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Phylogenetic Distribution Of An Endogenous Strain Of Dahlia Mosaic Virus In Members Of Asteraceae

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PHYLOGENETIC DISTRIBUTION OF AN ENDOGENOUS STRAIN OF DAHLIA MOSAIC VIRUS IN MEMBERS OF ASTERACEAE

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ABSTRACT

A newly discovered strain of Dahlia mosaic virus (DMV) called DMV-D10 was first observed in *Dahlia variabilis* in 2008. DMV-D10 does not induce visible symptoms of infection in the host plant, and is classified as an endogenous virus. Endogenous viruses like DMV-D10 have the ability to integrate their viral sequences into the host plant genome, which can be transmitted to offspring. No studies have examined the host range of DMV-D10 outside of the *Dahlia* genus. Because DMV-D10 has only been observed in *Dahlia*, the objective for this study was to determine if presence of DMV-D10 follows an evolutionary relationship among species closely related to *Dahlia*. It was hypothesized species in the same tribe (Coreopsideae) as *Dahlia* were more likely to be infected with DMV-D10 compared to species in other Asteraceae tribes. Ten tribes consisting of thirty-five species were collected and DNA was extracted to determine DMV-D10 infection. Polymerase chain reaction (PCR) results for a movement protein gene indicate DMV-D10 is widely spread across Asteraceae. Fragments of the DMV-D10 genome were present in thirteen species across seven tribes. Thirty-seven percent of species in this study contained DMV-D10 viral sequences. Additionally, six species across five tribes contained Dahlia common mosaic virus sequences, and three species across two tribes contained Dahlia mosaic virus sequences. Phylogenetic relationship of host plants does not necessarily determine DMV-D10 infection. This leads to questions of how this virus can move to species in other Asteraceae tribes. Some potential hypotheses include pollen transmission or possible plant-virus coevolution.

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PREFACE

This thesis follows the journal style for the *American Journal of Botany*.

INTRODUCTION

Evolution of plant detection and defense against pathogenic microorganisms is one of the most notable developmental successes of modern plants (Chisholm et al., 2006). The first terrestrial plants likely evolved under the presence of microbes several hundred million years ago (Dangl et al., 2013); therefore, land plants have continually been exposed to microorganisms throughout their evolutionary history. During this period, there has been a continual arms race between pathogenic microbes and plants, well explained by the concept of gene-for-gene resistance introduced in the early 1940's by Harold Henry Flor (Flor, 1942, 1947). However, even with progressive research in plant evolutionary virology, new viruses are continually emerging (Hull, 2009). Therefore, many areas of plant virology have not yet been explored.

For a microbe to become a pathogenic threat to a plant, the microorganism must first breach the external barriers of the plant body (Chisholm, et al., 2006; Jones and Dangl, 2006). Initial infection can be accomplished in several ways. Stomates, or modified stomates known as hydathodes, have been suggested as a primary entry for viral particles (Jones and Dangl, 2006). Pathogens and parasitic organisms can enter the wounds of a plant (Esau, 1977), which takes on a similar method of penetration into the plant body as in viral invasion of stomates. Insects and herbivores can also play a large role in the plant-virus relationship (Stout et al., 2006). For example, aphids can introduce viral particles directly into the plant vasculature via their stylet when feeding (Esau, 1961). Additionally, herbivores cause damage to the external plant body when eating, which results in a wound and potential entry for pathogenic invasion (Stout et al., 2006).

After infecting the plant, a virus can spread rapidly throughout the plant body (Curtis, 1935). Viral movement in a plant can be accomplished by cell-to-cell movement and long-distance systemic transport (Esau, 1961). Plant viruses can move cell-to-cell symplastically through plasmodesmata (Leisner and Howell, 1993). However, the size and shape of plasmodesmata can present a challenge to some viruses to access other plant cells (Wolf et al., 1989). Therefore, some successful invaders possess movement proteins, which can either alter the size exclusion limit of plasmodesmata (Leisner and Howell, 1993) or induce the removal of desmotubules and replace them with viral tubules to shuttle the virus directly to other cells (Hull, 2009). In the case of either strategy, these movement proteins allow the virus to move throughout the plant body at a rate of microns per hour (Dawson and Hilf, 1992). A plant's immune system can respond to these methods of infection by apoptosis, which hinders further viral infection by localized death of virus-infected cells as well as surrounding healthy cells (Coll et al., 2011). It has been suggested that relative susceptibility of plant species to particular viruses can be linked to the interaction of viral movement proteins with plasmodesmata characteristics of the host plant (Dawson and Hilf, 1992; Hull, 2009). Additionally, some viruses have the ability to move directly through cell walls of the host plant (Chisholm et al., 2006).

Some plant viruses rely on transport by vasculature to be distributed throughout the plant body (Hipper et al., 2013). This can include dispersal of viral particles via xylem or phloem (Esau, 1961). In particular, phloem can serve as a form of systemic transport for a virus to access all portions of the plant (Curtis, 1935). A virus can cross several cellular barriers (e.g., bundle sheaths, vascular parenchyma, companion cells) into

the sieve elements of phloem (Hull, 2009). Therefore, a virus can be passively transported with photoassimilates from one plant organ to another during source-to-sink flow (Hipper et al., 2013). This dispersal method can be successful in transporting the virus to areas of the plant to replicate and be transmitted to other hosts (Esau, 1961).

Once viral infection of the plant body is complete, a host plant may start to show visible symptoms of infection (Lucy et al., 1996). Some symptoms largely depend on whether the host was infected by a source-pathogen (i.e., pathogens which infect above-ground biomass, such as leaves) or a sink-pathogen (i.e., pathogens which infect below-ground biomass, such as roots) (Berger et al., 2007). For instance, infection of above-ground biomass, such as leaves, can result in an increased demand for assimilates in the plant, but also develop chlorotic or necrotic areas on leaves that decrease photosynthetic assimilate production (Berger et al., 2007). As a result, photosynthesis decreases along with a concomitant increased demand for assimilates, leads to source tissue being transformed into sink tissue. However, if the virus is able to use long-distance transport to infect the whole plant body, symptoms could vary throughout the plant (Hull, 2009).

Much emphasis in plant virology has been placed on the negative forms of plant-viral relationships, mostly due to the large accumulation of data on plant viruses that induce disease (Wren et al., 2006). Particular attention is given to viral symptoms in cultivated plants, such as corn, that can negatively influence crop yield (Muthukumar et al., 2009). However, there is importance in emphasizing mutualistic plant-viral relationships. Plant viruses can be credited with giving plants the ability to tolerate abiotic stresses (Hull, 2009). For instance, panic grass (*Dichanthelium lanuginosum*) has

the ability to grow in geothermal soils of Yellowstone National Park due to a three-way mutualism between the plant, its associated fungal endophyte, and a thermal-tolerant virus that infects the endophyte (Roossinck, 2011). Additionally, it has been hypothesized that some plants, which harbor endogenous viral sequences, can be immune to other plant pathogens (Roossinck, 2011). Given the high abundance and diversity of viruses, it is possible many viral species can be attributed to mutualistic, commensal, or neutral relationships with plants.

According to the International Committee for the Taxonomy of Viruses, known plant viruses make up about 73 genera belonging to 49 families (ICTV, 2016). The viral family Caulimoviridae is composed of eight genera (ICTV, 2016). Caulimoviridae is the only family of plant viruses with double-stranded DNA genomes (Hull, 2009), with some endogenous viruses in the family (Geering et al., 2010). Viruses belonging to Caulimoviridae are plant pararetroviruses, which have a reverse transcription step during viral replication (Stavolone et al., 2003; Abdel-Salam et al., 2010; Geering, 2014). Plant pararetroviruses are similar to retroviruses in mammals, such as HIV. However, several differences set them apart, such as circular double-stranded DNA in pararetroviruses compared to linear single-stranded RNA in retroviruses (Hull, 2009). Caulimoviridae viruses can be transmitted between plants in a variety of ways, such as aphid transmission (Abdel-Salam et al., 2010). Once a plant is infected, a Caulimoviridae virus uses movement proteins to replace the desmotubules of plasmodesmata with its own viral tubules to move viral particles from cell to cell (Hull, 2009). Notable pathogens in the Caulimoviridae family include Banana streak virus (*Badnavirus*), Cauliflower mosaic

virus (*Caulimovirus*), Petunia vein clearing virus (*Petuvirus*), as well as Dahlia mosaic virus (*Caulimovirus*) (Pappu and Druffel, 2009; Abdel-Salam et al., 2010).

Dahlia mosaic virus (DMV) is a viral pathogen belonging to the genus *Caulimovirus* in the family Caulimoviridae (Pappu and Druffel, 2009). Host plant symptoms associated with DMV include vein clearing in the leaves, flower-breaking, and stunted growth (Abdel-Salam et al., 2010). DMV is most commonly observed in horticultural varieties of *Dahlia variabilis* (Pappu et al., 2005; Eid, et al., 2011), but DMV can also occur in other members of the Asteraceae family, including *Zinnia* (Kitajima and Lauritis, 1969). A newly discovered strain of this virus called DMV-D10 (Figure 1) was first observed in *Dahlia*, and is one of the few endogenous viruses to be discovered in Caulimoviridae (Pahalawatta, et al., 2008). Endogenous viruses have the ability to integrate their viral sequences into the host plant genome, which can be inherited from parent to offspring (Geering et al., 2010). DMV-D10 is detected in every part of the plant (e.g., leaves, stems, roots, flower petals, seeds, pollen), which has caused additional concern about its method of transmission, especially with respect to clonal propagation of *Dahlia* (Pahalawatta et al., 2008). Additionally, it has been suggested pollen transmission could be a potential risk of infection in horticultural settings (Pahalawatta et al., 2008). Currently, no studies have examined the full extent of DMV-D10 host range and what effects, if any, infection may have on the host plant.

Given the limited availability of data concerning DMV-D10, the purpose of this study was to determine its host range within Asteraceae. Since DMV-D10 and closely related viruses have only been observed in *Dahlia*, an objective of this study was to

determine if there was a relationship among other infected plant species compared to *Dahlia* within the Asteraceae family. It was hypothesized if a phylogenetic relationship were present with respect to DMV-D10 infection, the virus would be observed in *Dahlia* varieties, as well as in Asteraceae tribes more closely related to the Coreopsideae tribe, which contains *Dahlia*. Furthermore, potential host plants were examined for presence or absence of the DMV-D10 movement protein as well as Dahlia common mosaic virus. The objective was not only to determine if host species were infected with DMV-D10, but also to examine if plants may be infected with a closely related virus. This was tested with a two-part study consisting of a greenhouse and field study encompassing 35 species from 22 genera representing 10 Asteraceae tribes.

MATERIALS AND METHODS

Greenhouse plant material and growing conditions—

Ten varieties of seven species belonging to six genera within Asteraceae were grown under greenhouse conditions for five weeks (Table 1). Seeds of *Callistephus chinensis* (Crego variety), *Centaurea cyanus* (Cyanus Double variety), *Cosmos bipinnatus* (Single Sensation variety), and *Tagetes erecta* (Crackerjack variety) were obtained from American Seed Plantation Products, LLC (Norton, MA). Seeds of *Dahlia variabilis* (Cactus and Dandy varieties) as well as *Zinnia elegans* (Cherry Queen, Giant Cactus, and Lilliput varieties) and *Zinnia marylandica* (Zahara Starlight Rose variety) were obtained from Outsidepride.com, Inc. (Independence, OR). All seeds were planted in 11 x 11 x 12 centimeter pots, with five to six seeds per pot. Plants were allowed to grow for five weeks from November to December 2015. The greenhouse received natural lighting. Greenhouse relative humidity ranged from 17 to 68% during daytime and 26 to 75% during nighttime hours.

Field collection—

Five regional botanical gardens were selected for this study (Figure 2). Samples from two to nine Asteraceae species mostly native to each region, as well as some horticultural species, were selected at each site. Altogether, 28 species representing 10 Asteraceae tribes were collected (Table 2). Two to three leaves were collected from three individuals of each species. Specifically, leaves were collected from individual plants that were spread across each flowerbed to prevent collection of identical offspring from

maternal plants. Leaf samples were placed in labeled coin envelopes on site, and kept in a cool location. Samples were then transported to Fort Hays State University (Hays, KS, USA), and transferred to a drying oven to dry for 24-72 hours at 50 °C. Duration of the drying process depended on individual factors of each sample, including thickness of leaves and leaf moisture content. Following this, samples were preserved in a desiccator until DNA extraction and analysis.

DNA isolation and extraction—

DNA was isolated from greenhouse and field samples with a Qiagen DNeasy Plant Mini Kit (Catalog #69104; Hilden, Germany). With greenhouse samples, one pot per variety was transferred from the greenhouse to the lab. Fresh tissue from three whole, healthy plants (i.e., roots, stem, and leaves) was harvested from each variety. Lysis of cells was accomplished by adding liquid nitrogen to the whole plant tissue in a mortar, and tissue was ground thoroughly into a fine powder with a pestle. With field samples, dried leaves from three individuals per species were ground into a powder with a mortar and pestle. Protocol for isolating DNA from plant tissue followed the Quick-Start guide included in the Qiagen kit. Following isolation, samples were frozen at -20 °C until further analysis.

DNA quantification and analysis—

Polymerase chain reaction (PCR) was conducted to quantify DNA samples for presence or absence of a DMV-D10 movement protein with an expected size of 900 base pairs (bp). Primers and PCR cycling conditions followed Abdel-Salam et al. (2010) (Table 3). PCR was conducted with a Phusion High-Fidelity PCR Kit (New England

Biolabs, Ipswich, Massachusetts, USA), and PTC-100 Programmable Thermal Controller. DMV-D10 movement protein primers and PCR program followed Abdel-Salam et al. (2011) (Table 3) Specifically, a program was designed to have a four minute denaturation period at 94 °C, 20 second annealing period at 50 °C, and one minute extension period at 72 °C for 50 cycles, followed by a seven minute extension period at 72 °C. Samples were then kept at 4 °C until electrophoresis.

Agarose gel electrophoresis was performed to separate DNA fragments from samples following PCR. A 1% agarose gel solution was prepared with TAE (i.e., Tris base, acetic acid, and Ethylenediaminetetraacetic acid) and SYBER Safe DNA Gel Stain (Thermo Fisher Scientific, Waltham, Massachusetts, USA), as well as a 1 kb DNA Ladder (Promega, Madison, Wisconsin, USA). After electrophoresis, the gel was transferred to a Kodak Gel Logic 100 Imaging System to determine presence or absence of bands at 900 bp for the DMV-D10 movement protein. Samples that indicated a positive result at 900 bp as well as those samples that had strong bands amplified by DMV-D10 movement protein primers that could be a related virus at another base pair size were prepared for DNA sequencing.

For those samples that had a positive result using the DMV-D10 movement protein primers, primers to detect Dahlia common mosaic virus (DCMV) were used to aid in further analysis of infection. DCMV coat protein primers were used to determine potential presence of DCMV in samples. Primers and PCR program for the DCMV coat protein followed Eid et al. (2009) (Table 3). Specifically, a PCR program was designed to have a four minute denaturation period at 94 °C, 20 second annealing period at 59 °C,

and 50 second extension period at 72 °C for 50 cycles, followed by a seven minute extension period at 72 °C. A gel was run with PCR products and bands around 1,517 bp were examined to indicate presence of DCMV. Samples that indicated a positive result at 1,517 bp as well as those samples that had strong bands that could be a related virus amplified by DCMV coat protein primers at another base pair size were prepared for DNA sequencing.

DNA Sequencing—

Once it was determined which samples could potentially possess DMV-D10 or an associated virus based on electrophoresis results, PCR products with positive results were cleaned with a Qiagen PCR clean-up kit (Hilden, Germany). Following this, 2 µL of each sample was measured for DNA concentration with a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Samples for this study were sent to GENEWIZ (South Plainfield, New Jersey, USA) for DNA sequencing. For sequencing, PCR samples were prepared at a template concentration of 2 ng μL^{-1} x kb with a total volume of 10 µL. Custom primers were also sent with samples. Primers were chosen according to the specifications of GENEWIZ, including 18-24 bases in length, T_m between 50 and 60 °C, and G or C nucelotide at the 3′ end. The DMV-D10 movement protein reverse primer was chosen with a T_m of 59.4 °C and initial concentration of 100 µM. The DCMV reverse primer was chosen with a T_m of 68.0 °C and initial concentration of 100 µM. The GENEWIZ Premixed option was chosen for preparing samples before shipping to the facility for sequencing. This included addition of the 5 µL of custom primer to the corresponding 10 µL of prepared sample.

DNA sequences from the resulting chromatograms were inspected and then analyzed in the program NIH Nucleotide BLAST program to determine whether DMV-D10 or other related viruses were present. Specifically, sequenced products from each plant species was analyzed for presence of DMV-D10, DCMV, and Dahlia mosaic virus (DMV) viral sequences using the alignment function through the NIH Nucleotide BLAST program. Additionally, results were compared to determine whether there was a correlation between the various viruses and members of the Asteraceae tribes (Figure 3).

RESULTS

There was no phylogenetic relationship among host plants infected with DMV-D10 viral fragments; furthermore, DMV-D10 had a wider host range than expected (Tables 4 and 5). Gel electrophoresis results with DMV-D10 specific primers suggested DMV-D10 was present in samples belonging to tribes other than Coreopsideae. Concerning the DMV-D10 movement protein, gel electrophoresis indicated positive results in the Anthemideae, Astereae, Cardueae, Helenieae, Heliantheae, Senecioneae tribes, as well as the Coreopsideae tribe. In contrast, use of primers to detect Dahlia common mosaic virus (DCMV) yielded mixed results. Overall, this indicated DMV-D10, a related virus (e.g., DCMV), or a different endogenous virus with similar sequences enhanced by the primers used in this study could have been present in the samples.

Clarification of these observations came from DNA sequencing of PCR products with the positive gel results (Tables 4 and 5), which confirmed fragments of the DMV-D10 movement protein (Figure 4) and other pieces of the DMV-D10 genome (Figure 5) were in species sampled from the Anthemideae, Astereae, Cardueae, Helenieae, Heliantheae, Senecioneae tribes, as well as the Coreopsideae tribe. In particular, the genus *Symphotrichum*, which belongs to the tribe Astereae, had fragments of DMV-D10 viral sequences from the movement protein gene and polyprotein gene, as well as other viral fragments from the DMV-D10 complete genome. It was initially hypothesized that Asteraceae tribes more closely related to Coreopsideae would be infected if DMV-D10 infection could be related to phylogeny of its host plants. However, DNA sequencing

results from this study suggest there may be a wider host range of DMV-D10, but with no clear phylogenetic relationship of infected hosts (Figure 6).

Fragments of the DCMV and Dahlia mosaic virus (DMV) genomes matched sequenced PCR products from species in several of the same tribes, but to an apparent narrower host range than DMV-D10. For instance, DCMV was detected in species representing the Anthemideae, Astereae, Cardueae, Coreopsideae, and Heliantheae tribes (Figure 7) whereas DMV was only in species representing the Coreopsideae and Heliantheae tribes of Asteraceae (Figure 8). As seen with results from potential DMV-D10 infection, there was no obvious phylogenetic relationship between Asteraceae tribes infected with DCMV or DMV.

DISCUSSION

Viral fragments of DMV-D10 were in thirteen out of thirty-five, or thirty-seven percent, of plant species sampled in this study. Furthermore, DMV-D10 viral fragments were widely distributed across Asteraceae species and tribes. Therefore, there was no phylogenetic relationship relative to DMV-D10 infection of Asteraceae members. This suggests either another mode of viral transmission or evidence of long-term coevolution between DMV-D10 and members of Asteraceae. In addition, viral fragments of Dahlia common mosaic virus (DCMV) and Dahlia mosaic virus (DMV) were in a few plant species sampled in this study, but to a lesser extent compared to DMV-D10.

Past studies indicate DMV-D10 spreads via vertical transmission from parent to offspring in *Dahlia* species (Pahalawatta et al., 2008). Therefore, the inconsistency of viral infection with relation to phylogeny and indications of a potentially wider host range of DMV-D10 in this study leads to speculations about how this virus could be transmitted other than from parent to progeny. Furthermore, questions are raised, given sequencing results indicated only fragments of DMV-D10 viral sequences were detected in DNA of plant species. The exception to this was observed in *Dahlia* samples, which contained longer and continuous sequences of the DMV-D10 viral genome compared to other species in this study. There are a few possibilities on how fragments of DMV-D10, DCMV, and DMV could have been transmitted to other tribes of Asteraceae, including horticultural cultivation practices, pollen transmission, or an unknown insect vector.

Additionally, results from this research indicate the possibility of plant-virus coevolution and evolutionary incorporation of viral sequences into Asteraceae species.

Horticultural cultivation practices—

Cultivation practices in horticultural and agricultural systems can be a source of viral spread to other plant hosts (Hull, 2009; Sastry, 2013). In particular, vegetative propagation of tubers, corms, bulbs, and cuttings, as well as grafting methods, can transmit viruses (Hull, 2009). Several viruses that infect agricultural crops are spread easily through vegetative propagation (Sastry, 2013). Studies indicate DMV can easily be transmitted during *Dahlia* cultivation and propagation, whereas DMV-D10 also can be spread by seed (Eid and Pappu, 2013). *Dahlia variabilis* varieties in this study were infected with DMV, DCMV, and DMV-D10. This could be due to propagation techniques in horticultural systems (Pappu et al., 2005), since varieties of *Dahlia variabilis* in this study were grown from seed. Since it has been documented in previous research, cultivation practices are a strong possibility for how DMV, DCMV, and DMV-D10 were transmitted to *Dahlia variabilis* in this study. However, cultivation practices do not explain how other species in this study became infected, but there are other possible transmission methods.

Unknown insect vector—

Most plant viruses are not able to enter their host directly without the assistance of vectors or other methods of infection (Power, 2000). For instance, many plant viruses rely on insects as vectors (Power, 2000). Given that fragments of DMV-D10 viral sequences were detected in host genomes of several Asteraceae species with no

phylogenetic relationship, this could be evidence of possible insect transmission. DMV-D10 has been documented in every part of the plant (Pahalawatta et al., 2008). Therefore, it is possible that an insect feeding on one plant infected with DMV-D10 could transmit to an uninfected plant. In particular, insects with sucking mouthparts that feed on phloem sap could inadvertently carry plant materials containing DMV-D10 viral sequences from an infected plant to an uninfected plant by mechanical introduction (Esau, 1961). Approximately 90% of plant pathogens are spread solely by insects with sucking mouthparts (Power, 1987). For instance, many viruses can be transmitted by aphids during feeding (Esau, 1961), but DMV-D10 is known to lack an aphid transmission factor (Pahlawatta et al., 2008). Given the seemingly wide distribution of DMV-D10 viral sequences in several species, it is possible DMV-D10 can be mechanically introduced to other species of plants by an insect vector that has yet to be discovered. However, given DMV-D10 lacks an aphid transmission factor, this seems less likely (Pahalawatta et al., 2008).

It has been suggested that mode of insect transmission is a stable evolutionary trait when comparing viral genera (Nault, 1997). In other words, there is great specificity which insect vectors are able to transmit particular viruses (Power, 2000). DMV, a relative of DMV-D10, is transmitted by several species of aphids, allowing DMV to have a wider range of infection than the *Dahlia* host genus (Pappu et al., 2005). For this reason, it is understandable why some species in this study were infected with DMV. All varieties of *Zinnia elegans* and *Zinnia marylandica*, as well as varieties of *Dahlia variabilis*, were infected with DMV. These results are consistent with previous studies

that have shown DMV to infect species in the genera *Zinnia* (Kitajima and Lauritis, 1969; Hull, 2009) and *Dahlia* (Eid et al., 2009). Therefore, it is possible DMV could have been transmitted by aphids between these two species.

DCMV is another distinct virus belonging to *Caulimovirus*, but related to DMV and DMV-D10 (Almeyda et al., 2015). Much like DMV-D10, the host range of DCMV or possible modes of transmission are not well studied. A mixed infection of DMV-D10, DMV, and DCMV in *Dahlia* is common (Eid et al., 2009), and reflects results of this study. However, *Artemisia frigida*, *Callistephus chinensis*, *Centaurea cyanus*, *Coreopsis tinctoria*, and *Zinnia elegans* also had fragments of DCMV in their DNA. Due to the lack of information about DCMV, it is possible this virus does have an aphid transmission factor, such as that of DMV, or there is an unknown insect factor transmitting DCMV to other species of plant hosts. Since several species of virus belonging to *Caulimovirus* are spread by insects with sucking mouthparts (Nault, 1997): either of these hypotheses are possible explanations of viral transmission.

Pollen transmission—

Pollination can serve as transportation for viruses to infect plants (Card et al., 2007). Viruses contained in pollen can either infect the embryo and, thus, the seedling that grows from the seed or can infect the maternal plant through the fertilized flower (Hull, 2009). Pollen transmission of viruses has been shown to occur between plants of different species, where the pollen tube from one species germinates through the stigma and penetrates the style tissue of another plant species to transmit the virus to the maternal tissue (Isogai et al., 2014). DMV-D10 has been detected in all parts of *Dahlia*,

including the pollen grains (Pahalawatta et al., 2008). Therefore, it is possible DMV-D10 could be transmitted to other plant species during pollination by transmitting directly to the maternal tissue.

Furthermore, it is possible the floral anatomy of the family Asteraceae encourages fertilization by different pollen donors. The head inflorescence of Asteraceae is made of several hundred individual flowers that mature at different times over a period of days and can be pollinated by pollen from different species in Asteraceae during this time. It is hypothesized this characteristic gave rise to the great diversity of the family (Barreda et al., 2015). Therefore, it is possible a combination of the reproductive biology of Asteraceae and DMV-D10's ability to infect every part of a plant shaped the genetic makeup of Asteraceae. Although, even if pollen may contain viral particles, it does not necessarily mean the virus is pollen transmitted; for instance, Tobacco mosaic virus is contained in pollen, but not pollen transmitted (Card et al., 2007). Therefore, further research is needed to determine if DMV-D10 is a virus that can be pollen transmitted and may help to understand the potential host range of this virus.

Plant-virus coevolution—

Perhaps one of the more perplexing questions regarding DMV-D10 is what evolutionary events were involved in integrating these viral sequences into the plant genome. It is possible the integration of this virus into the plant genome was a process of coevolution and can be aged based on distribution in related species (Geering et al, 2010). For instance, one study suggested the integration events of Badnavirus (Caulimoviridae) into the plant genome occurred more than 4.6 million years ago when two species of

Musa were derived from a common ancestor (Duroy et al., 2016). Similarly, results of this study hint a wider distribution of DMV-D10, which may be evidence of long-term coevolution between DMV-D10 and members of Asteraceae.

Even though there was no phylogenetic relationship between DMV-D10 viral infection and Asteraceae tribes in this study, it is possible that particular species harbored viral sequences due to an evolutionary advantage. For example, studies suggest the exchange and integration of genetic information between cyanophages (i.e., viruses that infect cyanobacteria) and their hosts have led to higher photosynthetic efficiency of cyanobacteria (Sullivan et al., 2006). Specifically, cyanobacteria appear to have obtained genes from cyanophages that code for components of photosynthetic proteins used in photosystem II (Sullivan et al., 2006). Considering cyanobacteria are the smallest and most numerous photosynthetic cells in marine systems (Sullivan et al., 2006), the coevolution of this particular virus and host was an important event. Similarly, the evolutionary benefit of possessing DMV-D10 viral sequences in particular Asteraceae species may be due to a significant evolutionary advantage. Conversely, it is possible presence of DMV-D10 viral sequences in members of Asteraceae is a neutral relationship.

Evolutionary incorporation of viral sequences—

As with any biological entity, genetic variation gives rise to viral diversity (Garcia-Arenal et al., 2001). Specifically, mutation, recombination, and reassortment are the variants that natural selection acts upon for evolution (Roossinck, 1997). Viral studies suggest natural selection favors plant viruses that have a wider host range and possess the

ability to use several different vectors for transmission (Roossinck, 1997). The heterogeneity of viral populations due to genetic variation allows mechanisms of evolution to shape the specificity of plant-viral relationships (Garcia-Arenal et al., 2001). Furthermore, evidence has supported the claim by scientists that viruses have played a larger role in shaping the evolution of biological organisms than previously thought (Hendrix, et al., 2000).

Longer and continuous sequences of DMV-D10, DMV, and DCMV were localized in both varieties of *Dahlia variabilis*. However, shorter fragments of these viruses were detected in other species of Asteraceae. Given the possible modes of viral transmission (e.g., pollen transmission, unknown insect vector, long-term coevolution), it is possible to hypothesize further that integration of DMV-D10 viral fragments, as observed in many species of this study, could be treated as potentially new viruses. DMV-D10, as with any endogenous virus, has the ability to integrate its viral sequences into the host genome (Eid and Pappu, 2013). Studies have shown viral fragments of other Caulimoviridae endogenous viruses became either rearranged or decayed when integrated into genomes of differing plant species (Geering et al., 2010). Therefore, it is possible that during transmission to other species, only fragments of DMV-D10 were compatible and integrated differently into the associated host genome of other plant species. Additionally, because studies have suggested DMV-D10 does not induce any physical symptoms of disease, integration of particular DMV-D10 viral fragments could have been considered advantageous to the host plant (e.g., the mutualistic relationship between cyanophages and cyanobacteria). It is possible mutation and recombination also

attributed to how these pieces of DMV-D10 viral sequences were integrated into the host genome, which could have influenced the evolution of Asteraceae.

Conclusions—

Many of the transmission strategies mentioned previously, including pollen transmission, an unknown insect vector, or coevolution between DMV-D10 and Asteraceae members, could be single or a combination of possibilities for how DMV-D10 was able to infect the thirteen species of Asteraceae in this study. Given these possibilities for viral transmission and previous knowledge of DMV-D10, this study suggests pollen transmission and plant-virus coevolution are perhaps the most plausible ways in which DMV-D10 is transmitted to host plant species outside the *Dahlia* genus. However, further research is needed to determine how DMV-D10 is spread to Asteraceae members. Studying this could provide us with a better understanding of the biology of this virus in relation to their host plants. Furthermore, given these results indicate only fragments of DMV-D10 (and DCMV and DMV) were present in some species other than *Dahlia*, more support is given to the idea that viral fragments observed in several plant species in this study are evidence of long-term coevolution between an ancestral virus and members of Asteraceae.

LITERATURE CITED

Abdel-Salam, A.M., M.M. Al Khazindar, S.G. Eld, and H.R. Pappu. 2010. Caulimoviral sequences in *Dahlia variabilis* in Egypt. *African Journal of Biotechnology* 9: 6835-6839.

Almeyda, C.V., G. Raikby, and H.R. Pappu. 2015. Characterization and comparative analysis of promoters from three plant pararetroviruses associated with *Dahlia* (*Dahlia variabilis*). *Virus Genes* 51: 96-104.

Barreda, V., L. Palazzesi, M.C. Telleria, E.B. Olivero, J.I. Raine, and F. Forest. 2015. Early evolution of the angiosperm clade Asteraceae in the Cretaceous of Antarctica. *Proceedings of the National Academy of Sciences* 112: 10989-10994.

Bent, A.F. 1996. Plant disease resistance genes: function meets structure. *Plant Cell* 8: 1757-1771.

Berger, S., A.K. Sinha, and T. Roitsch. 2007. Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany* 58: 4019-4026.

Card, S.D., M.N. Pearson, and G.R.G. Clover. 2007. Plant pathogens transmitted by pollen. *Australasian Plant Pathology* 36: 455-461.

Chisholm, S.T., G. Coaker, B. Day, and B.J. Staskawicz. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124: 803-814.

Coll, N.S., P. Epple, and J.L. Dangl. 2011. Programmed cell death in the plant immune system. *Cell Death and Differentiation* 18: 1247-1256.

Curtis, O.F. 1935. The Translocation of Solutes in Plants. McGraw-Hill Book Company, Inc., New York, New York, USA.

Dangl, J.L., D.M. Horvath, and B.J. Staskawicz. 2013. Pivoting the plant immune system from dissection to deployment. *Science* 341: 746-751.

Dawson, W.O. and M.E. Hilf. 1992. Host-range determinants of plant viruses. *Annual Review of Plant Physiology and Plant Molecular Biology* 43: 527-555.

Duroy, P., X. Perrier, N. Laboureau, J. Jacquemoud-Collet, and M. Iskra-Caruana. 2016. How endogenous plant pararetroviruses shed light on *Musa* evolution. *Annals of Botany* 117: 625-641.

Eid, S., K.L. Druffel, D.E. Saar, and H.R. Pappu. 2009. Incidence of multiple and distinct species of Caulimoviruses in Dahlia (*Dahlia variabilis*). *HortScience* 44: 1498-1500.

Eid, S., and H.R. Pappu. 2013. Expression of endogenous para-retroviral genes and molecular analysis of the integration events in its plant host *Dahlia variabilis*. *Virus Genes* 47: 998-1008.

Eid, S., D.E. Saar, K.L. Druffel, and H.R. Pappu. 2011. Plant pararetroviral sequences in wild *Dahlia* species in their natural habitats in Mexican mountain ranges. *Plant Pathology* 60: 378-383.

Esau, K. 1961. Plants, Viruses, and Insects. Harvard University Press, Cambridge, Massachusetts, USA.

Esau, K. 1977. Anatomy of Seed Plants. 2nd edition. John and Wiley, Inc, San Francisco, California, USA.

Flor, H.H. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32: 653-669.

Flor, H.H. 1947. Inheritance of reaction to rust in flax. *Journal of Agricultural Research* 74: 241-262.

Garcia-Arenal, F., A. Fraile, and J.M. Malpica. 2001. Variability and genetic structure of plant virus populations. *Annual Review of Phytopathology* 39: 157-186.

Geering, A.D.W., T. Scharaschkin, and P. Teycheney. 2010. The classification and nomenclature of endogenous viruses of the family Caulimoviridae. *Archives of Virology* 155: 123-131.

Geering, A.D.W. 2014. Caulimoviridae (plant pararetroviruses). eLA.

Hammond-Kosack, K.E. and J.D.G. Jones. 1997. Plant disease resistance genes. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 575-607.

Hendrix, R.W., J.G. Lawrence, G.F. Hatfull, and S. Casjens. 2010. The origins and ongoing evolution of viruses. *Trends in Microbiology* 8: 504-507.

Hipper, C., V. Brault, V. Ziegler-Graff, and F. Revers. 2013. Viral and cellular factors involved in phloem transport of plant viruses. *Frontiers in Plant Science* 4: 1-24.

Hull, R. 2009. Comparative Plant Virology. 2nd edition. Elsevier Academic Press, Burlington, Massachusetts, USA.

International Committee on Taxonomy of Viruses (ICTV). 2016. Website http://www.ictvonline.org.

Isogai, M., T. Yoshida, C. Nakanowatari, and N. Yoshikawa. 2014. Penetration of pollen tubes with accumulated *Raspberry bushy dwarf virus* into stigmas is involved in

initial infection of maternal tissue and horizontal transmission. *Virology* 452-453: 247-253.

Jones, J.D.G. and J.L. Dangl. 2006. The plant immune system. *Nature* 444: 323-329.

Kitajima, E.W. and J.A. Lauritis. 1969. Plant virions in plasmodesmata. *Virology* 37: 681-685.

Leisner, S.M. and S.H. Howell. 1993. Long-distance movement of viruses in plants. *Trends in Microbiology* 1: 314-317.

Letunic, I. and P. Bork. 2011. Interactive Tree of Life v2: Online annotation and display of phylogenetic trees made easy. *Nucleic Acids Research* 39: 475-478.

Lucy, A.P., M.I. Boulton, J.W. Davies, and A.J. Maule. 1996. Tissue specificity of *Zea mays* infection by maize streak virus. *Molecular Plant-Microbe Interactions* 9: 22-31.

Melotto, M., W. Underwood, J. Koczan, K. Nomura, and S. Yang He. 2006. Plant stomata function in innate immunity against bacterial invasion. *Cell* 126: 969-980.

Mukhtar, M.S. A.R. Carvunis, M. Dreze, P. Epple, J. Steinbrenner, J. Moore, M. Tasan, et al. 2011. Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science* 333: 596-601.

Muthukumar, V., U. Melcher, M. Pierce, G.B. Wiley, B.A. Roe, M.W. Palmer, V. Thapa, et al. 2009. Non-cultivated plants of the Tallgrass Prairie Preserve of northeastern Oklahoma frequently contain virus-like sequences in particulate fractions. *Virus Research* 141: 169-173.

Nault, L.R. 1997. Anthropod transmission of plant viruses: a new synthesis. *Annals of the Entomological Society of America* 87: 521-541.

Pahalawatta, V., K. Druffel, and H. Pappu. 2008. A new and distinct species in the genus *Caulimovirus* exists as an endogenous plant pararetroviral sequence in its host, *Dahlia variabilis*. *Virology* 376: 253-257.

Pappu, H.R., S.D. Wyatt, and K.L. Druffel. 2005. Dahlia mosaic virus: molecular detection and distribution in *Dahlia* in the United States. *HortScience* 40: 697-699.

Pappu, H.R. and K.L. Druffel. 2009. Use of conserved genomic regions and degenerate primers in a PCR-based assay for the detection of members of the genus *Caulimovirus*. *Journal of Virological Methods* 157: 102-104.

Power, A.G. 2000. Insect transmission of plant viruses: a constraint on virus variability. *Current Opinion in Plant Biology* 3: 336-340.

Power, A.G. 1987. Plant community diversity, herbivore movement, and an insect-transmitted disease of maize. *Ecology* 68: 1658-1669.

Roossinck, M.J. 1997. Mechanisms of plant virus evolution. *Annual Review of Phyotpathology* 35: 191-209.

Roossinck, M.J. 2011. The good viruses: viral mutualistic symbioses. *Nature Reviews: Microbiology* 9: 99-108.

Sastry, K. 2013. Seed-Borne Plant Virus Diseases: Plant virus transmission through vegetative propagules (asexual reproduction). Springer, USA.

Stavolone, L., A. Ragozzino, and T. Hohn. 2003. Characterization of *Cestrum yellow leaf curling virus*: a new member of the family *Caulimoviridae*. *Journal of General Virology* 84: 3459-3464.

Stout, M.J., J.S. Thaler, and B.P.H.J. Thomma. 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annual Review in Entomology* 51: 663:689.

Sullivan, M.B., D. Lindell, J.A. Lee, L.R. Thompson, J.P. Bielawski, and S.W. Chisholm. 2006. Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. *PLoS Biology* 4: 1344-1357.

Wolf, S., W.J. Lucas, C.M. Deom, and R.N. Beachy. 1989. Movement protein of tobacco mosaic virus modified plasmodesmatal size exclusion limit. *Science* 246: 377-379.

Wren, J.D., M.J. Roossinck, R.S. Nelson, K. Scheets, M.W. Palmer, and U. Melcher. 2006. Plant virus biodiversity and ecology. *PLoS Biology* 4: e80.

TABLES

Tribe	Genus	Species	Common name	Variety
Astereae	*Callistephus*	*chinensis*	Annual Aster	Crego
Cardueae	*Centaurea*	*cyanus*	Bachelor's Button	Cyanus Double
Coreopsideae	*Cosmos*	*bipinnatus*	Cosmos	Single Sensation
	Dahlia	*variabilis*	Dahlia	Cactus
				Dandy
Heliantheae	*Zinnia*	*elegans*	Zinnia	Cherry Queen
				Giant Cactus
				Lilliput
		marylandica		Zahara Starlight Rose
Tageteae	*Tagetes*	*erecta*	Marigold	Crackerjack

TABLE 1. Members of Asteraceae tested for DMV-D10 and Dahlia common mosaic virus (DCMV) in the greenhouse study. Seeds of *Callistephus chinensis* (Crego variety), *Centaurea cyanus* (Cyanus Double variety), *Cosmos bipinnatus* (Single Sensation variety), and *Tagetes erecta* (Crackerjack variety) were obtained from American Seed Plantation Products, LLC (Norton, Massachusetts, USA). Seeds of *Dahlia variabilis* (Cactus and Dandy varieties) as well as *Zinnia elegans* (Cherry Queen, Giant Cactus, and Lilliput varieties) and *Zinnia marylandica* (Zahara Starlight Rose variety) were obtained from Outsidepride.com, Inc. (Independence, Oregon, USA).

Tribe	Genus	Species	Common name	Location
Anthemideae	*Achillea*	spp. 'Cornation Gold'	Yarrow	Lauritzen Botanic Garden
Anthemideae	*Achillea*	*millefolium*	Common yarrow	Lauritzen Botanic Garden
Anthemideae	*Artemisia*	*frigida*	Prairie sagewort	Denver Botanic Garden
Anthemideae	*Artemisia*	*tridentata*	Big sagebrush	Denver Botanic Garden
Anthemideae	*Chrysanthemum*	spp. 'Superbum'	Shasta daisy	Powell Botanic Garden
Arctotideae	*Berkheya*	*Purpurea*	Berkheya	Missouri Botanical Garden
Astereae	*Erigeran*	*speciosus*	Aspen fleabane	Denver Botanic Garden
Astereae	*Solidago*	*drummondii*	Drummond's goldenrod	Powell Botanic Garden
Astereae	*Symphyotrichum*	spp. 'Wood's purple'	Aster	Lauritzen Botanic Garden
Astereae	*Symphyotrichum*	*laeve*	Smooth aster	Missouri Botanical Garden
Astereae	*Symphyotrichum*	*novae-angliae*	New England aster	Missouri Botanical Garden
Astereae	*Symphyotrichum*	*novi-belgii*	New york aster	Cheyenne Botanic Garden
Astereae	*Symphyotrichum*	*oblongifolius*	Aromatic aster	Cheyenne Botanic Garden
Cardueae	*Centaurea*	*macrocephala*	Giant knapweed	Cheyenne Botanic Garden
Cardueae	*Centaurea*	*montana*	Mountain coneflower	Cheyenne Botanic Garden
Coreopsideae	*Coreopsis*	*palmata*	Tickseed	Missouri Botanical Garden
Coreopsideae	*Coreopsis*	*tinctoria*	Plains coreopsis	Missouri Botanical Garden
Coreopsideae	*Coreopsis*	*verticillata*	Threadleaf coreopsis	Lauritzen Botanic Garden
Eupatorieae	*Eupatorium*	*altissimum*	Tall thoroughwort	Powell Botanic Garden
Eupatorieae	*Liatris*	*spicata*	Blazingstar	Lauritzen Botanic Garden
Gnaphalieae	*Antennaria*	spp. 'McClintock'	Pussytoes	Denver Botanic Garden
Helenieae	*Gaillardia*	*aristata*	Blanket flower	Denver Botanic Garden
Helenieae	*Hymenoxys*	*hoopesii*	Owl's claw	Denver Botanic Garden
Heliantheae	*Berlandiera*	*lyrata*	Chocolate flower	Denver Botanic Garden
Heliantheae	*Echinacea*	*purpurea*	Purple coneflower	Powell Botanic Garden
Heliantheae	*Echinacea*	*tennesseensis*	Tennessee coneflower	Missouri Botanical Garden
Heliantheae	*Helianthus*	*salicifolius*	Willowleaf sunflower	Powell Botanic Garden
Heliantheae	*Ratibida*	*columnifera*	Yellow coneflower	Denver Botanic Garden
Heliantheae	*Ratibida*	*pinnata*	Grey-head coneflower	Missouri Botanical Garden
Heliantheae	*Rudbeckia*	*fulgida*	Orange coneflower	Lauritzen Botanic Garden
Heliantheae	*Rudbeckia*	*hirta*	Black-eyed Susan	Lauritzen Botanic Garden
Heliantheae	*Rudbeckia*	*maxima*	Great coneflower	Powell Botanic Garden
Senecioneae	*Petasites*	*japonicus*	Fuki	Powell Botanic Garden
Senecioneae	*Senecio*	*spartioides*	Broom grounsel	Denver Botanic Garden

TABLE 2. Members of Asteraceae tested for DMV-D10 and Dahlia common mosaic virus (DCMV) in the field study.

Virus	Primer	Sequence (5′ to 3′)	Annealing Temperature (°C)	Extension Time (seconds)	Expected Size (bp)	Reference
DMV-D10	Movement protein (forward)	ATGGATCGTAAAGATT	50	60	900	Abdel-Salam, et al., 2010
DMV-D10	Movement protein (reverse)	CTGTTTTCTGTGTTTCTACTGG	50	60	900	
DCMV	Coat protein (forward)	GGATCCTCATTCTGAGTCTTCGTCTTC	59	50	1,517	Eid et al., 2009
DCMV	Coat protein (reverse)	CATATGGCCACCCAAATGACC	59	50	1,517	

TABLE 3. Details of primers used in detection of DMV-D10 and Dahlia common mosaic virus (DCMV) in Asteraceae species.

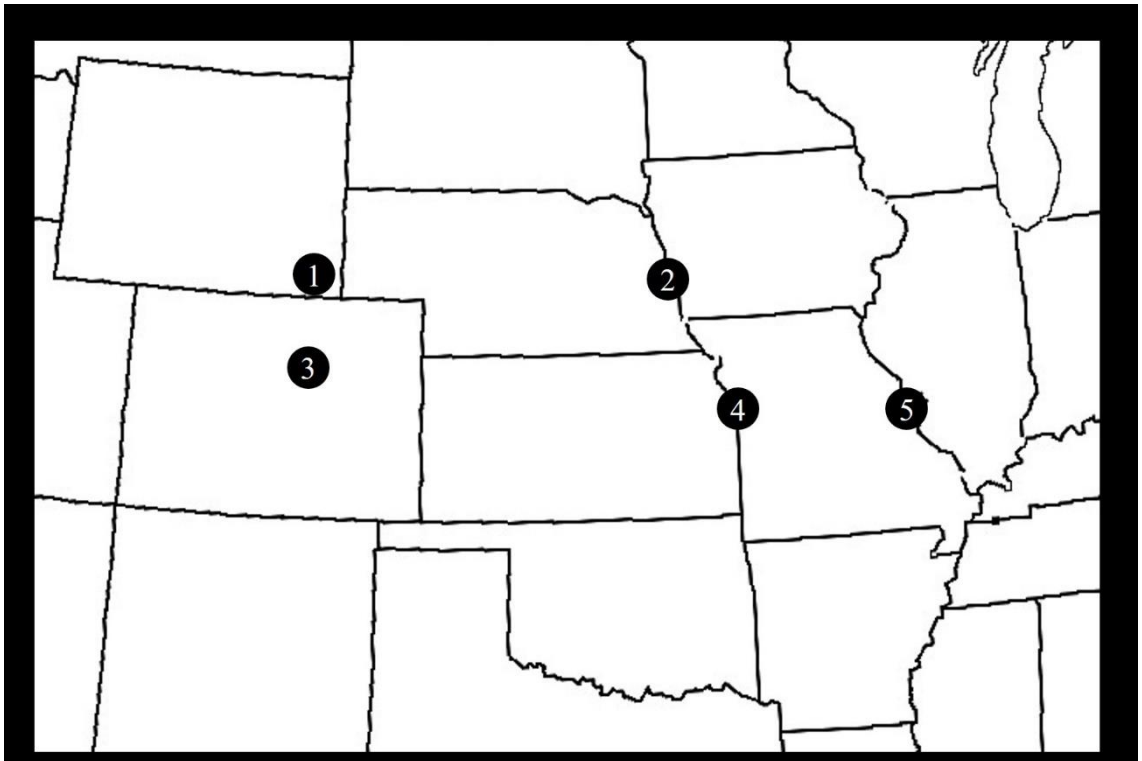
Tribe	Genus	Species	Common name	Variety	Viral fragments from sequenced PCR bands
Astereae	*Callistephus*	*chinensis*	Annual Aster	Crego	DMV-D10, DCMV
Cardueae	*Centaurea*	*cyanus*	Bachelor's Button	Cyanus Double	DMV-D10, DCMV
Coreopsideae	*Cosmos*	*bipinnatus*	Cosmos	Single Sensation	None
	Dahlia	*variabilis*	Dahlia	Cactus	DMV-D10, DCMV, DMV
				Dandy	DMV-D10, DCMV DMV
Heliantheae	*Zinnia*	*elegans*	Zinnia	Cherry Queen	DMV-D10, DCMV DMV
				Giant Cactus	DMV-D10, DCMV DMV
				Lilliput	DMV-D10, DMV
		marylandica		Zahara Starlight Rose	DMV-D10, DMV
Tageteae	*Tagetes*	*erecta*	Marigold	Crackerjack	None

TABLE 4. Sequencing results of PCR products with DMV-D10 and Dahlia common mosaic virus (DCMV) primers from Asteraceae species included in the greenhouse study.

Tribe	Genus	Species	Common name	Location	Viral fragments from sequenced PCR bands
Anthemideae	*Achillea*	spp. 'Cornation Gold'	Yarrow	Lauritzen Botanic Garden	None
Anthemideae	*Achillea*	*millefolium*	Common yarrow	Lauritzen Botanic Garden	None
Anthemideae	*Artemisia*	*frigida*	Prairie sagewort	Denver Botanic Garden	DMV-D10, DCMV
Anthemideae	*Artemisia*	*tridentata*	Big sagebrush	Denver Botanic Garden	None
Anthemideae	*Chrysanthemum*	spp. 'Superbum'	Shasta daisy	Powell Botanic Garden	None
Arctotideae	*Berkheya*	*purpurea*	Berkheya	Missouri Botanical Garden	None
Astereae	*Erigeran*	*speciosus*	Aspen fleabane	Denver Botanic Garden	None
Astereae	*Solidago*	*drummondii*	Drummond's goldenrod	Powell Botanic Garden	None
Astereae	*Symphyotrichum*	spp. 'Wood's purple'	Aster	Lauritzen Botanic Garden	DMV-D10
Astereae	*Symphyotrichum*	*laeve*	Smooth aster	Missouri Botanical Garden	DMV-D10
Astereae	*Symphyotrichum*	*novae-angliae*	New England aster	Missouri Botanical Garden	DMV-D10
Astereae	*Symphyotrichum*	*novi-belgii*	New York aster	Cheyenne Botanic Garden	None
Astereae	*Symphyotrichum*	*oblongifolius*	Aromatic aster	Cheyenne Botanic Garden	DMV-D10
Cardueae	*Centaurea*	*macrocephala*	Giant knapweed	Cheyenne Botanic Garden	DMV-D10
Cardueae	*Centaurea*	*montana*	Mountain coneflower	Cheyenne Botanic Garden	None
Coreopsideae	*Coreopsis*	*palmata*	Tickseed	Missouri Botanical Garden	None
Coreopsideae	*Coreopsis*	*tinctoria*	Plains coreopsis	Missouri Botanical Garden	DCMV
Coreopsideae	*Coreopsis*	*verticillata*	Threadleaf coreopsis	Lauritzen Botanic Garden	None
Eupatoriae	*Eupatorium*	*altissimum*	Tall thoroughwort	Powell Botanic Garden	None
Eupatorieae	*Liatris*	*spicata*	Blazingstar	Lauritzen Botanic Garden	None
Gnaphalieae	*Antennaria*	spp. 'McClintock'	Pussytoes	Denver Botanic Garden	None
Helenieae	*Gaillardia*	*aristata*	Blanket flower	Denver Botanic Garden	None
Helenieae	*Hymenoxys*	*hoopesii*	Owl's claw	Denver Botanic Garden	DMV-D10
Heliantheae	*Berlandiera*	*lyrata*	Chocolate flower	Denver Botanic Garden	None
Heliantheae	*Echinacea*	*purpurea*	Purple coneflower	Powell Botanic Garden	None
Heliantheae	*Echinacea*	*tennesseensis*	Tennessee coneflower	Missouri Botanical Garden	None
Heliantheae	*Helianthus*	*salicifolius*	Willowleaf sunflower	Powell Botanic Garden	None
Heliantheae	*Ratibida*	*columnifera*	Yellow coneflower	Denver Botanic Garden	None
Heliantheae	*Ratibida*	*pinnata*	Grey-head coneflower	Missouri Botanical Garden	None
Heliantheae	*Rudbeckia*	*fulgida*	Orange coneflower	Lauritzen Botanic Garden	None
Heliantheae	*Rudbeckia*	*hirta*	Black-eyed Susan	Lauritzen Botanic Garden	None
Heliantheae	*Rudbeckia*	*maxima*	Great coneflower	Powell Botanic Garden	None
Senecioneae	*Petasites*	*japonicus*	Fuki	Powell Botanic Garden	DMV-D10
Senecioneae	*Senecio*	*spartioides*	Broom grounsel	Denver Botanic Garden	None

TABLE 5. Sequencing results of PCR products with DMV-D10 and Dahlia common mosaic virus (DCMV) primers from Asteraceae species included in the field study.

FIGURE 1. DMV-D10 genome, which consists of 7,156 bp. Roman numerals correspond to open reading frames (ORF). Specifically, ORF I = movement protein gene, ORF III = nucleic acid binding protein, ORF IV = coat protein, ORF V = polyprotein gene, ORF VI = inclusion body protein, and ORF VII = protein of unknown function. Modified after Pahalawatta et al. (2008). Roman numerals of ORFs correspond to genomic mapping of viruses belonging to the *Caulimovirus* genus in Caulimoviridae.



1	Cheyenne Botanic Gardens	Cheyenne, WY
2	Lauritzen Gardens	Omaha, NE
3	Denver Botanic Gardens	Denver, CO
4	Powell Gardens	Kingsville, MO
5	Missouri Botanical Garden	St. Louis, MO

FIGURE 2. Map of sites for the field study where Asteraceae species were collected for detection of DMV-D10 and Dahlia common mosaic virus (DCMV).

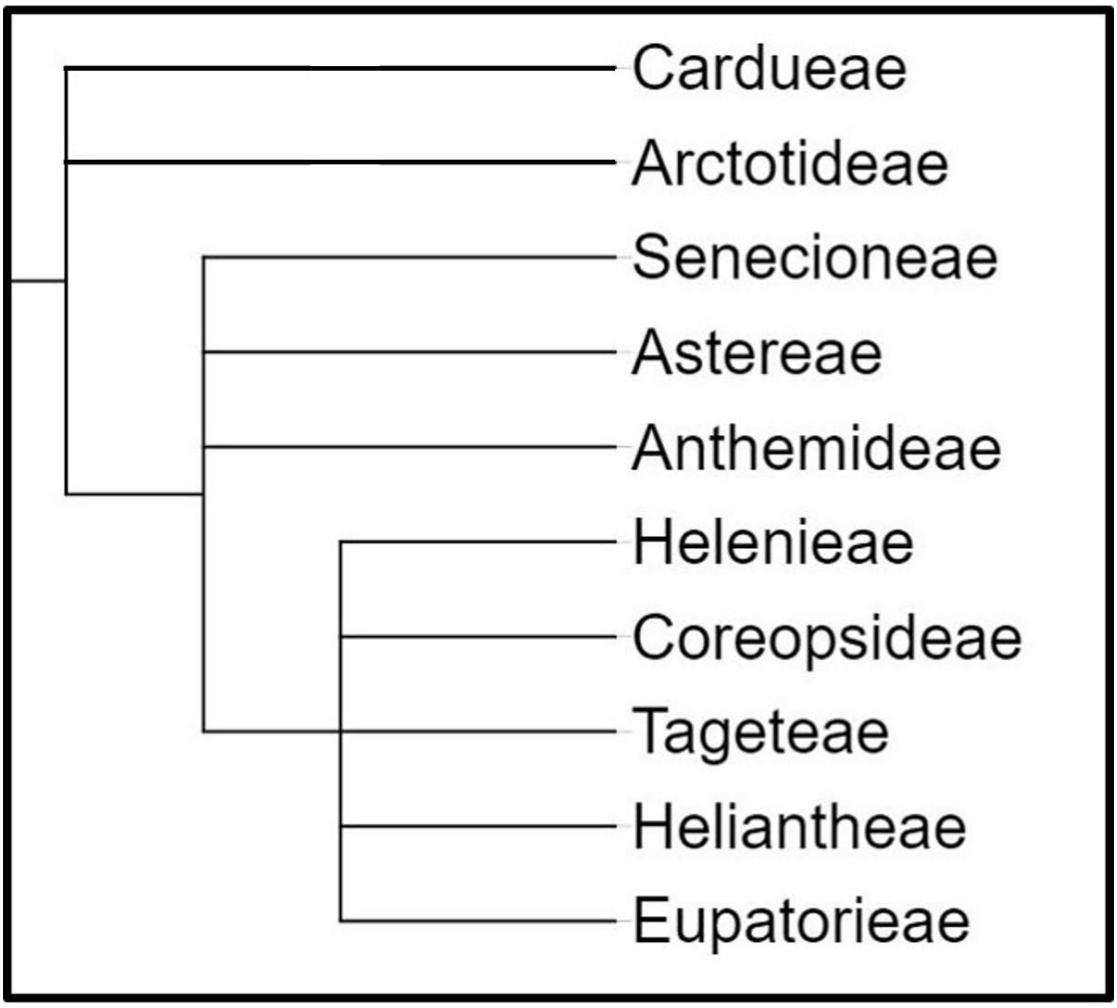


FIGURE 3. Cladogram of Asteraceae tribes included in the study for detection of DMV-D10 and Dahlia common mosaic virus (DCMV). Modified from Letunic and Bork (2011).

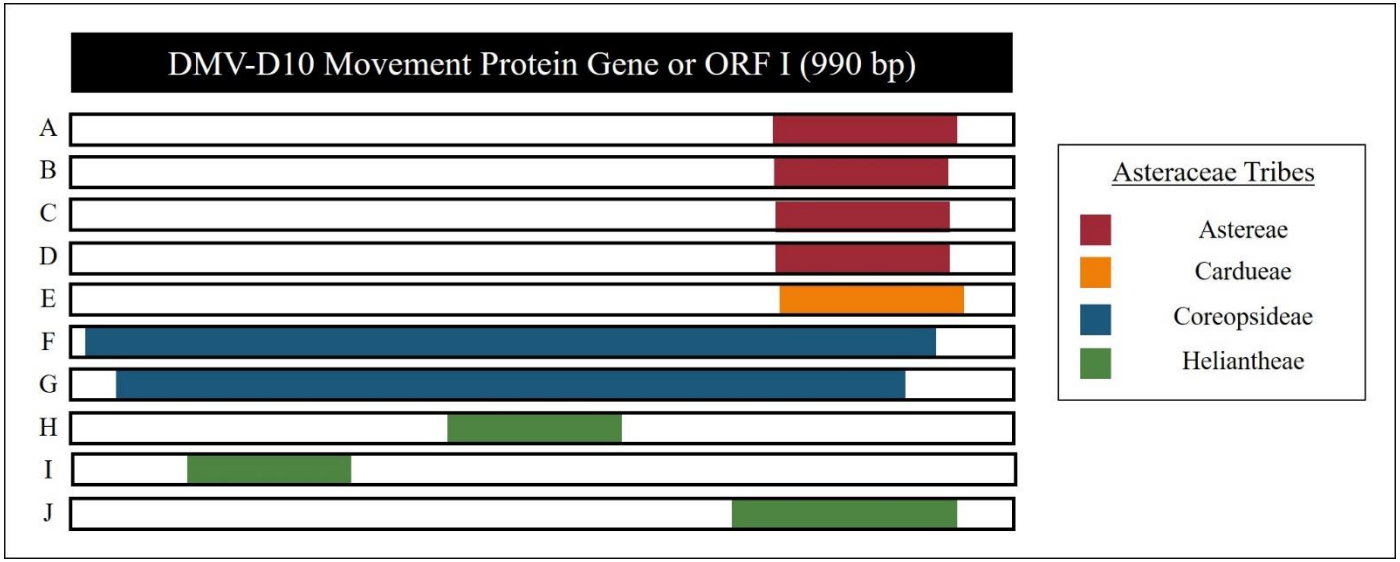


FIGURE 4. Asteraceae tribes and species with viral fragments of the DMV-D10 movement protein gene. Letters correspond to specific species. A = *Callistephus chinensis*, B = *Symphyotrichum* spp. 'Wood's Purple', C = *Symphyotrichum novae-angliae*, D = *Symphyotrichum oblongifolius*, E = *Centaurea cyanus*, F = *Dahlia variabilis* 'Cactus', G = *Dahlia variabilis* 'Dandy', H = *Zinnia elegans* 'Giant Cactus', I = *Zinnia elegans* 'Lilliput', and J = *Zinnia marylandica* 'Zahara Starlight Rose'. Sections highlighted in color indicate nucleotide sequence matches to the DMV-D10 movement protein gene.

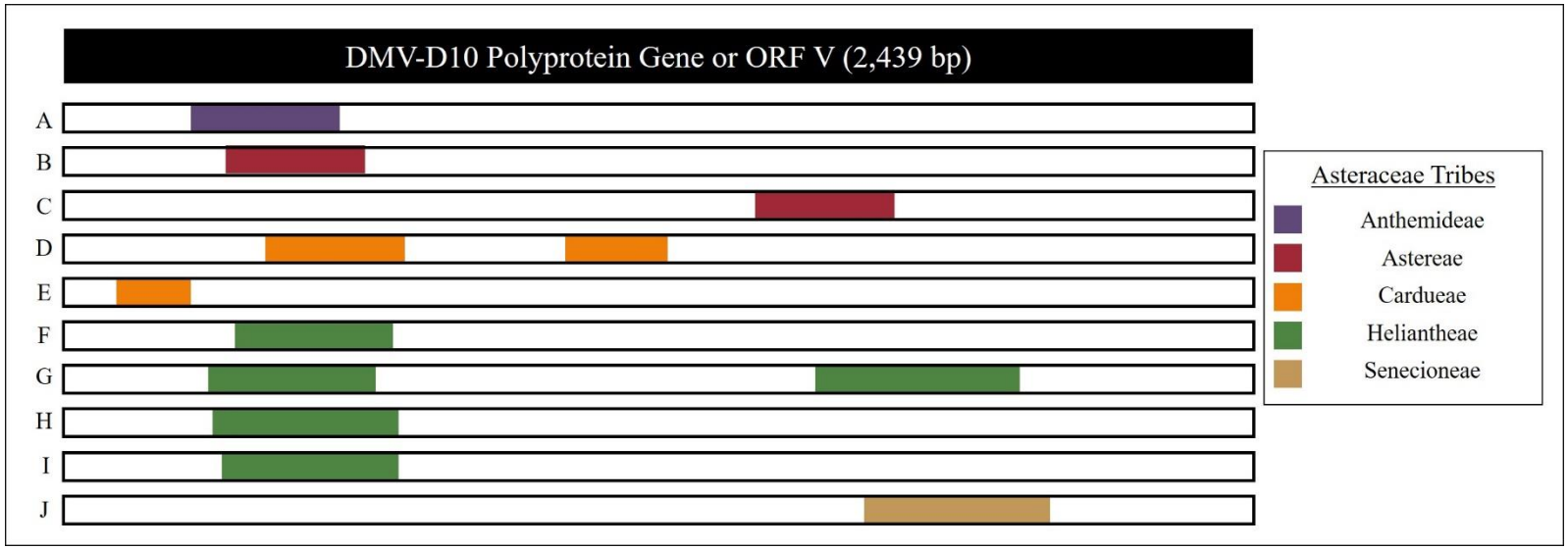


FIGURE 5. Asteraceae tribes and species with viral fragments of the DMV-D10 polyprotein gene. Letters correspond to specific species. A = *Artemisia frigida*, B = *Symphyotrichum laeve*, C = *Symphyotrichum oblongifolius*, D = *Centaurea cyanus*, E = *Centarea macrocephala*, F = *Zinnia elegans* 'Cherry Queen', G = *Zinnia elegans* 'Giant Cactus', H = *Zinnia elegans* 'Lilliput', I = *Zinnia marylandica* 'Zahara Starlight Rose', and J = *Petasites japonicas*. Sections highlighted in color indicate nucleotide sequence matches to the DMV-D10 polyprotein gene.

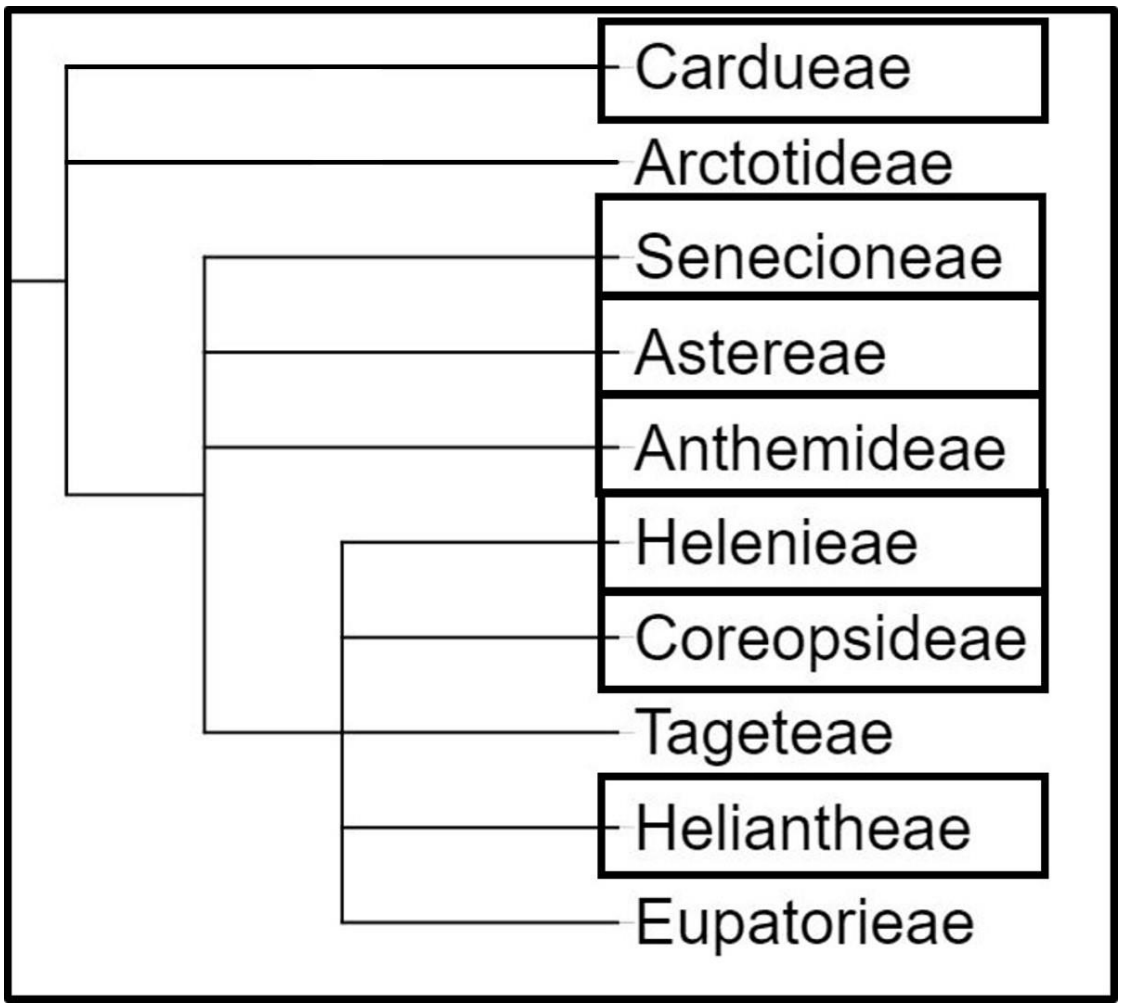


FIGURE 6. Asteraceae tribes infected with DMV-D10 from the study (surrounded in boxes). Modified from Letunic and Bork (2011).

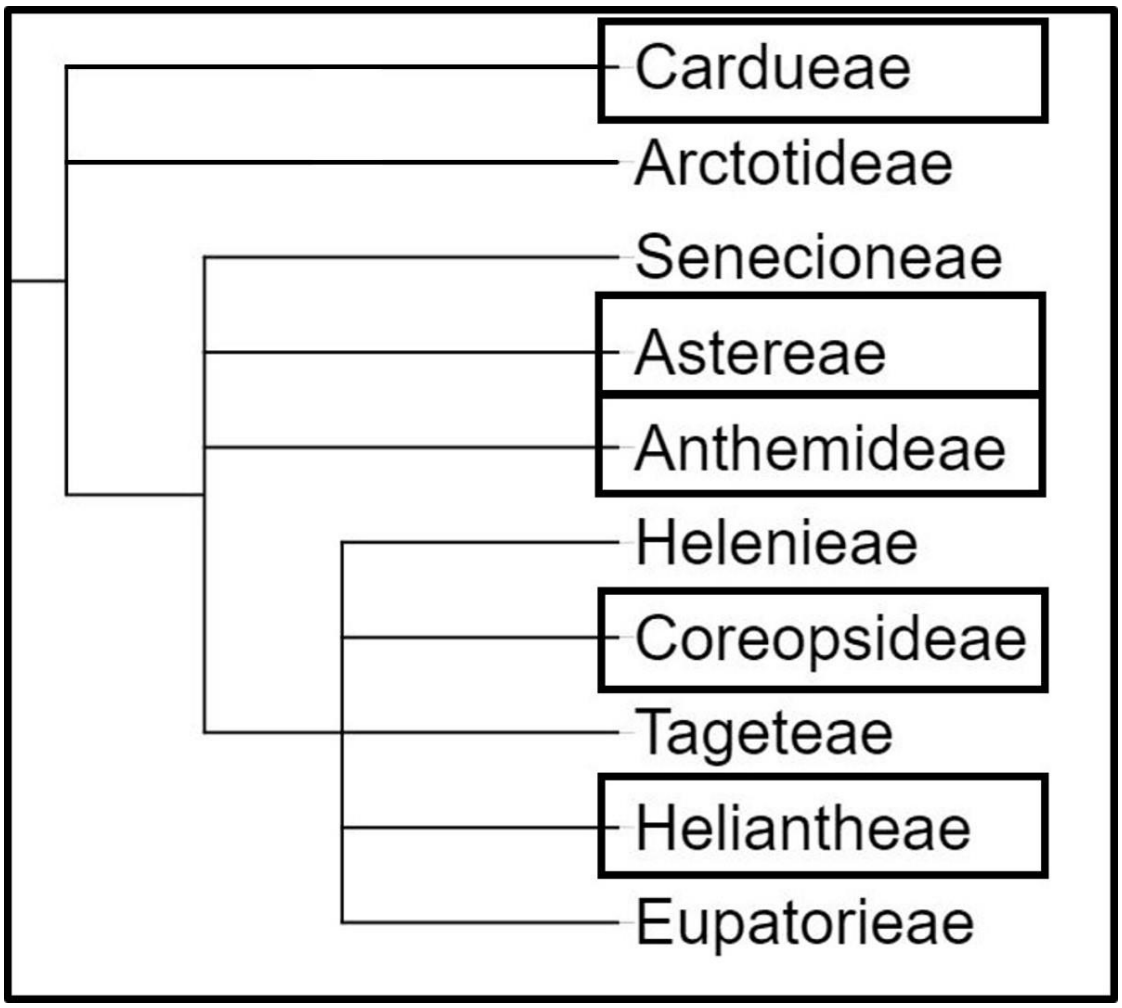


FIGURE 7. Asteraceae tribes infected with Dahlia common mosaic virus (DCMV) from the study (surrounded in boxes). Modified from Letunic and Bork (2011).

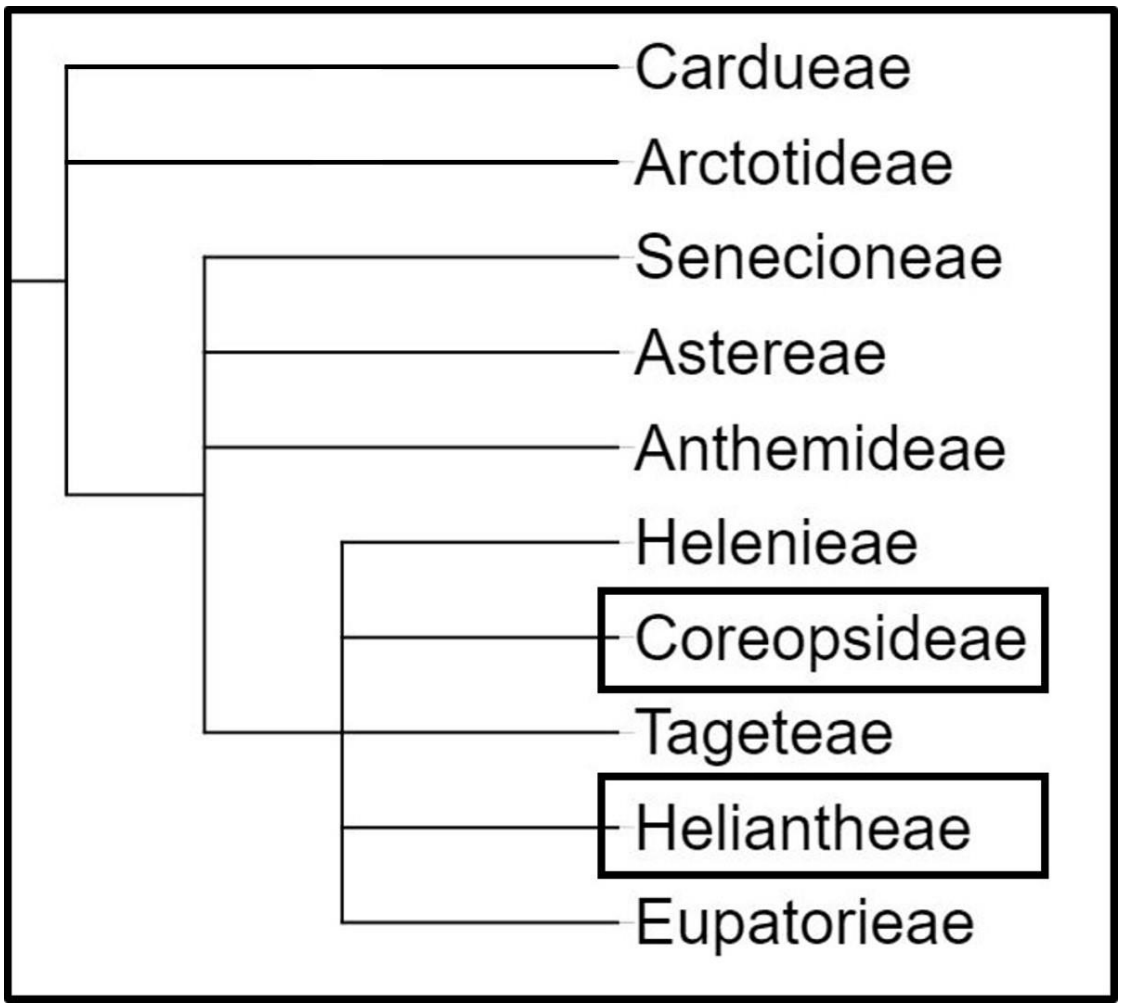


FIGURE 8. Asteraceae tribes infected with Dahlia mosaic virus (DMV) from the study (surrounded in boxes). Modified from Letunic and Bork (2011).

APPENDICES

NNNNNNNNNNNNTNNTNNNTNNTACTNNNTNNNNNNNGAGAGNNCATGTTG
GCGCTTTTGATTCCCACCCCCAGGTGAAANNGGNCTTACNTTGAAAATGCTG
ACCATGTTCAANGACTTGNATGATTTGACTTATAACAATTTGAGTNNNGTNA
AATCAGTACAAGTTACTGTTTCCCTTNNGGANGATGATGAAGACTACAATAC
AGACGTCACCGANNACGCCGATGTGGATCCCGATGATGACGAGTCTGATGAG
GGTAATGATGAACCAGTTCAANGAATCATGAACAATAATATCAAGATCCATG
AACAAGGCTCGNAGTTGNATGANGNNNCCATTTATGGCAAANATTTTTTTCT
ANTGCATATTTGCCTTTNTGNGNATNTTGNNACNANTNTTGTTGTCATTTGTT
GCCNTTTCCCCNTGNNATCTNGGCGNANNNACCATGAANACGNAGGTTGCAT
CTTGCTTTGANTTGGGAANTGATCACAACTTTTCTGATATGATGAATGTCTNC
AATTCATACCTANCCACCTTAATCATCTTCANATCTAAATACCTTTTTGTCACT
CCCGGATAAGTACAATATTAGGATTTATGCGCCTCTTACAGTAGGATATTCTT
AACTTCACTCCGGATAGGTACCCAGATTCTACCATTTCNAANCAAAATATCTC
GAGTATCCTTGGTGANNCTTTAACGTGNCCTTTTATCCTCTCTTTCTTCTCATC
ATTCTGGANAGCTACTCACTGANCTTCACAAATTTTATCAAAGNATTCGGTAG
AGACCACCATGGCTAACGATTTCACGATAAGAGGATAATGATGAACAAGTTC
NAN

APPENDIX 1. Sequenced PCR product with DMV-D10 movement protein primers for *Callitephus chinensis* ‘Crego’ variety.

NNNNNNNNNNNNNNNNNANNNNNNNNNTCAGTGNNNCNNGGNTTGNTGA
TGGTGAGATGATGNNNAACATGTTCTANTTGCCCNAGANAACNGGTGGGTGC
AGTTGGTGTTATGCAAAACTTGATTACTGGTTCAATTGGATAATGATGACCAA
ATTCAAAANTGNTTCTGTATTGCCCACAAAACCCCTTGAAATCTTGGCTTAAA
TTCAGCAAACCCCACNAGTNTCCCCGATTTNGCNTTCGTGATANGNCAATTA
AAAGTCNNGNTCCCNCTNAGAGTGGGTTAATCTCTAACAAAATCACGAGGAC
AATCGAGCGGCAATNNATGCTTATCAACCGATAATGANGAACAATTTCAANG
ATGGAAGATTCTAAGGTAATAAAGAGGCGTATTTTGAGCAGGGTACCGATCG
AAAGCCGGAAAAACCTCATCAAGAATAGTACGGACAACGAGTTCTATGAGGT
GGTGGAGCACACCATGCACAAAGATGATAATTTTAAGAGAAAGGTGACCGTC
ATTGAAGATCTACNTGAAATGACACGTANGGTTCTGCATTACTACAAAGGGG
ATTTCTTGGATGAACTACCCGGACTTGTGGACTTCTCGGTCTTTCTCAACCTC
NGCCCCAAGCAAAAACACGAGGTTTCAGAATTGAGAAAGTTATCAAAGAAA
TTCAAGATCAGTTCTGATGGAAGTGCAATTTATGTGCACCCATGGCTAANGTC
CCTCACAAAGAATACGGCTTCTANAGACAAAAACGATGACAACAGTAACAA
GATTGATGAGCTGCTCGAGAANCTGGATGANAGGNATGGAGTGAAAGCCAA
GTTTTTTCTGAATATGCTTCGGCTATGTGAATCTGGAGGAGAAAGGCTTTTAG
TCTTTNNNCAGTATCTGTTACCCCTAAANTTCCTGATGANATTGGCGATGAAA
GTTNNAANGNNNNNTCCNACNAGGANATTTTTATGANAACAGGAGATCATTG
ATATGATGAACNAGTTCAAA

APPENDIX 2. Sequenced PCR product with DMV-D10 movement protein primers for *Centaurea cyanus* ‘Cyanus Double’ variety.

TAATATGGTTGCGTGCGGTAGTATCTTCGATCGGTAAACCGCTGAAGTAATA
AACTTCTAGTGAAACAAACAAAGGATTTGTGTCAGACTACATGATTAATTCT
GATTATTTAGAAAAAATCATGAAGCTTAAGCTAAAGCTTGATACAAAACAGG
TTTTTAATCAACCTAGTAATTTACAGAGATTAGTTTCAAAAGCTTTCTCTAGA
AAAAATAATATCTTTTATTGCTTTAATACTGAAGAATTGTCAGTAGATATAAA
AGATACTACAGGTGAAGTGTATTTACCACTTCTAACAAAAGGAGAAATAGCC
CGAAGACTTCTGACTATTAAACCAGAATTAAGAAAAACCATGAATATGGTGC
ACATCGGAGCAGTAAAAATCCTTCTGAAGGCACAGTTCAGAGATGGAATTAA
CTTCCCGATAAAAATGGCTTTAGTTGATAACAGAACTATCAACAGGCAAGAC
GCTCTACTCGGAGCAGTTCAAGGAAATTTAGCATACGGTAAATTTATGTTTAC
TGTTTATCCTAAATTTGCATTACATCGAGATTCAAAAGATTTCGATAAAACCT
TAAGTTTCATACATCAGTGCGAAAGGACTGACCTCATGGGAACCAGGTAACA
AAGTATTTACGATTAATTATTTAATTTCGTATGCTTTGACAAATAGTACTCATT
CAATTGAGTATAAAGAAAAGGAGAGTATAACACTTGATGATGTATTCTCAGG
AATAGGTACTGTCGAAAGAAGCAAGTTCGCTGAACCCTTCTCAGATACAGGA
AAATTGGCGATTGACTATTGCTCGAGAGAAAACAACTCTAGGATTTCAACCC
TAGACATAGTTTTACAGGATCCTTTACAAATAGGGCGAGTCCAGTAGAAACA
CAGANANCAGA

APPENDIX 3. Sequenced PCR product with DMV-D10 movement protein primers for *Dahlia variabilis* ‘Cactus’ variety.

NNACATCGCTGACGTGCGGTACATCTTCGATCAGGAATCTCACTGAAGTGAT
AAACTTTCAGTGAAACATGACAAAGGCATTTGTATCAGACTACATGATTAAT
TCTGATTATTTAGAAAAAATCATGAAGCTTAAGCTAAAGCTTGATACAAAAC
AGGTTTTTAATCAACCTAGTAATTTACAGAGATTAGTTTCAAAAACTTTCTCT
AGAAAAAATAATATCTTTTATTGCTTTAATACTGAAGAATTGTCAGTAGATAT
AAAAGATACTACAGGTGAAGTGTATTTACCACTTCTAACAAAAGGAGAAATA
GCCCGAAGACTTCTGACTGTTAAACCTGAATTAAGAAAAACCATGAATATGG
TGCACATCGGAGCAGTAAAAATCCTTCTGAAGGCACAGTTCAGAGATGGAAT
TAACTTCCCGATAAAAATGGCTTTAGTTGATAACAGAATTATCAACAGGCAA
GATGCTCTACTCGGAGCAGTTCAAGGAAATTTAGCATACGGTAAATTTATGTT
TACTGTTTATCCTAAATTTGCATTACATCGAGATTCAAAAGATTTCGATAAAA
CCTTAAGTTTCATACATCAGTGCGAAAGGACTGACCTCATGGAACCAGGTAA
CAAAGTATTTACGATTAATTATTTAATTTCGTATGCTTTGACAAATAGTACTC
ATTCAATTGAGTATAAAGAAAAAGAGAATATAACACTTGATGATGTATTCTC
AGAAATAGGTAACTGTCGAAGGAAGCAAGTTCGCTGAACCTTCTCAGATACA
GGAAAATTGGGCGATTGACATTGCTCGAGAAAAACAAAACTCTAGGATTTCA
ACCTAGAAAATAGTTTTACAGGAATCCCTTTACAAAATAAGGTGACTCCAGT
AGAAACACAGGAAAAAAACAGA

APPENDIX 4. Sequenced PCR product with DMV-D10 movement protein primers for *Dahlia variabilis* ‘Dandy’ variety.

NNNNNNNNNNNNNNANNNNNNGNGCTGTTTTTCTGTGTTTCTACTGGCTGTT
TTTCTGTGTTTCTACTGGCTGTTTTTCTGTGTTTCTACTGGCTGTTTTTCTGTGT
TTCTACTGGCTGTTTTTCTGTGTTTCTACTGGCTGTTTTTCTGTGTTTCTACTGG
CTGTTTTTCTGTGTTTCAACTGCATAAAAACAGCCAGAAAAAAAACACAAAA
ACAGCCAGTAAAAACACAAAAAAACAGCCAGTAAAAAAACAGAAAAACAG
CCAGTAAAAAAACAAAAAAACAGCCAGTAAAAAAACAAAAAAACAGCCAGT
AAAAAAACAAAAAAACAGCCAGTAAAAAAACAAAAAAACAGCCAGTAAAA
AAACAAAAAAACAGCCAGTAAAAAAACAAAAAAACAGCCAGTAAAAACAC
AAAAAAACAGCCAGTAAAAAAACAAAAAAACAGCCAGTAAAAAAACAAAA
AAACAGACAGTAAAAAAACAAAAAAAAAGCCCGTAAAAACACAAAAAAAC
AGCCAGTAAAAACACANAAAAACNGCCAAAAAAAAAACAAAAAAACCGCC
AAAANAAACACAAAAAAACAGCCNGTAAAAACACAGAAAAACCNCCCNTAA
AAAAACAGAAAAACCGCCNGTAAAAANACANNANAACCGCCCTTTNAAANA
CNNNAAAACCCGCCNNTAAAAAAACCAGAAAAAACNNNNNNTNNAAACCCN
CNNAN

APPENDIX 5. Sequenced PCR product with DMV-D10 movement protein primers for *Zinnia elegans* ‘Cherry Queen’ variety.

GNANNNNNNNNNNNNNNNNTGNNCNNNNGATNNAGNNGCTTGTANCTTGG
GTGNNNCGTGTNATGCAGTAACTACGATTGNCGTCCGCAGCATCGCAGTAGT
GTCTTAGATTATTCGTGAGAGTGTGGATGCCGCACGGGGGAGCTAGGTTCCC
CTGAATCGTTCATAACAGTNATGTCCTTTTGCGGTGCTTTTGNNNTTCNTACG
CTTCTGAATTTTGNTGCCTTTTCTGACGCTCGCGTACCCCCTCCTTGATNGCTA
ATGTCATTGGACTCCCAGCCTTTTCNACATACCGACAAACAGAGACACAATT
GCANTCCCTATCTCGTCCNACACGCACTACTCAAAATCGACCCTCACGGCCCC
AATACGTCTACCAACATCNTCTACACCAAGCNNATGTGGTATTATGGTCCTAC
CAATCACGGTTCACGAAGCCATAAGAAACATCGNAAAACTGACGGCTACAA
CTNCTAACACAAGCCAACAACCCGAACCTCGCGAACAANCCGCACACCAACC
AGTCCAAACACANACAAACAGAATCAGACCATGAACAACGNACAACAGGAA
CAATATACGTTGCGGCCCAGTCATCGCATCATATTTATCATTATAAACACAGA
ANANCAGANAAAAAGCACCCCCCTTTTTTTTTCTTTGGNGAAACCATTAAAA
ATTTTTTTTTTAGGNAGTGAAGATNCATGGNNCNGCGACCCCCNNGGNCCCC
CCCNNGNAAANNANTTTNTTTTTGGNAACNNNNNNNACCNNCACCCCANATA
ANNAAAACNTTGNNNNNNNTGGGCNCNGGGNTGGCTCCAGCCNTAANTNTTCC
NNGCNNNGGNANTNTNNNNTNTTTTTCCNNAAAAAATCGGATTTTNNNNNNA
NNTTTTANANNAAAATTTNGCCAATNNNNGNAGNGNCTTNNCNAANCNNCTN
NNGNGATNNNNCCCNNAAAGGGGAATTTTGTNNCAANANNNGNGCCCCNCA
CCCCCNNNNNNGGAGNGNGNNNNNAAGTNNANTATNNNTTANNCCNNGNN
NNNATTNNGNGNANNNNNAAAAAATTGNGGGGGNNNNNNCTN

APPENDIX 6. Sequenced PCR product with DMV-D10 movement protein primers for *Zinnia elegans* ‘Giant Cactus’ variety.

NNNNNNNNNNNNNNNANNNNNGGCTGTTTTTCTGTGTTTNCTACTGGCTGTT
TTTCTGTGTTTATACTGGCTGTTTTTCTGTGTTTCNACTGGCTGTTTTTCTGTGT
TTCTACTGGCTGTTTTTCTGTGTTTCNACTGGCTGCTTNTCNGTGNGGNCACG
ATCNTTTTATGCNGGNCNNNNCNNNNCACNNTNNNAAAANNNGAGTTTGCN
ATNNNGATGTCCTCTACTGCGCCGGGNNNNTNCAAANTNTGTACCNGNNNGG
CNTAGNNTGAGNAANTTNNCATTCCAANCCTTNTTAGAGNNANANNCNCTAN
ACAATNNNNNCAANCGAGAAGGAAAATNAAGGTGNGTCGNGATANNCNTNA
TAGGGAAGACNCANTATGGANNANTNNACTANAATAAGGGCNTGAANNGNA
AANTNCCAANCANNCGTNNANNNACCTNGTCCGGNATTNNCTTTGAAAATGT
GNANATTCCNNTNATNTNTNGAATCTTGNANNNTACTGTATCNTTCNAANAC
ANNAAATCTGNNNNNCTCCATANGTAATCGNNNCTNNTAAANATACGNNGC
GGNNCAGTATNNATCNGNTNANNTTTTGAATCTNGAAANACAGANTATGGTA
TTCCNTTTTTCNNTTTTTGATTCTTATTTTNTNNTTTTNTTATTTTNTTATTTNN
TNNTTTTATTTCAAATTTNTCTTATNTAATTTATTTTTTTTCATTNNTATCTTNA
CTCTTTTTTNATTTTTTCTNNNTTTTTTTTTNTTTTTTNCTNNNNTTCTNCTTTTT
CCTTNTNNATTTTNTANAACTCNNTNTTTTCTNTTTANTTATTTTCATCTCTCTT
TGNTNNTATCATCNTTGTGGTCAGAATCTCTTAATGTCTATTGTTTTAANTGA
NCNCCCTCNNNTNTCTNTGTGTNCTCCNTTTTTTNTTNTNNTNTNNNNTNTNN
TCTNANTTTNTNTCTNNNNNNTNCTNNNNTNN

APPENDIX 7. Sequenced PCR product with DMV-D10 movement protein primers for *Zinnia marylandica* ‘Zahara Starlight Rose’ variety.

GNNNNNNNNNNNNNNNNNNNNANGGTNCCNANTGCNNNGTNNNNNANNA
TGNNNGCACCTGGTGAGTCTCCTCTTTGATTNGAATACNTGTNTTGTTCACCT
ACTTGTATATCTTACCTCCTTTTGCTNTGAACTGCCTTAAANTGCTTCACTNNT
CACTCTTTTGNATGAACCGGATGATGACACAGGTGTTAATGTTTCGACCACTA
GAACTGATGAAGGACCCTTNNNCCCCTCNCCNTNTGCTTCTNAAGAGAATGA
CCCTATNACTCTTACCTTCCTGTATGACTTACGTCTCACGTACTCACGGGCCG
TTGATGGTCTCTNGAAAGACCTTGCGCATACAAAAGTCTCTGTTTCAACTAAN
ATTGTCCAACTGCCGGGGAACGTTCAGGAGTTCCAAACNCANCTGGGACANA
GGTAATNNGNNGTTAGTGATTGTCNCCTATGACCACCAATTGNTCTGGCAAC
TGGGCCCCCTTGGTGTTCTGGTTGAGGTACCCCTGNNNACNNANGTNATTTCT
CCTATGATCNNGATGCTGACCCNTNATGGAAATCGAGATGACCCACGTTCCA
ACGAAGAAGAGAAGATCTTATCTGGACAAGGATGGGGCTGCTCCTTCGCATG
ATCATGCTCATGATGATGCTCATGATGAGGAGATGGACTGTGATTAGGAGTT
TCATGNNCNTCTTGANNNTNNTTGCGGANGNNNTTCNCATGANACGCANNNN
AANNANGTNNANGACCCTCACCCNGANCNNCTGAAGGATTNCCCTCANAGN
ACNCANNANANNAGGANNNGAGANGNNNGNNGATGGAANTACTNNNNNGG
GTNNNTNNNTNNTACCAANNTGGGNTGNGACGAGGAANTAANNNCGTTGTT
ATTCCNTCGANNNNAAAANAAAANAAGNGATCNGTGGNTCNCTTATCGTTNN
GNCAAGCCTCTCTGCTCNNCANCACCTNNTCNTCACCANATNNGNNNNNTCN
TCNGATANNANANNTNNNTNNNNTTGGANNAAGAAGGNNNNNGTNTNNCCT
TCNNNANATGNNNNNNTCNNNNNANGGNNNNN

APPENDIX 8. Sequenced PCR product with DMV-D10 movement protein primers for *Artemisia frigida*.

NNNNNNNNNNNNNNNNNNNNGCTGTTTTTCTGTGTTTCTACTGGCTGTTTTT
CTGTGTTTCTACTGGCTGCTTTTCTGTGTTTCNNCTGGCTGTTTTTCTGTGTTTC
TACTGGCCTTTCTTCNNACAGAAAAACTTTTAGANCCCTCAGGATTCCCTTT
CTTTTTTNATATGACTTTCGCCTCCCGTTCTTACTTCNNATTCGTCCNGCTCCA
TCGCTCCCCTGGNTTGCAGTACTATCCGTTCNAAAGTCTCTTGNCGTCCTTTTA
NTTCTTAAATCATTGAGAAAACCANGTTCTAAGTTTCATCTAACCATGAATG
TAATATAAGGCAGATTANTCAGAANAACATTCTTCTGTGGGTTATTTATCCCT
TTAATAAANCTTTGTANATTNNAAATTTCTTTGTATTATNAAATATGATAATG
TGTTGAAATGCTTTTNNTATTCCANTGTTCANNTNAATTGTNAGGTGGACTGA
ACNACACTAGANAAATGTATAGTATCAATTGTAANTGTAATGACNAATGNCA
AATAGTNTAATGTACNATGNTTAATTAGTATTCACTATTTCTGCACAGTAGAA
ACACCGACAAACAGANCCAAATCAATTAACCCATCTATTTTTTTGAATTTGAA
CCTACTTCTCANCCTAGGTGACTTTGGTAGCCTAATGAGTGGTGGCTTCTGAG
TTGGTTGTGATGATTGGTTTCTTTTTGGTCTAGGAGTAAAAACACAGAAAAAA
CAGAATTCCTTTGCNNNACNNNGGGAATATCGGTCGGATATAATTAAAAACA
ATTTANTGTAATATAATCCACTTTTTTGGNAATGTANTTGNAAAANTGGNTGN
NAAANGNNACTNAAACTNCNNGTGAANCNAAATTTTGTGGANGNTGNNANT
GAAANTNNNNGTTTNNCTNCTACTTTACCNGACACNGTNNTGGNAGCGTATN

APPENDIX 9. Sequenced PCR product with DMV-D10 movement protein primers for *Symphyotrichum oblongifolius*.

NNNNNNNNNNNNNNNNNNNANNNNNGATGNTCGTCTCANGATTGNGGAAA
TCTCATTTTCACCATTATAACCTGCCNCTCCATGGCATATATNACGAGTATTG
AGAAGCTTATGCATTGGGCTAGAGGAAACTAGGAAAGTCCCTGATTGCTCAA
ATTCNCAAGTNAAATTTCTGAAAAAGAANATCATGCTTCNNGGNNNTTTTGA
NNGCAGATNNTNAGGTGCTGATTTGTTCAGAAGATGCCCATAAAAGATAATN
TGATTGGCTTGCAANANATTCTCAGCAAATTTGACCACAGAAAAACNGANGG
CTTGCACAGCCCCTTAAATAGGATCTAACGGATGCACCTTAGTCAATTTATAC
NGTATNNCAAACTAGAAACTTGGATTCTNTTGAATATGAGTTTCTTGTTGATC
TGCGTTAGAAACAGAAATCAAAAGACAAAATGTGGCCTCTCAAGAAGACAG
AGTATGCCACGATGATGATTACTAANCTTATCNATGAATCTGATGCACTTTTT
GAGCCGGTTATTCAACCACCAAAAGGAAGACCGCCNAAACCAAAAAAGAAA
AGAGGAATAACTTCGTCAAGAAGAGACCCATCGAGGTTTGAGCATGTAGAAT
CATCACAAACACAAAACTCGTCAACATCTACTTGTGTCGAAAGAAACAGTGG
AACAACTAATGAATTTAGTTATATCTTTCATGACAACAATTCACTTGATTTAA
ATCTGTACTCAGATTTTTCAAGTGATTATATGTTGTTAGAGTAATAAGGTAGC
CTTGACTCTTGAACAATGAATTTTTGTATATTCNACAGGCACTTTTTGGAAAC
TTGTACGCTTTTAGCTTTGGATCGTATGTTTTGTACTCGTGTCCTAATCAAGTA
TAGATATANGCCTGTTGTGTGCTTTTGAAAGCTTGTTTTATGTAATAGCTTTTG
TTAATGTATTGAAACGGATGCATGTATGCACTTGAATTTTTATTTTATGGGNA
TTATTCNGCTAGTAATTNGACTGTAGNNNNANGTGTNANTTTTANTNANAAA
GAAAAATTGAANTATTANANNTAACNNNNTTTNNTNGNNGTTGNTTNNNANN
N

APPENDIX 10. Sequenced PCR product with DMV-D10 movement protein primers for *Centaurea macrocephala*.

NNNNNNNNNNNNNNNNNNNNNNNGNGNGNNCGTTCCTGCATNNGGNGGTC
TNAATCNTGACAGGTGGTTCACTTGACCTNCTCTTAAGTGTGCGATTCANTAT
CCACAGACTCGACTGATAGTGAAATNTATGAACTTTTCTAATGACCTGGATG
AAACTTNTGATTGCAGGAAAAATGTGGCTGAACTGCAGNAGATTACTCGCGN
GANNGTCTATCTNTGNNAATCTNAGAAATGAANATTTTTCAATTTANTTCTTT
CANCATATCTCTCNAATTTNNTGGNGANATTTTTTCNTNCAATAACCGCCNTT
NCATGGTTATTCTAAATTTTCAAATACTACNNCTGAGCTTCGGAGATTTTGAC
TGAAAGAGTGGGANANTGGAATATTCCCACCANACTCAAGTGTAAACCGCAT
TCTTGGATAAACCCAAGGAACTAAGTCCTTCTATTTCACTTCCTCAATCTATN
GNTGATAATGATGAGCACCCTTGGGAGGAAGNANTGTGTCTTGNNTGNNNAG
NNNNNNCCNCCATCTACANGNATTATCATCNTCGTATCGAAAGATGTTTGAG
ATGATNCTCCCANNAAACACACAAAAAAACNGAGAACANGAACNNCNTACA
NNNGNTNCAAAAANNTGNACNNNTGTCNNNNCGNAANNNATTANAACCGTA
ATGCTANCGNNNNCACNNNNNNNNCTNCCACCNANATCNCTTTGATGATGCA
TCGCAACTTANNAANNTNNGTANCGTTNNNCCTGTNTNNNACTCTCTGAANT
ANNNCAATNNNGTCCNNAAGTTCGNTNTTNNNNGAAAAACTCTNNAAANCCT
TCATAGAGTATCCTGAAANAGGTGATCNNAACGTCTNNNNCGCNGNNCNNN
NANCAAANGCNNTTNNNNCNNANNTANNGNNNNGANNTNNNCNANCCCNCC
NATGGCNTNGCG

APPENDIX 11. Sequenced PCR product with DMV-D10 movement protein primers for *Hymenoxys hoopesii*.

NNNNNNNNNNNNNNNNNCNTGNTACNNNCTTCTGATGATTTCTTTAGATAC
TTATACTGACTTATAAAGATACCAGTTGGCTTTTGCTTGACTTGTAACCCAAG
AAAGAATGTCAATTCTCCCATTGAACTCATTTTGAACTTTAAATGCATTAAAG
CTTCCAACTCTCGACATAACTCCTCTCTTGGAGAACCAAAAATGATATCATCT
ACATGTATTTGAACCAGTAGAATCTCANTCTAANCNANAAAGATTNTAAAGT
CTTATTAATTGCTCCTCTTTTGAANTTGTTTTCCAGTNGATTNCGNAAAANNG
NANTATCCTTGATCTCNTAAATNANCAAGGCTTTGTTATCTTCAAGGACAATA
AAAAAGGCCCTTTCAAAAATTTTAACTTCCGCCTGANTTTTCAAAAATTCATT
NGGAGGCTTTTGGGGGAAAAAAATNATGGCCTCCAAACTTTNNGNACCTTTT
TGTGTTTANTTTTTAANNNTCCCTGAAACCCACCAAATTAACAAAACCAGTAT
TTTTTGACTTTTTTTCNACTAATGCCCTGATATANCCCAAACTTAATCCAACAC
ACGAATTTTTTATGCATCCTCGTTGGTTTTCAACATGAAAACTTAAATTTCCA
GATCCAGAGCCNATGGNNCTGATACCAGTTGTGATCANCNGATANGGNTCGA
TGANTGNNTCGCCATGAGNNACTCATCAGTANAAACTGCTGCGGAGCNNTAC
NCNNNTTGNNCANNGTTTANCTGTTAAACNNNNNGNANNNANGNAAANNGG
NNNANGANGGGNNNTTGTTTTTCACGCNCCNCCNTCAAGCTGAAATATTGAT
NAATCNNAGNATGTAACAATNTTCANAANGTCCTATTATTTNNTCCTTGNGG
CTAACAANCCTNGTTTTCCANNNNAAAAGATTAANATGATNACNNNNTCANA
CTATCTTAATNNGNNCCCTNNNANCNNNGAATGNGGTACTACCATATTTACA
NGAACNGTANNGNNNAATTTCNCNANNANTNNNTGAN

APPENDIX 12. Sequenced PCR product with DMV-D10 movement protein primers for *Petasites japonicus*.