



# COLEGIO DE POSTGRADUADOS

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CAMPUS MONTECILLO

POSTGRADO DE FITOSANIDAD

ENTOMOLOGÍA Y ACAROLOGÍA

## **ABUNDANCIA Y DIVERSIDAD DE FITOSEIDOS (ACARI: PHYTOSEIIDAE) ASOCIADOS A ZARZAMORA (*Rubus* sp.), FRAMBUESA (*Rubus idaeus* L.) Y ARÁNDANO (*Vaccinium corymbosum* L.) EN MICHOACÁN, MÉXICO**

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# COLEGIO DE POSTGRADUADOS

INSTITUCIÓN DE ENSEÑANZA E INVESTIGACIÓN EN CIENCIAS AGRÍCOLAS

La presente tesis titulada: **ABUNDANCIA Y DIVERSIDAD DE FITOSEIDOS (ACARI: PHYTOSEIIDAE) ASOCIADOS A ZARZAMORA (*Rubus* sp.), FRAMBUESA (*Rubus idaeus* L.) Y ARÁNDANO (*Vaccinium corymbosum* L.) EN MICHOACÁN, MÉXICO**, realizada por el estudiante: **Ricardo Villalvazo Valdovinos**, bajo la dirección del Consejo Particular indicado, ha sido aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

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FITOSANIDAD  
ENTOMOLOGÍA Y ACAROLOGÍA

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**ABUNDANCIA Y DIVERSIDAD DE FITOSEIDOS (ACARI: PHYTOSEIIDAE)  
ASOCIADOS A ZARZAMORA (*Rubus* sp.), FRAMBUESA (*Rubus idaeus* L.) Y  
ARÁNDANO (*Vaccinium corymbosum* L.) EN MICHOACÁN, MÉXICO**

**Ricardo Villalvazo Valdovinos, M.C.  
Colegio de Postgraduados, 2022**

**RESUMEN**

Las berries son cultivos importantes en México. Los ácaros fitoseidos son enemigos naturales importantes de plagas agrícolas. Algunos de ellos son utilizados como agentes de control biológico en diferentes cultivos. A pesar de que se han reportado 2,557 especies en el mundo, se conoce muy poco acerca de su rol en la regulación de plagas agrícolas. La presente investigación tuvo como objetivos identificar a nivel morfológico y molecular los fitoseidos asociados a frambuesa, zarzamora y arándano; determinar la diversidad y abundancia de las especies presentes y analizar la relación de estas especies con el tipo de cultivo y el régimen de aplicación de plaguicidas. Se identificaron 11 especies: *Amblydromalus limonicus*, *Amblyseius swirskii*, *Amblyseius tamatavensis*, *Euseius hibisci*, *Euseius mesembrinus*, *Typhlodromalus peregrinus*, *Neoseiulus californicus*, *Neoseiulus mumai*, *Phytoseiulus persimilis*, *Metaseiulus negundinis* y *Typhlodromina subtropica*. Se comparó la diversidad de especies de ácaros Phytoseiidae entre zarzamora, frambuesa y arándano. Posteriormente, con las especies más abundantes, se estudió el efecto de las especies de berries y el régimen de aplicación de plaguicidas. La mayor diversidad de especies se encontró en frambuesa, seguida de zarzamora y arándano. Las especies más abundantes fueron *Typhlodromalus peregrinus* y *Neoseiulus californicus*. La abundancia de *T. peregrinus* se vio significativamente afectada por la aplicación de plaguicidas, pero no por las especies de berries. Por el contrario, la abundancia de *N. californicus* se vio significativamente afectada por las especies de berries, pero no por el régimen de plaguicidas.

**Palabras clave:** zarzamora, frambuesa, arándano, Phytoseiidae

**ABUNDANCE AND DIVERSITY OF PHYTOSEIDS (ACARI: PHYTOSEIIDAE)  
ASSOCIATED WITH BLACKBERRY (*Rubus* sp.), RASPBERRY (*Rubus idaeus* L.) AND  
BLUEBERRY (*Vaccinium corymbosum* L.) IN MICHOACÁN, MEXICO**

**Ricardo Villalvazo Valdovinos, M.C.  
Colegio de Postgraduados, 2022**

**ABSTRACT**

Berries are important crops in Mexico. Mites within the family Phytoseiidae are natural enemies of important agricultural pests, therefore used as biological control agents in many crops. Despite that 2,557 species have been reported, still there are many gaps in the knowledge about their role reducing pest populations. Our main objective was to identify Phytoseiidae mites present in raspberry, blackberry and blueberry using morphological attributes and molecular techniques, and determine if the diversity and abundance of Phytoseiidae mites were related to the berry species and/or frequency of pesticides applications. We identified 11 species: *Amblydromalus limonicus*, *Amblyseius swirskii*, *Amblyseius tamatavensis*, *Euseius hibisci*, *Euseius mesembrinus*, *Typhlodromalus peregrinus*, *Neoseiulus californicus*, *Neoseiulus mumai*, *Phytoseiulus persimilis*, *Metaseiulus negundinis* and *Typhlodromina subtropica*. The greatest species diversity of Phytoseiidae was found in raspberry, followed by blackberry and blueberry. The most abundant species were *Typhlodromalus peregrinus* and *Neoseiulus californicus*. The abundance of *T. peregrinus* was significantly affected by pesticide application but no berry species. In contrast, abundance of *N. californicus* was significantly affected of berry species but not pesticide regime

**Key words:** blackberry, raspberry, blueberry, Phytoseiidae

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## INTRODUCCIÓN GENERAL

Los fitoseidos son ácaros de vida libre que se encuentran comúnmente en el follaje de las plantas (Dhooria 2016), con un tamaño entre 200 y 600  $\mu\text{m}$  de longitud (Walter y Proctor 2013). Pasan por los estados de huevo, larva, protoninfa, deutoninfa y adulto; su ciclo de vida puede ser completado en cinco días (ej. *Phytoseiulus* spp.), aunque lo más común es de siete a diez días (McMurtry et al. 2015). Son los depredadores de plagas agrícolas más utilizados (Dhooria 2016). De acuerdo con Demite et al. (2022) se contabilizan 2,557 especies válidas a nivel mundial, representando el 20% de la diversidad de Mesostigmata (Walter y Proctor 2013). Su hábitat principal es la parte aérea de las plantas, aunque algunas especies también se pueden encontrar en el suelo y en la hojarasca (McMurtry et al. 2013). En plantas se localizan en tallos, ramas, flores, frutos y hojas, pero en mayor cantidad en estas últimas (Childers y Denmark 2011) con preferencia por la superficie abaxial (envés) (Sudo y Osakabe 2011). Se distribuyen de manera variable entre hojas internas y externas del dosel, dependiendo de la especie de fitoseido de que se trate (Villanueva y Childers 2005; Childers y Denmark 2011). La densidad de tricomas y el domacio en la planta influyen significativamente en la cantidad y especies presentes (Schmidt 2014), así como la presencia de agallas y erineos (McMurtry et al. 2013). Los fitoseidos pueden tener un ritmo circadiano distinto, de acuerdo con la época del año, aunque es más común la actividad diurna (Pérez-Sayas et al. 2017).

McMurtry y Croft (1997) clasificaron a las especies de la familia Phytoseiidae en cuatro categorías, basadas en sus hábitos alimentarios. Tipo I (especialistas en *Tetranychus* spp.), Tipo II (selectivos a especies de la familia Tetranychidae), Tipo III (depredadores generalistas), Tipo IV (especialistas en alimentarse con polen y depredadores generalistas). Posteriormente, McMurtry et al. (2013) agregaron información a la clasificación anterior y la subdividieron de acuerdo con el

hábitat y las plantas que prefieren habitar estos depredadores. Otras características como reproducción, desarrollo, mortalidad, fisiología, relación con el hospedero, dispersión, morfología externa de idiosoma y gnatosoma (Croft et al. 2004; Liu et al. 2017) contribuyen a la clasificación de los fitoseidos y por tanto a brindar información sobre su uso como agentes de control biológico. Por lo general, los especialistas están más directamente relacionados con su presa, mientras que los generalistas guardan una relación más estrecha con la planta hospedera (Schmidt 2014).

Los fitoseidos se han implementado para combate biológico de artrópodos plaga en todo el mundo (Tixier 2018). Las principales plagas contra las que se utilizan son ácaros fitófagos, trips y moscas blancas (McMurtry et al. 2013). Desde 1968, se han utilizado 23 especies distintas de fitoseidos en control biológico aumentativo, siendo las más importantes *Amblyseius swirskii*, *Neoseiulus cucumeris*, *Phytoseiulus persimilis* y *Neoseiulus californicus* (van Lenteren 2012; Knapp et al. 2018), las cuales representan dos tercios del mercado global de artrópodos usados para control biológico (Knapp et al. 2018). Alrededor de sólo 20 especies se ofertan comercialmente en el mundo (Knapp et al. 2018). Otras especies han demostrado tener potencial para su uso como agentes de control biológico, pero no se han utilizado de manera comercial (Zacarias et al. 2019). Aquellos especialistas funcionan mejor en el control biológico por aumento, mientras que los generalistas en el control biológico por conservación (Hoy 2011). Los generalistas son más sensibles a las aplicaciones de plaguicidas que los especialistas (Schmidt-Jeffris et al. 2021). Algunas especies comerciales han desarrollado cierta resistencia a los acaricidas más comunes, por lo que pueden persistir en los cultivos. En contraste, los fitoseidos nativos no logran sobrevivir ante el uso de acaricidas (Hoy 2011).

Sin embargo, los fitoseidos nativos juegan un rol importante en la supresión de plagas agrícolas (Tixier 2018). La conservación de depredadores nativos en un cultivo está en función de

tres acciones: mantenimiento de plantas adyacentes y circundantes al cultivo (Tixier 2018), utilización de plaguicidas selectivos (Duso et al. 2020; Tsuchida y Masui 2021) y suministro de polen como fuente alternativa de alimento para el depredador (Tsuchida y Masui 2020; Pascua et al. 2020; Malagnini et al. 2022).

La principal plaga contra la que se han utilizado ácaros fitoseidos en berries es *Tetranychus urticae*. En frambuesa se ha liberado a *N. californicus* y a *P. persimilis* porque son eficaces en el control de *T. urticae* (Charles et al. 1985; Bylemans et al. 2003; Linder et al. 2003). En zarzamora, el más utilizado es *N. californicus* (Akyazi y Liburd 2019). Y en el caso particular del arándano, la principal plaga son los trips, por lo que se han obtenido buenos resultados cuando se libera de forma preventiva a *N. cucumeris* para el control de *Frankliniella* spp. (Arévalo et al. 2009).

En México se encuentran registradas 131 especies de fitoseidos nativos (Demite et al. 2022), no obstante, sólo se ha reportado a *Euseius quetzali*, *N. californicus*, *Proprioseiopsis aetus*, *Typhlodromalus peregrinus* y *Typhlodromips josephi* en zarzamora cv. Tupy (Ayala-Ortega et al. 2019). En Michoacán se comercializan *Amblydromalus limonicus*, *Amblyseius swirskii*, *N. californicus* y *P. persimilis* (Koppert México 2022; Biobest 2022; BioBee 2022; SENASICA 2022) para el manejo de plagas en berries y otros cultivos.

Para México es muy importante el cultivo de berries ya que se cultivan comercialmente cuatro tipos: fresa (*Fragaria* spp.), zarzamora (*Rubus* sp.), frambuesa (*Rubus idaeus*) y arándano (*Vaccinium corymbosum*) (SIAP 2021). A nivel mundial, México es el cuarto productor en importancia en volumen de producción de fresa, el primero de zarzamora, el segundo de frambuesa y el quinto de arándano (FAOSTAT 2020). De acuerdo con datos del SIAP (2021), estas cuatro berries tienen un valor de producción de 41.7 mil millones de pesos, equivalente al 6.1% de todo

el valor de la producción agrícola mexicana, lo cual equivale a tan solo el 0.21% de la superficie agrícola cosechada del país.

La superficie cultivada de berries se ha incrementado en los últimos 20 años, y actualmente este aumento se ve especialmente reflejado en arándano (que pasó de 60 ha en 2002 a 4,909 ha en 2019), frambuesa (de 213 ha en 2002 a 9,279 ha en 2022) y zarzamora (de 1,116 ha en 2002 a 10,156 ha). El arándano se cultiva en 10 estados del país, pero la superficie se concentra en Jalisco (51%), Michoacán (15.5%) y Sinaloa (14.8%). En nueve estados se produce frambuesa, pero la mayor superficie cultivada está en Jalisco (73.7%), Michoacán (13.3%) y Baja California (10.4%). La zarzamora, por su lado, se cultiva en 12 entidades, pero principalmente en Michoacán (87.7%) y Jalisco (8.6%).

Dada la importancia que tiene la producción de berries en nuestro país, en términos de superficie cultivada y valor de la producción, actualmente se buscan diferentes tácticas de combate para las plagas que les afectan que sean más amigables con el ambiente, y menos dañinas a los enemigos naturales y al hombre. De ahí la importancia de conocer cuáles especies de fitoseidos se encuentran en campo en estas frutillas, ya que brinda información sobre su rol como enemigos naturales de ácaros e insectos fitófagos, así como de su potencial como agentes de control biológico.

## **OBJETIVOS E HIPÓTESIS**

### **Objetivo general**

Determinar la abundancia y diversidad de fitoseidos asociados a zarzamora, frambuesa y arándano en Michoacán, México.

### **Objetivos específicos**

- Identificar a nivel morfológico y molecular las especies de ácaros fitoseidos recolectados
- Estimar la diversidad y abundancia de las especies por cultivo.
- Analizar la relación de las especies predominantes con el cultivo y la frecuencia de aplicación de plaguicidas.

### **Hipótesis**

La abundancia y diversidad de fitoseidos es diferente dependiendo del cultivo y la frecuencia de aplicación de plaguicidas.

# CHAPTER I. BERRY SPECIES AND CROP MANAGEMENT AFFECTS SPECIES

## DIVERSITY AND ABUNDANCE OF PREDATORY MITES (ACARI:

### PHYTOSEIIDAE)<sup>1</sup>

#### 1.1 ABSTRACT

Berries are an important economic crop. Knowledge on their arthropod pests and biological control agents are important to develop more efficient integrated pest management programmes. Identification based on morphological attributes can be difficult and so molecular techniques should be incorporated. Here we studied the species diversity of predatory mites in the family Phytoseiidae, and how this diversity was affected by the berry species and crop management approaches, specifically pesticide application regimes. We sampled 15 orchards in the estate of Michoacán. Sites were selected based on berry species and pesticide regimes. Mite identification was achieved by combining morphological attributes and molecular techniques. The species diversity of Phytoseiidae mites was compared amongst blackberry, raspberry and blueberry. Using just the most abundant species, we studied the effect of berry species and pesticide regime on abundance. We identified 11 species. The greatest species diversity was found in raspberry, followed by blackberry and blueberry. The most abundant species were *Typhlodromalus peregrinus* and *Neoseiulus californicus*. The abundance of *T. peregrinus* was significantly affected by pesticide application but no berry species. In contrast, abundance of *N. californicus* was significantly affected of berry species but not pesticide regime.

**Keywords:** molecular taxonomy, diversity indices, blackberry, raspberry, blueberry

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<sup>1</sup> Villalvazo-Valdovinos R, Guzmán-Franco AW, Valdez-Carrasco JM, Martínez-Núñez M, Soto-Rojas L, Vargas-Sandoval M, Santillán-Galicia MT <sup>✉</sup> (2022) Berry species and crop management affects species diversity and abundance of predatory mites (Acari: Phytoseiidae). Exp Appl Acarol (**submitted**).



## 1.2 INTRODUCTION

Members of the family Phytoseiidae are free living mites that normally lives on plant leaves (Dhooria 2016). Their size is between 200 and 600  $\mu\text{m}$  in length (Walter and Proctor 2013). Typical life stages are egg, protonymph, deutonymph and adult, and their life cycle is completed in 7 - 10 days (McMurtry et al. 2015). Phytoseiidae are considered the most efficient and widely distributed predatory mites of agricultural pests (Dhooria 2016). Worldwide, there are approximately 2,557 species (Demite et al. 2022) representing 20% of Mesostigmata (Walter and Proctor 2013). A correct identification of Phytoseiidae is important for studies reporting their life cycles and implementation as biological control agents (Denmark and Evans 2011). However, there are many synonyms in Phytoseiidae identification (de Moraes et al. 2004). Therefore, a combination of morphological and molecular taxonomy is highly recommended for accurate identification of mites including Phytoseiidae (Dos Santos and Tixier 2018).

Mexico is the most important producer of blackberry (*Rubus* sp.) (Akyazi et al. 2021), second most important producer of raspberry (*Rubus idaeus*), and the fifth most important producer of blueberry (*Vaccinium corymbosum*) worldwide (FAOSTAT 2020). There have been studies reporting diversity of phytophagous and predatory mites in berry crops such as blackberry (e.g. Marchetti and Ferla 2011; Ayala-Ortega et al. 2019; Akyazi et al. 2021), raspberry (Kontschán and Ripka 2017; Marić et al. 2018) and blueberry (De Lillo and Duso 1996; Miñarro et al. 2005; Lopez and Liburd 2020). Most studies have focused on phytophagous mites because of the damage they cause to berries. However, considering the importance of mites in the family Phytoseiidae as biological control agents, studies on their diversity and abundance on specific habitats or production systems are increasingly important and will help to exploit them more efficiently. Species composition of Phytoseiidae can be affected by the geographical region and

crop species (Grafton-Cardwell et al. 2020), pesticide application (Schmidt-Jeffris et al. 2021) and prey and pollen availability (McMurtry y Croft 1997).

Based on this, we determined the species diversity of Phytoseiidae in three of the most important berries in Mexico: blackberry, raspberry and blueberry. Our aim was to determine the potential interaction between species diversity of Phytoseiidae, the berry species and pesticide regimes of different intensities. For accurate identification of mites, we used a combination of morphological attributes and sequencing of mitochondrial genes; the sequences obtained also support future taxonomic studies on Phytoseiidae. Our studies were done in the state of Michoacán, which has 89%, 18% and 15% of total blackberry, raspberry and blueberry production, respectively, in Mexico (SIAP 2020).

## 1.3 MATERIALS AND METHODS

### **Mite collection**

Mites were collected monthly from September to December 2021 in 15 orchards from six municipalities in the state of Michoacán (Table 1, Figure 1). Orchards were selected based mainly on berry species and differences in the frequency of pesticide applications (Table 1). From each location (orchards), 30 samples were collected and, depending on phenological stage of the plants present at the time of sampling, each sample contained between three and six leaves, two flowers and two fruits for raspberry and blackberry, and four of 10-cm long buds for blueberry. For all berry species, leaves were collected from the lower, mid and upper strata (Akyazi et al. 2021); while flowers and fruits were collected only from the mid and upper strata. Sampling sites within each orchard were selected randomly but covered the complete area. All samples (leaves, flowers, fruits) were placed in plastic bags, transported to the laboratory and maintained at 7 °C until processed, but never more than 7 days. Phytoseiidae mites were collected from the samples using a fine brush (000) under a stereomicroscope (Leiss® Stemi 305, Carl Zeiss Microscopy GmbH, Munich, Germany), and deposited in 70% ethanol for mounting. Approximately 50% of the individuals collected from each species were separated and deposited in DNA stabilizer (DNA/RNA Shield®, Zymo Research Corp, Irvine, Cal, USA) for molecular identification.

### **Identification of mites**

#### *Morphological identification*

For identification, mites were mounted in modified Berlese medium (Schuster and Pritchard 1963). Approximately ten specimens for each identified species, were mounted in Hoyer medium (Walter and Krantz 2009), which allowed a better contrast for photography. All slides

were maintained at 45 °C for 20 days before identifications were made. Identification was achieved using a phase contrast microscope (Olympus® BX41, Olympus Corporation of the Americas, PA, USA) and the taxonomic keys of Muma and Denmark (1969), Denmark (1982), Chant and Yoshida-Shaul (1983, 1984), Swirski et al. (1998), Denmark et al. (1999), Chant and McMurtry (2007), Denmark and Evans (2011), de Moraes et al. (2013), Lopes et al. (2015), Demite et al. (2016, 2019, 2021) and Argolo et al. (2017) were used. Images were taken by differential interference contrast photography using a Photomicroscope III (Carl Zeiss Microscopy GmbH, Munich, Germany) attached to a digital camera (Cannon EOS 5D, Canon Ōita Factory, Ōita, Japan).

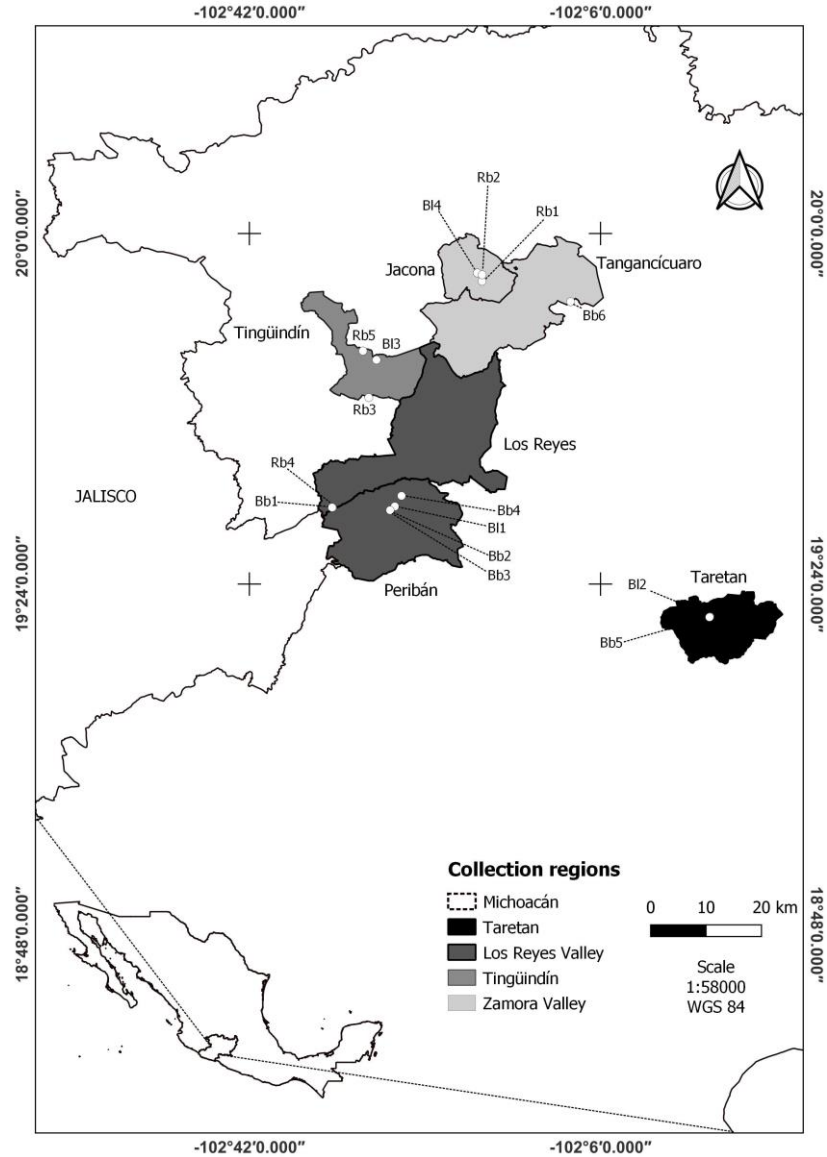
#### *Molecular identification*

To ensure a clear and direct link between morphological and molecular identification for the same specimen, the morphological identity of all specimens was made prior to DNA extraction; each specimen was first mounted temporarily in 85% to lactic acid for morphological identification, then rinsed with distilled water for 30 mins prior to DNA extraction (Tixier et al. 2010). DNA was extracted from individual specimens using the Tissue & Insect DNA MicroPrep kit (Zymo Research, Irvine, California, USA) following the manufacturer's instructions.

**Table 1** Collection sites in Michoacán state, Mexico. Pesticide application regime: H = high (more than 4 sprayings per month), M = medium (2-3 sprayings per month), L = low (one spraying per month)

Berry species	Orchard key	Coordinates	Pesticide application
blackberry	Bb1	19°31'51.87''N 102°33'31.30''W	H
	Bb2	19°31'33.75''N 102°27'35.14''W	L
	Bb3	19°31'35.00''N 102°27'34.49''W	H
	Bb4	19°33'03.62''N 102°26'24.28''W	H
	Bb5	19°20'36.03''N 101°54'49.77''W	H
	Bb6	19°52'59.60''N 102°09'03.87''W	M
raspberry	Rb1	19°55'05.72''N 102°18'08.85''W	L
	Rb2	19°55'45.98''N 102°18'08.37''W	L
	Rb3	19°43'08.51''N 102°29'45.82''W	M
	Rb4	19°31'51.13''N 102°33'28.20''W	M
	Rb5	19°47'56.29''N 102°30'21.73''W	L
blueberry	Bl1	19°31'58.27''N 102°27'05.54''W	H
	Bl2	19°20'38.41''N 101°54'46.92''W	H
	Bl3	19°47'01.45''N 102°28'58.22''W	H
	Bl4	19°55'57.11''N 102°18'37.59''W	L

Partial sequences from three genes were obtained using the following primers: LCOHCOF (TTTCAACWAATCATAAAGATATGG) and LCOHCOR (TAAACTTCWGGRTGWCCAAA RAATCA) of the Cytochrome Oxidase Subunit 1 (COI mtDNA), CytF (TAWRAARTATCAYTC DGGTTKRATATG) and CytR (CWTGAGGACAAATAWSWTTYTGAGG) of the Cytochrome Oxidase B (CytB mtDNA, and the 12SF (TACTATGTTACGACTTAT) and 12SR (AAACTAGG ATTAGATACCC) of the 12S Ribosomal RNA (12S rRNA) (Tixier et al. 2012). PCR reactions were made as described by Dos Santos and Tixier (2016); for the COI mtDNA, reactions were made in final volume of 25 µL containing 2.5 µL of 10X buffer (10X), 0.5 µL of MgCL<sub>2</sub> (25 mM), 0.5 µL of dNTPs (10 mM), 0.4 µL of each primer (10 µM), 0.13 µL of Taq polymerase (5U/ µL), and 5 µL of DNA (approx. 10 ng). For the CytB mtDNA and 12S rRNA, reactions were in final volume of 50 µL containing 5 µL of 10X buffer (10X), 0.5 µL of MgCL<sub>2</sub> (25 mM), 0.5 µL of



**Figure 1** Localities sampled in our study from five municipalities from Michoacán state.

dNTPs (10 mM), 0.4  $\mu$ L of each primer (10  $\mu$ M), 0.13  $\mu$ L of Taq polymerase (5U/  $\mu$ L), and 5  $\mu$ L of DNA (approx. 10 ng). Reactions were made in a MyCycler thermocycler (BIO-RAD Laboratories Inc., Hercules, CA, USA), with the thermal conditions described by Dos Santos and Tixier (2016). PCR products were stained with ethidium bromide (10mg/mL), and photographed to confirm successful amplification. Products were then sent to Macrogen Inc, in South Korea for direct sequencing.

All traces were edited using the software BioEdit v. 7.2.5. (Hall 1999). Multiple alignments were made with Clustal W implemented in the software Mega v. 10.2.6 (Kumar et al. 2018). Model tests and inference cladograms were done in IQ TREE using maximum likelihood analysis (Trifinopoulos et al. 2016). The robustness of branches was estimated by bootstrap approximation analysis with 5000 repeated samples using UFBoot2 (Hoang et al. 2018). Phylogenetic trees were edited and visualized using iTOL (Letunic and Bork 2021). All sequences were deposited in GenBank and accession number are shown in Table S1.

### **Estimation of species diversity**

Once all specimens were identified to species level the following specific diversity indexes were estimated: Margalef ( $\alpha$ ) (Margalef 1958), Shannon-Wiener ( $H'$ ) (Shannon and Weaver 1949) and equality of Pielou ( $E$ ). In addition, the faunistic indexes of dominance (D), abundance (A), frequency (F) and constancy (C) were estimated using the ANAFAU software (Moraes et al. 2003).

### **Effect of berry species plant and pesticide regime on Phytoseiidae abundance**

Only data from the most abundant species were used: *T. peregrinus* and *N. californicus*. The effect of host plant (blackberry and raspberry) and pesticide regime (high= four or more sprays per month, medium=between two and three sprays per month and low=one spray per month) was determined. Data from blueberry was excluded from the analysis as only one individual of each species was found in this host plant. Prior to analyses, data were transformed using Box-Cox with  $\lambda=0.29$  and  $0.20$  for *T. peregrinus* and *N. californicus*, respectively. Transformed data were analysed using a generalized linear model (GLM) with host plant and pesticide regime as fixed main effects and their interaction, and sampling time and orchard as random block effects.

Variance components were estimated using the restricted maximum likelihood method (REML) in the statistical software GenStat v. 8.0 (Payne et al. 2005).



## 1.4 RESULTS

### Species diversity

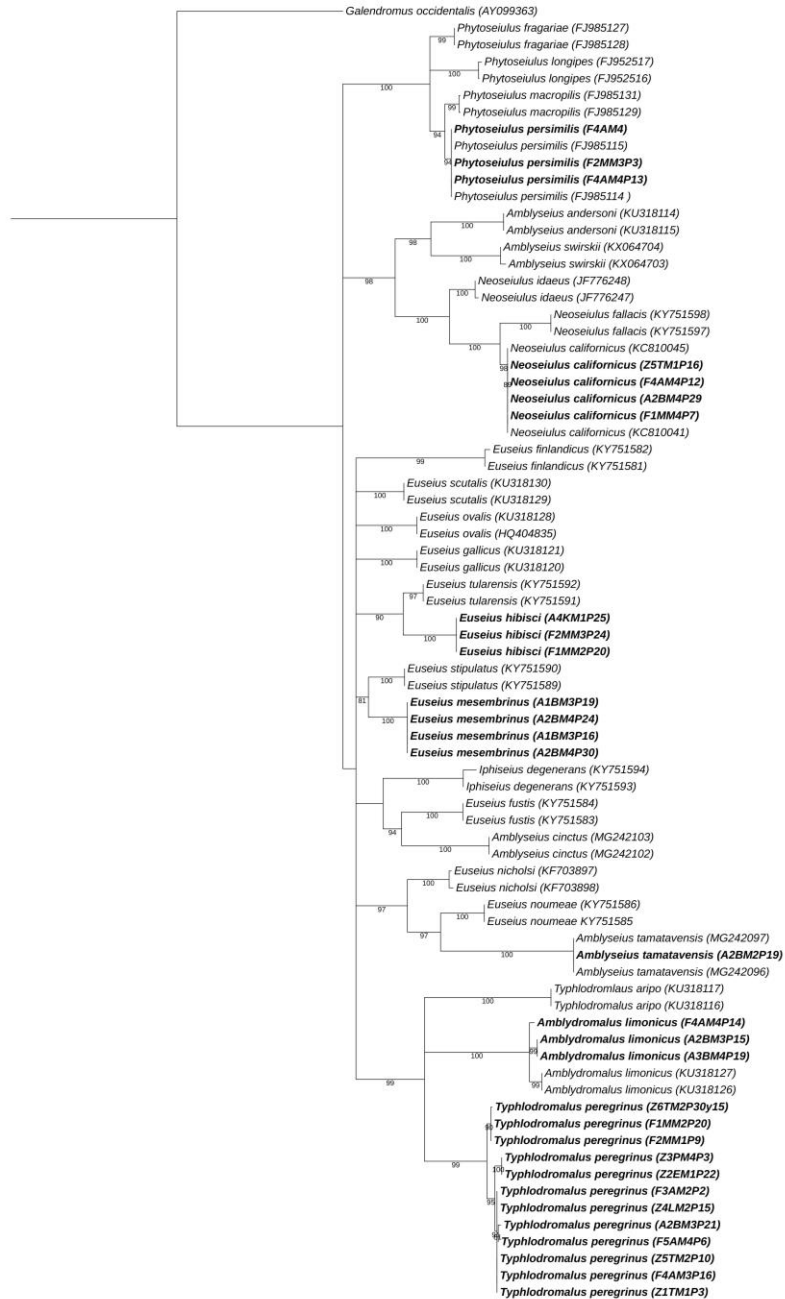
Eleven species from eight genera and two taxonomic subfamilies were identified. Raspberry (eight species) had the largest number of species found, followed by blackberry (seven species) and blueberry (six species) (Table 2). The main morphological characteristics we used for the identification of some of the mites are shown as supplemental figures: *Amblydromalus limonicus* (Figure S1 a-d); *Amblyseius tamatavensis* (Figure S2 a-d); *Euseius hibisci* (Figure S3 a-d); *Euseius mesembrinus* (Figure S4 a-d); *Typhlodromalus peregrinus* (Figure S5 a-d); *Neoseiulus mumai* (Figure S6 a-d); *Metaseiulus negundinis* (Figure S7 a-d), and *Typhlodromina subtropica* (Figure S8 a-d). The morphological identity of these species was confirmed using the partial sequence information from the 12S rRNA (Figure 2), CytB mtDNA (Figure 3) and COI mtDNA (Figure 4). The phylogenetic placement of all sequences confirming taxonomic identity always had bootstrap values above 90. Markers with the highest success rate for PCR amplification were the 12S rRNA with 30 sequences in total for seven species (Figure 2) and CytB mtDNA with 27 sequences for six species (Figure 3). For COI mtDNA we obtained only 17 sequences for six species. Partial sequences of the species *N. californicus*, *P. persimilis*, *E. mesembrinus* and *Amblydromalus limonicus* were successfully obtained with all three markers; for *E. hibisci* and *T. peregrinus*, only with primers for 12S rRNA and CytB mtDNA. Only one sequence was obtained for the species *A. tamatavensis* from the 12S rRNA gene (Figure 2), and for *T. subtropica* and *M. negundinis* from the COI mtDNA (Figure 4) gene. The species *Amblyseius swirskii* and *N. mumai*, both in raspberries, were only identified based on morphological attributes, because no PCR amplification could be obtained with the three molecular markers from the small number of specimens obtained.

Diversity indices estimated for each berry species (Table 3) confirmed that raspberry showed the largest diversity index ( $H'$ ) followed by blackberry and blueberry. The most common species were *T. peregrinus* and *N. californicus*, these species resulted as highly dominant and highly abundant in blackberry; dominant and very abundant in raspberry; and not dominant but common in blueberry (Table 3).

**Table 2** Species and number of mite specimens collected from blackberry, raspberry and blueberry orchards.

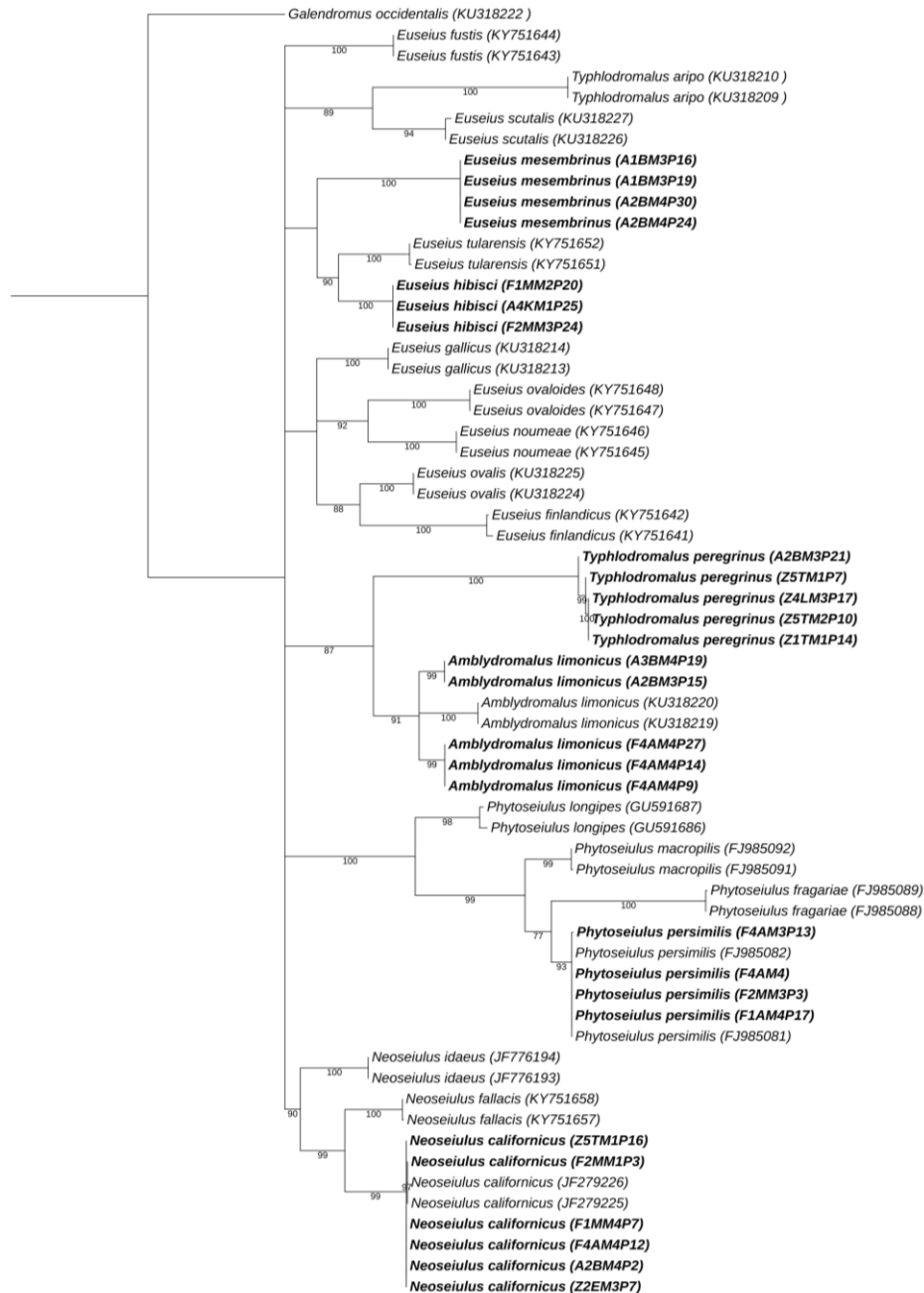
<b>Subfamily</b>	<b>Species</b>	<b>blackberry</b>	<b>raspberry</b>	<b>blueberry</b>
	<i>Amblyseius tamatavensis</i>	0	0	1
	<i>Amblyseius swirskii</i>	0	3	0
	<i>Amblydromalus limonicus</i>	0	39	7
	<i>Euseius hibisci</i>	1	202	16
Amblyseiinae	<i>Euseius mesembrinus</i>	2	0	5
	<i>Typhlodromalus peregrinus</i>	429	336	1
	<i>Neoseiulus californicus</i>	79	287	1
	<i>Neoseiulus mumai</i>	0	1	0
	<i>Phytoseiulus persimilis</i>	1	80	0
	<i>Metaseiulus negundinis</i>	17	0	0
Typhlodrominae	<i>Typhlodromina subtropica</i>	5	1	0

Tree scale: 0.1



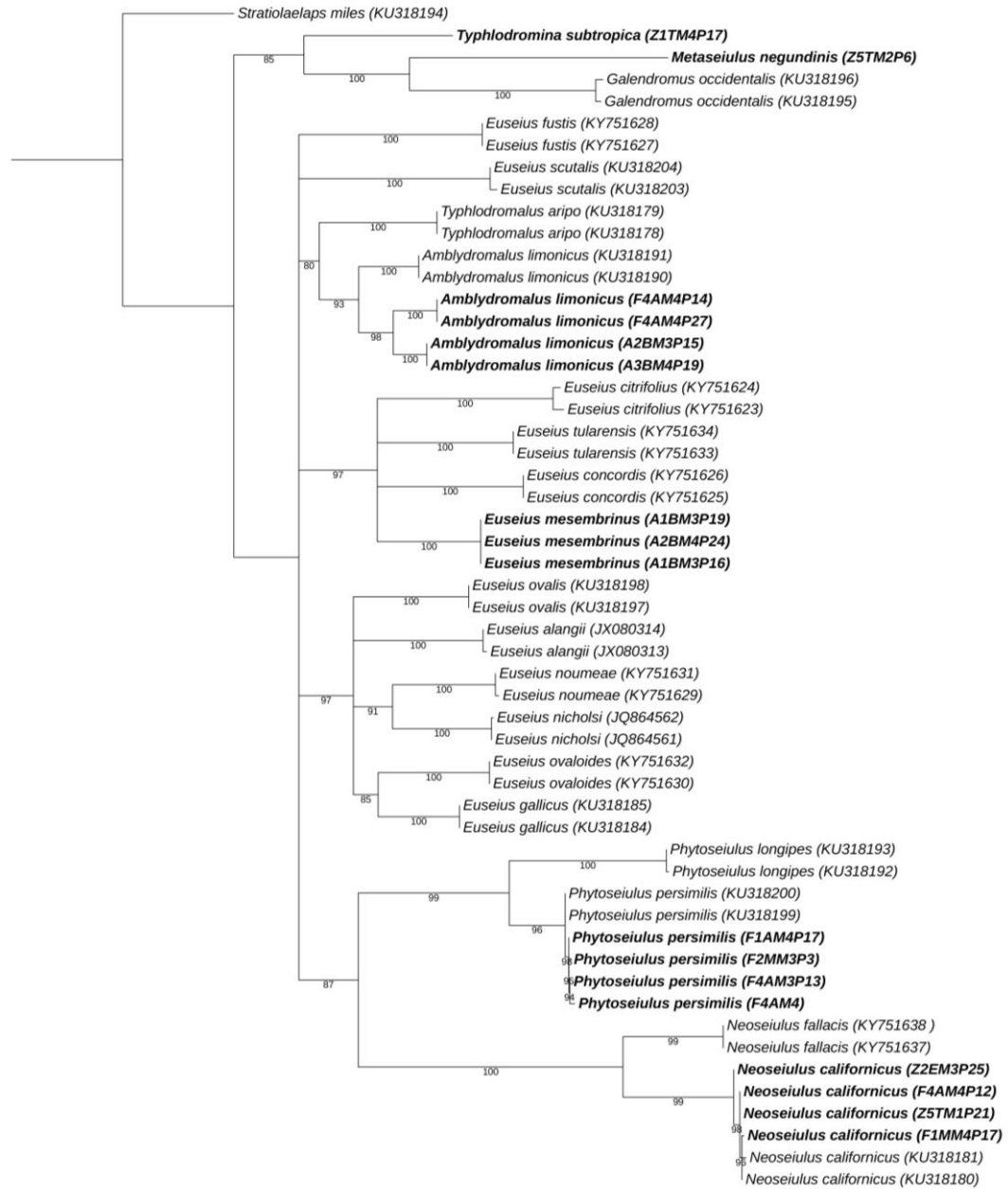
**Figure 2** Phylogeny of Phytoseiidae mites inferred from maximum likelihood analysis of partial sequences of the 12S rRNA gene. Sequences used as reference are labeled with GenBank accession numbers in brackets. Sequences in bold were obtained in this study and labels in brackets are specimen code. Genbank accession numbers for each specimen are shown in Supplemental Table S1. Only bootstrap values above 80% are shown.

Tree scale: 0.1



**Figure 3** Phylogeny of Phytoseiidae mites inferred from maximum likelihood analysis of partial sequences of the CytB mtDNA gene. Sequences used as reference are labeled with GenBank accession numbers in brackets. Sequences in bold were obtained in this study and labels in brackets are specimen code. Genbank accession numbers for each specimen are shown in Supplemental Table S1. Only bootstrap values above 80% are shown.

Tree scale: 0.1



**Figure 4** Phylogeny of Phytoseiidae mites inferred from maximum likelihood analysis of partial sequences of the COI mtDNA gene. Sequences used as reference are labeled with GenBank accession numbers in brackets. Sequences in bold were obtained in this study and labels in brackets are specimen code. Genbank accession numbers for each specimen are shown in Supplemental Table S1. Only bootstrap values above 80% are shown.

**Table 3** Diversity indices estimated for mites in each berry orchards after ANAFAU analysis. **D** = Dominance (SD = highly dominant, D = dominant, ND = not dominant); **A** = Abundance (sa = highly abundant, ma = very abundant, a = abundant, c = common, d = disperse); **F** Frequency (SF = highly frequent, MF = very frequent, F = frequent, FF = infrequent); **C** = Constancy (W = constant, Y = accessory).

Species	blackberry				raspberry				blueberry			
	D	A	F	C	D	A	F	C	D	A	F	C
<i>A. tamatavensis</i>	-	-	-	-	-	-	-	-	ND	c	F	Y
<i>A. swirskii</i>	-	-	-	-	ND	d	FF	W	-	-	-	-
<i>A. limonicus</i>	-	-	-	-	D	c	F	W	D	c	F	W
<i>E. hibisci</i>	ND	ma	F	Y	D	a	MF	W	D	ma	MF	W
<i>E. mesembrinus</i>	ND	ma	F	Y	ND	d	FF	Y	ND	c	F	W
<i>T. peregrinus</i>	SD	sa	SF	W	D	ma	MF	W	ND	c	F	Y
<i>N. californicus</i>	SD	sa	SF	W	D	ma	MF	W	ND	c	F	Y
<i>N. mumai</i>	-	-	-	-	ND	d	FF	Y	-	-	-	-
<i>P. persimilis</i>	ND	ma	F	Y	D	c	F	W	-	-	-	-
<i>M. negundinis</i>	D	ma	MF	W	-	-	-	-	-	-	-	-
<i>T. subtropica</i>	ND	ma	F	W	ND	d	FF	Y	-	-	-	-
Shannon-Wiener index(H')	1.043 (0.97-1.115)				1.438 (1.436-1.439)				1.304 (1.249-1.358)			
Margalef index ( $\alpha$ )	1.228				1.167				1.456			
Pielou evenness index (E)	0.648				0.654				0.728			

### Effect of berry species and pesticide regime on Phytoseiidae abundance

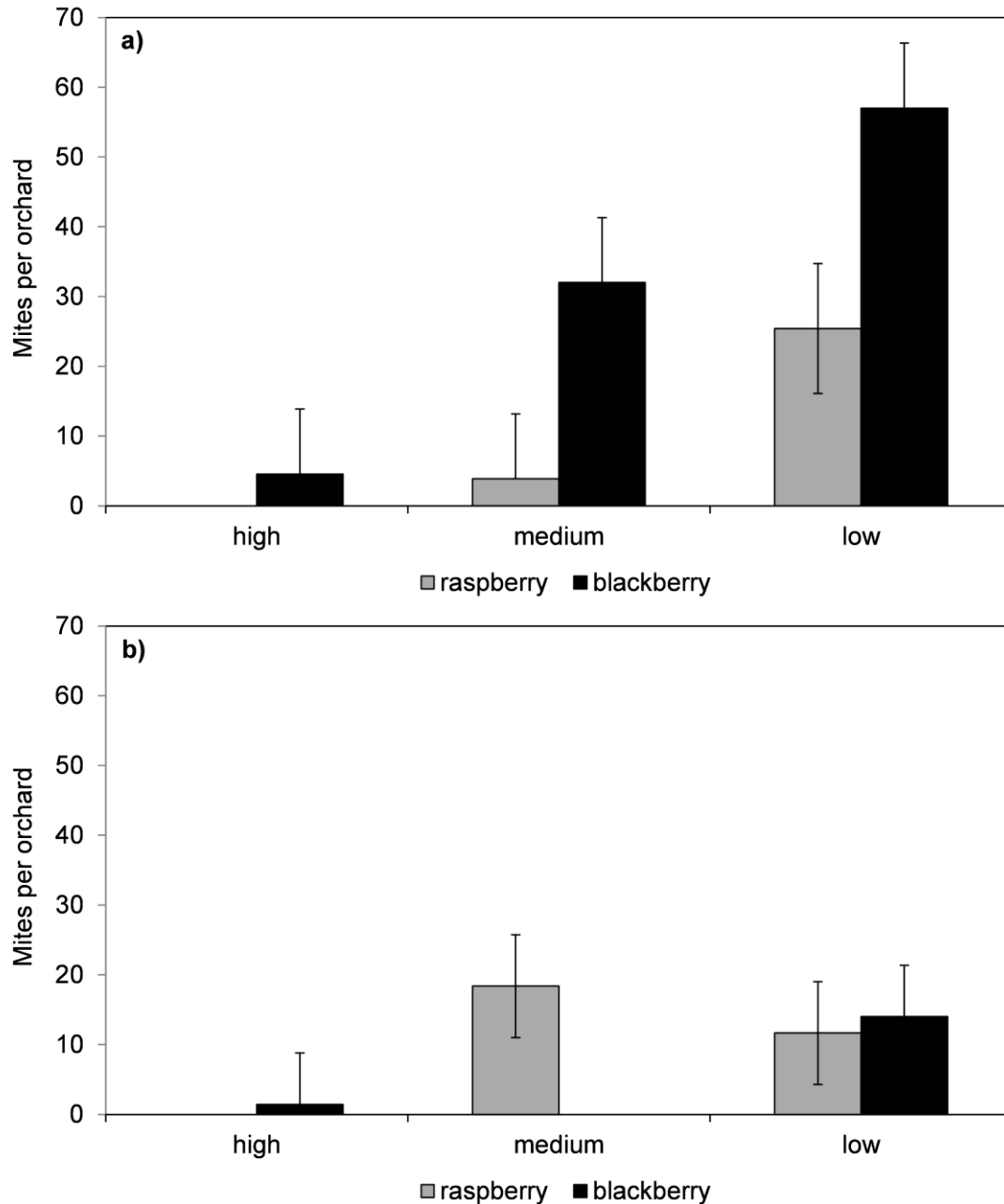
There was no significant effect of berry species on abundance of *T. peregrinus* ( $F_{1, 36.0}=0.07, P=0.792$ ), but there was a significant effect of pesticide regime ( $F_{2, 36.0}=21.78, P<0.001$ ), and this was regardless of berry species ( $F_{1, 36.0}=1.69, P=0.201$ ). The largest populations of *T. peregrinus* were found in orchards which had received the smallest number of pesticide applications (Figure 5a); although no significant differences could be detected between berry species, there was trend for more *T. peregrinus* individuals on blackberry than raspberry. The abundance of *N. californicus* was significantly affected by berry species ( $F_{1, 36.0}=4.81, P=0.035$ ) as more individuals were found in raspberry (Figure 5b). There was no effect of pesticide regime on abundance of *N. californicus* ( $F_{3, 36.0}=0.07, P=0.968$ ) but this was not consistent between the two berry species ( $F_{1, 36.0}=6.98, P=0.012$ ), as more individuals of *N. californicus* were found in

the medium pesticide regime on raspberry but none on blackberry, compared to the lowest pesticide regime where *N. californicus* was found on raspberry and blackberry, but practically none could be found in the highest pesticide regime (Figure 5b).

## 1.5 DISCUSSION

We found 11 species of Phytoseiidae mites in berry crops, all have been reported previously in this part of the Americas, with the exception of *Amblyseius tamatavensis* which has not been reported for this region (Demite et al. 2022). The complex requirements for the effective morphological identification of these species suggests that a combination of morphological and molecular techniques should perhaps be mandatory. For example, the morphological identification of *Euseius hibisci* could not be achieved using only the keys of Denmark and Evans (2011) and Lopes et al. (2015), so we also used the diagnostic characteristics described by McMurtry et al. (1985) from which the majority of the characteristics confirmed the identity of *E. hibisci*. The phylogenetic analyses done using partial sequences of 12S (Figure 2) and CytB (Figure 3), clearly grouped the sequences of *E. hibisci* together and separated from the other *Euseius* species with bootstrap values above 90%. A similar methodology was used for *E. mesembrinus*, where we needed to use keys developed by Denmark and Evans (2011), Lopes et al. (2015), Demite et al. (2016), Abou-Setta et al. (1991) and Cavalcante et al. (2021) for the morphological identification and confirmed with 12S (Figure 2) and CytB (Figure 3) partial sequences. Overall, all mite species could be successfully identified combining morphological and molecular techniques. From the three markers used, we suggest using the 12S rRNA and CytB mtDNA markers to identify and/or confirm the taxonomic status of Phytoseiidae mites, as both could be successfully amplified using DNA from all our species. In addition, our sequences deposited in GenBank, could now be used as references for accurate identification in future studies.





**Figure 5** Mean number of mites found in blackberry and raspberry orchards, and under three different pesticide applications regimes (**high**=more than four applications, **medium**=2-3 applications and **low**=one application) for *Typhlodromalus peregrinus* (a) and *Neoseiulus californicus* (b). Error bars represent  $\pm 1$  SEDM (standard error of differences).

The largest mite diversity was found on raspberry, followed by blackberry and blueberry (Table 3). Our results suggest that the main contributing factor for this was the associated pesticide regimes; the majority of blueberry orchards received four or more pesticide sprays during this

study (Table 1), whereas in raspberry, all orchards sampled received only one application (low) or between two and three sprays (medium). Although the negative effects of synthetic pesticides on diversity of predatory mite species has been reported previously in apple (Thistlewood 1991) and vineyards (Moth et al. 2021), more research documenting this is needed in other agricultural systems particularly economically important berry crops. The effect of pesticide regimes was very clear for *T. peregrinus* as the highest populations were found in the orchards (both raspberry and blackberry) receiving the fewest sprays, followed by the medium number and finally the highest number which had practically no individuals (Figure 5a). Despite this, *T. peregrinus* was found in practically all sampled orchards, which could be because this species is considered is a generalist predator that can feed on a range of mites (e.g. *T. urticae* [Fouly et al. 1995], *Panonychus citri* [McGregor] [Tetranychidae], *Phyllocoptruta oleivora* [Ashmead] [Eriophyidae] [Childers and Abou-Setta 1999; Fadamiro et al. 2009], *Polyphagotarsonemus latus* [Peña 1992; Silva et al. 2016], *Frankliniella* spp. [Thripidae] [Childers and Denmark 2011]), but also on different types of pollen (Peña 1992; Fouly et al. 1995; Villanueva and Childers 2004; Cañarte et al. 2020), which could enable this species to survive even when there were no potential prey. The effect of pesticide application regimes on *N. californicus* was not clear. Despite always being in lower abundance than *T. peregrinus*, a similar mean number of *N. californicus* were found in medium and low pesticide regimes particularly in raspberry; this could be due to commercial releases of this species in berry crops, making it difficult to assess their direct effect of pesticides. *N. californicus* is adapted to a wide range of climates (Demite et al. 2022), encouraging its wide release as biological control agent of various pests; despite being considered a specialist on Tetranychidae (McMurtry et al. 2013), it can also feed on thrips (Weintraub and Palevsky 2008; Rahmani et al. 2016), white flies (Shehawy et al. 2020), Tenuipalpidae (Silva et al. 2015; Vázquez-Benito et al. 2022),

Tarsonemidae (Easterbrook et al. 2001; Zhu et al. 2019) and also pollen (Soltaniyan et al. 2020), encouraging its presence in orchards in the absence of preys.

In blueberry, we found the lowest diversity of predatory mite species; although we believe this is mainly due to the larger number of pesticide sprays (high) than in raspberry or blackberry. Others factors could also be playing a minor role. Typically, *T. urticae* is not common in blueberry (Migeon and Dorkeld 2022), compared with raspberry and blackberry where it is very common (Márquez-Chavez et al. 2019); this could be why there are more predatory mites species in blackberry and raspberry than in blueberry. It is also possible that the glabrous and waxy leaves of blueberry prevent establishment of *T. peregrinus* or *N. californicus* (McMurtry et al. 2013). The species *Euseius hibisci* and *Amblydromalus limonicus*, were the most abundant in blueberry. *Euseius hibisci* has been reported as abundant in avocado and citrus orchards (Grafton-Cardwell et al. 2020) and is a generalist predator (McMurtry et al. 2013), which suggest it could be feeding on other pest mites present in blueberry. Recent studies reported that *Scirtothrips dorsalis* Hoot causes damages in blueberry (Ortiz et al. 2020), and *Amblydromalus limonicus* is an effective biological control agent for this pest (Schoeller et al. 2020, 2022); it is therefore possible that *A. limonicus* could be preying on *S. dorsalis*, as well as other mite and insect pests species (Knapp et al. 2013; Xu and Zhang 2015; Lam et al. 2019) and also pollen (Samaras et al. 2015, 2019, 2021), allowing it to establish in blueberry. In addition, *A. limonicus* prefers glabrous leaves as a host plant (McMurtry et al. 2013), which could also favour its establishment in blueberry.

In conclusion, when identifying predatory mites, a combination of morphological attributes and molecular techniques is important to obtain accurate results. This allowed us to draw more robust conclusions when comparing species diversity amongst berry crops. In this regard, species diversity of predatory mites in the family Phytoseiidae was affected by the interaction between

berry species and pesticide regimes. This interaction was complex and dynamic as pesticide regimes can drastically affect the diversity of species as seen for *T. peregrinus* and *N. californicus* in blackberry and raspberry. However, the high pesticide regime in blueberry, in combination with morphological characteristics of the leaves and the presence of suitable prey, seems to have a major role in determining the diversity of predatory species in blueberry.

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## CONCLUSIONES GENERALES

La zona productora de berries en Michoacán posee una diversidad importante de fitoseidos. Un total de 11 especies fueron identificadas: *Amblydromalus limonicus*, *Amblyseius swirskii*, *Amblyseius tamatavensis*, *Euseius hibisci*, *Euseius mesembrinus*, *Typhlodromalus peregrinus*, *Neoseiulus californicus*, *Neoseiulus mumai*, *Phytoseiulus persimilis*, *Metaseiulus negundinis* y *Typhlodromina subtropica*.

Las especies más abundantes en zarzamora y frambuesa fueron *Typhlodromalus peregrinus* y *Neoseiulus californicus*. En arándano las más abundantes fueron *Euseius hibisci* y *Amblydromalus limonicus*.

Las poblaciones de *T. peregrinus* se vieron significativamente disminuidas cuando el régimen de aplicaciones de plaguicidas fue más elevado. *T. peregrinus* no mostró diferencias significativas entre frambuesa y zarzamora.

Los efectos de las aplicaciones de plaguicidas no fueron significativos para *N. californicus*. No obstante, mayores poblaciones se presentaron en frambuesa en relación a zarzamora. Las liberaciones comerciales de esta especie en algunos huertos de frambuesa pudieron haber influido en estas diferencias.

Se requieren más estudios a cerca del rol que tiene *T. peregrinus* y *N. californicus* en la regulación de plagas en frambuesa y zarzamora, así como de la interacción de éstos en el agroecosistema. De igual manera, es necesario obtener más información sobre el potencial de *E. hibisci* y *A. limonicus* en la regulación de plagas en arándano.

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## ANEXOS

### Supplemental table

**Table S 1** GenBank accession numbers for partial sequences from three genes obtained for mites in family Phytoseiidae.

Species	label	12S rRNA	CytB mtDNA	COI mtDNA
	F4AM4			
<i>Phytoseiulus persimilis</i>	F2MM3P3			
	F4AM4P13			
	F1AMAP17			
	Z5TM1P16			
<i>Neoseiulus californicus</i>	F4AM4P12			
	A2BM4P12			
	A2BM4P29			
	F1MM4P7			
	F2MM1P3			
	Z2EM3P7			
	Z2EM3P25			
<i>Euseius hibisci</i>	A4KM1P25			

F2MM3P24

F1MM2P20

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A1BM3P19

A2BM4P24

*Euseius mesembrinus*

A1BM3P16

A2BM4P30

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*Amblyseius*

A2BM2P19

*tamatavensis*

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F4AM4P14

A2BM3015

*Amblydromalus*

A3BM4P19

*limonicus*

F4AM4P27

F4AM4P9

---

Z6TM2P30

*Typhlodromalus*

F1MM2P20

*peregrinus*

F2MM1P9

Z3PM4P3

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Z2EM1P22

F3AM2P2

Z4LM2P15

A2BM3P21

F5AM4P6

Z5TM2P10

F4AM3P16

Z1TM1P3

Z5TM1P7

Z4LM3P17

Z1TM1P14

---

*Typhlodromina* Z1TM4P17

*subtropica*

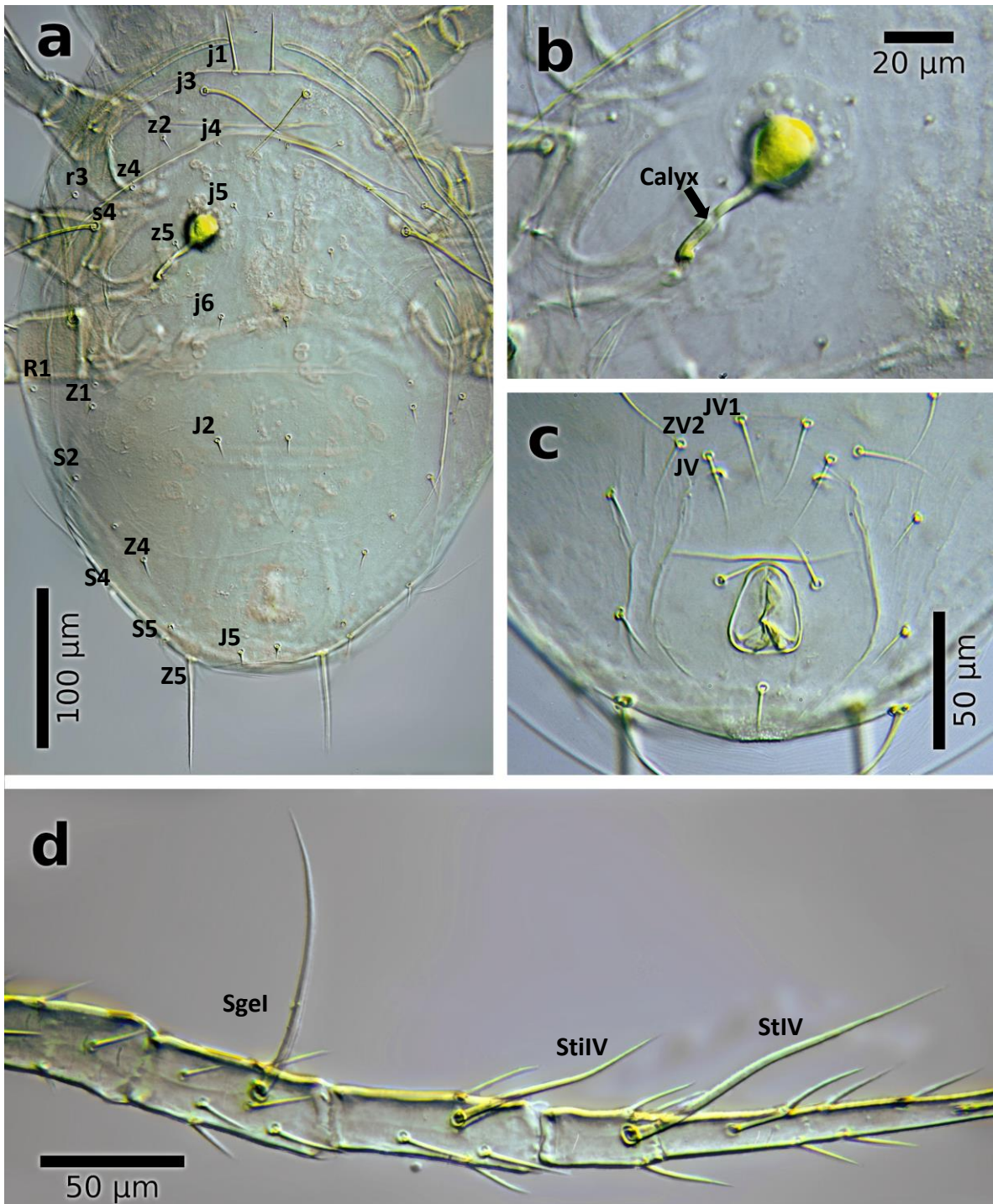
---

*Metaseiulus* Z5TM2P6

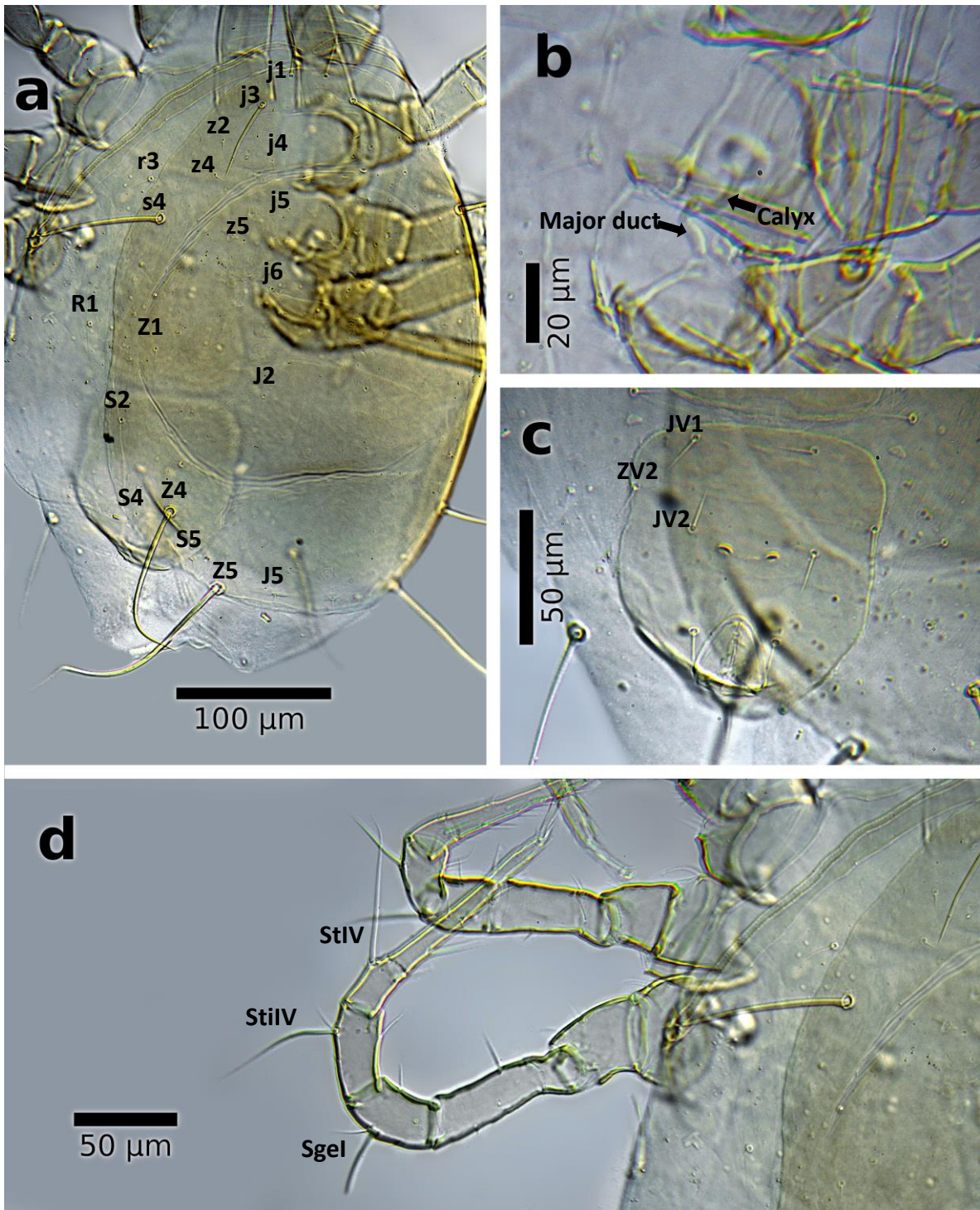
*negundinis*

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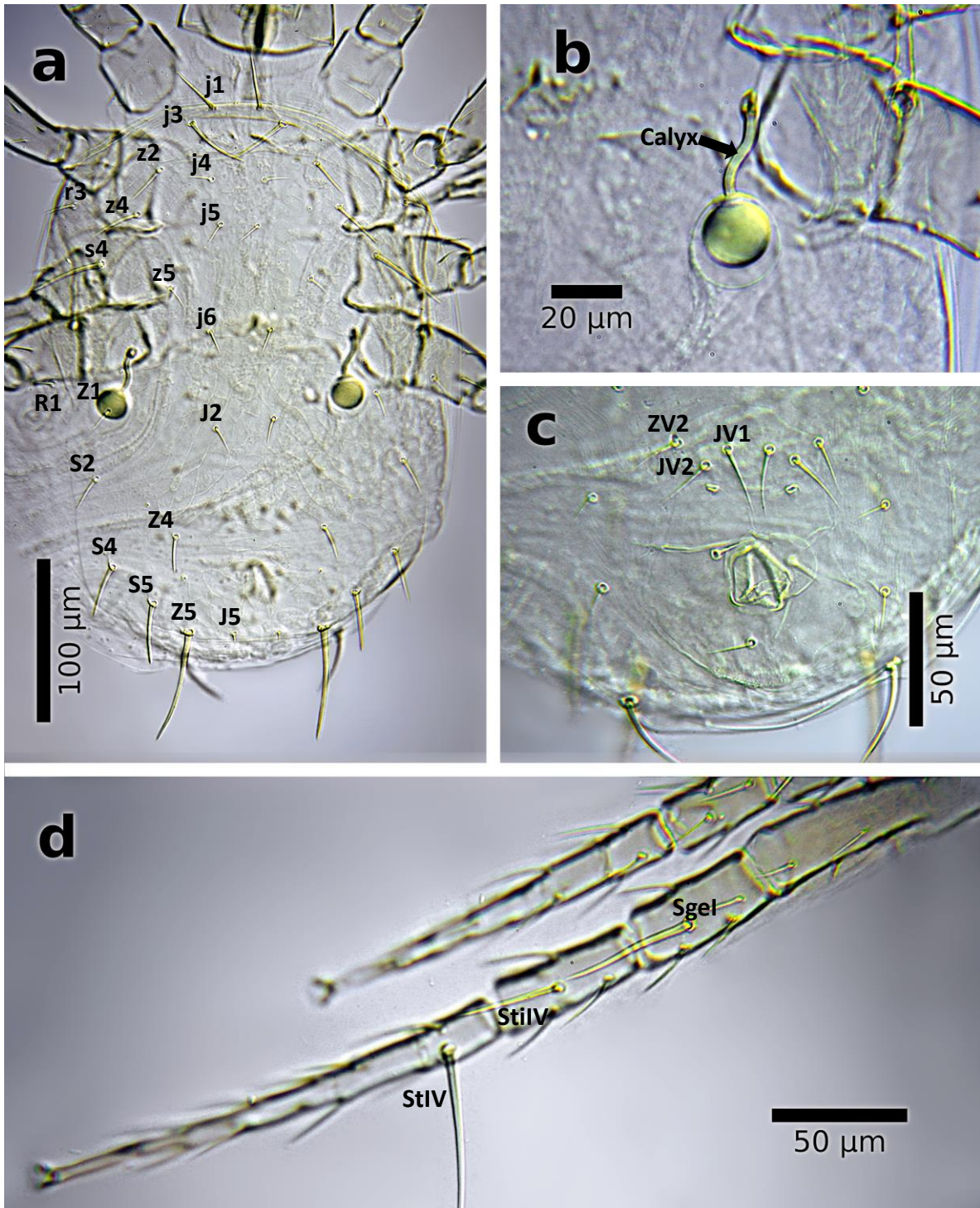
Supplemental figures



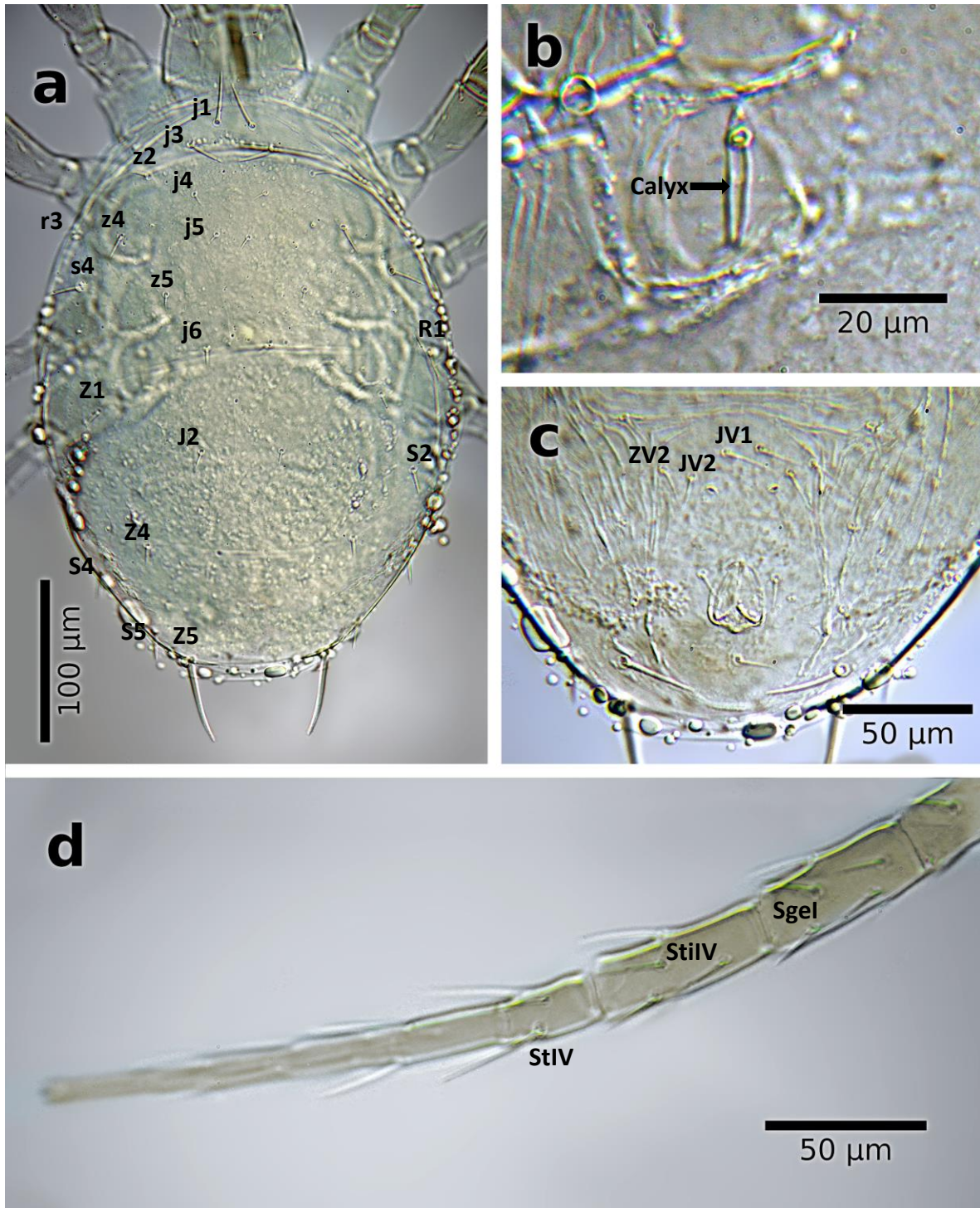
**Figure S 1** *Amblydromalus limonicus* (female). **a**) dorsal shield and dorsal setae, **b**) spermatheca, **c**) ventrianal shield, **d**) macrosetae from leg IV.



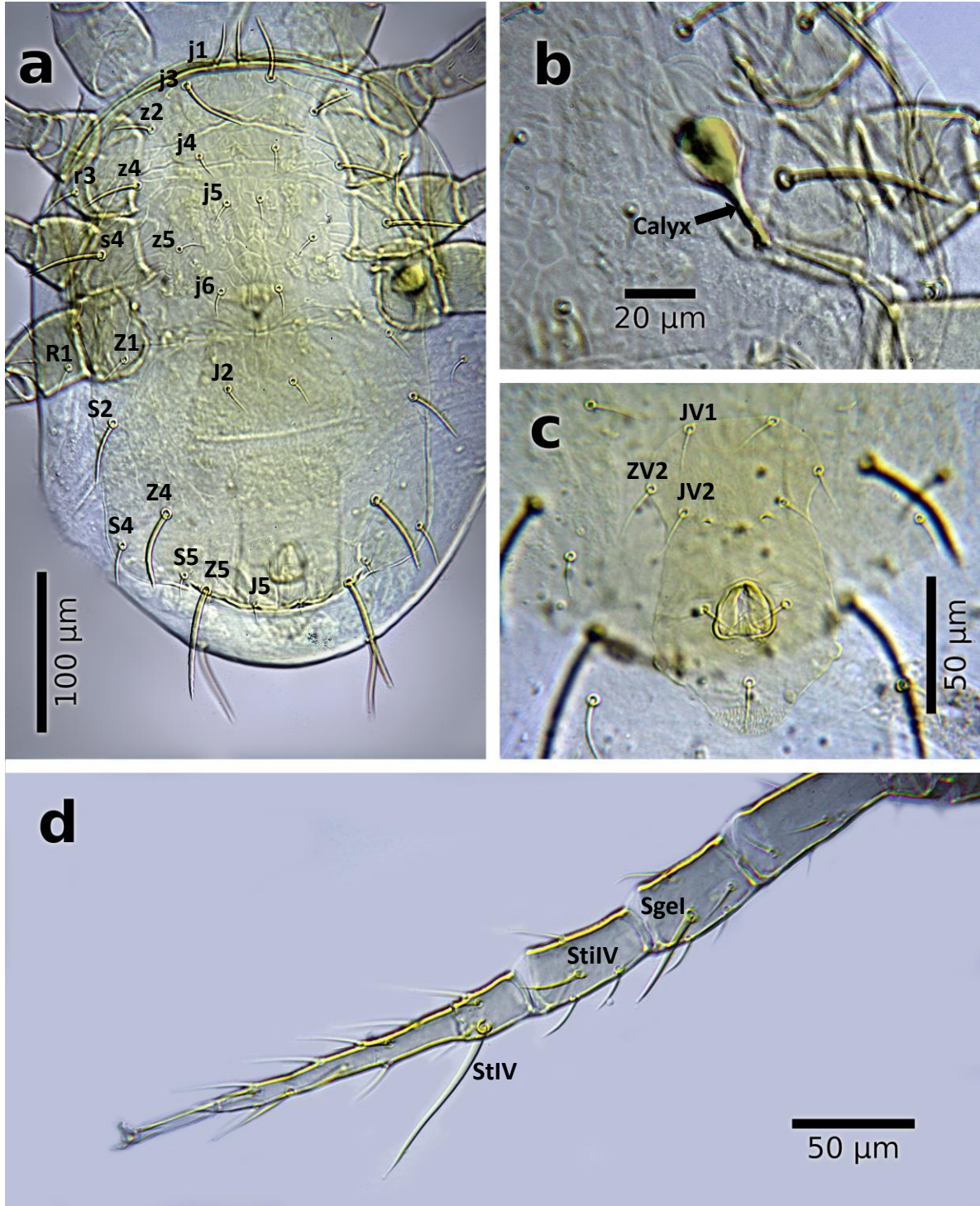
**Figure S 2** *Amblyseius tamatavensis* (female). **a)** dorsal shield and dorsal setae, **b)** spermatheca, **c)** ventrianal shield, **d)** macrosetae from leg IV.



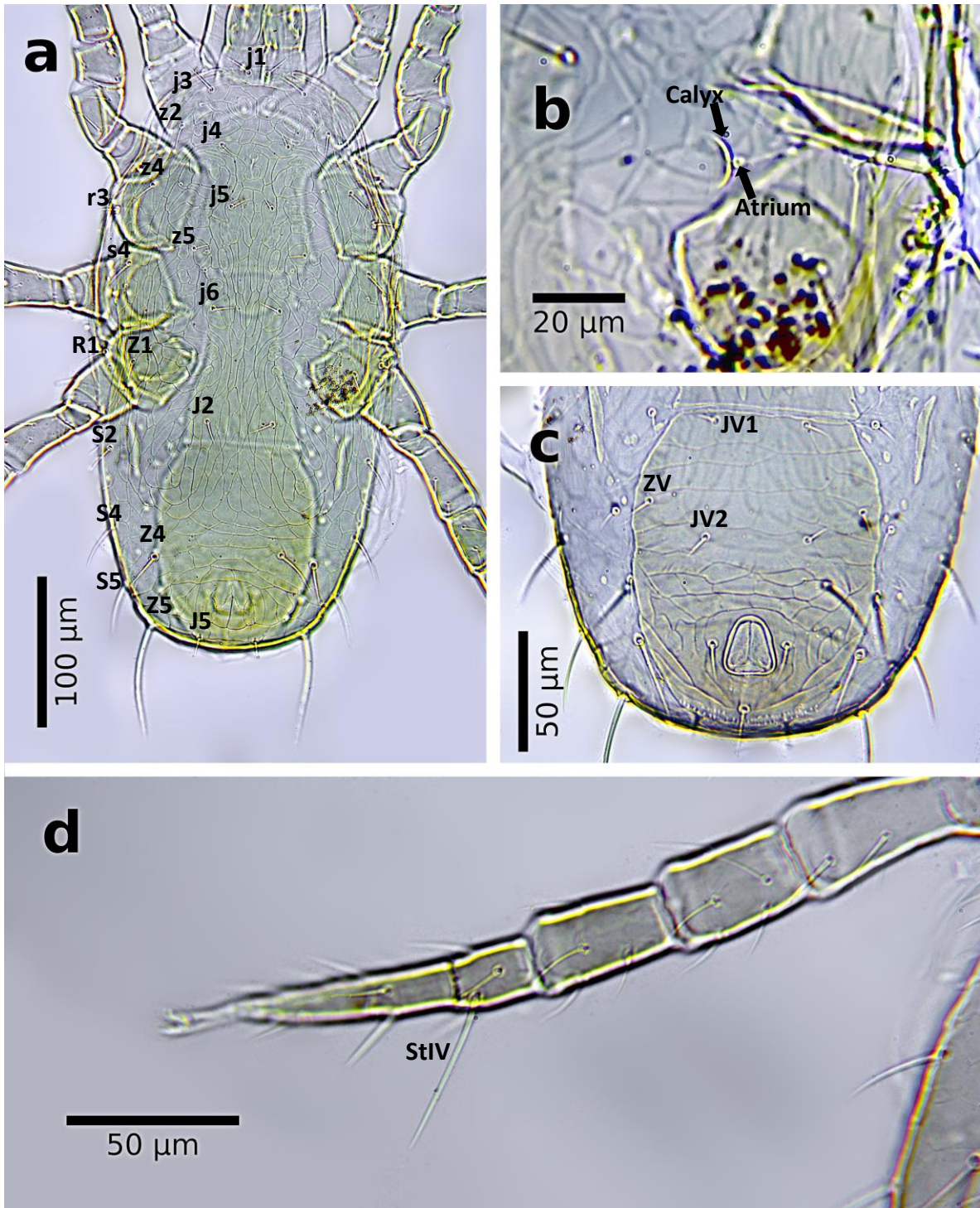
**Figure S 3** *Euseius hibisci* (female). **a**) dorsal shield and dorsal setae, **b**) spermatheca, **c**) ventrianal shield, **d**) macrosetae from leg IV.



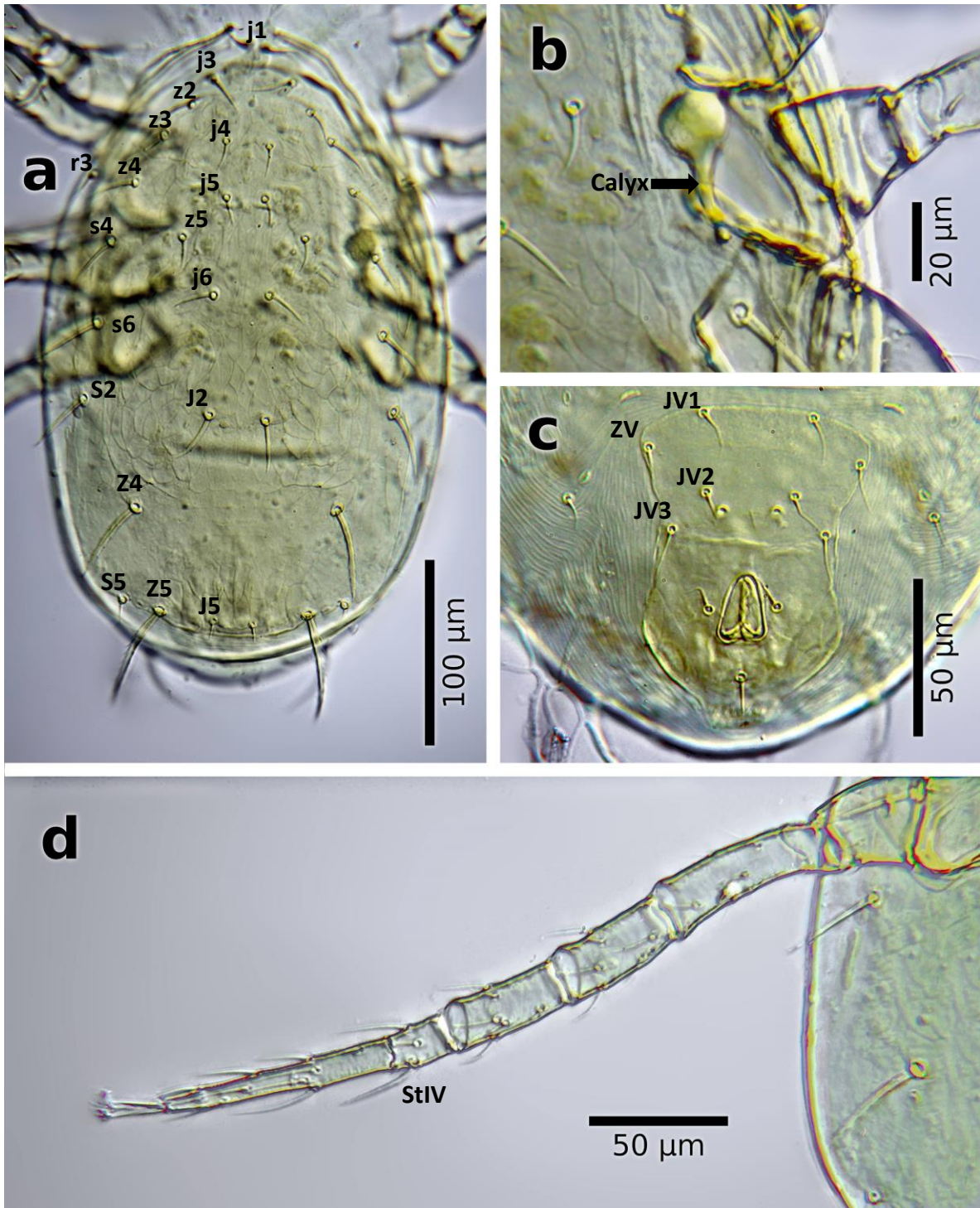
**Figure S 4** *Euseius mesembrinus* (female). **a)** dorsal shield and dorsal setae, **b)** spermatheca, **c)** ventrianal shield, **d)** macrosetae from leg IV.



**Figure S 5** *Typhlodromalus peregrinus* (female). **a)** dorsal shield and dorsal setae, **b)** spermatheca, **c)** ventrianal shield, **d)** macrosetae from leg IV.

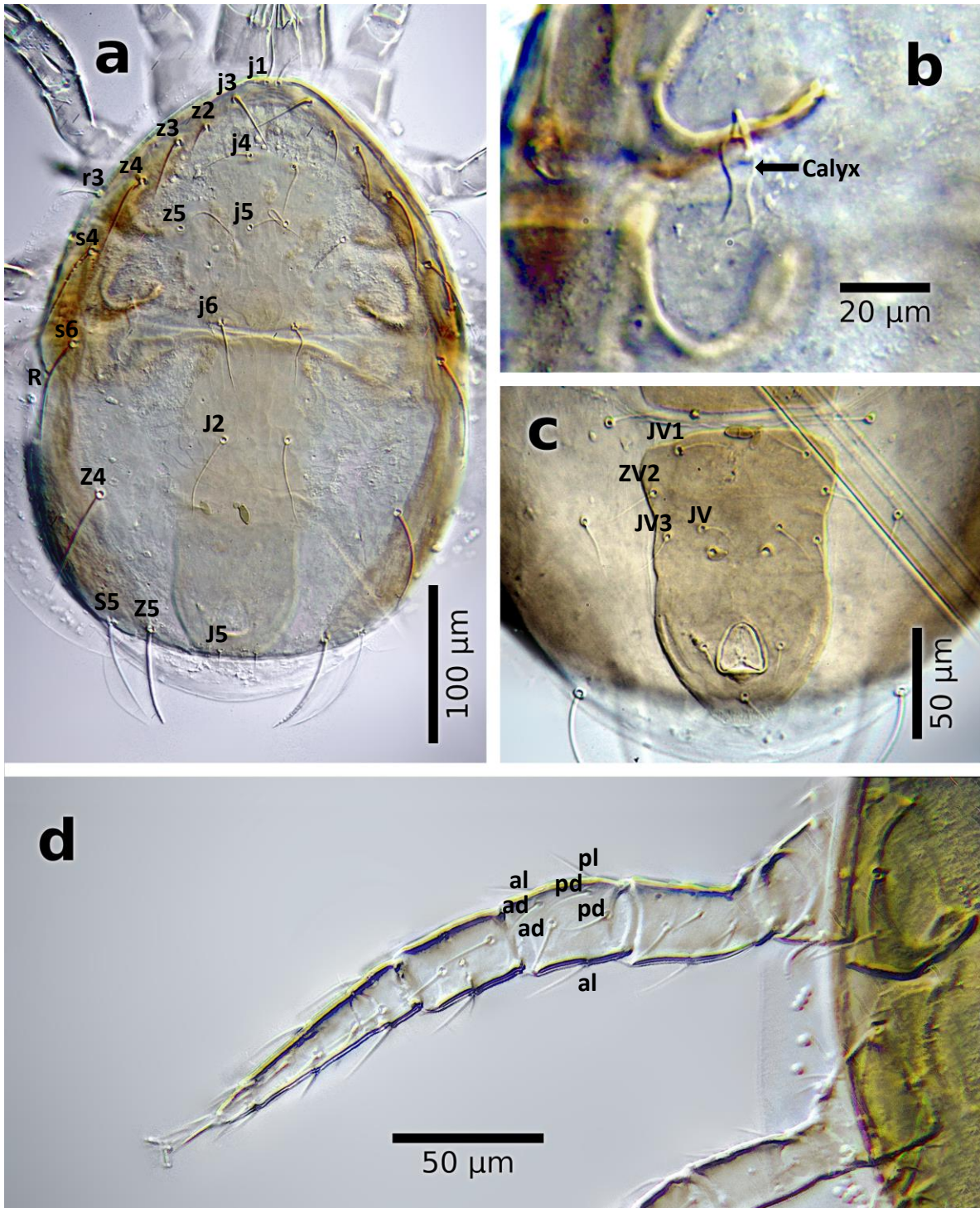


**Figure S 6** *Neoseiulus mumai* (female). **a)** dorsal shield and dorsal setae, **b)** spermatheca, **c)** ventrianal shield, **d)** macrosetae from leg IV.



**Figure S 7** *Metaseiulus negudinis* (female). **a)** dorsal shield and dorsal setae, **b)** spermatheca, **c)** ventrianal shield, **d)** macrosetae from leg IV.





**Figure S 8** *Typhlodromina subtropica* (female). **a)** dorsal shield and dorsal setae, **b)** spermatheca, **c)** ventrianal shield, **d)** macrosetae from leg IV.