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### First report of the *Brevipalpus*-transmitted (Trombidiformes: Tenuipalpidae) virus, *Orchid fleck virus* (Mononegavirales: Rhabdoviridae) infecting three ornamentals in Florida

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### Abstract

Several flat mite species, all from the genus *Brevipalpus* Donnadieu (Trombidiformes: Tenuipalpidae), are the only known vectors of 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 belongs to the family Orchidaceae, Asparagaceae (Nolinoidaea), and Rutaceae (*Citrus*). During June 2020, chlorotic ringspot symptoms on Giant Lilyturf (*Liriope* spp., cv. ‘Gigantea’) were observed in a landscape in Leon County, Florida. Later in the year, 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. The identification was also confirmed with the quantitative RT-PCR (RT-qPCR). Additional samples were taken from other Nolinoidaea, including *Liriope muscari*, *Ophiopogon japonicus*, *O. intermedius* and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) 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. One of these species is presumably responsible for OFV transmission. Florida has various mite species of *Brevipalpus* and a diverse array of susceptible native and introduced plant species in the landscape. We suggest that OFV already has a wide distribution in Florida which will continue to spread if unchecked, representing a potential threat for *Liriope* spp., *Ophiopogon* spp. and *Aspidistra elatior* which are commonly used in landscaping in Florida . In this study we are reporting three new hosts from the family Asperagaceae. To know the extended host range of OFVs, a survey in the citrus growing regions in Florida is essential with special emphasis to the plants belongs to the family 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 virus* (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). Other members of this genus are: *Citrus chlorotic spot virus*, *Citrus leprosis virus* N, *Clerodendrum chlorotic spot virus* and *Coffee ringspot virus* (Dietzgen, Freitas-Astúa, et al. 2018). Flat mites from the genus *Brevipalpus* Donnadieu (Trombidiformes: Tenuipalpidae) are the only known vector for dichorhaviruses (Maeda 1998), and *Brevipalpus californicus* (Banks) mites are the only mite known to transmit OFV in a persistent propagative manner (Kondo et al. 2003).

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

OFV was first described infecting *Cymbidium* orchids in Japan (Doi et al. 1977). Many countries have reported OFV and OFV-like rhabdoviruses infecting orchids worldwide (Kondo et al. 2003), including Asia: [China (Peng et al. 2017), Korea Zheng et al. (2013)], Africa: [South Africa (Blanchfield et al. 2001)], North America: [The United States (Blanchfield et al. 2001, Bratsch et al. 2015)], South America: [Brazil (Kitajima et al. 1974, Kitajima et al. 2001), Colombia (Kubo et al. 2009), Costa Rica (Freitas-Astúa et al. 2002), Paraguay (Ramos-González et al. 2015)], Europe: [Denmark (Begtrup 1972), France (Sauvêtre et al. 2018), Germany (Petzold 1971, Lesemann and Doraiswamy 1975)] and Oceania: [Australia (Lesemann and Begtrup 1971, Lesemann and Doraiswamy 1975, Gibbs 2000), Fiji (Pearson et al. 1993), Vanuatu (Pearson et al. 1993)]. 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), as well as Rutaceae: *Citrus* where it causes citrus leprosis-like symptoms (García-Escamilla et al. 2018, Beltran-Beltran et al. 2020, Roy et al. 2020). Mechanical transmission of OFV is possible under lab conditions to various Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013).

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 lilliod plants which are native to southeastern Asia (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 sold by the nursery industry in southeastern US (Mcharo et al. 2003).

Viral infections of suspected plant 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 tested negative for begomovirus, potyvirus, tospovirus as well as for Impatiens necrotic spot virus, Tobacco mosaic virus and Tomato spotted wilt virus. The infected materials were subsequently sent to the Florida Department of Agriculture and Consumer Services (FDACS). The presence of OFV was confirmed using OFV generic R2-Dicho-GF and R2-Dicho-GR primers (Roy et al. 2020) by one step conventional RT-PCR, amplifying ~800 nt of L-gene (RNA2) amplicon from an infected *Liriope* leaf sample. The RT-PCR amplicon was sequenced using Sanger sequencing and confirmed the presence of OFV in the tested samples. Sanger sequencing of RT-PCR amplicons shared 98% nucleotide identity with orchid strains of OFV (GenBank Accession numbers: AB244418 and LC222630) (Kondo et al. 2006, 2017). Further surveys of putatively OFV-infected plants were taken during subsequent visits to the initial site of collection. Plant samples included various cultivars of Nolinoid plants, including *Liriope* spp., *Ophiopogon* spp., and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) which were located at the same site as the original infected *Liriope* spp., cv ‘Gigantea.’ Samples were tested via RT-PCR at the NFREC to confirm the presence of OFV.

Further surveys of plants belonging to the subfamily Nolinoidaea in Florida have revealed more sites with symptomatic plants in both Leon and Alachua counties. In addition to the discovery of the OFV-infected plants, we collected mites from symptomatic plants in both Leon and Alachua counties. In addition to the discovery of the OFV-infected plants, we collected mites from symptomatic plants in Leon county which were observed with phase contrast microscopy. We encountered both an unidentified eriophyoid mite species on the *Liriope* spp., along with flat mites on all species tested (*Liriope* spp., *Ophiopogon* spp., and *A. elatior*). The flat mites were originally identified as *Brevipalpus californicus* (Banks) sensu lato, an identity which was confirmed by the FDACS via Differential Interference Contrast (DIC) microscopy. *Brevipalpus* mites have been previously associated with OFV (Dietzgen, Tassi, et al. 2018, García-Escamilla et al. 2018, Beltran-Beltran et al. 2020) as well as similar diseases (Kitajima et al. 2010) and are known to feed on a large variety of economically important plants (Childers, Rodrigues, and Welbourn 2003, Akyazi et al. 2017). Unfortunately, the *Brevipalpus* mite species complex is known to contain cryptic species (Childers and Rodrigues 2011) whose identification can be improved with molecular methods as well as more advanced microscopy techniques, such as cryo scanning electron microscopy (currently known as Cryo-SEM) (León and Nadler 2010, Beard et al. 2015, Skoracka et al. 2015). With that in mind, we collected additional samples of the mites from the same site as the original OFV detection and sent them to the USDA-ARS in Beltsville to observe the mites with Cryo-SEM techniques, which agreed with both prior identifications of *B. californicus* s.l. but revealed the presence of two other species: *B. obovatus* and *B. confusus*.

The first report of OFV in the United State 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 in the US was made by Bratsch et al. (2015), who 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. The authors did not make a conclusive species identification but suggested the mite species was *B. californicus* group as the vector 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). The Florida collected plants of *Liriope* spp., cv ‘Gigantea’ are thought to belong to either *Liriope muscari* or *Liriope gigantea*. In addition, we are not aware of any record other than this manuscript which reports OFV infection in *Ophiopogon* plants. Unfortunately, the Ophiopogonae group includes species of *Liriope* and *Ophiopogon* which are very similar in appearance and growth habit, with few useful morphological characters used for their classification (Fantz 2008). Furthermore, the horticultural industry has created a diverse array of cultivars of these plants, which are often mislabeled (Fantz 2008). Aside from the taxonomic confusion created by humans, natural hybrids between *Ophiopogon* and *Liriope* have created a natural source of error for reconstructing phylogenies (Zhou et al. 2009). Together, these factors make it difficult to differentiate and identify plants in the landscape by visual inspection alone. These obfuscations of species identity may be accounted for in the future via sequence comparisons of the OFV-infected plants (Rudall et al. 2000, Wang et al. 2014), but these comparisons are beyond the scope of our current report. Nonetheless, we are confident that ours is the first report of OFV infecting *Aspidistra elatior*; although (Zheng et al. 2013) mentions the association of *B. californicus* with *A. elatior* they make no mention of OFV symptoms in this plant.

### Conclusion

OFV is the type member of the genus *Dichorhavirus* (family *Rhabdoviridae*, order *Mononegavirales*) contain a bipartite, single-stranded and negative sense RNA genome. 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 but none of the citrus strain 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]. The first time leprosis has been observed in the Florida in 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, which are closely related to OFV strains (Roy et al. 2013). Association of a distant relative of OFV which was named as 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) highlights the threat of different strains of OFV; which will be a definite concern to the multi-billion dollar citrus industry. Lastly, some OFV isolates are known to be involved with citrus leprosis disease in Mexico (Roy et al. 2015), which may be a cause for concern for the 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, Rodrigues, Derrick, et al. 2003). All three mite species/complexes are present in Florida (Childers, Rodrigues, Derrick, et al. 2003, Akyazi et al. 2017). 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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### Figure captions

Fig. 1: Variety of symptoms expressed by plants infected with Orchid fleck dichorhavirus: (a) ringspot symptoms on *Liriope gigantea* (b) chlorotic flecking on *Aspidistra elatior*.

### Figures

