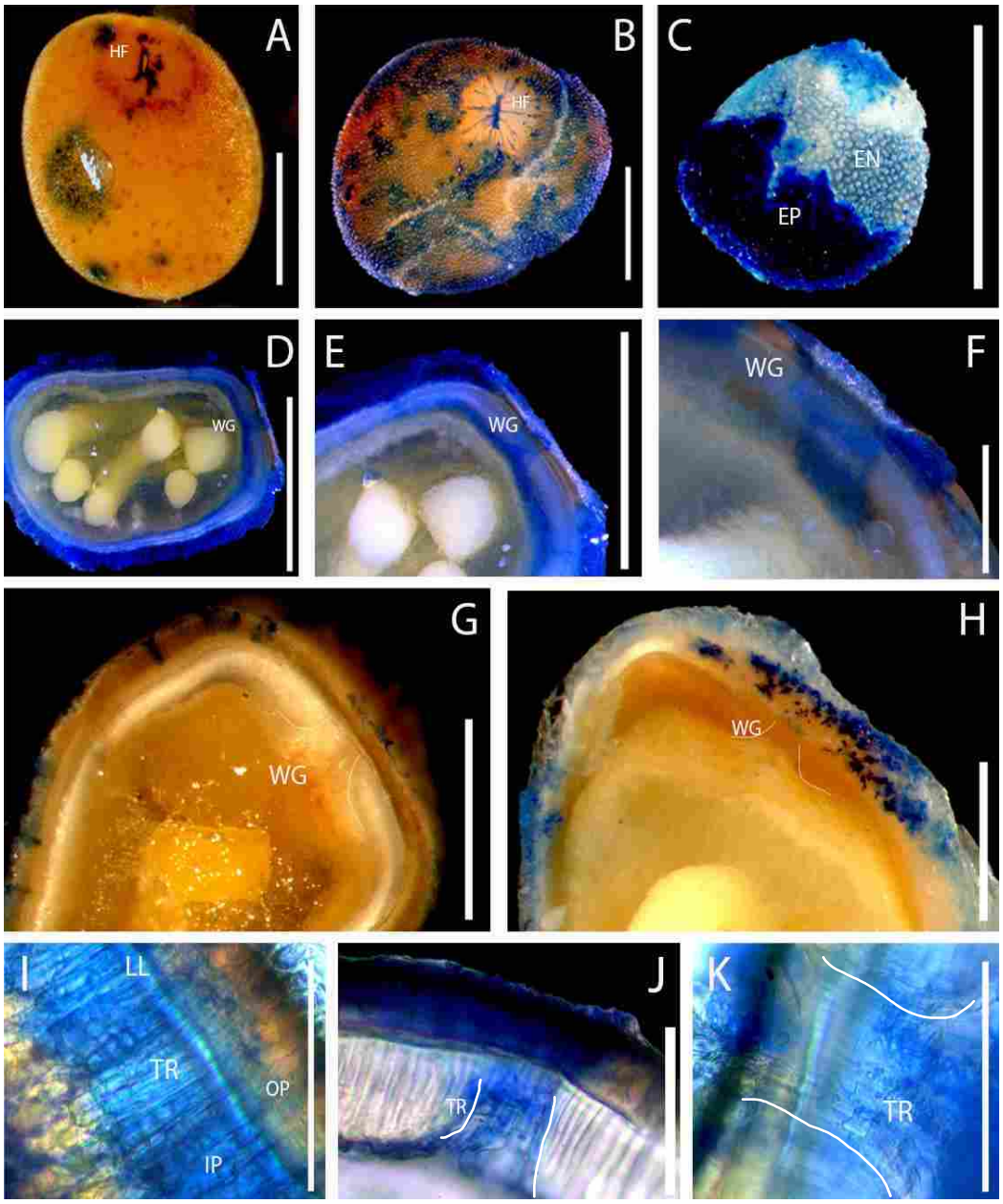

FIGURE 9. Water gap morphology and anatomy. A. Rehydrated (non-dormant) seed of ***C. tasmanica*** after 30 min in aniline blue solution (ABS). Hilar fissure (HF) is open, with dye concentrating around its opening. **B.** Rehydrated seed of ***C. gronovii*** after 15 min in ABS. Epidermal cells have taken up a significant amount of ABS; HF is open and completely saturated. **C.** Rehydrated seed of ***C. epithymum*** after 1h and 45 min in ABS. Half of the seed coat was removed to exhibit a completely saturated seed coat with the underlying endosperm (EN) unaffected, except at the hilar area. **D-E.** Longitudinal section of rehydrated ***C. sandwichiana*** seed after 30 min of in ABS; dye penetration exclusive to the water gap (WG). **F.** Longitudinal section of rehydrated ***C. gronovii*** seed after 1h and 15 min in ABS; dye enters through the evident WG region and surrounds the EN. **G.** Longitudinal section of rehydrated ***C. tasmanica*** seed with hilum painted after 1h in ABS with no dye penetration at the hilar area. **H.** Longitudinal section of rehydrated ***C. volcanica*** seed with hilum painted after 30 min in ABS; epidermal cells stained but no dye entered through the WG. **I.** Section of rehydrated ***C. gronovii*** seed after 30 min in ABS; tracheids (TR) are evident in the inner palisade layer (IP) and are most likely the route of water access. **J.** Hand section of rehydrated ***C. epithymum*** seed after 30 min in ABS with TR visible in the WG area. **K.** Hand section of ***C. sandwichiana*** seed after 30 min in ABS with the TR passing through the IP and outer palisade layer in the WG area. Scale bar: **A-B** = 1.00 mm; **C** = 500 µm; **D** = 1.00 mm; **E** = 500 µm; **F** = 250 µm; **G-H** = 500 µm; **I-K** = 100 µm**.**

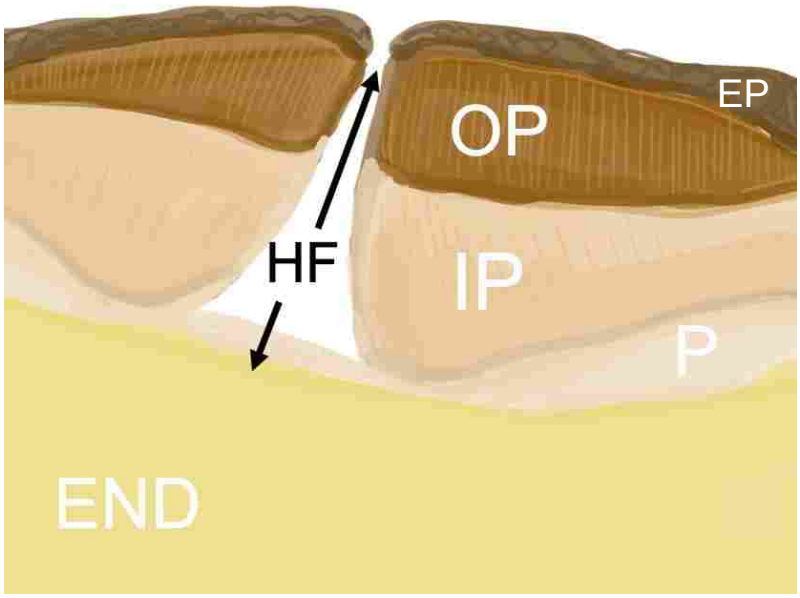

FIGURE 10. Schematic representation of section through water gap in *Cuscuta*. Outer palisade (OP) layer is present in all species under and around the hilar pad just below the epidermal layer (EP), inner palisade (IP) layer is present throughout entire seed coat. Remnants of parenchymal layers (P) are occasionally evident just below the inner palisade, and the endosperm is immediately below. In this area, both inner and outer palisade layers are the thickest in the seed coat. The hilar fissure (HF) passes through all the layers of the seed coat. END = endosperm.

5.6 FUNCTIONAL RELATIONSHIPS

Breeding systems and number of seeds per capsules in Cuscuta

A bipartite network graph was used to visualize the breeding system categories, the pollen/ovule ratios (P/O) and the average number of seeds per capsule (S/C) (Fig. 12). Further visualization was provided by graphing P/Os against the S/Cs and coloring the species points based on their breeding system categories (Fig. 11). Taxa categorized as facultatively autogamous appear to have on average a higher S/C. Fully xenogamous species seem to have amongst the lowest S/Cs. This visualization suggests a negative weak correlation. A Kendall's Tau correlation supported this preliminary assessment and indicated a weak negative correlation (Table 6; ρ= 0.21632). ANOVA results confirmed a statistically significant (p-value: 2e-16; Table 7) relationship between the S/C and the

breeding system categories determined by Cruden's P/Os. Fully autogamous species have the highest numbers of seeds per capsule whereas fully xenogamous have the smallest (Fig. 13).

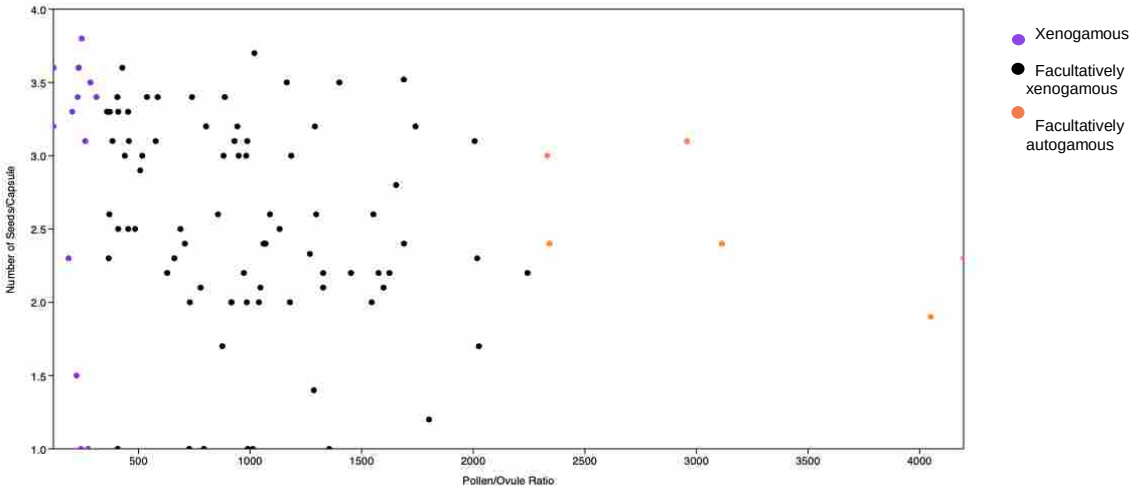

FIGURE 11. X-Y graph of the pollen/ovule ratios (P/Os) and the average number of seeds per capsule (S/C) for 98 taxa representing all four subgenera of *Cuscuta*. A weak negative trend is observed. **Purple dots.** 14 taxa categorized as facultatively autogamous have among the highest S/C averages as selfing provides reproductive assurance and therefore the highest seed counts. It is important to note that some taxa in this category have evolved to only produce one S/C. **Black dots.** Facultatively xenogamous category comprised of 77 species that show the greatest S/C variation, possibly indicating the degree to which some were selfing vs. outcrossing. **Orange dots.** Fully xenogamous, represented by only five species, have relatively low S/C as they are self-incompatible and cannot self-fertilize.

TABLE 6. Kendall's Tau correlation statistic (ρ). Pollen/Ovule ratio (P/O) and the number of seeds per capsule exhibit a weak negative correlation, indicating that as the pollen ovule ratio increases the average number of seeds per capsule (S/C) slightly decreases.

	Pollen/Ovule Ratio (P/O)	Number of Seeds per Capsule (S/C)
Pollen/Ovule Ratio (P/O)		0.0016005
Number of Seeds per Capsule (S/C)	-0.21632	

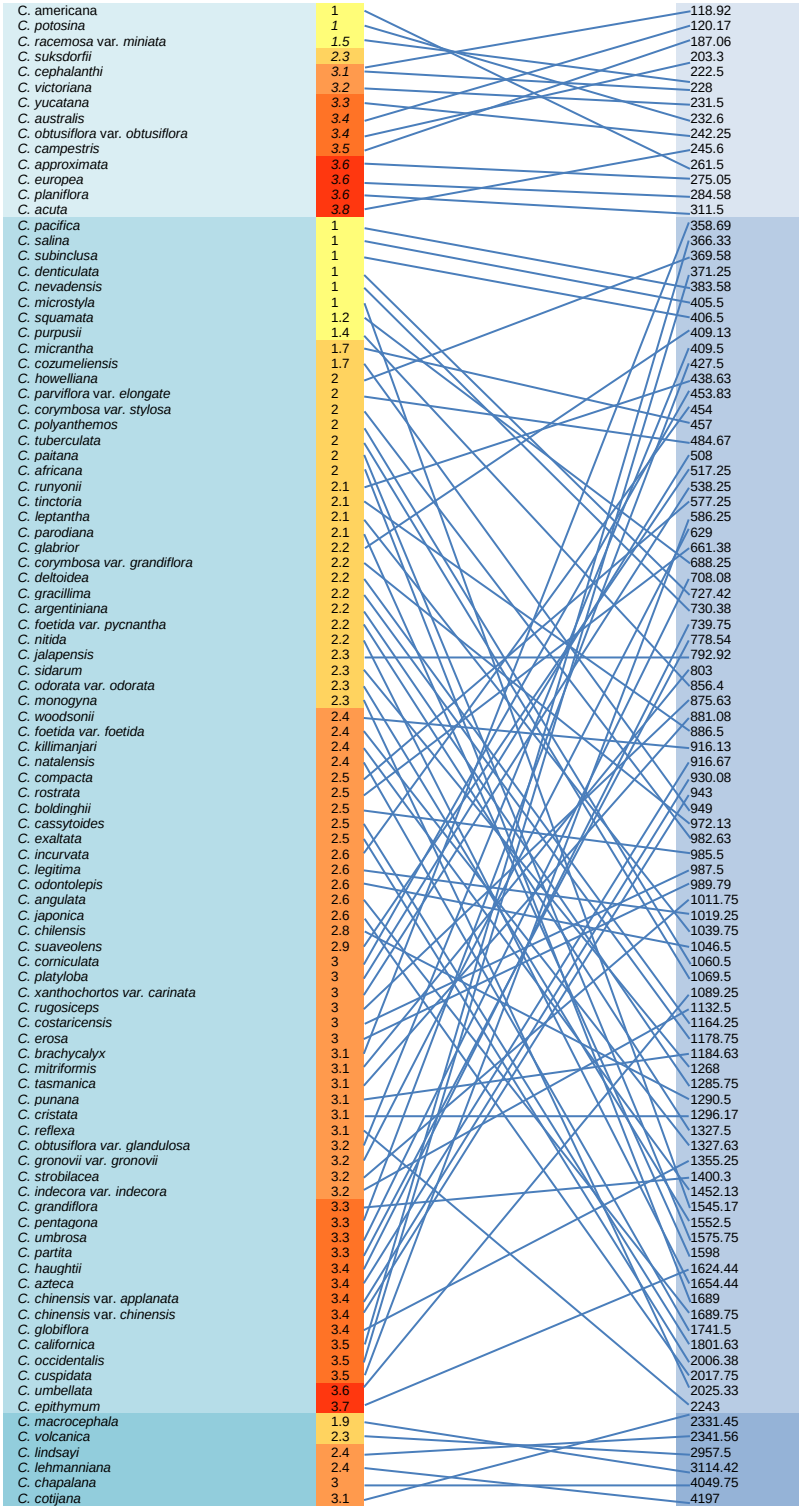

FIGURE 12. Bipartite network graph of breeding system categories indicated by pollen/ovule ratios (P/Os) and the average number of seeds per capsule (S/C). The first column indicates the breeding system category including a facultatively autogamous group with 14 taxa, facultatively xenogamous with 80 taxa and fully xenogamous category with 6 taxa. The second column includes the average S/C and was color - coded from yellow to red. The third column indicates the pollen ovule ratio and was arranged from the minimum to the maximum. Facultatively autogamous species have the lowest pollen/ovule ratios but amongst the highest S/C, while the inverse is true for fully xenogamous species.

TABLE 7. ANOVA results showing that the difference between means of pollen ovule ratios (P/Os) and the number of seeds per capsule (S/C) are significant.

	df	Sum sq	Mean sq	F value	Sig.
Ind	2	67733881	33866941	173.9	2e-16
residuals	294	57267255	194787		

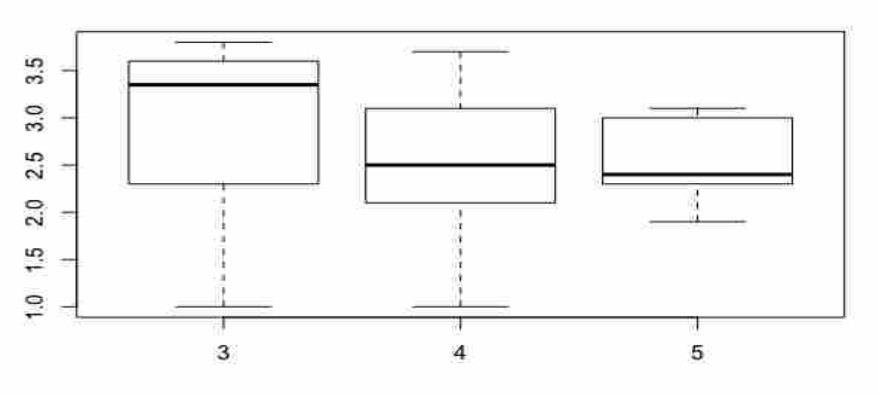

FIGURE 13. Boxplot of ANOVA results for the breeding system categories and the average number of seeds per capsule (S/C). 3. Facultatively autogamous taxa have the highest S/Cs averages but also the highest variation as two taxa have evolved to have only one S/C. **4.** Facultatively xenogamous group include six taxa with one S/C while the remainder species possess an intermediate S/C number between the other two categories. **5.** Fully xenogamous taxa have the lowest S/C average and the least amount of variation; species in this category are self-incompatible.

As a prediction model a general regression tree was constructed, with the response variable being the pollen/ovule ratios, the explanatory variables the breeding system categories and the average number of seeds per capsule (S/C) (Fig. 14). Each leaf is composed of a percentage of included taxa and their average P/Os. Each split in the tree is dictated by a statement; if the statement is true one follows the split to the left, and if the statement is false, one follows the branch to the right. The terminal leaves, at the very bottom of the tree, include all the taxa now divided into six leaves based on their S/C and their P/O ratios.

At the first split, the regression tree separated directly to the leaf of 14% facultative autogamous taxa (coded 3) from the remainder species, which lead to additional splits based on their S/C averages. The terminal leaves of these additional

splits divided the remaining 86% taxa into additional leaves, and illustrated a steady increase of P/O values as the S/C averages decrease. Overall, these results confirm the hypothesis about a relationship between the reproductive output (S/C) and breeding systems.

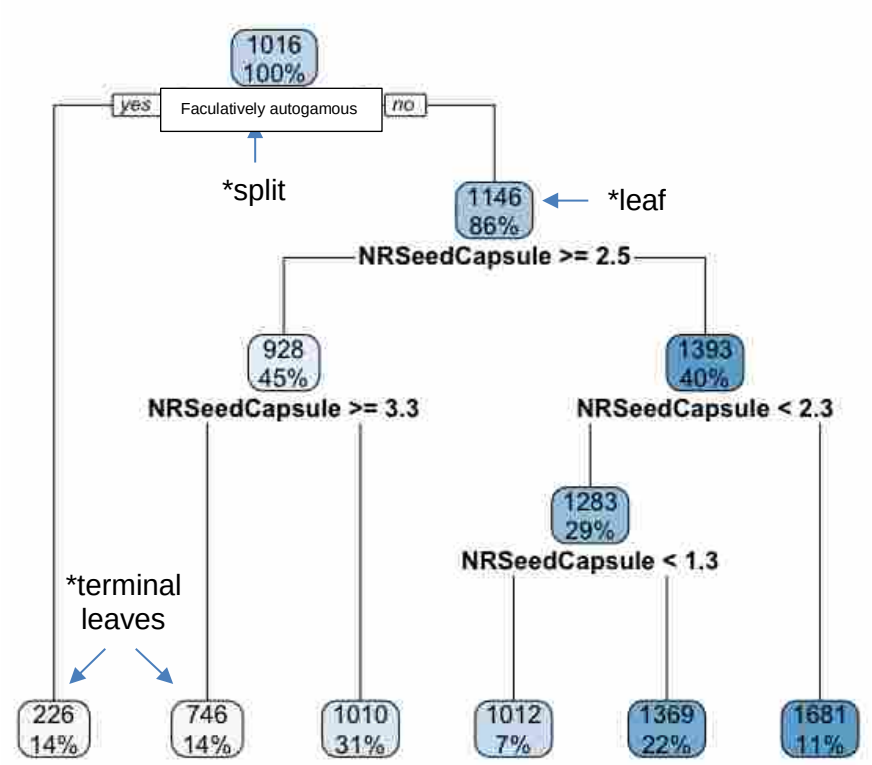

FIGURE 14. Regression tree analysis of number of seeds per capsule (S/C) and pollen/ovule ratios (P/O) used as an indicator of breeding systems. The first split separated directly the leaf of **14%** facultatively autogamous taxa with an average P/O of 226 (first leaf to the left). At the next node, the remainder species were divided depending on whether they had more or less than 2.5 S/C. **45%** of taxa had more than 2.5 S/C and were split again depending if they had more or less than 3.3 S/C. **14%** of taxa had more than 3.3 S/C and were placed in the *second terminal leaf*, with a P/O of 746. **31%** had less than 3.3 S/C and were separated in *the third terminal leaf,* with a P/O of 1010. Taxa with less than 2.3 S/C were found in the *sixth terminal leaf*, comprising 11% of the total, P/O of 1681. Taxa with more than 2.3 S/C were divided once more if they have more of less than 1.3 S/C. **7%** of the total had less than 1.3 S/C, P/O 1012, while **22%** had more than 1.3 S/C and P/O of 1369. **NRSeedCapsule = Number of seeds per capsule.**

Geographical distribution of subg. Grammica and the size of seeds

A Spearman's Rank correlation indicated a very weak correlation between the seed size and the total geographical distribution range of species (r = 0.094412, r^2= 0.0089136) revealing a non-monotonic relationship between the variables. This strongly suggests that seed size does not have an impact on the total geographical range of the species.

6. DISCUSSION

6.1 DIVERSITY OF MORPHOLOGY AND ANATOMY IN SEEDS

This study is in agreement with previous morphological (Knepper et al. 1990; Abdel Khalik 2006; Costea et al. 2005, 2006 a, b) and anatomical (Hutchison and Ashton 1979; Lyshede 1984,1992; Jayarusiya et al. 2008; Rodriguez-Pontes 2009) investigations on the seed coat and seed characters in *Cuscuta.* However, this research has produced the first genus - wide comparative survey of the seeds and provided a better resolution on several features, some of which had not been examined before.

Epidermal cell layer

Several studies (e.g. Hamed 2005; Abdul Khalik and Osman 2007) have suggested that similarities exist between the seed coat in *Cuscuta* and other genera in Convolvulaceae (e.g. *Convolvulus, Cressa, Evolvulus, Ipomoea, Merremia* and *Seddera*). Despite the fact that the epidermal cells of some Convolvulaceae are isodiametric and dome-like, resembling those of *Cuscuta* (Sripleng and Smith 1960; Hamed and Mourad 1994; Ketjarun et al. 2016), there is no evidence of an ability of these cells to reversibly shift from invaginated to papillose during imbibition (Gaertner 1950). Furthermore, a key for noxious and common weed species of Convolvulaceae

(*Convolvulus, Ipomea, Jacquemontia* and *Merremia*; Gunn 1969) indicated the presence of additional morphological features such as a prominent hilar ridge, a prominent keel and trichomes covering various parts of the seed, which are absent in *Cuscuta* species. Although their shape can be similar, the seeds of other Convolvulaceae tend to be larger, with only a few species exceptions in *Cressa* and *Convolvulus* (Abdel Khalik and Osman 2007) where seed size is similar to the sizes observed in *Cuscuta*. Albeit there are similarities between *Cuscuta* and the remaining genera of Convolvulaceae, as parasitism evolved, dodder seeds have apparently become less complex morphologically with the exception of the reversible pitted-papillose epidermis which seems to be an apomorphy in *Cuscuta*.

The invaginated and water-induced bulging in epidermal cells (Knepper et al. 1990; Costea and Tardif 2006), described as Type II in this study, were observed in the majority of *Cuscuta* species with the exception of subg. *Monogynella*, which exhibits the ancestral Type I cells. The interesting bulging hydrating feature has been attributed to physical forces assisted by hydrophilic pectic zones (Lyshede 1984,1990,1992), which are capable of attracting and retaining water, and to the flexible nature of the outermost layer of the epidermal cells (Gaertner 1950; Lyshede 1990).

Koch (1880) speculated that wind is responsible for dispersal of *Cuscuta* seeds and Lyshede (1984) agreed with this assumption based on alleged similarities with other small-seeded families like the Scrophulariaceae and the Orobanchaceae. However, the seeds of the latter families are smaller, often dust-like, and their epidermis has often a reticulate or “honey-comb” morphology which does not change depending on the amount of humidity available (Chang and Heckard 1972; Elisens and Tomb 1983; Eriksson and Kainulainen 2011). Accordingly, Costea and Tardif (2006) suggested that *Cuscuta* seeds do not possess “classical” adaptations for wind dispersal, and that the alveolate/papillate seed coat is more likely an adaptation related to the imbibition and

germination process. The results of this study support the latter hypothesis. The invagination of the epidermal cells likely enables the loose cell walls to contract when seeds desiccate and thus to be more protected while seeds are dormant. When hydration occurs, the epidermal cell becomes papillose allowing water retention within the epidermal layer.

The presence of pectin in the epidermal cell walls results in the creation of a mucilaginous layer around the seeds when these are hydrated (Grubert 1982; Lyshede 1984; Costea and Tardif 2006). This protects the seeds from water loss (Harper and Benton 1966) and aids in germination under low water conditions (Young and Evans 1973; Yang et al. 2012). Likely, the mucilage and the increase in seed surface also prevent the deeper displacement of pre-germinating seeds within the soil profile, as these require light and can germinate in high proportions at depths no greater than 5 mm (Allred and Tingey 1964; Dawson 1965; Hutchison and Ashton 1979; Benvenuti 2003; Benvenuti et al. 2005). This may also explain why smaller seeds tend to have the largest epidermal cells, while the opposite is true for larger seeds. By analyzing the ratio between the epidermal cell thickness and the seed size, as done in this study, a more relevant comparison could be made among taxa from different subgenera.

The Type I epidermal cells of subg. *Monogynella* have not been investigated to the same extent as Type II. The general morphology has been briefly commented on (Knepper et al. 1990; Kim et al. 2000; Abdel Khalik 2006; Costea et al. 2015), but without details on anatomy or comments on the function of this type of epidermal morphology. The characteristic orientation of these epidermal cells may optimize the mechanical resistance of this cell layer (Barton 1965; Bouman and Boesewinkel 1995). In any case, the ancestral character reconstruction revealed the likely primitive status for this type of epidermis, which is reasonable considering the functional advantages provided by the Type I seed epidermis.

Palisade layers

Convolvulaceae are distinguished by the special development of the three-outer cell-layers of integument and Corner (1976) argued that their presence in *Cuscuta* supported its position in Convolvulaceae. The ovular integument undergoes anticlinal or periclinal divisions forming the outer epidermal layer and the second cell layer which either remains as a single palisade layer or continues dividing (Sripleng and Smith 1960; Govil 1971; Corner 1976; López-Curto et al. 1990). In *Convolvulus,* the epidermal layer consists of thick-walled lignin cells with no starch, followed by a sub-epidermal layer of small, rectangular sclereids and a layer of palisade sclerenchyma. In *Cuscuta*, the outer palisade layer contains cells with lignified thickenings at their base, resembling those of Brassicaceae (Johri and Tiagi 1952; Lyshede 1984), while the inner palisade contains a light line (linea lucida) that coincides with seeds of other taxa with impermeable seed coats (e.g., Fabaceae) (Sripleng and Smith 1960; Bouman and Schier 1979; Lyshede 1984,1992; Jayasuriya et al. 2008; de Paula et al. 2012; Venier et al. 2012). Corner (1976) described the outer palisade layer of *Cuscuta* as being slightly lignified and the inner one as being strongly lignified. This is in contrast to observations of other authors that reported the outer cells had lignified or cellulosic walls while the inner ones were unlignified (Guttenberg 1909; Breymann 1914; Kamensky 1928; Lyshede 1984). Our results concur with Corner (1976); the outer palisade layer is less lignified, whereas the inner palisade exhibits increased lignification by comparison. The higher degree of lignification within the inner palisade layer and the presence of a linea lucida in this layer suggest that this is the layer predominately responsible for the water impermeability and mechanical resistance. The outer palisade layer adds extra protection, but it is less strong mechanically.

Anatomical studies on *Cuscuta* seeds have been limited mainly to species from subg. *Grammica*, with similar findings on the palisade layers by several authors

(Hutchison and Ashton 1979; Lyshede 1984, 1992; Jayarusiya et al. 2008; Behdarvandi 2014). Johri and Tiagi (1952) and Knepper et al. (1990) investigated seeds from subg. *Monogynella* but they provided little to no detail on the palisade layers. Species of subg. *Monogynella* have a different seed coat anatomy compared to most of the species in the remaining subgenera, and this study found the incomplete outer palisade layer to be likely ancestral. The addition of a complete external palisade layer to the seed coat in the remaining subgenera is likely derived and associated with the reversible pitted-bulging epidermis.

An impermeable palisade cell layer(s) or "hardseedness" has been associated with physical dormancy in many plants (Rolston 1978; Werker 1980; Kelly et al. 1992; Baskin and Baskin 2000). In subg. *Monogynella*, the inner palisade layer is clearly responsible for both physical dormancy and the mechanical resistance of the seed coat, as taxa exhibit an inner palisade layer that is thicker than the combined inner/outer palisade layers for species in the remainder of the genus. In subg. *Cuscuta,* combinational dormancy (physical + physiological) has been reported for *C. epithymum, C. epilinum, C. europaea and C. approximata* (Gaertner 1950, 1956; Tingey and Allred 1961; Meulebrouck et al. 2008) where physiological dormancy must be broken following the physical dormancy break. This may explain why the inner palisade layer of subg. *Cuscuta* is substantially thinner than that of *Grammica* and *Monogynella.* Most likely the outer palisade layer is related to specific Type II epidermal cells and coincides with their evolution or possibly with the physiological dormancy of many taxa.

Behdarvandi (2014) reported that *C. nevadensis* in sect. *Denticulatae* of subg. *Grammica* also exhibited an incomplete outer palisade layer like in subg. *Monogynella*. In this study, I found that the other two species of sect. *Denticulatae, C. veatchii* and *C. denticulata*, as well as *C. microstyla* from sect. *Subulatae* also exhibit a partial external palisade layer. The seeds of these species rehydrate almost immediately, suggesting

that most likely they do not exhibit physical dormancy. From an ecological perspective, these four *Grammica* species occur in areas with low precipitations (sect. *Denticulatae*- Southwestern deserts in the U.S.A. (Costea et al. 2005; García et al. 2018), *C. microstyla* - on either side of the Andes in Chile and Argentina) (Hunzinker 1949; Ho 2017). Species of sect. *Denticulatae* are viviparous; i.e. germination can take place while seeds are still in the capsules and the parasite is attached to the host (Costea et al. 2005). Vivipary has not been reported in *C. microstyla,* but its capsules are also indehiscent (Hunziker 1949). At least in the case of species from sect. *Denticulatae*, these seed traits (lack of dormancy and vivipary) allow the rapid germination during the scarce precipitation events and ensure host availability.

The palisade layers have also been suggested to mechanically restrict germination (Hutchinson and Ashton 1979) and on the basis of these results, I agree with Behdarvandi (2014) that seed germination in *Cuscuta* species is likely influenced negatively by the thickness of the seed coat layers.

Endosperm and embryo

Although the endosperm has only been studied in *Cuscuta* for relatively few species, results are controversial. The formation of the endosperm has been classified as nuclear by some (MacPherson 1921; Truscott 1966; Corner 1976; McDonald 1991), while others countered that endospermic tissue may begin as such, but ultimately becomes cellular. During early development there are many free nuclei formed, however once the change-over to wall formation occurs, wall formation gradually progresses centripetally, as reported by Johri (1987) and Johri et al. (1992).

The presence of both a perisperm (Des Moulins 1853; MacPherson 1921; Hunzinker 1949; Kuijt 1969) or alternatively a persistent aleuronic layer (Lyshede 1984;1990) has been described, but our findings do not match these results. The

perisperm derives from the body of the ovule as a persisting nucellar tissue prior to endosperm development (Houk 1938; Bouman 1984; Madrid and Friedman 2010; Zheng et al. 2010; López-Fernández and Maldonado 2013) and its presence characterizes plants of several families including Caryophyllaceae, Zingiberaceae and Piperaceae (Yuncker 1958; Bittrich 1993; Larson et al. 1998). Perispermic development does not occur in *Cuscuta* as the nucellus is rapidly reabsorbed prior to fertilization (Govil and Lavania 1980; Rodríguez-Pontes 2009). Moreover, the term “aleuronic layer” is generally specific to species of Poaceae (e.g., Hemdane et al. 2016). Although an aleurone layer in cereal grains resembles somewhat the cellularization of the endosperm in *Cuscuta,* ultimately the two are different. Both are characterized by prominent nuclei and thick cell walls, but *Cuscuta*’s peripheral layer is packed with starch granules whereas aleurone layers are rich in proteins (Jones and Jacobsen 1991). Furthermore, aleurone layer development is initiated by periclinal divisions that produce an initial cellular peripheral layer along with an interior layer and divisions are repeated until the entire endosperm becomes cellular. Upon cellularization, peripheral cell divisions are highly ordered, and the resulted aleuronic cells are cuboidal, while the internal cells are atypical in division (Kiesselbach 1949; Brown et al. 1994; Becraft and Yi 2010). In *Cuscuta,* only the peripheral layer of the endosperm becomes cellular. Therefore, our results coincide with Rodriguez-Pontes’ (2009) description of a peripheral parenchymal layer surrounding the endosperm.

This “membranous” peripheral layer of endosperm shows different degrees of thickness amongst taxa, varying from rather scanty to significantly thicker. The peripheral endosperm cell layer does not impede water from entering the endosperm, rather it may prevent water loss during initial stages of germination (Reid 1985). Among the ten species identified with a thicker endosperm peripheral layer, only three (*C. veatchii, C. nevadensis, C. denticulata* - Clade E; sect. *Denticulatae*) are found within the

same clade while the remainder are found throughout subg. *Grammica* and one species is in subg. *Pachystigma* (*C. nitida*). Behdarvandi (2014) reported that *C. nevadensis* had a membranous endosperm layer followed by the embryo, and we can confirm that this is the case for the aforementioned species and for *C. microstyla* (Clade O; sect. *Subulatae*) which shares Clade E's unique character set. In contrast, in the remaining taxa, the embryo is embedded within a gelatinized endosperm with a peripheral layer, even at seed maturity.

Additionally, taxa of Clade E (sect. *Denticulatae*) and *C. microstyla* (sect. *Subulatae*) were observed to have an embryo with an enlarged spherical or club-shaped proximal end (denoted as the Type II in this study). Lloyd (1908) was the first to note a "water-storage bulb" in an unknown *Cuscuta* species from Zacatecas, Mexico. Yuncker (1921,1932) first used this embryo characteristic to differentiate subsect. *Denticulatae* in which he included *C. denticulata, C. veatchii*, and interestingly, *C. microstyla*. What remains a mystery is how Yuncker (1932) discerned the inclusion of *C. microstyla* in this subsect. as only the type specimen was available at the time, which contained neither capsules nor seeds, thus preventing him from observing the defining embryo feature. Nor was there any previous report of the embryo type in *C. microstyla;* Engelmann (1859), who described and placed this species in section *Cleistogrammica* subsect. *Oxycarpae*, provided no information on the embryo. After Yuncker (1932), Hunzinker (1949) reported and illustrated the embryo of *C. microstyla* and also placed the species in subsect. *Denticulatae*. When subg. *Grammica* was reassessed phylogenetically using plastid and nuclear DNA sequences (Stefanović et al. 2007), *C. microstyla* was moved to sect. *Subulatae*, together with other South American species (not within sect. *Denticulatae* in NW N America).

Nevertheless, there is a morphological difference between the embryo of *C. microstyla*, in which the embryo enlargement tapers off gradually towards the distal end,

and those of sect. *Denticulatae* in which the enlargement is ball-like at the radicular end of the embryo and does not taper; instead the remaining quarter of the embryo resembles the filiform embryo of Type I. Costea et al. (2005) revised the taxonomy of sect. *Denticulatae* species and reported that the globular part of the embryo increases in volume during seed maturation and that the endosperm was entirely consumed at seed maturity. I agree with the authors Lloyd (1908) and Costea et al. (2005) that the enlarged embryo most likely has a function of water and nutrient storage which may replace the function of a gelatinized endosperm in extreme desert and dry environments.

Many authors have commented and investigated to some extent the filiform, thread-like embryo (Type I) (Hooker 1889; MacPherson 1921; Johri and Tiagi 1952; Truscott 1966; Lyshede 1989, 1992; Lee et al. 2000; Behdarvandi 2014). The thread-like embryo consists morphologically of a shoot apex and a radicular tip. The coiling of the embryo has been said to foreshadow the parasitic behavior of the plant as it coils around its host (Kuijt 1969). It is more conceivable, however, as Lee et al. (2000) suggested, that the coiling pattern allows for a longer embryo to develop within the limited space of the seed, and upon germination, the extra length gained through the straightening of the coils may represent an advantage for the seedlings which need to elongate rapidly for locating a host. The number of coils, and therefore the size of the embryo may have an inverse relationship with the amount of endosperm available to the embryo. A trade-off may exist between having a larger seedling and smaller endosperm compared to having shorter seedling with a greater endosperm. Further investigations are required to elucidate whether or not this relationship exists.

6.2 WATER GAP ANATOMY

A water gap has been described in 12 of the 16 families known to have physical dormancy (Baskin and Baskin 2000). Several different types of water gaps have been

described in these 12 families, as they can differ in origin, morphology and anatomy (Baskin and Baskin 2000, Geneve et al. 2018). In this study, it was shown that the anatomy of the water gap anatomy in *Cuscuta* is uniform despite some variations observed in the structure of the seed coat (e.g. epidermis type and number of palisade layers).

Originally, it was thought that the papillae on *Cuscuta* seed epidermis were responsible for the water entry (Hutchison and Ashton 1979; Lyshede 1984) and it was assumed that imbibing seeds would take up water through their entire seed coat. Our results disagree and confirm the findings of Jayasuriya et al. (2008) for *C. australis,* in that the point of water entry through intact seeds is through the hilar fissure. Johri and Tiagi (1952) commented on seed coat development and noted that a vascular strand with well-formed tracheids ran around the integument close to the outer margin corresponding to the vasculature of the funiculus. It is likely that remnants of this vasculature, represented by tracheids, are at least in part the avenue by which water enters and may likely explain why two palisade layers at this area are constant for the whole genus.

The water gap in *Cuscuta* seeds is different from that in other Convolvulaceae seeds (e.g. *Ipomoea, Merremia, Calystegia* - Baskin and Baskin 2004; Jayasuriya et al. 2007; 2009, Geneve et al. 2018) in which “bulges” adjacent to the micropyle initiate water entry into the seed. Though the hilar fissure has been recognized as the entry point for water in *Cuscuta*, the mechanism for its opening has not been elucidated. Jayasuriya et al. (2008) suggested that palisade cells in the hilar suture separate and the hilar fissure opens, but this still raises the question as to how the palisade cells separate.

Below we propose a water entry mechanism for undamaged seeds (when the seed coat layers are intact) and we suggest a second scenario for dormancy break under natural conditions stratification, zoochory or in artificially scarified seeds. When the

seed coat is intact, the turgidity of the hydrated epidermal cells may cause the initial separation of the palisade cells within the hilar fissure. Papillose epidermal cells have been observed on both dormant and non-dormant seeds that were being rehydrated, yet it was not until the hilar fissure opened that the remainder of the seed coat began to rehydrate. This suggests a prolonged imbibition action is required for the hilar fissure to open in intact seeds. This hypothesis does not explain the opening of the hilum in subg. *Monogynella*; however, it is possible that the increased hilar pad size may be assisting in the initial opening of the hilar fissure and dormancy break, but this requires additional investigation.

The second suggested mechanism involves gradual or abrupt structural changes within the seed coat caused by biotic and abiotic factors. Zoochory had been suggested for *Cuscuta* (Kuijt 1969; Kuhn 1868) and has been confirmed specifically for bird endozoochory (Costea et al. 2016). Passed seeds resembled those that were scarified in sulfuric acid (Gaertner 1950; Hutchinson and Ashton 1979; Costea personal communication), in which the seed coat damage was extensive. Generally, damage included the loss of the epidermal layer and the total or partial removal of the outer palisade layer. In addition, cracks opened within the second palisade layer reaching the endosperm (Costea et al. 2016; Costea, personal communication). Natural stratification in the field will produce at least seed coat fissures through cycles of freezing-thawing or temperature variations after one or multiple years. In this case, the water access will not be limited to the hilar fissure, and this has also been observed in other plants (Rolston 1978; Ma et al. 2004).

6.3 FUNCTIONAL RELATIONSHIPS

Mixed mating systems of Cuscuta and the average number of seeds per capsule

The evolution of mating systems is both an integral and dynamic facet of plant populations and taxa. Mixed mating systems have evolved combining the advantages of both reproductive strategies (selfing and outcrossing); the increase of genetic diversity while still providing the reproductive assurance of selfing (Vogler and Kalisz 2001; Goodwillie et al. 2005; Wright et al. 2011). High measures of reproductive success (e.g., fruit/flower ratio, seed/ovule ratio, brood size and number of seed/fruit) characterize annuals, while these measures are generally lower for perennials which are consistently outcrossing (Guo et al. 2002). Studies have confirmed this is the case for some angiosperm families (Amaranthaceae - *Alternanthera*; Asteraceae – *Adenostemma*; Convolvulaceae – *Calystegia*; Fabaceae – *Astragalus, Lupinus*) (Wiens 1984; McMullen 1987; Lyons and Antonovics 1991; Gallardo et al. 1994; Ushimaru and Kikuzawa 1999).

This is the first investigation into the relationship between the reproductive output (S/Cs) and breeding systems in *Cuscuta*. Wright et al. (2011) noted a wide range of mixed mating breeding systems. However, the degree to which individual species rely on selfing versus out-crossing was still unclear, especially for those categorized as facultatively xenogamous. In the Wright et al. (2011) study, 108 taxa were classified as self-compatible but primarily outcrossing with a large variation of P/Os. Our results provide some possible clarification within this breeding category. In the terminating leaf that included 31% of taxa, species have both higher values of S/C but mid-range P/O values (1010/<3.3>2.5). Considering the dense inflorescences of *Cuscuta*, the pollination of flowers by pollen from other flowers on the same plant via pollinators (geitonogamy) may be responsible for the higher numbers of S/C (Lloyd 1979; de Jong et al. 1993). Geitonogamy, however, has yet to be studied in *Cuscuta.* The remaining 29% of taxa in this category were split into two leaves with higher P/O values but lower S/C (1369, 1681/>2.5) suggesting these taxa were most likely mainly relying on xenogamy.

Taxa that were assigned by Wright et al. (2011) as facultatively autogamous had among the highest S/Cs confirming that selfing has an influential role in reproductive assurance. Self-compatible plants have an advantage for both colonization and persistence but are susceptible to inbreeding depression (Charlesworth and Charlesworth 1987; Uyenoyama et al. 1993; Carr and Dudash 2003) and pollen and seed discounting (Holsinger et al. 1984; Harder and Wilson 1998; Herlihy and Eckert 2002; Barrett 2003). Nevertheless, no taxa in *Cuscuta* are categorized as fully autogamous. Only six taxa were considered fully xenogamous and self-incompatible where insufficient pollination becomes a frequent limiter of seed production (Burd 1994; Ashman et al. 2004).

Seed size and geographical distribution in Cuscuta

Seed size is a key character that in some plants is strongly related to dispersal ability and other important life-history characteristics including their competitive ability, dormancy and seed bank persistence (Thompson and Grime 1979; Thompson 1987; Venable and Brown 1988; Michaels et al. 1988; Venable and Búrquez 1990; Reese 1995; Guo et al. 2000). Small-seeded species are hypothesized to have a greater dispersal capacity as not only are they produced in greater quantity but are more readily transported via abiotic and biotic factors (Venable and Brown 1988; Greene and Johnson 1993; Guo et al. 2000). When studies are conducted to test this hypothesis, authors are generally looking at seeds of many different species and families of varying orders of magnitude in regard to seed size (Guo et al. 1998b,1999; Westoby et al. 1990,1992; Greene and Johnson 1993) and not within families or genera with relatively minute differences.

Our results found no relationship between seed size and geographical distribution, which contradicts other family and genus level studies (e.g. Fabaceae, Tribe

Acacieae-*Acacia,* Brassicaceae*,* Myrtaceae, Tribe Eucalypteae-*Eucalyptus,* Fagaceae-*Quercus*) that reported a positive association (Aizen and Paterson 1990; Edwards and Westoby 1996; Willis et al. 2014b).

As in other plants, it is possible that seed size in *Cuscuta* is related to seedling survival. Seed size has been confirmed to have a positive correlation with higher seedling survivorship, as large-seeded species have more reserves for seedling survival when faced with seedling hazards (e.g., deep shade, drought, physical damage etc.) (Baker 1972; Venable and Brown 1988; Bekker et al. 1998). Considering the most crucial life-stage for these parasitic plants occurs when they are seedlings attempting to locate and attach to their hosts, seed size will affect their survival because they depend entirely on their seed reserves as they are unable to photosynthesize. In the case of *Cuscuta*, if seedlings cannot find a suitable host within a certain period of time, they will die (Dawson et al. 1994; Sandler et al. 1997). Several authors reported varying anecdotal survival times of *Cuscuta* seedlings, from several days up to seven weeks (Spisar 1910; Lyshede 1985; Parker and Riches 1993)

Behdarvandi et al. (2015) compared the seedling survival of *C. gronovii* and *C. campestris* in the presence or absence of arbuscular mycorrhiza fungi. Both colonized and uncolonized seedlings of *C. gronovii*, which has larger seeds, lived longer than the seedlings of *C. campestris* with smaller seeds. The same positive correlation was observed in the case of three other species of subg. Grammica — *C. campestris, C. epithymum* and *C. costaricensis* (Dilliott 2019), which strongly suggests that seed size in *Cuscuta* is related to seedling survival rather than the dispersal potential.

6.4 FUTURE DIRECTIONS

Based on this comprehensive overview of the Cuscuta seeds, many studies are possible in the future by zooming in on small groups of taxa to determine details of

development and ultrastructure as well as seed biology and ecology. Within subg. *Grammica,* sect. *Denticulatae* in particular with its unique character set requires additional investigation: first, to confirm that environmental conditions selected for a loss of the complete outer palisade layer, but at the same time the retention of the Type II epidermal cells. Second, to compare these findings to the South American species *C. microstyla*, which exhibits similar characteristics, but is found at high elevation in the Andes. And finally, the Type II embryo that evolved in these four species could additionally be studied, especially in regard to its development and viviparous nature and how it affects initial seedling growth and attachment, in both field and laboratory settings. Subgenus *Monogynella* was only accounted for in this study by five species as herbarium material was limited, but a more in-depth analysis into the subgenus would be necessary to confirm its unusual epidermal cell layer. A study into the function of epidermal cells could then either confirm our hypotheses or present new information as to the function of the pitted-papillose Type II seed coat. Additionally, investigations into the ultrastructure and chemical composition of the cell walls of Type II epidermal cells could further elucidate their function.

Concerning a possible relationship between anatomical characteristics and dormancy, the main obstacle encountered in this study was the reduced number of species with published data about dormancy. Thus, studies about germination and dormancy in more species of *Cuscuta* would be very useful. Germination trials could be performed and seed size as well the thickness of palisade layers could be related to the germination time of a substantial number of taxa. The understanding of zoochory, specifically endozoochory and its role in dispersal of *Cuscuta* is fairly novel, however, the work I have completed for this thesis could be of assistance when trying to further discern the role of the seed coat in protection and germination via natural scarification processes.

A study investigating the breeding systems in *Cuscuta* using molecular markers would be better suited than the P/O values used in this study. The precision of this study was limited by the broad taxon sampling and the estimation of breeding systems using Cruden's categories. It would be interesting to examine breeding systems of select species at the population level over several years and monitor if and how their reproductive output is affected by environmental and biological factors.

Another study is necessary to test on more species the hypothesis according to which seed size is related to seedling survival, which was only preliminarily investigated in Costea lab. Seed size may also be related to the type of host as apparently *Cuscuta* species growing on woody hosts have larger seeds than those parasitizing herbaceous plants.

7. SUMMARY

Cuscuta may develop one to four seeds per capsule, with the shape and compression dependent on the final number of seeds in the fruit. This study has revealed three general seed types based on morphology and anatomy: (1) seeds that have an epidermis with elongated, interlocking cells, incomplete outer palisade layer and a filiform embryo; (2) seeds that have an epidermis with isodiametric cells, complete outer palisade layer and a filiform embryo; and (3) seeds that have isodiametric epidermal cells, incomplete outer palisade layer and an enlarged "bulbous" embryo.

The diversity in morphology and anatomy of the seeds provides observable characters that are useful for species identification including the measurements of the hilar pad length and width, the length of the funicular scar, behaviour of epidermal cells, their cell diameters, cell thickness and width; presence and thickness of palisade layers, and the embryo type. The evolutionary advantage of the change in physiology from

pitted to papillose in the epidermal cells of subg. *Grammica, Pachystigma* and *Cuscuta* was narrowed down to two possibilities: (1) increased water retention to protect seeds from water loss and aid in germination and (2) mucilage and increased seed surface to prevent deeper displacement in the soil once the conditions are favourable for germination. The seed coat anatomy directly beneath the hilar pad is similar for the entirety of the genus, while in subg. *Monogynella* and four species in subg. *Grammica* the seed coat in the rest of the seed does not have an outer palisade layer. The inner palisade layer has been identified as the water impermeable and the mechanically resistant layer for the genus. Functionality of the outer palisade layer seems to be associated with the Type II epidermal cells that evolved in the same taxa.

The initial route of water entry into the seeds or the water gap was confirmed in seven taxa, representing subgenera *Grammica, Cuscuta,* and *Monogynella*. Two scenarios were proposed for the route of water entry and the commencement of breaking physical dormancy: (1) under natural conditions where the continued pressure of the epidermal cells allows for palisade cells at the hilar fissure to open and water to enter the seed and (2) involving gradual or abrupt structural changes within the epidermal and palisade layers caused by biotic and abiotic factors that allow water to enter the remainder of the seed.

Additionally, the investigations into the mixed mating systems of *Cuscuta* and the number of seeds per capsule revealed that taxa which are regularly outcrossing possess on average fewer number of seeds per capsule while those that are primarily autogamous tend to have more seeds per capsule. No relationship was revealed between the seed size and the geographical distribution. Instead, seed size is more likely a key feature in the survival of the seedlings.

8. INTEGRATIVE NATURE OF THIS THESIS

The interrelationships of organisms in environments having different levels of complexity make it difficult to answer simple biological questions (Barbault et al. 2004) using only one discipline, thus “integrative biology” is necessary (Wake 2003). This thesis has been an interdisciplinary approach to the biological research of the genus *Cuscuta*. It has allowed exploration of several questions from multiple perspectives that have bridged and been a collaboration of various disciplines and sub-disciplines of biology, across diverse taxa and over time scales. I used various microscopy techniques to study the comparative morphology and anatomy of a large number of species. I explored the evolutionary aspects and functional relationships of a multitude of characters and integrated plant morphology/anatomy, biogeography, dispersal, seed ecology and reproductive plant biology into my thesis. Based on seed characteristics, I constructed an identification key, which can be considered a practical application of this research.

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10. APPENDICES

APPENDIX A: LIST OF HERBARIUM VOUCHERS

Cuscuta acuta Engelm.: **ECUADOR, Galapagos.** *Anderson 1853* (S); Tower Island, 16 Jun 1932, *Howell 10140* (G); **Manabí.** Bahia de Caraquez, Hotel La Herradura, near sea shore, 15 Feb 1981, *Benkt Sparre 19700* (S). ***C. alata C. campestris*** Yunck.: **CANADA.** *Roosen 16637* (RSA). **USA, Utah.** *Arnow 4694* (RSA); *Arnow 4694* (NY). ***C. cephalanthi*** Engelm.: **USA, Indiana.** *Bauta s.n.* (IND); *Deam 51439* (IND). **Louisiana.** *Shinners 26667* (SMU). ***C. chapalana*** Yunck.: **MEXICO. Jalisco.** *Chazaro et al. 4408.* (IBUG); *Garcia-R &Harker 438* (IBUG); Mprio Jocotepec, Barrauca de Sayula, al SE de San Pedro Tesistan. Hierba annual, flores blaucas, bosque tropical caducifolio, Nov 1993, *Machuca 7026* (MICH). ***C. chilensis*** Ker Gawl: **ARGENTINA.** *Smith 1749* (S). **CHILE.** *Castillo 4* (SGO); *Laudrum 3073* (ASU). ***C. compacta*** Juss ex. Choisy: **ARGENTINA.** *Demaree 59628* (SMU); *Langley 70465* (RSA). **USA, St. Louis.** August 1845, *Engelmonn* (R). ***C. coryli*** Engelm.: *Dean 14856* (IND). **MAN** *Boivin & Champagne 13869* (UNB). **USA, West Virginia.** Ohio Co.: on bank of Ohio River just below Eight Street, Wheeling, 16 September 1951. *Bartholomew 0-923* (NY). ***C. corymbose var. corymbosa*** Ruiz & Pav.: **COSTA RICA.** *H.E Stork 1002* (F). **HONDURAS.** *Standley R Padilla 1938* (HERBARIUM). **MEXICO, Baja.** *Boyd 3491* (UCR). ***C. corymbosa var***

grandiflora Engelm. **MEXICO, Gulf of California.** Isla Partita in steep rocky draw facing the ocean, 22 September 1921. *Johnston 3222* (GH). **Jalisco.** Sierra de MAuanthlon Occidental with dacing slopes of deep cool valley at headwaters of Arroyo Lay Joyas, 1.5-2km. ENE of Zarza Mora cabins, ca. 2-3 km ESE of Las Joyas 19°35'15"-45N, 104°15' 30"-45"W, 1 Jan 1984. *Iltis & Guzman 29077* (MICH). **Temascaltepec.** Cumbre-Cruz, Alnus weeds, 15 March 1936. *Hinton et al. 8984* (GH). ***C. corymbosa var stylosa*** (Choisy) Engelm.: **MEXICO, Hidalgo.** Muprid. San Salvador: km 135-137 on Laredo Hwy between Actofran and Ixmiguilpan, 8 October 1943. *Gilly & Cany 5* (MICH). **Veracruz.** December 1915. *Purpus 7564* (GH). ***C. cristata*** Engelm.: **ARGENTINA, Catamarca.** *E.S. Riggs 100* (F). *Fres Cordolea 314* (KEW). ***C. cuspidata*** Engelm.: **USA, Indiana.** Posey Co.: low fallow field along the Wabash River, 2 mi S of New Harmony. 24 September 1920. *Deam 33.011*(NY); Posey Co.: low fallow field along the Wabash River, 2 mi S of New Harmony. 24 September 1920. *Deam 33.011*(IND). **Utah.** *Jones s.n* (RSA). ***C. denticulata*** Engelm.: **USA, California.** *Munz 11731* (SD). **Nevada.** *Parish 10299* (CAS). ***C. desmouliniana*** Yunck.: **MEXICO.** *Charlton et al. 3986* (OCR); *Pringle 105* (SMU). **Sonora.** Hwy W fom Hermosillo to Babia Kino, 12 mi W of the Hwy Jet., 800ft. 29 January 1963. *Dunn et al. 14130* (NY). ***C. epilinum*** Wiehe: **CANADA.** August 4, 1880. *Pringle 204788* (CAS). **Quebec.** Sainte-Hélène, Kamouraska, 15 August 1942. *Cayoutte s.n.* (QUE); Cté Shefford, Sainte-Alphonse, sur la ferue de M. Wilfrid Viar, 30 July 1941. *Cartier s.n.* (DAO). ***C. epithymum*** L.: **AUSTRALIA.** *Clarke 107955-212* (RSA). **USA, Idaho.** *Macbride & Payson 3219.* ***C. erosa*** Yunck.: **USA. Arizona.** *Jones 28731* (CAS); Pima Co.: Fresnal, 21 August 1932, 3000 ft on Fronseria. *Peebles 8998* (QUE). ***C. europaea*** L.: **BELGIUM.** Prov de Luxembourg Chassepierre, bord de la Semois en amout du village, 30 September 1975. *Duvigneauol & Laubinon 75B953* (QUE). **GEORGIA.** *Atha et al. 3294* (NY). **NETHERLANDS, Gelderland.** Base of lennee in the Ooypolder E of Nijmegen, near Tiengeboden, 12 September 1959.

Hekking 635 (NY). ***C. foetida*** Kunth: *Acosta Solis 5599* (F). ***C. gigantea*** Griff.: **INDIA, Jammu and Kashmir.** 1925, *Stewart 8249a* (NY). ***C. glabrior*** (Engelm.) Yunck.: **MEXICO, Coaluila.** Chojo Grande, 27 mi S of Saltillo, 16 July 1955. *Palmer 723* (GH); *Hendrickson 13676c* (RSA). **USA, Texas.** Deaf Smith Co.: 15 mi N & 15 mi W of Hereford, 23 July 1966. *Waller 962* (TEX, LL). ***C. glomerata*** Choisy: **USA, Indiana.** Lake Co.: marsh 2 mi N of Hobart 17 September 1930. *Deam 49686* (NY). **Kanasas.** Riley Co.: Horse Pasture, NW ¼ sec. 13, TIIS, R7E, 10 September 1979. *Freeman 293* (NY). **Nebraska.** *Hoperman 21141* (NMS). ***C. gracillima*** Engelm.: **MEXICO.** *Jones 22408* (UCR). ***C. grandiflora*** Kunth: **ECUADOR.** *Laegoord 54907A* (QCNE); *Tipaz et al. 1563* (QCNE). **PERU.** *Pennell 13613* (F). ***Cuscuta gronovii*** Willd: **CANADA, New Brunswick.** Charlotte Co.: Campobello isl., Ferry landing area for Deer is ferry. Low area; ca 44°13', 66°57', 28 Aug 1982, *Hinds 5639* (UNB). **Ontario.** Waterloo along the Grand River, Oct 2014, *Anna Ho s.n.* (WLU); Long Point, Oct 2014, *Anna Ho s.n (WLU);* Glengarry Co., Kenyon Twp., 10 km NE of Maxville, 7 Sept 2001, *Brownell& Catling s.n*. (DAO). **Quebec***.* Reserve faunique du lac Saint-Francis (45°02' N-74°30') i Conte de Huntington, 1 Sep 1988, *Dignard & Masson 1101*(QUE); Cte. D'Argeuteuil le long piste est-ouest de la presque'rle Robillorol on Laportea canadensis, 10 Aug 1986, *Guertin 1334* (QFA). **USA, Florida.** *Wilbur 69645* (BRIT). **North Carolina.** *Anderson 1858* (SMU). **Ohio.** Hocking Co.: Good Hope Twp. 4 mi W off t-122 on 374, 1.25 mi SW of center of Rockbridge, wet ditch along road, 16 Sept 1979, *Bryant 1016* (QFA). ***C. gymnocarpa*** Engelm.: **GALAPAGOS.** *Fagerline & Wibom 3658* (S); *Fagerline & Wibom 3641* (S); *Inga Eliasson 2079* (S). **C. harperi** Small: *Demaree 46295* (NY). ***C. haughtii*** Yunck.: *Asplund 5618* (G). *Asplund 5618* (KEW). *Asplund 15974* (UPS). ***C. howelliana*** P. Rubtzoff: **USA, California.** Peter Ahart Ranch, Honcut, Valley Grassland, elevation 50m, 7 April 1978. *Lowell Ahart 1804* (CHICO). ***C. iguanella*** Costea & I. Garćia: **MEXICO.** *Pringle 4529* (MEXU); *Pringle 4529* (F). **Jalisco.** Wooded hills ear

Guadalajara, flowers fragrant, 2 September 1893. *Pringle 4529* (GH). ***C. indecora*** Choisy: **USA, Nebraska.** *Vescio & Kruse 174* (NY). **New Mexico.** *Spellemberg et al. 3427* (NY). **Utah.** *Jones s.n.* (RSA). ***C. jalapensis*** Schltdl.: *Ton 603* (NY). **MEXICO, Chiapas.** Large moist pasture at Amatenaugo del Valle, 5800ft, 26 July 1966. *Breedlove 15669* (MICH); *10684* (MEXU). ***C. japonica*** Choisy: **CHINA.** 5-9 October 1986. *Bartholomew et al.* (RSA). **Bizen.** 7 October 1925. *Masamune s.n.* (NY). ***C. legitima*** Costea & Stefanović: **MEXICO, Sonora.** Cerro La Antenna, 1 km. north of Microondas La Cabana; Sinaloan thornscrub; 27°27'45"N, 109°46'20"W, 200m elevation. 19 September 1994. *T.R. Van Devender 94-603, D.A Yetman.* (MEXU). **USA, New Mexico.** *Spellemberg & Zucker 12966* (NMC); *Dunn 3850* (RSA). ***C. leptantha*** Engelm.: **MEXICO.** *Lindsay 2928* (SD). **Baja California.** Los Animas Bay, gravelly plain, 8 May 1928. *Johnston 3484* (GH); Between Santonrio and Puerto de Babia de los Muertos, 4 May 1931. *Wiggins 5625* (GH). ***C. liliputana*** Costea & Stefanović: **USA, New Mexico.** 9 September 1904. *Metcalfe 1290* (SD). **Texas.** Hildalgo Co.: low ground about 4.5 miles south of San Juan, February 6, 1969. *Correll D.S. 36759* (UC). ***C. macrocephala*** W. Schaffn ex. Yunck.: **MEXICO.** *Carter et al. 2186* (F). **Baja California.** Parasitic on several herbs and shrubs in semi-tropical serul 7.1 mi S of Cadauno, between Sauta Anita and La Paloma, 18 January 1959. *Wiggins 14726* (GH/UofW); La Paz, 20 January-5 February 1890. *Palmer 141* (GH). ***C. membranaceae*** Yunck.: **ARGENTINA.** *Hunziker 4823* (S)*; Hunziker 4833* (S)*; Hunkziker 4695* (S). ***C. microstyla*** *var bicolor* Hunz.: *Wilczek 388* (G). **ARGENTINA.** *Boelcke 10243* (CTES). **CHILE.** *Reiche 1802* (SGO). ***C. mitriformis*** Engelm.: **MEXICO, Michoacán.** Pine covered slopes and meadows, ea. 18 mi S of Pátecuaro, 8900-9000 ft, full sun, 20-25 November 1961. *King & Soderstorm 5214* (MICH); *King & Soderstorm 5214* (NY). **Nuevo Leon.** Galeana Hacineol Pabillo, 1 August 1936. *Taylor 38* (GH). ***C. monogyna*** Vahl: **KAZAKHSTAN.** 1914. *Kutscherovskaja 429* (NY). **UZBEKISTAN.** 19 July 1914.

Knowing 74 (NY). ***C. montana*** Costea and Stefanović: *Durango, Reveal & Atwood 3616* (NY). **MEXICO.** *Temorio 18058* (CHAPA). ***C. nevadensis*** I.M Johnst.: **USA, California.** *Angel 111* (SD). **Nevada.** *Bestley 9954* (RSA); *Peterson 888* (NY). ***C. nitida*** E. Mey.: **SOUTH AFRICA, Cape Peninsula.** Smitswinkel hillside, 11 January 1944. *R.H. Coptom 15508* (NBG). ***C. obtusiflora*** Kunth: **USA, Arkansas,** Ashley Co.: Lone Prairie. Prainies, 15 Septemver 1940. *Demaree 21543* (NY). *Stetermork 31988* (F). ***C. occidentalis*** Millsp.: *Howell et al. 50745* (NY). *Ahart 13199* (CHSC). **USA, California.** *Rowen 3720* (RSA). ***C. odontolepis*** Engelm.: **MEXICO.** *Leferriere 318* (ENCB). ***C. odorata*** Ruiz & Pav.: *MacBride 3993* (G). **ECUADOR.** *Camp 3027* (S). **PERU.** *Asplund 11177* (S). ***C. pacifica*** Costea & M.A.R Wright: **USA, California.** *Harris 1175* (B); Sausaloto, 5 November 1939, *JT Howell 15385* (UC). **Oregon.** Lane Co., Marshy tide land at Florence, Oregon, 28 October 1930. *Fletcher s.n.* (OSC). ***C. paitana*** Yuck.: **ECUADOR.** *Madsen 63790* (AAU). ***C. palustris*** Yunck.: *G. Carnevali & I Ramirez 7472* (CICY). ***C. parodiana*** Yunck.: **ARGENTINA.** *Novaro 6656* (S). ***C. partita*** Choisy: **BRAZIL.** *Eiten & Eiten 3961*(US). **Maranhão.** Municípo de Loreto: “Ilha de Balsas” region between the Blasas & Parnaíba Rivers. 35 km of Loreto. 22 Feb 1970. 7˚23’S. 45˚4’ W. Alt. 300m. South of main house of Fazenda Morros in open field cut out of former gallery forest “tabuleiro” transition. *George Eiten & Liene T. Eiten 10752* (US). **VENEZUELA.** En hombrillo afaltado y sustrato terrestre a orilla de la cerretera Bobare-Aguada Grande 17km antes del crusero a la ultima pablacíon Edo. Lara. 29 July 1981. *Marisela Ponce y Baltazar Trujillo 342* (VEN). ***C. pentagona*** Engelm.: **USA, Florida.** Levy Co.: border of salt marsh on Solidago stricta, Cedar key, 10 May 1958. *Godfrey 5650* (NY). **Massachusetts.** Tonset, 27 August 1901. *Edmondson 2777* (NY). **Virginia.** Beoford Co.: no location, no date except “Sept”. *Curtis s.n.* (NY). ***C. plantiflora*** Ten.: Palestina. *Musselman 10461* (RSA). Priva. *Selim Birgen 82* (S). ***C. polyanthemos*** W. Schaffn. Ex Yunck.: **MEXICO.** *Wiggins 13153* (SD). ***C. polygonorum*** Engelm.: *Deam*

48024 (IND); *Deam 15269* (IND). **USA, Arkansas**. St. Francise Co.: 5 mi S of Forrest City, 19 Sept 1959. *McDaniel 1419* (NY). ***C. purpurata*** Phil.: *Dillon & Teiller 5104* (MO). **CHILE, Antofagasta.** Quebrado de Taltal on various hosts this on *Dolis,* vicinity of Taltal, ca, lat. 25˚25'S, 25 Novemver 1925. *Ivan M. Johnston 5082* (US). **Atacama.** Desert of Atacama September-October 1890. *Thos. Morong 1143* (US). ***C. purpusii*** Yunck.: **MEXICO.** *Hinton et al. 20029* (MEXU); *Torres Colin 15864* (MEXU). **Nuevo Leon.** *Henrickson 6608* (RSA). ***C. reflexa*** Roxb.: **INDIA, Allahabad.** 23 January 1923. *Dudgeon s.n.* (NY); 25 January 1923. *Dudgeon s.n* (NY); 25 January 1923. *Dudgeon s.n.* (NY). ***C. rostrata*** Shuttlew. ex Engelm. & A. Gray: **USA, North Carolina.** On thickets along South Fork of New River 0.8 mi. south of Todd on Co. Rt. 1347. 20 September 1968. *S.W Leonard, A.E Radford, D. Culwell, K. Greenlee 2053;* Mitchell Co.: creekbottom 0.6 mi W SW of Hughes Gap on rd. to Buladean, 25 September 1958. *Ahles & Duke* 49884 (NY). **Virginia.** No precise location. 28 August 1939. *E.J Alexander s.n.* (NY). ***C. rugosiceps*** Yunck.: **MEXICO.** *Huetra 1129* (XAL). ***C. salina*** Engelm.: **USA, California.** Madera Co.: 4 mi SW of Chowchilla, 1 October 1936. *RF Hoover 1610* (UC7632156); Glen Co.: Locally abundant in upland along edge of dry vernal pools in Sacramento National Wildlife Refuge, freshwater marsh, 5 August 1993. *VH Oswald 5777* (UMSC); Fresco Co.: in plains. *Bacigalupi, Wiggins & Ferris 2667* (UC). ***C. sandwichiana*** Choisy: *Degener 24212* (RSA). **USA. Hawaii.** 4 October 1960. *Fujwana s.n.*(BISH). ***C. sidarum*** Liebm.: **MEXICO, Michoacán.** Municipio de Arteaga, along the road to Infiernillo, 6.2 km (by road) south-southeast of the junction with MEX 37, below the road along Arroyo Pasa de Chivo; ca. 18˚27'55" N 101˚58'40"W, 200m. 20 November 2003. *V.W Steinmann 3883* (IEB). ***C. squamata*** Engelm.: **USA, TEXAS.** *Hutchins 643* (SMU). *Wa(r)(n)nock 10275* (SMU). ***C. stenolepis*** Englem.: *Jeromillo & Caruojol 2307* (AAU). *Palacios & Tipaz 9636* (MO). **ECUADOR.** *Weydahl 407* (S). ***C. strobilaceae*** Liebm. **var pringlei** (Yunck.) Costea & I. García: **MEXICO, Guadalajara.**

La Barranca, 25 November 1930. *Jones 97347* (MICH); La Baranca, 23 November 1930. *Jones s.n.* (RSA). ***C. subinclusa*** Durand & Hilg.: **USA, Califronia.** *Abramas 6657* (CAS). ***C. suksdorfii*** Yunck.: **USA, California.** Plumas Co.: Lost Lake, ca. 2 mi (air) NNW of Humbug Summit. Abundant parasite on the dry strand of the lake, 22 August 1989. *V. Oswald & L. Ahart 3949* (CHICO). ***C. tasmanica*** Engelm.: **AUSTRALIA, New South Wales.** W shore of Lake Bathurst, c. 2km NE of Tarago, altitude 630m, 35˚03'S 149˚39'E, 28 Dec 1992. *Lepschi 908* (CANB). ***C. timida*** Costea & Stefanović: **MEXICO.** *Linares 4285* (MEXU). **Hildago.** Km 134.5 on limestone mountainside, near Yolotepec, 8 Julu 1943. *Lundell 12538* (MICH). ***C. tinctoria*** Mart. ex Englem.: *Parry & Palmer 631* (K). **MEXICO, Gerrero.** Vicinity of Taxco, 1936. *Abbott 119* (GH); Alaracon, 27 Feb 1931. *Hunnelwell 11798* (GH). ***C. tinctoria* var. floribunda** (Kunth) Costea: **MEXICO, Michoacán.** Jiquilpan**,** April 2018**,** *García Ruiz s.n.* (WLU/CHMN). ***C. tuberculata*** Brandegee: **MEXICO, Baja Califonia.** Gentle, N-facing slopes of Varro Gabilán, S of Portezuels de Gabilán, 25 ˚50 ¾'N 111˚25'W 750m, 2 October 1965. *Carter 5080* (MICH); *Donohue 73168* (RSA). ***C. umbrosa*** Hook**: CANADA, Manitoba.** Otterbourne, 4 August 1954, *Bernard 54/349* (QFA); Sent to G. Knowles, Field Husbandry, 419 Kingston Crescent, Growing on raspberries, 1950, *M.R. Mackenzie s.n.* (DAO); Sister de Saint-Boniface. Riviere Rouge, ecorre de la Rouge a La Fourde, 21 August 1960, *Boivin 13852* (DAO); 20 Aug 1953, *Lore& Lore 6199* (DAO)**;** Sept 1975, *Fields (578)* (unknown); Sans Souci, sur les Salix dans la fleur sabloueuse, 29 Jul 1956, *Bernard 56/5473* (QFA); Sister Selfirk, sans soucim rivage sablo neux du loc Winnipeg, 29 July 1956, *Bernard 5473* (DAO); **Saskatchewan.** *Breitung 1433* (DAO); *Budd& Lodge 1197* (DAO). **USA, New Mexico.** *Wooton 3488* (UNM); *Wooton& Standley 3488* (BRIT). **Utah.** *Jones 1914* (RSA). ***C. umbellata*** Kunth: **GUIANA.** *Hitchcock 16564* (S). **MEXICO.** *Calónico Soto 24009* (MEXU); *Pringle 6297* (S). ***C. vandervendri*** Costea & Stefanović: **MEXICO. Sonora.** Sierra Techurahui, southeastern Sonora, 26-28 October 1961, Pine

Oak Forest, alt 4000-4500 ft. *Gentry, Barclay & Arguelle 19423* (US). ***C. veatchii*** Brandegee: **MEXICO. Baja California.** Los Angelos Bay, 6 May 1921. *Johnston 3430* (GH); *Johnston 3430* (KEW). ***C. victoriana*** Yunck.: **AUSTRALIA, Australian Capital Territory.** Canberra, 6 mi N.W. of Mt. Swan Stations N.T, 22˚36 135˚02, 11 March 1953. *RA Perry 3329* (CANB). **South Australia.** Region 2: Lake Eyre, between Hough's Fam and Chapman"s Creek Tank, Dulkaninna Station, 29˚04'23" S 138˚37'28", uncommon, low shrubland, 9 April 1997. *H.T. Smyth 261* (CANB). ***C. volcanica*** Costea & I. Garćia: **MEXICO. Jalisco.** NE slopes of the Navada de Colima, below Canoa de Leoncito, steep cut-over mountainsides in fir zone at head of Barrauea de la Rosa, 2800m, 10 October 1952. *McVaugh 13419* (MICH); A 10 km sobre la desvición al Nevado de Colima, a partir del Fresnito. Mpio. de Zapotitlám. Alt. 2510 m, 6 April 1988. *Abisaí G. Mendoza et al. 3817* (MEXU). ***C. warneri*** Yunck. Brittonia 12: 38, 1960, Greene, vicinity of Flowell, 15min west of Fillmare, Millard County, Utah, 10 September 1957 [isotype]. *Llyod Warner s.n.* (NY). ***C. woodsoni*** Yunck.: *Vélix et al. 99.7506* (MO). ***C. yucatana*** Yunck.: *Nee & Taylor 29575* (MO). *Rzedowski 25728* (G). **MEXICO. Chiapas.** Steep slope near crest of ridge in the paraje of Banabil, Municipo of Tenejapa, elevation 9100ft. 10 October 1965. *DE Breedlove & Peter H. Raven 12912* (F).

APPENDIX B: Data Matrices and Additional Phylogenies

TABLE C1. Complete data set of characters scored for both morphology and anatomy. Each clade is colored. Character codes: AVG LENGTH - average seed length; Seed T(CS) - seed thickness, cross section; EC T – epidermal cell thickness; EC width – epidermal cell width; Outer palisade T – outer palisade thickness; Inner palisade T – inner palisade thickness; OP – presence of outer palisade layer; IP – presence of inner palisade layer; Width (F) – width of full seed; H position – hilar pad position; H compression – hilar pad compression; H length – hilar pad length; H width – hilar pad width; EC shape – epidermal cell shape; EC diameter – epidermal cell diameter; length of FL Hilum – length of funicular scar on the hilar pad.

Species	AVG LENGTH	Seed T (CS)	EC T	EC width	Outer palisade T	Inner palisade T	Embryo Type	OP	IP	Width (F)	Compression	Shape	H position	H compression	H length	H width	Dry Epidermis	H. Epidermis	EC shape	EC diameter	length FL Hilum
C. brachycalyx	1075.2	707.56	53.117	31.676	23.84	80.172	2	1	1	1019.8	1&2	1&3	1	1	154.47	138.73	1	1	2	37.517	57.98
C. occidentalis	1025.6	637.26	39.634	29.327	22.569	74.706	2	1	1	913.90	1&2&3	1&5	1&2	1&2	143.29	114.43	1	1	2	31.065	49.04
C. californica	1088.0	712.23	44.794	36.126	21.901	68.083	2	1	1	1042.3	1&2	1	1&2	1	167.01	146.41	1	1	2	41.002	60.70
C. salina var. salina	1022.0	684.43	37.481	30.538	24.223	49.008	2	1	1	984.03	1&2&3	1&4	1&2	1	182.81	141.37	1	1	2	41.55	61.58
C. pacifica	1367.1	998.09	47.540	32.002	22.409	60.011	2	1	1	1260.6	1&2&3	1&4	1&2	1	-	-	1	1	2	34.426	-
C. subinclusa	1571.5	820.06	40.218	29.866	26.556	75.943	2	1	1	1410.2	1	1	1&2	1	211.36	196.26	1	1	2	32.333	91.23
C. suksdorfii	999.75	725.1	53.888	35.875	26.36	60.305	2	1	1	978.35	2	1	1&2	1	249.7	217.6	1	1	2	44.046	81.91
C. howelliana	910.05	479.5	-	-	22.3	48.095	2	1	1	747.9	1&2	1	1	1	94.27	87.97	1	1	2	43.911	40.71
C. draconella	1237.1	768.83	37.662	27.809	16.133	52.103	2	1	1	1158.3	1&3	1	1&2	1	184.9	141.3	1	1	2	40.929	62.34
C. obtusiflora	1367.6	919.53	47.918	31.795	25.870	91.786	2	1	1	1337.4	1&2&3	1&4	1&2	1	283.25	240.88	1	1	2	35.401	92.05
C. australis	1270.6	777.32	36.207	26.618	22.808	89.183	2	1	1	1223.2	1&2	1&4	1	1	214.33	231.11	1	1	2	28.085	98.29
C. campestris	1273.0	884.33	45.204	32.666	25.287	89.976	2	1	1	1133.8	2	1	1	1	244.20	207.1	1	1	2	36.271	77.65
C. pentagona	1159.4	652.92	36.907	23.660	24.349	77.910	2	1	1	1135.6	1	1	1&2	1	224.66	170.83	1	1	2	28.299	74.74
C. harperii	973.27	630.47	26.936	20.348	21.557	76.722	2	1	1	1034.8	1&3	1	1&2	1	188.2	147.05	1	1	2	25.021	63.10
C. glabrior	1396.6	984.61	38.781	30.919	26.967	97.266	2	1	1	1388.8	1	1	1&2	1	228.25	184.60	1	1	2	31.872	98.16
C. stenolepis	1261.3	671.1	-	-	24.418	59.551	2	1	1	-	-	-	-	-	-	-	1	1	2	-	NA
C. plattensis	943.55	388.4	-	-	-	-	-	1	1	-	-	-	-	-	-	-	1	1	2	-	NA
C. sandwichiana	1640.3	1181.1	76.376	43.062	37.164	85.731	2	1	1	1408.7	1&2	1	2	1	312.96	245.73	1	1	2	37.7765	98.73
C. polygonorum	1132.8	819.21	41.983	24.968	25.624	71.325	2	1	1	1156.7	1&2	1&4	2	1	237.93	183.81	1	1	2	29.954	98.35
C. gymnocarpa	1202.6	687.75	39.012	26.410	22.765	92.765	2	1	1	1032.6	2	1&4	1&2	1	310.58	254.75	1	1	2	29.785	140.5

C. cuspidata	1515.9	975.88	41.190	30.779	24.513	97.689	2	1	1	1465.8	1&2	1&3&5	1&2	1	305.38	227.61	1	1	2	30.285	118.7
C. squamata	1401.2	742.71	47.730	33.880	23.095	77.153	2	1	1	1187	1&2	1&4	2	1	222.95	177.4	1	1	2	43.636	62.64
C. compacta	1981.4	1201.7	58.638	47.625	34.994	106.09	2	1	1	1790.9	1	1&4&5	1	1	409.44	268.12	1	1	2	40.548	177.4
C. rostrata	1860.7	1044.8	42.859	32.496	30.923	92.959	2	1	1	1700.3	2	1	1&2	1	429.94	317.99	1	1	2	42.338	211.8
C. gronovii	1767.0	1275.4	41.878	41.909	31.678	101.13	2	1	1	1578.8	2	1	2	1	387.74	355.62	1	1	2	58.450	156.6
C. cephalanthi	1345.3	977.58	38.882	32.472	25.101	89.007	2	1	1	1294.5	1&2	5	1	1	254.85	238.46	1	1	2	35.976	87.21
C. umbrosa	1984.5	1180.6	36.677	36.131	35.327	114.94	2	1	1	1900.9	1	1	2	1	352.07	356.13	1	1	2	41.615	179.2
C. glomerata	1251.1	816.85	43.387	30.640	23.674	86.947	2	1	1	1075.7	1&2	5	1	1	249.98	223.36	1	1	2	32.083	73.41
C. denticulata	811.69	539.31	26.673	20.024	18.73	42.27	1	0	1	698.95	1&2	3&4	1	1	143.67	132.1	1	1	2	26.989	39.51
C. veatchii	1001.4	655.39	35.227	28.805	19.59	43.912	1	0	1	887.57	3	1	1	1&2	169.75	179.35	1	1	2	50.979	48.75
C. nevadensis	998.81	847.02	34.629	25.415	19.43	43.71	1	0	1	990.78	3	1&4	1	1	182.96	144.26	1	1	2	-	61.63
C. haughtii	945.88	561.13	26.99	19.539	19.841	69.078	2	1	1	1532.9	1&2	1&5	1&2	1	184.06	150.96	1	1	2	26.406	64.10
C. partita	1181.6	815.3	33.587	25.996	23.696	76.75	2	1	1	1018.2	1&2	1	1&2	1	172.5	138.33	1	1	2	-	64.42
C. tinctoria	1472.1	849.85	51.096	32.671	25.306	94.457	2	1	1	1282.8	1&2	4&5	1&2	1	241.14	186.10	1	1	2	36.913	85.95
C. mitriformis	2020.9	1138.7	55.925	47.99	34.808	123.35	2	1	1	1640.6	1&2	1&5	1&2	1	357.86	300.73	1	1	2	44.005	139.2
C. jalapensis	1698.5	957.68	73.873	46.272	35.166	101.40	2	1	1	1700.4	1&2	1&4	1&2	1	340.75	301.04	1	1	2	45.956	90.83
C. rugosiceps	1791.6	1171.7	49.119	46.763	44.272	114.7	2	1	1	1405.2	1&2	1&4	2	1	279.73	238.66	1	1	2	39.966	94.7
C. woodsonii	2026.8	1200.9	116.56	56.234	40.21	143.2	2	1	1	1954.4	2	1	2	1	306.74	300.2	1	1	2	53.230	139.8
C. volcanica	2124.9	973.5	76.37	40.305	33.917	113.05	2	1	1	1659.3	2	1&5	1&2	1	522.65	400.85	1	1	2	50.060	147.9
C. tasmanica	1565.5	875.58	44.24	30.128	27.508	97.62	2	1	1	1378.8	1&2	2	2	1	260.42	216.22	1	1	2	36.704	86.50
C. iguanella	1386.1	749.04	56.997	35.836	18.161	84.785	2	1	1	1104.7	1&3	1&4&5	1&2	1	285.87	243.99	1	1	2	32.967	95.61
C. montana	1588.7	929.1	54.65	38.018	-	-	2	1	1	1253.7	1&3	4	1&2	2	NA	NA	1	1	2	-	-
C. timida	1414.8	907.15	32.365	27.620	25.63	56.196	2	1	1	1272.7	2	2&3	1&2	1	280.96	203.53	1	1	2	38.903	93.29
C. purpusii	1574.7	939.59	51.923	37.111	28.607	94.344	2	1	1	1318.7	1	1	1&2	1	264.21	208.12	1	1	2	42.204	95.56
C. victoriana	1231.2	801.58	-	-	21.632	60.301	2	1	1	1069.4	1&2	1	1&2	1	221.68	209.23	1	1	2	42.773	74.87
C. macrocephala	1469.5	848.74	52.739	36.613	27.282	93.380	2	1	1	1368.7	1&2	1&3&4	1&2	1	168.96	146.38	1	1	2	33.318	80.11
C. globulosa	1337.6	876.56	40.69	28.997	23.725	94.905	2	1	1	1280.1	1	3	1&2	1	247.89	257.38	1	1	2	29.773	76.18
C. americana	1294.9	851.61	44.275	35.920	24.816	81.804	2	1	1	1157.4	1&2	1&3&4	1	1	228.32	218.13	1	1	2	39.384	60.99
C. azteca	1065.5	723.51	27.115	25.979	24.380	76.441	2	1	1	1024.1	1&2	1&3&5	1	1	194.35	175.12	1	1	2	26.335	65.70
C. yucatana	1030.2	695.72	-	-	24.789	68.478	2	1	1	921.30	1&2&3	1	2	1	199.51	157.06	1	1	2	30.201	65.95

C. corymbosa	1431.3	895.52	37.570	27.001	29.134	96.846	2	1	1	1381.8	1	1&3&4	1	1	221.55	186.82	1	1	2	28.099	72.74
C. corymbosa var. grandiflora	1669.9	817.66	40.081	29.120	30.413	103.40	2	1	1	1497.6	1&2	1	1	1&2	221.80	187.97	1	1	2	25.998	81.32
C. corymbosa var. stylosa	1127.4	585.65	25.041	22.713	20.657	61.540	2	1	1	992.41	1&2	1	1	1	179.78	138.69	1	1	2	24.445	60.05
C. chapalana	1661.3	835.4	53.443	37.768	33.460	102.83	2	1	1	1309.1	1&2&3	5	1	1	230.48	192.73	1	1	2	40.351	86.99
C. strobilaceae	1523.9	858.89	45.459	28.581	25.274	63.953	2	1	1	1500.0	2	4	1&2	1	270.05	203.83	1	1	2	35.348	86.50
C. erosa	1217.4	704.02	52.347	33.594	17.445	94.55	2	1	1	1031.3	2	1&4	1&2	1	216.48	173.85	1	1	2	31.977	82.67
C. boldinghii	981.35	637.23	39.564	32.534	24.493	82.85	2	1	1	857.96	2	3&4	1	1	162.8	168.05	1	1	2	33.970	66.05
C. costaricensis	1144	720.45	51.586	32.348	23.405	79.44	2	1	1	1032.9	1	1	2	1	257.7	196.95	1	1	2	44.686	83.88
C. bonafortuna	1363.7	742.65	34.99	26.818	26.485	132.4	2	1	1	1029.5	2	5	2	1	236.9	191.95	1	1	2	40.541	53.06
C. odentolepis	1109.0	862.9	NA	NA	22.705	59.365	2	1	1	856.53	2&3	1&5	1&2	1	165.4	164.7	1	1	2	31.822	66.36
C. legitima	1001.3	600.63	38.001	24.969	18.444	64.046	2	1	1	813.4	1&2	1&4&5	1&2	1	169.05	147.54	1	1	2	27.374	52.59
C. tuberculata	886.43	528.38	32.667	19.377	13.842	45.736	2	1	1	759.39	1&2&3	1	1&2	1	168.20	136.22	1	1	2	23.386	66.88
C. umbellata	1064.0	671.29	45.431	26.946	18.382	56.662	2	1	1	973.13	1&2&3	1&4	1&2	1	194.40	176.13	1	1	2	27.057	55.55
C. desmouliana	861.41	543.83	26.396	20.836	15.476	45.131	2	1	1	760.09	1&2	3&4&5	1&2	1	143.30	122.95	1	1	2	26.683	69.10
C. acuta	972.46	622.06	29.908	26.151	22.569	69.654	2	1	1	853.63	1&2	3&4&5	1	1	195.81	180.47	1	2	2	33.876	38.24
C. leptantha	784.70	505.91	37.043	26.429	14.759	54.785	2	1	1	679.96	1&2&3	1&3	1&2	1	116.86	99.915	1	1	2	22.013	32.06
C. polyanthemos	930.20	431.43	41.088	21.249	22.24	54.223	2	1	1	668.27	2	5	2	1	110.22	104.34	1	1	2	17.504	47.82
C. liliputana	939.2	522.6	20.611	16.613	18.741	62.132	2	1	1	848.57	1&2	1&4	2	1	136.34	104.36	1	1	2	25.272	76.64
C. alata	943.40	708.74	38.43	24.452	18.142	69.71	2	1	1	832.37	1&2	1&4	1	1	171.02	154.35	1	2	2	30.702	70.43
C. palustris	1099.9	749.22	39.402	27.111	28.396	61.47	2	1	1	1155.7	1&3	1&5	1&2	1	209.25	193.4	1	1	2	37.014	91.39
C. membranaceae	1075.8	600.31	37.818	28.576	23.758	66.824	2	1	1	958.33	1&2	1&3&4&5	1&2	1	197.06	180.03	1	1	2	35.115	-
C. warnerii	1362.2	911.95	50.316	29.083	28.07	69.77	2	1	1	1599	1	1&5	2	1	238.25	171.3	1	1	2	41.026	78.23
C. indecora	1442.5	800.56	48.489	35.477	35.906	113.32	2	1	1	1544	1&3	1&5	1&2	1	298.61	208.36	1	1	2	40.926	66.25
C. gracillima	1047.9	-	-	-	-	-	2	1	1	977.86	1&2	1&3	2	1	183.55	158.77	1	1	2	29.776	76.48
C. sidarum	814.5	501.5	18.0225	13.6133333	18.7	53.815	2	1	1	752.85	3	1	1	1	142.25	119.05	1	1	2	34.778	51.44
C. vandervendri	1034.2	564.9	38.801	24.808	22.544	61.89	2	1	1	967.32	2&3	1	2	1	166.13	169.73	1	1	2	33.938	-
C. odorata	1347.6	-	-	-	-	-	2	1	1	1391.6	1&2	1&4&5	2	1	294.05	273.57	1	1	2	40.933	74.35
C. chilensis	1503.7	867.46	92.043	55.506	26.473	93.596	2	1	1	1374.8	1&2	1&5	1	1	249.53	213.44	1	1	2	40.163	99.98
C. purpurata	1610.6	1053.7	81.815	46.066	33.235	116.14	2	1	1	1469.8	1&2	1&4&5	2	1	281.65	237.94	1	1	2	48.917	80.12

C. foetida	1502.6	1234.2	66.762	43.672	34.714	103.31	2	1	1	1359.3	1&2	1&3&5	1&2	1	315.36	274.93	1	1	2	42.141	143.1
C. grandiflora	1887.3	972.65	66.300	54.589	36.952	96.065	2	1	1	1776.9	1&2	1&2&3&4&5	2	1	329.87	286.57	1	1	2	50.546	100.1
C. parodiana	1455.2	793.1	46.58	38.129	24.62	80.575	2	1	1	1328	2	1&4	1&2	1	256.63	198.06	1	1	2	49.987	116.2
C. cristata	1439.0	1066.2	43.687	34.586	25.85	96.772	2	1	1	1332.2	1&2	1&4&5	1	1	363.06	290.32	1	1	2	36.637	-
C. microstyla	1193.1	831.93	51.224	44.411	35.736	NA	1	1	0	1050.5	3	3	1	2	NA	NA	1	1	2	55.132	-
C. acutiloba	900.25	714.31	40.504	34.816	17.115	81.253	2	1	1	818.42	1&2	1&3	1	1	140.7	133.56	1	1	2	-	114.4
C. boliviana	1769.0	1143.1	65.681	55.500	33.402	110.41	2	1	1	1672.9	1&2&3	1&4&5	1	1&2	323.93	282.44	1	1	2	55.969	54.06
C. paitiana	704.55	519.7	26.082	17.662	10.4	51.18	2	1	1	1021	1&3	1	1	NA	134.4	114	1	1	2	24.894	-
C. goyaziana	1343.7	576.7	61.963	39.616	19.7	75.715	2	1	1	1313.5	1	1	2	1	259	249.6	1	1	2	44.345	65.45
C. nitida	1072.2	679.27	50.253	29.355	16.602	67.975	2	1	1	881.15	1	1	2	1	231.9	202.3	1	1	2	33.218	58.32
C. planiflora	1052.7	627.03	57.392	35.624	22.4	69.78	2	1	1	852	1&2	1	2	1	199.36	163.5	1	1	2	48.493	103.5
C. europea	1277.6	906.82	54.546	35.617	20.045	59.565	2	1	1	1101.8	1&2	1&4&5	1&2	1	284.73	238.71	1	1	2	42.215	103.4
C. epilinum	1241.6	811.89	79.831	40.874	24.857	58.700	2	1	1	1076.8	1&2	1&3&4&5	1&2	1	282.89	226.21	1	1	2	49.822	53.67
C. approximata	1096.5	781.13	46.936	34.305	20.660	70.208	2	1	1	858.24	2&3	1	1	1	167.08	166.62	1	1	2	40.206	56.85
C. epithymum	896.28	615.51	45.256	28.687	16.334	54.130	2	1	1	754.46	1&2	1&4&5	1&2	1	171.4	142.13	1	1	2	38.044	52.11
C. babylonica	839.38	506.52	49.944	34.836	18.618	49.977	2	1	1	744.34	2	1&3	1	1&2	142	136.15	1	1	2	38.468	-
C. monogyna	2533.91	1479.5	22.963	23.832	-	111.72	2	0	1	2151.4	1	4	1	1&2	488.77	359.08	2	2	1	-	575.3
C. japonica	2457.4	1746.45	34.2255	28.939	-	150.48	2	0	1	2078.5	1&2&3	1&5	1	1	715.76	520.66	2	2	1	-	539.3
C. lupuliformis	2656	1820.5	-	-	60.505	108.54	2	0	1	2389.5	3	4&5	1	1	647.6	659.9	0	1	1	-	44.06
C. reflexa	3158.3	2133.16	47.432	36.453	41.305	162.99	2	0	1	2910.0	1&2	1&4	1&2	1	774.91	505.13	0	0	1	-	506.2
C. gigantea	2860	1488.5	27.34	30.128	42.215	111.75	2	0	1	2434	1&2	1&5	2	1	811.25	637.4	1	1	1	-	-

TABLE B2: Ratios created between various characters and the average number of seeds/capsule.

Species	Ratio of epidermal cell diameter to seed size	Ratio of epidermal cell thickness to seed size	Ratio of outer palisade to inner palisade layer	Average number of seeds/capsule
C. brachycalyx	0.03489	0.049401	0.297360	3.1
C. occidentalis	0.0302	0.038643	0.30211	3.5
C. californica	0.03768	0.041169	0.321693	3.5
C. salina var salina	0.04065	0.036671	0.49426	1
C. pacifica	0.02518	0.0347	0.37342	1
C. subinclusa	0.02057	0.02559	0.3496	1
C. suksdorfii	0.044057	0.053902	0.437	-
C. howelliana	0.04825	-	0.463	-
C. draconella	0.03308	0.030442	0.30964	-
C. obtusiflora	0.02588	0.03503	0.28184	3.4
C. australis	0.02210	0.02849	0.255748	3.4
C. campestris	0.0284	0.03550	0.28104	3.4
C. pentagona	0.02440	0.03183	0.312528	3.3
C. harperii	0.0257	0.02767	0.280980	1.2
C. glabrior	0.0228	0.02776	0.27724	2.2
C. stenolepis	-	0.04656	0.410033	-
C. plattensis	-	0.0370	-	-
C. sandwichiana	0.0230	0.03243	0.433498	-
C. polygonorum	0.02644	0.03503	0.35925	-
C. gymnocarpa	0.02476	0.02849	0.24541	-
C. cuspidata	0.0199	0.02717	0.25093	3.5
C. squamata	0.0311	0.03406	0.2993	1.2
C. compacta	0.02046	0.029594	0.32984	2.5
C. rostrata	0.02275	0.02303	0.3326	2.5
C. gronovii	0.03307	0.02370	0.31322	3.2
C. cephalanthi	0.02674	0.02890	0.2820	3.1
C. umbrosa	0.0209	0.01848	0.307342	3.3
C. glomerata	0.0256	0.03468	0.27228	2.4
C. denticulata	0.0332	0.032861	0.44318	1
C. veatchii	0.05090	0.03517	0.44611	-
C. nevadensis	0.02803	0.0346	0.44452	1
C. haughtii	0.0279	0.0285	0.28723	2.4
C. partita	-	0.0284	0.30875	3.3
C. tinctoria	0.0250	0.034	0.2679	2.6
C. mitriformis	0.0217	0.02767	0.2821	3.1
C. jalapensis	0.0270	0.04349	0.34680	2.3
C. rugosiceps	0.0223	0.02741	0.385	3
C. woodsonii	0.02626	0.057510	0.280	2.4
C. volcanica	0.023558	0.03593	0.300	2.3
C. tasmanica	0.0234	0.02825	0.2817	3.1
C. iguanella	0.0237	0.0411	0.2142	-
C. montana	-	0.0343	-	-
C. timida	0.027496	0.02287	0.4560	-
C. purpusii	0.026801	0.03297	0.30322	-
C. victoriana	0.03474	-	0.35873	-
C. macrocephala	0.0226	0.03590	0.292166	-
C. globulosa	0.02225	0.030419	0.24998	-
C. americana	0.03041	0.034191	0.3033	1
C. azteca	0.02471	0.025446	0.318949	3.4
C. yucatana	0.02931	-	0.362000	3.3
C. corymbosa	0.019632	0.02624	0.3008	-
C. corymbosa var. grandiflora	0.01556	0.02400	0.2941	2.2
C. corymbosa var. stylosa	0.021683	0.022211	0.3356	2
C. chapalana	0.02428	0.032168	0.325378	3
C. strobilaceae	0.02319	0.02983	0.395203	3.2
C. erosa	0.02626	0.0429	0.18450	3
C. boldinghii	0.0346	0.04031	0.2956	2.5
C. costaricensis	0.039061	0.04509	0.294	-
C. bonafortuna	0.029728	0.025657	0.20003	-
C. odentolepis	0.02869	-	0.382464	2.6
C. legitima	0.027336	0.03794	0.2879	2.6
C. tuberculata	0.0263	0.03685	0.30266	2
C. umbellata	0.0254	0.04269	0.3244	3.6

C. desmouliana	0.03097	0.03064	0.342	2.6
C. acuta	0.03483	0.0307	0.32402	3.8
C. leptantha	0.02805	0.0472	0.2693	2.1
C. polyanthemos	0.018817	0.04417	0.41015	2
C. liliputana	0.02690	0.02194	0.301633	2
C. alata	0.0325	0.04073	0.26024	-
C. palustris	0.033651	0.03582	0.46194	-
C. membranaceae	0.032640	0.03515	0.3555	-
C. warnerii	0.030116	0.03693	0.40232	-
C. indecora	0.02837	0.03361	0.31684	3.2
C. gracillima	0.02841	-	-	-
C. sidarum	0.04269	0.0221	0.3474	-
C. vandervendri	0.032814	0.037	0.3642	3.6
C. odorata	0.03037	-	-	-
C. chilensis	0.02670	0.06121	0.28284	2.8
C. purpurata	0.03037	0.05079	0.2861	3.2
C. foetida	0.02804	0.0444	0.3359	2.4
C. grandiflora	0.02678	0.03512	0.384	3.3
C. parodiana	0.03037	0.03200	0.30555	2.1
C. cristata	0.02670	0.0303	0.26717	3.1
C. microstyla	0.03037	0.04293	-	1
C. acutiloba	0.02804	0.044991	0.2106	-
C. boliviana	0.02678	0.0371	0.302524	-
C. paitiana	0.030375	0.03702	0.20320	-
C. goyaziana	0.0330	0.046112	0.2601	-
C. nitida	0.03097	0.04686	0.244244	2.2
C. planiflora	0.04606	0.05451	0.321008	-
C. europea	0.033041	0.04269	0.336523	3.6
C. epilinum	0.040124	0.06429	0.423457	3.6
C. approximata	0.036667	0.042804	0.294277	3.6
C. epithymum	0.042446	0.05049	0.301755	3.7
C. babylonica	0.045829	0.0595	0.372534	-
C. monogyna	-	0.00906	-	2.3
C. japonica	-	0.01392	-	2.6
C. lupuliformis	-	0.01347	-	-
C. reflexa	-	0.01501	-	3.1
C. gigantea	-	0.0095	-	-

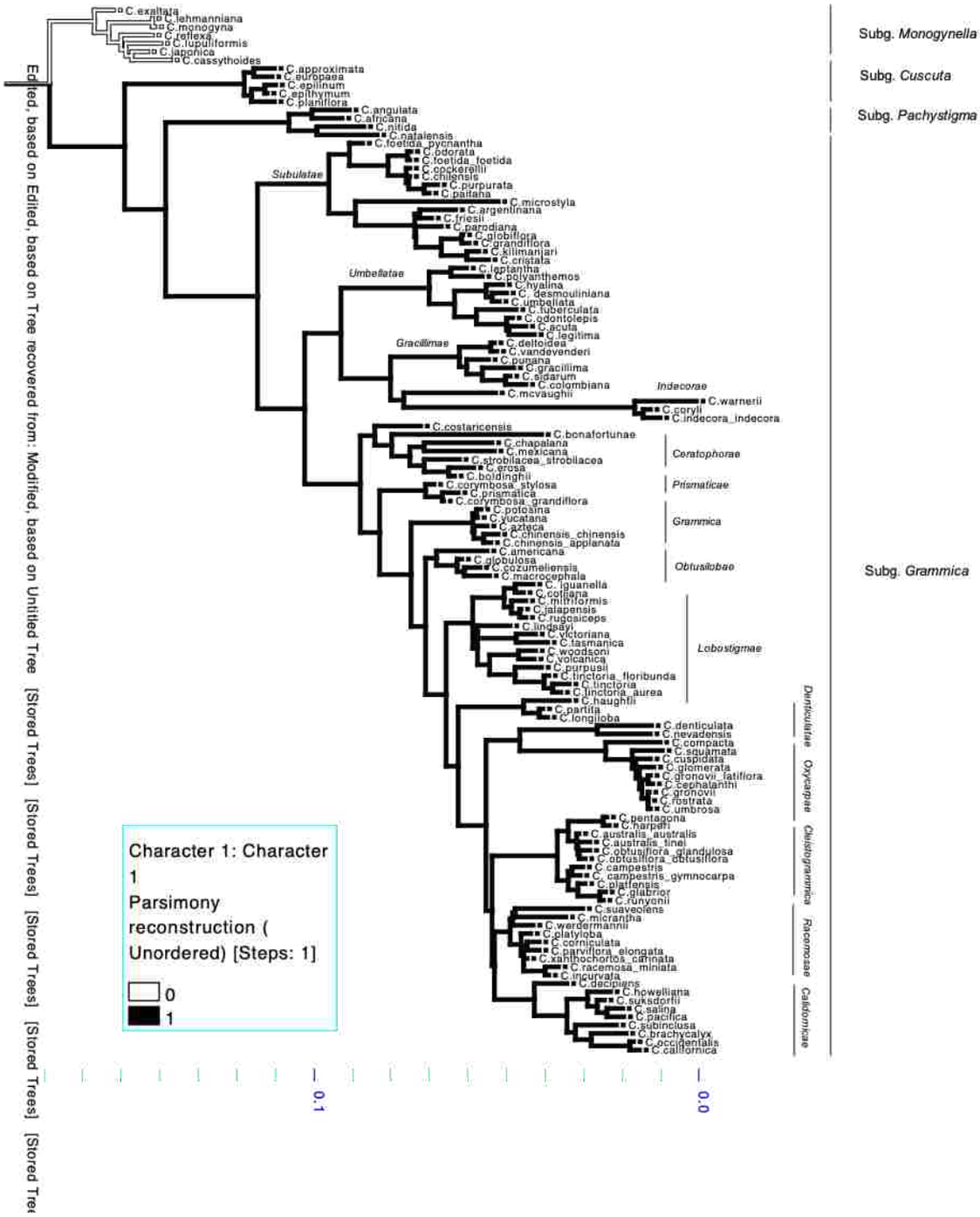

FIGURE B3. Ancestral character state reconstruction of epidermal cell types in *Cuscuta* mapped onto a genus phylogeny based on rbcL and nrLSU sequences (García et al. 2014). Characters scored "0" and colored white are the ancestral character state of elongated interlocking linear epidermal cells characteristic to subg. *Monogynella* (Type I). The remaining subgenera scored "1" and colored black, *Grammica, Pachystigma* and *Cuscuta* evolved the Type II with ± isodiametric epidermal cells. Type II is morphologically affected by surrounding humidity and is the derived character state.

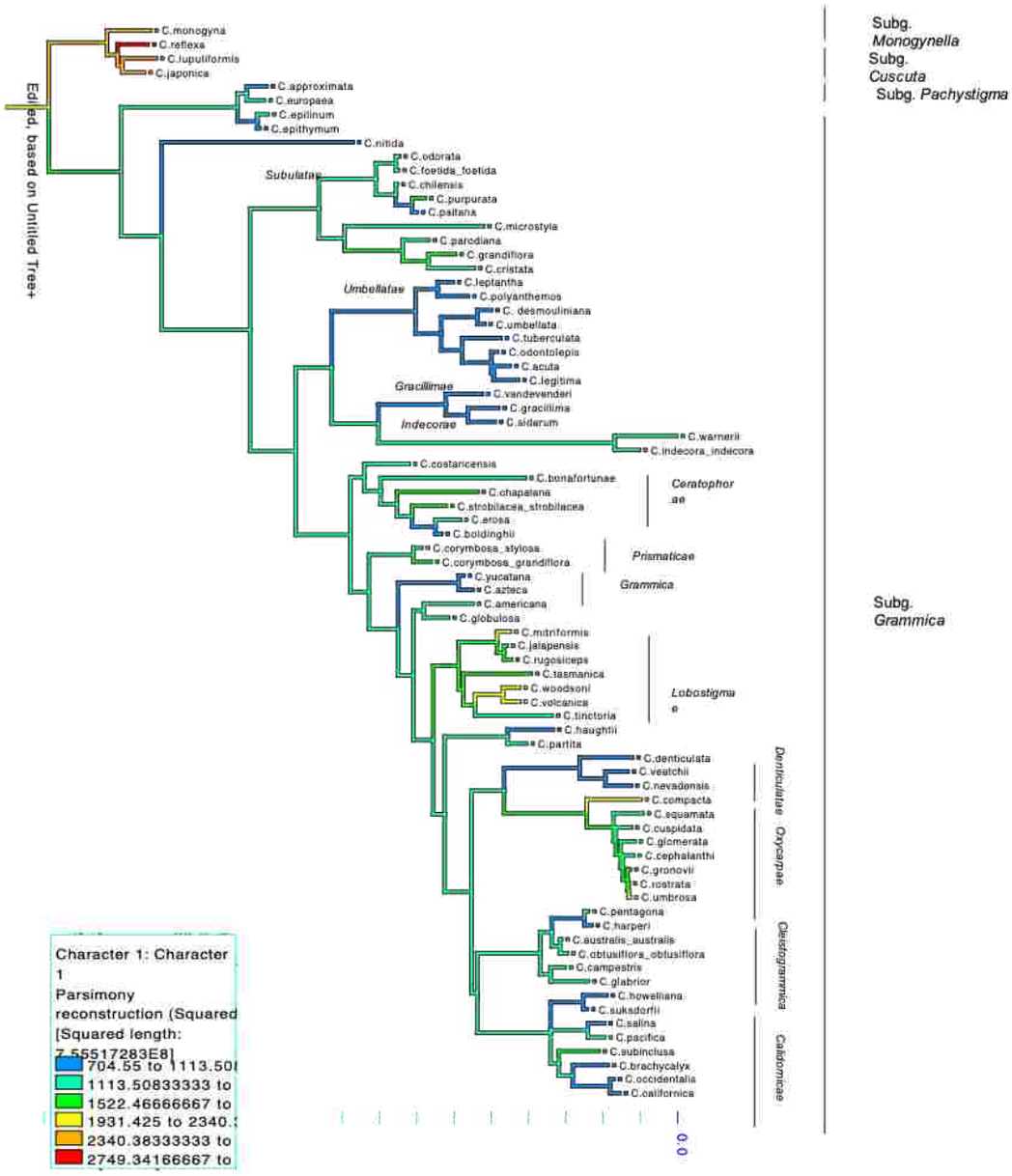

FIGURE B4. Ancestral character state reconstruction of "seed size" in *Cuscuta* mapped onto a genus phylogeny based on rbcL and nrLSU sequences (García et al. 2014). Characters are colored from smallest to largest (blue to red).

APPENDIX C: R Codes with Annotations

Breeding Systems

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>PollenOvule<c(187.06,311.5,284.58,228,222.5,261.5,118.92,242.25,203.3,275.05,245.6,120.17,232.6,2
31.5,987.5,1689,985.5,1400.25,406.5,792.92,989.79,972.13,439.63,803,409.5,1046.5,438.63,369.58,202
5.33,916.67,517.25,508982.63,688.25,1164.25,1290.5,1132.5,1801.63,371.25,1011.75,727.42,405.5,453
.83,1268,383.5.8,1285.75,949,930.08,1598,1069.5,739.75,538.25,586.25,875.63,1624.44,1545.17,409.1
3,881.08,1184.63,1741.5,1296.17,778.54,1552.5,1178.75,916.13,427.5,943,629,2243,457,661.38,1575.7
5,1654.44,577.25,708.08,1327.5,886.5,358.69,1060.5,1355.25,2017.75,1039.75,1327.63,1019.25,1452.1
3,730.38,1089.25,1689.75,484.67,454,856.4,366.33,2006.38,2957.5,3114.42,4197,4049.75,2331.45,234
1.56)
>NRSeedCapsule<c(2.3,3.4,3.5,3.4,1.5,3.1,3.2,1,3.3,1,3.8,3.6,3.6,3.6,3.1,3.52,2,3.5,1,1,1,2.2,3,3.2,3.3,2.
1,3,2.6,1.7,2,3,2.9,3,2.5,3.5,3.2,2.5,1.2,3.3,1,1,3.4,3.3,2.33,3.1,1.4,3,3.1,2.1,2.4,3.4,3.4,3.4,1.7,2.2,2,2.5,
3,3,3.2,2.6,2.1,2.6,2,2,3.6,3.2,2.2,2.2,3.1,2.3,2.2,2.8,3.1,2.4,2.2,3.4,3.3,2.4,1,2.3,2,2.1,3.7,2.2,2,2.6,2.4,2.
5,2.5,2.6,2.3,3.1,3.1,2.4,2.3,1.9, 3, 2.4)
>BreedingSys<c(3,3,3,3,3,3,3,3,3,3,3,3,3,3,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4
,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,5,5,5,5,5,5)
>Combined<-data.frame(cbind(PollenOvule,NRSeedCapsule,BreedingSys))
>Stacked<-stack(Combined)
>Anova_results<-aov(values~ind,data=Stacked)
>summary(Anova_results)
>boxplot(PollenOvule,NRSeedCapsule,BreedingSys)
>boxplot(NRSeedCapsule~BreedingSys,col='skyblue")
>install.packages("tree")
>library(tree)
>combined<-data.frame(cbind(NRSeedCapsule,BreedingSys))
>model<-tree(PollenOvule~.,combined)
>print(model)
>library(rpart)
>model<-rpart(PollenOvule~.,data=combined)
>BreedingSys<-as.factor(BreedingSys)
>plot(model)
>text(model)
>
```

```
>library(rpart)
>fit <- rpart(PollenOvule~BreedingSys + NRSeedCapsule, method="anova")
>printcp(fit) # display the results
>plotcp(fit) # visualize cross-validation results
>summary(fit) # detailed summary of splits
```

11. GLOSSARY

Apomorphy	A novel evolutionary trait that is unique to a particular species and all its descendants and which can be used as a defining character for a species or group in phylogenetic terms
Autogamous	Self-fertilization, especially the self-pollination of a flower.
Anatropous	(of an ovule) Inverted at an early stage of growth, so that the micropyle is turned toward the funicle
Basal Placentation	Where the placenta develops at the base of the ovary
Bet-Hedging strategy	Organisms sacrifice short-term success
Circumscissile dehiscent	Splitting or opening of the fruit along a built-in circumference, a line of weakness in a plant structure in order to release its contents
Delimitation	Formal statement in taxonomy that defines and limits the characters of a taxon
Ephemeral	Lasting for a very short time
Geitonogamy	The fertilization of a flower by pollen from another flower on the same (or a genetically identical) plant.
Indehiscent	Fruit not opening at maturity
Infrageneric	Pertaining to division or sub-classification within a biological genus of organisms
Megasporogenesis	The formation and development of megaspores. A diploid cell in the ovule, megaspore mother cell, undergoes meiosis and gives rise to four haploid megaspores. One megaspore goes on to develop into a megametophyte within the ovule, while the other three disintegrate.
Physical dormancy	Dormancy caused by water-impermeable seed or fruit, dormancy break occurs by a formation of an opening in the water impermeable seed or fruit coat through a physical break in a specialized morphoanatomical area called the water gap
Physiological dormancy	Dormancy that prevents embryo growth and seed germination. It is relieved by periods of moist chilling and stratification, normally from wintering under/in ground over winter thus synchronizing germination in the spring
Sections	A taxonomic rank below the genus, but above the species. The subgenus, if present, is higher than the section and the rank of series, if present, is below the section
Tenuinucellate	Absence of parietal cells, megaspore mother cell lies directly below the nucellar epidermis
Unitegmic	Referring to an ovule having a single integument
Xenogamous	The transfer of pollen grains from the anther to the stigma of a different plant. This is the only type of pollination which during pollination brings genetically different types of pollen grains to the stigma