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First report of the *Brevipalpus*-transmitted (Trombidiformes: Tenuipalpidae) *Orchid fleck dichorhavirus* infecting three ornamentals in the United States

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Abstract

Several flat mite species, all from the genus *Brevipalpus* Donnadieu (Trombidiformes: Tenuipalpidae), are the only known vectors of cileviruses and dichorhaviruses. The *B. californicus* species group exclusively can transmit Orchid fleck dichorhavirus (OFV) in a persistent propagative manner. OFV is the type species for the genus *Dichorhavirus* and infects more than fifty plant species belonging to the family Orchidaceae, Asparagaceae (Nolinoidaea), and Rutaceae (*Citrus*). During the summer of 2020, chlorotic ringspot symptoms were seen on Giant Lilyturf (*Liriope* spp., cv. 'Gigantea,' Asparagaceae: Nolinoidaea) and leaf chlorosis was observed on leaves of *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) in a landscape in Leon County, Florida. In both cases, the presence of OFV was confirmed using OFV specific conventional reverse transcription polymerase chain assay (RT-PCR) assay and Sanger sequencing. RT-PCR amplicons had a 98% identity with the known OFV sequences available in the Genbank (Add an accession number….). The identification was also confirmed with the quantitative RT-PCR (RT-qPCR). Additional samples were taken from other Nolinoidaea, including Aztec Grass (*Ophiopogon intermedius* cv. 'Argenteomarginatus' D. Don, Asparagaceae: Nolinoidaea), Mondo Grass *O. japonicus* and Lilyturf *L. muscari*, with ringspot symptoms were observed in Leon and Alachua counties. Identification of partial genome sequence confirmed the presence of both the orchid strains (OFV-Orc1 and OFV-Orc2) in Florida. Three mite species were recovered from OFV-infected plants: *Brevipalpus californicus* sensu lato, *B. obovatus* Donnadieu and *B. confusus* Banks. At least one of these species is presumably responsible for OFV transmission. Florida has various *Brevipalpus* susceptible native and introduced plant species in the landscape. In

this study we are reporting three new hosts from the family Asperagaceae from multiple locations. Our data suggested that the OFV is widely distributed in Florida and might be a potential threat for *Liriope* spp., *Ophiopogon* spp. and *Aspidistra elatior* which are commonly used in landscaping in the southeastern US, if not controlled. As both the orchid strains of OFV are known to infect citrus and cause citrus leprosis disease, a survey in citrus growing regions of Florida is essential, emphasizing plants within the families Orchidaceae, Rutaceae and Asparagaceae.

Keywords:

False spider mite, flat mite, *Brevipalpus*-transmitted viruses, *Liriope*, Nolinoideae, *Ophiopogon*, Ruscaceae, Rutaceae, Asparagaceae, orchid, Orchidaceae, pests, ornamental plants, orchid fleck virus.

Orchid fleck dichorhavirus (OFV) is the type member for the genus *Dichorhavirus*, family Rhabdoviridae; a bacilliform, nuclear rhabdovirus composed of two segments of single-stranded, negative-sense RNA which infects plants (Dietzgen et al. 2014, Walker et al. 2018, Amarasinghe et al. 2019). Flat mites from the genus *Brevipalpus* Donnadieu (Trombidiformes: Tenuipalpidae) are the only known vectors for cileviruses and dichorhaviruses (Maeda 1998), and *B. californicus* (Banks) group of mites are the only known to transmit OFV in a persistent propagative manner (Kondo et al. 2003).

OFV-infected plants exhibit various symptoms depending on the infected plant species as well as the strain of the OFV associated with the infection (Kubo et al. 2009), but symptoms typically appear as chlorotic flecks, which ultimately coalesce into larger spots or ringspot patterns (Fig. 1, Fig. 2).

OFV was first described infecting *Cymbidium* orchids in Japan (Doi et al. 1977). OFV and OFV-like rhabdoviruses have been reported infecting orchids in Asia, Africa, North America, South America, Europe, and Oceania. The prevalence of OFV and its mite vector is thought to be associated with the movement of infected orchids (Dietzgen, Freitas-Astúa, et al. 2018).

OFV naturally infects more than fifty species of Orchidaceae (Kitajima et al. 2010, Peng et al. 2013), some Asparagaceae (Nolinoidaea) (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), and Rutaceae: (*Citrus*), where it causes citrus leprosis-like symptoms (Roy et al. 2015, 2020, Cook et al. 2019, Olmedo-Velarde et al. 2019). Mechanical transmission of OFV is possible to some plants belonging to the plant families Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013), under laboratory conditions.

During June 2020, chlorotic ringspot symptoms were observed on Giant Lilyturf *Liriope* spp., cv. ‘Gigantea’ in a landscape of Leon County, Florida (Fig. 1). *Liriope* belong to a group of plants in the family Asparagaceae, subfamily Nolinoidaea, which includes a diverse array of various monocotyledonous southeastern Asian native liliod plants (Chase et al. 2009, Meng et al. 2021). *Liriope* and the closely related *Ophiopogon* Ker Gawler (Asparagaceae: Nolinoidaea) are considered the most important ground cover nursery plants in southeastern US (Mcharo et al. 2003).

Viral infections of suspected leaf samples were initially tested at the Plant Disease Diagnostic Clinic at the North Florida Research and Education Center (NFREC) in Quincy, FL. All the samples were tested with RT-PCR and were found negative for begomovirus, carlavirus, potyvirus, tospovirus as well as for Cucumber mosaic virus, Impatiens necrotic spot virus, Tobacco mosaic virus and Tomato spotted wilt virus.

The initial site of collection was visited two more times during 2020 to gather plants for identification of the unidentified plant pathogen. These surveys were conducted on July 28th and August 19th, to collect more putatively infected *Liriope* spp. and a suspected new member of the family Asparagaceae; *Aspidistra elatior* Blume with chlorotic leaves (Fig. 2), respectively. Upon collection, these new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) for identification.

The presence of OFV was confirmed using OFV generic R2-Dicho-GF and R2-Dicho-GR primers by one step conventional RT-PCR (Roy et al. 2020), amplifying ~800 nt of L-gene (RNA2) amplicon from an infected *Liriope* leaf sample. Sanger sequencing of RT-PCR amplicons shared 98% nucleotide identity with orchid strains of OFV: OFV-Orc1 and OFV-Orc2 (GenBank Accession numbers: AB244418 and LC222630) (Kondo et al. 2006, 2017).

Subsequent surveys of plants belonging to the subfamily Nolinoidaea in Florida have revealed more sites with Asparagaceae putatively infected with OFV in Leon and Alachua counties (Table 1). Mites were collected from symptomatic plants in Leon county and observed with phase contrast microscopy. Tenuipalpid mites (flat mites or false spider mites) were commonly found in abundance on the Asparagaceae which tested positive for OFV. These flat mites were originally identified as *Brevipalpus californicus* (Banks) sensu lato and later confirmed by the FDACS via Differential Interference Contrast (DIC) microscopy. The *Brevipalpus* mite species complex is known to contain cryptic species (Childers and Rodrigues 2011) that require advanced microscopy techniques, such as cryo scanning electron microscopy (Cryo-SEM) for species identification (León and Nadler 2010, Beard et al. 2015, Skoracka et al. 2015). Additional mite samples were collected from the original OFV detection site, and examined under Cryo-SEM (Fig. 3). The determinations approved prior identifications of *B. californicus* s.l. but revealed the presence of two other species *B. obovatus* and *B. confusus* (Fig. 4).

The first report of OFV in the United States is thought to be Ko et al. (1985), who describes nuclear inclusions caused by an undescribed bacilliform rhabdovirus in *Brassia* orchids. The significance of this report is their reference to spoke-wheel configurations of the viral particles (Ko et al. 1985), a sign typically associated with OFV infection (Chang et al. 1976). Unfortunately, Ko et al. (1985) made no mention of mites or further investigations of this virus. The first certain report of OFV was from Hawaii in

2001 (Blanchfield et al. 2001), while the first report from the continental US was by Bratsch et al. (2015). In that publication, the authors confirmed the presence of OFV in *Phalaenopsis* hybrids in the US, using TEM of ultrathin sections of plant tissue as well as molecular sequence analysis and its association with *Brevipalpus* mites (Bratsch et al. 2015). The authors did not make a conclusive species identification but suggested the mite vector was within the *B. californicus* group, as referred by Kondo et al. (2003).

OFV has been reported in other Nolinoidaea in Australia (Mei et al. 2016, Dietzgen, Tassi, et al. 2018), including *Liriope spicata* (Thunb.) Lour. (Mei et al. 2016) but not in the United States. The Florida collected plants of *Liriope* spp., cv 'Gigantea' are thought to belong to either *Liriope muscari* or *Liriope gigantea*. We are not aware of any previous report of OFV infection in *Ophiopogon* plants. Although Zheng et al. (2013) mentions the association of *B. californicus* with *A. elatior* but they did not mention the presence of OFV symptoms in this plant. However, our finding will be notified as the first report of OFV in the United States on ornamentals and among them *A. elatior* is a new natural host of OFV.

Conclusion

The dichorhavirus that infects citrus in Hawaii, Mexico, Colombia, and South Africa are identical to the OFV in gene order, content, and the genome sequence. According to the International committee on Taxonomy of Viruses (ICTV) classification, OFV consist of two orchid strains (OFV-Orc1 and OFV-Orc2) and two citrus strains (OFV-Cit1 and OFV-Cit2). Both the orchid strains of OFV infects citrus (Roy et al. 2020), but none of the citrus strains have been reported from any orchid species. Detecting OFV in Florida represents a concern for horticulturists who grow orchids, *Liriope*, *Ophiopogon*, or other susceptible Asparagaceae species which are commonly used in landscaping. Florida is also home to a plethora of native and naturalized orchid species, many of which are threatened, including cultivating *Vanilla* in southern Florida (Chambers et al. 2019) and the famous Ghost Orchid, [*Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe]. Citrus leprosis was present in Florida during the 1860's and eradicated in the mid-1960s. In fact, Kitajima et al. (2011) found that the Citrus Leprosis virus (CiLV) which previously affected Florida citrus was a nuclear type of citrus leprosis closely related to OFV strains (Hartung et al. 2015, Roy et al. 2020). Association of a distant relative of OFV named Citrus leprosis dichorhavirus-N0 (CiLV-N0) was confirmed in relation to the leprosis disease outbreak in Florida (Hartung et al. 2015). The recent detection of OFV-Orc1 in South Africa (Cook et al. 2019) in *C. sinensis* (Navel and Valencia orange) and OFV-Orc2 in Hawaii (Velarde et al. 2021) in *C. reticulata* (mandarin) and *C. jambhiri* (rough lemon) associated with leprosis-like symptoms highlights the threat of different strains of OFV on citrus; which will be a definite concern to the multi-billion dollar citrus industry. *B. californicus* and *B. yothersi* are both known vectors of Dichorhaviruses (OFV) and Cileviruses (Citrus Leprosis) (Knorr 1968, Kondo et al. 2003, Beltran-Beltran et al. 2020) and *B. obovatus* is a suspected vector as well (Childers et al. 2003). All three mite species/complexes are present in Florida (Childers et al. 2003, Akyazi et al. 2017) (Fig. 4). Therefore, it is critical to identify the vector of OFVs in Florida and monitor its spread to determine the risk this virus represents for the native plants, agriculture and the ornamental/landscaping industries of Florida and the surrounding regions.

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Table

Scientific Name	Common Names	Symptoms Observed
Liriope muscari Bailey	Lilyturf, Orchardgrass, Monkeygrass	Ringspots, Chlorotic Flecking, Necrotic Lesions
*Liriope gigantea** Hume	Giant Lilyturf	Ringspots, Chlorotic Flecking, Necrotic Lesions
Ophiopogon japonicus Ker Gawl.	Dwarf Lilyturf, Mondo Grass, Snake’s beard	Ringspots, Chlorotic Flecking, Necrotic Lesions
*Ophiopogon intermedius*** Don	Aztec Grass, ‘Argenteomarginatus’	Ringspots, Chlorotic Flecking, Necrotic Lesions
Aspidistra elatior Blume	Cast Iron Plant, Bar-room Plant	Chlorosis, Chlorotic Flecking, Necrotic Lesions

Table 1: List of plants with symptoms of Orchid fleck dichorhavirus found in northern Florida. * *L. gigantea* have been traditionally classified as seperate from *L. muscari* by Broussard (2007) and Fantz et al. (2015), although this distinction has been challenged by Wang et al. (2014) and Masiero et al. (2020). * * *O. intermedius* is sometimes misclassified as *Liriope muscari* 'Variegated Evergreen Giant' Fantz (2009) or 'Grandiflora White' (Fantz 2009).

Figure captions

Fig. 1: Variety of symptoms expressed by *Liriope* spp. infected with Orchid fleck dichorhavirus (OFV): (a) ringspot symptoms on *Liriope gigantea* (b-c) Details of ringspot symptoms on *Liriope gigantea* (d) chlorotic ringspots *Liriope muscari* cv. 'Silvery Sunproof'

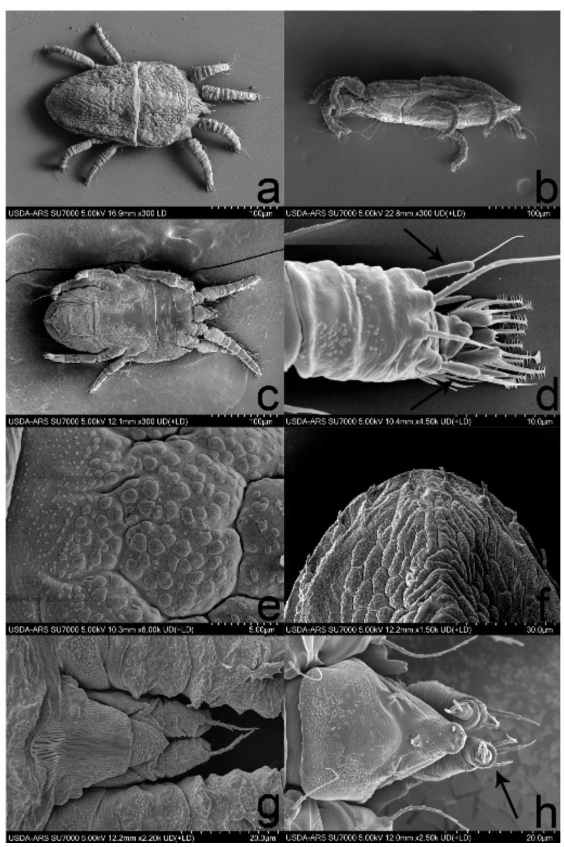
Fig. 2: Symptoms expressed by *Aspidistra elatior* infected with Orchid fleck dichorhavirus (OFV): (a) Detail of leaf chlorosis (b) Chlorosis caused by OFV appears similar to sunburn damage (c-d) Chlorotic ringspots may indicate early symptoms of OFV

Fig. 3: Cryo-SEM images of *Brevipalpus californicus* sensu lato displaying various characters used for identification (Baker and Tuttle 1987, Beard et al. 2012) (a) Dorsum (b) Lateral view (c) Venter (d) Close up of distal end of leg 2, with arrows indicating paired solenidia, characteristic of the genus *Brevipalpus* (e) Enlargement of the microplates of the mite cerotegument (f) Dorsal view of the distal portion of mite abdomen (g) Dorsal view of the mite rostrum (h) Ventral view of mite rostrum, observe 3 distal setae.

Fig. 4: Florida is home to other common pest species of *Brevipalpus* which are potential vectors of *Orchid fleck dichorhavirus*: (a) *B. yothersi*, dorsal (b) *B. yothersi*, lateral (c) *B. obovatus*, dorsal.

Figures


a
b
c
d


a
b
c
d
e
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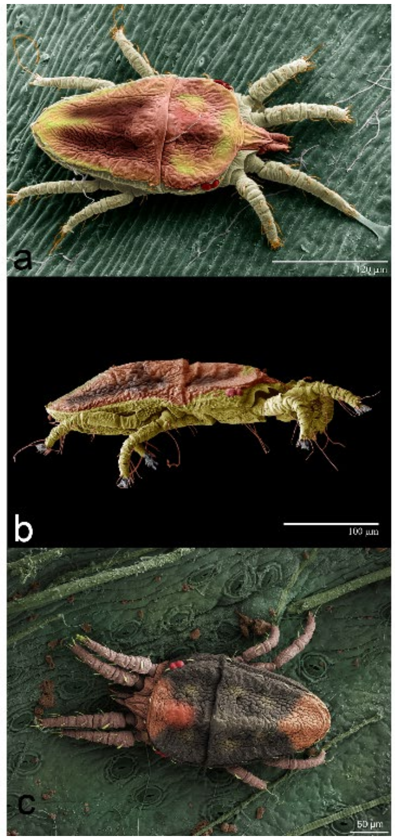
g
h


a
b
100 µm
c
50 µm