

Survey of tenuipalpid mites (Acariformes: Tenuipalpidae) in orchid species with the presence of *Cilevirus* in Colombia

Estudio de ácaros tenuipalpidos (Acariformes: Tenuipalpidae) en especies de orquídeas con presencia de *Cilevirus* en Colombia

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Abstract: To understand and manage viruses causing citrus leprosis it is necessary to identify their alternate host plants and know their distributions. Currently, the citrus leprosis disease is associated with several viruses of the genera *Cilevirus* and *Dichoravirus*. In Colombia, symptoms of citrus leprosis have been related to citrus leprosis virus C (CiLV-C - *Cilevirus leprosis*) and citrus leprosis virus C2 (CiLV-C2 - *Cilevirus colombiense*). To detect leprosis-associated viruses in plants of the family Orchidaceae and identify the possible associated vectors, inspections and samplings were carried out on garden plants and orchid crops, and molecular detection of viruses and the taxonomic identification of mites were accomplished. As a result, *Brevipalpus californicus* s. l., *B. phoenicis* s.s., and *B. essigi* (Prostigmata: Tenuipalpidae) are recorded for the first time on orchid plants from Colombia. A taxonomic key is provided to identify mites of the family Tenuipalpidae on orchids worldwide. The implications of these mites for the phytosanitary protection in citrus crops of Colombia are discussed.

Keywords: Biodiversity, *Brevipalpus* spp., CiLV-C, CiLV-C2, CiLV-C2H, Orchidaceae, vectors.

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Resumen: Para comprender y manejar los virus que causan leprosis en cítricos, es necesario identificar sus plantas hospedantes alternas y conocer su distribución. Actualmente, la enfermedad de leprosis de los cítricos es asociada con varios virus de los géneros *Cilevirus* y *Dichoravirus*. En Colombia los síntomas de la leprosis en cítricos han sido asociados al virus de la leprosis C (CiLV-C, *Cilevirus leprosis*) y virus de la leprosis C2 (CiLV-C2, *Cilevirus colombiense*). Para diagnosticar los virus asociados con la leprosis en plantas de la familia Orchidaceae e identificar los posibles vectores asociados, se realizaron inspecciones y muestreos en plantas de jardín y cultivos de orquídeas en busca de material vegetal con lesiones típicas, por ejemplo, manchas necróticas. Los virus fueron detectados por técnicas moleculares y los ácaros rojos planos fueron identificados taxonómicamente. Como resultado, *Brevipalpus californicus* s.l., *B. phoenicis* s.s., y *B. essigi* (Prostigmata: Tenuipalpidae) se registran por primera vez en plantas de orquídeas de Colombia. Se proporciona una clave taxonómica para identificar ácaros de la familia Tenuipalpidae en orquídeas en el mundo. Se discuten las implicaciones de estos ácaros para la protección fitosanitaria de cultivos de cítricos en Colombia.

Palabras clave: Biodiversidad, *Brevipalpus* spp., CiLV-C, CiLV-C2, CiLV-C2H, Orchidaceae, vectores.

Introduction

Colombia has the largest number of orchids known, with 4,270 species, and, 1,572 endemics of about 30,000 estimated in the world (Ministerio del Medio Ambiente [Minambiente], 2019). Orchids are highly attractive as ornamental plants, and the global trade associated with them is estimated at around 6 billion dollars (Anderson, 2018). In Colombia, the leading orchid growers have traditionally been carried out by associations and collectors who grow them mainly as a pastime (Guzman-Pinilla, 2016). However, over the last two decades, the production and commercialization process has been optimized. The national and international orchid trade in Colom-

bia, although small, has increased considerably during this period (Guzman-Pinilla, 2016; Jiménez, 2018)

Despite the potential value of some Colombian species of orchids as an export crop, the study of phytosanitary protocols has not developed along with production. Two red flat mites in particular, *B. californicus s.l.* and *Tenuipalpus pacificus* Baker, have been considered cosmopolitan pests on orchids (Labanowski & Soika, 2011; Ochoa *et al.*, 1994). Of the twelve species of Tenuipalpidae mites known on orchids in the neotropics (Castro *et al.*, 2022), only two have been reported from Colombia (Mesa & Valencia, 2013). There is a notable research gap regarding this group of important plant-feeding mites.

Red flat mites of the genus *Brevipalpus* (Acariformes: Tenuipalpidae) can cause damage to orchids by feeding (Childers *et al.*, 2003) and are also associated with virus transmission (de Lillo *et al.*, 2021; Rodrigues *et al.*, 2008). Among the viruses that affect orchids that are transmitted by mites of the genus *Brevipalpus*, members of the genus *Cilevirus* have been reported (Freitas-Astua *et al.* 1999; Kitajima *et al.*, 2010). The most recognized of these viruses is OFV (Orchid Fleck Virus), of which there are two types: nuclear and cytoplasmic, as well as CiLV, which causes the disease called Citrus Leprosis (Kubo *et al.*, 2009). Viruses causing citrus leprosis belong to several species distributed in two families of viruses, the genus *Cilevirus*, family *Kitaviridae* and the genus *Dichorhavirus*, family *Rhabdoviridae* where it is hypothesized that a convergent evolution with the same group of mite vectors resulted in similar symptomatology on plants (Freitas-Astua *et al.*, 2018). Those viruses could be transmitted to some families of wild plants, ornamentals, or crops (Dietzgen *et al.*, 2018; Garita *et al.*, 2014; Kitajima *et al.*, 2010; Ramos-González *et al.*, 2023; Roy *et al.*, 2015). In Colombia, citrus leprosis virus C (CiLV-C) was detected for the first time in 2006 (León *et al.*, 2006), and a *Dichorhavirus* affecting citrus, orchid fleck virus-citrus (OFV-C) in 2014 (Roy *et al.*, 2014). In Colombia, citrus leprosis is known to be transmitted primarily by *B. yothersi* (Beard *et al.*, 2015; León, 2012; Roy *et al.*, 2015). Citrus leprosis virus C2 (CiLV-C2, *Cilevirus colombiense*) was reported in Colombia for the first time in 2015 (Roy *et al.*, 2015) whereas CiLV-C2H strain from *Hibiscus* in 2018 (Roy *et al.*, 2018).

Recently, in South Africa the presence of orchid fleck virus in fruits, leaves and branches of Valencia and Navel orange trees *Citrus sinensis* was reported; these infected plants were linked to the presence of orchids belonging to the genus *Brassia* on which infestations with *B. californicus s.l.* were also observed on both hosts, suggesting that the orchid was the possible link with the infection of citrus plants (Cook *et al.*, 2019).

Olmedo-Velarde *et al.* (2021) reported the presence of OFV in rough lemon *Citrus jambhiri* and mandarin orange (*Citrus reticulata*) in the United States, being the first appearance of a citrus leprosis-related virus since it disappeared from Florida in the 1960s. Padmanabhan *et al.* (2022), using High Throughput sequencing (HTS) protocols for the detection of *Cilevirus*, found in Colombia positive infections for CiLV-C2, in *Citrus sinensis*, *Swinglea glutinosa*, and *Hibiscus rosa-sinensis* associated with *Brevipalpus* mites. Also, the presence of CiLV-C2 in a sample of non-citrus plants (León *et al.*, 2023), confirmed the presence of this virus in 52 species from 22 plant families, including Orchidaceae. These recent

reports highlight the increasingly complicated relationship between mites, viruses and host plants which represent a high risk to the global citrus industry with many questions still unanswered. The objective of this work was to identify mites of the Tenuipalpidae family on orchids sampled for molecular analysis of viruses of the species *Cilevirus colombiense* in Colombia.

Materials and Methods

Plant and Mite Sampling. In 2020 and 2021 two orchid crops were visited. These spaces are not used for commercial purposes through the production of ornamental plants or cut flowers but are relatively small spaces about 200 square meters, which are used for the conservation and research of regional orchid biodiversity, that is, with academic and tourist purposes by members of the Santander Orchidology Society. All plants in these crops were inspected. Complementary collections were carried out in gardens of the urban and rural areas of the municipalities of Charalá, Girón, and Bucaramanga in the department of Santander and the city of Sandona in the department of Nariño (Table 1). Collections were not planned according to seasonality. Plants with virus symptoms transmitted by red flat mites of the genus *Brevipalpus*, which are characterized by chlorotic or necrotic lesions or ring spots on leaves and stems, were searched (Kubo *et al.*, 2009). Leaves with symptoms of damage by mites were also collected in plastic containers with 70 % ethanol; whole leaves were collected and examined. These samples were taken to the laboratory of La Suiza Research Center of the Colombian Agricultural Research Corporation Agrosavia.

Tenuipalpid mites were carefully collected under a stereo microscope and mounted on Hoyer's medium for identification. These mites were not analyzed with molecular techniques to detect leprosis viruses, due to the low number of specimens collected in each sample. The plant material was selected, carefully packaged, and coded to be sent to the Corporación Colombiana de Investigación Agropecuaria – Agrosavia, La Libertad research center, where molecular diagnoses for viruses of the species *Cilevirus colombiense* were performed. Samples of vegetal material collected in Colombia were also sent to the Maryland National Plant Germplasm Inspection Station USDA, APHIS, PPQ. The methodology and results are detailed by León *et al.* (2023).

Taxonomic Identification of Tenuipalpid Mites. The interactive key to red flat mites of the world was consulted (Beard *et al.*, 2012); also, the key to the *B. phoenicis* species complex (Beard *et al.*, 2015) and other taxonomic literature (Baker, 1949; Baker & Tuttle, 1987; Castro *et al.*, 2016; Mairena & Ochoa, 1989). The dichotomous taxonomic key for the identification of tenuipalpid mites in orchids was elaborated and modified from Baker (1949), Baker and Tuttle (1987), Castro *et al.* (2016) and, Mairena and Ochoa (1989). All the arthropod and plant materials were collected under the collection framework permit 1466 of 2014 granted by the ANLA to the Colombian Agricultural Research Corporation. This is the license required by Colombian legislation for the collection of animal and plant materials. The mites were deposited in the “Luis María Murillo” National Taxonomic Collection of Insects (CTNI).

Table 1. Sampling locations for tenuipalpid mites on orchids

Municipality / Department	County/Locality	Latitude	Longitude	Meters a.s.l
Girón / Santander	Urban area	06°59'33"N	73°10'08"W	803
Bucaramanga/ Santander	Urban area	07°07'45,2"N	73°07'01"W	1101
Bucaramanga/ Santander	La Maraña	07°08'11"N	73°05'50"W	1341
Charalá/ Santander	Violín	06°06'26"N	73°11'52"W	1746
Sandona/ Nariño	Urban area	01°16'37"N	77°28'21"W	1802

Results and Discussion

Taxonomic Identification of Tenuipalpid Mites. Five species of tenuipalpid mites were found (Table 2), in addition to *Brevipalpus obovatus* previously reported by Mesa and Valencia (2013) in Colombia. This is the first report of *Brevipalpus californicus s.l* and *B. phoenicis s.s.* for Colombia. Mesa and Valencia (2013) reported *B. phoenicis* on 44 plants, including two orchids, from 15 locations in the department of Valle del Cauca, Colombia. However, this material should be re-examined based on the revision by Beard *et al.* (2015). *Brevipalpus essigi* represents the first record for South America since this species has only been reported for Greece, India, New Zealand, Mexico, and North America (Baker, 1949; Baker & Tuttle, 1987; Castro *et al.*, 2022; Nagrare & Barman, 2009). The red flat mite, *B. essigi*, is also polyphagous like other species reported in this document (Baker & Tuttle, 1987; Hatzinikolis, 1986). In Colombia, it was found on hybrid orchids of *Miltoniopsis* sp., and although *B. essigi* has previously been reported on Orchidaceae, it has only been mentioned on *Dendrobium nobile* plants (Nagrare &

Barman, 2009). This mite species has not been reported to cause economically significant damage to crops of agricultural importance or as a vector of the leprosis virus (Childers & Rodrigues, 2011). Therefore, it may have been undetected for a long time. However, a recent introduction through the importation of plant material from one of its hosts, such as chrysanthemums and orchids, cannot be ruled out.

Material Examined

Tenuipalpus pacificus Baker. 1♀. COLOMBIA. Santander, Bucaramanga, vereda Malaña, 07°08'11"N, 73°05'50"W, 1341 m a.s.l., *ex* orchid *Phalaenopsis* sp., 18-nov-2020, Montes, J. [CTNI].

Brevipalpus californicus s.l. Baker. 1♀. COLOMBIA. Santander, Bucaramanga, vereda Malaña, 07°08'11"N, 73°05'50"W, 1341 m a.s.l., *ex* orchid *Maxillaria* sp., 18-nov-2020, Montes, J. [CTNI]. 1♀. COLOMBIA. Santander, Charalá, Virolín, 06°06'26"N, 73°11'52"W, 1746 m a.s.l., *ex* orchid *Ada* sp., 3-Mar-2021, Montes, J. [CTNI]. 1♀. COLOMBIA. Santander, Charalá, Virolín, 06°06'26"N, 73°11'52"W, 1746

Table 2. Tenuipalpid mites and virus detection in orchid plant material.

Locality	Orchid Specie	<i>Cilevirus colombiaense</i> analysis *	Tenuipalpid Mite	Symptoms
Girón, urban area	<i>Arundina graminifolia</i>	Positive	<i>Brevipalpus yothersi</i>	Fig. 2A
Bucaramanga, urban area	<i>Spathoglottis plicata</i>	Positive	<i>Brevipalpus yothersi</i>	Fig. 2B
Bucaramanga, Malaña	<i>Stanhopea</i> sp.	Negative	No mites	
	<i>Maxillaria</i> sp.	Positive	<i>Brevipalpus californicus s.l.</i>	Fig. 2C
	<i>Phalaenopsis</i> sp.	Positive	<i>Tenuipalpus pacificus</i>	Fig. 2D
Charalá, Virolín	<i>Eriopsis biloba</i>	Positive	No mites	
	<i>Maxillaria</i> sp.	Positive	No mites	Fig. 2E
	<i>M. desvauxiana</i>	Positive	No mites	Fig. 2F
	<i>Ada</i> sp.	Positive	<i>Brevipalpus californicus s.l.</i>	Fig. 2G, 2H
	<i>Sobralia</i> sp.	Positive	No mites	Fig. 2I
	<i>Phragmipedium</i> sp.	No analysis	<i>Brevipalpus phoenicis s.s.</i>	No analysis
	<i>Elleanthus</i> sp.	No analysis	<i>Brevipalpus phoenicis s.s.</i>	No analysis
	<i>Oncidium</i> sp.	No analysis	<i>Brevipalpus californicus s.l.</i>	No analysis
	<i>Oncidium isthmi</i>	No analysis	<i>Brevipalpus californicus s.l.</i>	No analysis
Sandoná, urban area	<i>Miltoniopsis</i> , hybrid	No analysis	<i>Brevipalpus essigi</i>	No analysis

*The results of molecular analyzes of plant material are taken from León *et al.* (2023)

m a.s.l., *ex* orchid *Oncidium isthmi*, 3-Mar-2021, Montes, J. [CTNI].

***Brevipalpus yothersi* Baker.** 1♀. COLOMBIA. Santander, Bucaramanga, 07°07'45"N, 73°07'01"W, 1011 m a.s.l., *ex* orchid *Spathoglottis plicata*, 4-Nov-2020, Montes, J. [CTNI]. 1♀. COLOMBIA. Santander, Chocoita, 06°59'33"N, 73°10'08"W, 803 m a.s.l., *ex* orchid *Arundina graminifolia*, 31-Oct-2020, Montes, J. [CTNI].

***Brevipalpus phoenicis sensu stricto* (Geijskes).** 1♀. COLOMBIA. Santander, Charalá, Virolin, 06°06'26"N, 73°11'52"W, 1746 m, *ex* orchid *Phragmipedium* sp., 3-Mar-21, Montes, J. [CTNI]. 1♀. COLOMBIA. Santander, Charalá, Virolin, 06°06'26"N, 73°11'52"W, 1746 m a.s.l., *ex* orchid *Elleanthus* sp., 3-Mar-21, Montes, J. [CTNI].

***Brevipalpus essigi* Baker** 1♀. COLOMBIA. Nariño, Sandona, 01°16'37"N, 77°28'25"W, 1848 m a.s.l., *ex* orchid *Miltoniopsis* sp., 15-Ene-2021, Sierra, J. [CTNI].

Key for the identification of Tenuipalpidae mite species reported on orchids in the world based on adult females.

- 1. With one to three segments on the palps2
- 1'. With four segments on the palps6
- 2. H2 elongated, with two pairs of anal setae, pregenital setae, and dorsal setae variable3
- 2'. H2 not elongated, with a pair of anal setae, pregenital setae separated by less than the width of the genital plate, dorsal seta on femur II shorter or not much longer than this segment.....4

***Dolichotetranychus vandergooti* (Oudemans, 1927)**

- 3. Dorsum with a pair of lateral projections associated with seta C3.....4
- 3'. Dorsum without lateral projections associated with seta C3.....5

4. Central prodorsum with a sculpture of rhomboid to hexagonal cells like a honeycomb.....

***Tenuipalpus rhyssus* Baker & Pritchard, 1953**

4'. Central prodorsum with rough and striated sculpture.....

***Tenuipalpus bakeri* McGregor, 1949**

5. With two pairs of IC3 and one pair of IC4. Ventral lateral cuticle anterior to coxa III and lateral to setae 3a1 and 3a2 with characteristic pebble-like pattern.....

***Tenuipalpus pacificus* Baker, 1945**

5'. With one pair of IC3 and two pairs of IC4. Ventral lateral cuticle without characteristic pebble-like pattern.....

***Tenuipalpus orchidifilo* Moraes & Freire, 2001**

- 6. Setae f2 present (Figure 1A).....7
- 6'. Setae f2 absent10
- 7. Two solenidia in tarsus II (Figure 1B). Spermatheca circular, with an internal bubble (Figure 1A and 1C).....

***Brevipalpus californicus s. l.* Baker**

7'. One solenidion in tarsus II. Spermatheca with variable shape and never with an internal bubble8

8. The two pairs of ventral hysterosoma setae of the same size.....

***Brevipalpus oncidii* Baker, 1949**

8'. Pair of anterior ventral hysterosoma setae notably shorter than posterior pair9

9. Reticulate pattern throughout the ventral plate (Figure 1D). Propodosoma and hysterosoma with reticulations evenly distributed over the entire area (Figure 1E) Propodosoma with dorsal setae strongly serrate.....

***Brevipalpus essigi* Baker, 1949**

9'. Reticulate pattern of the ventral plate is present only laterally. Propodosoma and hysterosoma with reticulations are not present over the entire area. Propodosoma with dorsal setae slightly serrate.....

***Brevipalpus russulus* Boisduval, 1867**

10. One solenidion on tarsus II.....

***Brevipalpus obovatus* Donnadieu, 1875**

10'. Two solenidia on tarsus II.....11

11. Dorsal seta on femurogenu setiform, cuticle on dorsal opisthosoma between setae e1-e1 and h1-h1 with V-shaped folds, oval spermatheca vesicle with a marked distal duct. Ventral plate cuticle uniformly verrucose, with small, rounded "warts" that are sometimes weakly impressed centrally.....

***Brevipalpus yothersi* Baker, 1949**

11'. Dorsal seta on femurogenu broad and flat (Figure 1F), cuticle on dorsal opisthosoma between setae e1-e1 and h1-h1 with few transverse folds (Figure 1G), spermatheca vesicle not visible, duct sometimes visible. Ventral plate cuticle usually with some "warts" laterally that are fused medially to form transverse bands.....

***Brevipalpus phoenicis sensu stricto* Geijskes, 1939.**

Lesions typically caused by leprosis virus (Figures 2a and 2b), coincide with the presence of *B. yothersi*. The red flat mite *B. yothersi* is polyphagous and confirmed as the main vector of the leprosis virus in Colombia (León *et al.*, 2006; Roy *et al.*, 2015). *Brevipalpus yothersi* has been reported as a frequent carrier of CiLV-C and OFV in citrus crops (Beltran-Beltran *et al.*, 2020; Olmedo-Velarde *et al.*, 2021). Considering that these viruses can produce symptoms in orchids, it could be possible that *B. yothersi* is the vector of *Cilevirus colombianaense* virus in orchids from Colombia. However, it is necessary to include the molecular analysis and experimental transmission assays of CiLV-CH2 in *B. yothersi* affecting orchids in future research to confirm the vector-virus-host relationship in Orchidaceae.

Brevipalpus californicus s.l., which can be the carrier of CiLV-C and OFV (Beltran -Beltran *et al.*, 2020; Roy *et al.*, 2015), is now accepted as a complex of seven species (Tassi *et al.*, 2022). (Beard *et al.*, 2012). Given this development, it is necessary to verify the transmission capacity of the leprosis viruses by each member of this mite complex.

It is noteworthy that although citrus leprosis-causing viruses are present in orchids, symptoms have not been reported on citrus plants by farmers or extension workers even though periodic monitoring of the phytosanitary status of citrus crops is carried out by trained personnel from the Colombian Agricultural Institute (ICA), looking for symptoms of leprosis, HLB, and other pests and quarantine diseases.

Citrus leprosis-causing viruses have had little impact in the Santander citrus growing region, possibly due to several factors influencing their survival and dispersal. Firstly, there are differences between citrus species and cultivars that vary in susceptibility to leprosis (Freitas-Astua *et al.*, 2008). The predominant citrus crops in the Santander region are Tahiti lime and tangerine, which are less susceptible than oranges (Childers & Rodrigues, 2011). For example, CiLV-C is more

important and causes more damage in the state of Sao Paulo, which produces 80 % of the oranges in Brazil (Bastianel *et al.*, 2010). Secondly, regarding orange production, the most susceptible citrus species is generally found in agroforestry systems with coffee and cacao but not in monoculture, making virus dispersion more difficult. Thirdly, the citrus industry in Santander, Colombia, is mainly based on small producers with small areas that are less than four hectares; these relatively small plots are mixed in a matrix with other crops, fallow land, and natural areas. Bastianel *et al.* (2010) mentioned that even though citrus leprosis is present in some regions of Bra-

zil, it does not cause significant damage because it is not farmed in large monocultures and its environmentally friendly management favors a reasonable natural mite control. Another factor could be that a large proportion of the crops, especially tangerine, are grown in areas with good humidity and rainfall above 2000 mm per year which has an inversely proportional relationship with mite proliferation (Solano *et al.*, 2008).

Other factors related to the biology and ecology of the mite to prevent the proliferation of citrus leprosis are: (i) the acquisition of the virus occurs in parts of the plant with lesions because the virus is not systemic (Locali *et al.*, 2004);

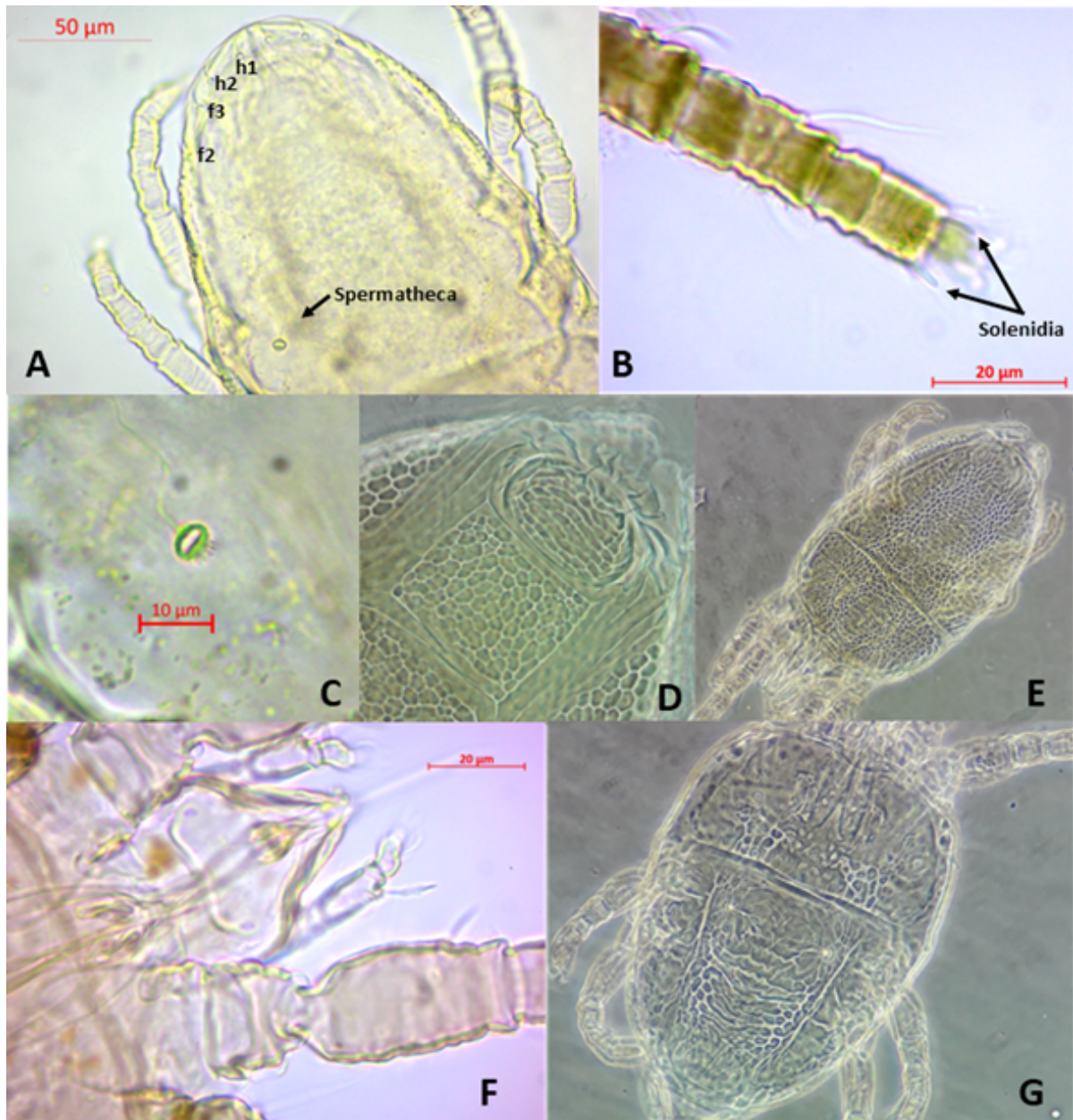


Figure 1. A-C. *Brevipalpus californicus* s.l. A. Opisthosoma. B. Tarso II. C. Spermatheca. D-E. *Brevipalpus essigi* s.s. D. Ventral and genital plate. E. Dorsum. F-G. *Brevipalpus phoenicis* s.s. F. Palps. G. female dorsum. Photos: A-C, F by J. Montes; D, E, G by A. Sierra.

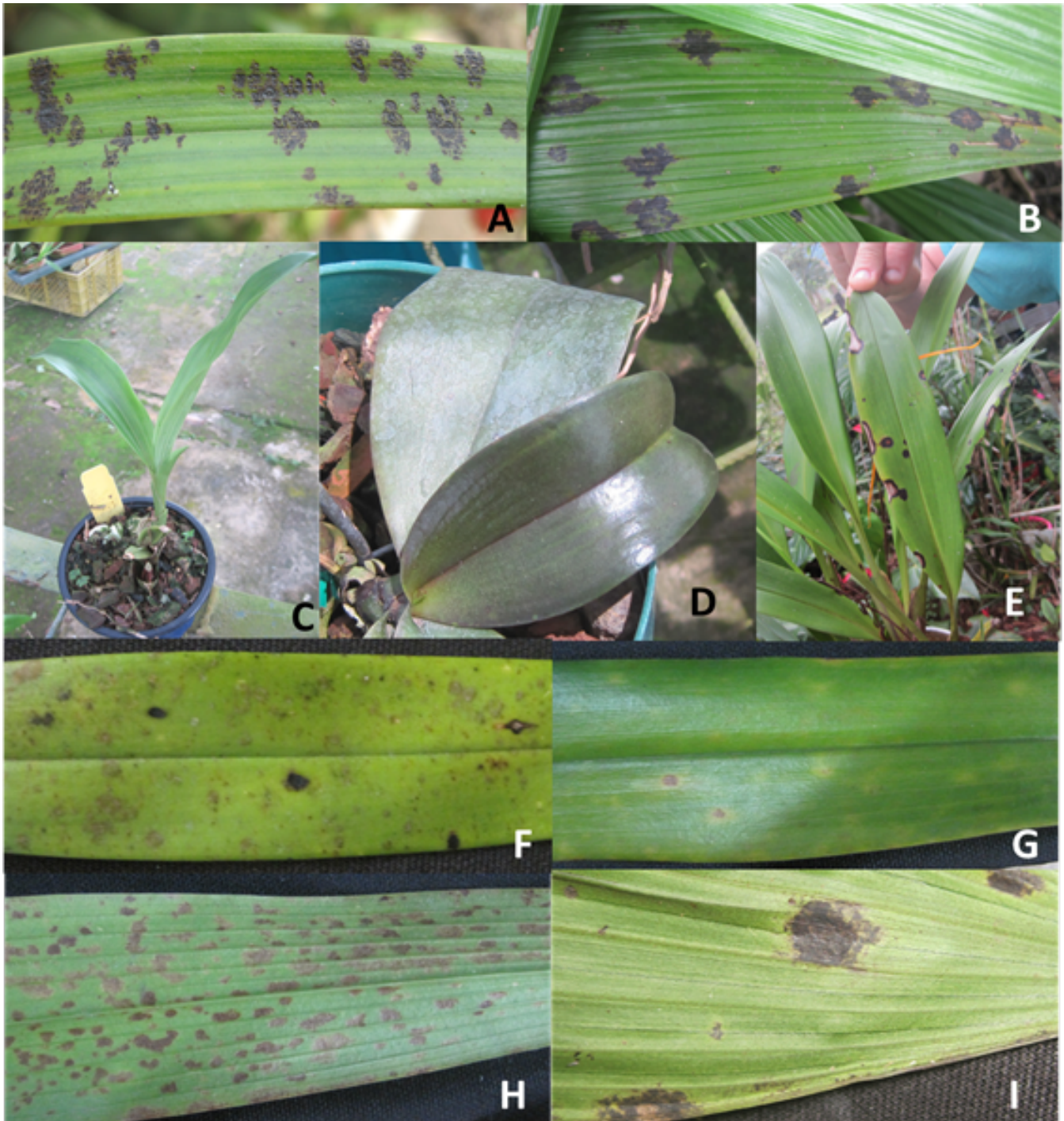


Figure 2. Symptoms and damage of tenuipalpid mites on orchids infected by *Cilevirus colombiaense* virus. **A.** *Arundina graminifolia*. **B.** *Spathoglottis plicata*. **C.** *Maxillaria* sp. **D.** *Phalaenopsis* sp. **E.** *Maxillaria* sp. **F.** *Maxillaria desvauxiana*. **G-H.** *Ada* sp. **G.** Leaf dorsal side. **H.** Leaf ventral side. **I.** *Sobralia* sp. Photos by J. Montes.

(ii) there is no transovarial transmission of the leprosis virus (Boaretto *et al.*, 1993) and (iii) reduction in the reproductive potential of mites fed on leprosis-affected tissue has been recorded (Salinas-Vargas *et al.*, 2019). In Brazil, Bastianel *et al.*

(2010) reported that the disease is found in several citrus-productive regions in low incidence and severity and that farmers only become concerned when the damage becomes visible and appears to spread rapidly.

Conclusions

Three new reports of species of tenuipalpid red flat mites of the genus *Brevipalpus* are recorded for the first time from Colombia. Of the red flat mite species now recorded in Colombia, two species have a high capacity to transmit leprosis virus, namely, *B. yothersi* and *B. californicus s.l.* Orchids are a group of host plants of *Cilevirus colombiaense* virus in Colombia and because these plants are often found near or within citrus groves, they should be monitored for leprosis-causing viruses as well as their mite vectors.

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References

- Anderson, M. (2018). La atracción por las orquídeas salvajes persiste en Colombia. Mongabay. Periodismo ambiental independiente en Latinoamérica. <https://1bestlinks.net/UuclE>
- Baker, E., W., & Tuttle, D., M. (1987). *The False Spider Mites of Mexico (Tenuipalpidae: Acari)*. Beltsville (MD): Department of Agriculture. (Technical Bulletin No. 1706).
- Baker, E. W. (1949). The genus *Brevipalpus* (Acarina: Pseudoleptidae). *The American Midland Naturalist*, 42(2), 350-402. <https://doi.org/10.2307/2422013>
- Bastianel, M., Novelli, V. M., Kitajima, E. W., Kubo, K. S., Bassanezi, R. B., Machado, M. A., & Freitas-Astúa, J. (2010). Citrus leprosis: Centennial of an unusual mite-virus pathosystem. *Plant Disease*, 94(3), 284-292. <https://doi.org/10.1094/PDIS-94-3-0284>
- Beard, J., J., Ochoa, R., Bauchan, G., R., Trice, M., D., Redford, A., J., Walters, T., W., & Mitter, C. (2012, May 15). Flat Mites of the World Edition 2.
- Beard, J. J., Ochoa, R., Braswell, W. E., & Bauchan, G. R. (2015). *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae): A closer look. *Zootaxa*, 3944, 1-67. <https://doi.org/10.11646/zootaxa.3944.1.1>
- Beltran-Beltran, A. K., Santillán-Galicia, M. T., Guzmán-Franco, A. W., Teliz-Ortiz, D., Gutiérrez-Espinoza, M. A., Romero-Rosales, F., & Robles-García, P. L. (2020). Incidence of Citrus leprosis virus C and Orchid fleck dichorhavirus Citrus Strain in mites of the genus *Brevipalpus* in Mexico. *Journal of Economic Entomology*, 113(3), 1576-1581. <https://doi.org/10.1093/jee/toaa007>
- Boaretto, M. A. C., Chiavegato, L. G., & Silva, C. D. (1993). Transmissão da leprose através de fêmeas de *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae) e de seus descendentes, em condições de laboratório. *Científica*, 21(2), 245-253.
- Castro, E. B., Feres, R. J., Ochoa, R., & Bauchan, G. R. (2016). A new species of *Tenuipalpus sensu stricto* (Acari: Tenuipalpidae) from Brazil, with ontogeny and a key to the known species. *Zootaxa*, 4088(3), 355-378. <http://dx.doi.org/10.11646/zootaxa.4088.3.3>
- Castro, E. B., Mesa, N. C., Feres, R. J., Moraes, G. J., de Ochoa, R., Beard, J. J., & Demite, P. R. (2022, June 30). Tenuipalpidae Database. 2016-2022. Sao Pablo (Brazil): UNES Universidad Estadual Paulista “Julio de Mesquita filho”. <http://www.tenuipalpidae.ibilce.unesp.br>
- Childers, C. C., French, J. V., & Rodrigues, J. C. V. (2003). *Brevipalpus californicus*, *B. obovatus*, *B. phoenicis*, and *B. lewisi* (Acari: Tenuipalpidae): a review of their biology, feeding injury and economic importance. *Experimental & Applied Acarology*, 30, 5-28. <https://doi.org/10.1023/B:APPA.0000006543.34042.b4>
- Childers, C. C., & Rodrigues, J. C. (2011). An overview of *Brevipalpus* mites (Acari: Tenuipalpidae) and the plant viruses they transmit. *Zoosymposia*, 6, 180-192. <https://doi.org/10.11646/zoosymposia.6.1.28>
- Cook, G., Kirkman, W., Clase, R., Steyn, C., Basson, E., Fourie, P. H., Moore, S. D., Grout, T. G., Carstens, E., & Hattingh, V. (2019). Orchid fleck virus associated with the first case of citrus leprosis-N in South Africa. *European Journal of Plant Pathology*, 155(4), 1373-1379. <https://doi.org/10.1007/s10658-019-01854-4>
- De Lillo, E., Freitas-Astúa, J., Kitajima, E. W., Ramos-Gonzalez, P. L., Simoni, S., Tassi, A. D., & Valenzano, D. (2021). Phytophagous mites transmitting plant viruses: update and perspectives. *Entomologia Generalis*, 41(5), 439. <https://doi.org/10.1127/entomologia/2021/1283>
- Dietzgen, R. G., Freitas-Astúa, J., Chabi-Jesus, C., Ramos-González, P. L., Goodin, M. M., Kondo, H., Tassi, A. D., & Kitajima, E. W. (2018). Dichorhavirus in their host plants and mite vectors. *Advances in Virus Research*, 102, 119-148. <https://doi.org/10.1016/bs.aivir.2018.06.001>
- Freitas-Astúa, J., Rezende, J. A. M., & Kitajima, E. W. (1999). Incidence of orchid viruses in the state of São Paulo, Brazil. *Fitopatologia Brasileira*, 24(2), 125-130.
- Freitas-Astúa, J., Fadel, A. L., Bastianel, M., Novelli, V. M., Antoniolli-Luizon, R., & Machado, M. A. (2008). Resposta diferencial de espécies e de híbridos de citros à leprose. *Pesquisa Agropecuária Brasileira*, 43(7), 809-814. <https://doi.org/10.1590/S0100-204X2008000700004>
- Freitas-Astúa, J., Ramos-Gonzalez, P. L., Arena, G. D., Tassi, A. D., & Kitajima, E. W. (2018). *Brevipalpus*-transmitted viruses: parallelism beyond a common vector or convergent evolution of distantly related pathogens?. *Current Opinion in Virology*, 33, 66-73. <https://doi.org/10.1016/j.coviro.2018.07.010>
- Garita, L. C., Tassi, A. D., Calegario, R. F., Freitas-Astúa, J., Salarioli, R. B., Romão, G. O., & Kitajima, E. W. (2014). Experimental host range of Citrus leprosis virus C (CiLV-C). *Tropical Plant Pathology*, 39(1), 43-55. <https://doi.org/10.1590/S1982-56762014005000004>
- Guzman-Pinilla, J. (2016; June 15). Colombia hace negocios con 4200 especies de orquídeas. Agronegocios.
- Hatzinikolis, E. N. (1986). The genus *Brevipalpus* in Greece (Acari: Tenuipalpidae). *Entomologia Hellenica*, 4(2), 37-48. <http://dx.doi.org/10.12681/eh.13931>
- Jiménez, H. (2018, June 15). Exportaciones de orquídeas crecieron 14,1 % y su principal destino fue EE.UU. Agronegocios.
- Kitajima, E. W., Rodrigues, J. C. V., & Freitas-Astua, J. (2010). An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. *Scientia Agricola*, 67(3), 348-371. <https://doi.org/10.1590/S0103-90162010000300014>
- Kubo, K. S., Freitas-Astua, J. D., Machado, M. A., & Kitajima, E. W. (2009). Orchid fleck symptoms may be caused naturally by two different viruses transmitted by *Brevipalpus*. *Journal of General Plant Pathology*, 75, 250-255. <https://doi.org/10.1007/s10327-009-0167-z>
- Labanowski, G., & Soika, G. (2011). False spider mites (Acari: Tenuipalpidae) as pests on orchids (hybrids) in Poland. *Biological Letters*, 48(2), 167-175. <https://doi.org/10.2478/v10120-011-0015-7>
- León, M. (2012). Current status of the Citrus leprosis virus (CiLV-C) and its vector *Brevipalpus phoenicis* (Geijskes). *Agronomía Colombiana*, 30(2), 242-250. <http://www.scielo.org.co/pdf/agc/v30n2/v30n2a12.pdf>

- León, M. G., Realpe, C. E., Garzón, P. A., Rodríguez, J. A., Moreno, P. M., Childers, C. C., Achor, D., Freitas-Astua, J., Antonioli-Luiz, R., Salaroli, R. B., Mesa, N. C., & Kitajima, E. W. (2006). Occurrence of Citrus leprosis virus in Llanos Orientales, Colombia. *Plant Disease*, 90(5), 682-682. <https://doi.org/10.1094/PD-90-0682C>
- León M., G. A., Campos P., J. C., Guevara, Y. A., & Roy, A. (2023). Distribución, plantas hospederas y ácaros vectores del virus de la leprosis de los cítricos en Colombia. Corporación Colombiana de Investigación Agropecuaria (Agrosavia). <https://doi.org/10.21930/agrosavia.investigacion.7406429>
- Locali, E. C., Freitas-Astua, J., & Machado, M. A. (2004). Leprose dos citros: biologia e diagnóstico do vírus. *Laranja*, 25(1), 53-68.
- Mairena, H., & Ochoa, R. (1989). Revisión del género *Tenuipalpus* (Acari: Tenuipalpidae) en Costa Rica: *Tenuipalpus bakeri* McGregor nuevo representante. *Manejo Integrado de Plagas y Agroecología*, 11, 75-80. <https://repositorio.catie.ac.cr/handle/11554/6786>
- Mesa, N. C., & Valencia, M. O. (2013). Diagnóstico taxonómico de la familia Tenuipalpidae (Acari: Tetranychoidae) en el Valle del Cauca (Colombia). *Caldasia*, 35(1), 199-207. <https://revistas.unal.edu.co/index.php/cal/article/view/39106/40969>
- [Minambiente] Ministerio del Medio Ambiente. (2019, May 16). Colombia, segundo país con mayor diversidad de plantas del planeta. <https://www.minambiente.gov.co/bosques-biodiversidad-y-servicios-ecosistemicos/colombia-segundo-pais-con-mayor-diversidad-de-plantas-del-planeta/>
- Nagrare, V. S., & Barman, D. (2009). Pests associated with orchid *Dendrobium nobile* under mid-altitude of Sikkim. *Environment and Ecology*, 27(2), 560-562. <https://drive.google.com/file/d/1EUqP8rGKBOeGZYxoTtq4G-ec3t07mcGN/view>
- Ochoa, R., Aguilar, H., & Vargas, C. (1994). Phytophagous mites of America Central: An illustrated guide (No. 6). CATIE.
- Olmedo-Velarde, A., Roy, A., Padmanabhan, C., Nunziata, S., Nakhla, M. K., & Melzer, M. J. (2021). First report of Orchid fleck virus associated with Citrus leprosis symptoms in rough lemon (*Citrus jambhiri*) and mandarin (*C. reticulata*) the United States. *Plant Disease*, 105(8), 2258. <https://doi.org/10.1094/PDIS-12-20-2736-PDN>
- Padmanabhan, C., Nunziata, S., Rivera, Y., Mavrodieva, V. A., Nakhla, M. K., & Roy, A. (2022). High-throughput sequencing application in the detection and discovery of viruses associated with the regulated citrus leprosis disease complex. *Frontiers in Plant Science*, 13, 1-22. <https://doi.org/10.3389/fpls.2022.1058847>
- Ramos-González, P. L., Dias Arena, G., Tassi, A. D., Chabi-Jesus, C., Watanabe Kitajima, E., & Freitas-Astua, J. (2023). Kitaviruses: A window to atypical plant viruses causing nonsystemic diseases. *Annual Review of Phytopathology*, 61, 97-118. <https://doi.org/10.1146/annurev-phyto-021622-121351>
- Rodrigues, J. C. V., Antony, L. M., Salaroli, R. B., & Kitajima, E. W. (2008). *Brevipalpus*-associated viruses in the central Amazon Basin. *Tropical Plant Pathology*, 33(1), 12-19. <https://doi.org/10.1590/S1982-56762008000100003>
- Roy, A., León, M. G., Stone, A. L., Schneider, W. L., Hartung, J. S., & Brlansky, R. H. (2014). First report of citrus leprosis virus nuclear type in sweet orange in Colombia. *Plant Disease*, 98(8), 1162-1162. <https://doi.org/10.1094/PDIS-02-14-0117-PDN>
- Roy, A., Hartung, J. S., Schneider, W. L., Shao, J., León, G., Melzer, M. J., Beard, J. J., Otero-Colina, G., Bauchan, G. R., Ochoa, R., & Brlansky, R. H. (2015). Role bending: Complex relationships between viruses, hosts, and vectors related to citrus leprosis, an emerging disease. *Phytopathology*, 105(7), 1013-1025. <https://doi.org/10.1094/Phyto-12-14-0375-FI>
- Roy, A., Stone, A. L., León-Martínez, G., Hartung, J. S., Wei, G., Mavrodieva, V. A., Nakhla, M. K., Schneider, W. L., & Brlansky, R. H. (2018). First Report of Hibiscus-infecting cilevirus in *Citrus sinensis* in Meta and Casanare, Colombia. *Plant Disease*, 102(8), 1675-1675. <https://doi.org/10.1094/PDIS-01-18-0150-PDN>
- Salinas-Vargas, D., Santillán-Galicia, M. T., Guzmán-Franco, A. W., Mora-Aguilera, G., Ortega-Arenas, L. D., Hernández-López, A., & Sánchez-Soto, S. (2019). Development and reproduction of *Brevipalpus yothersi* on orange fruits infected with Citrus leprosis virus C. *International Journal of Acarology*, 45(4), 209-213. <https://doi.org/10.1080/01647954.2019.1586993>
- Solano, D. A., Alvarez-Herrera, J. G., & Rodríguez, J. A. (2008). Distribución espacial de *Brevipalpus phoenicis*, vector de la leprosis de los cítricos en el cultivo de naranja Valencia (*Citrus sinensis*) en Yopal, Casanare (Colombia). *Agronomía Colombiana*, 26(3), 399-410. <https://revistas.unal.edu.co/index.php/agrocol/article/view/11471>
- Tassi, A. D., Ochoa, R., Ramos-González, P. L., Kitajima, E. W., Freitas-Astua, J., & Carrillo, D. (2022). Revisiting *Brevipalpus californicus*: a species complex under review and its potential role in the transmission of dichorhavirus. *Zoosymposia* 22: 72-72.

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Author contribution

The first author participated in the sampling and taxonomic identification of mites, sampling of plant material, contributed to the analysis of the data and the writing of the final document.

The second author participated in the sampling and taxonomic identification of mites, contributed to the analysis of the data and in the writing of the final document.

The third author stated the objectives of the research, obtained funding, processed the plant material, contributed to the analysis of the data and to the writing of the final document.

The fourth author participated in the taxonomic identification of mites, contributed in the analysis of the data and in the writing of the final document.

Conflict of interest

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