El Colegio de la Frontera Sur

Ácaros *Brevipalpus* spp asociados al cultivo del café (*Coffea arabica* L. y *Coffea canephora* Pierre ex Froehner) en el Soconusco, Chiapas, México

Tesis

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Con orientación en Entomología Tropical

Por

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Las personas abajo firmantes, miembros del jurado examinador de:

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Hacemos constar que hemos revisado y aprobado la tesis titulada

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Para obtener el grado de **Maestro en Ciencias en Recursos Naturales y Desarrollo Rural.**

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DEDICATORIA

A DIOS, porque sus planes son planes de bien y no para mal.
Gracias por ser ese amigo fiel que está siempre en cada momento y etapa de mi vida y por permitir que pueda confiar en ti, Jehová es mi pastor y nada me faltará. Salmos 23:1.

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CONTENIDO

I. RESUMEN

Los ácaros del género *Brevipalpus* de la familia Tenuipalpidae, son de gran importancia económica y cuarentenaria, por el daño que producen y por ser vectores de virus fitopatógenos en cafetales y otros cultivos. En esta investigación, nuestros objetivos fueron identificar las especies del género *Brevipalpus*, verificar el estatus de los virus asociados (CoRSV, CiLV-C, CiLV-C2, CiLV-N y CiLV-N *sensu novo)*, y monitorear su fluctuación poblacional durante un año, en cafetales del Soconusco, Chiapas, México. De un total de 635 ácaros del complejo *Brevipalpus phoenecis* sensu lato recolectados, 509 (80.2%) fueron *Brevipalpus papayensis* y 126 (19.8%) *Brevipalpus yothersi*, basados en sus características morfológicas y moleculares. Sus poblaciones fueron generalmente bajas y sin presencia de virosis (CoRSV, CiLV-C2, CiLV-N y CiLV-N *sensu novo*). La mayor abundancia de ácaros se presentó en agosto y septiembre ($\bar{x}$=0.5, EEM 0.05; $\bar{x}$= 0.4, EEM 0.05) y la menor en febrero y marzo ($\bar{x}$=0.01, EEM 0.005; $\bar{x}$=0.005, EEM 0.003). Se encontró una relación entre la abundancia de las especies descritas y las condiciones de sombra y la altitud en la que se encuentran cultivadas las variedades de café. *Brevipalpus papayensis* fue más abundante en *Coffea arabica* variedad Bourbon en sistema de producción con sombra (80%) a una altitud de 1300 ($\bar{x}$=0.267, EME 0.026). *Brevipalpus yothersi* fue mayor en cafetales de *Coffea canephora,* sin sombra y a baja altitud ($\bar{x}$=0.897, EEM 0.016). Consideramos que, hasta el momento, *B. papayensis* y *B. yothersi* no representan riesgos para la producción de café en las plantaciones estudiadas. Sin embargo, dado que las regiones productoras de café en México son ecológicamente diversas, será importante estudiar el estatus de las poblaciones de *Brevipalpus*.

Palabras claves: Ácaros fitófagos, Tenuipalpidae, *Brevipalpus*, virus.

II. INTRODUCCIÓN

El café es un arbusto originario de África que pertenece al género *Coffea* de la familia Rubiaceae. Este género consta de más de 124 especies, de las cuales sólo dos, *C. arabica* L. (café arábica) y *C. canephora* Pierre ex A. Froehner (café robusta) se cultivan para la producción comercial de café (Vega et al. 2015; Davis et al. 2019). En 2019, se produjeron alrededor de 168,711 millones de sacos (60 kg de capacidad) de café a nivel mundial, de los cuales el arábica representa 96,215 millones de sacos, y robusta 72,496 millones de sacos (57 y 43%, respectivamente) (International Coffee Organization (ICO) Report 2020). En México, el café es uno de los cultivos más importantes desde el punto de vista económico y social, ocupa el onceavo lugar en producción a nivel mundial, siendo el estado de Chiapas quién presenta la mayor superficie cultivada, con 252,744 ha y una producción de 367,789 toneladas durante el año 2019 (SIAP 2017; CENACAFE 2020).

El incremento de plagas y enfermedades en el mundo cafetalero ha repercutido en la disminución de calidad y rendimiento; dentro de estas plagas se incluyen los ácaros (Arachnida: Acari), que son pequeños organismos que pueden encontrarse en casi cualquier hábitat de la naturaleza, en ambientes terrestres y acuáticos, incluyendo aguas termales. Es un grupo diverso y complejo y presenta diferencias muy marcadas en morfología y hábitos (Ochoa et al. 1991). De hecho, ocupan todos los nichos de alimentación, desde depredadores, fitófagos, descomponedores y fungívoros (Krantz 2009), siendo los fitófagos y depredadores los de mayor interés por su importancia como plagas y como enemigos naturales, respectivamente (Moraes y Flechtmann 2008). Van Leeuwen et al. (2015) mencionan que los ácaros fitófagos son plagas importantes en muchos cultivos agrícolas en todo el mundo, lo que significa una amenaza real para la producción de alimentos, si no se mantienen bajo umbrales de daño económico. En particular, muchas especies de ácaros pertenecientes a las familias Tetranychidae, Tenuipalpidae, Tarsonemidae y Eriophyidae son de importancia económica real o potencial, considerando el daño que pueden causar, ya sea por alimentación directa o por ser transmisores de patógenos. Además, la importancia de los ácaros como especies invasoras ha crecido en los últimos años debido a que el número de especies que se mueven es cada vez mayor, por lo que sus efectos negativos sobre las especies nativas

y la economía humana son muy grandes (Pérez et al. 2014). Los daños provocados por los ácaros en plantas de importancia económica no habían sido debidamente reconocidos hasta la década de los 80´s del siglo pasado, cuando las políticas de la agricultura tendientes a producir cultivos tradicionales se cambiaron para generar aquellos que representaban mejores perspectivas para la exportación, entre los que están las plantas ornamentales y frutales. Además, la siembra tecnológica e intensiva de los cultivos, la alta intensidad y cambios al ambiente producidos por todas las practicas efectuadas, han convertido a los ácaros en plagas importantes (Ochoa et al. 1991).

Entre las familias de ácaros fitófagos que comúnmente se han encontrado asociadas al cultivo del café a nivel mundial se encuentran las familias Tetranychidae, Tenuipalpidae y Tarsonemidae (Meza y Rodríguez 2012).

Los ácaros pertenecientes a la familia Tenuipalpidae se conocen como ácaros falsos o ácaros planos. Esta familia contiene más de 1,100 especies válidas pertenecientes a 38 géneros (Vacante 2015). Estos ácaros dañan directamente a las plantas mediante la alimentación, y algunas especies también actúan como vectores de virus de plantas. Dentro de esta familia de ácaros fitófagos, se ubican algunas especies de mayor importancia económica en el mundo, especialmente las del género *Brevipalpus* Donnadieu con más de 300 especies (Jeppson et al. 1975; Childers y Rodrigues 2011; Beard et al. 2015). Estos ácaros causan daño directo al insertar sus partes bucales en los tejidos de las plantas y chupar los contenidos celulares. También inyectan su saliva tóxica en la planta durante la alimentación (Childers y Rodrigues 2011). Además, varios de estos ácaros transmiten virus a las plantas huésped (Mesa et al. 2009; Salinas-Vargas et al. 2016). Su importancia como plagas agrícolas ha aumentado, principalmente debido a su papel en la transmisión de algunas virosis en plantas cultivadas, lo que ha llevado a la necesidad de establecer medidas cuarentenarias para evitar su extensión geográfica (Ochoa et al. 1994; Childers y Derrick 2003; Childers et al. 2003; Gerson 2008; Kitajima et al. 2010; Rodrigues y Childers 2013; Alberti y Kitajima 2014). Las tres especies de la familia Tenuipalpidae más abundantes son *Brevipalpus californicus* (Banks), *Brevipalpus obovatus* Donnadieu y *Brevipalpus phoenicis* (Geijskes), las cuales han sido determinadas como plagas importantes en una gran variedad de plantas (Childers et al.

2003). En una revisión reciente del estado taxonómico de *B. phoenicis* sensu lato se determinó que es un grupo compuesto por ocho especies (Beard et al. 2015).

B. phoenicis es vector del Virus de la Mancha Anular del Café (CoRSV) que afecta a hojas y frutos en *C. arabica*. Este virus está reportado en varias regiones en Brasil (Chagas et al. 2003; Kitajima et al. 2003); fuera de dicho país, solamente en Costa Rica (Rodrigues et al. 2002). En las plantaciones de café en México se desconoce la acarofauna presente, en específico las especies pertenecientes al género *Brevipalpus*; dentro de este grupo, *B. yothersi* (Baker), recientemente reinstalada como especie válida (Beard et al. 2015), se reporta como la especie más abundante y ampliamente distribuida en huertos mexicanos en una amplia gama de especies de cítricos y es la responsable de trasmitir el virus de la leprosis de los cítricos C (CiLV-C), el cual es un patógeno económicamente importante y el principal agente causal de la enfermedad de la leprosis (Sánchez-Velázquez et al.2015; Salinas-Vargas et al. 2016; Gómez-Mercado et al. 2019).

En este sentido, esta investigación tuvo como objetivos conocer las especies existentes de ácaros de la familia Tenuipalpidae asociadas al cultivo del café a diferentes altitudes en la región del Soconusco, Chiapas, México, así como la detección de la presencia de virus patógenos que pudieran ser los responsables de los daños hacia la producción y cosecha de este cultivo, como pudieran ser mancha anular del café, (CoRSV), leprosis de los cítricos citoplásmica (CiLV-C), leprosis de los cítricos citoplásmica 2 (CiLV-C2), entre otros, además determinar su distribución espacial en la estructura de la planta y su fluctuación poblacional.

III. ARTÍCULO CIENTÍFICO

Brevipalpus **mites associated with coffee plants (*Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner) in Chiapas, Mexico.**

Artículo enviado a Experimental and Applied Acarology

Experimental and Applied Acarology

Brevipalpus mites associated with coffee plants (*Coffea arabica* L. *Coffee canephora* Pierre ex Froehner) in Chiapas, Mexico.

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Abstract:	Tenuipalpid mites of the genus *Brevipalpus* are of significant economic and quarantine importance in agriculture. They can damage and vector phytopathogenic viruses in coffee plantations and other crops. In this research, we focused on the following objectives: to identify species of the genus *Brevipalpus*, to assess the spread of *Brevipalpus* -associated viruses (CoRSV, CiLV-C, CiLV-C2, CiLV-N y CiLV-N sensu novo), and to monitor mite population fluctuations over the course of one year. The study was conducted in coffee plantations in Soconusco, a coffee-producing region in Chiapas, Mexico. The collected mites of the *Brevipalpus phoenicis* sensu lato species complex (635) were identified as *Brevipalpus papayensis* (80.2%) and *Brevipalpus yothersi* (19.8%) based on morphological and molecular characteristics. Their population abundance was low and there were no indications for virosis. The highest mite abundance was recorded in August-September and the lowest in February-March. An interaction was observed between mite abundance and coffee species in open-grow and shaded cultivation at various altitudes. *Brevipalpus papayensis* was more abundant in *Coffea arabica* var. Bourbon, in shaded (80%) growing conditions at an altitude of 1300 m.a.s.l. *Brevipalpus yothersi* was more abundant in *Coffea canephora*, in open-grow cultivation conditions at low altitude. Weare of the opinion that, at this moment, *B. papayensis* and *B. yothersi* do not present risks to the production of coffee for the studied plantations. However, as the coffee producing regions of Mexico are ecologically diverse, it will be important to continue examining the status of *Brevipalpus* mite populations in other regions in Mexico.

Brevipalpus mites associated with coffee plants (*Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner) in Chiapas, Mexico.

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Abstract

Tenuipalpid mites of the genus *Brevipalpus* are of significant economic and quarantine importance in agriculture. They can damage and vector phytopathogenic viruses in coffee plantations and other crops. In this research, we focused on the following objectives: to identify species of the genus *Brevipalpus*, to assess the spread of *Brevipalpus*-associated viruses (CoRSV, CiLV-C, CiLV-C2, CiLV-N y CiLV-N *sensu novo*), and to monitor mite population fluctuations over the course of one year. The study was conducted in coffee plantations in Soconusco, a coffee-producing region in Chiapas, Mexico. The collected mites of the *Brevipalpus phoenicis* sensu lato species complex (635) were identified as *Brevipalpus papayensis* (80.2%) and *Brevipalpus yothersi* (19.8%) based on morphological and molecular characteristics. Their population abundance was low and there were no indications for virosis. The highest mite abundance was recorded in August-September and the lowest in February-March. An interaction was observed between mite abundance and coffee species in open-grow and shaded cultivation at various altitudes. *Brevipalpus papayensis* was more abundant in *Coffea arabica* var. Bourbon, in shaded (80%) growing conditions at an altitude of 1300 m.a.s.l. *Brevipalpus yothersi* was more abundant in *Coffea canephora*, in open-grow cultivation conditions at low altitude. We are of the opinion that, at this moment, *B. papayensis* and *B. yothersi* do not present risks to the production of coffee for the studied plantations. However, as the coffee producing regions of Mexico are ecologically diverse, it will be important to continue examining the status of *Brevipalpus* mite populations in other regions in Mexico.

Key words: phytophagous mites, Tenuipalpidae, *Brevipalpus*, virus.

Introduction

Pests and diseases have been responsible for inflicting yield and quality-reducing damage in coffee plantations. As one of the most diverse groups of arthropods, acarines (Arachnida: Acari) can certainly be counted among these pests. Phytophagous as well as predatory species have aroused much interest for their significance as pests and natural enemies, respectively (Moraes and Flechtmann 2008). On a global level, members of the acarine families Tetranychidae (spider mites), Tenuipalpidae (flat mites) and Tarsonemidae (thread-footed mites) - all of them phytophagous - have the highest incidence in coffee plantations (Mesa and Rodríguez 2012). However, there is currently only limited information available on the diversity of the acarofauna in Mexican coffee plantations, in particular data regarding the genus *Brevipalpus* (Tenuipalpidae).

Flat mites are a globally distributed group of phytophagous acarines with a substantial economic impact. Especially the genus *Brevipalpus* Donnadieu, which encompasses more than 300

species, presents extensive challenges (Jeppson et al. 1975; Childers and Rodrigues 2011; Beard et al. 2015). Within the family Tenuipalpidae, the three most abundant species - *Brevipalpus californicus* Banks, *Brevipalpus obovatus* Donnadieu and *Brevipalpus phoenicis* Geijskes – are regarded as serious pests on several crops (Childers et al. 2003). In a taxonomic revision, Beard et al. described *Breviplpus phoenicis* sensu lato as a species complex constituted by: *Brevipalpus azores* Beard & Ochoa, *Brevipalpus feresi* Ochoa & Beard, *Brevipalpus ferraguti* Ochoa & Beard, *Brevipalpus hondurani* Evans, *Brevipalpus papayensis* Baker; *Brevipalpus phoenicis* (Geijskes) sensu stricto, *Brevipalpus tucuman* Beard & Ochoa, and *Brevipalpus yothersi* Baker (Beard et al. 2015). Their importance as agricultural pest has increased significantly, in particular because of their ability to serve as vector of plant viruses, which has resulted in the quarantine of many crops to geographically contain the spread of disease (Ochoa et al. 1994; Childers and Derrick 2003; Childers et al. 2003; Gerson 2008; Kitajima et al. 2010; Rodrigues and Childers 2013; Alberti and Kitajima 2014). Within this group, *B. yothersi* was reported as one of the most abundant and widely distributed flat mites in Mexico among different citrus species, where it is often responsible for the transmission of the economically important citrus leprosis virus cytoplasmic (CiLV-C), which causes leprosis disease in citrus (Beard et al. 2015; Sánchez-Velázquez et al. 2015; Salinas-Vargas et al. 2016; Gómez-Mercado et al. 2019).

Coffea arabica L. (Arabica coffee) is affected by the coffee ringspot virus (CoRSV), which is transmitted by *B. phoenicis* and characterized by the appearance of its namesake annular spots on infected leaves. The virus has been reported in various regions in Brazil (Chagas et al. 2003; Kitajima et al. 2011) and Costa Rica (Rodrigues et al. 2002). The topic of the present investigation is the identification of species from the genus *Brevipalpus* associated with *Coffea arabica* L. var. Bourbon and *Coffea canephora* Pierre ex Froehner in coffee plantations (open-grow as well as shaded conditions along an altitudinal gradient) in the Soconusco region, Chiapas, Mexico. Such an investigation is warranted given the importance of mites as vectors of viral diseases and the major economic impact they may have. Soconusco is a tropical agricultural region in the south-eastern tip of Mexico on the border with Guatemala, and coffee has been an important crop ever since its introduction in the late 19th century. In 2018, Chiapas was Mexico's coffee production leader, accounting for 41% of Mexico's coffee production (SADER, 2018).

The presence of several viruses in *Brevipalpus* mites was assessed for coffee ringspot virus (CoRSV), citrus leprosis virus cytoplasmic (CiLV-C) and cytoplasmic type 2 (CiLV-C2), citrus leprosis virus nuclear (CiLV-N) and nuclear *sensu novo* (CiLV-N *sensu novo*). In addition, *Brevipalpus* mite population fluctuation and spatial distribution on the plant were both monitored over the course of one year.

Material and methods

Sampling sites

Mites were collected at three different coffee plantations of approximately 20 years old in Soconusco, Chiapas, Mexico. The plantations cultivated *C. arabica* var. Bourbon and *C. canephora* in shaded and open-growth conditions along an altitudinal gradient (690-1300 m.a.s.l.). Site 2M is an open-grow plantation of *C. canephora* located in Ejido Dos de Mayo, municipality of Cacahoatán (15°01'41.34" N, 92°08'58.03" W, 690 m.a.s.l.). Site SD is an open-grow plantation of *C. arabica* var. Bourbon located in Ejido Santo Domingo, municipality of Unión Juárez (15°02'03.31" N, 92°06'43.39" W, 832 m.a.s.l.). Site PL is a shaded (80%) plantation of *C. arabica* var. Bourbon located in Ejido Pico de Loro, municipality of Unión Juárez (15°03'04.40" N, 92°05'55.94" W, 1300 m.a.s.l.). The dominant tree species at this site were *Terminalia amazonia* (J. F. Gmel.) Exell (locally known as *guayabo volador*) and *Inga jinicuil* Schltdl. & Cham. Ex G. Don (locally referred to as *jinicuil*). Samples were collected in the course of one year - from August 2015 to August 2016 - at one month intervals.

Sample preparation and morphological identification of Brevipalpus mites

A 2-ha plot was chosen in each plantation and two 20 m line transects were set out (100 m distance in-between). Ten plants were randomly chosen and each plant was divided into three sections, namely a bottom, middle, and top section, which were at 30, 60, and 120 cm above ground level, respectively. Four branches (one *per* cardinal point) were selected from each section and two leaves were randomly picked from the middle and distal part of the branch (16 leaves *per* plant *per* sampling). Four coffee berries were collected from the middle and distal part of the branch (32 coffee berries *per* plant *per* sampling) if the plant was carrying fruit (Spongoski et al. 2005). Collected samples were placed in an airtight plastic bag and transported to the laboratory (*Salud Forestal de El Colegio de la Frontera Sur*, Tapachula, Chiapas) for further examination.

The acarines were extracted by washing the samples with a 1% Tween 20 solution. The washing was then filtered through a column of sieves with decreasing mesh sizes (500 (25 µm), 325 (45 µm), 100 (150 µm) and 40 (425 µm)) to isolate the mites. They were finally collected from the 25 µm sieve (washed with 70% alcohol; washing bottle) and stored into labelled vials. Acarine families were determined based on morphological characteristics under a dissecting microscope (Carl Zeiss Stemi 2000 C at 40x). Tenuipalpid mites were placed in a staining plate (cavities: 16 mm diameter, 2.3 mm depth) and covered with 1% lactic acid (cleaning agent) for one week. They were then mounted on glass slides using Hoyer's medium: the mite was placed into a droplet of Hoyer's medium on the centre of a microscopic slide and covered with a coverslip (1 x 1 cm). The slide was then placed in an oven until it was dry (45°C, 7 days). After the drying stage, the coverslip

was sealed with insulating paint. Each slide was labelled with origin (sampling site), host, collecting date and collector (Krantz and Walter 2009).

The mites were identified using a phase-contrast microscope (Carl Zeiss Axio Lab.A1 at 100x) according to the morphological criteria established by Beard et al. (2012 and 2015).

Molecular identification of Brevipalpus mites based on Cytochrome Oxidase I

For the molecular identification of *Brevipalpus* mites, a total of 80 mites *per* site were collected in August 2019 (month in which we identified the highest *Brevipalpus* population abundance in this region during the period 2015-2016), by direct inspection of the leaves and coffee berries. Of these specimens, one half was stored in RNALater (Invitrogen CA USA) and maintained at 4°C for future analysis, while the other half was used to confirm their identity based on morphological characteristics.

Samples for molecular identification from each site were ground in liquid nitrogen before being processed through DNA/RNA Purification Micro Kit (Norgene Bioteck Corp, ON Canada) according to the manufacturer's instructions. In this protocol, DNA and RNA are processed simultaneously but eluted separately (end-volume 40 µL).

PCR amplification of Cytochrome Oxidase I (COI) was performed using the oligonucleotide primers COI_F (5'-TGATTTTTTGGTCACCCAGAAG-3') and COI_R (5'-TACAGCTCCTATAGATAAAAC-3'), previously described by Navajas et al. 1996. The reaction was set up using PCR Master Mix 2x (Promega, WI USA), 2 pM of each primer and 2 µL of DNA template in a final volume of 20 µL. Touchdown PCR conditions were employed as follows: 95°C for 5 min (initial denaturation), then 10 cycles of 95°C for 30 sec (denaturation), followed by 58°C for 30 sec (annealing; decrease in the annealing temperature at a rate of 1°C per cycle), 75°C for 1 min (elongation), then 35 cycles of 95°C for 1 min (denaturation), followed by 48°C for 30 sec (annealing), 72°C for 1 min (elongation), and ending at 72°C for 5 min (final elongation). The obtained PCR products were purified using the DNA Clean and Concentrator kit (ZYMO Research, CA USA) according to manufacturer protocol.

Purified PCR products were then cloned into the pJet1.2 plasmid vector using a CloneJET PCR Cloning Kit (Thermo Scientific, MA USA), which was then used to transform competent *Escherichia coli* DH5α cells. Plasmids were extracted from the transformed cells and their construction verified by PCR with the COI primers and by Sanger sequencing (Macrogen, Seoul, South Korea). The obtained sequences were cleaned and processed using the MEGA X program (Kumar et al. 2018), and compared against KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.kegg.jp) and the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) with BLASTN.

Detection of CorSV and other viruses transmitted by Brevipalpus mites

cDNA was synthesized from 40-80 ng acarine RNA by reverse transcription using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, MA USA) according to the supplier's instructions. Next, the cDNA was used as a template for Nested End-Point PCR with primers specific for the coffee ringspot virus (CoRSV), citrus leprosis virus cytoplasmic (CiLV-C) and cytoplasmic type 2 (CiLV-C2), citrus leprosis virus nuclear (CiLV-N) and nuclear *sensu novo* (CiLV-N *sensu novo*), and the MP-gene (movement protein involved in cell-to-cell movement of the citrus leprosis virus) (Table 1). The reaction was performed using PCR Master Mix 2x (Promega, WI USA), 0.2 pM of each primer and 1 µL of cDNA in a final volume of 20 µL. The following PCR conditions were used: 95°C for 5 min (initial denaturation), then 35 cycles of 95°C for 30 sec (denaturation), followed by *x*°C for 40 sec (annealing; *x* is 64 for site SD, and 62 for sites 2M and PL), 72°C for 90 sec (elongation), and ending at 72°C for 10 min (final elongation). A plasmid (1 µL) containing a fragment of the CiLV-C gen (donated by *Centro Nacional de Referencia Fitosanitaria* (CNRF-SENASICA) was used as positive control. Amplicons were visualized by electrophoresis on a 1% agarose gel and a band at 400 pb was excised, purified using the Wizard SV Gel and PCR Clean-Up System kit (Promega, WI USA), and sent to Macrogen (Seoul, South Korea) for Sanger sequencing. The obtained sequences were cleaned and edited using the MEGA X program (Kumar et al. 2018), and compared against KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.kegg.jp) and the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) with BLASTN.

Statistical analysis

Data were analysed in the statistical computing environment R (version 4.0.2; R core Team 2020) with a generalized linear model and type II analysis of variance, while the χ^2-test was used for the comparison of means.

Results

In total, 1614 acarine specimens were collected during the sampling period (from August 2015 to August 2016), of which 1445 (89.5%) were found on leaves and 169 (10.5%) on coffee berries. Of the total number of collected specimens, 635 (39.3%) were classified into the family Tenuipalpidae, genus *Brevipalpus* spp. The remaining mites (60.7%) were placed into the families Tetranychidae and Phytoseiidae. No mite-attributable direct damage was observed at any sampling site.

Morphological identification of Brevipalpus mites

The key characteristics that allowed us to separate *B. papayensis* were the following: prodorsal central cuticle with strongly defined areolae; dorsal opisthosoma in *c1-c1* to *d1-d1* is smooth to

192 slightly wrinkled, with some oblique folds adjacent to setae *d1*. The cuticle of the ventral plate is
193 covered with separately formed rounded warts; the cuticle of the genital plate has narrow and
194 irregular transverse bands. The spermathecal duct is fairly long and thick and ends in a small,
195 sclerotized spherical vesicle, with a crown of minute projections (Fig. 1 A-D).
196 The main criteria used to separate *B. yothersi* were: prodorsal central cuticle with well-defined
197 areolae, dorsal opisthosoma between setae *c1-c1* to *d1-d1* is smooth to slightly wrinkled; in-
198 between setae *e1-e1* to *h1-h1*, cuticle with V-shaped folds becoming weaker towards *h1*. The
199 cuticle of the ventral plate is covered with small rounded warts (central part); the cuticle of the
200 genital plate has large cells formed by fused warts. The spermathecal duct is long, narrow and
201 convoluted and ends in a sclerotized oval vesicle with thick distal stipe (Fig. 1 a-d) (Beard et al.
202 2012; Beard et al. 2015).
203 Of the 635 mites that were classified as *Brevipalpus* spp., 509 (80.2%) were identified as
204 *Brevipalpus papayensis* and 126 (19.8%) as *Brevipalpus yothersi*.
205 *Molecular identification of Brevipalpus mites based on Cytochrome Oxidase I*
206 Fragments of approximately 450 bp of the COI gene were obtained from collected mites. Their
207 sequences were deposited in GenBank and assigned accession numbers MW587268 to
208 MW587269. Sequences were aligned and compared against the GenBank database with
209 BLASTN, which revealed that one isolate (2M13, *Brevipalpus papayensis*, 453 bp, deposited as
210 MW587268) showed 99.76% similarity to *Brevipalpus obovatus* (DQ450492.1), whereas a second
211 isolate (2M16, *Brevipalpus yothersi*, 453 bp, deposited as MW587269) produced 98.54 and
212 99.78% similarity matches to *Brevipalpus yothersi* (KP180426.1) and *Brevipalpus californicus*
213 (DQ450499.1), respectively (Table 2).
214 *Detection of CorSV and other viruses transmitted by Brevipalpus mites*
215 Acarine RNA obtained from specimens collected by direct inspection (*C. canephora* and *C.*
216 *arabica* var. Bourbon) did not yield amplification products with the primers for CoRSV, CiLV-C2,
217 CiLV-N and CiLV-N *sensu novo*, even though various PCR parameters were evaluated and
218 optimized. However, a ~400-pb fragment was amplified with the CiLV-C primer in samples from
219 the sites SD and PL (Fig. 2a; open-grow and shaded *C. arabica* var. Bourbon, respectively).
220 Samples collected at site 2M (*C. canephora*) showed two other fragments (Fig. 2b). Sequences
221 (amplified with CiLV-C primer) were aligned and compared against the KEGG and GenBank
222 database with BLASTN, revealing a partial alignment (24/25 nt for site SD, 24/24 nt for site PL)
223 with the MP-coding region of the CiLV-C genome (NC_008170.1).

Following a direct alignment approach revealed partial alignment with different regions distributed throughout the reference sequence (DQ352195), separated by gaps. The identification of CiLV-C was, therefore, not considered conclusive.

Population fluctuation of Brevipalpus spp.

An analysis of variance (type II) indicated that the abundance of *Brevipalpus* spp. fluctuated significantly over the year (df 12, χ^2 491.01, Pr < $2.2e^{-16}$), and that there was a highly significant interaction between mite abundance and coffee species (df 2, χ^2 44.54, Pr<2.12e-10).

One of the observations made during the monitoring of *Brevipalpus* mites was that the highest mite abundance was encountered in August and September ($\bar{x}$ 0.5, SEM 0.05 and $\bar{x}$ 0.4, SEM 0.05, respectively), whereas the lowest abundance was recorded in February and March ($\bar{x}$ 0.01, SEM 0.005 and $\bar{x}$ 0.005, SEM 0.003, respectively). No specimens were observed in December and January (Fig. 3). The mites did not exhibit a preference for a particular section of the plant.

Figure 4 shows there is an interaction between mite abundance (*B. papayensis* and *B. yothersi*) and coffee species (open-grow and shaded, different altitudes). In the case of *B. papayensis*, the highest abundance was recorded for *C. arabica* var. Bourbon in a shaded (80%) cultivation system at an altitude of 1300 m.a.s.l. ($\bar{x}$ 0.267, SEM 0.026). In an open-grow coffee plantation at 832 m.a.s.l., its population was significantly lower ($\bar{x}$ 0.166, SEM 0.022). The population of *B. yothersi*, on the other hand, was higher in an open-grow coffee plantation growing *C. canephora* at low altitude ($\bar{x}$ 0.897, SEM 0.016). The *B. papayensis* population remained low for nearly the entire sampling period and apparently preferred shaded growing conditions and higher altitudes, which is where *C. arabica* is cultivated. The highest growth rate for the *B. papayensis* population was observed at 1300 m.a.s.l. in the period from July to November, whereas its population levels remained low in an open-grow coffee plantation at 832 m.a.s.l. (Figs. 4 and 5).

Discussion

The taxonomic identification of quarantine and economically important species is key to the successful design of a preventive and control strategy. Moreover, owing to the occurrence of cryptic species in the *Brevipalpus* genus, it is pertinent to combine morphological characteristics with molecular analysis (mitochondrial COI gene markers) for identification (Groot and Breeuwer 2006; Navia et al. 2013; Beard 2015). Di Palma et al. (2020) suggest to include also the morphological and ultrastructural details of the insemination system among our mite identification tools. It was by implementing all these criteria that the presence of *B. yothersi* in coffee plantations in Chiapas could be substantiated. Its morphological identification was confirmed by virtue of molecular analysis, which produced a match (99.78% similarity) with a GenBank accession of the same species (KP180426.1). Sequences obtained from specimens that were morphologically

classified in this study as *B. papayensis* gave a match (99.76% similarity) with *B. obovatus* (DQ450492.1). The fact that two apparently well-characterized species are misidentified is certainly a cause of preoccupation. It appears that the identity of the specimens from which the sequences were derived and submitted to GenBank was incorrectly established. Beard et al. (2012 and 2015) included *B. yothersi* as well as *B. papayensis* in the *B. phoenicis* species complex and clearly separated them from *B. obovatus*. *Brevipalpus papayensis* has two solenidia on tarsus II (one adaxial and another one abaxial), whereas *B. obovatus* has one distal solenidion on tarsus II (antiaxal). According to Beard et al. (2015), many specimens that previously had been identified as *B. phoenicis* were actually *B. obovatus* and vice versa. Moreover, even though the sequence was submitted to GenBank (DQ450492.1), there seems to have been no publication to support or explain the submitters' rationale for the identification of their specimens as *B. obovatus*.

The mites *B. papayensis* and *B. yothersi* were detected on *C. arabica* var. Bourbon and *C. canephora*. Apparently, *B. papayensis* populations had a preference for *C. arabica* var. Bourbon sheltered by umbrageous trees, particularly *T. amazonia* and *I. jinicuil*. In their population study of mites on arabica coffee plants, Mineiro et al. (2019) encountered the same species even though they were more abundantly present in open-grow (conventional) coffee plantations compared to plantations within native forest fragments for the surveyed region (Monte Alegre do Sul, São Paulo, Brazil). Spongoski et al. (2005), on the other hand, found that *B. phoenicis* was the more abundant species in coffee plantations (Minas Gerais, Brazil) at low altitudes in the dry as well as in the rainy season. Given, however, that our study has identified *B. yothersi* as the dominant species in low altitude *C. canephora* plantations, and also considering that the *Brevipalpus phoenicis* group B reclassification by Beard et al. (2015) was published later than the investigation by Spongoski et al. (2005), it might be possible that the latter's identification of *B. phoenicis* should be reassessed as *B. yothersi*.

There are several factors that may be responsible for the observed fluctuation in the abundance of *Brevipalpus* mites during our study: coffee species (*C. canephora* and *C. arabica* var Bourbon), cultivation method (open-grow and shaded), geographical location of the sites, climate, and soil, among others. It is likely that a combination of these factors contributes to the distinct composition of these species. Results of a study by Teodoro et al. (2009) indicate that mite abundance in coffee plantations was higher in simple-shade agroforests compared to complex-shade and abandoned agroforests. According to González et al. (2002), fluctuations in the abundance of mites are brought on by a confluence of factors - among them variable seasonal conditions such as temperature and precipitation. They also indicate that the presence of other phytophagous arthropods and their natural enemies plays a role, and even though their presence was not

quantified in our investigation, it is very well expected that they have an impact on the local coffee plantation acarofauna. The type of agronomic management and the presence of other arthropod pests in crops can cause changes in plant morphology and physiology, which, as Quiroz et al. (2005) argue, also could lead to fluctuations in the *Brevipalpus* mite population.

In this study, *B. papayensis* and *B. yothersi* did not prevail in any particular part of the plant and their distribution was random. This finding is in agreement with Gómez-Mercado et al. (2019), who reported that the distribution of *B. yothersi* in orange trees (*Citrus* x *sinensis* (L.) Osbeck) is random. Both mite species are also known to be vectors for viral disease in coffee and citrus plants (Salinas-Vargas et al. 2016; Nunes et al, 2017). In Brazil, Nunes et al. (2017) were able to confirm that *B. papayensis* is capable to transmit CoRSV under field as well as laboratory conditions. The mite *B. yothersi*, moreover, which is the most abundant mite species encountered in Mexican and Brazilian citrus orchards (Sánchez-Velázquez et al. 2015; Salinas-Vargas et al. 2016) and in coffee plantations in São Paulo, Brazil (Mineiro et al. 2019), is also considered the principal vector for citrus leprosis virus cytoplasmic and cytoplasmic type 2 (CiLV-C and CiLV-C2, respectively), citrus leprosis virus nuclear (CiLV-N) and hibiscus green spot virus 2 (HGSV-2) (Beard et al. 2012; Roy et al. 2015). Even so, cDNA synthesized from field-collected mites did not produce amplification products with the primers for CoRSV, CiLV-C2, CiLV-N and CiLV-N *sensu novo*. Combining this fact with the absence of virosis in the examined plants suggests that these viral diseases were not present yet at the time of study.

Samples from *B. papayensis* generated an amplification product of ~400 bp for CiLV-C. However, the segments with similarity to the CiLV-C sequence were small (~24 nt), distributed throughout the sequence and separated by many gaps because of which a conclusive identification is not possible. Since the number of identified viruses transmitted by *Brevipalpus* mites as well as the reported number of possible host plants of these mites (~40) are on the rise (Kitajima et al. 2010; Castro et al. 2020), we cannot discard the possibility that we are dealing with a yet unreported CiLV variant. Clearly, more investigations are required to corroborate the identity of this virus.

Moreover, only adult specimens were included in the identification analyses of the virus, and only in *B. papayensis* were indications for a CiLV-C-related virus. Nevertheless, Tassi et al. (2017) confirmed that *B. yothersi* could become a vector of viral disease by lesions in the leaves, fruits and petioles, and was capable of transmitting CiLV-C in every developmental stage. Nunes et al. (2017) reported that *B. papayensis* can also serve as vector for CoRSV and CiLV-C.

Conclusion

The current study allowed us to document the population status of *Brevipalpus* mites and their associated viruses in coffee plantations of the Soconusco region, Chiapas, Mexico. *Brevipalpus*

papayensis and *Brevipalpus yothersi*, both members of the *Brevipalpus phoenicis* sensu lato species complex, were identified only in low numbers and lacked virosis. On that account, they are not likely to currently pose a significant threat to local coffee production. However, since coffee growing regions in Mexico are ecologically very diverse, it remains important to investigate and evaluate the population behaviour of *Brevipalpus* mites in these regions.

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Author contribution

- ✓ Julio Domínguez Gabriel and Rebeca González Gómez conceived of the present study and designed the experiments.
- ✓ Julio Domínguez Gabriel carried out the experiments, analysed the data and drafted the manuscript.
- ✓ Rebeca González Gómez supervised the project, analysed the data and managed the development of the manuscript.
- ✓ Karina Guillén Navarro contributed to the molecular analysis and provided critical input on the manuscript.
- ✓ Gabriel Otero Colina performed the taxonomic classificiation of the studies acarines and provided critical comments on the manuscript.
- ✓ Javier Franciso Valle-Mora assisted with the statistical analysis.
- ✓ All authors have read and approved the final version of the manuscript.

Competing interest

The authors declare that no competing interests exist.

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Figures

Fig. 1 A-E) *Brevipalpus papayensis*, adult female, **A)** areolae in the centre of the prosoma and oblique folds between setae *d1*; **B)** oblique folds between setae *d1*, transverse grooves between setae *e1* and longitudinal grooves in the basal part; **C)** ventral plate, irregular design, diagonals in the anterior part, transverse bands in anal plate; **D)** spermatheca; and **E)** two solenidia on tarsus. **a-e)** *Brevipalpus yothersi*, adult female, **a)** areolae in the centre of the prosoma; **b)** V-shaped grooves in opisthosoma; **c)** verrucose ventral plate; **d)** spermatheca; and **e)** two solenidia on tarsus II.

Fig. 2 Visualization of amplified fragments (primers MPF/MPR for MP-coding region of CiLV-C) by electrophoresis (1% agarose gel). (a) Obtained amplicons and (b) purified bands sent for sequencing (Macrogen, Seoul, South Korea).

1% Agarose gel, TBE buffer 1x, 90 V/35 min, M = molecular weight marker of 100 pb, (-) negative control, (+) positive control for CiLV-C (SENASICA), SD = site Santo Domingo, PL = site Pico de Loro, 2M = site Dos de Mayo.

Fig. 3 *Brevipalpus* mite population fluctuation over the course of our experiment (August 2015-August 2016).

Fig. 4 Mite abundance of *Brevipalpus papayensis* and *Brevipalpus yothersi* for the three sampling sites (coffee plantations, Soconusco, Chiapas).

Fig. 5 Cumulative growth curve of *Brevipalpus papayensis* and *Brevipalpus yothersi* populations.

494 **Fig. 1**

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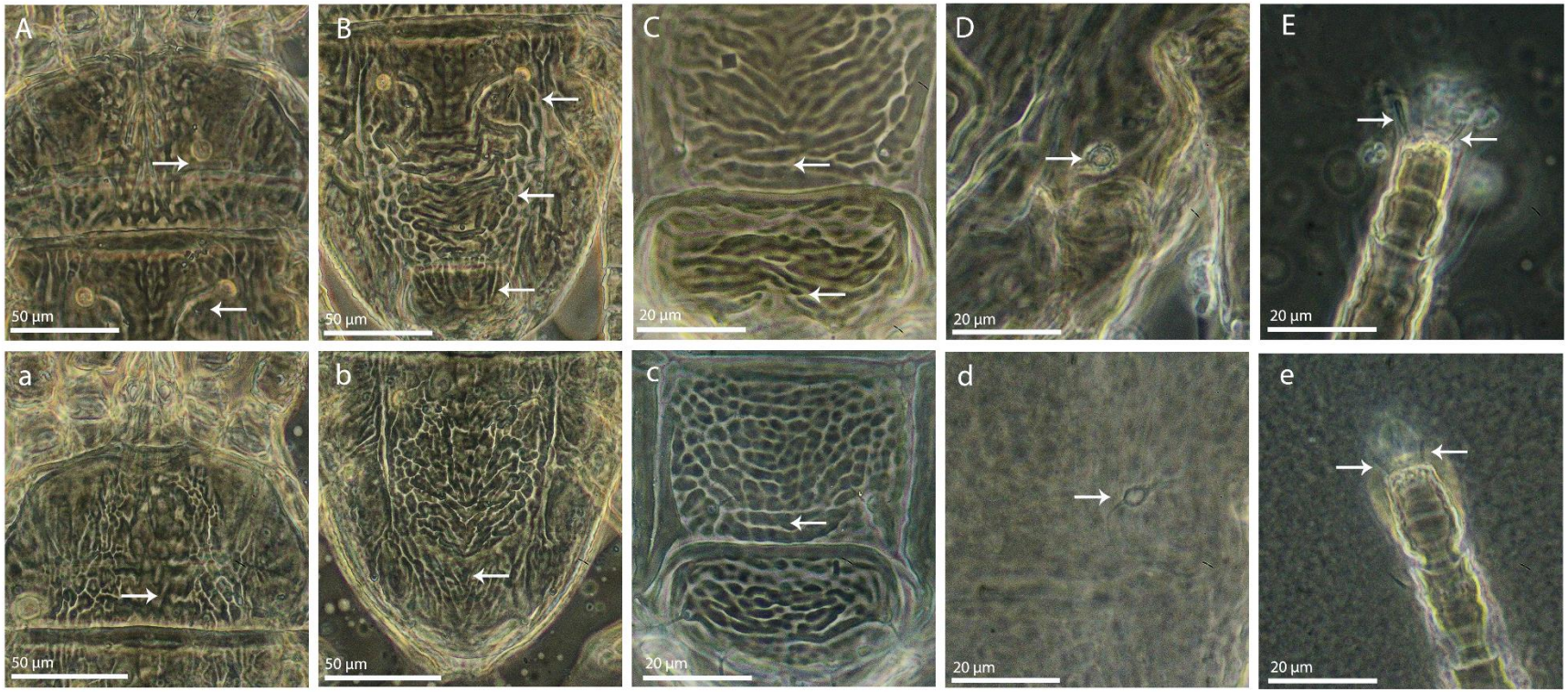

497 **Fig. 2**

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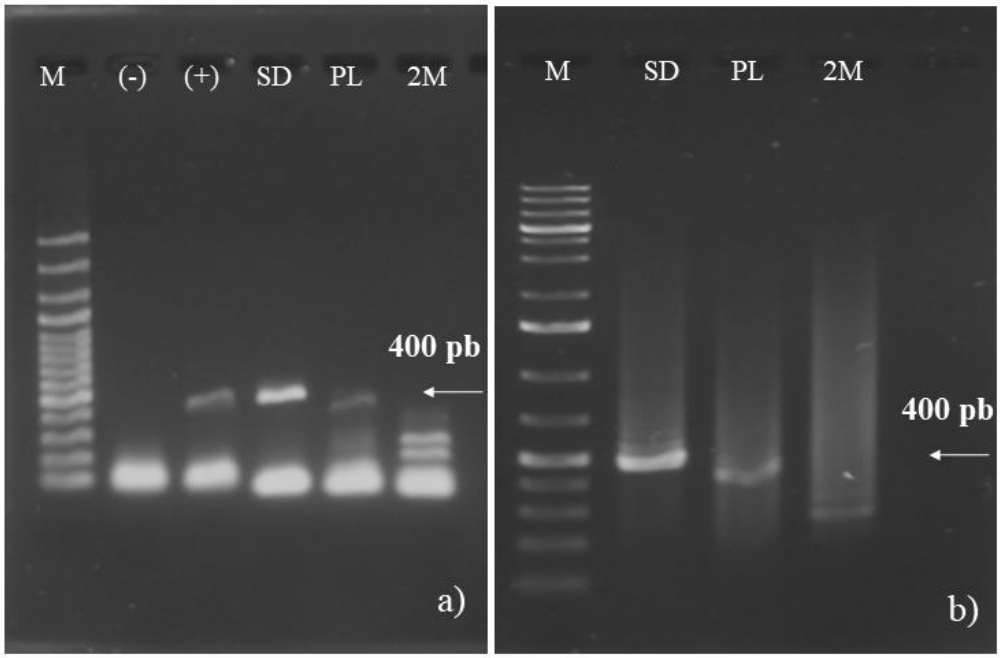

499 **Fig. 3**

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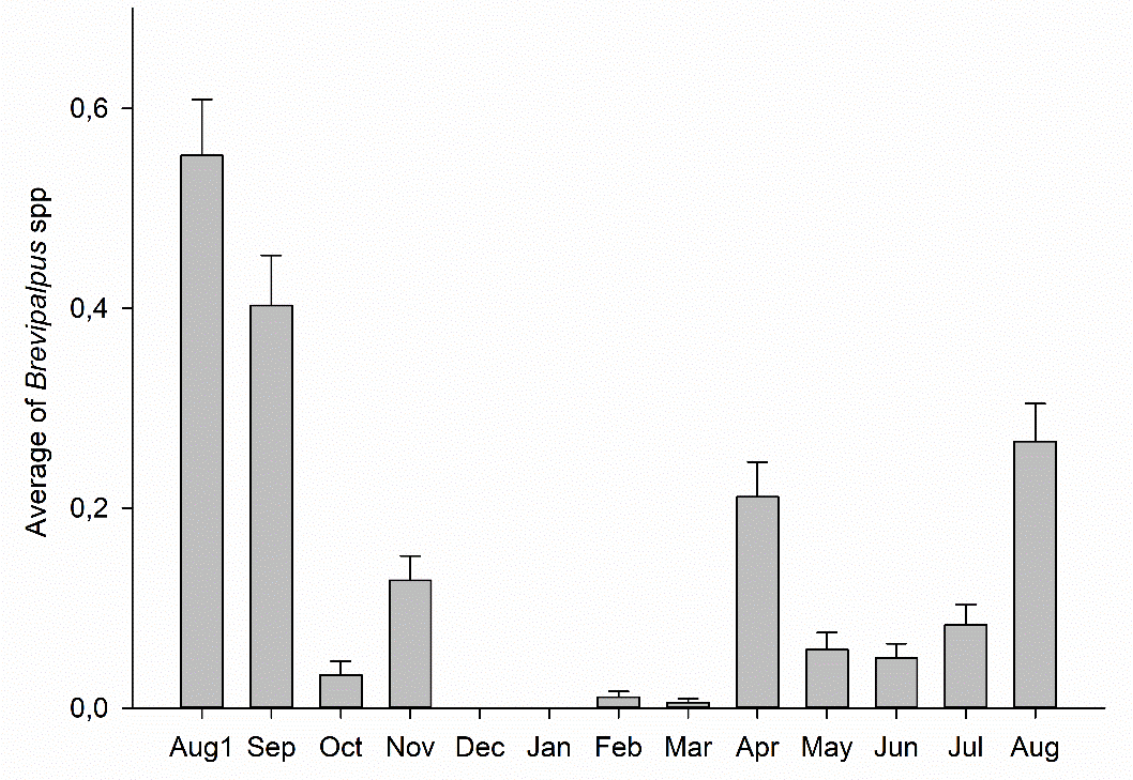

502 **Fig. 4**

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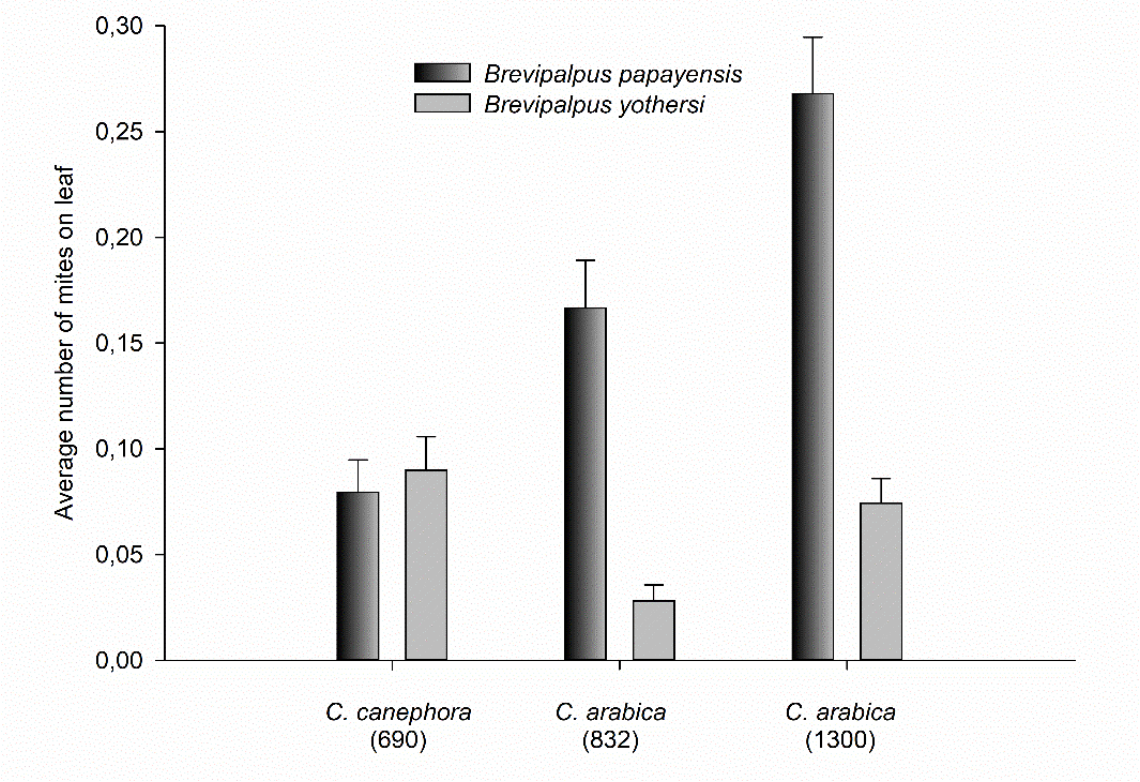

505 **Fig. 5**

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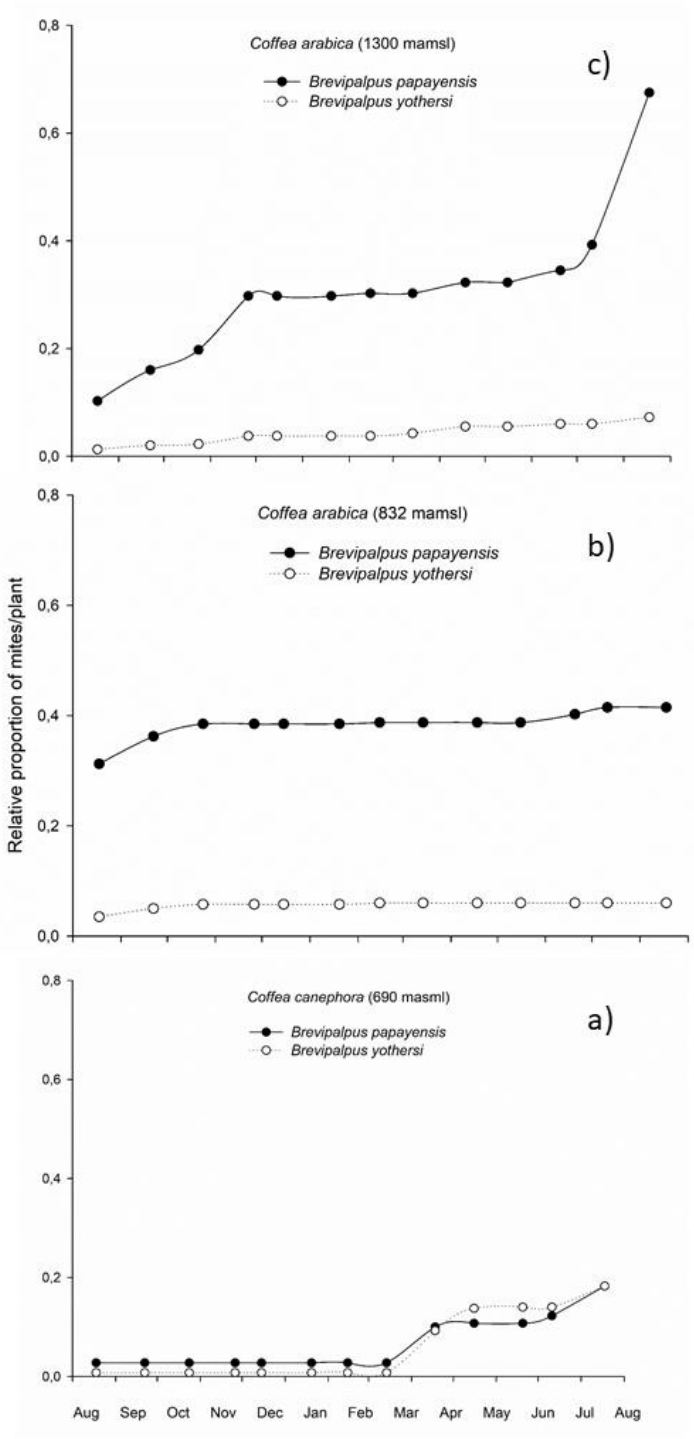

Table 1. Primers used in this study.

Primers				
Marker	**Orientation**	**Sequence**	**Expected amplicon size (nt)**	**Reference**
COI	F	TGATTTTTTGGTCACCCAGAAG	340-390	Navajas et al. 1996
	R	TACAGCTCCTATAGATAAAAC		
CoRSV	CoRSVF	GGACCATGAGACAGGAGGTG	389	Kitajima et al. 2011
	CoRSVR	CTCTGCCAGTCCTCAATGTG		
CiLV-C	MPF	GCGTATTGGCGTTGGATTTCTGAC	339	Locali et al. 2003
	MPR	TGTATACCAAGCCGCCTGTGAACT		
CiLV-C2	CiLV-C2-CPG-F	ATGAGTAACATTGTGTCGTTTTCGTTGT	794	Roy et al. 2013
	CiLV-C2-CPG-R	TCACTCTTCCTGTTCATCAACCTGTT		
CiLV-N	CiLV-N-NPF	ATGGCTAACCCAAGTGAGATCGATTA	681	Roy et al. 2014
	CiLV-N-NPR	AGTTGCCTTGAGATCATCACATTGGT		
CiLV-N *sensu novo*	N-DC_Br-Fwd	CCGTACCCATTGTGAAAATA	420	Ramos-González et al. 2017
	N-DC_Br-Rev	GAACCCCTTTGAGGAATG		

COI: cytochrome c oxidase subunit 1; CoRSV: coffee ringspot virus; CiLV-C: citrus leprosis virus cytoplasmic; CiLV-C2: citrus leprosis virus cytoplasmic type 2; CiLV-N: citrus leprosis virus nuclear; CiLV-N *sensu novo*: citrus leprosis virus nuclear *sensu novo*; F: Forward; R: Reverse.

Table 2. Results of BLASTN analysis regarding mite identification for different sampling sites in the Soconusco region, Chiapas.

Sequences from sites	Best Match	Score	Cover (%)	E-value	Identity/ Similarity	Accession Number
Sampling site Dos de Mayo (2M)						
Clone 1 2M16_pJET1.2 (453pb)	*Brevipalpus obovatus haplotype O11 cytochrome oxidase subunit 1 gene, partial cds; mitocondrial*	752	89	0	99.76%	DQ450492.1
Clone 2 2M12_pJET1.2 (456 pb)	*Brevipalpus yothersi isolate 92 cytochrome c oxidase subunit I (COI) gene, partial cds; mitocondrial*	832	100	0	99.78%	KP180426.1
Sampling site Santo Domingo (SD)						
Clone 1 SD2_pJET1. (434 pb)	*Brevipalpus obovatus haplotype O11 cytochrome oxidase subunit 1 gene, partial cds; mitocondrial*	750	94	0	99.76%	DQ450492.1
Sampling site Pico del Loro (PL)						
Clone 1 PL12_pJET1.2 (449 pb)	*Brevipalpus californicus haplotype C01 cytochrome oxidase subunit 1 gene, partial cds; mitocondrial*	730	91	0	98.78	DQ450499.1
Clone 2 PL14_pJET1.2 (429 pb)	*Brevipalpus obovatus haplotype O11 cytochrome oxidase subunit 1 gene, partial cds; mitocondrial*	750	95	0	99.76	DQ450492.1

IV. CONCLUSIONES

Se identificaron las especies de ácaros *Brevipalpus papayensis* y *Brevipalpus yothersi*, a través de características morfológicas y moleculares.

No se encontró la presencia de los virus: Coffee ringspot virus (CoRSV), Citrus leprosis virus cytoplasmic type 2 (CiLV-C2), Citrus leprosis virus nuclear (CiLV-N) y Citrus leprosis virus nuclear *sensu novo* (CiLV-N *sensu novo*), en los ácaros obtenidos en este estudio.

La presencia del virus de la leprosis de los cítricos C (CilV-C) en ácaros provenientes de los sitios de muestreo Santo Domingo y Pico de Loro no es concluyente, ya que solo se encontró similitud con un pequeño fragmento del genoma de CiLV-C, de 24/25nt y 24/24 nt para las secuencias respectivas; mientras que la secuencia de la muestra del sitio Dos de Mayo no tuvo similitud con este virus.

Brevipalpus papayensis fue más abundante en *Coffea arabica* variedad Bourbon en sistema de producción con sombra (80%) a una altitud de 1300, posiblemente debido a la diversidad de especies arbóreas (Pico de Loro). Sin embargo, en sistemas de producción sin sombra y a 832 msnm (Santo Domingo), su población fue significativamente menor. Mientras que la población de *B. yothersi* fue mayor en cafetales de *C. canephora,* sin sombra y a baja altitud (Dos de Mayo).

Los ácaros *B. papayensis* y *B. yothersi*, se encuentran distribuidos en los tres estratos (bajo, medio y alto) de la planta de café, y durante todo el estudio se detectaron de forma permanente en poblaciones bajas.

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