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

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Abstract

We describe the first outbreaks of *Orchid fleck dichorhavirus*, belonging to the orchid-infecting subgroup (OFV-Orc), from three unreported hosts: *Liriope muscari*, cv. 'Gigantea' (Decaisne) Bailey, *Ophiopogon intermedius* Don and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea) in Leon and Alachua Counties, FL. Strains of OFV-Orc infect over 50 plant species belonging to the plant families Orchidaceae, Asparagaceae (Nolinoidaea), and infects *Citrus* (Rutaceae) as citrus leprosis disease. The only known vectors of OFV-Orc are flat mites, genus *Brevipalpus* (Trombidiformes: Tenuipalpidae). Florida has various plants in the landscape which *Brevipalpus* spp. feed on, which are susceptible to infection by OFV-Orc.

Chlorotic ringspots and flecking were seen affecting Liriopogons (*Liriope* and *Ophiopogon* spp.) in Leon County, FL. Nearby *A. elatior* also appeared chlorotic. Local diagnostics returned negative for common plant pathogens, therefore new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) and USDA-ARS for identification.

Two orchid-infecting strains of Orchid fleck virus were detected via combinations of conventional RT-PCR, RT-qPCR, Sanger sequencing and High Throughput Sequencing. Amplicons shared 98% nucleotide identity with OFV-Orc1 and OFV-Orc2 available in NCBI GenBank. Coinfections were seen in each county, but single strains of OFV-Orc were seen in *L. muscari* (Alachua, OFV-Orc2) and *A. elatior* (Leon, OFV-Orc1).

Three potential mite vectors were identified via cryo-scanning electron microscopy (Cryo-SEM): *Brevipalpus californicus* (Banks) sensu lato, *B. obovatus* Donnadieu, and *B. confusus* Baker.

In conclusion, *Orchid fleck dichorhavirus* is present in northern Florida, representing a risk for susceptible plants in the southeastern US.

Keywords:

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

Orchid fleck dichorhavirus, commonly referred to as Orchid Fleck Virus (OFV), is the type member for the genus *Dichorhavirus*, family *Rhabdoviridae*. OFV is 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* (Trombidiformes: Tenuipalpidae) are the only group known to transmit dichorhaviruses (Maeda 1998), and *Brevipalpus californicus* (Banks) *sensu lato* are the only mites which do so 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. 2009a), but symptoms typically appear as chlorotic flecks, which ultimately coalesce into ringspot patterns.

OFV was first described as 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 et al. 2018a).

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 et al. 2018b), and Rutaceae, where it causes citrus leprosis-like symptoms (Roy et al. 2015, 2020, Cook et al. 2019, Velarde et al. 2021). Mechanical transmission of OFV is possible under laboratory conditions 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).

Virus Detection

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, comprised of grass-like monocotyledonous liliod plants native to southeastern Asia (Chase et al. 2009, Meng et al. 2021). *Liriope* and the closely related *Ophiopogon* (Asparagaceae: Nolinoidaea) are considered the most important ground cover plant in the southeastern United States (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 one step conventional RT-PCR, and were found negative for begomovirus, carlavirus, potyvirus, tospovirus, *Cucumber mosaic virus* and *Tobacco mosaic virus*.

As initial diagnostics were inconclusive, new samples were collected during July and August of 2020 to collect more of these putatively infected plants with ringspot symptoms. The plants collected included *Liriope* spp. and *Ophiopogon* spp., as well as *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea). *A. elatior* was suspected to be infected, due to both its proximity to infected *Liriope* and the presence of unusually chlorotic leaves (Fig. 2). Upon collection, the new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) for identification.

The FDACS determined eventually that the pathogen was *Orchid fleck dichorhavirus* using previously published primers and methods to conduct RT-PCR and Sanger sequencing (Kubo 2006a, 2006b, Kubo et al. 2009b, Kubo et al. 2009a, Ramos-González et al. 2015). Orchid subgroup 1, OFV-Orc was identified following the methods described in Kondo et al. (2017). Sequencing demonstrated a shared 98% nucleotide identity with the orchid strain subgroup, OFV-Orc (isolates So and Br with GenBank Accession numbers: AB244418 and MK522807, respectively) (Kondo et al. 2006, 2017).

These samples from FDACS were subsequently retested by the USDA-ARS, in conjunction with tests of fresh samples from both Alachua and Leon counties. The USDA used RT-PCR, RT-qPCR, and High Throughput Sequencing (HTS) in sequence to reconfirm the presence of *Orchid fleck dichorhavirus*. RT-PCR and qPCR with Generic R2-Dicho-GF and R2-Dicho-GR primers amplifed ~800 nt of L-gene (RNA2) amplicon (Roy et al. 2020), and OFV-Orc1 and OFV-Orc2 were detected in both *O. intermedius* and *A. elatior* from Leon County.

HTS reaffirmed the presence of OFV-Orc1 and OFV-Orc2 strains in Leon and Alachua counties (Table 1). HTS results from Leon County revealed that *L. muscari* were coinfected with both strains (OFV-Orc1 and OFV-Orc2), while *A. elatior* were solely infected with OFV-Orc1. HTS of *L. muscari* from Alachua County revealed infections with the OFV-Orc2 strain.

After the initial identification by FDACS of OFV-Orc strains, mite samples were collected from symptomatic Asparagaceae in Leon County. Most mites collected were Tenuipalpid mites (flat mites or false spider mites), a known pest of ornamental plants, some of which are known to act as vectors for plant viruses (Childers et al. 2003, Childers and Rodrigues 2011).

Mite Description

Mite taxonomy is complicated by cryptic species complexes which occur in many plant-feeding groups of the Acari (Umina and Hoffmann 1999, Skoracka and Dabert 2010, Arthur et al. 2011, Skoracka et al. 2013), including tenuipalpid mites from the genus *Brevipalpus* (Navia et al. 2013). The commonly used phase-contrast microscopy is insufficient to detect some diagnostic characters for separation of cryptic species, instead best practices recommend the combination of Differential Interference Contrast (DIC) Microscopy and Scanning Electron Microscopy along with molecular methods to separate cryptic species (Beard et al. 2015).

The flat mites collected were initially suspected to belong to *B. californicus* after inspection with phase contrast microscopy. Subsequent observation via DIC microscopy at FDACS agreed with this tentative identification. Unfortunately, the *B. californicus* s.l. species group, *sensu* Baker and Tuttle (1987) is suspected to contain cryptic species (Childers and Rodrigues 2011, Rodrigues and Childers 2013). New mite samples were collected from symptomatic liriopogons and *A. elatior* in Leon County and sent to USDA-ARS's Electron and Confocal Microscopy Unit for analysis. Three mite species were recovered and examined under cryo-scanning electron microscopy (Cryo-SEM): *B. californicus* s.l. (Fig. 3), *B. obovatus* Donnadieu and *B. confusus* Baker (Fig. 4).

The first report of OFV in the US 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 description of the spoke-wheel configurations of the viral particles (Ko et al. 1985), a sign typically associated with OFV infection (Chang et al. 1976). Unfortunately, this article made no mention of mites or further investigations of the virus. The first report of OFV in the continental US was Bratsch et al. (2015), who confirmed the presence of OFV in *Phalaenopsis* hybrids using Transmission Electron Microscopy of ultrathin sections of plant tissue as well as molecular sequence analysis. They also discuss the association of OFV with *Brevipalpus* mites, but the authors did not make a conclusive species identification beyond suggesting that the mite vector belonged to the *B. californicus* group, referring to Kondo et al. (2003)'s publication (Bratsch et al. 2015).

Later reports of OFV described OFV infecting a previously undescribed Nolinoidaea hosts in Australia (Mei et al. 2016, Dietzgen et al. 2018b), including *Liriope spicata* (Thunb.) Lour, a different species of liriopogon than those identified from the Florida sites. We are not aware of any reports of OFV infecting liriopogons, *A. elatior* nor other Nolinoidaea in the US. Although Zheng et al. (2013) had mentioned an association between *B. californicus* and *A. elatior*, they never reported symptoms of OFV-Orc in this plant. We believe that our findings indicate the first report of OFV-Orc infecting ornamental Nolinoidaea in Florida, and possibly the US. This publication also marks the first reports of *A. elatior* and *Ophiopogon* spp. as natural hosts of OFV-Orc.

OFV consists of two orchid strains (OFV-Orc1 and OFV-Orc2) and two citrus strains (OFV-Cit1 and OFV-Cit2) (Beltran-Beltran et al. 2020, Roy et al. 2020). The OFV strains detected in Florida are identical in gene order, content, and genome sequence to the orchid strains of OFV infecting citrus in Hawaii, Mexico, Colombia, and South Africa

(Beltran-Beltran et al. 2020, Roy et al. 2020). Both OFV-Orc1 and OFV-Orc2 infect citrus (Roy et al. 2020), but none of the citrus strains have been reported from any orchid species. The *Brevipalpus* mites collected from liriopogons and *A. elatior* in Leon County were abundant on OFV-infected plants very near to citrus trees, some plants even surrounding the trunk. *B. californicus* s. l. has been reported as a pest of citrus (Childers et al. 2003) and are often collected from citrus rinds (Baker 1949, Baker and Tuttle 1987). The proximity of these mite vectors to citrus raises the question: why these trees are not currently infected with OFV-Orc? It is important to note the uncertainty surrounding the vector for OFV-Orc. There are three mite species which have been recovered from OFV-Orc infected plants: *B. californicus* s.l. (the most likely culprit), *B. obovatus*, and *B. confusus*. Each species has its own unique biology, and all have been implicated with a variety of different hosts. This suggests that the spread of OFV-Orc would be a function of various combinations of several potential factors, including host preferences, vectorial capacity, viral propagation/circulation in the vector, viral acquisition times, and feeding times required for transmission. Some of these factors have been tested: *Brevipalpus* vectors have demonstrated such virus-vector specificity with *Citrus leprosis virus N*–another dichorhavirus which causes citrus leprosis (Roy et al. 2013)–in studies done by Ferreira et al. (2020) and García-Escamilla et al. (2018). In these studies, *Brevipalpus* mites were not able to transmit more than one virus. This could mean that the *B. californicus* which we find on liriopogons and *A. elatior* are not actually the same species as those found on citrus, or at least are not able to transmit OFV to citrus.

Best practices for integrated pest management have not been created for controlling *Brevipalpus* mites on these ornamentals, but methods designed to control *Brevipalpus* in other systems may be applicable. The most common method used to control *Bervipalpus* are synthetic acaricides (Andrade et al. 2010, 2019). Unfortunately, some acaricides and their residues can harm beneficial predatory mites as well (Fernández et al. 2017), even at low doses (Havasi et al. 2021), and mixing different chemistries can be detrimental for mite control (Vechia et al. 2018). In addition, pesticide resistance has been reported in various *Brevipalpus* populations (Alves et al. 2000, Omoto et al. 2000, Campos and Omoto 2002, Rocha et al. 2021), due to exposure to pesticides used to control other arthropod pests (Vechia et al. 2021). In addition, predatory mites (Chen et al. 2006, Argolo et al. 2020), entomopathogenic fungi (Magalhães et al. 2005, Rossi-Zalaf et al. 2008, Peña et al. 2015, Revynthi et al. 2019) have shown promise for controlling other *Brevipalpus* mites. Moreover, it is often possible to integrate different control techniques for improved management, such as combining predatory mites with compatible acaricides and entomopathogenic fungi (Reddy 2001, Midthassel et al. 2016, Andrade et al. 2019).

In conclusion, 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 cultivated *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 almost eradicated by the mid-1960s (Knorr 1968, Knorr et al.

1968, Childers et al. 2003). An examination of herbarium specimens of Florida citrus found that this historical virus, Citrus leprosis dichorhavirus-N0, is distantly related to the modern strains of OFV (Kitajima et al. 2011, Hartung et al. 2015, Roy et al. 2020). 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 US multi-billion-dollar citrus industry already impacted by the Huanglongbing disease. *B. californicus*, as well as *B. yothersi*, are both known vectors of dichorhaviruses (OFV) (Kondo et al. 2003, García-Escamilla et al. 2018, 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), and are difficult to identify by non-experts, or without advanced methodologies. DNA barcoding (Armstrong and Ball 2005) or a similarly simple and accurate method for identification of these mite complexes is vital to determine which of these species are responsible for transmission of OFV-Orc, and therefore which mite populations need to be monitored or controlled. By doing so, we can determine the risk OFV-Orc represents for the native plants, agriculture and the ornamental/landscaping industries of Florida and the surrounding regions.

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Table 1: List of Asparagaceae (Nolinoidaea) species with verified cases of *Orchid fleck dichorhavirus*, collected from the landscape of northern Florida

Scientific Name	Common Names	County	Strains
Liriope muscari cv. 'Gigantea'* (Decaisne) Bailey	Lilyturf, Orchardgrass, Monkeygrass	Alachua & Leon	OFV-Orc1 & OFV-Orc2

*Ophiopogon intermedius*** Don	Aztec Grass, 'Argenteomarginatus'	Leon	OFV-Orc1 & OFV-Orc2
Aspidistra elatior Blume	Cast Iron Plant, Bar-room Plant	Leon	OFV-Orc1 & OFV-Orc2

Table 1: * *Liriope muscari* cv. 'Gigantea' has been traditionally classified as *L. gigantea* Hume 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*: (a) ringspot symptoms on *Liriope muscari* cv. 'Gigantea' (b-c) Details of ringspot symptoms on *L. muscari* cv. 'Gigantea' (d) rust colored spots on *Ophiopogon intermedius*

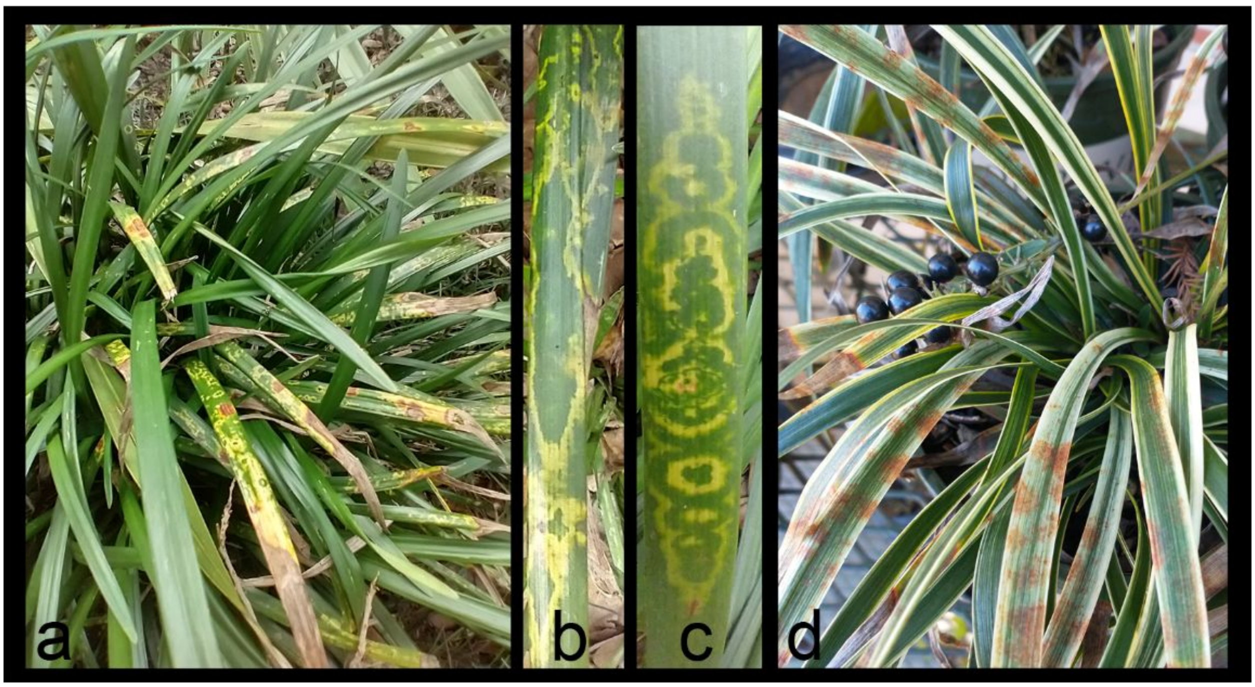
Fig. 2: Symptoms expressed by *Aspidistra elatior* infected with *Orchid fleck dichorhavirus*: (a) Detail of leaf chlorosis (b) Chlorosis appears similar to sun damage (c-d) Chlorotic ringspot may indicate early symptoms of OFV

Fig. 3: Cryo-SEM images of *Brevipalpus californicus sensu lato* displaying various characters used for identification (Baker and Tuttle 1987, Beard et al. 2015) (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. phoenicis*, dorsal (b) *B. yothersi*, lateral (c) *B. obovatus*, dorsal.

Figures




a
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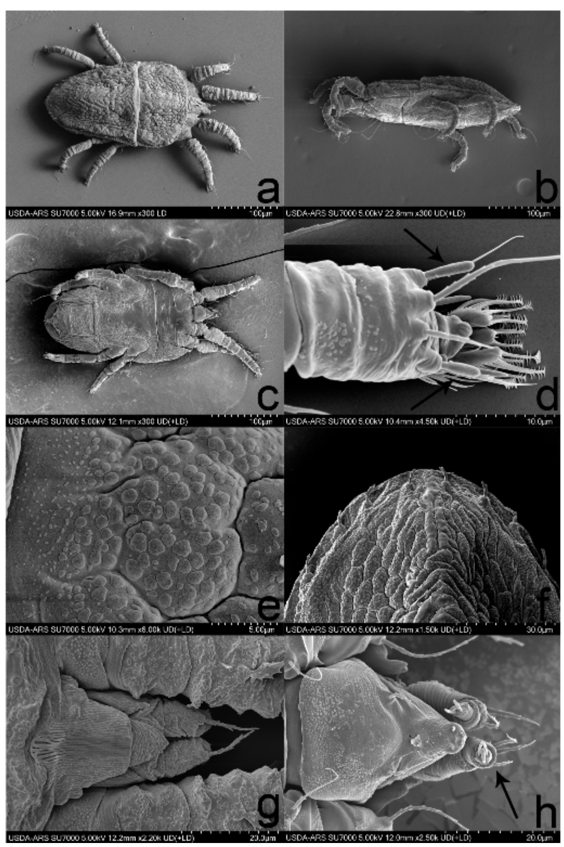
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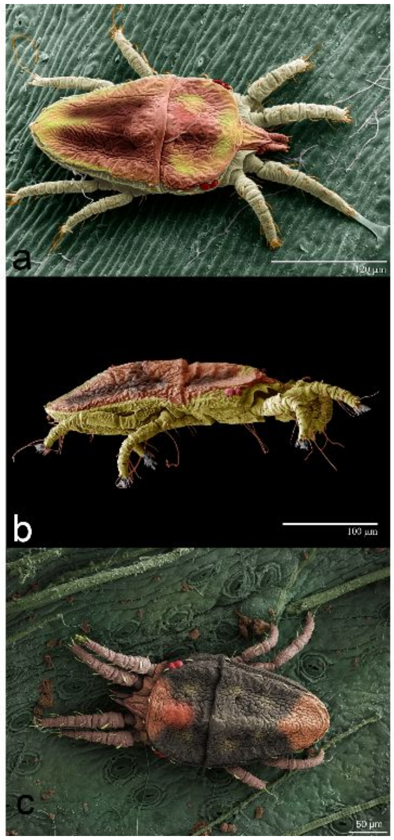

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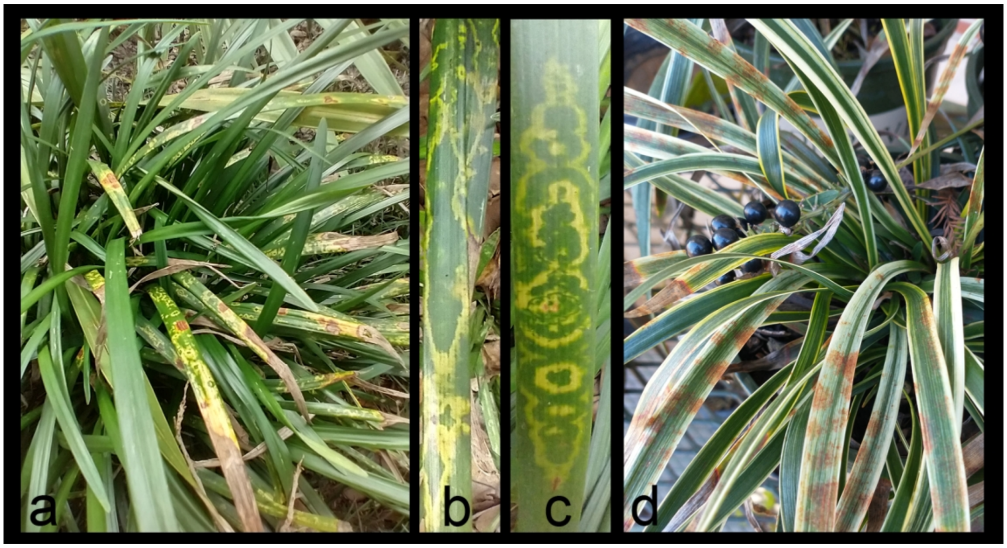

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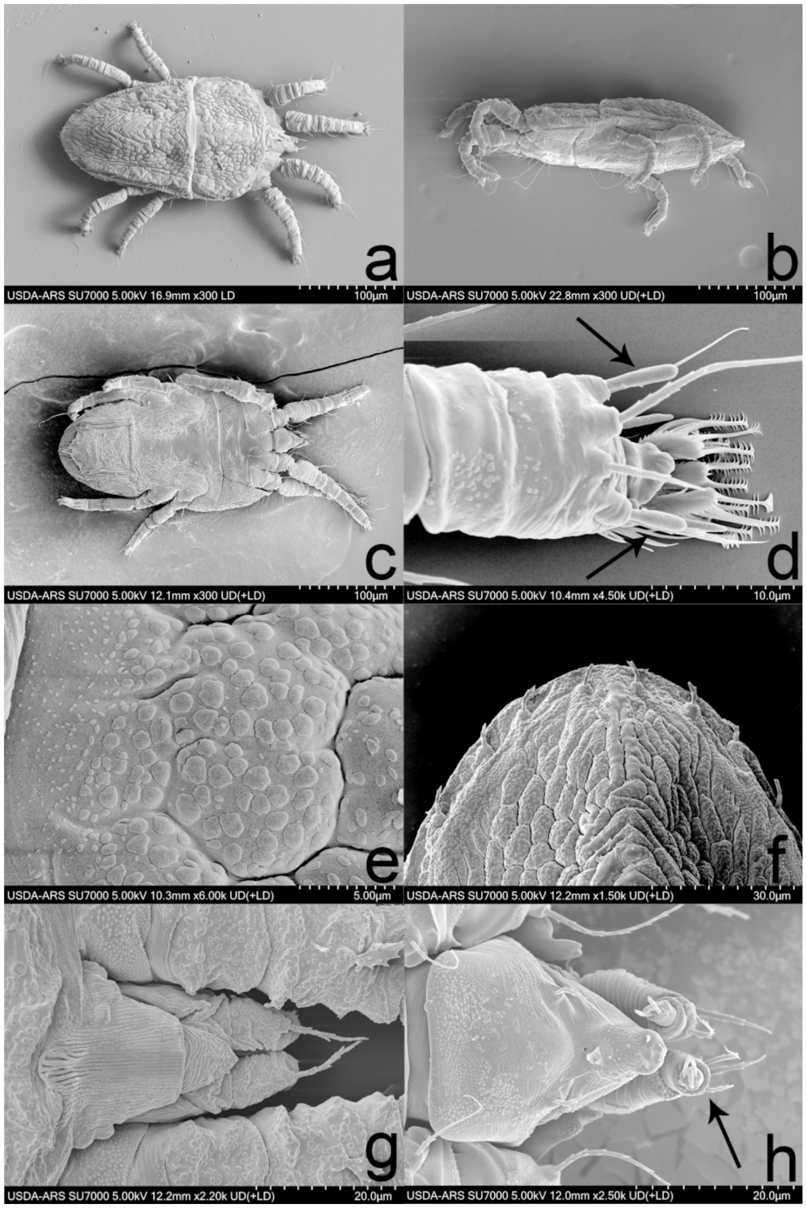

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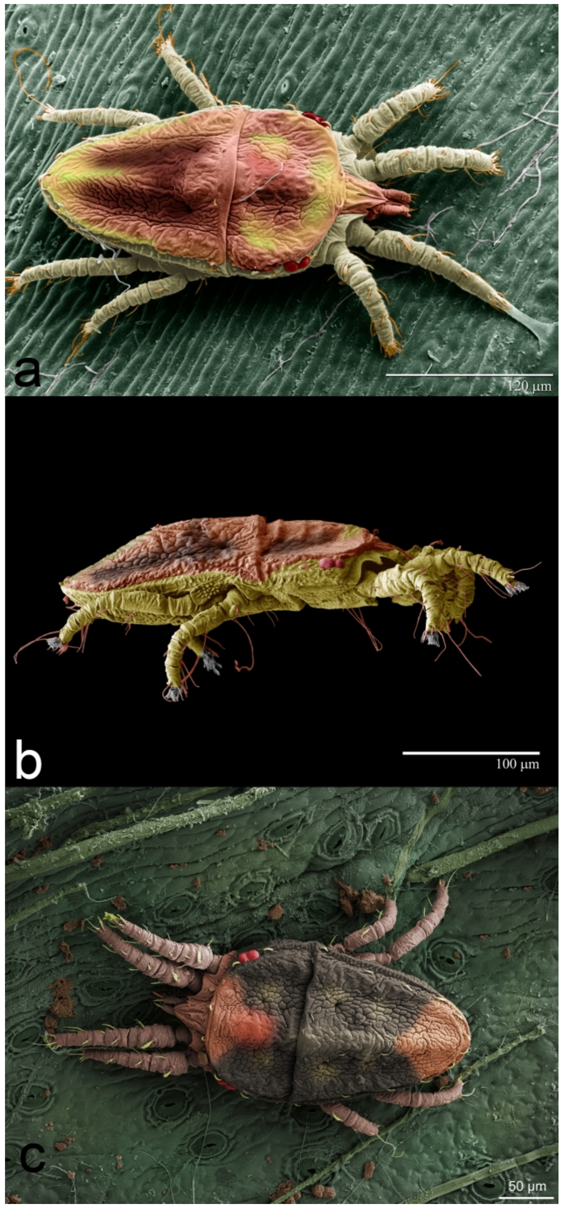

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