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DIVERSITY AND EVOLUTION OF FRUITS IN *CUSCUTA* (DODDERS; CONVOLVULACEAE)

By

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ABSTRACT

Cuscuta (dodder) is a genus of roughly 200 species of obligate stem parasites with sub-cosmopolitan distribution. The fruit, generally regarded as a capsule, has a thin pericarp containing one to four seeds and opening at the base (circumscissile dehiscence; DE), or remaining closed (indehiscent; IN). IN has evolved multiple times in *Cuscuta* from DE, and is most common in the North American clades of subgenus *Grammica*. In addition, some species produce fruits that open irregularly. Characteristics pertaining to the fruits of *Cuscuta* are important as their seeds contribute most to their distribution and prevalence across the globe, and their reduced vegetative organs limit the morphological variation available for species' identification. In this thesis, I examined the structural mechanism behind DE to elucidate fruit types and their evolution. I surveyed fruit morphological traits to determine their systematic significance and functional correlations with dehiscence/indehiscence. Finally, I explored the putative evolutionary advantage(s) of fruit indehiscence by examining distribution, floatability, germination, and infructescence architecture. Pericarp structure revealed three distinct fruit types: DE fruits with an abscission zone (AZ), IN fruits with a uniform pericarp, and fruits that dehisce irregularly via the thinning of endocarp cell walls (IrA). IN fruits that break open irregularly (IrB) may also be an evolutionary fruit trait and were considered as such. Most qualitative fruit traits were polymorphic and their evolution involved multiple transitions to each state. Differences in quantitative traits were not consistent among fruit types, however IrB fruit species generally have a large interstylar aperture and large fruits with more seeds. IrB fruit species have a larger geographical range and more northern latitudinal limit than DE fruit species, and their infructescences slightly more compact than IN fruit species; which along with fruit traits may contribute to their irregular dehiscence. Capsules of *C. gronovii* were capable of floating for at least one week longer than their seeds. Seeds of *C. gronovii* exhibit a delayed germination when not removed from their IN fruits; a strategy known as bet-hedging. These results suggest that the evolution of IN in *Cuscuta* has provided certain species with heterodiaspory, and enhanced their dispersal and germination strategies.

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1. INTRODUCTION

1.1 THE GENUS *CUSCUTA*: OVERVIEW

Cuscuta, commonly known as dodders, is a parasitic genus that has evolved in the morning glory family, Convolvulaceae (Stefanović et al., 2002; Stefanović and Olmstead, 2004). The genus is sub-cosmopolitan in distribution and comprises nearly 200 species (García et al., 2014; Costea et al., 2015) that depend entirely on their hosts for water and nutrients (Dawson et al., 1994). *Cuscuta* species are capable of parasitizing a wide variety of host plants from numerous habitats (Dawson et al., 1994; Costea and Tardif, 2006; Costea et al., 2015), though host specificity may range considerably (Engelmann, 1842; Gaertner, 1950) from "generalist" species, such as *C. campestris* and *C. gronovii*, to "specialists" such as *C. jepsonii* and C. *warneri* (Costea and Stefanović, 2009a). Parasitism by dodders involves specialized organs called haustoria capable of circumventing hosts' defenses, penetrating their stems, and connecting to their vascular tissues to allow for the diversion of water and nutrients from host to parasite (Yuncker, 1932; Dawson et al., 1994). The effect of dodders on their host varies considerably, but since the parasite acts as a powerful sink, in general, the growth and fitness of the host are negatively affected (e.g., Dawson et al., 1994).

As with typical members of Convolvulaceae, *Cuscuta* species are annual vines (though in some situations can "behave" as perennial; Muelebrouck et al., 2009) containing laticifers in all the organs (Riviere et al., 2013), and their flowers actinomorphic, hermaphroditic, and hypogynous (Stefanović et al., 2002; García et al., 2014). However, *Cuscuta* is the only parasitic genus within the morning glory family, and the species have a limited photosynthetic capacity (Stefanović et al., 2002; Stefanović and Olmstead, 2004;

García et al., 2014). Dodders are herbaceous, glabrous, with slender, twining, yellow, orange or purple-reddish stems and alternate leaves reduced to scales (Yuncker, 1932; Dawson et al., 1994). The embryo and seedling are devoid of cotyledons and the root is vestigial and ephemeral, disintegrating entirely at seedling stage prior to the host attachment (Yuncker, 1932; Dawson et al., 1994; Sherman et al., 2008; Behdarvandi et al., 2015). Under optimal growing conditions, the stems of *Cuscuta* spp. intertwine and form large, dense masses that cover the hosts (Dawson et al., 1994). Stems of a single *Cuscuta* plant are capable of growing up to 750 m long in a vegetation season (Dean, 1942) and parasitize simultaneously multiple host species from different families (Costea and Tardif, 2006). Though dodder stems are easily recognized from other plants, they lack characteristics for identification at the species level, thus researchers must rely on the flowers, inflorescences, and fruits (Yuncker, 1932; Kuijt, 1969; Dawson et al., 1994; Stefanović et al., 2007).

Since its proposal by Engelmann (1859), it has been universally accepted that *Cuscuta* must be divided into three subgenera based on style and stigma morphology: *Monogynella*, with one style and various stigma shapes; *Cuscuta*, with two styles and acute/clavate stigma; and *Grammica*, with two styles and globose/capitate stigma (Peter, 1891; Mirande, 1900; Yuncker, 1921, 1932, 1965; Wright et al., 2011). More recent molecular studies have revealed that these subgenera are for the most part evolutionary lineages: *Monogynella* is the sister to the rest of the genus, and *Cuscuta* and *Grammica* are sister to each other (Stefanović et al., 2002; Stefanović and Olmstead, 2004). However, subgenus *Cuscuta* was found to be paraphyletic and the delimitation of a fourth infrageneric lineage containing only five species from South Africa, "*Pachystigma*", emerged as a necessity (García and Martín, 2007; McNeal et al., 2007; Stefanović et al., 2007; García et al., 2014). In the most recent phylogeny of *Cuscuta*, *Pachystigma* is sister to *Grammica* (García et al., 2014). A formal infrageneric classification of *Cuscuta* proposed by Costea et al. (2015) recognized *Pachystigma* as a fourth subgenus and subdivided the four subgenera into a total of 18 monophyletic sections of which 15 are well-supported major clades within subg. *Grammica* (labeled informally A-O in Stefanović et al., 2007; Stefanović and Costea, 2008; García et al., 2014). Subgenus *Grammica* is the largest infrageneric group (includes over 75% of *Cuscuta* species diversity) and has evolved in the Americas (Stefanović et al., 2007; García et al., 2014; Costea et al., 2015).

1.2. SIGNIFICANCE OF *CUSCUTA*

Dodders are among the earliest referenced parasitic plants (Costea and Tardif, 2004). Their peculiar appearance and parasitic nature, particularly on crop plants, sparked an interest in early botanists to study their taxonomy, physiology and anatomy (e.g., Choisy, 1841; Engelmann, 1842; 1859; Mirande, 1900). However, although a vast collection of literature has accumulated on *Cuscuta*, researchers continue to study the genus especially due to its ecological and economic impact (Kuijt, 1969; Nickrent and Musselman, 2004; Costea and Tardif, 2006).

Similar to other parasitic plants, dodders are keystone species because they have the ability to alter plant community structures (Pennings and Callaway, 2002; Smith, 2008; Spasojevic and Suding, 2011) and the abiotic environment (Press and Phoenix, 2005; Ridenour et al., 2014) even when present in low abundances (Pennings and Callaway, 1996; Costea and Stefanović, 2009a). They can act as vectors for plant viruses

(Hosford, 1967 and references therein; Dawson et al., 1994; Dobson and Crawley, 1994) and they are capable of horizontal gene transfer through the haustorial connections (Mower et al., 2004). Their detrimental effect on host and associated ecological significance have inspired scientists to explore the use of *Cuscuta* advantageously as a biocontrol agent and remediation tool for native plant communities invaded by foreign species (Epstein and Hill, 1999; Yu et al., 2008; Li et al., 2012; 2015).

The economic importance of *Cuscuta* is mostly detrimental as roughly 15-20 dodder species attack numerous agricultural and horticultural crops worldwide, reducing their yield and/or lowering their commercial value (Dawson et al., 1994; Costea and Tardif, 2006). For example, *C. campestris*, perhaps the most widely distributed species worldwide and also the most damaging agricultural pest of the genus, was reported as a weed of 25 crops in 55 countries (Holm et al., 1997). Small-seeded forage legumes such as alfalfa (*Medicago sativa*) and clover (*Trifolium* spp.) are the crops that are most commonly infested by *Cuscuta* as they are favourable hosts to several species and their seeds are difficult to distinguish from dodder seeds once contaminated. Due to the difficulties of identifying species through seeds, the entire genus of *Cuscuta* is included on many governmental lists of noxious and/or invasive weeds and commercial seed shipments found to contain dodder seeds at the border are confiscated and destroyed (Dawson et al., 1994; Costea and Tardif, 2006).

Some limited positive economic significance exists, as several species have been used in traditional Asian medicine (e.g., *C. chinensis* and *C. reflexa*). These species are currently investigated for their medicinal properties (Pal et al., 2006; Wong et al., 2006; Yen et al., 2007).

1.3. RESEARCH FOCUS

This study explores the diversity and evolution of fruit in *Cuscuta*. More specifically, it focuses first on elucidating the structural mechanisms of different modes of fruit dehiscence/indehiscence. Second, it analyzes the morphological diversity, character evolution and systematic significance of fruit traits. Third, this research explores the evolutionary advantage(s) of fruit indehiscence, a trait that has evolved multiple times especially in subgenus *Grammica*. This section serves to: (a) introduce the problems arising from the current fruit categorization in *Cuscuta*; (b) explain why the fruit is potentially important for the systematics of the genus, and (c) introduce hypothetical selective advantage(s) of indehiscent fruits in *Cuscuta.* The following chapters will provide additional background, as well as the research objectives and hypotheses.

(a) *Clarifying fruit types in Cuscuta by determining the structural and ultrastructural basis of different modes of dehiscence/indehiscence*

Generally regarded as a capsule, the fruit of *Cuscuta* has a thin, membrane-like pericarp and contains one to four seeds. In some species, the capsules open at the base along a circular line (circumscissile dehiscence) while in others they remain closed (indehiscent). This characteristic was first noted by Engelmann (1842) and later used by Yuncker (1921; 1932) to classify the species within subgenus *Grammica* into two sections: *Cleistogrammica*, with indehiscent capsules, and *Eugrammica*, with dehiscent capsules. Yuncker (1932) also attempted to interpret the evolution of this trait, regarding dehiscence as derived from indehiscence. However, two recent large scale phylogenetic studies indicated that dehiscence is the primitive trait, while indehiscence has evolved multiple times in subg. *Grammica* (Stefanović et al., 2007; Garcia et al., 2014).

Unfortunately, circumscissile dehiscence and indehiscence are not always clear-cut character states. A literature review revealed that some species were described as having capsules "circumscissile but sometimes irregularly bursting" (e.g., *C. umbellata*; Engelmann, 1859), others as simply "irregularly circumscissile" (e.g., *C. kilimanjari*, *C. sacharrata*; Yuncker, 1932) or "tardily/late and irregularly circumscissile" (e.g., *C. gracillima*, *C. incurvata*, *C. hyalina*; Yuncker, 1932). Additionally, in some species with normally indehiscent capsules, some fruits may break irregularly (described as "indehiscent or irregularly dehiscent"; e.g., *C. gronovii*, *C. umbrosa,* and *C. indecora*; Costea et al., 2006a; 2006b). It is clear that an "irregular" attribute is shared by all these examples, but the "irregular" dehiscence may not be morphologically and developmentally similar among species. In the absence of a carpological study, the fruits of species with irregular dehiscence were treated as either dehiscent or indehiscent in character evolution studies (Stefanovic et al., 2007; Garcia et al., 2014). For example, the latter three species (*C. gronovii*, *C. umbrosa,* and *C. indecora*) were considered as having indehiscent fruits, while those of the remaining species mentioned above, as dehiscent (Stefanovic et al., 2007; Garcia et al., 2014). In order to elucidate the fruit categories and their evolution in *Cuscuta*, it is critical to also examine the "irregularly dehiscent" forms to understand the ontogeny and structural/ultrastructural mechanisms of dehiscence/indehiscence. Such a carpological study will improve the delimitation of fruit types in *Cuscuta*, eliminating any categorization ambiguities and providing new character states for the character evolution study, which will allow for a better understanding of how dehiscence/indehiscence modes evolved in the genus.

(b) *Morphological diversity of fruit traits and their taxonomic significance*

The fruit of *Cuscuta* has been a part of the species descriptions since Engelmann's studies (1842; 1859) because, in addition to dehiscence/indehiscence, it provides other morphological characters that are useful for species delimitation. This is significant because similar to other obligate parasitic plants (reviewed by Kuijt, 1969; Heide-Jorgensen, 2008), *Cuscuta* has fewer morphological characters available for systematics/taxonomy compared to green plants (Stefanovic et al., 2007; Costea et al., 2015). Although subsequent authors have used fruit morphology to distinguish various dodder species (Yuncker, 1921; 1932; 1965; Costea et al., 2005; 2006a; 2006b; 2006c; 2006d; 2008a; 2008b; 2009; 2011a; 2011b; 2012; 2013; Costea and Stefanovic, 2009a; 2009b; 2010), no character evolution study has been conducted to examine the diversity and systematic significance of fruit traits.

(c) *Assessing the selective advantage(s) of indehiscent fruit in Cuscuta*

The convergent evolution scenario of fruit indehiscence in subg. *Grammica* in North America (García et al., 2014; Costea et al. 2015) raises questions about its evolutionary advantage(s). Fruit indehiscence, in general, is involved in seed protection and affects dispersal (Roth, 1977; Bazzaz et al., 2000; Baskin and Baskin 2014). To what extent protection and/or dispersal roles are selected for is often reflected in the morphology/structure of the fruit wall (pericarp) and seed coat (Roth, 1977). Since the pericarp in *Cuscuta* is essentially a thin membrane (Engelmann, 1856; Yuncker, 1932;

Wright et al., 2011), it is unlikely the fruit plays a significant role in seed protection. Seeds of *Cuscuta* species with circumscissile fruit disperse individually, whereas the seeds within indehiscent fruits disperse as units with up to 4 seeds. When diaspores contain multiple seeds, indehiscence has usually been associated with reduced dispersal ability (e.g., Augspurger and Hogan, 1983; Snijman and Linder, 1996; Willis et al., 2014) However, in some cases, indehiscence may coevolve with dispersal-enhancing traits (Wilis et al., 2014) or as a trade-off with life-history traits that are not directly related to dispersal. For instance, in the latter case, indehiscence can influence dormancy and germination (e.g., Hu et al., 2009; Andrade et al., 2015; Lu et al., 2015). Whether the dehiscence/indehiscence modes, and therefore diaspore type, are reflected in the dispersal ability and geographical distribution of *Cuscuta* species, or if indehiscence affects other life history traits is unknown.

Since dispersal capability plays a significant role in the size of geographical ranges (reviewed by Gaston et al., 2003; Lester et al., 2007), the distribution of subgenus *Grammica* species in North America will be used to determine whether a geographical pattern exists among species with different modes of dehiscence/indehiscence. Whether there is a potential ability for indehiscent capsules and seeds to disperse by water will be determined by comparing their floating capability. Lastly, the effect of fruit on germination will be studied as indehiscent fruits (pericarp present) may alter germination and therefore seedling establishment and population persistence.

“Indehiscent or irregularly dehiscent" capsules are apparently present in species with dense, glomerulate infructescences (e.g., *C. campestris, C. gronovii*; Costea et al., 2006a; 2006c). Because the pericarp is very thin, in such species the capsules which are normally indehiscent may break open irregularly. Although irregular breaking occurs only in some of the capsules within each infructescence, its consequence is that some of the capsules will release their seeds, while others will remain closed. This allows the seeds of the same plant to disperse both independently as well as within capsules, which may be advantageous. Also, if seeds germinate differently when enclosed within the fruit versus when they are dispersed individually, indehiscent and irregularly dehiscent capsules may provide additional benefits, as in the case of certain Brassicaceae with heterodiaspory (plants produce two or more types of diaspores; reviewed by Baskin and Baskin, 2014; section 2.5). To reveal a possible relationship between the dehiscence/indehiscence modes and infructescence density, a comparative study of the evolution of different modes of dehiscence/indehiscence and the infructescence architecture in subgenus *Grammica* will be performed.

2. BACKGROUND

2.1 DEHISCENCE/INDEHISCENCE IN DRY FRUITS

Dry fruits are defined as having a fruit wall (pericarp) that dries out at maturity (Spjut, 1994) and are classified as either dehiscent (e.g., various types of capsules) or indehiscent (e.g., the achene, caryopsis, etc.) depending on whether they open or remain closed (Spjut, 1994; Leins and Erbar, 2010). Dry indehiscent fruits have evolved from dehiscent fruits numerous times in various angiosperm families, both in mono- and polycarpellate families (Stone, 1973; Roth, 1977; Hoot, 1991; Cronquist, 1988). Among the best studied are Brassicaceae, in which certain genera containing dry dehiscent fruits (i.e., silicle and silique) have evolved indehiscent or partially dehiscent fruit forms (Mühlhausen et al., 2013).

Circumscissile dehiscence — capsules opening along a basal circular dehiscence line — is not a unique trait to *Cuscuta*; it is present in other genera of Convolvulaceae (e.g. *Operculina*, *Dichondra,* and *Merremia*; Van Oostroom and Hoogland, 1953; Woodson et al., 1975; Rhui-Cheng and Staples, 1995; Austin, 1998; Felger et al., 2012), as well as in other angiosperm families. This type of capsule that opens through a lid is called a pyxidium and has evolved in at least 17 flowering plant families (Spjut, 1994). Well known examples include *Portulaca* (Portulaceae; Rethke, 1946; Subramanyam and Raju, 1953), *Hyoscyamus* (Solanaceae; Rethke, 1946), *Plantago* (Plantaginaceae; Subramanyam and Raju, 1953; Lamba and Gupta, 1981) and *Amaranthus* (Amaranthaceae; Costea et al., 2001).

Fruit dehiscence/indehiscence is typically a genus trait. Genera with both dehiscent and indehiscent versions of dry fruits (in different species) are extremely rare. For example, *Lepidium* (Brassicaceae) has species with either dehiscent or indehiscent silicles, and the latter have evolved from the former (Mummenhoff et al., 2009). Also, in *Amaranthus* (Amaranthaceae) some species possess circumscissile dehiscence while others have indehiscent fruits (Costea et al., 2001), but in this case, it is unknown which of the two character states is primitive. Certain cultivated plants such as *Sesamum indicum* (Pedaliaceae), *Linum ussitatissium* (Linaceae), *Euphorbia lagascae* (Euphorbiaceae), which normally have (dehiscent) valvicide capsules, also possess forms with indehiscent capsules, but these are the result of artificial selection (Fahn and Werker, 1972; Ashri, 1988; Muir and Westcott, 2003; Verdolini et al., 2004; Fu, 2011).

The structural mechanism of dehiscence in other angiosperm genera with circumscissile dehiscence, such as *Sesuvium* (Aisoaceae); *Allmania*, *Amaranthus*, *Celosia*, *Chamissoa* (Amaranthaceae), *Plantago* (Plantaginaceae), *Portulaca* (Portulacaceae), and *Hyoscyamus* (Solanaceae) involves the controlled development of a circular "weakness zone" within the pericarp at the base of the fruit (Rethke, 1946; Subramanyam and Raju, 1953; Lamba and Gupta, 1981; Costea et al., 2001; Oyama et al., 2010). The weakness zone functions as an abscission zone (AZ), which is also present in other types of dry dehiscent fruits and abscised organs more generally (Sexton and Roberts, 1982; Roberts et al., 2000; 2002; Patterson, 2001; Leslie et al., 2007). The anatomical studies mentioned above reported that various lignified/sclerified tissues within the pericarp surrounding the AZ also contribute to the dehiscence of fruit. The enlarging seeds within the fruit, together with the shrinking of the pericarp as the fruit dries at maturity will ultimately trigger the dehiscence along the circular weakness zone (Subramanyam and Raju, 1953; Costea et al., 2001; Wright et al., 2011).

Cuscuta is one of the rare angiosperm genera in which species are characterized by different modes of dehiscence/indehiscence. Engelmann (1842) noted first that in some species capsules are circumscissile, while in others they remain indehiscent (which he called “baccate capsules”). More recently two large-scale phylogenetic studies have shown that species bearing indehiscent fruits have evolved 8 times from those with dehiscent fruits in subg. *Grammica* (Stefanovic et al., 2007; García et al., 2014). As previously indicated, this convergent evolution scenario of indehiscence is complicated by the apparent existence of species possessing “irregularly” dehiscent fruits. In some of these latter species, capsules were reported to open late through an “irregular” or “jagged” line at the base (e.g., Yuncker 1921; 1932; Costea and Stefanović, 2010), while in others the pericarp tears irregularly “by cracks that spread in different directions” (termed “foraminicidal” capsules by Spjut, 1994; Costea et al., 2006a). In the absence of a carpological study, the fruits of species with irregular dehiscence have been treated as either dehiscent or indehiscent (Engelmann 1859; Yuncker 1932; Stefanovic et al., 2007; García et al., 2014). In order to elucidate the fruit categories and their evolution in *Cuscuta*, it is critical to also include the irregularly dehiscent forms, and for all the fruit types, study the ontogeny and structural/ultrastructural mechanisms of dehiscence/indehiscence.

2.2. FRUIT CHARACTERS AND SYSTEMATICS OF *CUSCUTA*

General fruit classifications were early elaborated (e.g., Linnaeus, 1751; Gaertner, 1788–1792; de Candolle, 1813) and fruit morphology figured prominently in the first comprehensive classification systems of angiosperms (e.g., Linnaeus, 1753; de Candolle and de Candolle, 1864). The case of *Cuscuta* is particular because as a result of the evolution to obligate parasitism, the leaves which have been much used in the separation of flowering plants species have undergone drastic reductions (Kuijt, 1969; Stefanović et al., 2007). As a consequence, the species level taxonomy of dodders has relied heavily on flower morphology (Choisy, 1841; Engelmann, 1859; Yuncker, 1932; reviewed by Costea et al. 2015). More recently, extensive character evolution studies of floral parts and their function have been conducted to reveal their phylogenetic/systematics significance (pollen, Welsh et al., 2010; gynoecium, Wright et al., 2011; perianth and pollen/ovule ratios, Wright et al., 2012; infrastaminal scales, Riviere et al., 2013; stomatiferous protuberances, Clayson et al., 2014).

In contrast, the fruit has received comparatively less attention in *Cuscuta*. Choisy (1841), the first monographer of the genus, used gynoecium characters but did not mention the fruit in species descriptions. Engelmann (1859) used the dehiscence/indehiscence of capsules to describe six of the nine sections within the three major infrageneric "groups" that are currently accepted as subgenera *Monogynella*, *Cuscuta* and *Grammica* (Costea et al. 2015). Yuncker (1921; 1932) also used the dehiscence/indehiscence to classify the largest subgenus, *Grammica,* into two sections: *Cleistogrammica*, with indehiscent capsules, and *Eugrammica* with dehiscent capsules. However, because of the repeated evolution of indehiscence in subg. *Grammica*, these two sections are not "natural" (monophyletic) lineages (García et al., 2014; Costea et al. 2015). The diversity and evolution of other fruit characteristics besides dehiscence/indehiscence have not been studied despite being used in species description since Engelmann (Yuncker, 1921; 1932; 1965; Costea et al., 2005; 2006a; 2006b; 2006c; 2006d; 2008a; 2008b; 2009; 2011a; 2011b; 2012; 2013; Costea and Stefanović, 2009a; 2009b; 2010). Exploring the morphological diversity and reconstructing ancestral character states for the fruit traits would be useful for the systematics of the genus.

2.3 DEHISCENCE/INDEHISCENCE MODES AND SPECIES DISTRIBUTION

The morphology of the dispersal unit (diaspore) affects both population level processes such as adaptive divergence and geographic isolation (e.g., Levin et al., 2003; Cousens et al., 2008), as well as species level patterns of distribution and diversification (Howe and Smallwood, 1982; Fernández et al., 2002; Cousens et al., 2008). "Dispersal syndromes" have been defined to connect morphological attributes of diaspores with dispersal vectors, such as wind, water, gravity or animals (e.g., van der Pijl, 1982; Tiffney, 1984). Angiosperm diaspores consisting of dry indehiscent fruits often contain only one seed (e.g., the achene and caryopsis; Spjut, 1994; Leins and Erbar, 2010), which maximizes the dispersal potential. Indehiscent diaspores with multiple seeds have been associated with a loss of dispersal ability (e.g., Augspurger and Hogan, 1983; Willis et al., 2014). In this latter case, the evolution of indehiscence has been interpreted as a trade-off with other beneficial life-history strategies such as the retention within favourable maternal sites (Friedman and Stein, 1980), the protection of seeds against environmental factors (Ellner and Shmida, 1981) or the modulation of dormancy/germination (Zhou et al., 2015). However, one recent study in Brassicaceae

(Willis et al., 2014), in which indehiscence is also derived from dehiscence, has suggested that indehiscence evolved in association with compensatory traits that ultimately enhanced the dispersal ability, such as joint abscission, certain pericarp characteristics, and a reduction of the number of seeds per propagule.

Whether the dehiscence/indehiscence modes, and therefore the diaspore type, are reflected in the dispersal ability and geographical distribution of *Cuscuta* species, or if indehiscence affects other life history traits is unknown. Diaspores of *Cuscuta* species with circumscissile capsules are the individual seeds, whereas diaspores of species with indehiscent capsules are the fruits containing one to four seeds. Indehiscent fruits can be dispersed individually or as fruit clusters (Costea et al. 2016).

The artificial dispersal of weeds and invasive dodders species has been well documented; it has occurred through seed contamination of commercial seed crops, particularly of forage legumes (Knepper et al., 1990; Dawson et al., 1994; Costea and Tardif, 2006). In contrast, the natural means of dispersal are little known. Dispersal of *Cuscuta* seeds has been considered “unspecialized” (Kuijt, 1969; Costea and Tardif, 2006) because dodders seeds do not possess morphological adaptations that match any of the known dispersal syndromes (Costea et al., 2016). Similar to other parasitic plants (Hughes, 1994), annual dodder diaspores (seeds or fruits) fall in the immediate vicinity of the mother plant and host(s), which may facilitate the establishment on the host in the next year. Natural vectors for *Cuscuta* seed dispersal are poorly known. Lyshede (1992) suggested wind as a possible dispersal agent of *C. campestris* and *C. pedicellata* because their seeds are very small (ca. 1 mm long) and their epidermis is finely alveolate (when seeds are dry). Subsequent authors, however, have indicated that the seed epidermis of many *Cuscuta* species — alveolate when seeds are dry — and papillate when seeds are hydrated, is more likely connected with the seed imbibition and germination rather than with the dispersal (Costea and Tardif, 2006; Jayasuriya et al., 2008). Seeds sink in the water (Verdcourt, 1948) though in some species (e.g., *C. gronovii*) they were reported to float "at least for a while" (Costea and Tardif, 2006). According to Verdcourt (1948), capsules of C. cf. *campestris* containing the seeds can float for up to two days. Most recently, Costea et al. (2016) reported that waterfowl birds such as the northern pintail (*Anas acuta*) can disperse the seeds of *C. campestris* and *C. pacifica* along their migratory pathway in North America. The authors noted that both *Cuscuta* species retrieved from the digestive system of pintails had indehiscent capsules, and that the clusters of capsules fallen on the ground or water were compatible with the feeding habits of the pintails (Costea et al., 2016). Because dispersal questions are complex and require extensive testing of hypotheses in the field, for this thesis, only the ability of capsules and seeds to float under the lab conditions will be examined.

The capacity of species to disperse is often positively correlated with the size of their distribution ranges (Hanski et al., 1993; Brown et al., 1996; Gaston, 1996; 2003; Birand et al., 2011). Assuming that dehiscence/indehiscence modes affect dispersal because they result in different types of diaspores, their influence on dispersal may be reflected in the geographical distribution of dodder species. Since indehiscence has evolved mostly in subgenus *Grammica* in North American clades (Stefanovic et al., 2007; Garcia et al., 2014), it is best to compare the geographical ranges of species with different dehiscence/indehiscence modes on this continent. If indehiscence limits dispersal, this should be reflected in species' ranges. A strongly supported phylogeographical scenario is available for subgenus *Grammica* (Stefanovic et al., 2007; Garcia et al., 2014), and if range differences will be revealed among species with different modes of dehiscence, these patterns will be interpreted in a biogeographical context.

2.4. INDEHISCENCE/DEHISCENCE AND SEED GERMINATION

The pericarp of dry indehiscent fruits protects the seed(s) during dispersal and/or influences seed dormancy and germination. An obvious seed protection role is provided in the case of species with hard pericarp dispersed through endozoochory (e.g., *Prosopis*; Baes et al., 2002) or diszoochory (e.g., *Corylus*, *Quercus*, *Fagus*; van der Pijl, 1982; Vander Wall, 2001). Germination inhibition or delay have been documented, for example, in *Atriplex spp.* (Garvin and Meyer, 2003; Li et al., 2008), *Hedysarum scoparium* (Hu et al., 2009), *Raphanus raphanistrum* (e.g., Cousens et al., 2009), *Rapistrum rugosum* (e.g., Ohadi et al., 2011), and *Isatis violascens* (Zhou et al., 2015). Because the pericarp in *Cuscuta* is very thin, it cannot provide protection when indehiscent fruits are ingested by birds (Costea et al., 2016) or when low mechanical forces are applied (Costea et al., 2005). Therefore, it is possible that the pericarp of indehiscent fruits has an effect on *Cuscuta* germination, which will be preliminarily explored in this thesis.

Newly produced seeds of *Cuscuta* are not dormant and can readily germinate (Gaertner, 1950). However, after a few days up to 95% of seeds become “hard” and dormant (Tingey and Allred, 1960; Allred and Tingey, 1964; Dawson, 1965; Hutchinson and Ashton, 1980; Lyshede, 1984). Dormancy of *Cuscuta* seeds is physical, imposed by the seed coat, which becomes hard and impermeable as the seeds dry out. Impermeability and hardness are ensured by the two schlerenchymatic palisade cell layers of the seed coat (Hutchinson and Ashton, 1979; 1980; Lyshede, 1984; Jayasuriya et al., 2008). For example, in the seeds of *C. campestris* and *C. pacifica* ingested by northern pintails, protection was provided by the inner palisade layer, while the epidermis and external palisade layer (in addition to the pericarp) were completely or partially removed during the digestion process (Costea et al., 2016).

Dodder seeds can remain viable up to 50 years in dry storage and at least 10 years under field conditions, which enables the formation of a persistent seed bank (Gaertner, 1950; reviewed by Costea and Tardif, 2006). Under natural conditions, in temperate regions, physical dormancy of *Cuscuta* seeds is broken by the cold period during the winter (Hutchinson and Ashton, 1980; Benvenuti et al., 2005; Meulebrouck et al., 2008). Thus, *Cuscuta* species undergo a cyclical dormancy/dormancy-break pattern similar to other annual plants in temperate regions (Baskin and Baskin, 2004; reviewed by Baskin and Baskin, 2014). Dormancy of *Cuscuta* seeds can also be broken artificially through cold stratification, mechanical (e.g., abrasion) or chemical treatments (e.g., with sulfuric acid; reviewed by Costea and Tardif, 2006). The optimal temperature for the germination of most species is over 25°C, which ensures that seedlings emerge later in the growing season once host plants have already established (Dawson et al., 1994; Costea and Tardif, 2006).

Since *Cuscuta* species are annual, germination is of paramount importance for seedling establishment and population dynamics (e.g., Crawley et al., 1990; Fenner, 2000). If seeds enclosed in indehiscent fruits differ in their germination behaviour from those released individually, then fruit indehiscence may influence establishment in *Cuscuta*, possibly advantageously.

2.5. INFRUCTESCENCE ARCHITECTURE AND ITS POTENTIAL CONNECTION TO DEHISCENCE/INDEHISCENCE MODES

The inflorescence is an organized system of branches (axes) that bear flowers (Weberling, 1992; Prenner et al., 2009). The role(s) of the inflorescence gravitate(s) around reproductive biology; displaying flowers in the most favorable position for pollination, as well as ensuring a dynamic architecture that increases the chances of reproductive success (Weberling, 1992; Prusinkiewicz et al., 2007). The three-dimensional patterns of various inflorescences have been correlated with pollination “syndromes” and specific pollinator behaviours (Harder et al., 2004; Harder and Prusinkiewicz, 2012). Inflorescences that persist at fructification are termed infructescences and in some plants, they act as diaspores (Hintze et al., 2013).

Cuscuta flowers develop in inflorescences that are characteristic to each species (Yuncker, 1932; Costea et al., 2015). Dodder inflorescences are loose to dense monochazial cymes that are further grouped in more complex inflorescences: either thyrses (subg. *Monogynella*) or larger (compound) cymose inflorescences (subgenera *Cuscuta, Pachystigma* and *Grammica*) that are glomerulate, spiciform, racemiform, paniculiform, corymbiform, umbelliform or fasciculate (Yuncker, 1932; 1965; Costea et al., 2015). Dodder inflorescences persist at fructification and become infructescences which remain attached to the host (Clayson et al., 2014; Costea et al., 2016). In the case of dehiscent fruits, seeds are released and dispersed in a seed shadow in the vicinity of the hosts. Infructescences of indehiscent species remain attached to the stems of the host and eventually they detach and fragment into clusters which also fall in the vicinity of the host during late fall-winter (Costea et al., 2016).

A preliminary review of the genus monograph (Yuncker, 1921; 1932) and taxonomic revisions of clades within subg. *Grammica* (Costea et al., 2005; 2006; 2008; 2011; 2013; Costea and Stefanovic, 2009; 2010), suggests that species with indehiscent fruits often have flowers/fruits growing in glomerulate inflorescences which are typically very dense, while species with dehiscent fruits possess more lax inflorescences. If this relationship is significant, then infructescence architecture may be connected to fruit dehiscence/indehiscence modes and possibly dispersal and/or germination. Costea et al. (2006) indicated that indehiscent capsules of species within the *C. pentagona* clade, one of the most recently derived clades within subg. *Grammica* (section *Cleistogrammica*; Garcia et al., 2014; Costea et al., 2015), may break irregularly because of the pressures generated by developing capsules within the same dense glomerulate inflorescences. Thus, some capsules release the seeds while others enclose them. If the fruit affects germination (see section 2.4), dispersing seeds both individually and within the fruit may result in a more diverse germination behaviour which may be advantageous for seedling establishment. In this case, such fruits could be regarded as *functionall*y heterodiasporous; two types of diaspores produced by the same plant differing in seed biology and ecological function (reviewed by Baskin and Baskin, 2014). If a relationship exists between dehiscence/indehiscence modes and infructescence compactness, it may be that fruit type is influenced by infructescence architecture in *Cuscuta*.

3. OBJECTIVES AND HYPOTHESES:

Structure and ultrastructure of Cuscuta fruit

1) My first objective is to determine the structural and ultrastructural basis of dehiscence/indehiscence modes and to analyze the data in a phylogenetic context as a means to distinguish fruit "types" and their developmental and evolutionary relationships. My hypothesis is that the circumscissile dehiscence mechanism in *Cuscuta* is similar to that encountered in other pyxidium fruits, involving a "weakness zone" and specialized thickenings in the adjacent pericarp. I also predict that the irregular dehiscent fruit forms are not similar ontogenetically and morphologically.

Systematic significance of Cuscuta fruit

2) My second objective is to: (a) survey the morphological diversity and reconstruct ancestral character states for fruit traits in *Cuscuta*, (b) investigate possible relationships between dehiscence/indehiscence modes and other fruit traits, and (c) discuss the usefulness of capsule characters for the systematics and taxonomy of *Cuscuta*. This objective is not hypothesis-driven but I anticipate that at least some of the fruit characters (other than dehiscence/indehiscence modes) have a systematic significance.

Evolutionary significance of indehiscence in Cuscuta

3) My third objective is to (a) analyze the geographical distribution of North American *Grammica* species in relation to their fruit dehiscence/indehiscence modes and evolution, and (b) determine in the lab the floating ability of *Cuscuta* indehiscent fruits and seeds to see if water is a potential dispersal vector. For the first part of this objective,

I hypothesize that dehiscent and indehiscent species exhibit different distribution ranges, but cannot predict *how* they will differ [multi-seed diaspores are associated with loss of dispersal ability *but* some other traits may enhance their dispersal. For the second part of this objective, I hypothesize that capsules can float for longer periods of time than seeds.

4) Preliminarily determine if seeds within indehiscent capsules have a different germination behaviour compared to seeds released from the capsules to determine if this can constitute a potential advantage. My hypothesis is that indehiscent capsules delay germination of enclosed seeds.

5) Examine a possible relationship between the infructescence architecture and the dehiscence/indehiscence modes. The hypothesis is that such a relationship exists and that species with indehiscent fruits and with very dense inflorescences also produce a form of irregularly dehiscent capsules.

4. MATERIALS AND METHODS

4.1 STRUCTURAL AND MORPHOLOGICAL DIVERSITY OF CAPSULES

Structure and ultrastructure of fruit

Pericarp structure was documented in 14 species selected (based on abundance and availability of material) to represent the four currently accepted subgenera of *Cuscuta*: *C. monogyna,* C. *japonica* (dehiscent; subg. *Monogynella*), *C. planiflora*, *C. approximata* (dehiscent; subg. *Cuscuta*), *C. nitida, C. africana* (dehiscent; subg. *Pachystigma*), *C. campestris*, *C. gronovii* (indehiscent to irregularly dehiscent; subg. *Grammica*), *C. chilensis, C. costaricensis*, *C. cotijana*, *C. chapalana*, *C. purpurata* (dehiscent; subg. *Grammica*) and *C. umbellata* (circumscissile but sometimes irregularly bursting; subg. *Grammica*). Developing and mature fruits of *C. campestris*, *C. costaricensis, C. cotijana*, *C. chapalana*, and *C. gronovii* were fixed directly in the field using 3% glutaraldehyde + 2% paraformaldehyde in 0.025M sodium phosphate buffer at pH 6.8. *Cuscuta monogyna*, *C. purpurata,* and *C. chilensis* were grown in a greenhouse from seeds collected in Israel and Chile. These species were examined both with light and transmission electron microscopy (TEM). Species for which fresh/fixed material was not available (*C. umbellata, C. japonica*, *C. planiflora*, *C. approximata, C. nitida* and *C. africana*) were obtained from herbarium specimens and their pericarp anatomy analyzed only with light microscopy using the protocol developed by Wright et al. (2011) for rehydration. Ten fruits were examined for each species. Samples were embedded using a modified Spurr's Resin protocol (Riviere et al., 2013). For light microscopy, Spurr blocks were sectioned at 2 µm with a Sorvall MT-1 ultra-microtome and stained with toluidine blue O (pH 4.4) for 2 minutes. Observation and imaging was conducted on Nikon Eclipse

50i brightfield and Nikon Eclipse E600 epifluorescence microscopes using a PaxCam digital arc camera and Pax-it 7.8 software. For transmission electron microscopy (TEM), blocks were cut with a diamond ultra-knife at 80–100 nm and mounted onto formvar and carbon-coated copper grids which were then post-stained with 5% uranyl acetate for 10 min, and then stained with Reynolds lead citrate for 5 minutes. Observations and images were taken with a Gatan Ultrascan digital camera and 'Digital Micrograph' software on a JEOL 2011 Transmission Electron Microscope at 200 kv (Gatan Inc. 2007, Pleasanton, CA). All herbarium vouchers used can be found in Appendix A.

Comparative morphological diversity of fruits

The morphology of mature fruits was examined in 126 taxa (118 species and 8 varieties) using ca. 400 herbarium specimens (Appendix A). Mature fruits were considered those that contained mature seeds. Dried fruits removed from herbarium specimens were placed in 50% ethanol, heated to boiling point and allowed to rehydrate for several minutes. When possible, ten fruits per specimen were examined with a Nikon SMZ1500 stereomicroscope and imaged with a PaxCam Arc digital camera equipped with Pax-it 7.8 software (MIS Inc., Villa Park, IL). Images were deposited in the Digital Atlas of *Cuscuta* (Costea, 2007-onwards). To determine whether endocarp cell walls are thickened, capsules were cut in longitudinal strips which were stained with toluidine blue O (pH 4.4) for 2 min and examined with light microscopy (endocarp cells facing up).

For scanning electron microscopy (SEM), hexamethyldisilazane (HMDS) was used as an alternative for critical point drying (Wright et al., 2011). Rehydrated herbarium samples were dehydrated using a series of ethanol (70%, 80%, 95% and 100%; each step one hour), immersed for 1 hour in 1:1 ethanol and HMDS, and passed through an overnight change of 100% HMDS. Samples were air dried and coated with 30 nanometers of gold using an Emitech K 550 sputter coater. Examination, measurements and pictures were taken at 10 kV using a Hitachi SU1510 variable pressure scanning electron microscope.

Ancestral character state reconstruction and data analysis

Fourteen characters were selected after a review of characters used in previous taxonomic studies of *Cuscuta* (Engelmann, 1859; Yuncker, 1932; Costea et al., 2005; 2006a; 2006b; 2006c; 2006d; 2008a; 2008b; 2009; 2011a; 2011b; 2012; 2013; Costea and Stefanović, 2009a; 2009b; 2010). Measurements and character states can be found in Table 1. Arbitrary numbers were assigned to each character state, and the number of states for each character was determined by the number of distinguishable phenotypic classes. Character states were mapped onto the recent genus phylogeny based on *rbcL* and nrLSU sequences (García et al., 2014). Analysis of character polarity in *Cuscuta* using formal outgroup analysis is hindered by the unresolved position of *Cuscuta* in Convolvulaceae (Stefanović and Olmstead, 2004). Thus, to reconstruct ancestral character states in *Cuscuta*, the distribution of character states was analyzed in-group as with previous character evolution studies (i.e. Welsh et al., 2010; Wright et al., 2011; Riviere et al., 2013; Clayson et al., 2014). Adding putative outgroup Convolvulaceae and coding them with a different character state than the in-group *Cuscuta* (García et al., 2014) produced similar results (not shown). Scenarios of character evolution were analyzed using the parsimony reconstruction method implemented in Mesquite 3.2

Table 1. Fruit characters and their representative codes and states used for surveying fruit morphology of 126 *Cuscuta* taxa.

Character	Character states
Categorical characters	
1. Dehiscence/indehiscence	0 = capsules indehiscent; 1 = capsules regularly circumscissile; 2 = capsules irregularly dehiscent type A; 3 = irregularly dehiscent type B. See "Results" for explanation of character states.
2. Fruit shape	1 = spherical (globose); 2 = depressed; 3 = ellipsoid; 4 = ovoid; 5 = obovoid; 6 = thimble-shaped
3. Position of persistent corolla on capsule	1 = corolla topping the capsule; 2 = surrounding the capsule (3/4); 3 = at the base of capsule (1/3–1/4)
4. Pericarp epidermis papillae	0 = absent; 1= present; see text (data not shown)
5. Pericarp translucent	0 = absent; 1 = present
6. Laticifers visible in the pericarp	0 = not visible under the stereomicroscope; 1 = visible
7. Interstylar aperture	0 = absent; 1 = interstylar aperture (IA) present but not thickened or raised; 2 = IA margins thickened but not raised; 3 = IA raised in a collar around the styles; 4 = IA with irregular distal protuberances (2–5); 5 = distal part of the ovary forms a rostrum under the styles
8. Style morphology at fruit stage	0 = styles remain ± cylindrical at fruit stage; 1 = styles become enlarged at the base, subulate (data not shown).
Continuous characters	
9. Fruit length	mm
10. Fruit width	mm
11. Fruit Ratio L/W	nr.
12. Interstylar aperture length	mm
13. Interstylar aperture width	mm
14. Average number of seeds/capsule	nr.

(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. Four qualitative, non-polymorphic characters (pericarp translucence, visible laticifers, papillae, and style morphology; Table 1) were also analyzed with the likelihood reconstruction method provided by the same software.

The analysis of possible relationships between the dehiscence/indehiscence modes and other fruit traits is complicated by the polymorphism and non-binary nature of most qualitative characters. In order to visualize the similarity of all the characters examined (Table 1), a PCoA (Principal Coordinates Analysis) and an NMDS (non-metric multidimensional scaling) were conducted in two dimensions using Gower's coefficient for mixed data (Gower, 1971) available from the statistical software PAST version 3.15 (Øyvind Hammer, 2017). The matrix used for the character evolution was rearranged in such a way that characters become the Operational Taxonomic Units and polymorphic characters are reduced to only include the most frequently occurring trait—except for dehiscence/indehiscence modes where different combinations of fruit types are treated as fruit types. After revealing the similarity of characters, a Kruskal-Wallis rank sum test and a Dunn's test for multiple comparisons (R package 'dunn.test'; Dinno, 2016) was used to determine the relationship between quantitative fruit characters and dehiscence/indehiscence modes using the statistical program R version 3.3.3 (R Core Team, 2017).

4.2. BIOGEOGRAPHY OF NORTH AMERICAN SPECIES OF SUBG. *GRAMMICA*

Sampling, data collection and mapping

Sampling included 76 *Cuscuta* taxa of subgenus *Grammica* native to North America because this is the major infrageneric group and geographical area in which most of the indehiscent species have evolved. The geographical distribution was estimated using locality data from specimens obtained from the following herbaria after their taxonomic identity was verified/determined: AAU, ABH, ALTA, ARAN, ARIZ, ASU, B, BAB, BC, BCN, BM, BOL, BORD, BR, BRIT, CAL, CANB, CAS, CEN, CHR, CHSC, CIIDIR, CIMI, COI, CTES, DAO, E, F, FT, G, GH, H, HAM, HUFU, HUJ, IAC, IEB, IND, J, JACA, JE, JEPS, K, L, LAU, LD, LE, LL, LP, LPB, LPS, M, MA, MACB, MAF, MEL, MERL, MEXU, MGC, MICH, MO, MT, MTMG, MPU, MSTR, NAP, NBG, NFLD, NMC, NSPM, NY, OAC, OKLA, OSC, OXF, P, PACA, PRE, QCNE, QFA, QUE, RB, RBG, RNG, RSA, S, SALA, SAM, SASK, SD, SEV, SFS, SGO, SI, SPF, TEX, TRT, TRTE, UA, UB, UBC, UCR, UC, UCT, UNB, UNM, UPRRP, UPS, US, UWO, VAL, W, WAT, WIN, WIS, WTU and XAL. Additional specimens with verified identity were obtained from *Cuscuta* systematics papers (Costea et al., 2005; 2006a; 2006b; 2006c; 2006d; 2008b; 2009; 2011a; 2011b; 2012; 2013; Costea and Stefanović, 2009a; 2009b; 2010) to increase sample size and minimize omission errors (ca. 7000 herbarium records used).

Coordinates were taken directly from herbarium specimens or assigned *post facto* and mapped as point data onto a base map with a Web Mercator projection and World Geodetic System 1984 datum using ArcGIS 10.3.1 for Desktop (ESRI, Redlands, USA). Each species was mapped for their North American range size and their northern latitudinal limit. The size of a species range was defined by the area (km^2) of its extent of occurrence using a convex hull minimum bounding geometry tool available with the software.

In addition, Mesquite Cartographer Package version 1.5 (Maddison and Maddison, 2017) was used to display the northernmost limit of each species together with the ancestral character reconstruction of fruit dehiscence/indehiscence modes. The fruit character tree was plotted onto a base map displayed in an Albers Conic Equal Area projection with the projection center at 36 North and 92 West. Calibration points were added manually to optimize map parameters.

Statistical analyses

The range size (km^2) and northernmost latitude of North American *Grammica* species were compared using fruit dehiscence/indehiscence modes as a covariate. Species with no range size, known from only one or two localities, were excluded from the range size analysis but were included in the latitudinal analysis. The statistical software used was R version 3.3.3 (R Core Team, 2017) and all annotations are available in Appendix D. Tests were performed using both the previous dehiscent/indehiscent fruit types retrieved from Garcia et al. (2014) and the new categorization proposed in chapter 5.1. Prior to determining whether there are differences between range size and northernmost latitudes for dehiscent and indehiscent species, a Shapiro-Wilk's test was performed to determine normality. Since the previous categorization has only two variables (dehiscent/indehiscent), a two-sample Wilcoxon test (Mann-Whitney) was used to determine whether there was a difference in range size, and a Student’s t-test was used to determine whether there was a difference in northernmost latitude between North American species with dehiscent fruits and those with indehiscent fruits. With the new fruit categorization, the difference in species range size was analyzed using a Kruskal-Wallis rank sum test, and a Dunn's test as the post-hoc analysis (R package 'dunn.test'; Dinno, 2016). Differences among the northernmost latitude of species were analyzed using an Analysis of Variance (ANOVA) and a Tukey Honest Significant Differences (TukeyHSD) as the post hoc analysis. Species or varieties that were not examined in section 4.1 and as a result do not have an updated dehiscence/ indehiscence mode character, were excluded from the analyses.

Northernmost latitude values were also mapped into the phylogeny of subg. *Grammica* (Stefanović et al. 2007; García et al. 2014) and analyzed using the parsimony reconstruction method implemented in Mesquite 3.2 (Maddison and Maddison, 2011). Two bins were created, a southern bin including species distributed between 15° to 35°N in North America and a northern bin for corresponding species occurring at latitudes over 35°N. The northernmost latitude tree was then mirrored with the parsimony ancestral reconstruction of the dehiscence/indehiscence modes to visualize possible relationships.

4.3 FLOATABILITY OF *CUSCUTA GRONOVII* FRUITS AND SEEDS

Mature fruits of *C. gronovii* (a species with typically indehiscent capsules) were collected from Long Point Provincial Park (42°35'43.34"N, 80°27'2.66"W) in October 2014 and dry stored in a fridge before the experiment was carried out. Intact capsules were randomly selected and counted, their pericarp broken to release seeds for treatments that require it. Each treatment consisted of 300 intact capsules (IC), 300 dried seeds (DS), and 300 imbibed seeds (IS). To allow for imbibition, dry seeds were placed in sterile petri dishes with No.1 Whatman filter paper saturated with deionized water for 24 hours. Seeds, imbibed seeds and intact capsules were each placed inside 600 ml beakers and 400 ml of DI water was added to them. Beakers were placed on a Barnstead Lab-Line Max Q 2000 E-Class platform shaker set to 65 rpm. Floatability trials were performed in the lab at ~23^{o}C and the number of seeds or capsules that remained floating was recorded every 12 hours, with 0 hours being the initial number of seeds or capsules that remained floating immediately after DI water was added. A survival (or time-to-event) analysis was used to compare the differences in the time it took for seeds or capsules to sink (R package ‘survival’; Therneau, 2015).

4.4 FRUIT EFFECT ON THE GERMINATION OF *CUSCUTA GRONOVII*

Mature indehiscent fruits of *C. gronovii*, were collected from plants parasitizing *Solidago canadensis* from two Ontario populations in October 2014. The first population is located near Long Point Provincial Park (42°35'43.34"N, 80°27'2.66"W) and the second population is situated along the Grand River in Waterloo (Claude Dubrick Trail, 43°30'12.02"N, 80°29'37.97"W). Collection was done by cutting the host’s stems in such a way that the attached infructescences of *Cuscuta* were obtained intact. Fruits were kept outdoors for one week before the start of the experiment. During this time, capsules that were not irregularly open or damaged by insects were carefully detached from their infructescence, counted, and placed in beakers for their eventual use.

The main idea of this experiment was to generate an “artificial dehiscence” by removing the seeds from indehiscent capsules, and, under natural conditions, to compare their germination with that of seeds left inside the indehiscent fruits. Thus, fruits collected from each sampling site were subject to two separate treatments: seeds within capsules (W), and seeds without capsules (WO). Each of the experimental units contained either 10 capsules, or seeds removed from 10 capsules gently pressed onto the surface of potting soil mix inside a square pot (7 cm^2). Each pot was placed in 1 of 4 transparent bins that were previously perforated at the base and half-filled with sand to ensure drainage. Each bin contained 30 pots (5 x 6 cm) with their positions randomized within the bins in order to reduce bias.

The experiment was installed outdoors in Waterloo, Ontario in early November 2014 to allow the seeds undergo their natural dormancy cycle. The area selected was uniformly shaded and exposed to the elements. Bins were covered with stainless steel mesh wire (mesh size 6.35 x 6.35 mm) to prevent predation by rodents or birds. Once the snow began to melt in the spring of the next year, the experiment was continuously monitored for signs of germination. The number of seeds that germinated was recorded and seedlings were removed from the pots every day. Pots and bins were randomized again in November 2015, allowed to pass through a second winter in identical conditions, and monitored again for germination in 2016.

The numbers of seeds that germinated each year and by the end of the experiment were compared among treatments and sampling sites using the parametric Student's t-test, or the non-parametric Wilcoxon rank-sum test after checking for departure from normality and unequal variances. Although it is useful to know whether there is a difference in number of seeds germinated in each treatment, in this case it would be even more interesting to know how germination is affected over time, and whether there are differences in patterns of germination between the treatments (McNair et al., 2012). For this reason, a survival analysis (or time-to-event analysis) was performed on the data using the ‘survival’ package from R (Therneau, 2015; see Appendix D for R codes).

4.5 FRUIT DEHISCENCE AND INFRUCTESCENCE ARCHITECTURE

Infructescence compactness indicator

Inflorescence density/compactness is usually ascertained on living plants by determining the inflorescence volume and number of flowers per inflorescence (Shavrukov et al., 2003; Friedman and Harder, 2005). In other cases, inflorescence compactness is characterized qualitatively (Djè et al., 2004; Healy and Gillespie, 2004; Keshavarzi et al., 2008), or is estimated using inflorescence weight divided by its length (Pavan et al., 2009). For this study, only herbarium specimens were available and since pressed specimens do not retain their three-dimensional architecture, it was not feasible to accurately determine inflorescence volume. Therefore, a composite metric formula, a “compactness indicator” was formulated specifically for *Cuscuta* in order to quantify the architecture variables — the length of inflorescence axes and fruit diameters — which contribute to the infructescence compactness:

$$IC = \left(\frac{lt+p}{No}\right)\left(\frac{1}{d*Na*MaxNa}\right)$$

Where *lt* = total length of inflorescence measured continuously from the base of the first bract to the base of the last node; *p* = length of the longest pedicel measured from the base of a flower/ovary to its insertion point at the base of a node; *No* = highest number of orders (or nodes) in the inflorescence; *d* = average diameter of a capsule (capsule width) retrieved from section 5.1; *Na* = number of axes at the base of the inflorescence (first node); and *MaxNa* = maximum number of axes present at a single node (Figure 1; see Appendix B for details on how the formula was derived).

Data collection and analysis

The infructescence architecture was examined in 75 taxa of subgenus *Grammica* (71 species and 4 varieties). Initially I had envisioned an identical sampling to include all the *Grammica* species used in the fruit morphological diversity study (chapter 4.1). However, entire inflorescences were not available for some taxa. Although not all taxa were sampled, at least half the species present in each clade were sampled and were used as representatives of the clade. At least three inflorescences/infructescences were examined for each specimen, and at least three specimens were examined for each taxon (except for taxa with very little material). Inflorescences/infructescences removed from herbarium specimens were placed in 50% ethanol heated to boiling point and allowed to rehydrate for at least a few minutes. Rehydrated samples were disentangled and laid out in concave slides filled with 50% ethanol and examined under a Nikon SMZ1500 stereomicroscope. Imaging was done with a PaxCam Arc digital camera equipped with Pax-it 7.8 software (MIS Inc., Villa Park, IL). Measurements were performed on the images taken, exported onto a spreadsheet, and entered into the compactness indicator formula for each sample. The average diameter of capsules was obtained from results in chapter 5.1.

Similar to previous sections, the results were analyzed with both the previous fruit categorization (dehiscence/indehiscence) as well as the new fruit types (chapter 5.1) as categorical covariates. Infructescence compactness based on dehiscence types were first checked for deviations from a normal distribution and unequal variances before comparing their differences. A Wilcoxon rank sum test was used to test the differences between dehiscence modes from the previous fruit categorization, and a Kruskal-Wallis rank sum test was used for the new categorization with a Dunn's test post hoc analysis (R package 'dunn.test'; Dinno, 2016).

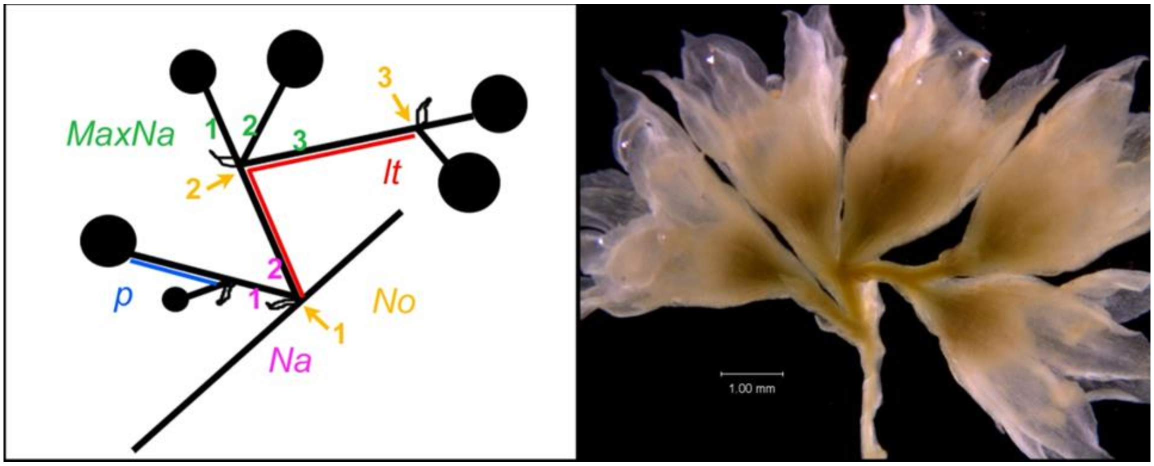

Figure 1. Data collected for determining infructescence compactness in *Cuscuta*. Measurements are taken for *p* (length of longest pedicel; blue), and *lt* (total length of inflorescence; red). Count data are collected for *Na* (number of axes at the first node; magenta), *MaxNa* (maximum number of axes at a single node; green), and *No* (highest number of orders/nodes; yellow). Images were taken using PaxCam Arc digital camera equipped with Pax-it 7.8 software (MIS Inc., Villa Park, IL), and measurements were exported directly from the software.

5. RESULTS

5.1 STRUCTURAL AND MORPHOLOGICAL DIVERSITY OF CAPSULES

General morphology, structure and ultrastructure of fruit

The fruit of *Cuscuta* develops from a 2-locular ovary with an incomplete septum (fused to the center of a single mass of placental tissue originating from the base of the ovary). Young fruits, after fertilization, are green but change in color to brown or become translucent as seeds mature. Based on the morphology, structure and ultrastructure of the dehiscence/indehiscence modes, three morphological/anatomical fruit types are distinguished: regularly dehiscent circumscissile (DE), indehiscent (IN), and irregularly dehiscent type A (IrA). In addition, a fourth “functional” type, the irregularly dehiscent type B (IrB), was observed (see below).

Regular circumscissile dehiscence (DE) is one of the first morphological traits that become apparent during fruit development because the dehiscence line differentiates at the base of the ovary before fertilization takes place. Morphologically, the dehiscence “line” of mature fruits appears more as a distinct area or ring surrounding the base of the fruit above the nectary (Fig. 2 A-B). The dehiscence area is an abscission zone (AZ) consisting of 4--8 layers of more or less isodiametric cells in subg. *Monogynella* and the sections *Subulatae* and *Lobostigmae* of subg. *Grammica*, and 2--4 layers in species of subgenera *Cuscuta*, *Pachystigma* and the remaining sections of subg. *Grammica*. Above the dehiscence ring, the pericarp has a simple and relatively consistent structure throughout the genus (Fig. 3). The epicarp consists of a cuticularized epidermis, and papillae present in some species. The mesocarp consists of 5–8 layers of parenchymatic cells in species of subg. *Monogynella* (Fig. 3 E-F) and 2–5 cell layers in subgenera

Cuscuta, *Pachystigma* and *Grammica* (Fig. 3 J-K). Laticifers are present in the mesocarp of all the species (Fig. 3 K-L), most commonly isolated, and only rarely articulated, in groups of 2–3 cells. The mesocarp of the two *Monogynella* species examined is thicker than in the other subgenera, with an additional layer of rectangular, thin-walled cells that differentiate adjacent to the endocarp (Fig. 3F). Mesocarp cells of young fruits contain large amounts of chloroplasts which convert to amyloplasts as the fruits mature. The endocarp consists of a single layer of cells that exhibit a “horse-shoe” pattern of lignified cell walls in the subgenera *Cuscuta*, *Pachystygma* and *Grammica* (Fig. 3 I-L; Fig. 4 K-L), and with additional bands of lignin only in the external periclinal cell walls in subg. *Monogynella* (Fig. 3 F-G). Pericarp vasculature consists of two larger collateral vascular bundles that correspond to the carpel midveins which run through the pericarp and reach the base of the styles. Smaller branches consisting mostly of phloem diverge from them especially in the vicinity of the nectary.

Cells of the AZ are smaller than the rest of pericarp cells; their cell walls are thin, cellulosic (Fig. 3 B-E; I-J; Fig. 4 A-J); one or two vacuoles are present and the cytoplasm is dense with plastids, mitochondria, and an endomembrane system, consisting of endoplasmic reticulum (ER) and Golgi apparatus (Fig. 4 A-J). Simple plasmodesmata are present in the cell walls between neighboring AZ cells (Fig. 4J). Early in the ontogeny of fruit, cells walls of neighboring cells begin to separate schizogenously at the middle lamella, forming intercellular spaces (Fig. 4 A-G). Subsequently, the degrading of the middle lamella accentuates, leading to more cells separations, which together with the breakdown of cell walls, cause the dehiscence of the fruit along the AZ and the shedding of the fruit part above it (Fig. 4, H-J).

Indehiscent capsules (IN) lack an AZ (Fig. 2, C-D; Fig. 3M;). Pericarp of indehiscent fruits has a uniform structure from the stylar area to the nectary ring, uninterrupted by a dehiscence area (Fig. 3 N-P). The anatomy of pericarp is similar to that of circumscissile capsules; however, the thickenings of endocarp cells are more localized, occurring mostly in the external periclinal walls (Fig. 3 N-P; Fig. 4 Q-S). In addition, the epidermis cell walls also become lignified. The thickened endocarp extends in the entire fruit, including at the base of the fruit, above the nectary where the AZ is present in DE fruits. Plastids of young fruit are preponderantly chloroplasts with thylakoids arranged in 2-3 “ministacks” with little or no separation between adjacent grana (Fig. 4 M-N). As seeds mature, plastids become amyloplasts with numerous starch grains.

Capsules irregularly circumscissile dehiscent type A (IrA; Fig. 2E; Fig. 5) — lack an AZ, but their endocarp is not uniform in the mature fruit: its cells walls are more lignified distally and become thinner towards the base of the fruit where they are cellulosic (Fig. 2E; Fig. 5 B-D). Thus, even though an AZ does not differentiate, because the endocarp cell walls are thin at the base of the fruit a zone of relative weakness results in this area. Dehiscence takes place later than in regularly circumscissile capsules, along a basal jagged line (Fig. 2E).

Capsules irregularly dehiscent type B (IrB; Fig. 2F)—Are developmentally, morphologically and structurally indistinguishable from indehiscent capsules. Such capsules have a uniform pericarp with thickened endocarp throughout the entire fruit and lack an AZ. This form of irregular dehiscence is not the consequence of intrinsic structural fruit traits, but rather the functional result of external factors such as the mechanical pressures created among ripening fruits within dense infructescences. The pericarp may tear irregularly (but not at the base of the capsule) through longitudinal cracks that extend toward the interstylar aperture (Fig. 2F). In such species, the majority of fruits are indehiscent (IN) but a few capsules break irregularly (IrB). Based on field observations conducted on the two populations of *C. gronovii* (Long Point and Grand River), only 0.5 to 2% of capsules within infructescences of the same plant break irregularly. The relationship between the inflorescence architecture and IrB capsules will be explored in chapter 5.4.

Comparative morphological diversity of fruits and character evolution

Immediately after fertilization, the immature fruit of *Cuscuta* are quite similar morphologically among species. Soon, however, the AZ becomes visible in species with DE capsules; fruits grow in size and acquire a particular shape, and the apical septum of the ovary enlarges into an interstylar aperture with variable morphology. In the end, the pericarp becomes translucent or remains opaque-brown. As a result of these changes, mature fruits exhibit a suite of subtle morphological characters (Table 1; Fig. 6; see Appendix C for data matrix).

Indehiscence/dehiscence modes — The circumscissile dehiscent fruit (DE) is the ancestral character state for *Cuscuta*, with at least ten independent transitions to irregularly dehiscent type A (IrA) and indehiscent fruits (IN) occurring in subg. *Grammica* (Fig. 7). Reversals to dehiscence took place in sections *Partitae* and *Racemosae*. Although most species are characterized by either DE or IN capsules, some species may also have individuals/populations that exhibit IrA capsules. Species examples with IN + IrA capsules include *C. cristata* (sect. *Subulatae*), *C. acuta* (sect.

Umbellatae), *C. yucatana* (sect. *Grammica*), *C. longiloba*, *C. platyloba* (sect. *Racemosae*), while DE + IrA capsules are found in *C. colombiana* (sect. *Gracillimae*), *C. umbellata* (sect. *Umbellatae*), and *C. incurvata* (sect. Racemosae). In the extreme case of two species in sect. Racemosae, C. *xanthochorthos* var. *carinata* and *C. corniculata*, in which specimens with DE, IN, or IrA capsules were documented. IrB appear to have evolved in four *Grammica* clades from strictly IN capsules. All species examined in clade B (sect. *Cleistogrammica*) have largely IN fruits and a small number of IrB fruits within the same infructescence. As previously indicated, IrB fruits are not developmentally or structurally different from IN capsules.

Fruit shape (Fig. 6 A-I) is quite polymorphic in *Cuscuta* (see Appendix C for data matrix). The most common fruit shape is spherical (globose), followed by depressed; however, transitions between these two shapes are often present within the same species and on the same individual. Ovoid and ellipsoid shapes are less common, but also present together in the same species. “Thimble-shaped”, conical capsule shape is the ancestral character state and characterizes species of subg. *Monogynella*; however this shape also transitions within certain species into ellipsoid. Fruit shape is apparently not associated with the dehiscence mode but rather with the number of seeds it contains: globose and depressed capsules have 2--4 seeds, while ovoid and ellipsoid seeds contain 1--2 seeds. Irregular dehiscence type B (IrB) is more common in species with depressed-globose or obovoid capsule shape.

Persistent corolla (Fig. 6 J-N) capping the capsule is the ancestral state while corolla surrounding or localized at the bottom of the capsules are derived and have evolved multiple times (tree not shown). Although exceptions exist, corolla capping the capsules is most commonly present in species with dehiscent fruits and likely contributes to the removal of the capsule lid after circumscissile dehiscence takes place.

Papillae (Fig 6O) have evolved as an epidermal feature of the ovary/fruit in species of four clades within subgenus *Grammica* (not included in data matrix): *C. pacifica* var. *papillata*, *C. californica* var. *papillata*, *C. jepsonii* (sect. *Californicae*); *C. glabrior, C. runyonii* (sect. *Cleistogrammica*); *C. desmouliniana* (sect. *Umbellatae*); *C. argentinana*, (sect. *Subulatae*).

An opaque pericarp is reconstructed as the ancestral character state for the entire genus by the maximum likelihood reconstruction, but its polarity in relationship to a translucent pericarp is ambiguous in the parsimony analysis; either way, many transitions towards both states occurred throughout the genus. Most species with an opaque pericarp have visible laticifers. Although exceptions were noted (e.g., subg. *Monogynella*), DE capsules tend to be translucent, while IN or IrB capsules are opaque.

Interstylar aperture has evolved in subgenera *Cuscuta*, *Pachystigma* and *Grammica,* but only in the latter infrageneric group has it become morphologically more diverse, with an apparent thickened ancestral state from which the “collar” and “protuberances” states are derived (Fig. 6 Q-V). All the species with indehiscent capsules have a form of thickened interstylar aperture. Among the clades with preponderantly dehiscent capsules, only sect. *Umbellatae* exhibits thickened interstylar apertures with protuberance. A distinct rostrum in the distal part of the fruit evolved only in *C. rostrata* (sect. *Oxycarpae*) and *C. cotijana* (sect. *Lobostigmae*). In the former subsection, *C.*

compacta, C. umbrosa, and *C. gronovii,* which normally have a “collar”, may exhibit morphological forms approaching *C. rostrata.*

Styles distinctly enlarged at the base (subulate; Fig. 6W) have evolved only in some species from two clades of subg. *Grammica* (not included in data matrix): *C. mitriformis*, *C. jalepensis*, *C. rugosiceps* and *C. woodsonii* (sect. *Lobostigmae*), *C. boldinghii*, *C. chapalana*, *C. erosa* and *C. strobilacea* (sect. *Ceratophorae*).

Of the two multidimensional scaling analyses conducted, both PCoA and NMDS resulted in similar groupings of fruit characters based on their similarity/dissimilarity. The dehiscence mode character was separated from all the other fruit traits. The characters that were grouped closest among themselves were the translucence of pericarp, laticifers, length/width of interstylar aperture and length to width ratio of fruits, and the characters most similar to dehiscence/indehiscence were the interstylar aperture morphology, and position of the persistent pericarp (see Appendix C for both graphs/analyses).

The size of capsules (length, width), though insignificant among most dehiscence/indehiscence modes, is significantly longer in IrB fruit species than DE fruit species (Dunn’s test, Z statistic = -1.78795, p-value = 0.03369) and wider in IrB fruit species than IN fruit species (Dunn’s test, Z statistic = -1.90098, p-value = 0.0287) but also wider in DE species than IN species (Dunn’s test, Z statistic = 1.8381, p-value = 0.0330; see Table 2 for summary statistics). IrB fruit species are essentially IN species with large fruit. Results for fruit ratio indicate that DE species generally have more depressed capsules that are wider than long, whereas IN species have capsules that are rounder and longer (Dunn's test, Z statistic = -2.358, p-value = 0.0092).

An interstylar aperture (ISA) evolved in subgenera *Cuscuta*, *Pachystigma*, and *Grammica* as a consequence of the evolution of two styles. Its size (length and width) increases in subg. *Grammica* and although exceptions exist, dehiscent fruit species have smaller interstylar apertures than most indehiscent fruit species. ISA is especially larger in IN fruit species than DE species (Dunn's test, ISA length: z-statistic = -1.8018, p-value = 0.0358; ISA width: z-statistic = -2.905, p-value = 0.0018), and even larger lengthwise in IrB fruit species than strictly IN species (Dunn's test, z-statistic = -3.201, p-value = 0.0007, see Table 2). IrB fruit species have the largest interstylar aperture.

One to four seeds develop within each capsule and although the number may vary somewhat from capsule to capsule even within the same infructescence, the average is relatively constant within each species/variety. Capsules with one seed evolved in seven *Grammica* clades and are more common in IN fruit species than DE. Capsules with more than one seed can be DE, IN, IrA or IrB, and species with IN and IrA or IrB fruits generally have a higher number of seeds than do DE fruit species or strictly IN fruit species (Dunn's test; DE – IN+IrA: z-statistic = -2.2092, p-value = 0.0136; IN – IN+IrA: z-statistic = -2.3642, p-value = 0.0090; DE – IN+IrB: z-statistic = -1.8538, p-value = 0.0319; IN – IN+IrB: z-statistic = -1.93286, p-value = 0.0266, see Table 2).

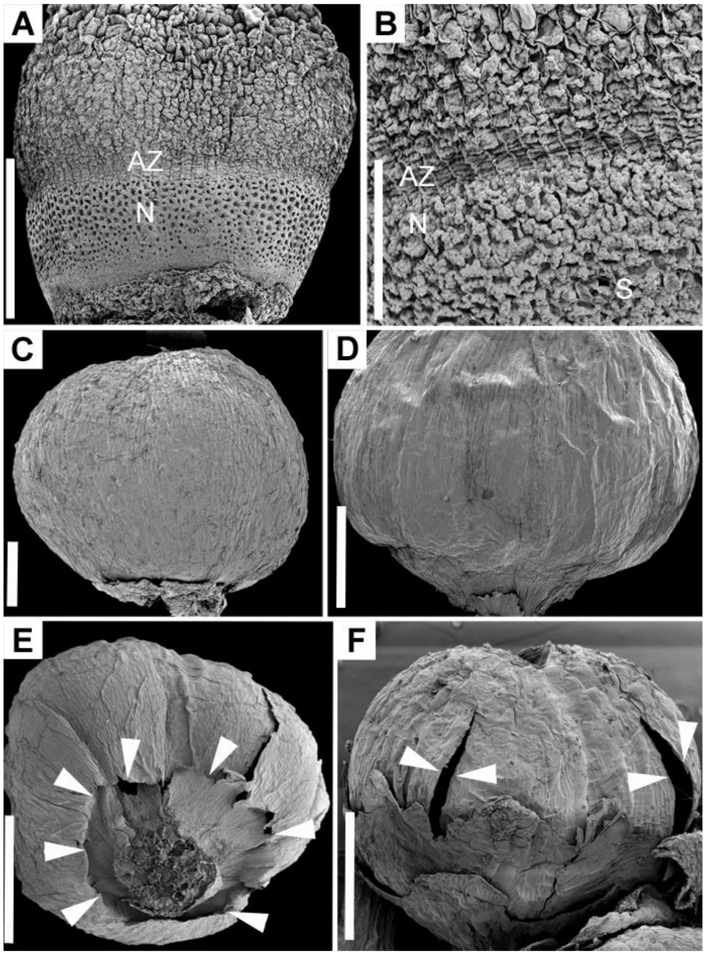

Figure 2. **Scanning electron microscopy morphology of different types of capsules in *Cuscuta*.** A–B. Circumscissile dehiscent capsules (*C. chilensis*); note the clearly differentiated AZ. C–D. Indehiscent capsule (*C. gronovii*); no AZ is present. E. Irregularly dehiscent capsule type A (C. xanthochorthos); no AZ develops but because the endocarp is thin and cellulosic at the base, capsules will break eventually in this region. F. Irregularly dehiscent capsule type B are structurally indehiscent but may break through longitudinal lines because of the pressures exercised by neighboring fruits in dense infructescences. AZ = abscission zone, N = nectary; S = nectary stomata. Scale bars: A = 0.5 mm; B = 150 μm C–F = 1mm.

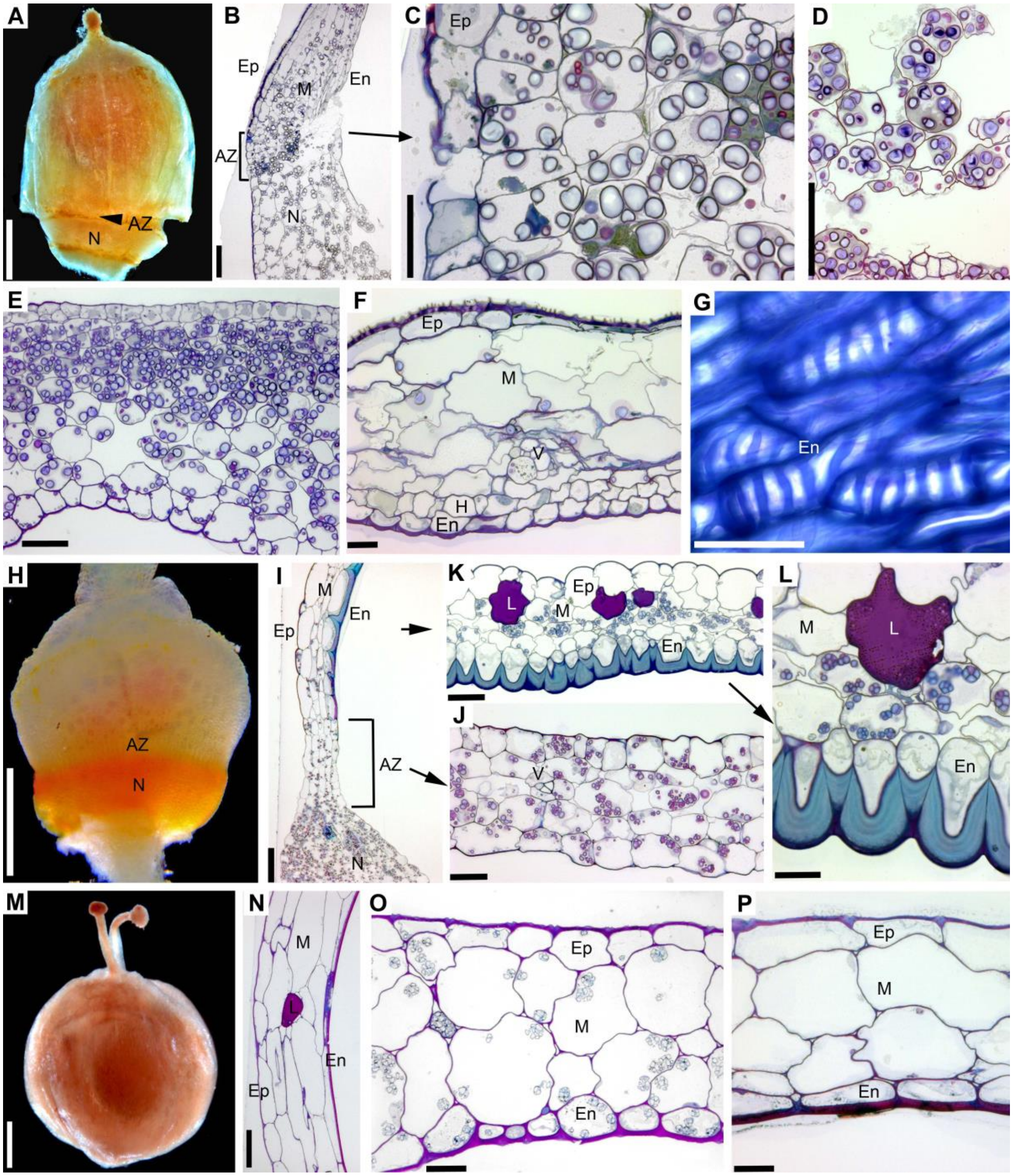

Figure 3. Anatomy of pericarp. A–G. *Cuscuta monogynella* (subg. *Monogyna*). A. Circumscissile capsule at dehiscence. B–C. Longitudinal sections through the capsule base showing the formation of the dehiscence zone. B. Pericarp begins to rupture along the abscission zone (AZ). C. Detail of the AZ. Note the AZ cells with thin cell walls and numerous amyloplasts. D–E. Cross-sections through the AZ right before dehiscence. D.

AZ cells losing cohesion. E. Overview of the AZ before the dehiscence. F. Transversal section through the pericarp above the dehiscence area. The mesocarp is thicker than in the other subgenera (see next), with an additional layer of rectangular, thin-walled cells under the endocarp (hypoderm). G. Perpendicular view of the endocarp cells with lignin bands in the external periclinal walls. H–L. *Cuscuta purpurata*. H. Circumscissile capsule before dehiscence. Nectary under the AZ is yellow-orange. I. Longitudinal section through the capsule base illustrating the structure of nectary, AZ and pericarp above the AZ. J. Transversal section through the AZ before dehiscence. Cell walls of epicarp, mesocarp and endocarp are thin; mesocarp cells have not begun to lose cohesion. K–L. Structure of pericarp above the dehiscence zone. K. Overview. L. Detail. Endocarp cells with "horse-shoe" pattern of lignified cell walls. Laticifers are present in the mesocarp. M–P. *Cuscuta gronovii*. M. Indehiscent capsule. N. Longitudinal section through the base of the capsule. No AZ develops; endocarp cells lignified to the base of the capsule. O. Transversal section through young capsule. P. Transversal section through mature capsule. AZ = abscission zone; Ep = epicarp; H= hypoderm; M = Mesocarp; En = Endocarp; N = Nectary; L = Laticifer; V = vascular bundle. Scale bars: A, H, M = 1 mm; B, I, N = 100 μm; C–G, J, O, P = 50 μm; K, L = 20 μm.

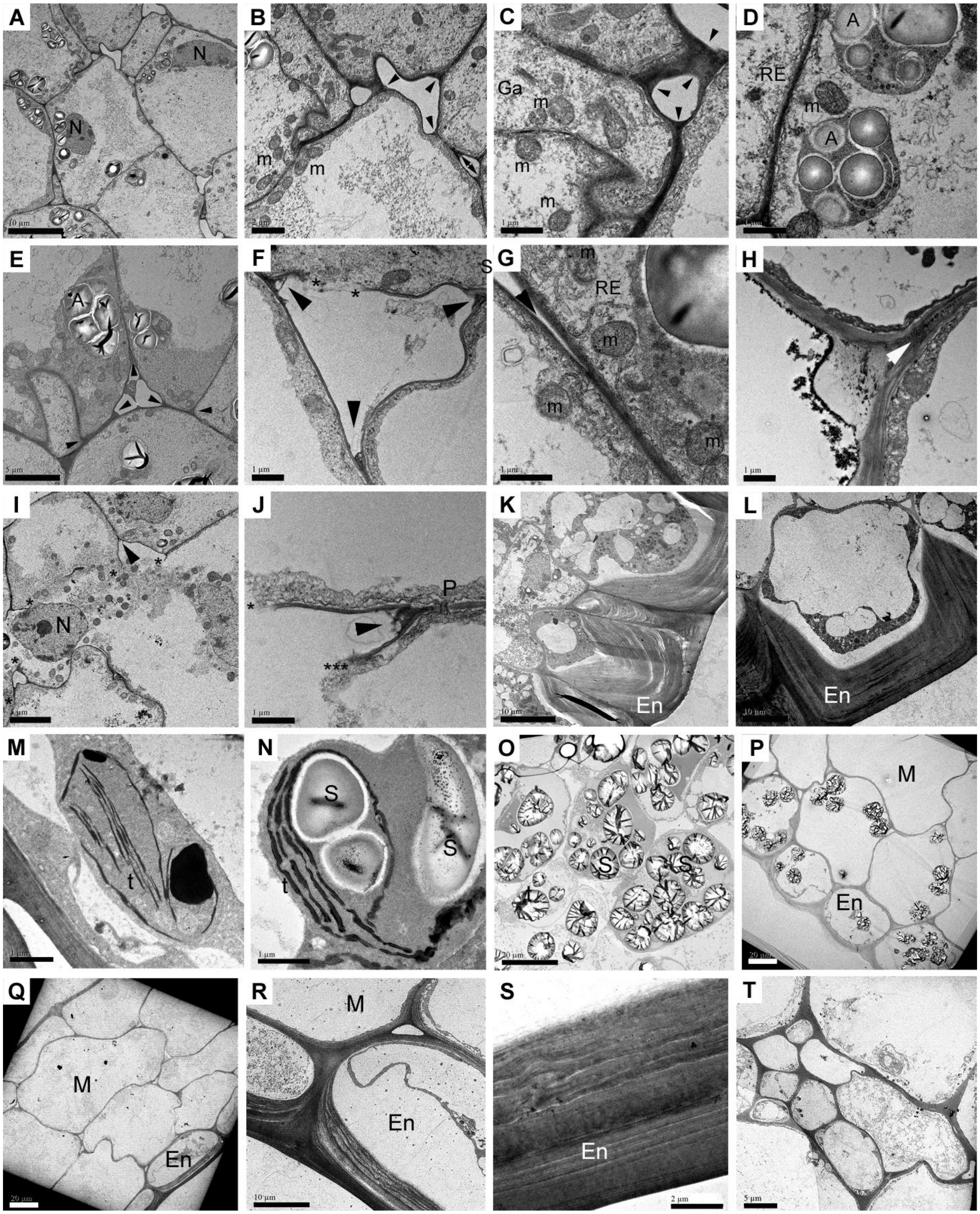

Figure 4. Ultrastructure of pericarp dehiscence/indehiscence. A–L. Circumscissile capsule (*Cuscuta purpurata*). A–E. Transversal sections through the abscission zone (AZ); incipient stages of cell walls separation and formation of intercellular spaces. F–J. More advanced stage in which cell walls continue to "unzip" schizogenously (F–H) and

break down (I–J). Cytoplasm of AZ cells is dense with numerous organelles. K–L. Transversal sections through the endocarp above the AZ; anticlinal and internal periclinal cell walls thickened with lignin (“horse-shoe” pattern). M–T. Indehiscent capsule (*Cuscuta gronovii*). M–P. Transversal sections practiced through the base of a young capsule (pericarp is still green). M–N. Detail of chloroplasts encountered in the mesocarp; thylakoids are arranged in 2-3 “ministacks” with little or no separation between adjacent grana. O. View of endocarp and adjacent mesocarp cells; endocarp cells are still relatively thin, cellulosic and contain starch. Q–T. Transversal sections practiced through the base of a mature capsule (pericarp is brown). Q. View of endocarp and adjacent mesocarp cells. Starch has disappeared. R–S. Thickenings of endocarp cells. R. External periclinal cell walls are more thickened than the anticlinal walls. S. Layers of lignin in the external periclinal wall. T. Vascular bundle in the mesocarp. A = amyloplast with starch (S); En = endocarp; Ga = Golgi apparatus; m = mitochondria; M = mesocarp; N = nucleus; P= plasmodesma; RE = rough endoplasmic reticulum; t = thylakoids. Arrows indicate directions of cell wall separation; “*” point to disintegrating cells walls. Scale bar values included in the figures.

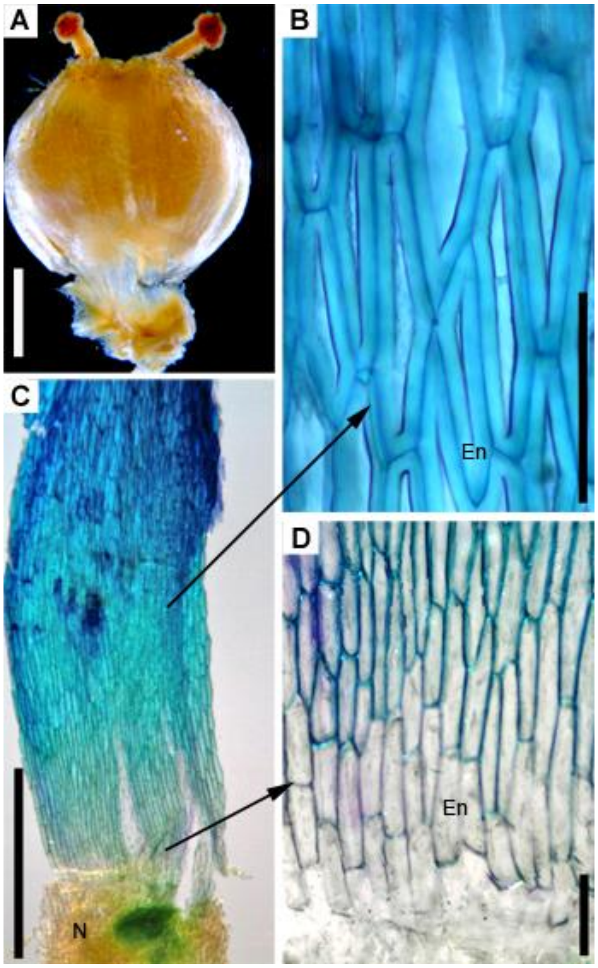

Figure 5. Irregularly dehiscent type A capsule (*C. corniculata*). A. AZ does not form, but the pericarp ruptures irregularly at the base of the capsule. B–D. Longitudinal strip of pericarp illustrating the unequal thickening of endocarp cells: thick and lignified throughout most of the fruit (C), but becoming thin and cellulosic at the base of the capsule (D). Since pericarp is very thin, the strip was placed on the slide with the endocarp up and optical images were acquired through transparency. En = endocarp; N = nectary. Scale bars: A, C = 1 mm; B, D = 100 µm.

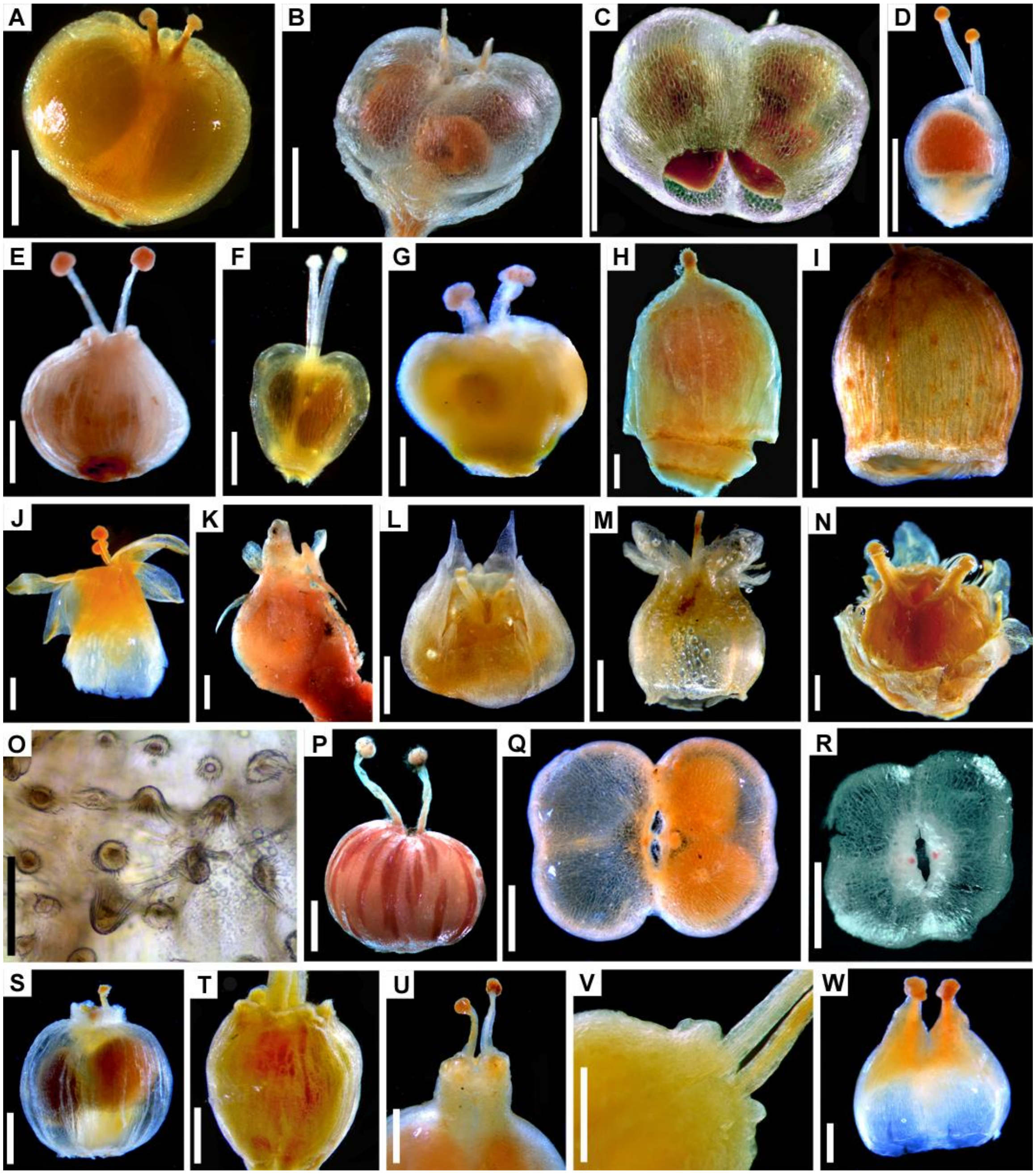

Figure 6. Morphology of capsules. A. Spherical (globose), *C. campestris*; B–C. Depressed, circumscissile dehiscent, *C. umbellata*: lateral view (B); view from the bottom (C). D. Ellipsoid, *C. nevadensis*. E. Ovoid, *C. indecora*. F–G. Obovoid; F. *C. californica*; G. *C. australis*. H–I. "Thimble-shaped": H. *C. monogyna*; I. *C. japonica*. J–K. Corolla topping the capsules: J. *C. purpusii*; K. *C. cephalanthi*. L–M. Corolla

surrounding the capsule: L. *C. occidentalis*; M. *C. epithymum*. N. Corolla at the base of the capsule, *C. mitriformis*. O. Papillae on capsule epicarp, *C. glabrior*. P. Laticifers visible in the pericarp, C. peruviana. Q–R. Interstylar aperture with thickened but not raised margins: Q. *C. victoriana*; R. *C. umbellata* form with irregular dehiscent type A capsule. S–T. Interstylar aperture raised in a collar around the styles: S. *C. warneri*; T. *C. corniculata*. U. Distal part of the ovary forms a rostrum under the styles, *C. rostrata*. V. Interstylar aperture with irregular distal protuberances, *C. acuta*. W. styles become enlarged at the base, subulate, *C. rugosiceps*. Scale bars = 1 mm; except O in which scale bar = 100 μm.

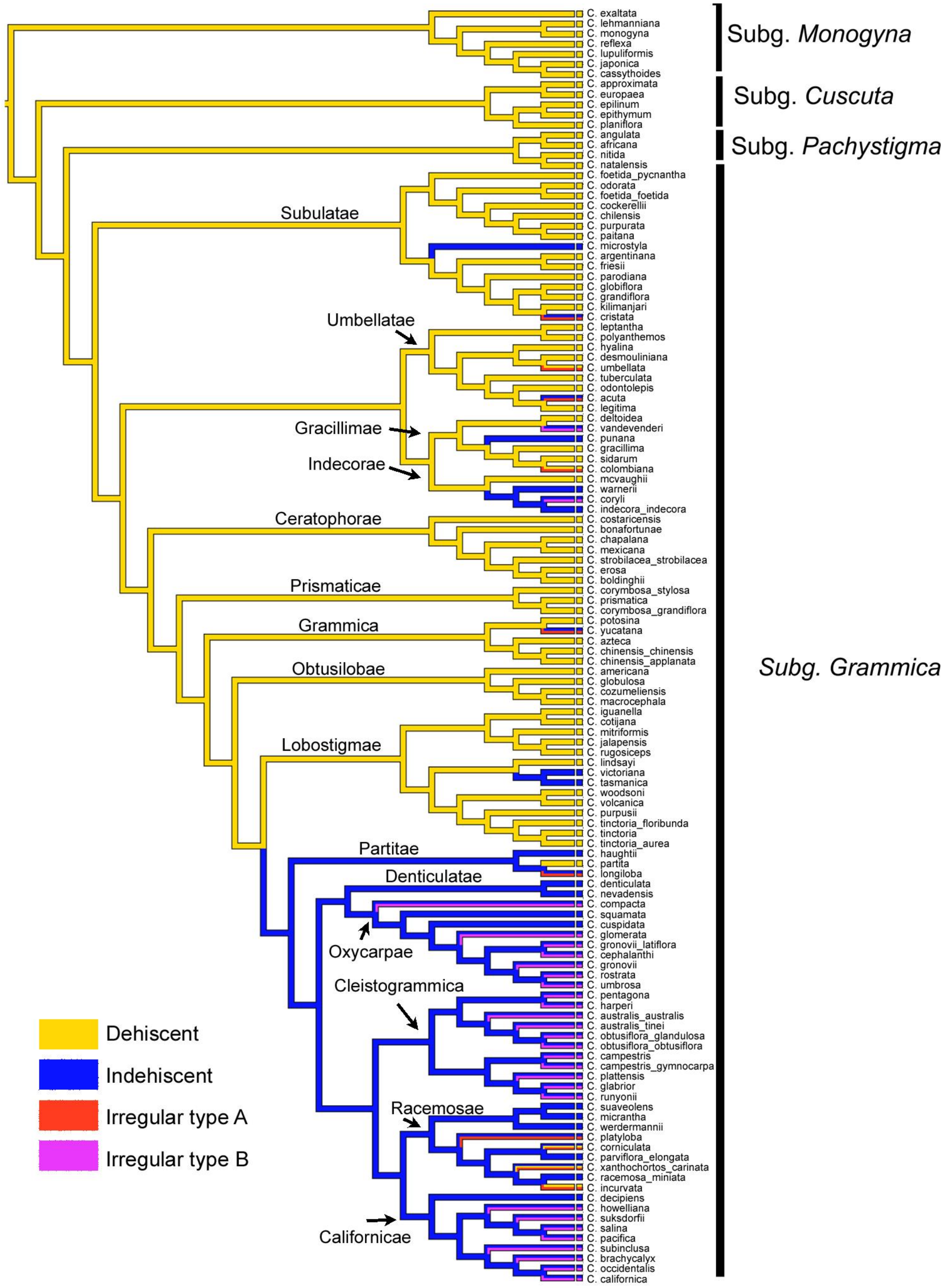

Figure 7. Ancestral character state reconstruction of fruit dehiscence modes in *Cuscuta* mapped onto the recent genus phylogeny based on rbcL and nrLSU sequences

(García et al, 2014). Dehiscence modes are polymorphic; some species have multiple fruit types. Regular circumscissile dehiscence is the ancestral character state for *Cuscuta*, with at least ten independent transitions to irregularly dehiscent and indehiscent fruits occurring in subg. *Grammica*. Irregular type B capsules are structurally and developmentally identical to indehiscent capsules but appear to be derived from them.

Table 2. Summary statistics for quantitative fruit characters of *Cuscuta* species with dehiscent (DE), indehiscent (IN), or indehiscent fruits that break irregularly (IrB). 126 taxa were surveyed and analyzed. Taxa with fruit type IrA were excluded from this table.

Fruit Trait	Fruit Type	Mean	Median	Std. error	95% CI	Min	Max	*n*	Shapiro (p-value)
Length (mm)	DE	2.33	1.90	0.154	2.030, 2.631	0.90	6.28	71	1.47e-08
	IN	2.024	1.850	0.148	1.727, 2.320	1.23	3.09	17	0.1741
	IN+IrB	2.552	2.275	0.224	2.131, 2.979	1.01	5.22	28	0.00194
Width (mm)	DE	2.89	2.60	0.140	2.627, 3.143	0.90	6.10	71	0.00167
	IN	2.274	2.110	0.208	1.859, 2.690	0.90	3.71	17	0.3986
	IN+IrB	2.912	2.805	0.204	2.504, 3.321	1.12	5.41	28	0.4066
Fruit L/W ratio	DE	0.8157	0.7900	0.022	0.773, 0.859	0.30	1.44	71	0.0018
	IN	0.9856	0.8750	0.079	0.829, 1.143	0.46	1.51	17	0.213
	IN+IrB	0.8741	0.7950	0.049	0.778, 0.972	0.50	1.59	28	0.0066
ISA length (mm)	DE	0.3245	0.225	0.040	0.246, 0.402	0.06	1.89	71	2.56e-09
	IN	0.4524	0.530	0.064	0.325, 0.580	0.10	0.96	17	0.2156
	IN+IrB	0.8146	0.765	0.056	0.703, 0.926	0.40	1.39	28	0.2104
ISA width (mm)	DE	0.1881	0.120	0.027	0.138, 0.240	0.01	1.21	71	4.2e-12
	IN	0.3006	0.280	0.038	0.224, 0.377	0.10	0.60	17	0.2694
	IN+IrB	0.4307	0.455	0.034	0.363, 0.499	0.10	0.78	28	0.6714
Nr. Of Seeds	DE	2.527	2.400	0.073	2.386, 2.668	1.00	3.70	71	0.01803
	IN	2.253	2.300	0.241	1.787, 2.714	1.00	3.50	17	0.00798
	IN+IrB	2.722	3.100	0.159	2.424, 3.020	1.00	3.60	28	0.00041

5.2. DISTRIBUTION OF *GRAMMICA* SPECIES IN NORTH AMERICA

The data collected for North American *Grammica* species' range size and northern limit are provided in Appendix C and summarized in tables 3 and 4, respectively. Range size distribution was determined to be non-normal for all fruit types regardless of fruit categorization data used (Table 3). Although indehiscent fruit species cover more area in North America than species with dehiscent fruits (1.11×10^7 km^2 vs. 3.1×10^6 km^2; Fig. 8 A-B), there was no significant difference in the average range size between dehiscent and indehiscent fruit species (Wilcoxon rank sum test, W = 509, p-value = 0.06919) when using previous fruit categorization. With the updated fruit categories defined in chapter 5.1, differences in range size was observed between species of different fruit types (i.e., dehiscent [DE], indehiscent [IN], and indehiscent with type B irregular dehiscence [IN+IrB]; Kruskal-Wallis rank sum test, K-W chi-squared = 6.8836, df = 2, p-value = 0.03201; Fig. 8C). Other fruit types (i.e. dehiscent with irregular dehiscence type A [DE+IrA], and indehiscent with irregular dehiscence type A [IN+IrA]), were excluded from the analysis because the sample size was insufficient (only two species in North America have the fruit types mentioned), though their populations were included in Fig. 8D. Between DE, IN, and IN+IrB, a pairwise multiple comparisons test indicates that species with IN+IrB type capsules have a larger range size than species with regularly circumscissile capsules (DE; Dunn's test, z test = -2.613893, p-value = 0.0045), but not when compared to species that are strictly indehiscent (IN; Dunn's test, z test = -0.714192, p-value = 0.2376). Between DE and IN species, the differences remained insignificant (Dunn's test, z test = -0.974516, p-value = 0.1649, see Appendix D for R codes used).

Data collected on the species northernmost limits based on previously categorized dehiscence/indehiscence modes strongly indicates that indehiscent fruit species have a higher latitudinal limit (Two Sample t-test, t = -9.1165, df = 75, p-value = 8.941e-14; Table 4; Fig. 9 A-B). When incorporating the new fruit types, similar results were obtained (Table 4 and Fig. 9 C-D). A Tukey multiple comparison of means shows that there is no significant difference in the northern limits between species with both indehiscent and irregularly dehiscent type B capsules (IN+IrB) and those with strictly indehiscent capsules (IN; diff = 2.346837, p-value = 0.6819874), though IN species still have a higher northern limit than do DE species (diff = 15.300374, p-value < 0.0001) and IN+IrB species have a higher northern limit than DE species (diff = 17.647211, p-value < 0.0001; Fig. 9C-D). Both native IN and IN+IrB fruit species can be found as far north as Canada, whereas DE fruit species can only be found in southern U.S.A. and Mexico (Fig. 9D). Of the 42 species with IN fruits examined, only two are endemic to Mexico: *C. vandevenderi* (IN+IrB; Clade N, sect. *Gracillimae*), and *C. yucatana* (IN+IrA; Clade H, sect. *Grammica*). Of the 34 species with DE fruits examined, only 10 species (including DE+IrA fruit species: *C. umbellata*, Clade L, sect. *Umbellatae*) are present north of Mexico. Figure 10 displays the northernmost distribution of these species with their phylogeny and dehiscence character history. A parsimony reconstruction of the latitude values using phylogeny of North American *Grammica* species determined that species evolved an increase in latitudinal limit (tree shown in Appendix C).

Table 3. Summary statistics for the range size (km^2) of 73 *Grammica* species in North America based on their fruit dehiscence/indehiscence modes using data from old fruit categorization retrieved from Garcia et al. (2014), and new fruit categorization from the results in section 5.1. Species with IrA fruit type were excluded from this table. Fruit types shown are dehiscent (DE), indehiscent (IN), and indehiscent fruits that may break irregularly (IN+IrB).

Data Type	Fruit Type	Mean	Median	Standard error	95% CI	Min	Max	*n*	Shapiro (pvalue)
Old	DE	956900	256600	356208.1	266668, 1634534	0	8776000	32	3.46e-09
	IN	2476000	554000	665599.5	1237884, 3769184	0	18750000	41	7.57e-09
New	DE	741400	244900	276536.6	244428, 1278715	0	6647000	29	1.33e-08
	IN	2778000	652500	2036706	0, 6402044	1	14770000	7	0.00003
	IN+IrB	3255000	1437000	942790.4	1526227, 5014368	0	18750000	24	2.81e-05

Table 4. Summary statistics for the northernmost limit (decimal degrees) of 76 *Grammica* species found in North America based on their fruit dehiscence/indehiscence modes using data from old fruit categorization retrieved from Garcia et al. (2014), and new fruit categorization from the results in section 5.1. Species with IrA fruit type were excluded from this table. Fruit types shown are dehiscent (DE), indehiscent (IN), and indehiscent fruits that may break irregularly (IN+IrB).

Data Type	Fruit Type	Mean	Median	Standard error	95% CI or BCI	Min	Max	*n*	Normality (p-value)
Old	DE	26.51	26.37	1.086969	24.33958, 28.68746	15.44	38.84	34	0.05805
	IN	41.34	41.50	1.163646	39.01005, 43.66464	20.69	53.54	42	0.2552
New	DE	25.70	23.28	1.070519	23.55566, 27.83773	15.44	38.03	31	0.06833
	IN	41.22	38.97	2.891838	35.43395, 47.0013	31.87	51.40	7	0.4599
	IN+ IrB	43.56	44.40	1.407892	40.74868, 46.38025	28.38	53.54	24	0.1447

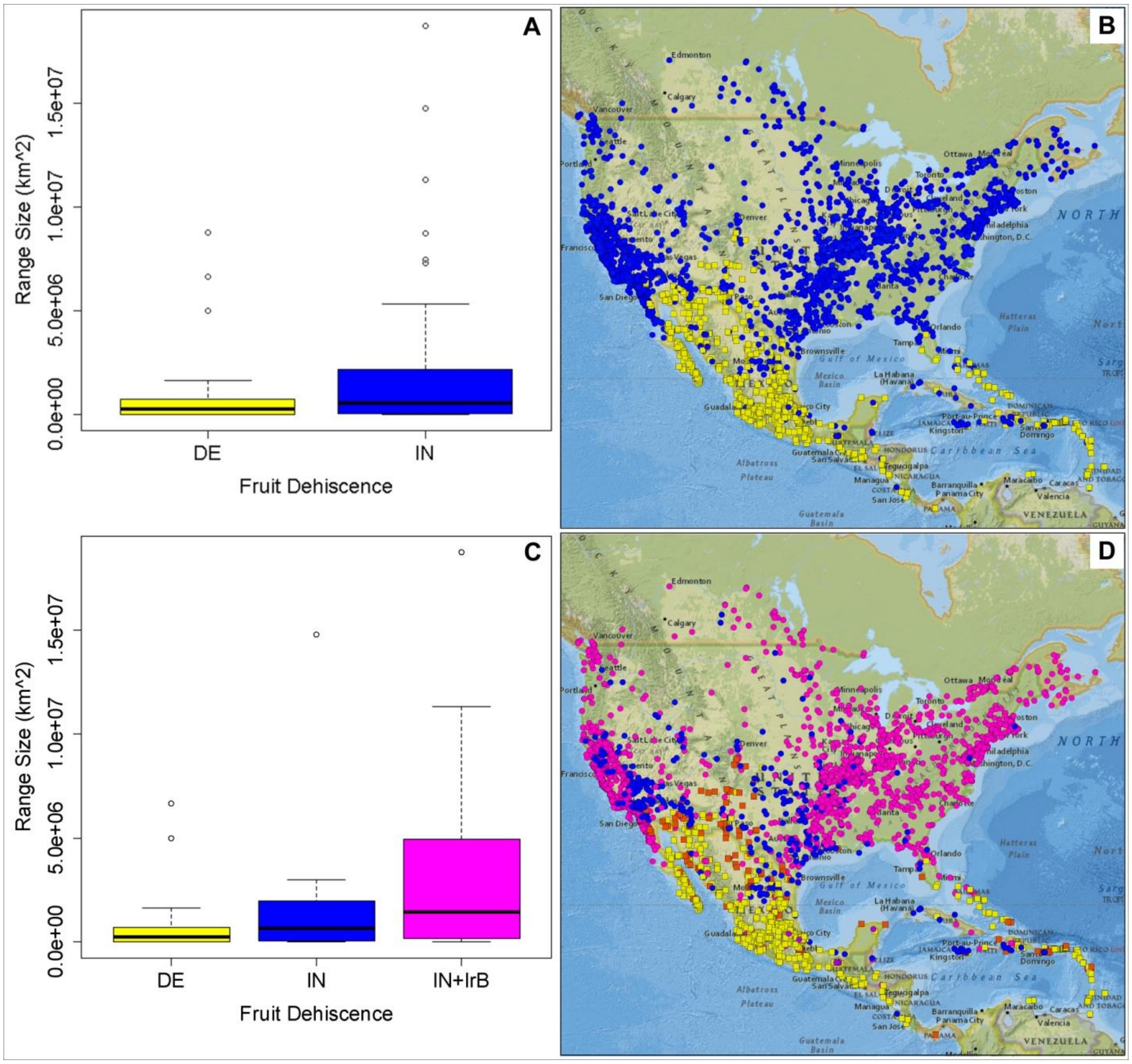

Figure 8. Extent of occurrence (EOO) of *Grammica* species native to North America categorized by fruit dehiscence/indehiscence. Boxplots display the statistical distribution of the data; boxes represent the middle 50% of distributions, horizontal lines within boxes represent the median, circles represent outliers (values that are >1.5 times the interquartile range), and whiskers represent the min and max values that are not outliers. A. The distributions of EOO of dehiscent and indehiscent fruit species without considering IrB fruits a true fruit type. B. Map displaying geographical distribution of

data; each point represents a population of a *Cuscuta* species that either has indehiscent (IN; blue), or regularly circumscissile (DE; yellow) fruits. Species with IN fruits are distributed over a larger area although there is no difference in EOO between species of different fruit types. C. With IrB species included as a separate fruit type differences were observed between IrB and DE species but not with IN. D. Geographical distribution of data, including species with irregular dehiscence, DE+IrA species shown in orange, IN+IrA species shown in purple, and IN+IrB species shown in magenta.

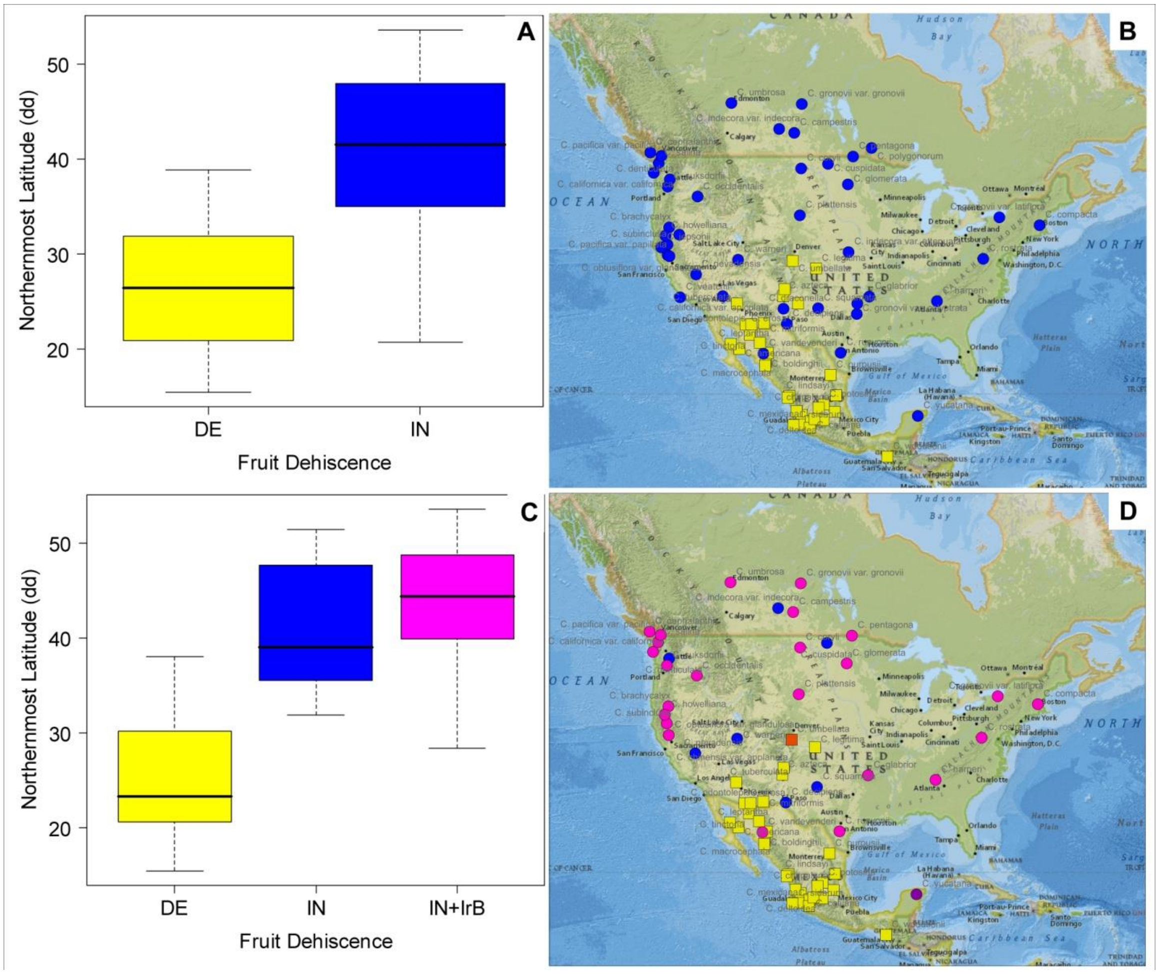

Figure 9. Distributions of the northernmost latitudes of North American *Cuscuta* species based on fruit dehiscence modes. A-B. Without considering IrB fruits as an evolutionary trait. C-D. With IrB fruits considered as an evolutionary fruit trait. A, C. Boxes display the middle 50% of the northernmost latitude distributions for each type of fruit dehiscence, and the horizontal lines within the boxes represent the median. Circles represent the outliers (values that are >1.5 times the interquartile range), and the whiskers represent the min and max values that are not outliers. B, D. Each point on the maps represents the northernmost population of a *Cuscuta* species that either has indehiscent fruits (IN; blue), regularly circumscissile dehiscent fruits (DE; yellow), indehiscent and

irregularly dehiscent type B fruits (IN+IrB; magenta), dehiscent and irregularly dehiscent type A fruits (DE+IrA; orange), or indehiscent and irregularly dehiscent type A fruits (IN+IrA; purple).

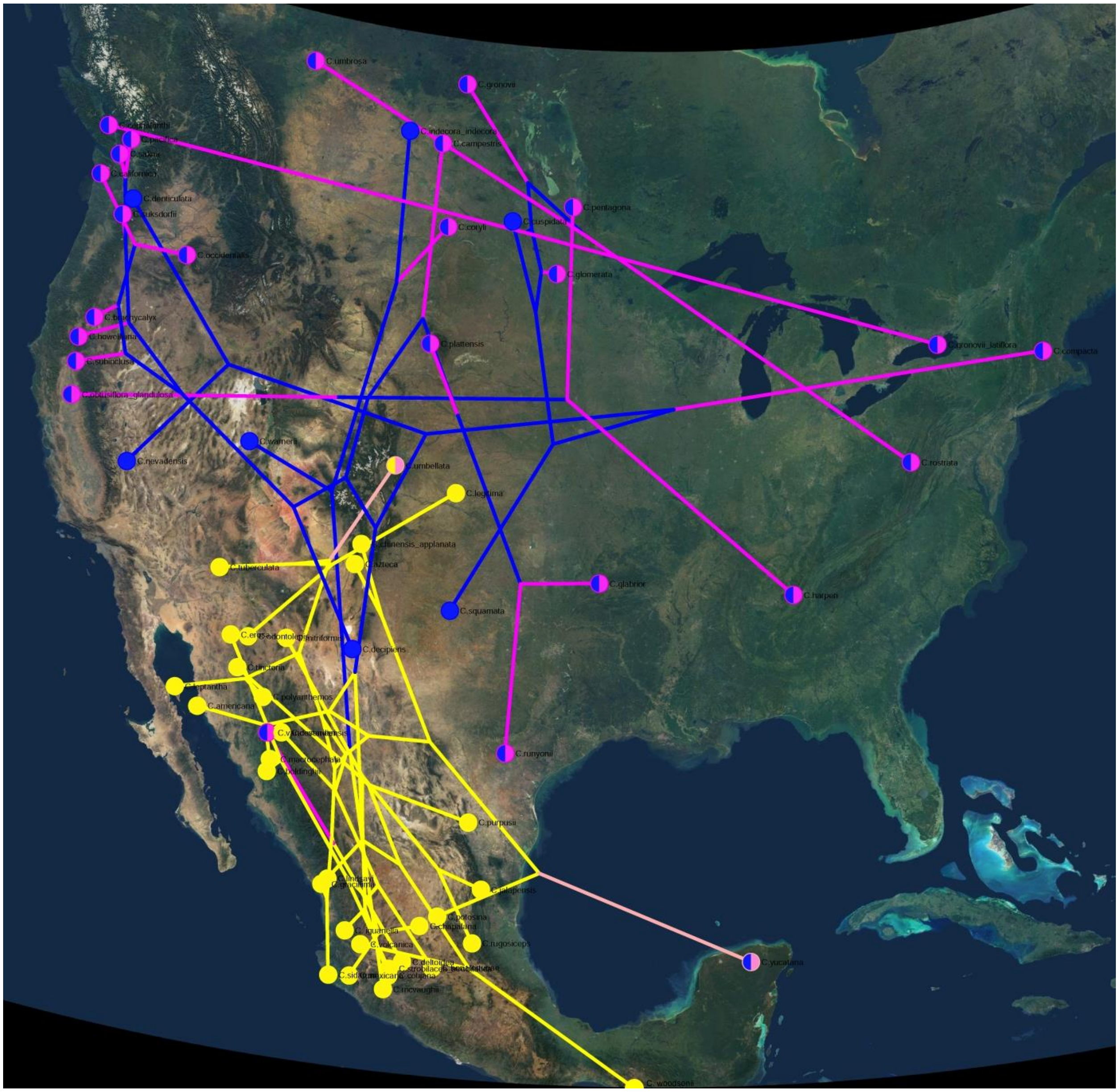

Figure 10. Geographical plotting of subgenus *Grammica* phylogeny in North America. Northernmost latitudes of species and history of fruit dehiscence/indehiscence

modes are traced. Ancestral nodes of the tree were plotted with Cartographer Mesquite (2017) which uses an algorithm that involves squared-change parsimony. Yellow dots = circumscissile dehiscent (DE) species; blue dots = indehiscent (IN) species; half magenta dots = species with IN and irregularly dehiscent type B (IrB) fruits, and half pink dots = species that also have populations with irregularly dehiscent type A (IrA) capsules. Note the southern latitudinal distribution of DE species. Only IN and IN + IrB species are found at the northernmost latitudes.

5.3. FLOATABILITY OF *CUSCUTA GRONOVII* FRUITS AND SEEDS

Imbibed seeds did not float when deionized water was added to the beakers. Approximately 71% of dried seeds floated after water was added to the beakers, but after 12 hours, the number of seeds that remained floating decreased to less than 20% (Fig. 11). After 36 hours, all dried seeds (except for one) were at the bottom of the beaker. Capsules, however, were capable of floating for over 9 days. The first capsules to sink were observed at 36 hours, though they represent <1% of all capsules tested. By the experiment end date, 42% of capsules remained floating; though the water level had decreased drastically and as a result, the experiment was discontinued. It may be worthwhile to note that the seeds in this study imbibed and germinated readily compared to those floating inside their capsules.

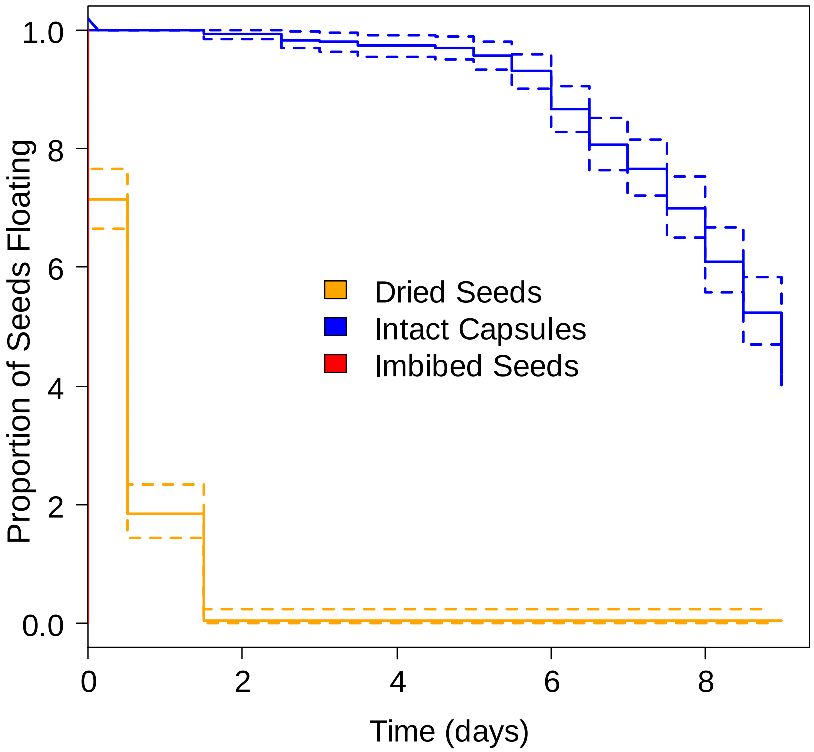

Figure 11. Kaplan-Meier survival curves displaying the proportion of seeds or capsules that remained floating over time. The initial number of seeds that float after water was added to the experiment was recorded at time 0. Each step in the curves represent an event in which seed(s) sink. Dotted lines represent the upper and lower 95% confidence intervals. Imbibed seeds (red) did not float, whereas the majority of dried seeds (yellow) floated immediately. No seeds remained floating after 36 hours, whereas 42% of capsules remained floating for over 9 days.

5.4 FRUIT EFFECTS ON THE GERMINATION OF *CUSCUTA GRONOVII*

In 2015, germination of *C. gronovii* seeds was first observed on May 5th and was recorded for a total of 98 days. In 2016, the experiment was monitored again starting from May 5th for 99 days. Table 5 provides summary statistics for the results obtained. In the first year, germination between treatments was significantly different (Two Sample t-test: df = 118, p-value = 0.00034) with more seeds germinating on average without capsules (WO) than within capsules (W), and with no difference in germination between the populations (Wilcoxon rank sum test, W = 2060.5, p-value = 0.1708; Fig. 12A). In the second year alone, differences were observed between the populations (Wilcoxon rank sum test, W = 1288.5, p-value = 0.0062), but not between the treatments (Wilcoxon rank sum test, W = 1798, p-value = 0.9936; Fig. 12B). When comparing the total number of seeds that germinated by the end of the experiment, again, more seeds germinated when removed from their capsules than when capsules were left intact (Welch's Two Sample t-test, df = 109.81, p -value = 0.0003), and there was no difference in germination between the two populations (Welch's Two Sample t-test, df = 102.33, p-value = 0.6531; Fig. 12C).

Not only did the total number of seeds that germinated by the end of the experiment differ between treatments, but the rate at which they germinated throughout the study was also different (log-rank test, chisq = 41.9, df = 1, p-value = 9.8e-11). The germination (survival) curves of the two treatments for each growing season are displayed in Figure 13. As this type of analysis requires the total number of seeds used, the average number of seeds from 10 capsules was determined to be 26 seeds for site LP, and 27 seeds for site GR. Seeds without capsules (WO) were the first to germinate each year and generally germinated at a higher rate throughout the season except during the first 4 weeks of the experiment where the rate of germination was the same for both treatments. The majority of seeds germinated in the first year between weeks 4 to 6 for both treatments. By the end of the first growing season, 77% of W seeds remained, whereas 68% WO seeds remained. In the second year, most of the germination took place within the first 3 weeks, and by the end of the season, an additional 10% of W seeds (total = 33% germination), and 14% WO seeds (total = 46% germination) germinated (Fig. 13).

Table 5. Summary statistics of *Cuscuta gronovii* seed germination data collected over two growing seasons. Seeds and capsules were collected from two sampling sites (Grand River, GR, and Long Point, LP), and treatments for each site include seeds with their capsules intact (W), or seeds removed from their capsules (WO). Each treatment contained 30 replicates.

Year	Site or Treatment	Mean	Median	Standard error	95% CI or BCI	Minimum	Maximum	Normality (p-value)
2015	W	7.767	8.000	0.56517	6.63633, 8.89701	0.000	21.000	0.285
	WO	11.05	10.50	0.68619	9.67761, 12.4224	0.00	28.00	0.3582
	GR	9.967	10.000	0.56896	8.82876, 11.1046	1.000	21.000	0.3993
	LP	8.85	9.50	0.73984	7.423, 10.346	0.00	28.00	0.01474
2016	W	2.10	1.00	0.264362	1.583, 2.619	0.00	8.00	8.252e-06
	WO	2.483	1.000	0.376943	1.742, 3.200	0.000	12.000	1.735e-07
	GR	1.5	1.0	0.192369	1.128, 1.874	0.0	6.0	5.392e-06
	LP	3.083	2.000	0.393659	2.360, 3.834	0.000	12.000	1.169e-05
Total	W	9.867	9.000	0.590636	8.685394, 11.04794	1.000	23.000	0.5199
	WO	13.53	13.00	0.781664	11.97001, 15.09666	0.00	30.00	0.3345
	GR	11.47	11.00	0.571185	10.3243, 12.60904	2.00	23.00	0.7622
	LP	11.93	11.00	0.863532	10.20627, 13.6604	0.00	30.00	0.0662

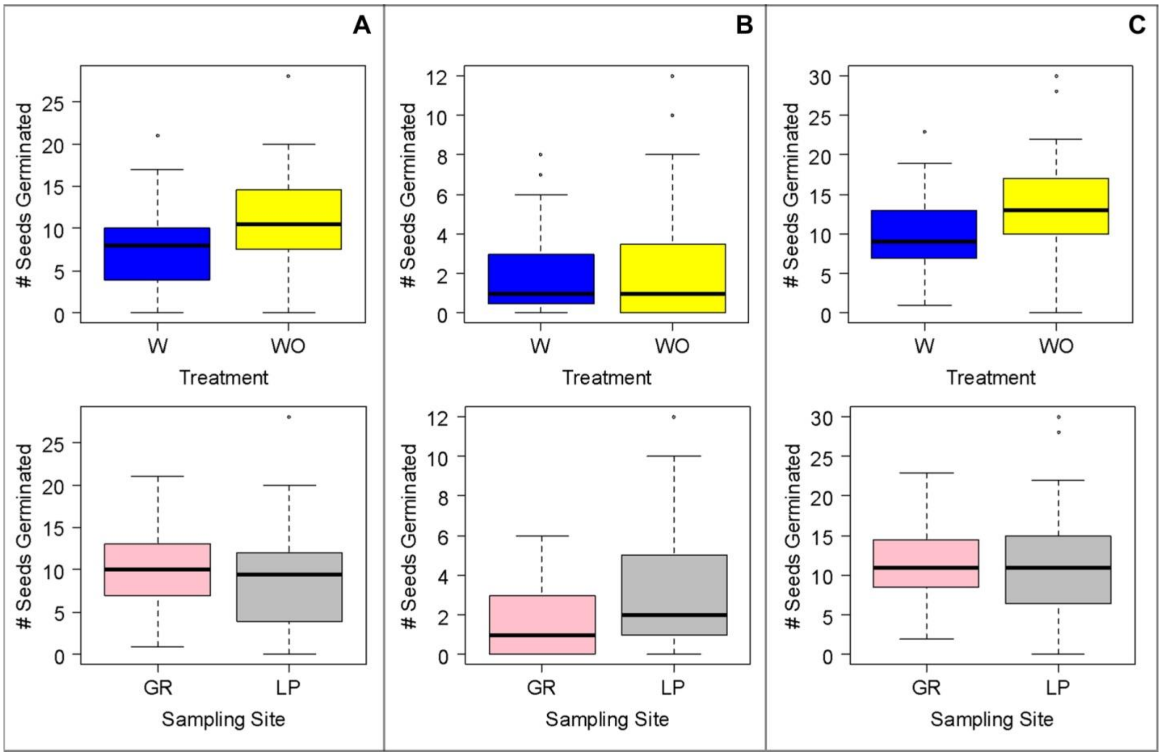

Figure 12. *C. gronovii* seed germination distributions. Capsules collected from two sampling sites (Grand River, GR; Long Point, LP) were used to compare the germination of seeds within capsules (W) to those removed from their capsules (WO). A. In the first year, treatment WO had a higher germination count than treatment W and germination was not different between sampling sites. B. In the second year, no differences were observed between the treatments. C. Overall, the total number of seeds that germinated was higher in treatment WO than W and not between the sampling sites.

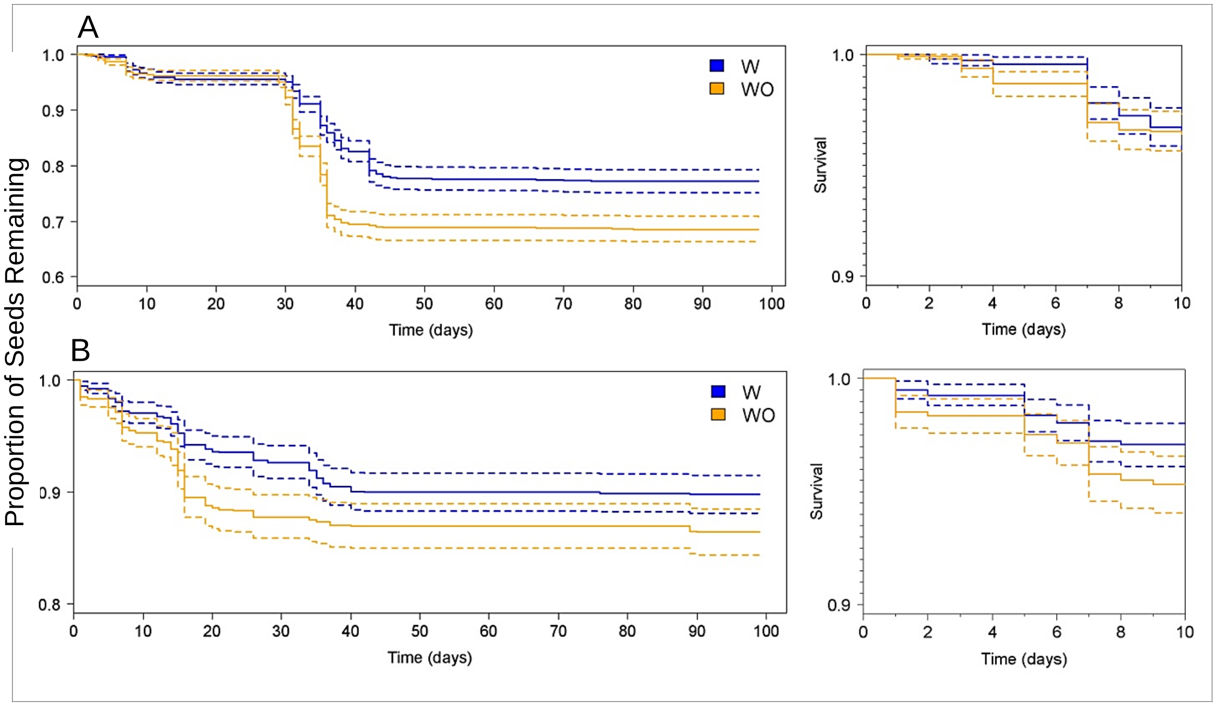

Figure 13. Kaplan-Meier survival curves displaying the proportion of seeds that remained in the study. A. Germination curves for the first growing season. B. Germination curves for the second growing season. Survival curves for the first ten days of each season is enhanced on the right. At the start of the experiment, all treatments have a survival rate of 1.0 or 100%. Steps indicate the time at which an event has occurred (in this case, germination). Dotted lines represent the upper and lower 95% confidence intervals for each curve. Throughout the study, seeds removed from their capsules (WO) germinated at a higher rate than seeds kept inside their capsules (W). Most germination occurred in the first season between days 30 and 45.

5.5 FRUIT DEHISCENCE AND INFRUCTESCENCE ARCHITECTURE

The data collected for measuring infructescence compactness indicates that within subgenus *Grammica*, although indehiscent fruit species generally have a more compact infructescence than dehiscent fruit species when using previous fruit categorization (Fig. 14A), this difference is not consistent (Wilcoxon rank sum test, W = 725, p-value = 0.4688). When using the new fruit categorization obtained from section 5.1 (Fig. 14B), significant differences were only observed between DE+IrA fruit species and IN+IrB fruit species (Dunn's test for multiple comparisons, z-value = 1.8766, p-value = 0.0303), with DE+IrA fruit species having the least compact infructescences and IN+IrB fruit species having the most compact infructescences (Fig. 14B). Although not statistically significant, IN species that also contain IrB fruits tend to have more compact infructescences than strictly IN species, and strictly IN species have lax infructescences compared to DE fruit species, though IN+IrB fruit species tend to have more compact infructescences than DE fruit species. Table 6 provides the summary statistics for the calculated infructescence compactness of species based on their fruit type.

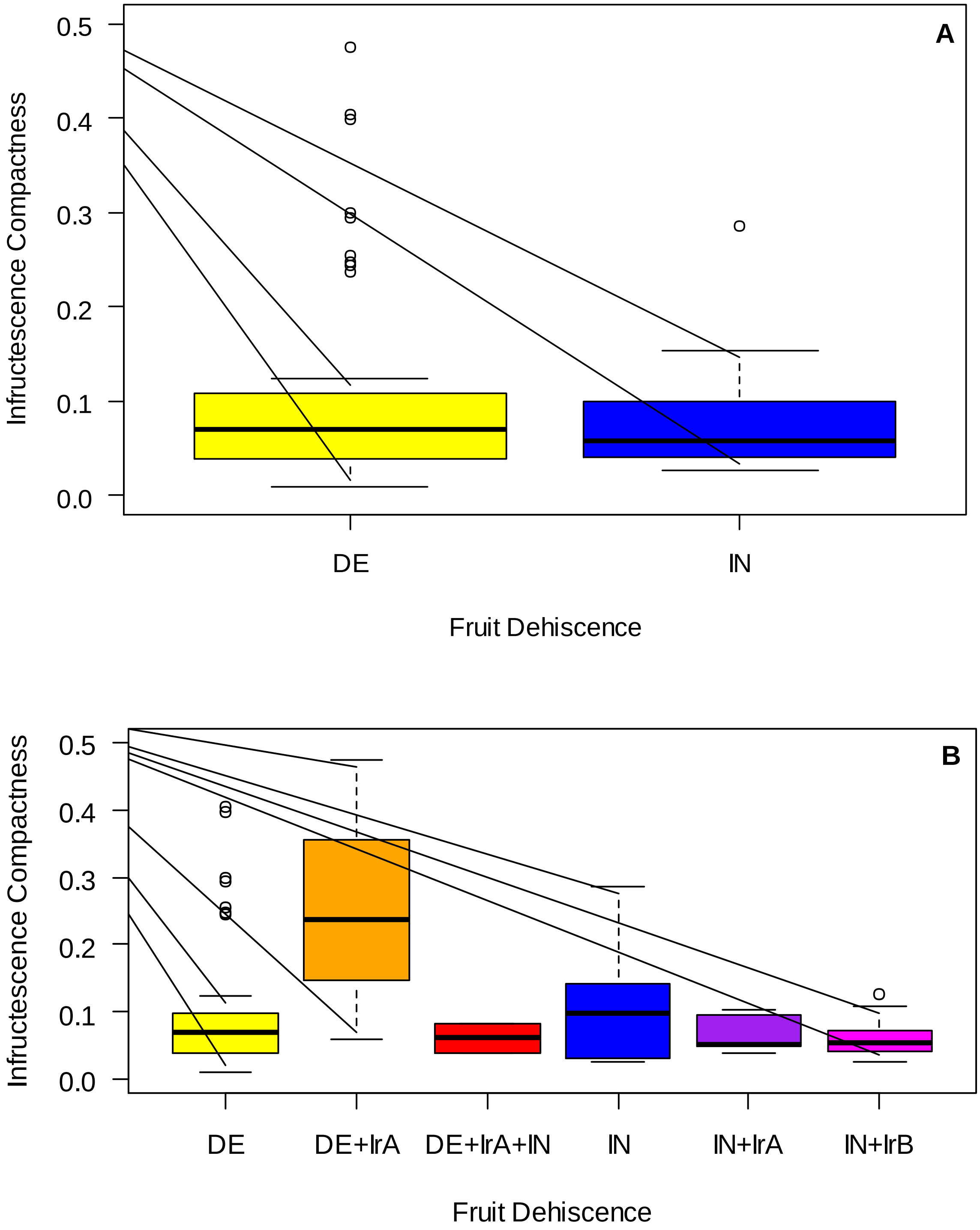

Figure 14. Distribution of infructescence compactness among 76 *Grammica* species of various dehiscence modes. A. Using the old fruit categorization retrieved from Garcia et al. 2014), indehiscent (IN) fruit species appear to have more compact infructescences

than dehiscent (DE) fruit species, although this difference is not statistically significant (Wilcoxon rank sum test, W = 725, p-value = 0.4688). B. Using the new fruit categorization proposed in section 5.1. IrB fruit species are included separately from strictly IN species. DE+IrA fruit species have the least compact infructescences, whereas species with IN+IrB capsules generally have the most compact infructescences. Among all fruit types, only DE+IrA and IN+IrB are statistically significant (Dunn’s test, z-value = 1.8766, p-value = 0.0303). Boxes display the middle 50% of the infructescence compactness distributions for each type of fruit dehiscence, and the horizontal lines within the boxes represent the median. Circles represent the outliers (values that are >1.5 times the interquartile range), and the whiskers represent the min and max values that are not outliers.

Table 6. Summary statistics for infructescence compactness of 76 *Grammica* species based on compactness indicator formula created in section 4.6. Infructescence compactness was compared among species with different fruit dehiscence modes, and based off old fruit categorization retrieved from Garcia et al. (2014), as well as new fruit categorization from results in section 5.1. A smaller value represents a more compact infructescence.

Data Type	Fruit Type	Mean	Median	Standard Error	95% CI or BCI	Min	Max	*n*	Shapiro (p-value)
Old	DE	0.10930	0.07057	0.01626	0.0781, 0.1408	0.00906	0.47480	47	5e-08
	IN	0.07463	0.05753	0.01028	0.0559, 0.0948	0.02543	0.28560	28	2.7e-05
New	DE	0.10110	0.06938	0.01544	0.0706, 0.1304	0.00906	0.40350	42	1.6e-07
	DE+IrA	0.25660	0.23720	0.12077	0.0628, 0.4499	0.05781	0.47480	3	0.8462
	DE+IrA +IN	0.06048	0.06048	0.02246	0.0292, 0.0900	0.03801	0.08294	2	N/A
	IN	0.10570	0.09636	0.03121	0.0454, 0.1639	0.02575	0.28560	8	0.1186
	IN+IrA	0.06687	0.05006	0.01314	0.0439, 0.0896	0.03950	0.10290	5	0.13
	IN+IrB	0.06063	0.05302	0.00721	0.0467, 0.0746	0.02543	0.12620	15	0.1236

6. DISCUSSION

6.1 STRUCTURAL AND MORPHOLOGICAL DIVERSITY OF CAPSULES

Structural and ultrastructural basis of different modes of dehiscence/indehiscence; types of fruits in Cuscuta and their evolution

This study confirms previous results (Stefanović et al. 2007; García et al. 2014) suggesting that indehiscence is derived in *Cuscuta*. This research, however, revealed a more complex situation than the “to dehisce or to not dehisce” scenario used in the previous character evolution studies (García et al. 2014). In addition to regularly circumscissile dehiscent (DE) and indehiscent (IN) capsules, *Cuscuta* possess a third morphological type, the irregularly dehiscent type A (IrA), as well as a functionally irregularly dehiscent fruit (IrB). IrA fruits do not develop an AZ, but have a thin endocarp at the base, which in conjunction with the growing of seeds and drying pericarp will cause most of the fruits to dehisce (but later than DE capsules). IrB capsules occur always in species with IN fruits; they are developmentally and structurally indistinguishable from indehiscent ones but some may break irregularly due to external factors. Spjut (1994) defined *Cuscuta* capsules as foraminicidal (“opening by cracks that spread in different directions”). It seems that only the IrB capsules may loosely fit this description, but considering that these fruits are developmentally indehiscent, their characterization as foraminicidal is not appropriate. The results of this study support the hypothesis that irregular dehiscence forms in *Cuscuta* are not homologous. Although the structural and ultrastructural differences between the DE, IrA and IN consist mainly of different thickenings of endocarp cell walls, the loss of the AZ is not sufficient to acquiring indehiscence. From both an ontogenetic and evolutionary point of view, IrA capsules can be regarded as an intermediary evolutionary stage between circumscissile dehiscence and indehiscence. Considering that indehiscence is ontogenetically gained through the loss of the AZ and special pericarp thickenings, and conversely, that the return to dehiscence requires a reversed sequence, the presence of IrA capsules in certain species indicates an incompletely acquired indehiscence or dehiscence, respectively.

The structural mechanism of opening of circumscissile capsules in *Cuscuta* is similar to that reported in other angiosperm genera with a pyxidium fruit such as *Sesuvium* (Aisoaceae); *Allmania*, *Amaranthus*, *Celosia*, *Chamissoa* (Amaranthaceae), *Plantago* (Plantaginaceae), *Portulaca* (Portulacaceae), *Hyoscyamus* (Solanaceae), in which various lignified/sclerified tissues also contribute to the dehiscence of fruit in addition to the AZ (Rethke, 1946; Subramanyam and Raju, 1953; Lamba and Gupta, 1981; Oyama et al., 2010). IrA capsules in *Cuscuta*, however, show that dehiscence is possible even if an AZ does not develop. The dehiscence mechanism in fruits with circumscissile dehiscence is mechanically less complex than in other types of dry dehiscent fruits where dehiscence requires the morphological formation of valves (e.g., Fahn and Werker, 1972; Addicott, 1982; Meakin and Roberts, 1990; Mummenhoff et al., 2009).

The ultrastructural characteristics of the AZ in *Cuscuta* revealed similarities with the dehiscence zone of the silique in *Arabidopsis* (Rajani and Sundaresan, 2001) and with abscised organs in general (Sexton and Roberts, 1982; Roberts et al., 2000; 2002; Patterson, 2001; Leslie et al., 2007). The cell separation in the AZ of *Cuscuta* is due to the dissolution of the middle lamella and the degradation of cell walls. The presence of DE or IN capsules together with IrA capsules in some of the *Cuscuta* species provides an ideal natural system in which to study the developmental genetic mechanisms that control dehiscence/indehiscence. Such studies have been conducted in Brassicaceae (e.g., *Lepidium*, Mühlhausen et al., 2013; and *Arabidopsis*, Lenser and Theißen, 2013) or Fabaceae (soybeans; Dong et al., 2014) which have more complicated structural mechanisms of dehiscence. *Cuscuta* provides a simplified model for the evo-devo study of dehiscence/indehiscence modes.

Taxonomic significance of fruit characters in *<u>Cuscuta</u>*

As in the case of all the other morphological characters in *Cuscuta* — perianth features (Wright et al., 2012); shape, size and reduction of infrastaminal scales (Riviere et al., 2013), pollen morphology (Welsh et al., 2010), gynoecium characteristics (Wright et al., 2011; García et al., 2014), multicellular protuberances with stomata (Clayson et al., 2014) — fruit traits are highly polymorphic and insufficient to reconstruct phylogenetic relationships among dodder clades (e.g., among the 15 major clades of subg. *Grammica*) because of extensive convergent evolution. For example, *Cuscuta yucatana* of Sect. *Grammica* ("clade H"; Costea et al., 2011b) exhibits a similar fruit morphology as *C. acuta* from Sect. *Umbellatae* ("clade L"; Costea and Stefanović, 2010). Both species have IN or IrA capsules in clades with predominantly DE capsules.

Nevertheless, taking into consideration the scarcity of morphological characters available for the systematics of *Cuscuta*, the diversity of fruit morphology is important for taxonomic revisions at species level *within* each clade. Although IN, IrA and IrB have evolved multiple times and have little bearing on the genus phylogeny and classification (see also Costea et al., 2015), they provide readily observable traits in clades that also have circumscissile species. The value of fruit morphology increases when they are added to gynoecium traits that have been studied elsewhere (Wright et al., 2011; García et al., 2014). For example, the styles/stigmas are persistent on the fruit and some of their characters, such as the number of styles and the shape of stigmas (Wright et al., 2011), provide important morphological characters for the current infrageneric classification of *Cuscuta* with four subgenera (Costea et al., 2015).

Differences in quantitative traits were observed among the major fruit types which allow for some generalizations to be made. Capsules of IN+IrB fruit species are relatively large, with a large interstylar aperture, and a high number of seeds per capsule compared to other fruit types. Whether this means that the IrB fruit type is an evolutionary trait derived from IN is still undetermined. Since these results were obtained from herbarium specimens, it is possible that IN species with larger fruits may be prone to breakage from the handling and preparation of their specimens. Thus, it would be beneficial to examine these species in the field, to determine if this is truly a case of heterodiaspory. Capsules of IN fruit species are somewhat smaller than DE capsules but form a much larger interstylar aperture. DE capsules have a relatively small interstylar aperture, if any. Other than the presence of an abscission zone seen in DE fruits, no difference in qualitative fruit traits exist between IN+IrA fruit species and DE+IrA fruit species.

6.2 DEHISCENCE/INDEHISCENCE MODES, THE GEOGRAPHICAL DISTRIBUTION OF NORTH AMERICAN SPECIES OF SUBGENUS *GRAMMICA*, AND THEIR DISPERSAL

No statistically significant differences in range size were observed between IN and DE North American *Grammica* species when using either the data of García et al. (2014) or the fruit categories generated in this study. However, the separation and inclusion in the analysis of species with both IN and IrB capsules resulted in significant differences of geographical range size (between IN + IrB and DE). Species with indehiscent fruit capsules (including IN+IrB) are distributed over a larger area in North America than species with DE fruits (1.11 x 10^7 km^2 vs. 3.1 x 10^6 km^2). Three species with IN or IN + IrB capsules have the largest ranges in N America: *C.* campestris, *C. indecora* and C. *gronovii*. Interestingly, a species with DE and irregular type A dehiscence (IrA, late dehiscence), *C. umbellata*, has the 4th largest range size.

Dehiscence/indehiscence modes displayed a clear latitudinal pattern in North\ America, with most DE species being restricted to the southern part of the U.S.A. and Mexico (northern most latitude usually under 35°N) and the IN + IrB species reaching the highest latitudes (53.5°N). Although several IN and IN + IrB species have exclusively southern ranges (e.g., *C. decipiens*, *C. warneri*, C. *vandevenderi*, *C. runyonii*, *C. glabrior*, *C. harperi*), the southern distribution of the majority of IN or IN + IrB species is only a part of broader distribution ranges.

Bayesian Binary Method (BBM) and Statistical dispersal–vicariance analysis (S-DIVA) (García et al., 2014) indicated that Mexico and the adjacent regions are the ancestral diversification area for *Grammica* clades A to K, with local radiations followed by independent dispersals both to North America and South America. Dispersal to North America was followed by the diversification of clades A-E (García et al., 2014), while a long-distance dispersal event to South America was the ancestor of clade C (García et al., 2014). This is significant because clades A-E are the North American groups in which IN or IN + IrB have evolved in most of the species. Thus, the latitudinal pattern of dehiscence/indehiscence modes follows the radiation of subg. *Grammica* species in North America: *IN and IN + IrB evolved from south to north*. Clade C in South America is also the group with most IN or IrA species (although indehiscence also evolved earlier in South America in clade O), but since no taxonomic revision has been conducted for this group yet (Costea et al., 2015), and geographical data is scarcer than for North American *Grammica* clades, a possible geographical pattern could not be examined.

The tendency of species geographical ranges to increase with latitude is known as the “Rapoport's rule” (Stevens, 1989; Gaston et al., 1998). This much-debated hypothesis is currently considered to apply mainly to the higher latitudes of the Northern Hemisphere (Rohde, 1996; Gaston et al., 1998; Gaston, 2003; Ruggiero and Werenkraut, 2007). As previously indicated, in North America, IN and IN + IrB species possess the largest distribution ranges with the northernmost limits, while most DE species are restricted to Mexico and the adjacent areas. Although the intention of this study was not to test a latitudinal increase of species ranges, Rapoport’s rule seems to describe adequately the situation of subgenus *Grammica* species in North America.

The size of geographical ranges of subg. *Grammica* species in North America cannot be reconciled with the potential reduction in dispersal capacity posited for IN capsules. One-seeded capsules have evolved in seven *Grammica* clades and are prevalent in species with IN capsules, which could be regarded as a trend to increase their dispersal capability (e.g. Augspurger and Hogan, 1983; Willis et al., 2014). However, two species with DE capsules, *C. americana* and *C. potosina*, also have one-seeded capsules and numerous species with IN or IN + IrB capsules possess two or more seeds per capsule.

Two explanations can be formulated to account for the broad geographical ranges observed in species with IN and IN + IrB capsules: 1) the dispersal ability may not be the only factor modulating the size of geographical ranges in *Cuscuta* species; and 2) indehiscence may provide enhancing dispersal traits.

Dispersal ability may not be the only factor modulating the size of geographical ranges in Cuscuta species

The dispersal capability plays a significant role in the size of geographical range of plants (e.g., Oakwood et al., 1993; Brown et al., 1996; Edwards and Westoby, 1996; Lloyd et al., 2003; Lowry and Lester, 2006) because it influences their ability to colonize new areas and affects speciation rate (reviewed by Gaston et al., 2003; Lester et al., 2007). However, the positive relationship between dispersal distance and geographical extent is not universal (Lester et al., 2007; Gove et al., 2009; Slatyer et al., 2013). Niche breadth size — the totality of environments or resources that a species can inhabit or use (Gaston et al., 1997) — has also been shown to greatly influence the size of geographical ranges (e.g., Thompson et al., 1999; Broennimann et al., 2006; Slatyer et al. 2013). Species with broader tolerances to abiotic factors may be less affected by the local variation in availability of resources and environmental conditions because they can persist in multiple habitats, which is also reflected in their wider geographical distribution/ranges (Brown, 1984; Baltzer et al., 2007). In the case of *Cuscuta*, abiotic factors play a role only during the non-parasitic stage, at germination and prior to the attachment of seedlings to the host (Costea and Tardif, 2006; Dawson et al., 1994;

Behdarvandi et al., 2015). Once successfully attached to the host, this becomes the “niche”, providing everything the parasite needs to complete its life cycle (Costea and Tardif, 2006; Dawson et al., 1994). Thus, host specificity predefines the spatial limits across which a parasitic plant can occur and expand, both at the level of the ecosystem and at a geographical scale. *Cuscuta* species with large host ranges (“generalists”) have also large geographical ranges, while dodders with narrow host ranges (“specialists”) tend to have small ranges (Costea and Stefanović, 2009a). Not surprisingly, this apparent relationship between the host range and geographical range size is reflected in the rarity or commonness of various species, their extinction, or invasiveness (Costea and Stefanović, 2009a). In general, *Cuscuta* species with IN or IN + IrB fruits are more broadly distributed in North America than DE ones; those that are more localized geographically also have narrow host ranges. For example, the rarity of *C. warneri* (Costea et al., 2006b), *C. decipiens, C. draconela* (Costea and Stefanović, 2009b), *C. jepsonii* (Costea and Stefanović, 2009a), *C. plattensis,* and *C. harperi* (Costea et al., 2006a) is likely not caused by the indehiscence of their fruits but by their host specificity.

The evolution of indehiscence in North American plants has been commonly associated with deserts plants in which the more reduced dispersal capacity was hypothesized to be offset by reproductive advantages such as the protection of seeds against environmental factors (Ellner and Shmida, 1981), modulation of dormancy/germination (reviewed by Lu et al., 2015), and retention within favourable maternal sites (Friedman and Stein, 1980). This tendency is not apparent in *Cuscuta* because, a mixture of DE and IN species are found in arid and semi-arid habitats in North America, which shows that both strategies offer advantages.

Indehiscence may provide enhancing dispersal traits

This study provided the first experimental data on the floatability of capsules and seeds of *Cuscuta* using *C. gronovii*, which is the third most widely distributed dodder species in North America (Costea and Tardif, 2006; Costea et al., 2006c). Capsules can float for over 9 days which is probably a sufficient time to allow long distance dispersal by water under certain conditions. In contrast, hydrated seeds do not float at all and dry seeds float only until imbibition has taken place. Certain *Cuscuta* sp. in North America (e.g., *C. gronovii*, *C. obtusiflora*, *C. polygonorum*, *C. pacifica*) occur preferentially in wetlands, and possessing IN or IN + IrB capsules, they are likely to be dispersed by water. Since a significant part of the geographical ranges of many IN or IN + IrB *Grammica* species is in the temperate areas of North America, dispersal by water may also play a significant role after melting of the snow or during the spring rains. Both seeds and capsules fall in the vicinity of mother plants; as seeds imbibe, they rapidly lose their floating capability, while capsules (or clusters of capsules) may float for extended periods of time. The floating capability of IN capsules may enhance the dispersal of species with IN or IN + IrB capsules, but this will have to be tested under field conditions. If this is correct, indehiscence may explain in part the tendency of *Cuscuta* species' geographical ranges to increase with latitude.

Recently, Costea et al. (2016) documented the first case of endozoochory in *Cuscuta.* Viable seeds of *C. campestris* and *C. pacifica* were found in the rectum of northern pintails (*Anas acuta;* dabbling ducks) at Suisun Marsh in California. These two dodder species have IN + IrB capsules and the authors suggested that fruits or infructescences are more suitable than seeds for the feeding of pintails (Costea et al., 2016). The authors also suggested that long distance dispersal by other migratory waterbirds may explain the transoceanic historical cases of long distance dispersal documented in the evolution of *Cuscuta* (reviewed by García et al., 2014). Cuscuta infructescences in species with DE + IrA, IN and IN + IrB capsules may also be dispersed by wind (chapter 5.4). In the last decade, it has become commonly accepted that most plant species are dispersed by more than one dispersal vector (Poschlod et al., 2005; Hintze et al., 2013). Even if a plant exhibits traits specific to a particular dispersal mode, these traits may enable or be compatible with other dispersal modes (Hintze et al., 2013). Possessing several dispersal modes and vectors will increase the chances of effective dispersal both in terms of distance and habitat suitability (Webb, 1998). Even if IN capsules in *Cuscuta* may result in a loss of dispersal capability when diaspores contain more than one seed, both the hydrochory and zoochory of capsules act as enhancers of dispersal and may create opportunities for long distance dispersal, which do not exist for the seeds alone. Traits that enhanced dispersal were also recently observed in the evolution of Brassicaceae with indehiscent fruits (Willis et al., 2014).

Future studies will have to comparatively study in the field the dispersal of species with IN or DE species occurring in similar habitats and at the same latitudes.

6.3 DEHISCENCE/INDEHISCENCE AND SEED GERMINATION

This research has shown that although the pericarp has simple structure, it is able to significantly alter the germination behavior of seeds. The germination pattern of *C. gronovii* seeds enclosed within capsules (W) was different from those that were removed from the fruits (WO). Although both W and WO seeds germinated in largely coinciding seasonal peaks, WO seeds germinated earlier and in greater abundance. The germination of seeds within indehiscent capsules (W) was delayed and their germination rate was lower compared to WO seeds. Thus, species with IN capsules employ a bet-hedging strategy: fewer seeds germinate in the peaks and overall, and the germination is delayed both during each vegetation season and throughout the years.

Evolutionary bet-hedging (Slatkin, 1974) is the theory according to which evolution may unfold via forfeiting the average (or expected) fitness to reduce the *variance* in fitness of a life-history strategy or genotype (Seger and Brockmann, 1987; Philippi and Seger, 1989). Cohen's (1966) classical model of (diversified) bet-hedging predicts that annual plants in deserts or highly disturbed environments (Cohen, 1968) can reduce their fitness variance by ensuring that only a portion of seeds germinate in a certain year. In other words, in highly variable environments, a portion of the seeds produced remains dormant as a hedge against the risk of total reproductive failure. Bet-hedging via delayed germination in annual plants has been the subject of numerous theoretical (Kalisz and McPeek, 1993; Rees, 1994; Venable and Brown, 1988; Evans et al., 2007; Childs et al., 2010; Gremer and Venable, 2014; reviewed by Baskin and Baskin, 2014) and empirical studies (Thompson and Grime, 1979; Thompson et al., 1998; Venable, 2007; reviewed by Baskin and Baskin, 2014). Delayed germination can also function as a strategy to avoid sibling competition (reviewed by Baskin and Baskin, 2014).

To understand this finding in *Cuscuta* we must first consider the paramount significance of seedlings in the life cycle of dodder plants. In annual plants, in general, seedling establishment is crucial because it affects the persistence and dynamics of their populations (Grubb, 1977; Harper, 1977). In *Cuscuta*, seedlings face similar abiotic and biotic challenges as the green plants (e.g., Maun, 1994; Maestre et al., 2003; Isselstein et al., 2002), however, unlike green plants, dodders also have to locate compatible hosts, circumvent their defenses, and successfully establish a haustorial connection with them (Dawson et al., 1994; Costea and Tardif, 2006). If seedlings cannot attach to a suitable host within a short window of time, mortality ensues (Dawson et al., 1994; Costea and Tardif, 2006; Behdarvandi et al., 2015). Thus, the seedling stage in *Cuscuta* represents a stronger ontogenetic filter than is the case in annual heterotrophic plants.

The high temperature germination requirements of *Cuscuta* species (Hutchinson and Ashton, 1980; Benvenuti et al., 2005; Costea and Tardif, 2006) ensure that germination takes place when the seedlings or shoots of suitable hosts are already established or present (Dawson et al., 1994; Costea and Tardif, 2006). This is probably because unlike other holoparasitic plants (e.g., Orobanchaceae; Matusova et al., 2005; Fernández-Aparicio et al., 2009), *Cuscuta* seeds cannot sense host-derived strigolactones and trigger their germination when suitable hosts “appear” in their vicinity. Dodders possess sophisticated methods of host detection only *after* seedlings emerge (Dawson et al., 1994; Costea and Tardif, 2006; Runyon et al., 2006), but if no suitable host is located in the vicinity of seedlings, they cannot survive. Considering the uncertainties of host availability at the moment of germination, bet-hedging is advantageous because it spreads the risk among different individuals of the same genotype and increases the chances that eventually some seedlings will successfully attach to a compatible host. In the case of IN capsules, bet-hedging is also advantageous because it reduces potential sibling competition of seedlings originating from the same capsule for the same host plant.

Diaspores of species with IN + IrB fruits consist mostly of IN capsules, but a small percentage of seeds are also dispersed individually. This resembles the heterodiaspory condition, in which two or more different morphological types of diaspores differing also in ecological function are produced by the same plant (reviewed by Baskin and Baskin, 2014). Unlike other plants in which heterodiaspory is generated by intrinsic morphological and physiological characteristics of the diaspores (Roth, 1977; reviewed by Baskin and Baskin, 2014), in *Cuscuta*, heterodiaspory is apparently functional because the IrB capsules are indistinguishable from the IN capsules within the same inflorescence. Heterodiaspory results because a small percentage of capsules break irregularly and release their seeds, which have a different germination behaviour compared to those that remained enclosed in the capsules. In a species with IN + IrB fruits, germination will be more gradual compared to a species with exclusively IN fruits, thus potentially creating more opportunities for the seedlings to forage in the plant community (Kelly, 1990; Press and Phoenix, 2005). Overall, this maximizes the parasites' chances of interaction with compatible hosts while maintaining a reserve of seeds as a potential safeguard. At the same time, the two types of diaspores, individual seeds and seeds within IN fruits, will likely have different dispersal ability. Capsules are morphologically adapted to be dispersed at potentially longer distances compared to the unspecialized seeds.

The most important limitation of this germination study is that dehiscence was generated artificially in one single species (*C. gronovii*) with IN + IrB fruits. It is unknown how bet-hedging, which is common in annual desert plants (Gremer and Venable, 2014 and references cited therein), functions in *Cuscuta*. For this reason, future studies will have to comparatively study not only the dispersal of species with IN or DE species at lower latitudes in North America (there are no native species with DE fruits in temperate North America), but their germination as well.

6.4 FRUIT DEHISCENCE AND INFRUCTESCENCE ARCHITECTURE

As indicated in the introduction, the inflorescence is most commonly associated with the reproductive biology of flowers (e.g., Weberling, 1992; Harder et al., 2004; Prusinkiewicz et al., 2007; Harder and Prusinkiewicz, 2012). In some cases, however, inflorescences persist at fructification (infructescences), and they also play a role in the dispersal (Hintze et al., 2013). Infructescence diaspores are often “wind-tumblers” or “rollers”, dispersal taking place through eolic drive or rolling on the ground (anemogeochory or chamaechory; van der Pijl, 1982; van Oudtshoorn and van Rooyen, 1999). This dispersal mechanism has been more studied when the entire plant, not only the infructescence, participates in the dispersal as in the case of the “tumbleweeds” (e.g., many Chenopodiaceae; van der Pijl, 1982; Borger et al., 2007; Poaceae; Cheplick, 1998; see more examples in van der Pijl, 1982; van Oudtshoorn and van Rooyen, 1999). Although anemogeochory involving infructescences has been less studied, it has been reported in genera from Amaryllidaceae (Snijman and Linder, 1996), Campanulaceae (Maier et al., 1999), Poaceae (e.g., Carey and Watkinson, 1993), and it is likely to occur in many other plant families with infructescences (e.g., Lamiaceae, Asteraceae). Traits common to anemogeochory diaspores are their spherical shape and increased volume, which facilitate rolling on the ground (van der Pijl, 1982; Snijman and Linder, 1996). In *Cuscuta*, anemogeochory cannot play a role in the dispersal of species with dehiscent capsules because their seeds are released before the breakup of the infructescences. However, anemogeochory is possible for species with IN, IrA and IrB fruits. The results of this study demonstrate that species with IN, IrA and IrB capsules possess globular-glomerulate infructescences, which are consistent with the anemogeochory morphology (van der Pijl, 1982; Snijman and Linder, 1996; Maier et al., 1999).

Interestingly, inflorescence compactness differences were not observed between DE and IN species, nor between IN and IN + IrB species as was predicted. Infructescence compactness may have been selected to generate the spatial conditions that cause irregular dehiscence in some capsules, however, this was not supported by the results in this study. Only slight differences were observed among all fruit types. The reason for these insignificant results may be due to the compactness indicator formula—the formula may be oversimplified, thus not compensating for true differences in compactness between each species. Another reason for the lack of differences observed may be because inflorescence/infructescence architecture in *Cuscuta* is associated with traits other than fruit dispersal.

SUMMARY

This fruit evolution study has revealed three structurally distinct fruit types in *Cuscuta*: (1) regularly circumscissile [DE] fruits that dehisce through an abscission zone containing small, cellulosic cells, (2) indehiscent [IN] fruits with a uniform pericarp uninterrupted by an abscission zone, and (3) irregularly dehiscent [IrA] fruits that, although do not have an abscission zone, will dehisce tardily and irregularly through a zone of weakness created by the thinning of the endocarp cell walls near the base of the fruit. Indehiscent capsules may also break irregularly [IrB] if enough pressure is applied on the fruit, and since this phenomenon was observed in some indehiscent fruit species but not all, it may be considered a functional trait that has evolved in the genus. DE fruits are ancestral, as determined in previous studies, and IN fruits, along with IrA and IrB fruits, are derived. Since the evolution from dehiscence to indehiscence is gained through the loss of an abscission zone, IrA capsules can be regarded as an intermediary evolutionary stage between DE and IN.

Fruit traits are highly polymorphic within *Cuscuta* and insufficient to reconstruct phylogenetic relationships among dodder clades. Nevertheless, the diversity of fruit morphology provides easily observable characters useful for species' identification. Few characters are associated with fruit dehiscence modes, though in general, IN fruit species have a much larger interstylar aperture than DE fruit species, and species with IrB fruits have an even larger interstylar aperture. IrB fruit species also have larger fruits, and more seeds per capsule than DE or strictly IN fruit species. These fruit traits along with infructescence compactness contribute to the irregular dehiscence seen in IrB capsules; the only type of dehiscence that can occur on a plant already producing IN capsules, thus allowing the plant to have multiple diaspores.

The evolutionary advantage of fruit indehiscence in *Cuscuta* was narrowed down to two possibilities: (1) it enhances the dispersal of a species, and (2) provides species with a bet-hedging strategy of seed germination. This study demonstrates that IN capsules containing multiple seeds is not associated with the reduction of dispersal ability as commonly understood. The range size of *Cuscuta* species with IN capsules is no different from that of DE fruit species in North America. As it happens, IN fruit species that also produce IrB fruits have an even larger range size than DE fruit species. IN capsules of *C. gronovii* are also capable of floating in water for much longer than their seeds which readily germinate and sink as soon as they become imbibed. The preliminary germination study of *C. gronovii* seeds with and without their capsules determined that seeds inside capsules experience a delayed germination. This ensures that seeds have time to disperse and reduces the risk of reproductive failure. Furthermore, IrB fruit species appear to have evolved from IN fruit species and their heterodiaspory could only enhance these attributes as it provides the species with both the dispersal and germination strategies of individual seeds and seeds within capsules.

INTEGRATIVE NATURE OF THIS THESIS

Although the topic of this thesis is on the evolutionary biology of *Cuscuta* fruits, it incorporates many branches and sub-disciplines of biology. The structure and ultrastructure study in this thesis integrates cell biology and anatomy as I examined cell structures and their functions in the dehiscing fruit. For the biodiversity study, I examined fruit morphology and their evolution, including phylogenetics and systematics. As I explored the evolutionary advantage of fruit indehiscence, I looked at species distributions, water dispersal potential, and seed germination, thus integrating biogeography, dispersal biology, and seed ecology in my work. This thesis also integrates disciplines related to biology such as microscopy and biostatistics as both were largely used throughout the study.

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APPENDICES

APPENDIX A: LIST OF HERBARIUM VOUCHERS

A1. Fruit Anatomy/Morphology

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. africana*** Willd.: **SOUTH AFRICA.** *Muir 156* (GRA); *Oliver 11852* (NBG). ***C. americana*** L.: **MEXICO, Alamos.** Arroyo el Mentidero at El Chinal Rd, 11.3 km S of Álamos, 26º54'45"N, 108º55'05"W, 240 m, 15 Mar 1994, *Van Devender 94-176* (UCR). **Guerrero.** Acapulco and vicinity, Oct 1894, Mar 1895, *Palmer 341* (GH). **Jalisco.** Mpio. La Huerta, near Chamela, 15 Apr 1982, *Lott 994* (MICH). **Sonora.** Mpio. Villa Pesqueira, southern foot of Sierra Pinta, about 33 miles S Moctezuma, ca 29º35'N, 110º01'W, ca. 2400', 15 Sep 1996, *Shortman 96-71* (ARIZ). **USA, Florida.** Lower Matecumbe Key, 3 Jan 1925, *Small et al. 11596* (NY); Pinelands, Buena Vista, Miami, Jan 1930, *Mosier s.n* (NY). ***C. angulata*** Engelm.: **SOUTH AFRICA.** Near Farm “Alwee,” on road from Swellendam to Infanta, norths slope of Pottberg in an area of sandy soil and shale chips, 24 Sep 1973, *Carlquist 4691* (RSA); *Beyers 12-1985* (NBG); *Williams 2690* (NBG). ***C. approximata*** Bab.: **CANADA, British Columbia.** Cherry Creek, Kamloops Lake, 30 Jul 1993, *Lomer 93-204* (UBC); Spences Bridge, 27 Sep 1992, *Lomer 92-306* (UBC). **USA, California.** *Abrams 457* (CAS). **Nevada.** *Hillman s.n* (RSA). **Utah.** Salt Lake City, 24 Sep 1905, 4300ft, *Jones s.n* (RSA). ***C. argentinana*** Yuncker: **ARGENTINA, Corrientes.** Quebrada de Escoipe, ayo. Malcanto, 1940 m, Ruta 33, 10 Apr 1980, *Krapovickas & Schinini 36049* (CTES). **Salta.** Chicoana, La Zanja, 10 km N de la Quebrada de Escoipe (Ruta 33), 2700 m, 10 Apr 1980, *Krapovickas & Schinini 36757* (CTES). ***C. australis var. australis*** R. Br.: **AUSTRALIA, New South Wales.** Warrabah National Park, flood area for Namoi River, 30°34'S, 150°55'E, 425m, 13 Mar 1994, *J. R. Hosking 938* (CANB). **Queensland.** Maryborough, Wallum not far from sea, 23 Oct 1948, *Clemens s.n* (RSA). **CHINA, Guangxi.** Near Guilin, Yangshan, Guangxi Institute of Botany, 2 Sep 1988, *Sykes CH99* (CHR). **NEW CALEDONIA.** Nouméa, Feb 1907, *Bonati 737* (S). ***C. australis var. tinei*** (Insenga) Yunck.: **HUNGARY.** Tisza-Koff. *Simonkai 2635* (NY). ***C. azteca*** Costea & Stefanovic: **MEXICO, Mexico City.** Federal District, Pedrigal, Valley of Mexico, 8 Oct 1896, *Pringle 6575* (S). **Puebla.** 18°10'48"N, 97°27'00"W, 9 Jul 1908, *Purpus 3554* (MO). **Querétaro.** 13.8 km SE MEX 120 on the road to Bucareli, Mpio. Piñal de Amóles, *Ramírez-Amezcua et al. 1099* (IEB). **San Luis Potosi.** Charcas, Jul - Aug 1934, *Lundell 5193* (S). ***C. boldinghii*** Urb.: **HAITI.** Massif de la Hotte, close to Dame Marie, on the beach, 1 Aug 1928, *Ekman 10455* (S). **MEXICO, Jalisco.** 8 km E of Chamela, 30-50m, lowland forest, 8-10 Dec 1970, *McVaugh 25140* (MICH). ***C. bonafortunae*** Costea & I. García: **MEXICO, Guanajuato.** Santiago de Maravatío, close to Ojo de Agua, 1900 m, 15 Oct 1989, *Rzedowski 49127* (IEB). **Michoacán.** Zamora, same location as the type, 11 Sep 2010, *García Ruiz & García 8375* (CIMI, WLU); 16 Oct 2010, *García Ruiz 8391* (CIMI, WLU). ***C. brachycalyx*** (Yunck.) Yunck.: **USA, California.** Butte County, west of Lunt Road, about 1/2 mile north-west of Highway 70, 1800 ft, 10 Aug 1988, *Ahart 6170* (CAS); Kern County, 1.5

mi N of Kernville, 2670 ft, 28 Sep 1962, *Howell 38877* (CAS); Santa Clara County, between Sunnyvale and Alviso, lower end of San Francisco Bay, 22 Aug 1933, *Keck 2473* (CAS). ***C. californica*** (Hook. & Arn.): **USA, California.** Colusa County, dry slopes along upper reaches of Dark Hollow Creek, Snow Mt., 6600 ft, 10 Sep 1974, *Heckard 3888* (JEPS); Los Angeles County, ridge south of Swartout Valley, 8200 ft, 31 Aug 1923, *Munz 7689* (RSA); Coastal Sage Scrub, Wash outside RSABG, 9 Oct 1965, *Massey 1005* (SMU). ***C. campestris var campestris*** Yunck.: **USA, California.** San Bernardino County, near pond, North Verde Ranch, near the Mojave River east of Victorville, 2800 ft, 10 Sep 1961, *Raven 16637* (RSA); San Bernardino Mountains, Los Rios Rancho at Oak Glen Forest Falls, 4800 ft, 11 Nov 1999, *Sanders 23249* (UCR). **Florida.** Dade County, 14000 SW 8th Road, Miami, 12 Nov 1974, *Corell 43759* (NY). **Utah.** Salt Lake County, Wasatch Range ca. 9 ½ mi. E of Salt Lake City, 5 mi. E of mouth of Emigration Canyon, 5600 ft, 1 Sep 1975, *Arnow 4694* (NY); Wayne County, Caineville, 4500 ft, 20 Jul 1894, *Jones 5653* (RSA). ***C. campestris*** Yunck. ***var. gymnocarpa*** Engelm.: **ECUADOR, Galapagos.** Espanola, undated, *Fagerlind & Wibom 3641* (S); *Fagerlind & Wibom 3658* (S); Isla Floreana, W side, along the trail from Black Beach towards the highland, 260 m, 6 May 1967, *Eliasson 2079* (S); Tortoise Country, about 8 mi. w. of Academy Bay, Indefatigable Is., 400 ft, 11 Apr 1930, *Svenson 242* (F); James Bay, James Island, 4 Jun 1932, *Howell 9642* (KEW). ***C. cassytoides*** Nees ex Engelm.: **SOUTH AFRICA, Zululand.** Mtunzini, ‘Twinstreams’ Farm, Apr 1977, *Garland s.n.* (NU); *Drege s.n.* (MO). ***C. cephalanthi*** Engelm.: **USA, Indiana.** Newton County, Near the Kankakee River N of Lake Village, 22 Sep 1927, *Deam 455* (NY); Sullivan County, Along a dredged ditch 6 mi southwest of Carlisle, 4 Oct 1931, *Deam 51439* (IND). **Michigan.**

Wayne County, Oakwood, 15 Sep 1918, *Farwell 5124* (NY). **Minnesota.** St. Louis County, Low shore ledges of Sand Point Lake at Harrison's Narrows international boundary, 11 Sep 1952, *Lakela 15846* (DAO). **Utah.** Salt Lake County, Salt Lake City, 4300 ft., 22 Aug 1880, *Jones s.n* (RSA). ***C. chapalana*** Yunck.: **MEXICO, Jalisco.** Rancho "Las Papas de Arriba", 4.5 km northeast of Guadalupe Victoria, 21°43′48″N, 101°39′48″W, 2260 m, 14 Oct 2000, *García & Harker 438* (IBUG); Mpio. Jocotepec: north face of Cerro Viejo, 2200 m, 17 January 1987, *Chazaro et al. 4408* (IBUG, XAL); Barranca de Sayula, al SE de San Pedro Tesistan, Nov 1993, *Machuca 7026* (MICH). ***C. chilensis*** Ker Gawl.: **ARGENTINA, Mendoza(?).** 1890-91, *Smith 1749* (S). **CHILE, Santiago.** Barnechea in Las Condes, hills near Nido de Aquilas school, 550 m, 21 Jan 1978, *Landrum 3073* (ASU). **Valparaíso.** Uspallata-Pass der chilenischen Hochcordillere, Juncal, auf den Bergen, 16 Jan 1903, *Buchtien s.n* (UPS). ***; C. chinensis var. applanata*** (Englm.) Costea and Stefanović: **MEXICO, Chihuahua.** Santa Eulalia, Buena Tierra Mine, ca. 28°37'N, 105°53'W, 6200 ft, 21 Aug 1948, *Hewitt 323* (GH). **Coahuila.** Near tip of Sierra de San Marcos, *Lewis s.n* (NY); Western Coahuila near Rosario, a railroad station 45 km W of Cuatro Cienagas, ca. 1100 m, 3-4 Sep 1941, *Johnston 8826* (GH); ca. 25 mi E of Americanos, 22 Aug 1937, *Wynd 754* (NY). **USA, New Mexico.** Albuquerque, 28 Sep 1932, *Casteller 7339* (UNM). ***C. chinensis*** Lam. **var. *chinensis***: **AUSTRALIA.** Western Australia, at NE end of airstrip One Arm Point, N Dampier Peninsula, W Kimberley, 16°27'S, 123°04'E, 9 Apr 1993, *Carter 628* (CANB, PERTH). **CHINA, Shantung.** Tsinanfu, 4 Sep 1930, *Chiao 3033* (GH, NY). **NORTH KOREA.** Rikiho, Sep 1930, *Dorsett & Morse 6336* (NY). ***C. cockerellii*** Yunck.: **ARGENTINA.** *Vargas 2600* CUS); *Vargas 19383* (CUS); *Nunez 28* (USM). *C.*

colombiana Yunck.: **COLOMBIA, Magdalena.** Near Riohacha, 30 m, 23 Dec 1944, *Haught 4535* (KEW, S). ***C. compacta*** Juss. ex Choisy: **USA, Alabama.** Dekalb County, Westfork Branch, De Soto State Park, Fort Payne, 850 ft, 13 Sep 1962, *Demaree 46247* (NY). **Arkansas.** Pulaski County, Des Moulins, 27 Sep 1931, *Demaree 8260* (NY). **Florida.** Alachua County, Along Hawthorn Rd. in small swamp ca. 4 mi E of Gainesville, 20 Nov 1943, *Rhoads & West s.n* (NY). **Indiana.** Crawford County, on Pilot knob, about 4 mi S of Marengo, 2 Sep 1937, *Deam 58335* (NY); Posey County, in a dried slough about ½ mi S of Half Moon Pond which is 10 mi SW of Mt. Vernon, 26 Sep 1920, *Deam 33,043* (NY). **Oklahoma.** McCurtain County, along creek in woods, 3 mi N of Broken Bow, 13 Oct 1957, *Waterfall 14770* (RSA). ***C. corniculata*** Engelm.: **BRAZIL, Bahia.** Piatá, Gerais da Inúbia, 22-26 km de Catolés, 1400 m, 10 Mar 1992, *Stannard et al. H51861* (G). **COLOMBIA.** Intendencia Meta Villavicencio, 450 m, 26-31 Aug 1917, *Pennell 1453* (GH). ***C. coryli*** Engelm.: **CANADA, Manitoba.** District de Provencher entre Saint-Pierre et Otterburne, 27 Aug 1956, *Bernard 5669* (DAO); District de Selkirk, Rivière Rouge, entre l'ile au Sucre et l'embouchure de la rivière qui Déboule, 23 Aug 1960, *Boivin & Champagne 13869* (DAO, UNB). **Saskatchewan.** Ravine S of Qu'Appelle Valley 4 m N of Stoney Beach, 20 m NE of Moose Jaw, *Ledingham 3160* (USAS). **USA, Indiana.** Perry County, top of wooded flood plain bank of the Ohio River just below Derby, 11 Oct 1931, *Deam 51589* (IND). **Missouri.** Barry County, Eagle Rock, 28 Sep 1896, *Bush 202* (MO, NY). **New York.** Tioga County, Long Island, sea cliff, 24 Sep 1928, *Ferguson 7181* (NY). **West Virginia.** Ohio County, on bank of Ohio River just below Eight Street, Wheeling, 16 Sep 1951, *Bartholomew 0-923* (NY). ***C. corymbosa*** Ruiz & Pav. **var. *grandiflora*** Engelm.: **MEXICO, Baja California Sur.**

Gulf of California, isla Partita in steep rocky draw facing the ocean, 22 Apr 1921, *Johnston 3222* (GH). **Jalisco.** Sierra de Manantlán Occidental W facing slopes of deep cool valley at headwaters of Arroyo Las Joyas, 19º35’15”-45”N, 104º15’30”-45”W, 1 Jan 1984, *Iltis & Guzman 29077* (MICH). **Tamascaltepec.** Cumbre-Cruz, Alnus woods, 15 Mar 1936, *Hinton et al. 8984* (GH). ***C. corymbosa*** Ruiz & Pav. **var. *stylosa*** Engelm.: **MEXICO, Hidalgo.** Mpio. San Salvador, km 135-137 on Laredo Hwy between Actopan and Ixmiquilpan, 8 Oct 1943, *Gilly & Cany 5* (MICH). **Mexico.** Vertiente sur del Cerro Tlapacoya, mpio. Chalco, 7 Nov 1971, *Rzedowski 28752* (MEXU). **Veracruz.** Zacualpan, Dec 1915, *Purpus 7564* (GH). ***C. costaricensis*** Yuncker: **MEXICO, Chihuahua.** Las Gallinas, +/- 15 km west of Yepachi, 28º27’ N 108º31’W, 1500 m, 22 Oct 1984, *Levin 1440* (SD). **Durango.** El Saltito, Nombre de Dios, 13 Sep 1984, *Jienez & Acevedo 35* (MEXU); San José de Parrilla, +/- 4 km al W. 23º43’99”N 104º8’99”W, 2150 m, 25 Oct 1983, *González & Acevedo 2758* (MEXU). **Jalisco.** Isla Alacranes (Chapala), Maleza, 1500 m, 17 Sep 1964, *Cota 77* (MEXU). ***C. cotijana*** Costea & I. García: **MEXICO, Michoachán.** Cotija, Los Gallineros, ca. 1900 m, 19 Apr 1991, *Ruiz & Olmos 3289* (UCR) isotype. ***C. cozumeliensis*** Yuncker: **GUATEMALA.** *Kellerman 6589* (F). **MEXICO.** *Calzade & Nievea 9427* (XAL); *Vazquez 176* (MEXU). ***C. cristata*** Engelm.: **ARGENTINA, Buenos Aires.** Baradero, Estancia “Los Alamos”, Fundación Figueroa Salas, Final de la Reserva, 27 Oct 2003, *Robles 1511* (MO). **Córdoba.** Río Tereero, barranca, 10 Jan 1940, *Burkart 10399* (MO); *Lossen 314* (KEW). Rio III, entre Almafuerte y el Embalse del Rio III[2], 27 Feb 1944, *Hunziker 4927* (S). **Corrientes.** Esquiina, Isla frente a Esquina, 30 Nov 1974, *Krapovickas et al. 26901* (G). **La Rioja.** Sañogasta, Villa Bustos, 10 Dec 1951, *Pentzell 19170* (SI). ***C. cuspidata*** Engelm.: **USA,**

Indiana. Posey County, low fallow field along the Wabash River ca. 2 mi S of New Harmony, 24 Sep 1920, *Deam 33011* (IND, NY). **Kansas.** Meade County, park, 2460 ft, 18 Sep 1944, *Horr E476* (BRIT, SMU). **Missouri.** St. Louis, 11 Aug 1891, *Eggert s.n* (CAS). **New Mexico.** Clayton, 24 Sep 1907, *Evans s.n* (NMC). **Texas.** Gray County, 15 mi S of Lefors, Ste. 291, 28 Sep 1969, *Correll 38056* (CAS, NY); Robertson County, SW ¼ Round Prairie Quad., U.S.G.S. 1966, Tx. Hwy. 7 and the Navasota River, 9 Oct 1981, *Starbuck 1198* (BRIT). ***C. decipiens*** Yunck.: **MEXICO, Zacatecas.** Ca. 92 air miles NE of Cd. Zacatecas, 89.6 road miles (144.5 km) NE of Hwy 45 along Hwy 54 to Saltillo, about 2 miles NW of highway opposite turnoff to Majoma, (25 miles S of San Tiburcio) then to the first gate in the fence (to right) into the flats near a small milpa, near 23°52'N, 101°44'W, 1950 m, 19 Oct 2001, *Henrickson 22781* (RSA). ***C. deltoidea*** Yunck.: **MEXICO, Michoacan.** Monte Leon, 11 Nov 1892, *Pringle 5350* (NMC). ***C. denticulata*** Engelmann: **USA, California.** Inyo County, steep canyon leading into Saline Valley from the south, 36°34'N 117°35'W, about 5000-6000 feet, 16 Sep 1960, *Thomas 8904* (RSA); Riverside County, Morongo Wash at the San Bernardino County line, 2400 feet, 11 Oct 1932, *Wolf 4282* (BRIT); San Diego, Borego Valley, on Larres, 7 Apr 1940, *Howe 991* (SD). ***C. desmouliniana*** Yunck.: **MEXICO, Baja California**. Chavez Ranch airstrip, about 5 mi. NW of Mulegé, 22 Oct 1962, *Wiggins 18130* (MEXU). **Sonora.** Hills near Altar, 26 Aug 1884, *Pringle s.n* (NY); Low basaltic hills 15 mi S of La Palina, between Hermosillo & Guayanas, 2 Sep 1941, *Wiggins & Rollins 232* (GH); Hwy W from Hermosillo to Bahía Kino, 12 mi W of the Hwy Jct. in Hermosillo, 800 ft, 29 Jan 1963, *Dunn et al. 14130* (NY); At coast on north side of headland about 10 mi. due S of Desemboque, 22 Mar 1978, *Spellenberg 4943* (MEXU, NMC); Terrace above arroyo at mouth of Nacopuli Canon, southeast end of Cerros Las perinolas, north of San Carlos, 28°01’N lat., 111°03’W long, 26 Apr 1985, *Burgess 6949* (MEXU). ***C. epilinum*** Weihe: **CANADA, Quebec.** Shefford, Saint-Alphonse, sur lin, ferme de M. Wilfrid Viau, 30 Jul 1941, *Cartier s.n* (DAO, QFA); St. Alphonse, 30 Jul 1941, *Barabe 16914* (BRIT, SMU); Sainte-Hélène, Kamouraska, 15 Aug 1942, *Cayouette s.n* (QUE). **Lower Canada.** Flax fields, 4 Aug 1880, *Pringle 204788* (CAS). **SWEDEN, Angermanland.** Suecia, Paroecia Arnäs, Idbyn, 22 Aug 1937, *Samuelsson 1317* (RSA). ***C. epithymum*** (L.) L.: **CANADA, British Columbia.** Kootenay Lake, Balfour Bay, 16 Aug 1947, *Turner 5983* (ALTA). **MEXICO, Mexico.** Valley of Mexico, 7800 ft, 27 Jun 1901, *Pringle 8514* (NMC). **USA, Idaho.** Custer County Challis, edge of alfalfa field, 5400 ft, 15 Jul 1916, *Macbride & Payson 3219* (DS, RSA). ***C. erosa*** Yunck.: **MEXICO, Sonora.** Bank of water course 15 mi SE of Magdalena on rd. to Cucurpe, 11 Sep 1934, *Wiggins 7123* (NY); Agua Prieta, ca. 7.5 km (by air) southwest of Agua 31°15’34”N, 109°36’34”W, 1233 m, 3 Oct 2004, *Van Devender et al. 2004-1199* (WLU). **USA, Arizona.** Pima County, Baboquivari Canyon, 3500-4500 ft., *Kearney & Peebles 10377* (CAS, RSA); *Kearney & Peebles 10422* (CAS, RSA); Baboquivari Mts, 21 Aug 1932, *Peebles 8998* (RSA); Baboquivari Mts, 19 Sep 1931, *Jones 28731* (CAS). ***C. europaea*** L.: **BELGIUM, Luxembourg.** Chassepierre, bord de la Semois en amont du village, 30 Sep 1975, *Duvigneaud & Lambinon 75B953* (QUE). **DENMARK, Dyrehaven.** Copenhagen, 6 Aug 1970, *Svendsen 329* (NY). **FINLAND, Kemiö**. Stenhol, 60°05’N, 22°45’E, Cliff near the old limestone quarries, 16 Aug 1978, *Alava et al. s.n* (NY, RSA). **GERMANY, Alsfeld.** Kestrich, Sengesweg, 12 Aug 1972, *Hupke s.n* (USAS). **Thüringen.** 20 Aug 1900, *Rudolph s.n* (RSA). **NETHERLANDS, Gelderland.** Base of levee in the Ooypolder E of

Nijmegen, near Tiengeboden, 12 Sep 1959, *Hekking 635* (NY). **SWEDEN.** Blekinge County, Sölvesborg, 23 Jul 1932, *Holmgren 19784* (SD). ***C. exaltata*** Engelm.: **USA, Texas.** Aransas County, Goose Island State Park, 1 Oct 1975, *Snyder 472* (SMU); Dallas, Sep 1885, *Reverchon 663* (CAS); Hays County, Stagecoach Ranch subdivision off Ranch Road 3238, S of Hamilton Pool Preserve, 24 Sep 1992, *Westlund s.n* (CAS); Johnson County, 97°36'N 32°15'W, Cleburne State Park, about 12 mi (19 km) WSW of Cleburne, incised valley along West Fork of Camp Creek at end of Park Road 21.6 mi (10 km) SW of US Hw 67, 27 Oct 1997, *Sanders 4270B* (BRIT); San Patricio County, local, E edge of perimeter road on W side of installation, SW of jct. F. M. 2725 and F. M. 1069, Naval Station Ingleside, Port Ingleside Quadrangle, 25ft, 11 Sep 1992, *Carr 12341* (BRIT); Val Verde County, Dry Devils River, E of crossing of road from "big house" N to mouth of Jane Hollow, Dolan Falls Ranch, Dolan Springs Quadrangle, 1530 ft, 30 Sep 1992, *Carr 12418* (SMU). ***C. foetida*** Hook. & Arn. **var. *foetida***: **ECUADOR, Azuay.** Laguna Llaviuco (Surucuchu) W of Cuenca, 3150 m, 25 Feb 1993, *Harling & Ståhl 26675* (S); **Canar.** Parroquia Bayas, Valley of rio Tabaca, ca. 15 mi NE Azugues, 8000 ft, 27 Sep 1944, *Prieto & Camp P-111* (S). **Chimborazo.** En el camio de Sibambe a Alausí, Reg. Interandina, 2500 m.s.m, 25 Aug 1943, *Solís 5599* (F). ***C. foetida*** Kunth ***var. pycnantha*** Yuncker: **ECUADOR, Cotopaxi.** Cantón Sigchos, Triunfo Grande, entrada a la comunidad "El Valle", 2765 m, 00°34'02"S 78°57'41"W, 31 Jul 2003, *Ramos et al. 6775* (MO). **Pichincha.** West of Nono, 2700 m, 12 Jun 1968, *Harling et al. 10258* (US). **PERU.** *Plowman et al. 14291* (F). ***C. friesii*** Yunck.: **ARGENTINA, Tucumán.** Infiernillo, 37 km de Tafí del Valle, 2650 s.m., 18 Mar 1972, *Krapovickas & Maruñak 21898* (CTES). ***C. glabrior*** (Engelm.) Yunck.: **MEXICO, Coahuila.** Parras de la Fuente, Sierra de Parras, Rancho El Tunal 31.2 km (19.5 mi) al E de Parras por carretera, 4.8 km (3 mi) al S por terracería hasta la Puerta del sur del rancho, 1870 msnm, 21 Aug 1982, *Cowan 3644* (MEXU); ca 35 (air) miles SSW of Cuatro Cienegas, in northern slope of limestone Sierra de Los Alamitos, ca 9.2 (rd) miles S of El Hundido, in Izotal, 26º30'N, 102º17'W, 4650 ft,, 29 Sep 1973, *Henrickson 13676c* (RSA); Chojo Grande, 27 mi SE of Saltillo, State of Coahuila, 16 Jul 1905, *Palmer 723* (GH, MO). **USA, Texas.** Deaf Smith County, 15 mi N & 15 mi W of Hereford, 23 Jul 1966, *Waller 962* (TEX/LL). ***C. globiflora*** Engelm.: **ARGENTINA, Catamarca.** Andalgalá, El Condado, 25 Feb 1916, *Jorgeusen 1613* (SMU). **Jujuy.** Tumbaya, ruta hacia Tilcara, Barcena, a 1700 m s. n., 14 Mar 1994, *Múlgura et al. 1199* (MO). **BOLIVIA.** Andian reg, Cotaña am Llimain, 2450 m, undated, *Buchtien 133* (F). **Cinti.** Sucre, Puca Rhasa prope Tacaquira, 21-27 Mar 1934, *Hammarlund 341* (S). **Murillo.** La Paz, below Obrajes, ca 3300m, 27 Jan 1921, *Asplund 2088* (S, UPS). ***C. globulosa*** Benth.: **CUBA, Oriente.** El Cobre in fructicetis communis, 6 Oct 1916, *Ekman 7839* (S). **PUERTO RICO, Culebra.** Playa Flamenco, at end of road along beach, 1m, 15 Jul 1989, *Axelrod 1154* (UPRRP). **Guayama.** Bo. Palmas, Rte 712, km 16.7, 1 km along farm track following ridge S of road, ca. 350-400 m, 21 Jan 1991, *Axelrod 1875* (UPRRP). ***C. glomerata*** Choisy: **USA, Indiana.** Lake County, marsh 2 mi N of Hobart, 17 Sep 1930, *Deam 49868* (NY). **Kansas.** Riley County, Horse Pasture, NW ¼ sec. 13, T 11 S, R 7 E, 10 Sep 1979, *Freemon 293* (NY). **Nebraska.** Minden, Sep 1996, *Hapeman 21141* (NMC). ***C. gracillima*** Engelm.: **MEXICO, Mexico.** 3km W of Ixtapan del Oro on rd. to Zitacuaro, Mich, 19º15'N, 100º16'W, 1900m, 3 Dec 1983, *Solheim et Benz 1073* (NY); *Hinton 2497* (F). **Sinaloa.** Mazatlan, approx. 23º12'N, 106º25'W, 0-30m/0-100ft, 20 Nov 1926, *Jones 22408*

(UCR). ***C. grandiflora*** Kunth: **ECUADOR, Carchi.** Tulcan Canton, Colonia Huaqueña, Loma El Corazón, Bosque remanente, Suelos volcánicos, Bosque muy húmedo Montano, 77º42’W 00º35’N, 3000 m, 8 Jul 1992, *Tipax et al. 1563* (QCNE, MO); Ca. 2 km along the road El Angel-Tulcán, hedges along the road, 77º55’W, 0º38’N, alt. 3150-3300 m, 14 May 1973, *Holm-Nielsen et al. 5214* (AAU); Km 3 on old road El Angel-Tulcan, 77º55’W 00º39’N, alt. 3200 m, 13 Aug 1985, *Laegaard 54907A* (QCA). **PERU, Cuzco.** Cuzco, Mar 1929, *Herrera 2354a* (F). **Huerta.** Conima, 3900 m, 6 Mar 1948, *Aguilar s.n.* (MO). **Paucartambo.** Cusco, along Río Paucartambo, S of Paucartambo, 13º18’S 71º40’W, 3 Oct 1995, *Croat 78149* (BRIT, MO). **San Sebastián.** Cusco, rocky slope of canyon, alt. 3300-3400 m, 25 Apr 1925, *Pennell 13613* (F). ***C. gronovii var. gronovii*** Willd. ex Schultes: **CANADA, Quebec.** Cté de Chauveau, St-Augustin, face au Séminaire, 14 Aug 1975, *Pérusse 75-39*1 (QFA); Comté de Lotbinière, Sainte-Croix-de-Lotbinière, 2 km au sud-est de la Pointe au Platon, 46º39’10”N 71º49’30”W, 24 Oct 1989, *Garneau et Roy 89-626-M* (QFA); Ottawa Distr. Near Gatineau Point, 2 mi N of Ottawa, 14 Sep 1952, *Dore & Erskine 14109* (DAO). **New Brunswick.** Kent County, St. Louis Parish, 2.6 mi into Kouchibouguac National Park on Cap St. Louis Rd. along shore past gate, 9 Sep 1977, *Munro & Cody 1786* (DAO). **Ontario.** near Long Point Provincial Park, 42°35'43.34"N, 80°27'2.66"W, 19 Oct 2014, *Costea & Ho s.n.* (WLU); Waterloo, Grand River, Claude Dubrick trail, 43°30'12.02"N, 80°29'37.97"W, 17 Oct 2014, *Costea & Ho s.n.* (WLU). ***C. gronovii var. latiflora*** Engelm.: **USA, Indiana.** *Yatskievych 84-162* (IND). **Texas.** Wood County, 5 mi NE of Crow, Border of mat, Lake Ellis, 2 Sep 1942, *Lundell 11721* (SMU). ***C. harperi*** Small: **USA, Alabama.** Marion County, sandstone outcrop area by North Fork Creek S. of Hackleburg on US 43, 7 Sep 1968, *Kral 32878*

(SMU); undated, *Damaree 46295* (NY). ***C. haughtii*** Yunck.: **ECUADOR, Guayas.** Salinas, La Puntilla, 6 Apr 1939, *Asplund 5618* (G, KEW, S). **Manabi.** Manta, 6 Apr 1955, *Asplund 15974* (G, S, UPS). ***C. howelliana*** Rubtzoff: **USA, California.** *True 6716* (CAS); Shasta County, between Goose Valley and Burney Valley, ca. 3.5 mi NNW from Burney, 3200 ft, 8 Aug 1988, *Taylor 10026* (CAS). ***C. hyalina*** Roth.: **INDIA, Delhi.** Delhi University ridge, 8 Aug 1954, *Mkhanno 249* (ARIZ); Delhi University campus, 690 ft, 12 Feb 1961, *Pushpander s.n.* (CANB). **South West Africa.** Mar 1931, *Bosch 25022* (BOLUS). ***C. iguanella*** Costea & I. García: **MEXICO, Jalisco.** Wooded hills near Guadalajara, 2 Sep 1893, *Pringle 4529* (F, GH, MEXU, S). ***C. incurvata*** Progel: **PARAGUAY, North Paraguay.** In regione cursus superioris fluminis Apa, Dec 1901, *Hassler 8178* (F); Zwischen Rio Apa und Aquidaban, 1908/1909, *Fiebrig 5083* (K). **Caaguazú.** 4 km al N de Yhú en cerrado, suelo arenoso, 6 Feb 2003, *López et al. 243* (CTES). ***C. indecora*** Choisy: **HONDURAS.** Malfredi Lagoon, 100 ft, 1, May 1933, *Schipp 1161* (S). **USA, Arizona.** Gila County, along roadside north of Winkelman, south of Globe, 5000 ft, 16 Aug 1973, *Moldenke 27920* (AAU); Pinal County, near Peppersauce Campground on N side of Santa Catalina Mountains, ca. 15 miles SE of Oracle, 4700 ft, 30 Aug 1989, *Austin 7599* (RSA). **Nebraska.** Arthur County, Arapaho Prairie, T18N R39W Sect 31, 32, ca. 1200 m, 27 Jul 1977, *Vescio & Kruse 174* (NY). **Nevada.** Nye County, U.S. Atomic energy commission's Nevada test site and vicinity, Abandoned field, Rt 52 near Rt 16 jnct., Cent. Pahrump, 2600 ft, 26 Sep 1970, *Beatley s.n.* (RSA). **New Mexico.** *Spellenberg et al. 3427* (NY). **Utah.** Salt Lake City, 4200 ft, 25 Aug 1879, *Jones s.n.* (RSA). ***C. jalapensis*** Schlechtendal: **GUATEMALA, Totonicapán.** Region of Chui-quisís, above Totonicapán on Rd. to Desconsuelo, 2500-

2800 m, 23 Jan 1948, *Standley 84397* (NY). **MEXICO, Chiapas.** Amatenango del Valle, 5800 ft, 26 Jul 1966, *Breedlove 14669* (MICH); San Cristóbal de Las Casas, Santa Cruz in San Felipe, 15 Nov 1986, *Ton & Lopez 9826* (GH); Tenejapa, Paraje Shohleh, 2560 m, 12 Jan 1966, *Ton 603* (NY). **Hidalgo.** El Chico, alrededores de Peña del Cuervo, 5 km al SE de El Chico, 2800 m, 20 Jul 1986, *Medina 3102* (MICH). **Puebla.** Near Huauchi-nango, 5000 ft, 27 Mar 1945, *Sharp 45380* (NY). ***C. japonica*** Choisy: **CHINA, Bizen.** 7 Oct 1925, *Masamune s.n.* (NY). **Guizhou.** Songtaoi Xian, vicinity of Lengjiaba in the vicinity of the confluence of the Xiaohe and Dahe rivers, NE side of the Fanjing Shan mountain range, 820-1120 m, 5-9 Oct 1986, *Bartholomew et al. 2309* (RSA). **Shaanxi.** Foping county, 500 m, 16 Oct 1998, *Weiqing 619* (MO). **JAPAN, Hondo.** 17 Oct 1952, *Hashimoto 853* (NY). **Honshu.** Kyoto, Anshu, Yamashina-ku, Kyoto-shi, 70 m, 25 Oct 1998, *Tsugaru et al. 27202* (MO). ***C. killimanjari*** Oliv.: **MALAWI.** C. region, Lilongwe Nature Sanctuary, 1050 m, dry mixed woodland near river, 29 Jun 1987, *LaCroix 4559* (MO). **ZIMBABWE.** *Eyles 352* (J). ***C. legitima*** Costea & Stefanović: **MEXICO, Baja California.** On Amaranthus sp. Along arroyo 3 ½ mi S of La Paz, about 24º09’N, 100º15’W, 3 Nov 1959, *Wiggins 15294* (MEXU). **Sonora.** Cerro La Antena, 1 km north of Microondas La Cabana; Sinaloan thornscrub, 27º27’45”N, 109º46’20”W, 200 m, 19 Sep 1994, *Van Devender 94-603* (ARIZ). **USA, Arizona.** Douglas, 11 Sep 1948, *Jones s.n.* (RSA). ***C. lehmanniana*** Bunge: **KAZAKHSTAN.** Turkestan, 12 Feb 1910, *Fedtschenko 1* (NY). **UZBEKISTAN.** Samarkand, 30 Jul 1914, *Knorring 104* (NY); Syr-Darja, Tashkent, 1 Aug 1924, *Vvedensky 153* (NY). ***C. leptantha*** Engelm.: **MEXICO, Baja California.** 10 miles inland from Bahia de Los Angeles near Agua Armaga, near 29º00’N 113º45’W, 22 May 1960, *Lindsay 2928* (SD); On arid hills near

Club Aereo airport, Mulegé, 9 Apr 1963, *Wiggins & Wiggins 18219* (K); Bahia de Los Angeles, southwest shore, near intersection of a road leading to the beach, along roadside, 14 Mar 1992, *Fritsch & Fritsch 1337* (RSA). ***C. lindsayi*** Wiggins: **MEXICO, Sinaloa.** On talus slope 55.7 miles E of Villa Union, 6210 ft, 18 Mar 1955, *Wiggins 13185* (MO). ***C. longiloba*** Yunck.: **BOLIVIA, Azero.** Chuquisaca, Estsoión Experimental Zootecnica "El Salvador (27 km NW de Carandaytí, 63°13'W, 20°45'S, 500 m, 800 m NE de la entrada a la Est. Exper., 13 Apr 1977, *Krapovickas & Schinini 31255* (CTES). **PARAGUAY, Chaco.** Parque Nacional Defensores del Chaco, a 30 km de Aguarrica en dirección a Lageren za, 21 Oct 1980, *Casas & Molero s.n.* (MO). ***C. lupuliformis*** Krock.: **AUSTRIA.** Ca. 1.2-1.25 km SE Baumgarten an der March, 14 Aug 2004, *Barta 2004-177* (NY); ca. 0.25 km ENE Sierndorf an der March, 28 Jul 2004, *Barta 2004-302* (NY). **HUNGARY.** Prope Budam, 18 Aug 1898, *Degen s.n.* (RSA). ***C. macrocephala*** Schaffner: **MEXICO, Baja California.** Along road to El Valle Perdido 4 mi. E of La Paz-Todos Santos road, about 23°43'N 110°10'W, 350 m, 6 Nov 1959, *Wiggins 15323* (K). **Sinaloa.** Cosalá, 24°24'16"N 106°41'26"W, 4 Jan 2006, *Van Devender 2006-15* (WLU); *Van Devender 2006-16* (WLU). **Sonora.** Álamos, 27°06'34"N 108°42'58"W, 5 Oct 2006, *Van Devender 2006-1240* (WLU); Arivechi, 28°49'15"N 109°10'24"W, 15 Sep 2006, *Van Devender 2006-872* (WLU); San Javier, 28°34'48"N 109°40'15"W, 31 Aug 2001, *Van Devender 2001-758* (WLU); Yécora, 28°25'48"N 109°11'31"W, 1 Sep 2001, *Van Devender 2001-774* (WLU); 28°25'53"N 109°09'34"W, 16 Sep 2006, *Van Devender 2006-901* (WLU); 28°20'50"N 109°07'17"W, 21 Sep 1998, *Van Devender 98-1524* (WLU). ***C. macvaughii*** Yunck.: **MEXICO, Michoacán.** Churumuco, 18°37'09"N 101°36'48"W, 8 Sep 2007, *Steinmann & Ramírez*

5870 (IEB, WLU); Apatzingan, 19º01’40”N 102º17’43”W, 13 Mar 2010, *García-Ruiz & Alvarez 8335* (CIMI, WLU). ***C. mexicana*** Yunck.: **MEXICO, Jalisco.** Autlan, 16 May 1990, *Cuevas & Núñez 3834* (IEB, ZEA). ***C. micrantha*** Choisy: **CHILE, Atacama.** Llano de Churque, S de Copiapó, 27º38’S 70º28’W, 7 Oct 1987, *Teillier 894* (MO); Huasco, Isla Guacolda, 5-15 m, 26 Oct 1938, *Worth & Morrison 16235* (MO). ***C. microstyla*** Engelm.: **ARGENTINA.** *Boelcke et al. 10243* (CTES). ***C. mitriformis*** Engelm.: **MEXICO, Chihuahua.** Wooded canyons, Sierra Madre, 2 Oct 1887, *Pringle 1342* (MO). **Coahuila.** Ca 22 (air) miles WNW of Cuatro Cienegas, in upper portio of limestone Canyon de la Hacienda, below 1st lumber camp in Sierra de la Madera, 5000-6000 ft, near 27º04’N 102º25’W, 28 Sep 1973, *Henrickson 13638* (RSA). **Michoacan.** Mountains, near Lake Chapala, 18 Nov 1892, *Pringle 4330* (S). **Nuevo Leon.** Hacienda Pablillo, Galeana, 8 Jan 1936, *Taylor 38* (F). ***C. monogyna*** Vahl.: **CANADA, Ontario.** Mississauga, grown in greenhouse at U of T, 13 Jul 2015, *Stefanovic s.n.* (TRTE, WLU). **UZBEKISTAN, Andijan.** 25 Jul 1911, *Knorring & Minkwitz 1590* (NY). ***C. natalensis*** Baker: **BRAZIL, Rio Grande do Norte.** Natal, *Rudatis s.n.* (NBG). ***C. nevadensis*** I. M. Johnston: **USA, California.** Inyo County, lower end of Westgaard Grade near Big Pine, 5400 ft, 10 Jul 1938, *Jaegar s.n.* (RSA). **Nevada.** Rye County, sands W of Lathrop Wells, 2500 ft, Amargosa drainage basin, 19 Jun 1969, *Beatley s.n.* (RSA). ***C. nitida*** Meyer: **SOUTH AFRICA.** At memorial along contour trail to summit of 11:30 Peak, Clock Peaks, near Swellendam, 2000 ft, 11 Dec 1973, *Carlquist 5082* (RSA). ***C. obtusiflora*** H.B.K. ***var. glandulosa*** Engelm.: **MEXICO, Tamaulipas.** At edge of lake, 600 ft, 24 Jul 1939, *Chase 7563* (MO). **USA, Texas.** Dallas, undated, *Reverchan 2878* (MO); Dallas County, bordering lake, Bachmans Dam, 24 Aug 1944, *Lundell 11626*

(SD); Rio Grande, unknown date 1848, *Wright s.n.* (MO); Bastrop County, Sep 1937, *Tharp s.n.* (MO); Austin, 14 Aug 1934, *Tharp s.n.* (UC1). ***C. obtusiflora var. obtusiflora*** Kunth: **ARGENTINA, Corrientes.** Concepción, 21 Dec 1977, *Tressens & Sesa 12026* (MO); near San Cosme, 29 Jan 1970, *Pedersen 9628* (MO); Ituzaingó, Isla Apipé Grande, huerto San Antonio, 19 Nov 1976, *Guaglianone et al. 138* (SI). **USA, Delaware.** Bank of creek, Middletown, 6 Aug 1911, *Churchill 672* (MO). ***C. occidentalis*** Millspaugh: **USA, California.** Los Angeles County, San Clemente Island, Sand dunes of coastal terrace at West Cove, SW of the new landing field, 20 feet, 17 May 1991, *Ross et al. 5087* (RSA); Marin County, Mt. Tamalpacs, 1200 ft, 8 May 1922, *Munz 6445* (RSA); Siskiyou County, Siskiyou Mountains, Lily Pad Lake, 21 Aug 1958, *Wheeler 7417* (RSA). ***C. odontolepis*** Engelm.: **MEXICO.** Unknown locality, 1851-1852, *Wright 1624* (K). **Sonora.** Near a deserted Rancho on rocky hill sides, 15 Sep 1851, *Wright 529* (MO). ***C. odorata*** Ruiz & Pavon: **ECUADOR, Chimborazo.** Cañon of the río Chanchan near Huigra, 4000 – 4500 ft, 7-14 May 1945, *Camp 3027* (S). **PERU, Huarochiri.** Lima, San Mateo, 3200 m, 28 May 1940, *Asplund 11177* (S); Lima, along Rio Chillón, above Obrajillo, Open rocky slopes, 2800 – 3200 m, 13-23 Jun 1925, *Pennell 14382* (S). ***C. pacifica*** Costea & M. A. R. Wright: **USA, California.** Humboldt County, Humboldt Bay near Table Bluff, 28 Aug 1941, *Harris 1175* (B); Santa Cruz, 30 Jun 1881, *Jones 13467* (MO); Thorne, 8 Aug 1965, *Gveaelt 75280* (UC). ***C. paitana*** Yunck.: **PERU, Paita.** Piura, 150 m, 16-17 Mar 1927, *Weberbauer 7762* (F); Pariñas Valley about 6 miles inland, growing on *Cryptocarpus cordifolia* (F-63), 26 Dec 1928, *Haught F-100* (F). ***C. parodiana*** Yuncker: **ARGENTINA, de Salta.** La Caldera, Yacones, Laderas de cerros al W del camino y rio, 1700 m, 29 Apr 1990, *Novara & Bruno 9821* (S). **Jujuy.** Ledesma, camino de Fraile pintado a El Aibal, 13 Nov 1992, *Kiesling 8236* (MO); illegible locality, 26 Feb 1901, *Kurtz 11792* (S). ***C. partita*** Choisy: **BRAZIL, Maranhão.** Lorêto, Ilha de Balsas region, between the Balsas & Parnaíba Rivers, About 35 km S of Lorêto, 100 m NE of main house of Fazenda Morros, 7°23'S, 45°4'W, 300 m, 3 Apr 1962, *Eiten & Eiten 3961* (US). **Piaui.** Corrente. BR-135, 500 m S da ponte sobre o rio Corrente, 10°27'S, 45°9'W, 460 m, monte abierto, 3 Apr 1983, *Krapovickas et al. 38723* (CTES). **COLOMBIA.** Forest, Rincon Hondo, Magdalena Valley, 5 Aug 1924, *Allen 267* (F, MO). **VENEZUELA, Lara.** En hombrillo asfaltado y sustrado terrestre a orilla de la carretera Bobare-Aguada Grande 17 km antes del crusero a la ultima población, Edo. Lara, 29 Aug 1981, *Ponce & Trujillo 342* (OAC). ***C. parviflora*** Engelm. ***var. elongata*** Engelm.: **BRAZIL, Goiás.** *Oliveira et al. 745* (US). ***C. pentagona*** Engelm.: **USA, Florida.** Levy County, border of salt marsh on *Solidago stricta*, Cedar Key, 10 May 1958, *Godfrey 56580* (NY). **Indiana.** Cass County, in a sandy fallow field about 1 ½ mi NW of Lake Cicott (P. O.), 1 Oct 1940, *Deam 60219* (IND); Starke County, Abundant in a very sandy fallow field 2 ½ mi SE of North Judson, 18 Jul 1930, *Deam 49139* (IND). **Kansas.** Trego County, 19 mi S and 2 mi W of Collier, 6 Aug 1952, *David & Harr 4136* (NY). **Massachusetts.** Tonset, 27 Aug 1901, *Edmondson 2777* (NY). **Michigan.** Kalamazoo County, Fort Custer, 12 Aug 1945, *Hanes 4541* (NY). **Texas.** Hunt County, 7.4 mi E of Greenville, fine sandy clay roadside, 8 Jun 1953, *Shinners 15030* (TEX/LL). **Virginia.** Beoford County, undated, *Curtiss s.n* (NY). ***C. planiflora*** Ten.: **PALESTINA.** Lentil field, lentils secondary host, near village of Kesan, near Tekoa, 31°35'N, 35°15'E, 22 May 1987, *Musselman 10461* (RSA). Unknown locality, undated, *Priva 82* (S). ***C. plattensis*** A. Nelson: **USA, Wyoming.** Goshen County, T25N R60W Sec 31 S ½ SW ¼, 3 mi. NE of Torrington, 4200 ft, 15 Aug 1993, *Dorn 5470* (MO); Platte Canon, 27 Aug 1896, *Nelson 2741* (MO). ***C. platyloba*** Progel: **ARGENTINA, Misiones.** San Ignacio, Balneario Teyú Cuaré, 27º16'S 55º35'W, 23 Nov 1995, *Guaglianone et al. 3025* (K). **BRAZIL, Rio Grande Do Sul.** Osório, 3 m, 19 Jan 1951, *Sehnem 5597* (B). **COLOMBIA.** Intendencia meta Villavicencio, grassy plains E of V, 450 m, 26-31 Aug 1917, *Pennell 1453* (MO). ***C. polyanthemos*** Schaffner ex Yunck.: **MEXICO, Sonora.** Monctezuma, 29º39'44"N 109º37'13"W, 14 Sep 2006, *Van Devender 2006-809* (WLU); 31 mi NW Queriego, 6 Mar 1933, *Wiggins 6457* (US). ***C. potosina*** Schaffner: **MEXICO, Estado de Mexico.** Pedrigal (lava beds), Valley of Mexico, 7300 ft, 8 Oct 1896, *Pringle 6575* (MO). **Guanajuato.** Mpio. De San Diego de la Unión, 11 Sep 1997, *Pérez et al. 3707* (IEB). ***C. prismatica*** Pav. ex Choisy: ***C. punana*** Costea & Stefanović: **ECUADOR, Guayas.** Isla Puná, Río Hondoto la Florida, 02º49'S, 80º01'W, 0 m a.s.l., 7 Jun 1987, *Madsen 63850* (AAU); El Placer, 0 – 5 km on path toward Río Hondo, 02º48'S, 80º00'W, 8 Sep 1987, *Madsen 63936* (AAU). ***C. rugosiceps*** Yunck.: **GUATEMALA, Quiché.** San Miguel Uspantan, Apr 1892, *Heyde & Lux 2912* (GH). **MEXICO, Oaxaca.** Sierra Madre del Sur, 20 Jun 1962, *Webster 11561* (GH). **Queretaro.** Jalpan, 21°28'11"N 99°09'02"W, 3 Sep 1005, *Pérez-Calix 4603* (IEB, WLU). ***C. runyonii*** Yuncker: **USA, Texas.** Hidalgo County, off U.S. 83, 2 miles east of Sullivan City on gravelly hill, 31 Mar 1941, *Lundell & Lundell 9827* (BRIT). ***C. salina*** Engelm.: **CANADA, British Columbia.** Vancouver Island, vicinity of Victoria, 24 Jul 1893, *Macoun 695* (K). **USA, Nevada.** Caliente, Lower Temperate life zone, 27 Aug 1912, *Jones s.n.* (RSA). ***C. sidarum*** Liebm.: **MEXICO, Yucatán.** Chocholá, 4 km al E de la población de Chocholá, saliendo del libramiento del pueblo desde la caarretera Mérida-

Campeche, a lo largo del camino de terracería Chocholá-Yaxcopoil, unos 25 km al SO de Mérida, approx. 20º44’30”N, 89º47’20”W, 20-50 m, 7 Nov 2001, *Carnevali et al. 6425* (CICY); unknown locality, 1841-1843, *Liebmann 02359* (S). **NICARAGUA, Managua.** Near Parque de Las Madres; ca 12º08’N, 86º16’W, 80 m, 30 Nov 1981, *Stevens 20950* (RSA). ***C. squamata*** Engelm.: **USA, New Mexico.** Doña Ana Co., White Sands Missile Range, 3 mi E of Main Post, East Dry Lake Playa near Range Road 3 beweeen LC 33 and C Statio, 3900 ft, undated, *Anderson & Brice 8057* (NMC); Collected on the Mesa west of the Organ Mountains, undated, *Wooton s.n.* (NMC). **Texas.** El Paso, 10 Sep 1883, *Jones 4170* (RSA). ***C. strobilacea var. strobilacea*** Liebm.: **MEXICO, Jalisco.** Hillsides near Guadalajara, 10 Oct 1889, *Pringle 2472* (K, MEXU). **Morelos.** Along Hwy 115 D (Autopista toll road) between Cuautla and Cuernavaca, NW of Cuautla, 3.9 miles SE of junction with Hwy 95 D (between Cuernavaca and Mexico City), 18º59’N, 99º06’W, 1960 m, 24 Feb 1987, *Croat & Hannon 65757* (MO). ***C. suaveolens*** Seringe: **USA, California.** Humboldt County, Myers Ranch, in alfalfa field, South Fork of Eel River 8 miles above the mouth, 200 ft, 29 Sep 1918, *Tracy 5113* (JEPS, UC1); Kern County, Rosedale, 30 Sep 1894, *Abrams 458* (RSA). ***C. subinclusa*** Durand & Hilgard: **USA, California.** Kern County, Kernville, 2650 ft, 25 Sep 1970, *Howell 47416* (NY); Riverside County, San Gorgonio wash at S. P. RR, San Gorgonio Pass, 2100 ft, 1 Dec 1933, *Wheeler 2284* (RSA); San Diego County, chaparral, 4 mi W of Hwy 94 on road to Otay Reservoir, N base of San Ysidro Mountains, 800 ft, 20 Aug 1952, *Munz & Balls 17942* (NY); San Luis Obispo County, Rinconada district, below Santa margarita and Pégo, 15 Sep 1946, *Hoover 6401* (RSA). ***C. suksdorfii*** Yuncker: **USA, California.** Mariposa Co. Yosemite National Park, NAD27 Zone 11 280152E 4189262N, 8710 feet, 20 Jul 2004, *Colwell AC 04-159* (UC1); Tuolumne County, Mineral spring near John Muir Trail in Lyell Canyon 1.4 km E of Rafferty Creek, Yosemite National Park UTM, Zone 11 297096E 4193313N, 2670 m, 8 Sep 2005, *Colwell et al. AC05-233* (UC1); Siskiyou County, South side of Preston Peak, Rattlesnake Meadow, 25 Aug 1963, *Wheeler 8269* (RSA). ***C. tasmanica*** Engelm.: **AUSTRALIA, Victoria.** Volcanic Plain, By Causeway between Lke Corangamite and Lake Martin, W of Berrybank- Cundare Road, 38^{o}06’S, 142^{o}33’E, Towards Lake Corangamite on N side of causeway, 29 Jan 1991, *Walsh 3045* (MEL). ***C. tinctoria var. aurea*** (Liebm.) Costea: **MEXICO, San Luis Potosi.** Unknown locality, 12-16 Sep 1902, *Palmer 89* (S). ***C. tinctoria var. floribunda*** (Kunth) Costea: **MEXICO, Veracruz.** Maltrata, Jan 1883, *Kerber s.n.* (KEW). ***C. tinctoria var. tinctorial*** Martius: **MEXICO, Hidalgo.** 4 km al SE de Tolcayuca, 21 Nov 1978, *Ortega s.n.* (MEXU). **Mexico.** 1 km al N de San Juan Citlatepec, mpio. De Zumpango, 2240 m, *Rzedowski ME-22* (MEXU). **Puebla.** Cuapiaxtla, cerca de Tepeaca, 2000 m, 6 Aug 1965, *Rzedowski 20380* (MEXU); 1 km al SE de San Hipól.- to Xochiltenango, 11 Sep 1961, *Sousa s.n.* (MEXU). **San Luis Potosi.** Unknown locality, 1877, *Schaffner 781* (MEXU). ***C. tuberculata*** Brandegee: **MEXICO, Baja California Sur.** Hwy. 1, KP 20, 12 miles NE Villa Insurgentes El. 250’, 7 Sep 1983, *Donahue 73168* (RSA); Arroyo de Santa Agueda, southwest of Santa Rosalía on road to Santa Agueda, 27^{o}15’N, 112^{o}24’W, 3 Oct 1951, *Carter & Kellogg 3085* (NY, RSA). **Sonora.** Bavispe, 3850 ft, 19 Oct 1890, *Lumholtz 179* (GH). ***C. umbellata*** Kunth: **GUIANA, Georgetown.** Seacoast east of sea wall, 29 Oct 1919, *Hitchcock 16564* (S). **MEXICO, Guerrero.** Coahuayutla de Guerrero, La Corva, 5.46 km al N, 18^{o}32’8”N, 101^{o}50’28”W, 20 Oct 1999, *Soto 17626* (MEXU). **Jalisco.** ca. 4.5 mi from Zacoalco on low moist areas

beside rd. to Acatlán, 1350-1375 m, *Dieterle 3471* (MICH). **Oaxaca.** Cuicatlán, San Juan Bautista Cuitcatlan, 10.2 km al NW del Chilar, 17º47'21"N, 96º59'31"W, 665 m, 1 Oct 2002, *Soto 24009* (MEXU). **Puebla.** Calcareous hills near Tehuacan, 5500 ft, 24 Dec 1895, *Pringle 6297* (S); Tehuacán, 9 Aug 1938, *Kenoyer A307* (F). **USA, New Mexico.** Doña Ana County, NW of Doña Ana Community College on ground next to corral, Las Cruces, 15 Oct 1990, *Silversmith s.n.* (NMC). ***C. umbrosa*** Beyr. ex Hook.: **CANADA, Manitoba.** Distr. De Saint-Boniface. Rivière Rouge, ecorre de la Rouge à La Fourche, 21 Aug 1960, *Boivin 13852* (DAO); Otterbourne, 4 Aug 1954, *Bernard 54/349* (QFA); Sans Souci, sur la Salix dans la place sablouuense, 21 Jul 1956, *Bernard 56/5473* (QFA); Winnepeg, sent to G. Knowles, Field Husbandry by M. R. Mackenzie 419 Kingston Crescent, 1950, *Mackenzie s.n.* (DAO); 13 Aug 1954, *Bernard 497* (DAO). **Saskatchewan.** Little Manitou Lake on Mentha arvensis and Urtica dioica in damp wooded ravine bottom N side of the lake, 20 Aug 1992, *Hudson 5082* (USAS). **USA, Colorado.** Dome Rock in Platte Canyon, 7000 ft, 8 Aug 1878, *Jones 571* (RSA). ***C. vandevenderi*** Costea & Stefanović: **MEXICO, Sonora.** Álamos, El Palmarito, 27°03'04"N 108°45'51"W, 1 Oct 2006, *Van Devender 2006-983* (WLU); Yécora, 28°22'40"N 109°09'W, 20 Sep 1998, *Van Devender 98-1434* (WLU). ***C. victoriana*** Yuncker: **AUSTRALIA, Northern Territory.** 6 miles NW of Mt. Swan Station, ca. 22º36'S 135º02'E, 11 Mar 1953, *Perry 3329* (CANB). **South Australia.** Lake Eyre Region, between Hough's Dam and Chapman's Creek Tank, Dulkaninna Station alongside track from homestead to Chapman's Creek, 29º04'23"S, 138º37'28"E, 9 Apr 1997, *Smyth 261* (CANB). **Western Australia.** Rear of Bullgarra cell, Karratha, Jul 1987, *Glennon 379* (CANB). ***C. volcanica*** Costea & I. García: **MEXICO, Jalisco.**

Tonila, Volcán Nevado de Colima, bosque de Abies religioda, 3100 m, *Zamudio 4274* (MICH). **Puebla.** Azumbilla, Puerto del Aire, carr. A Orizaba, 3 Jan 2002, *Tenorio 21748* (MEXU); Caltepec, El Ojo de Agua, al E de Caltepec, 18º4'N 97º25'W, 1250 m, 28 Dec 2001, *Tenorio & Kelly 21688* (MEXU). ***C. warnerii*** Yuncker: **USA, New Mexico.** Sierra County, Pedro Armendaris Grant, 15.6 miles north of Engle, east of Red Lake, 4800 feet, 24 Sep 1998, *Peterson 98-699* (NMC). **Utah.** Millard County, Vicinity of Flowell, 15 miles west of Fillmore, 10 Sep 1957, *Warner s.n.* (UC1) isotype. ***C. werdermanii*** Hunz.: **CHILE.** Unknown locality, unknown date, *Reiche s.n.* (SGO). ***C. woodsonii*** Yuncker: **MEXICO, Hidalgo.** 3.5 km NW of Zimapan on road to La purism, hard limestone hills, 1800 m, 20º46'N 99º25'W, 9 Oct 1985, *Spellenberg 8334* (NMC); along Mexico Highway 85 between Tamazinchale and Zimapan, 90 km S of border with San Luis Potosi, 40 km N of Zimapan, 10 Oct 1985, *Spellenberg et al. 8359* (NMC). **PANAMA, Chiriqui.** Lava field and slopes between Volcan de Chiriqui and Cerro Aquacate, 6500-7200 ft, 16 Jan 1971, *Wilbur et al. 13316* (MO); Road to top of El Baru from Boquete, east side of Baru, 7600-9000 ft, 17 Mar 1979, *D'Arcy et al. 12622* (MO). ***C. xanthochortos*** Mart. ex Engelm. ***var. carinata*** (Yunck.) Yunck.: **PARAGUAY, Cordillera.** Cerrado forest, 25º07'S 57º19'W, 1 Jun 1993, *Zardini & Guerrero 35961* (MO, AS, WLU). ***C. yucatana*** Yunck.: **MEXICO,** Unknown locality, unknown date, *Rzedowski 25728* (G). **Chiapas.** Steep slope with *Quercus, Drimys,* and *Magnolia* near crest of ridge in the paraje of Banabil, Tenejapa, 9100 feet, 10 Oct 1965, *Breedlove & Raven 12912* (F). **Puebla.** Laguna El Salado, along dirt road 3 km. W of hwy. Mex. 140, 6 km N of Laguna de Alchichica, Tepeyahualco, 19º28'N 97º25'W, 20 Feb 1984, *Nee & Taylor 29575* (MO).

A2. Infructescence Architecture

Cuscuta acuta Engelm.: **ECUADOR, Galapagos.** Darwin Bay, Tower Island, 16 Jun 1932, *Howell 10140* (GH); Sulivan Bay, James Island, 13 Jun 1932, *Howell 10048* (GH); Tower Island, Wm. 19 Apr 1923, *Wheeler 21* (GH). **PERU, Piura.** Talará. 1926, *Haught 154a* (GH). ***C. americana*** L.: **MEXICO, Michoacan.** Apatzingan, 19°02'29"N 102°18'43"W, 21 Sep 2007, *Carranza & Silva 7374* (IEB, WLU). **Sinaloa.** Concordia, El Capomito, 23°18'10"N 105°56'12"W, 7 Jan 2006, *Reina & Van Devender 2006-117* (WLU). **Sonora.** Álamos, Río Cuchujaqui, 27°02'56"N 108°44'00"W, 8 Oct 2006, *Van Devender 2006-1303* (WLU); Soyopa, 28°34'33"N 109°33'02"W, 7 Jan 2001, *Van Devender 2001-16* (WLU). ***C. argentinana*** Yunck.: **ARGENTINA, Salta.** Chicoana, La Zanja, 10 Apr 1980. *Krapovickas y Schinini 36757* (CTES); Guachipas, Pampa Grande, 29 Apr 1942, *Hunziker 1775* (US); 27 Apr 1942, *Hunziker 1729 ½* (US); Rosario de Lerma, 11 Jan 1929, *Venturi 8556* (US). ***C. australis*** R. Br.: **PAPUA NEW GUINEA, Morobe.** Markham Valley, 12 Nov 1959, *Brass 32508* (NY). ***C. azteca*** Costea & Stefanović: **MEXICO, Aguascalientes.** La Luz, El Llano, N 21.990081 W -101.994180, 31 Aug 2012, *Romero 3350*12P0050052* (WLU); San Antonio, Tepezala, N 22.22909 W -102.23662, 04 Oct 2012, *Romero 33530*12P0050047* (WLU). **Queretaro.** Ezequiel Montes, 8 Sep 1990, *Rzedowski 49994* (IEB, WLU). ***C. boldinghii*** Urb.: **BONAIRE.**

North of OPEC, 27 Feb 1999, *Proosdij 859* (AAH). **HONDURAS.** Cortés, San Pedro Sula, 3 Aug 1887, *Thieme 5366* (US); Yoro, Near Progreso, 24 Jan 1928, *Standley 55040* (US). **MEXICO, Sonora.** Guiroba crossing of Rio Cuchujaqui, 26°56'15"N 108°53'W, 28 Jan 1992, *Van Devender 92-31* (ARIZ). **Yucatán.** X-Can, Lat: 20.86279 Long: -87.66470, 20 Apr 2011, *López 2011-00766* (WLU). ***C. brachycalyx*** Yunck.: *Stefanović 15-18* (WLU); *Stefanović 13-41* (WLU); ***C. californica*** Hook. & Arn.: **USA, California.** San Bernardino County, Bristol Mountains, 19 Nov 2005, *Andre 5631* (UCR); Cleghorn Canyon, 34°17'N 117°26'W, 21 Jul 1993, *White 1771* (UCR). ***C. californica*** Hook. & Arn. var. ***papillosa*** Yuncker.: **USA, California.** Riverside County, Colorado Desert, Coachella Valley, 33°53'N 116°15'W, 20 Apr 1995, *Sanders 16587* (UCR); Pushawalla Wash, 7 Dec 1946, *Roos 3184* (UCR); San Bernardino County, Devil Canyon, 12 Jun 1987, *Sanders 7125* (UCR); Union Flat, 28 Aug 1978, *Krantz s.n* (UCR). ***C. campestris*** Yuncker.: **CANADA, Ontario.** Lat: 12.92793 Long: -78.92624, 15 Aug 2007, *Oldham & Brinker 34876* (WLU). **USA, California.** Butte County, 39°21'51.0"N 121°29'21.0"W, 1 Oct 2006, *Ahart 13,388* (CHICO). Los Angeles County, Devels Gate Dam, Pasadena, 6 Oct 1965, *Wheeler s.n* (UCR); SE of Alameda St. & San Diego (405) Fwy, E of Dominguez Flood Control Channel, 8 Jun 1973, *Wheeler s.n* (UCR); NE end of Santa Susana Mtns, 34°25'07"N 118°38'13"W, 6 Aug 2003, *Sanders 27126* (UCR); San Gabriel Valley, 34°00'36"N 117°51'33"W, 5 Jun 1973, *Clarke s.n* (UCR); Yolo County, Sacramento Valley, 7mi W of Davis, 38°33.7'N 121°55'W, 29 Sep 1958, *Crampton 5117* (UCR). **MEXICO, Tlaxcala.** Tetlatlahuca, Santa Isabel, N 19.20423 W -98.28179, 10 Oct 2011, *Flores 2011-0131* (WLU). **TÜRKMENISTAN.** Ashgabat, 16 Jun 1975, *Ivanov 45598* (CHICO). ***C. cephalanthi*** Engelm.: **CANADA, Ontario.** Rainy River

County, Seine River, Lat: 48.69902 Long: -92.60554, 23 Aug 2002, *Oldham 28012* (NHIC, WLU). ***C. chapalana*** Yuncker.: **MEXICO, Jalisco.** Jocotepec, 20°17'727 N 103°29' 935 W, 8 Nov 2008, *Ruiz 8169* (CIMI, WLU). **Michoacan**, Huiramba, 5 Sep 1986, *Escobedo 1223* (IEB, WLU). ***C. chilensis*** Ker Gawl.: **CHILE**. Copada, O'Higgins, 25 Jan 1925, *Pennell 12261* (US); Casilla, Valdivia, Valparaiso, 1895, *Buchtien 4508* (US); Uspallata Pass, Feb 1903, *Buchtien 4507* (US); Valparaiso, Cerro Las Vizcaches, Ramayama Copper Mine, 8 Dec 1951, *Hutchison 116* (US). ***C. chinensis*** Lam. **var. *chinensis***: **AUSTRALIA**. 9.9km N of Long Spring, 14°48'20"S 128°39'0"E, 9 Mar 1989, *Keighery 11100* (PERTH, WLU). ***C. chinensis*** **var. *apiculata*** (Engelm.) Costea & Stefanović: **MEXICO, Durango.** Mapimí, 25°47'N 103°46"W, 8 Sep 1983, *Torrecillas 237* (CIIDIR, WLU). **Sonora.** 2.5mi S of Llano, 15 Sep 1934, *Wiggins 7221* (US); Onavas, Rancho La Mula, 28°28'50"N 109°22'W, 30 Aug 2000, *Van Devender 2000-506* (ARIZ). ***C. colombiana*** Yunck.: **COLOMBIA.** Magdalena, near Riohachsa, 23 Dec 1944, *Haught 4535* (US). ***C. corniculata*** Engelm.: **COLOMBIA.** Meta, Villavicencio, 31 Aug 1917, *Pennell 1453* (GH). ***C. corymbosa*** Ruiz & Pav. **var. *grandiflora*** Engelm.: **MEXICO, Michoacan.** Real de Otzumatlán, 20 Nov 1986, *Rzedowski 41882* (IEB, WLU); Queréndaro, 26 Feb 1989, *Rzedowski 48303* (IEB, WLU). ***C. corymbosa*** **var. *stylosa*** (Choisy) Engelm.: **MEXICO, Chalco.** Cerro Tlapacoya, 7 Nov 1971, *Rzedowski 28752* (UCR). **Oaxaca.** Nacaltepec, 21 Sep 1895, *Smith 829* (GH). **Queretaro.** 10km S of Querétaro, 3 Jul 1992, *Rzedowski 51448* (IEB, WLU). ***C. costaricensis*** Yunck.: **MEXICO, Guanjuato.** El Volcancito, Yuriria, 2 Oct 1986, *Zamudio 4655* (IEB, WLU). **Michoacan.** Coeneo, 29 Nov 1985, *Escobedo 730* (IEB, WLU). **Sonora.** Álamos, 27°07'08"N 108°43'18"W, 2 Oct 2006, *Reina 2006-1049* (WLU); Yécora, Río Yepachic, 28°27'10"N 108°32'15"W, 27 Sep 1998, *Van Devender 98-1789* (WLU); 28°29'44"N 108°41'37"W, 14 Sep 1999, *Van Devender 99-563* (WLU); 28°21'48"N 108°55'56"W, 16 Sep 2006, *Van Devender 2006-888* (WLU). ***C. cotijana*** Costea & I. García: **MEXICO, Colima.** Queseria, 14 Mar 2010, *García 8338* (CIMI, WLU); *García 8337* (CIMI, WLU). **Jalisco.** Quitupan, 9 Sep 2008, *García Ruiz 8089* (CIMI, WLU); 19°42'42.1"N 102°55'42.2"W, 10 Aug 2009, *García Ruiz 8263* (CIMI, WLU). ***C. cristata*** Engelm.: **ARGENTINA, Buenos Aires**, Punta Lara, Feb 1942, *Hunziker 2487* (US); *Crovetto 46* (CTES). **Córdoba.** San Esteban, 4 Jan 1938, *Nicora 1575* (CTES). **La Rioja**. Sanagasta, 18 Feb 1944, *Hunziker 2487* (US). ***C. decipiens*** Yunck.: **MEXICO, Coahuila.** Filipinas, Oct 1910, *Purpus 4973* (GH). ***C. deltoidea*** Yunck.: **MEXICO, Michoacan.** Monte Leon, 11 Nov 1892, *Pringle 5350* (US). ***C. denticulata*** Engelm.: **USA, California.** Inyo, Hwy 178, 3mi E of Shoshone, 2013, *Stefanović 13-28* (WLU); San Bernardino, Hwy 66, 3mi E of Amboy, 2013, *Stefanović 13-22* (WLU); Hwy 95, 15mi W of Essex, 15mi E of Amboy, 2013, *Stefanović 13-19* (WLU). ***C. erosa*** Yunck.: **MEXICO, Sonora.** 4mi E of Willard, 5 Sep 1941, *Wiggins 288* (ARIZ); Cucurpe, 30°19'46"N 110°42'18"W, 22 Aug 2001, *Reina 2001-748* (WLU); Nogales, 31°11'49"N 111°05'46"W, 6 Sep 2005, *Van Devender 2005-1226* (WLU); Opodepe, 30°03'14"N 110°03'31"W, 21 Aug 2001, *Van Devender 2001-737* (WLU). ***C. foetida*** Kunth. var. ***foetida***: **ECUADOR, Pichincha.** Cantón Mejía, 00°30'S 78°25'W, 23 May 1988, *Zac 3700* (MO, WLU); El Pongo, 00°15'S 78°40'W, 24 Jul 1987, *Zac 2257* (MO, WLU). **PERU.** Amazonas, Luya, 06°25'S 77°55'W, 9 Apr 2001, *Van der Werff 16851* (MO, WLU). ***C. friesii*** Yunck.: **ARGENTINA.** Catamarca, Los Varela, Ambato, 28 Mar 1995, *Toledo 12993* (CTES); Salta, Cachi, 19 Mar 1972, *Krapovickas 21964* (CTES); Tucumán, Tafí, Infiernillo, 18

Mar 1972, *Krapovickas 21898* (CTES). ***C. glabrior*** (Engelm.) Yunck.: **MEXICO, Coahuila.** 18mi NE Saltillo, 6 Aug 1957, *Waterfall 13240* (US); Saltillo, 25°00'N 101°05'W, 5 Jun 1990, *Villarreal 5676* (ARIZ); Buenavista, El Bajío, 12 Oct 1981, *Carranza 1396* (ARIZ). ***C. globiflora*** Engelm.: **BOLIVIA, Murillo.** La Paz, Valle de la Luna, 27 Feb 1975, *Davis 5196* (GH); 16°32'S 68°05'W, 9 Jan 1982, *Solomon 6681* (MO, WLU); 16°40'S 68°01'W, 28 Mar 1982; *Solomon 7400* (MO)**.** ***C. globulosa*** Benth.: **CUBA, Santiago.** El Cobre, 24 Feb 1902, *Palmer 393* (GH). **GRENADA.** Lowthers Lane, 12 Jun 1908, *illegible author 77* (GH). **MONTSERRAT.** Plymouth, 8 Sep 1935, *Potter 5558* (GH). **ST. VINCENT.** Dorsetshire Hill, near Kingstown, 4 Apr 1947, *Morton 4712* (GH). **TRINIDAD.** St. Augustine, 23 Mar 1920, *Britton 947* (GH). **US VIRGIN ISLANDS.** Saint Croix, Feb 1955, *Hunnewell 20,145 left specimen* (GH). ***C. gracillima*** Engelm.: **MEXICO, Sinaloa.** Concordia, 23°19'54"N 105°56'29"W, 8 Jan 2006, *Van Devender 2006-182* (WLU); *Van Devender 2006-166* (WLU); Río Panuco, 23°19'16"N 105°57'45"W, 1 Mar 2007, *Van Devender 2007-215* (WLU)**.** ***C. grandiflora*** Kunth.: **BOLIVIA, Murillo.** La Paz, 16°32'S 68°03'W, 21 Mar 1982, *Solomon 7217* (MO, WLU). **PERU, Cusco.** Calca, Lares, Suyo, 12°28'S 72°00'W, 17 Jun 2005, *Valenzuela 5713* (MO, WLU); Urubamba, Yucay, 13°18'S 72°04'W, 22 Jul 2003, *Huamantupa 3668* (MO, WLU). ***C. gronovii*** Willd. ex Schult.: **CANADA, Ontario.** Waterloo, Grand River, 18 Sep 2014, *Costea s.n* (WLU). **SANTO DOMINGO.** Cordillera Central, La Vega, Constanza, 9 Nov 1929, *Ekman 14062* (AAH)**.** ***C. gronovii var. latiflora*** Engelm.: **CANADA, Ontario.** Peterborough, Otonabee, Lat: 44.23881 Long: -78.15329, 5 Aug 2002, *Oldham 27653* (NHIC, WLU). **DOMINICAN REPUBLIC, Trujillo.** Repressa Dam, Maná, 26 Nov 1947, *Allard 17186* (US); *Allard*

17184 (US). ***C. haughtii*** Yunck.: **ECUADOR, Guayas.** Salinas, 2°13'S 81°W, 5 Mar 1941, *Svenson 11,281* (GH); 26 Mar 1941, *Svenson 11074* (US). **PERU, Piura.** Huancabamba, 30 Apr 1949, *Ferreyra 6030* (US); Talara, 11 Oct 1925, *Johnston 3514* (GH). ***C. incurvata*** Prog.: **PARAGUAY.** Caaguazú, 4km N of Yhú, 6 Feb 2003, *López 243* (CTES). ***C. indecora*** Choisy: **MEXICO, Chihuahua.** Jiménez, R. el Capricho, N 27.36752 W -104.87734, 22 Jul 2011, *Domínguez 2011-01261* (WLU). López, Salaices, N 27.02717 W -105.19731, 1 Aug 2011, *Domínguez 2011-01256* (WLU). **Querétaro.** San Juan del Rio, Vistha, Lat: 20°45093 Long: -100°00113**,** 30 Jun 2011, *Monrreal 2011-00670* (WLU). **Zacatecas.** Loreto, El Tepetate, N 22.36323 W -102.08333, 26 Oct 2011, *Ibarra 23475-11P0038310* (WLU). ***C. jalapensis*** Schltdl.: **MEXICO, Guaajuato.** Arroyo Seco, 21 Nov 1995, *Pérez 3261* (IEB, WLU). **Michoacan.** Indaparapeo, 30 Sep 2006, *Rzedowski 54246* (IEB, WLU). **Queretaro.** Landa, 16 Mar 1987, *Rzedowski 42811* (IEB, WLU); Pinal de Amoles, San Gaspar, 13 Sep 1989, *Zamudio 7495* (IEB, WLU). ***C. legitima*** Costea & Stefanović: **MEXICO, Sonora.** Agua Prieta, 31°18'21"N 109°34'55"W, 13 Sep 2006, *Van Devender 2006-757* (WLU); Soyopa, 28°35'39"N 109°35'33"W, 16 Aug 2006, *Van Devender 2006-586* (WLU); *Van Devender 2006-606* (WLU); *Van Devender, 2006-607* (WLU); Tónichi, 28°35'55"N 109°33'50"W, 17 Aug 2006, *Van Devender 2006-627* (WLU). **USA, Arizona.** Pima County, Tucson, 32°17'25"N 110°57'35"W, 17 Nov 2005, *Van Devender 2005-1661* (WLU). ***C. leptantha*** Engelm.: **MEXICO, Baja California.** Bahia de Los Angeles, 14 Mar 1992, *Fritsch 1337* (UCR); Between Santonio and Puerto de Bahia de los Muertos, 4 May 1931, *Wiggins 5625* (US). **Sonora.** Hermosillo, 28°52'28"N 112°01'20"W, 31 Dec 2000, *Van Devender 2000-933* (WLU)**. USA, New Mexico.** Hidalgo County, Animas Valley, 32°15'06"N

108°52'45"W, 20 Aug 2004, *Ballmer s.n* (UCR). ***C. lindsayi*** Wiggins: **MEXICO, Jalisco.** Mazamitla, 17 Feb 2007, *García Ruiz 7571* (CIMI, WLU); 19°51'33.6"N 102°59'38.3"W, *García Ruiz 7569* (CIMI, WLU); Quitupan, 12 Apr 2008, *García Ruiz 8076* (CIMI, WLU). ***C. longiloba*** Yunck.: **BOLIVIA.** Chuquisaca, 63°13'W 20°45'S, 13 Apr 1977, *Krapovickas 31255* (CTES)**.** ***C. macrocephala*** W. Schaffn. ex Yunck.: **MEXICO, Sinaloa.** Cosalá, 24°24'16"N 106°41'26"W, 4 Jan 2006, *Van Devender 2006-15* (WLU); *Van Devender 2006-16* (WLU). **Sonora.** Álamos, 27°06'34"N 108°42'58"W, 5 Oct 2006, *Van Devender 2006-1240* (WLU); Arivechi, 28°49'15"N 109°10'24"W, 15 Sep 2006, *Van Devender 2006-872* (WLU); San Javier, 28°34'48"N 109°40'15"W, 31 Aug 2001, *Van Devender 2001-758* (WLU); Yécora, 28°25'48"N 109°11'31"W, 1 Sep 2001, *Van Devender 2001-774* (WLU); 28°25'53"N 109°09'34"W, 16 Sep 2006, *Van Devender 2006-901* (WLU); 28°20'50"N 109°07'17"W, 21 Sep 1998, *Van Devender 98-1524* (WLU). ***C. mcvaughii*** Yunck.: **MEXICO, Michoacán.** Apatzingan, 19°01'40"N 102°17'43"W, 13 Mar 2010, *García Ruiz* ***8335*** (CIMI, WLU); Churumuco, 18°37'09"N 101°36'48"W, 8 Sep 2007, *Steinmann 5870* (IEB, WLU)**.** ***C. mitriformis*** Engelm.: **MEXICO, Coahuila.** Cañón de San Lorenzo, 25°20'N 100°59'W, 17 Nov 1983, *Villarreal 1991* (ARIZ). **Durango.** Durango, 23°36'53"N 105°9'24"W, 11 Sep 2001, *Carrillo 356* (CIIDIR, WLU). **Guanajuato.** Atarjea, El Coporito, 4 Jun 1991, *Ventura 9215* (IEB, WLU). ***C. nevadensis*** I. M. Johnst.: **USA, California.** Inyo, Hwy 178, 1mi E of Shoshone, 2013, *Stefanović 13-27B* (WLU); Hwy 127, 5mi N of Death Valley Jct., 2013, *Stefanović 13-48* (WLU). **Nevada.** Nye, Hwy 373 intersection with road to Ash Meadows, 2013, *Stefanović 13-47.* ***C. obtusiflora*** Kunth.**: COLOMBIA, Putumayo.** Valle de Sibundoy, 29 Apr 1963, *Bristol 892* (GH). **JAMAICA.** St. Catherine Parish, Charlton, 1m W Ewarton, 5 Nov 1958, *Proctor 18327* (AAH); Trelawny, Windsor estate, 22 Aug 1955, *Proctor 10531* (US). ***C. obtusiflora var. glandulosa*** Engelm.: **MEXICO, Jalisco.** San Diego (Lázaro Cárdenas), 19°54'44.1"N 102°49'34.8"W, 8 Aug 2009, *García Ruiz 8256* (CIMI, WLU); 19 Aug 2007, *García Ruiz 7752* (CIMI, WLU); 18 Dec 2007, *García Ruiz 8054* (CIMI, WLU). **Michoacan.** Huaniqueo, 10 Sep 1990, *Barriga 6471* (IEB, WLU). ***C. occidentalis*** Millsp.: *Stefanović 15-09* (WLU). ***C. odontolepis*** Engelm.: **MEXICO, Sonora.** Arivechi, 28°49'15"N 109°10'24"W, 15 Sep 2006, *Van Devender 2006-869* (WLU); Fronteras, 30°42'41"N 109°35'15"W, 13 Aug 2006, *Van Devender 2006-467* (WLU). ***C. odorata*** Ruiz & Pav.: **PERU, Apurimac.** Abancay, 13°36'S 72°54'W, 16 Aug 2002, *Valenzuela 291* (MO, WLU). **Contumazá.** Cajamarca, 1 May 1981, *Sagástegui 9769* (MO, HUT, WLU); 26 May 1981, *Sagástegui 9936* (MO, HUT, WLU). ***C. pacifica*** Costea & Wright: *Stefanović 15-03B; Stefanović 15-03A; Stefanović 15-24.* ***C. paitana*** Yunck.: **PERU, Paita.** Piura, 30km SE of Talara, 4 Mar 1939, *Horton 11575* (GH); Pariñas Valley, 1925, *Haught 11* (US). ***C. parodiana*** Yunck.: **ARGEINTINA, Salta.** Cuesta del Gallinato, Caldera, 29 May 1980, *Pedersen 12841* (CTES). **Jujuy.** Calilegua, 3 Jun 1943, *Bartlett 20344* (US). **Tucumán.** Capital, Muñecas, 22 Mar 1922, *Venturi 3880* (US); Siambon, 25 Apr 1925, *Venturi 3880* (US). ***C. partita*** Choisy: **BRAZIL, Ceará.** Guaramiranga, 1938, *Eugenio 1017* (GH). **COLOMBIA, Magdalena.** Riohacha, 11 Nov 1944, *Haught 4435* (US, GH). ***C. platyloba*** Prog.: **ARGENTINA, Misiones.** Candelaria, Bonpland, 19 Jan 1910, *Jorgensen-Hansen 31784* (CTES, BAB). **BRAZIL, Paraná.** Jaguariaíva, 23 Jun 1910, *Dusén 10005* (GH). **Rio de Janeiro.** Gavea Pequena, 8 Mar 1931, *Brade 10651* (GH). ***C. polyanthemos*** Schaffn. ex Yunck.: **MEXICO, Sonora.** 31 mi NW Queriego, 6 Mar

1933, *Wiggins 6457* (US); Moctezuma, 29°39'44"N 109°37'13"W, 14 Sep 2006, *Van Devender 2006-809* (WLU). ***C. prismatica*** Pav. ex Choisy: **ECUADOR, Guayas.** Guayaquil, 26 Jun 1923, *Hitchcock 20141* (US, GH)**. *C. purpurata*** Phil.: **CANADA, Ontario.** Mississauga, grown in greenhouse at U of T, 13 Jul 2015, *Stefanović s.n* (TRTE WLU). **CHILE, Antofagasta.** Near Paso Malo, N of Taltal, 28 Nov 1925, *Johnston 5170* (US). **Copiapó.** 26°54'S 70°47'W, 14 Oct 1965, *Ricardi CONC47838* (CTES). ***C. purpusii*** Yunck.: **MEXICO, Queretaro.** Cadereyta, 20°54'27"N 99°58'57"W, 18 Sep 2006, *Zamudio & Alcalá, s.n* (IEB, WLU). ***C. rugosiceps*** Yunck.: **GUATEMALA, Quiché.** San Miguel Uspantan, Apr 1892, *Heyde & Lux 2912* (GH). **MEXICO, Oaxaca.** Sierra Madre del Sur, 20 Jun 1962, *Webster 11561* (GH). **Queretaro.** Jalpan, 21°28'11"N 99°09'02"W, 3 Sep 1005, *Pérez-Calix 4603* (IEB, WLU). ***C. salina*** Engelm.: **USA, California.** Riverside County, 3mi NE Elsinore, 6 Aug 1969, *Clarke s.n* (UCR); San Bernardino County, Rabbit Springs, 1mi NW Lucerne Valley Town Ctr., 7 Jun 1978, *Vasek s.n* (UCR). **Nevada**. Churchill County Stillwater Range, 39°51.128'N 118°13.968'W, 14 Jul 2015, *Tiehm 17087* (UNLV, WLU). ***C. sidarum*** Liebm.: **MEXICO, Michoacan.** Arteaga, 18°27'55"N 101°58'40"W, 20 Nov 2003, *Steinmann 3883* (IEB, WLU); Lázaro Cárdenas, 4 Feb 2008, *Carranza & Silva 7451* (IEB, WLU); 18°01'23"N 103°21'19"W, *Carranza & Silva 7458* (IEB ,WLU). ***C.*** *squamata* Engelm.: **MEXICO, Chihuahua.** Jiménez, N 27.18376 W -104.84068, 28 Jun 2011, *Calderón Domínguez 2011-01255* (WLU); López, Salaices, N 27.03560 W -105.17870, 12 Aug 2011, *Calderón Domínguez 2011-01259* (WLU); Valley of the Rio Grande, Paso del Norte, 14 Sep 1886, *Pringle 785* (US). ***C. strobilacea*** Liebm.: **MEXICO, Durango.** Sierra Tres Picos, 20 Dec 1939, *Gentry 5291* (ARIZ). **Morelos.** 18°58'48"N 99°06'W, 24

Feb 1987, *Croat 65757* (MO, WLU). **Nayarit.** Tepic, 21°35'31.1"N 104°51'35.8W, 12 Aug 2009, *García Ruiz 8274* (CIMI, WLU). ***C. suaveolens*** Ser.: **ARGEINTINA, Medoza.** Luján, 15 Apr 1945, *Semper 591* (ARIZ)**. CHILE, Rancagua.** May 1828, *Bertero 20* (GH). ***C. subinclusa*** Durand & Hilg.: **USA, California.** Kern County, 3mi S of Havilah to Caliente, 24 Sep 1970, *Thomas 47408* (UCR); San Bernardino County, Colton, 34°02'45"N 117°21'W, 2 Jul 2000, *Provance 2263* (UCR); San Diego County, Blue Sky Ecological Reserve, 33°00'57"N 117°00'51"W, 5 Oct 2005, *Sanders 31698* (UCR); San Mateo Creek, 32°24'N 117°35'W, 7 Jun 1986, *Prigge 7215* (UCR). ***C. tinctoria*** Mart. ex Engelm.: **MEXICO, México.** Temascaltepec, 29 Dec 2009, *García Ruiz 8323* (CIMI, WLU); Temascalcingo, 3 Apr 2010, *García Ruiz 8341* (CIMI, WLU). **Michoacán.** Briseñas, 20°15'41.1"N 102°33'03.9"W, 14 Aug 2009, *García Ruiz 8279* (CIMI, WLU); San Pedro (Venustiano Carranza), 20°06'44.4"N 102°39'32.7"W, 20 Feb 2007, *García Ruiz 7575* (CIMI, WLU). **Oaxaca**. 5-6km SSE carretera Ejutla-Miahuatlán, 7 Apr 2010, *García Ruiz 8344* (CII, WLU). **Sonora.** Magdalena de Kino, 30°37'N 110°57'30"W, 29 Dec 1994, *Van Devender 94-1008* (WLU)**, *C. tuberculata*** Brandegee: **MEXICO, Sonora.** Moctezuma, 29°49'40"N 109°40'28"W, 13 Aug 2006, *Van Devender 2006-457* (WLU); 29°39'30"N 109°36'37"W, 14 Aug 2006, *Van Devender 2006-514* (WLU); Villa Pesqueira, 29°35'N 110°01'W, *Shortman 96-51* (ARIZ). ***C. umbellata*** Kunth.: **MEXICO, Guanajuato.** León, 7 Jul 2001, *Ocampo 1067* (IEB, WLU); Yuriria, 15 Jun 2006, *Carranza & Silva 7191* (IEB, WLU); *Carranza & Silva 7190* (IEB, WLU). ***C. vandevenderi*** Costea & Stefanović: **MEXICO, Sonora.** Álamos, El Palmarito, 27°03'04"N 108°45'51"W, 1 Oct 2006, *Van Devender 2006-983* (WLU); Yécora, 28°22'40"N 109°09'W, 20 Sep 1998, *Van Devender 98-1434* (WLU). *C.* ***victoriana***

Yunck.: **AUSTRALIA.** Coondiner Pool, ca. 71km N of Newman, 22°43'0"S 119°38'0"E, 30 Mar 1984, *Newbey 10073* (PERTH, WLU); ca. 10km E of Boolaloo Homestead, Mt. Stuart Station homestead, 22°34'36"S 115°53'10"E, 15 May 1997, *Mitchell 4734* (PERTH, WLU); East Island, Ashmore Reef, 12°15'34.90"S 123°5'53.5"E, 9 Sep 2004, *Williams 8235* (PERTH, WLU)**.** ***C. volcanica*** Costea & I. García: **MEXICO, Jalisco.** Mazamitla, 19°51'27.3"N 102°58'59.4"W, 17 Feb *2007, García Ruiz 7568* (CIMI, WLU); Quitupan, 19°52.778'N 102°57.328'W, 10 Aug 2009, *García Ruiz 8262* (CIMI, WLU). **Michoacán.** Sirio, Patamban, 31 Mar 2009, *García Ruiz 8072 Type* (CIMI, WLU); Tancítaro, 19°21'53.4"N 102°15'27.8"W, 15 Dec 2007, *García Ruiz 8029* (CIMI, WLU). ***C. xanthochortos*** var. ***carinata*** Yunck.: **PARAGUAY, Caazapá.** 26°16'26"S 55°45'47"W, 14 Dec 1999, *Zardini 52948* (MO, AS, WLU). **Cordillera**, Cerrado forest, 25°07'S 57°19'W, 1 Jun 1993, *Zardini 35961* (MO, AS, WLU). **San Pedro**, 24°42'07"S 56°30'31"W, 24 Jun 2001, *Zardini 56666* (MO, FACEN, WLU). ***C. yucatana*** Yunck.: **MEXICO, Chiapas.** San Cristobal, 7 Aug 1967, *Gittins 4202* (UCR).

APPENDIX B: INFRUCTESCENCE COMPACTNESS FORMULA

$$IC = \left(\frac{lt + p}{No}\right)\left(\frac{1}{d * Na * MaxNa}\right)$$

The infructescence compactness formula included in the text is indicated above.

As *lt* (total length of inflorescence) and *p* (pedicel length) increases, inflorescence decreases in compactness.

As *No* (number of orders/nodes), *d* (fruit diameter), *Na* (number of axes at the first node), *MaxNa* (maximum number of axes present at a single node) increases, inflorescence increases in compactness.

The first half of the equation considers the average length of nodes in an inflorescence.

Therefore, as *IC* approaches 0, inflorescence compactness increases.

APPENDIX C: DATA MATRICES AND ADDITONAL RESULTS

Table 1. Fruit evolution data matrix. For character states refer to Table 1 in the text. 1 = dehiscence, 2 = fruit shape, 3 = position of persistent pericarp, 4 = translucence, 5 = laticifers visible, 6 = interstylar aperture morphology, 7 = fruit length, 8 = fruit width, 9 = ratio L/W, 10 = interstylar aperture length, 11 = interstylar aperture width, 12 = number of seeds.

Species	1	2	3	4	5	6	7	8	9	10	11	12
C.brachycalyx	0&3	1&2&5	1	1	0	2	1.5	2.2	0.68	0.4	0.1	3.1
C.occidentalis	0&3	1&2	2	1	0	2	1.73	2.41	0.71	0.67	0.26	3.5
C.californica	0&3	1&2	2	1	0	2	2.5	2.84	0.88	0.84	0.44	3.52
C.salina	0&3	3&4	1&2	1	1	2&3	1.7	1.3	1.3	0.51	0.15	1
C.pacifica	0&3	3&4	2	0	1	2&3	2.25	1.52	1.48	0.65	0.45	1
C.suksdorfii	0&3	1&4	3	0	1	2	1.82	2.09	0.87	0.56	0.26	2.3
C.subinclusa	0&3	1&3&4	1	0	1	2&3	3.88	2.47	1.59	1.1	0.15	1
C.howelliana	0&3	1&2	1	1	0	2	1.6	2.02	0.79	0.41	0.18	2
C.decipiens	0	1&3&4	2	0	1	2&3	2.3	1.65	1.39	0.21	0.21	1
C.obtusiflora_glandulosa	0&3	1&2	3	0	1	2	1.4	2.65	0.52	0.86	0.58	3.2
C.obtusiflora_obtusiflora	0&3	1&2	3	0	1	2	1.81	2.34	0.5	0.81	0.51	3.4
C.australis_tinei	0&3	1&2	3	0	1	2	2.2	2.98	0.73	1.1	0.53	3.4
C.australis_australis	0&3	1&2	3	0	1	2	2.6	3.6	0.72	1.39	0.61	3.4
C.campestris	0&3	1&2	3	0	1	2	2.43	3.44	0.77	1.26	0.56	3.5
C. campestris_gymnocarpa	0&3	1&2	3	1	1	2	2.2	3.01	0.73	1.1	0.46	3.3
C.pentagona	0&3	1&2	3	0	1	2	1.55	2	0.775	0.72	0.46	3.3
C.harperi	0&3	1&2	3	1	1	2	1.01	1.12	0.9	0.42	0.23	1.2
C.plattensis	0&3	1&2	2	0	1	2	2.8	3.62	0.77	0.96	0.46	2.8
C.runyonii	0&3	1&2	2	0	1	2	2	2.6	0.76	0.58	0.32	2.1
C.glabrior	0&3	1&2	2	0	1	2	2.3	2.8	0.82	0.67	0.36	2.2
C.micrantha	0	3&4	2	1	1	2	1.54	1.54	1.2	0.16	0.1	1.7
C.xanthochortos_carinata	0&1&2	1&4	2	1	1	3	2.07	2.36	0.87	0.62	0.48	3
C.corniculata	0&1&2	1&4	2	1	1	3	2.1	2.44	0.86	0.77	0.62	3
C.suaveolens	0	1	2	1	1	2	2.2	2.6	0.84	0.61	0.42	2.9
C.werdermanii	0	1	2	1	1	2	2.46	2.7	0.91	0.62	0.28	3.2
C.parviflora_elongata	0	1&4	2	1	1	2	1.85	2.19	0.82	0.62	0.6	2

C.racemosa_miniata	0	1	2	1	1	2	1.45	1.82	0.79	0.45	0.23	1.5
C.platyloba	0&2	1&4&5	2	1	1	2&3	2.62	2.72	0.96	0.61	0.31	3
C.incurvata	1&2	1&4&5	2	1	1	2&3	2.2	2.49	0.88	0.67	0.62	2.6
C.cuspidata	0	1&2&3&4	1	1	1	3	2.18	3.26	0.66	0.59	0.47	3.5
C.squamata	0	3&4	1	1	1	3	3.09	2.04	1.51	0.3	0.28	1.2
C.compacta	0&3	1&3&4	1	0	1	3	4.95	4.56	1.03	0.51	0.48	2.5
C.rostrata	0&3	1	2	0	1	5	4.92	4.07	1.2	0.7	0.64	2.5
C.gronovii_gronovii	0&3	1&4&5	2	0	1	2&3	4.82	5.41	0.89	1.23	0.62	3.2
C.gronovii_latiflora	0&3	1	3	0	1	2	2.64	2.98	0.8	0.9	0.41	3.3
C.cephalanthi	0&3	1&2	1	0	1	2	2.67	3.5	0.91	0.93	0.44	3.1
C.umbrosa	0&3	1&2&4&5	2	0	1	3	5.22	5.18	1	1.36	0.78	3.3
C.glomerata	0&3	1	1	0	1	3&5	3.08	2.81	1.09	0.72	0.56	2.4
C.denticulata	0	1&3&4	1	1	0	2	1.69	1.27	1.33	0.1	0.1	1
C.nevadensis	0	3&4	1	1	0	2	1.53	1.23	1.24	0.1	0.1	1
C.haughtii	0	1&2	3	0	1	2	1.41	2.11	0.66	0.53	0.36	3.4
C.partita	1	1&2	2	0	1	2	1.56	2.45	0.62	0.7	0.36	3.3
C.longiloba	0&2	1	2	0	1	2	1.75	2.14	0.82	0.68	0.38	3.1
C.lindsayi	1	1	2	0	1	2	3.4	4.1	0.82	0.23	0.1	2.4
C.tinctoria_tinctoria	1	1&2	1	1	0	2	2.46	2.8	0.67	0.11	0.11	2.6
C.mitriformis	1	1&4	3	0	1	2	3.92	4.11	0.97	1.11	0.9	3.1
C.jalapensis	1	1&4	2	0	1	2	4.6	4.7	0.97	1.32	1.21	2.33
C.rugosiceps	1	1&2	2	0	1	2	3.9	4.2	0.92	0.86	0.82	3
C.tinctoria_aurea	1	1	1	1	0	2	2.2	2.6	0.84	0.1	0.1	2.1
C.tinctoria_floribunda	1	1	1	1	0	2	2.52	3.2	0.78	0.1	0.1	2.3
C.woodsonii	1	1&4	3	0	0	2	4.22	5.8	0.72	1.89	0.44	2.4
C.volcanica	1	1	1&2	0	0	2	4.33	5.5	0.78	0.9	0.78	2.3
C.purpusii	1	1	1	1	0	2	2.72	3.02	0.9	0.1	0.1	1.4
C.victoriana	0	1&2	3	1	0	2	1.74	3.71	0.46	0.96	0.26	3.2
C.tasmanica	0	1&4	2	1	0	2	3.08	3.32	0.92	0.81	0.36	3.1
C.cotijana	1	1&4	2	0	1	3	3.2	3.61	0.88	0.15	0.1	3.1
C. iguanella	1	1&4	1	1	0	2	2.1	3.9	0.53	0.22	0.16	2.8
C.potosina	1	4	1	1	0	2	1.3	0.9	1.44	0.07	0.05	1
C.azteca	1	1&2	2	1	0	2	1.3	2.11	0.61	0.44	0.21	3.4
C.yucatana	0&2	1&2	2	1	0	2	1.4	2.33	0.6	0.4	0.22	3.3
C.chinensis_applanata	1	1&2	1&2	1	0	2	1.52	2.33	0.65	0.38	0.18	3.4
C.chinensis_chinensis	1	1&2	1&2	1	0	2	1.44	2.22	0.64	0.41	0.2	3.4
C.macrocephala	1	1&4	1	1	0	2	2.6	3.3	0.78	0.2	0.1	1.9
C.cozumeliensis	1	1	1	0	0	2	1.48	1.83	0.8	0.2	0.1	1.7
C.globulosa	1	1	1	0	1	2	1.62	2.52	0.64	0.22	0.1	2

C.americana	1	3&4	1	0	1	2	1.99	2.2	0.9	0.08	0.08	1
C.corymbosa_stylosa	1	1&2	1	1	0	2	1.31	1.72	0.76	0.21	0.13	2
C.prismatica	1	1	1	1	0	2	1.33	1.54	0.86	0.18	0.11	2
C.corymbosa_grandiflora	1	1&2	1	1	0	2	1.51	1.88	0.8	0.15	0.12	2.2
C.chapalana	1	1&2	1	1	0	2	1.55	1.76	0.87	0.1	0.09	3
C.mexicana	1	3&4	1	0	1	2	1.5	3.36	1.04	0.13	0.06	1.2
C.strobilacea_strobilacea	1	1&2	1	0	1	2&3	2.6	3.3	0.78	0.12	0.09	3.2
C.erosa	1	1	1&2	0	0	2	1.85	2.5	0.74	0.15	0.07	3
C.boldinghii	1	1&2	1	0	0	2	1.7	2.4	0.7	0.1	0.04	2.5
C.costaricensis	1	1&2	1&2	1	0	2	2.1	3.2	0.65	0.16	0.06	3
C. bonafortunae	1	1&2	2&3	1	0	2	1.8	2.3	0.78	0.43	0.03	2.3
C.odontolepis	1	1&2	1&2	1	0	2&3	1.9	2.25	0.84	0.21	0.15	2.6
C.legitima	1	1&2	1&2	1	0	2&4	1.65	2.3	1.04	0.35	0.21	2.6
C.tuberculata	1	1	1	1	0	2&4	1.51	2.01	0.75	0.18	0.11	2
C.umbellata	1&2	1&2	1&2	1	1	2&4	1.62	2.09	0.77	0.62	0.27	3.6
C. desmouliniana	1	1&2	1	1	1	2&4	1.52	2.05	0.74	0.25	0.15	2.6
C.hyalina	1	1&2	2	1	1	2&4	1.59	2.01	0.79	0.32	0.18	3.1
C.acuta	0&2	1	2	1	1	2&4	2	2.5	0.8	0.72	0.4	3.8
C.leptantha	1	1&2	1	1	1	2&4	1.5	1.8	0.83	0.24	0.12	2.1
C.polyanthemos	1	1&2	1	1	1	2&4	1.2	2.25	0.88	0.35	0.23	2
C.indecora	0	1&2	1&2	0	1	3	2.7	3.2	0.84	0.65	0.47	3.2
C.coryli	0&3	1&2	1&2	0	1	2	2.77	4.23	0.65	0.99	0.75	3.1
C.warnerii	0	1	1	0	1	3	2.65	3.3	0.8	0.56	0.52	2.3
C.deltoidea	1	1	1	1	1	2	0.9	1.2	0.75	0.07	0.01	2.2
C.vandevenderi	0&3	1&2&4	3	0	1	2	1.1	1.8	0.61	0.46	0.31	3.6
C.sidarum	1	1&2	1	0	1	2	1.2	1.4	0.85	0.06	0.05	2.3
C.colombiana	1&2	1&2	1	0	1	2&3	0.9	1.2	0.75	0.05	0.02	2.1
C.gracillima	1	1&2	2	1	0	2	1.3	1.5	0.86	0.06	0.05	2.2
C.punana	1	2	1	1	0	2	1.23	1.82	0.64	0.1	0.1	3.1
C.macvaughii	1	1&2	1	1	0	2&3	1.23	1.63	0.75	0.11	0.06	2.2
C.cockerellii	1	1&2	1&2	1	0	2	2.2	2.57	0.85	0.16	0.09	2.3
C.chilensis	1	1	1&2	1	0	2	3.21	4.06	0.79	0.35	0.16	2.8
C.odorata	1	1&2	1&2	1	0	2	2.1	2.87	0.73	0.34	0.15	2.3
C.purpurata	1	1	1&2	1	0	2	1.52	2.57	0.59	0.36	0.18	3.2
C.foetida_foetida	1	1	1&2	1	0	2	2	2.8	0.71	0.34	0.15	2.4
C.paitana	1	1	1&2	1	0	2	1.8	2.6	0.69	0.21	0.14	2
C.foetida_pycnantha	1	1&2	1&2	1	1	2	2.1	2.9	0.7	0.33	0.12	2.2
C.globiflora	1	1&2	1&2	1	0	2	1.97	3.56	0.55	0.18	0.14	3.4
C.grandiflora	1	1&2	1&2	1	0	2	1.1	3.6	0.3	0.54	0.42	3.3

C.parodiana	1	1&2	1&2	1	0	2	2.1	2.9	0.79	0.51	0.38	2.1
C.kilimanjari	1	1&2	1&2	1	0	2	2.2	3.1	0.7	0.14	0.06	2.4
C.cristata	0&2	1&2	1&2	0	0	2	2.11	3.11	0.67	0.85	0.71	3.1
C.argentinana	1	1&2	1&2	0	0	2	2.12	3.27	0.64	0.38	0.21	2.2
C.friesii	1	1	1&2	0	1	2	2.2	4.45	0.49	0.77	0.26	2.8
C.microstyla	0	1&2	2&3	0	1	2	1.3	0.9	1.4	0.32	0.25	1
C.natalensis	1	1&2	2	0	1	1	1.4	1.8	0.88	0.23	0.1	2.4
C.nitida	1	1	3	0	0	1	1.3	1.7	0.9	0.21	0.1	2.2
C.angulata	1	1&2	3	0	0	1	1.27	1.65	0.85	0.18	0.09	2.6
C.africana	1	3&4	2	0	1	1	1.24	1.66	0.83	0.23	0.18	2
C.europaea	1	1&2	1	1	0	1	2.2	2.79	0.78	0.26	0.15	3.6
C.epilinum	1	1&2	1	1	0	1	2.3	2.85	0.8	0.36	0.13	3.6
C.approximata	1	1	1	1	0	0	1.72	2.22	0.77	0.37	0.16	3.6
C.epithymum	1	1	1	1	0	0	1.53	1.72	0.88	0.34	0.15	3.7
C.planiflora	1	1	1	1	0	0	1.34	1.72	0.76	0.26	0.1	3.6
C.exaltata	1	6	1	0	1	0	5.88	5.47	1.07	0	0	2.5
C.lehmanniana	1	3&6	1	0	1	0	6	4.51	1.33	0	0	2.4
C.monogyna	1	3&6	1	0	1	0	4.9	4.6	1.06	0	0	2.3
C.japonica	1	3&6	1	0	1	0	5.14	4.45	1.17	0	0	2.6
C.reflexa	1	3&6	1	0	1	0	6.28	5.17	1.24	0	0	3.1
C.cassythoides	1	3&6	1	0	1	0	4.99	6.1	1.15	0	0	2.5
C.lupuliformis	1	3&6	1	0	1	0	4.2	3.78	1.17	0	0	2.1

Table 2. Biogeography data matrix

Species/variety	Type1	Type2	Area (km2)	Max Y	Max X
C. californica var. californica Hook. & Arn.	IN	IN+IrB	1516447.01	47.52525	-124.04628
C. californica var. apiculata Engelm.	IN	IN	4985.35	35	-114.6504
C. californica var. papillosa Yuncker	IN	IN	218793.15	41.55792	-120.57495
C. occidentalis Millsp.	IN	IN+IrB	1052370.01	45.31972	-118.0879
C. brachycalyx Yunck.	IN	IN+IrB	151766.82	42.28303	-121.94433
C. subinclusa Durand & Hilg.	IN	IN+IrB	269373.01	40.5385	-122.174
C. salina Engelm.	IN	IN+IrB	1508262.63	48.42161	-123.35919
C. pacifica var. pacifica Costea & Wright	IN	IN+IrB	553965.92	49.06514	-122.99284
C. pacifica var. papillata (Yunck.) Costea & Wright	IN	IN	138500.7	39.44639	-122.14944
C. suksdorfii Yunck.	IN	IN+IrB	230878.31	46.25611	-122.14927
C. howelliana Rubtzoff	IN	IN+IrB	65733.42	41.43912	-122.43115
C. draconella Costea & Stefanović	IN	IN	3725.61	33.59061	-106.42084
C. jepsonii Yunck.	IN	IN	357.17	40.24451	-123.00882
C. decipiens Yunck.	IN	IN	58183.12	31.87483	-105.97844
C. obtusiflora var. glandulosa Engelm.	IN	IN+IrB	7286798.29	39.30936	-121.88212
C. pentagona Engelm.	IN	IN+IrB	5352161.32	49.00257	-96.98198
C. harperi Small	IN	IN+IrB	16800.28	34.45278	-85.60288
C. runyonii Yunck.	IN	IN+IrB	30346.91	28.48102	-98.67303
C. glabrior (Engelm.) Yunck.	IN	IN+IrB	7481331.81	34.9601	-94.78473
C. plattensis A. Nelson	IN	IN+IrB	0	43.47932	-104.20646
C. campestris Yunck.	IN	IN+IrB	18754484.08	51.06808	-104.95837
C. polygonorum Engelm.	IN	IN	1655871.03	49.75695	-94.47699
C. rostrata Shuttlew. ex Engelm.	IN	IN+IrB	240147.99	39.04364	-79.3181
C. gronovii Willd. ex Schult *var. gronovii*	IN	IN+IrB	11308257.5	53.44883	-103.93558
C. gronovii var. latiflora Engelm.	IN	IN+IrB	1429684.63	43.28971	-77.14383
C. gronovii var. calyptrate Engelm.	IN	IN	0	33.01022	-96.48279
C. umbrosa Beyr. Ex Hook.	IN	IN+IrB	4559418.67	53.53937	-113.48878
C. cephalanthi Engelm.	IN	IN+IrB	8728811.79	49.36611	-124.49833
C. glomerata Choisy	IN	IN+IrB	1443970.54	46.44769	-97.68477

C. *cuspidate* Engelm.	IN	IN	2977803.05	48.33407	-100.39056
C. *squamata* Engelm.	IN	IN	652504.26	33.65196	-101.72261
C. *compacta* Juss.	IN	IN+IrB	2195210.46	42.51075	-71.68475
C. *denticulate* Engelm.	IN	IN	916667.25	46.91738	-121.85494
C. *nevadensis* I. M. Johnst.	IN	IN	74530.51	37.374	-118.2924
C. *veatchii* Brandegee	IN	IN	72153.35	34.88297	-120.45891
C. *mitriformis* Engelm.	DE	DE	1008963.49	31.9723	-109.0506
C. *tinctoria* Mart. ex. Engelm.	DE	DE	591644.48	30.62084	-110.99073
C. *purpusii* Yunck.	DE	DE	93565.41	25.79288	-100.03639
C. *woodsonii* Yunck.	DE	DE	0	15.44376	-92.33125
C. *volcanica* Costea & I. García	DE	DE	42047.35	20.83326	-103.95889
C. *cotijana* Costea & I. García	DE	DE	805.31	19.73258	-102.69137
C. *jalapensis* Schltdl.	DE	DE	506561.88	23.28211	-99.27656
C. *rugosiceps* Yunck.	DE	DE	57963.56	21.21217	-99.46956
C. *lindsayi* Wiggins	DE	DE	3895.88	23.19528	-105.63887
C. *iguanella* Costea & I. García	DE	DE	1706.8	21.30003	-104.65071
C. *americana* L.	DE	DE	6646790.15	28.95577	-112.40807
C. *macrocephala* W. Schaffn. ex yunck.	DE	DE	783646.76	27.40115	-108.84166
C. *azteca* Costea & Stefanović	DE	DE	884853.14	35.02615	-106.44652
C. *chinensis* Lam var. *applanata* (Engelm.) Costea & Stefanović	DE	DE	1354494.11	35.7934	-106.27625
C. *dentatasquamata* Yunck.	DE	DE	0.09	31.82585	-110.77478
C. *yucatana* Yunck.	IN	IN+IrA	231861.81	20.68673	-88.20294
C. *potosina* Schaffn. Ex. Yunck.	DE	DE	37094.8	22.14026	-100.97218
C. *erosa* Yunck.	DE	DE	204936.66	31.78768	-111.58188
C. *boldinghii* Urb.	DE	DE	5001285.31	26.93754	-108.88337
C. *strobilacea* Liebm.	DE	DE	268309.12	19.99305	-102.70583
C. *chapalana* Yunck.	DE	DE	28322.83	21.72996	-101.66149
C. *mexicana* Yunck.	DE	DE	0	19.58776	-104.2478
C. *costaricensis* Yunck.	DE	DE	682515.97	28.45266	-108.53686
C. *bonafortunae* Costea & I. García	DE	DE	0.27	20.17315	-100.99198
C. *legitima* Costea & Stefanović	DE	DE	1643466.02	38.02705	-102.01064
C. *odontolepis* Engelm.	DE	DE	37199.89	31.81858	-110.78445
C. *tuberculata* Brandegee	DE	DE	421539.71	34.18582	-112.72763

C. umbellata Kunth var. *umbellata*	DE	DE+IrA	8775886.53	38.83808	-105.17435
C. leptantha Engelm.	DE	DE	564653.23	29.53172	-113.56779
C. polyanthemos Schaffn. ex. Yunck.	DE	DE	23132.43	29.66634	-109.62659
C. liliputana Costea & Stefanović	DE	DE	345434.51	34.1983	-104.43574
C. indecora Choisy var. *indecora*	IN	IN	14768729.62	51.40304	-107.01234
C. indecora var. *longisepala* Yunck.	IN	IN	1321669.65	34.17268	-96.41723
C. indecora var. attenuata	IN	IN	294600.39	39.74889	-97.5943
C. coryli Engelm.	IN	IN+IrB	3933418.79	47.92154	-104.02605
C. warneri Yunck.	IN	IN	1.42	38.96811	-112.59258
C. mcvaughii Yunck.	DE	DE	1669.79	19.19171	-102.84578
C. sidarum Liebm.	DE	DE	360331.55	19.53512	-105.07224
C. gracillima Engelm.	DE	DE	244942.71	22.96688	-105.85656
C. deltoidea Yunck.	DE	DE	2920.37	20.31057	-102.2108
C. vandevenderi Costea & Stefanović	IN	IN+IrB	9464.4	28.37778	-109.15001

Table 3. Infructescence compactness data matrix.

Clade	Type1	Type2	Species ep	Final
A	IN	IN	decipiens	0.153081
A	IN	IN+IrB	brachycalyx	0.063195
A	IN	IN+IrB	california	0.062032
A	IN	IN+IrB	occidentalis	0.080131
A	IN	IN+IrB	pacifica	0.107062
A	IN	IN+IrB	salina	0.126204
A	IN	IN+IrB	subinclusa	0.052574
B	IN	IN+IrB	australis	0.033753
B	IN	IN+IrB	campestris	0.043105
B	IN	IN+IrB	glabrior	0.067022
B	IN	IN+IrB	obtusiflora	0.053023
B	IN	IN+IrB	obtusiflora var glandulosa	0.040857
C	DE	DE+IrA	incurvata	0.05781
C	IN	IN	suaveolens	0.029261
C	IN	IN+IrA	platyloba	0.050065
C	DE	DE+IrA+IN	corniculata	0.038014
C	DE	DE+IrA+IN	xanthocortos var. carinata	0.082941
D	IN	IN	squamata	0.025746
D	IN	IN+IrB	cephalanthi	0.039236
D	IN	IN+IrB	gronovii	0.025431
D	IN	IN+IrB	gronovii var. latiflora	0.038219
E	IN	IN	denticulata	0.070095
E	IN	IN	nevadensis	0.28558
F	DE	DE	partita	0.118526
F	IN	IN	haughtii	0.122627
F	IN	IN+IrA	longiloba	0.102906
G	DE	DE	cotijana	0.048198
G	DE	DE	jalapensis	0.021129
G	DE	DE	lindsayi	0.058671
G	DE	DE	mitriformis	0.031255
G	DE	DE	purpusii	0.254435
G	DE	DE	rugosiceps	0.025355
G	DE	DE	tinctoria	0.044374
G	DE	DE	volcanica	0.009056
G	IN	IN	victoriana	0.030633
H	DE	DE	azteca	0.053171
H	DE	DE	chinensis	0.246919
H	DE	DE	chinensis var. applanata	0.051321
H	IN	IN+IrA	yucatana	0.039498
I	DE	DE	americana	0.044912

I	DE	DE	globulosa	0.068199
I	DE	DE	macrocephala	0.070569
J	DE	DE	corymbosa var. grandiflora	0.055525
J	DE	DE	corymbosa var. stylosa	0.086358
J	DE	DE	prismatica	0.123347
K	DE	DE	boldinghii	0.036289
K	DE	DE	chapalana	0.061664
K	DE	DE	costaricensis	0.034522
K	DE	DE	erosa	0.097334
K	DE	DE	strobilacea	0.032798
L	DE	DE	legitima	0.081259
L	DE	DE	leptantha	0.294304
L	DE	DE	odontolepis	0.025827
L	DE	DE	polyanthemos	0.403477
L	DE	DE	tuberculata	0.078763
L	DE	DE+IrA	umbellata	0.474826
L	IN	IN+IrA	acuta	0.094285
M	DE	DE	macvaughii	0.12059
M	IN	IN	indecora	0.128902
N	DE	DE	deltoidea	0.299626
N	DE	DE	gracillima	0.078063
N	DE	DE	sidarum	0.082431
N	DE	DE+IrA	colombiana	0.237181
N	IN	IN+IrB	vandevenderi	0.077618
O	DE	DE	argentinana	0.072461
O	DE	DE	chilensis	0.030744
O	DE	DE	foetida	0.040036
O	DE	DE	friesii	0.079792
O	DE	DE	globiflora	0.025325
O	DE	DE	grandiflora	0.244247
O	DE	DE	odorata	0.038507
O	DE	DE	paitana	0.397783
O	DE	DE	parodiana	0.091013
O	DE	DE	purpurata	0.089353
O	IN	IN+IrA	cristata	0.047578

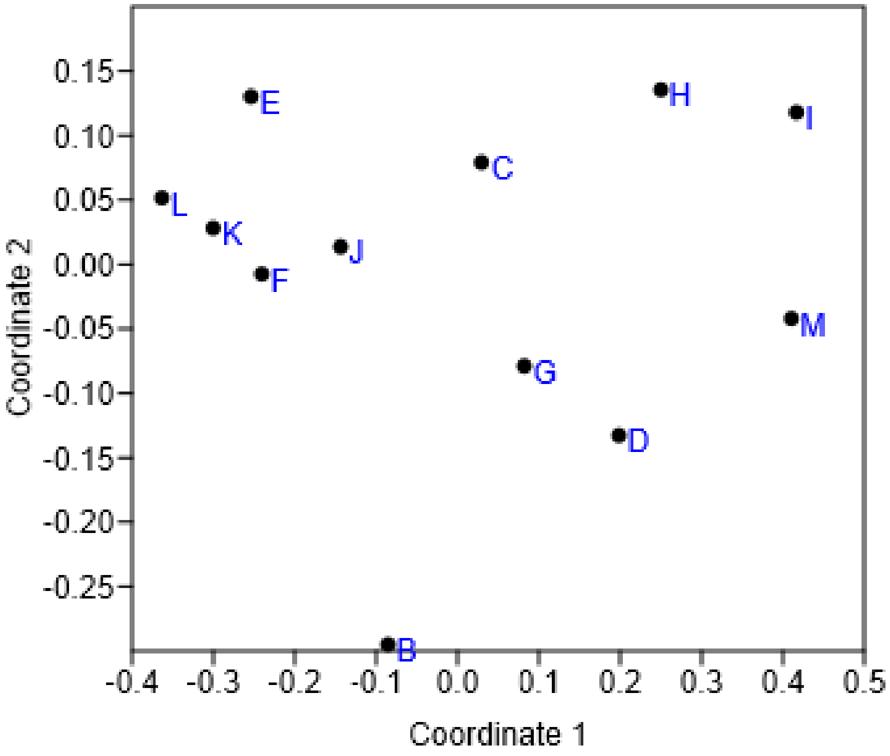

Figure 1. NMDS scatter plot displaying similarity/dissimlarity for all fruit traits (excluding pericarp papillae and style morphology). B = dehiscence/indehiscence modes, C = fruit shape, D = position of corolla, E = translucence, F = laticifers, G = interstylar aperture, H = fruit length, I = fruit width, J = ratio L/W, K = ISA length, L = ISA width, M = number of seeds per capsule. Stress value = 0.09453.

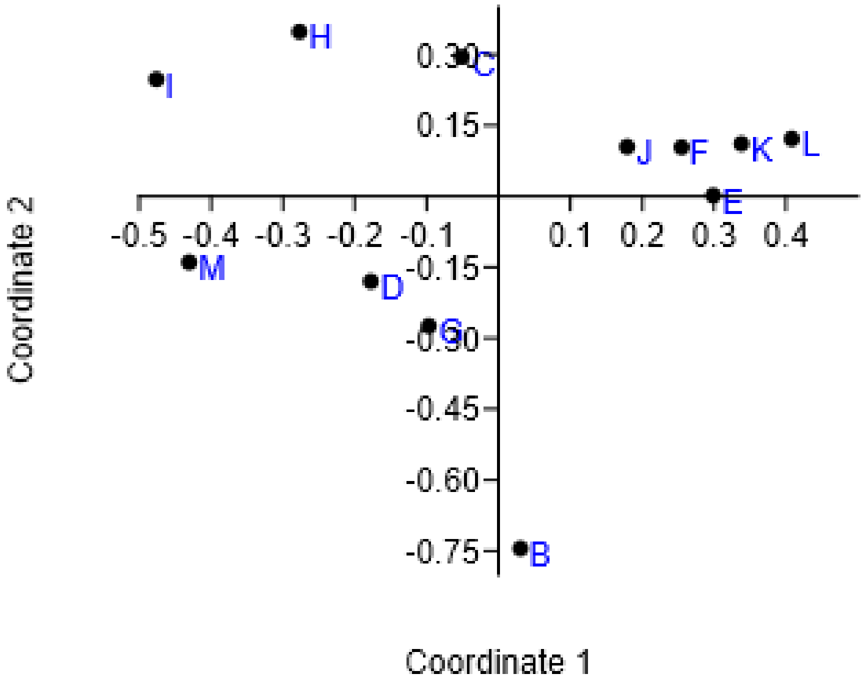

Figure 2. PCoA plot displaying similarity/dissimilarity for all fruit traits (excluding pericarp papillae and style morphology). B = dehiscence/indehiscence modes, C = fruit shape, D = position of corolla, E = translucence, F = laticifers, G = interstylar aperture, H = fruit length, I = fruit width, J = ratio L/W, K = ISA length, L = ISA width, M = number of seeds per capsule.

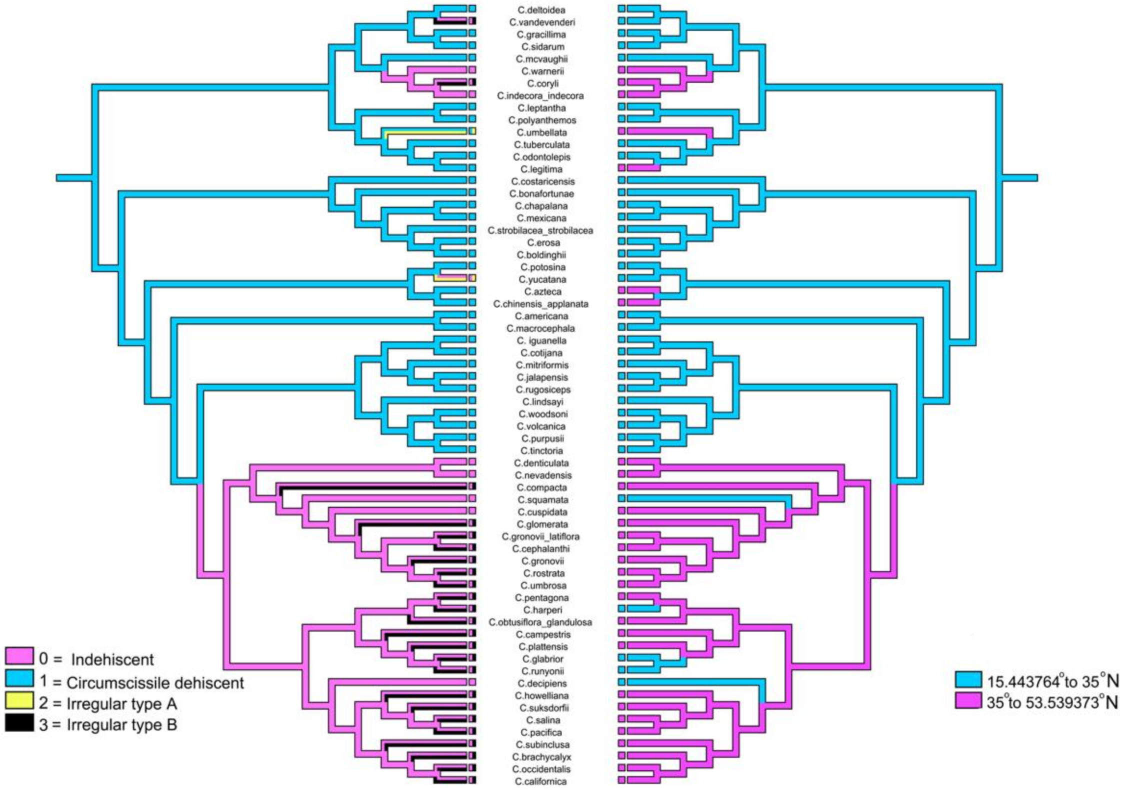

Figure 3. Mirror tree displaying fruit dehiscence/indehiscence character history (left) and evolution of species latitudinal limits above and below 35^{o}N.

APPENDIX D: R CODES WITH ANNOTATIONS

Fruit morphology

```
> data<-read.csv(“Fruit characters.csv”)
> attach(data)
> head(data)
> #test for normality (only fruit length shown)
> shapiro.test(Fruit.length[which(Dehiscence==”DE”)])
> shapiro.test(Fruit.length[which(Dehiscence==”IN”)])
> shapiro.test(Fruit.length[which(Dehiscence=="DE+IrA")])
> shapiro.test(Fruit.length[which(Dehiscence=="IN+DE+IrA")])
> shapiro.test(Fruit.length[which(Dehiscence=="IN+IrA")])
> shapiro.test(Fruit.length[which(Dehiscence=="IN+IrB")])
> leveneTest(Fruit.length~Dehiscence)
> kruskal.test(Fruit.length, g=Dehiscence, var.equal=TRUE)
> dunn.test(Fruit.length, g=Dehiscence, list=TRUE)
> summary(Fruit.length[which(Dehiscence==”DE”)])
> length(Fruit.length[which(Dehiscence==”DE”)])
> # standard error of means
sem=sqrt(var(Fruit.length[which(Dehiscence=="DE")])/length(Fruit.length[which(Dehisc
ence=="DE")]))
> sem
> #bootstrapped confidence intervals for non normal data
> library(boot)
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Fruit.length[which(Dehiscence=="DE")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")
> summary(Fruit.length[which(Dehiscence==”IN”)])
> length(Fruit.length[which(Dehiscence==”IN”)])
>
sem=sqrt(var(Fruit.length[which(Dehiscence=="IN")])/length(Fruit.length[which(Dehisc
ence=="IN")]))
> sem
> leftCI<-mean(Fruit.length[which(Dehiscence=="IN")])-(2*sem)
> rightCI<-mean(Fruit.length[which(Dehiscence=="IN")])+(2*sem)
> leftCI
> rightCI

>
sem=sqrt(var(Fruit.length[which(Dehiscence=="IN+IrB")])/length(Fruit.length[which(D
ehiscence=="IN+IrB")]))
> sem
```

```
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Fruit.length[which(Dehiscence=="IN+IrB")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")

>
sem=sqrt(var(Fruit.width[which(Dehiscence=="DE")])/length(Fruit.width[which(Dehisce
nce=="DE")]))
> sem
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Fruit.width[which(Dehiscence=="DE")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")

>
sem=sqrt(var(Fruit.width[which(Dehiscence=="IN")])/length(Fruit.width[which(Dehisce
nce=="IN")]))
> sem
> leftCI<-mean(Fruit.width[which(Dehiscence=="IN")])-(2*sem)
> rightCI<-mean(Fruit.width[which(Dehiscence=="IN")])+(2*sem)
> leftCI
> rightCI
>
sem=sqrt(var(Fruit.width[which(Dehiscence=="IN+IrB")])/length(Fruit.width[which(De
hiscence=="IN+IrB")]))
> sem
> leftCI<-mean(Fruit.width[which(Dehiscence=="IN+IrB")])-(2*sem)
> rightCI<-mean(Fruit.width[which(Dehiscence=="IN+IrB")])+(2*sem)
> leftCI
> rightCI

> #cannot use same sem codes for standard errors with missing values, must remove
> library(FSA)
> se(ISA.length[which(Dehiscence=="DE")], na.rm=TRUE)
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(ISA.length[which(Dehiscence=="DE")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")

> sem=se(ISA.length[which(Dehiscence=="IN")], na.rm=TRUE)
> sem
> leftCI<-mean(ISA.length[which(Dehiscence=="IN")])-(2*sem)
> rightCI<-mean(ISA.length[which(Dehiscence=="IN")])+(2*sem)
```

```
> leftCI
> rightCI
> sem=se(ISA.length[which(Dehiscence=="IN+IrB")], na.rm=TRUE)
> sem
> leftCI<-mean(ISA.length[which(Dehiscence=="IN+IrB")])-(2*sem)
> rightCI<-mean(ISA.length[which(Dehiscence=="IN+IrB")])+(2*sem)
> leftCI
> rightCI
> sem=se(ISA.width[which(Dehiscence=="DE")], na.rm=TRUE)
> sem
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(ISA.width[which(Dehiscence=="DE")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")
> sem=se(ISA.width[which(Dehiscence=="IN")], na.rm=TRUE)
> sem
> leftCI<-mean(ISA.width[which(Dehiscence=="IN")])-(2*sem)
> rightCI<-mean(ISA.width[which(Dehiscence=="IN")])+(2*sem)
> leftCI
> rightCI
> sem=se(ISA.width[which(Dehiscence=="IN+IrB")], na.rm=TRUE)
> sem
> leftCI<-mean(ISA.width[which(Dehiscence=="IN+IrB")])-(2*sem)
> rightCI<-mean(ISA.width[which(Dehiscence=="IN+IrB")])+(2*sem)
> leftCI
> rightCI
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Nr.of.seeds.per.capsule[which(Dehiscence=="DE")], mean.fun,
R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> boot.out<-boot(Nr.of.seeds.per.capsule[which(Dehiscence=="IN")], mean.fun,
R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> boot.out<-boot(Nr.of.seeds.per.capsule[which(Dehiscence=="IN+IrB")], mean.fun,
R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> sem=se(Nr.of.seeds.per.capsule[which(Dehiscence=="IN+IrA")], na.rm=TRUE)
> sem
> leftCI<-mean(Nr.of.seeds.per.capsule[which(Dehiscence=="IN+IrA")])-(2*sem)
> rightCI<-mean(Nr.of.seeds.per.capsule[which(Dehiscence=="IN+IrA")])+(2*sem)
> leftCI
> rightCI
```

Biogeography

```
> #Range size (previous categorization, excludes populations with 0 area)
> data<-read.csv("AreaIN.csv")
> attach(data)
> shapiro.test(Area[which(Dehiscence=="DE")])
> sqrt<-sqrt(Area[which(Dehiscence=="DE")])
> shapiro.test(sqrt)
> log<-log(Area[which(Dehiscence=="DE")])
> shapiro.test(log)
> #data not normal, can’t transform to normal
> library(car)
> leveneTest(Area~Dehiscence)
> #p-value = 0.065, variance the same between two groups
> wilcox.test(Area~Dehiscence, var.equal=TRUE)
> #no difference between indehiscent and dehiscent species (range size), p-value =
0.07798
> boxplot(Area~Dehiscence,xlab="Fruit Dehiscence",ylab="Range Size
(km^2)",las=0,col=c("yellow", "blue"), cex.lab=1.5, cex.axis=1.5, lwd=1.5)
>

> #summarize data
> library(boot)
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Area[which(Dehiscence=="Dehiscent")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")
> #summary
> summary(Area[which(Dehiscence=="DEt")])
> #sample size
> length(Area[which(Dehiscence=="DE")])
> #standard error
>
sem=sqrt(var(Area[which(Dehiscence=="DEt")])/length(Area[which(Dehiscence=="DE"
)]))
> sem

> #codes for indehiscent species are the same except Dehiscence==”IN”

> #Range size (new categorization)
> detach(data)
> data<-read.csv(“AreaEXinadea.csv”)
> attach(data)
> shapiro.test(Area[which(Dehiscence2=="DE")])
> sqrt<-sqrt(Area[which(Dehiscence2=="DE")])
> shapiro.test(sqrt)
```

```
> log<-log(Area[which(Dehiscence2=="DE")])
> shapiro.test(log)
> #not normal, can’t be transformed
> kruskal.test(Area~Dehiscence2)
> #p-value = 0.03643, difference in range size between species with different fruit types
> library(dunn.test)
> dunn.test(Area, g=Dehiscence2)
> dunn.test(Area, g=Dehiscence2)
  Kruskal-Wallis rank sum test

data: Area and Dehiscence2
Kruskal-Wallis chi-squared = 6.6246, df = 2, p-value = 0.04



                    Comparison of Area by Dehiscence2
                           (No adjustment)
Col Mean-|
Row Mean |         DE         IN
---------+----------------------
      IN |  -0.969883
         |    0.1661
         |
     INB |  -2.564525  -0.696641
         |    0.0052     0.2430

> boxplot(Area~Dehiscence2,xlab="Fruit Dehiscence",ylab="Range Size
(km^2)",las=0,col=c("yellow", "blue", "magenta"), cex.lab=1.5, cex.axis=1.5, lwd=1.5)
>
> #summary of range data
> #range data not normal
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Area[which(Dehiscence2=="DE")], mean.fun, R=1000,
sim="ordinary")
> boot.ci(boot.out, type="norm")
> length(Area[which(Dehiscence2=="DE")])
>
sem=sqrt(var(Area[which(Dehiscence2=="DE")])/length(Area[which(Dehiscence2=="D
E")]))
> sem
> summary(Area[which(Dehiscence2=="DE")])
> #codes for IN and INB summaries are essentially the same



> #Max Latitude (previous categorization)
```

```
> detach(data)
> data<-read.csv("MaxIN.csv")
> attach(data)
> shapiro.test(Max.X[which(Dehiscence=="Dehiscent")])
> shapiro.test(Max.X[which(Dehiscence=="Indehiscent")])
> #normal
> var.test(Max.X~Dehiscence)
> #p-value = 0.3063, variances equal
> t.test(Max.X~Dehiscence, var.equal=TRUE)
> p-value = 9.051e-14, significantly different
> boxplot(Max.X~Dehiscence, las=1, ylab="Northernmost Latitude
(dd)",xlab="Dehiscence", col=(c("yellow","blue")))
>
> #summary statistics northernmost distribution
> summary(Max.X[which(Dehiscence=="Dehiscent")])
>
sem=sqrt(var(Max.X[which(Dehiscence=="Dehiscent")])/length(Max.X[which(Dehiscen
ce=="Dehiscent")]))
> sem
> leftCI<-mean(Max.X[which(Dehiscence=="Dehiscent")])-(2*sem)
> rightCI<-mean(Max.X[which(Dehiscence=="Dehiscent")])+(2*sem)
> leftCI
> rightCI

> summary(Max.X[which(Dehiscence=="Indehiscent")])
>
sem=sqrt(var(Max.X[which(Dehiscence=="Indehiscent")])/length(Max.X[which(Dehisce
nce=="Indehiscent")]))
> sem
> leftCI<-mean(Max.X[which(Dehiscence=="Indehiscent")])-(2*sem)
> rightCI<-mean(Max.X[which(Dehiscence=="Indehiscent")])+(2*sem)
> leftCI
> rightCI
> length(Max.X[which(Dehiscence=="Indehiscent")])

> #Max Latitude (new categorization without INA and DEA because sample size too
small)
> detach(data)
> data<-read.csv("MaxEXinadea.csv")
> attach(data)
> shapiro.test(Max.X[which(Dehiscence2=="DE")])
> shapiro.test(Max.X[which(Dehiscence2=="IN")])
> shapiro.test(Max.X[which(Dehiscence2=="INB")])
> #all normal
> bartlett.test(Max.X, g=Dehiscence2)
> # p-value = 0.633, variances equal, parametric: ANOVA
```

```
> aov=aov(Max.X~Dehiscence2)
> summary(aov)
> #pvalue = 3.2e-14, test for effect size
> TukeyHSD(aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Max.X ~ Dehiscence2)

$Dehiscence2
            diff       lwr       upr     p adj
IN-DE  15.520932  8.957158 22.084706 0.0000013
INB-DE 17.867769 13.603088 22.132450 0.0000000
INB-IN  2.346837 -4.390960  9.084633 0.6814385

> #difference between IN-DE and INB-DE but not between INB-IN
> boxplot(Max.X~Dehiscence2, las=1, ylab="Northernmost Latitude
(dd)",xlab="Dehiscence", col=(c("yellow","blue", "magenta")))
>

> #code for summary statistics essentially the same as old categorization northernmost
limit codes (normally distributed)
```

Floatability

```
> data<-read.csv("dispersal survival.csv")
> attach(data)
> head(data)
> km.fit1<-survfit(Surv(Time, Status)~Treat, data=data, type="kaplan-meier")
> plot(km.fit, col=c("orange","blue","red"), xlab="Time (days)", ylab="Survival",
conf.int=TRUE, cex.axis=1.5, cex.lab=1.5, lwd=2, las=1)
> legend("center", legend=c("Dried Seeds", "Imbibed Capsules", "Imbibed Seeds"),
fill=c("orange","blue","red"), bty="n", pt.cex=1, cex=1.5)
>
> float<-survdiff(Surv(Time, Status)~Treat)
> float
> #pvalue= 0, difference in survival curves
> coxph1<-coxph(Surv(Time, Status)~Treat)
> summary(coxph1)
> #p-values extremely low between all treatments

> summary(km.fit)
Call: survfit(formula = Surv(Time, Status) ~ Treat, data = data, type = "kaplan-meier")
```

```
               Treat=DS
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
  0.0    300      86  0.71333 0.02611     0.663955       0.7664
  0.5    214     159  0.18333 0.02234     0.144384       0.2328
  1.5     55      54  0.00333 0.00333     0.000471       0.0236

               Treat=IC
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
  1.5    300       2    0.993 0.00470        0.984        1.000
  2.5    298       3    0.983 0.00739        0.969        0.998
  3.0    295       1    0.980 0.00808        0.964        0.996
  3.5    294       2    0.973 0.00930        0.955        0.992
  4.5    292       1    0.970 0.00985        0.951        0.989
  5.0    291       4    0.957 0.01176        0.934        0.980
  5.5    287       8    0.930 0.01473        0.902        0.959
  6.0    279      19    0.867 0.01963        0.829        0.906
  6.5    260      18    0.807 0.02280        0.763        0.853
  7.0    242      12    0.767 0.02442        0.720        0.816
  7.5    230      20    0.700 0.02646        0.650        0.754
  8.0    210      27    0.610 0.02816        0.557        0.668
  8.5    183      26    0.523 0.02884        0.470        0.583
  9.0    157      31    0.420 0.02850        0.368        0.480

               Treat=IS
        time       n.risk      n.event     survival      std.err lower 95% CI
           0          300          300            0          NaN           NA
upper 95% CI
          NA

>
```

Germination

```
> #Comparison of number of seeds germinated (30 reps of 10 capsules/10-40 seeds)
> data<-read.csv("germfinalanova.csv")
> attach(data)
> #test distribution 2015
> shapiro.test(Total15[which(Treat=="W")])
> #normal (p-value=0.285)
> shapiro.test(Total15[which(Treat=="WO")])
> #p-value = 0.3582, normal
> shapiro.test(Total15[which(Site=="GR")])
> #p-value = 0.3993, normal
> shapiro.test(Total15[which(Site=="LP”)])
> #p-value = 0.01474, not normal
> var.test(Total15~Treat)
```

```
> #variances equal p-value=0.139
> t.test(Total15~Treat, var.equal=TRUE)
> #differences between treatments p-value=0.0003367
> levene.test(Total15~Site)
> #variances equal, p-value = 0.1428
> wilcox.test(Total15~Site, var.equal=TRUE)
> #no difference between sites, p-value = 0.1708
> summary(Total15[which(Treat=="W")])
> summary(Total15[which(Treat=="WO")])
> summary(Total15[which(Site=="GR")])
> summary(Total15[which(Site=="LP")])
> semW=sqrt(var(Total15[which(Treat=="W")])/length(Total15[which(Treat=="W")]))
> semW
> leftCI
> rightCI
>
semWO=sqrt(var(Total15[which(Treat=="WO")])/length(Total15[which(Treat=="WO")]
))
> semWO
> semGR=sqrt(var(Total15[which(Site=="GR")])/length(Total15[which(Site=="GR")]))
> semGR
> semLP=sqrt(var(Total15[which(Site=="LP")])/length(Total15[which(Site=="LP")]))
> semLP
> leftCI<-mean(Total15[which(Treat=="W")])-(2*semW)
> rightCI<-mean(Total15[which(Treat=="W")])+(2*semW)
> leftCI
> rightCI
> leftCI<-mean(Total15[which(Treat=="WO")])-(2*semWO)
> rightCI<-mean(Total15[which(Treat=="WO")])+(2*semWO)
> leftCI
> rightCI
> leftCI<-mean(Total15[which(Site=="GR")])-(2*semGR)
> rightCI<-mean(Total15[which(Site=="GR")])+(2*semGR)
> leftCI
> rightCI
> #bootstrap LP for normalized confidence intervals
> library(boot)
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Total15[which(Site==”LP”)], mean.fun, R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
                BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
Based on 1000 bootstrap replicates

CALL :
boot.ci(boot.out = boot.out, type = "norm")
```

```
Intervals :
Level      Normal
95%   ( 7.423, 10.346 )
Calculations and Intervals on Original Scale
> #differences in 2016:

> shapiro.test(Total16[which(Treat=="W")])
> shapiro.test(Total16[which(Treat=="WO")])
> shapiro.test(Total16[which(Site=="GR")])
> shapiro.test(Total16[which(Site=="LP")])
> #all NON normal

> levene.test(Total16~Treat)
> #variances equal, p-value = 0.2556
> levene.test(Total16~Site)
> #variances unequal, p-value = 0.0001538
> wilcox.test(Total16~Treat, var.equal=TRUE)
> #no difference between treatments, p-value = 0.9936
> wilcox.test(Total16~Site, var.equal=FALSE)
> #difference between sites, p-value = 0.006167

> summary(Total16[which(Treat=="W")])
> summary(Total16[which(Treat=="WO")])
> summary(Total16[which(Site=="GR")])
> summary(Total16[which(Site=="LP")])

> semW=sqrt(var(Total16[which(Treat=="W")])/length(Total16[which(Treat=="W")]))
> semW
>
semWO=sqrt(var(Total16[which(Treat=="WO")])/length(Total16[which(Treat=="WO")]
))
> semWO
> semGR=sqrt(var(Total16[which(Site=="GR")])/length(Total16[which(Site=="GR")]))
> semGR
> semLP=sqrt(var(Total16[which(Site=="LP")])/length(Total16[which(Site=="LP")]))
> semLP
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(Total16[which(Treat=="W")], mean.fun, R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> boot.out<-boot(Total16[which(Treat=="WO")], mean.fun, R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> boot.out<-boot(Total16[which(Site=="GR")], mean.fun, R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> boot.out<-boot(Total16[which(Site=="LP")], mean.fun, R=1000, sim="ordinary")
```

```
> boot.ci(boot.out, type="norm")

> #comparing total number of seeds germinated
> shapiro.test(Total[which(Treat=="W")])
> shapiro.test(Total[which(Treat=="WO")])
> shapiro.test(Total[which(Site=="GR")])
> shapiro.test(Total[which(Site=="LP")])
> #all normal
> var.test(Total~Treat)
> #variances unequal, pvalue = 0.0332
> var.test(Total~Site)
> #variances unequal, p-value = 0.00182
> t.test(Total~Treat, var.equal=FALSE)
> #difference between treatments, p-value = 0.0002916
> t.test(Total~Site, var.equal=FALSE)
> #no difference between populations, pvalue = 0.6531

> summary(Total[which(Treat=="W")])
> summary(Total[which(Treat=="WO")])
> summary(Total[which(Site=="GR")])
> summary(Total[which(Site=="LP")])
> semW=sqrt(var(Total[which(Treat=="W")])/length(Total[which(Treat=="W")]))
> semW
> semWO=sqrt(var(Total[which(Treat=="WO")])/length(Total[which(Treat=="WO")]))
> semWO
> semGR=sqrt(var(Total[which(Site=="GR")])/length(Total[which(Site=="GR")]))
> semGR
> semLP=sqrt(var(Total[which(Site=="LP")])/length(Total[which(Site=="LP")]))
> semLP
> leftCI<-mean(Total[which(Treat=="W")])-(2*semW)
> rightCI<-mean(Total[which(Treat=="W")])+(2*semW)
> leftCI
> rightCI
> leftCI<-mean(Total[which(Treat=="WO")])-(2*semWO)
> rightCI<-mean(Total[which(Treat=="WO")])+(2*semWO)
> leftCI
> rightCI
> leftCI<-mean(Total[which(Site=="GR")])-(2*semGR)
> rightCI<-mean(Total[which(Site=="GR")])+(2*semGR)
> leftCI
> rightCI
> leftCI<-mean(Total[which(Site=="LP")])-(2*semLP)
> rightCI<-mean(Total[which(Site=="LP")])+(2*semLP)
> leftCI
> rightCI
```

```
> #Survival Analysis
> library(survival)
> data<-read.csv("germ2015surv.csv")
> attach(data)
> head(data)
  Bin Pos Site Treat Rep Pot Days Status
1 iii  D5   LP     W   1   1    7      1
2 iii  D5   LP     W   1   1    8      1
3 iii  D5   LP     W   1   1   31      1
4 iii  D5   LP     W   1   1   32      1
5 iii  D5   LP     W   1   1   42      1
6 iii  D5   LP     W   1   1   45      1
>
> #fit a survival curve
> km15<-survfit(Surv(Days, Status)~Treat, data=data, type="kaplan-meier")
> #check for differences between treatments
> first<-survdiff(Surv(Days,Status)~Treat)
> first
Call:
survdiff(formula = Surv(Days, Status) ~ Treat)

            N Observed Expected (O-E)^2/E (O-E)^2/V
Treat=W  1590      363      449      16.5      35.4
Treat=WO 1590      501      415      17.8      35.4

 Chisq= 35.4  on 1 degrees of freedom, p= 2.69e-09
>
> #difference in survival between treatments, plot curves

> plot(km15, col=c("blue","orange"), xlab="Time (days)", ylab="Survival",
ylim=c(0.6,1), conf.int=TRUE, cex.axis=1.5, cex.lab=1.5, lwd=2, las=1)
> axis(side=1, at=c(10,20,30,40,50,60,70,80,90,100), cex.axis=1.5, cex.lab=1.5)
> legend("topright", bty="n", legend=c("W","WO"), fill=c("blue","orange"), cex=1.75)
>
> plot(km15, col=c("blue","orange"), xlab="Time (days)", ylab="Survival",
ylim=c(0.9,1), xlim=c(0,10), conf.int=TRUE, cex.axis=1.5, cex.lab=1.5, lwd=2, las=1,
yaxt="n")
> axis(side=2, at=c(0.9,1.0), cex.axis=1.5, cex.lab=1.5, las=1)
> library(Hmisc)
> minor.tick(ny=4)

> detach(data)
> data<-read.csv("germ2016surv.csv")
> attach(data)
> km16<-survfit(Surv(Days1, Status)~Treat, data=data, type="kaplan-meier")
```

```
> second<-survdiff(Surv(Days1, Status)~Treat)
> second
Call:
survdiff(formula = Surv(Days1, Status) ~ Treat)

           N Observed Expected (O-E)^2/E (O-E)^2/V
Treat=W  1228      125      147      3.18      6.93
Treat=WO 1089      148      126      3.69      6.93

 Chisq= 6.9  on 1 degrees of freedom, p= 0.00848
>
>  plot(km16, col=c("blue","orange"), xlab="Time (days)", ylab="Survival",
ylim=c(0.8,1), conf.int=TRUE, cex.axis=1.5, cex.lab=1.5, lwd=2, las=1, yaxt="n")
> axis(side=2, at=c(0.8, 0.9,1.0), cex.axis=1.5, cex.lab=1.5, las=1)
> axis(side=1, at=c(10,20,30,40,50,60,70,80,90,100), cex.axis=1.5, cex.lab=1.5)
> legend("topright", bty="n", legend=c("W","WO"), fill=c("blue","orange"), cex=1.75)
>
> plot(km16, col=c("blue","orange"), xlab="Time (days)", ylab="Survival",
ylim=c(0.9,1), xlim=c(0,10), conf.int=TRUE, cex.axis=1.5, cex.lab=1.5, lwd=2, las=1,
yaxt="n")
> axis(side=2, at=c(0.9,1.0), cex.axis=1.5, cex.lab=1.5, las=1)
> minor.tick(ny=4)
>
```

D5. Infructescence compactness

```
> #Infructescence Architecture (previous categorization)
> data<-read.csv("infl stats.csv")
> attach(data)
> shapiro.test(final[which(Type=="IN")])
> #p-value = 2.2e-16
> sqrtin=sqrt(final[which(Type=="IN")])
> shapiro.test(sqrtin)
> login=log(final[which(Type=="IN”)])
> shapiro.test(login)
>#indehiscence cannot be transformed to normality, NON PARAMETRIC
> leveneTest(final~Type)
> #p-value = 3.171e-05 ***, variances unequal
> wilcox.test(final~Type, var.equal=FALSE)
> # p-value = 0.02777, within Grammica, there is a significant difference in
infructescence architecture between dehiscent and indehiscent species
> plot(final~Type, las=1, ylab="Infructescence Compactness", xlab="Fruit Dehiscence",
col=(c("yellow", "blue")))
>
#summary statistics inflorescence architecture old categorization
> summary(final[which(Type=="DE")])
```

```
> shapiro.test(final[which(Type=="DE")])
> #data not normal, bootstrap for confidence intervals
> sem<-sqrt(var(final[which(Type=="DE")])/length(final[which(Type=="DE")]))
> sem
> mean.fun<-function(dat,idx)mean(dat[idx],na.rm=TRUE)
> stderr<-function(x)sd(x)/sqrt(length(x))
> boot.out<-boot(final[which(Type=="DE")], mean.fun, R=1000, sim="ordinary")
> boot.ci(boot.out, type="norm")
> #sample size
> length(final[which(Type=”DE”)])
> #indehiscence cannot be transformed, same R codes used except Type==”IN”
```

```
> #Infructescence Architecture (new categorization)
> data<-read.csv("infl stats.csv")
> attach(data)
> shapiro.test(final[which(Type2==”DE”)])
> fligner.test(final~Type2)
> kruskal.test(final~Type2)
> dunn.test(final, g=Type2,  list=TRUE)
> plot(final~Type, las=1,ylab="Infructescence Compactness", xlab="Fruit Dehiscence",
col=(c("yellow", "blue")), cex.axis=1.25, cex.lab=1.25, lwd=1.25, ylim=c(0,1))
>
```