Phyllocoptes parviflori is a distinct species and a vector of the pervasive blackberry leaf mottle associated virus

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Abstract

Several viruses are transmitted by eriophyid mites (Acariformes: Eriophyoidea) including blackberry leaf mottle-associated emaravirus (BLMaV) (*Emaravirus rubi*). BLMaV is transmitted by an unidentified eriophyid species and is involved in blackberry yellow vein, a devastating disease in the southeastern United States. In this study, we assessed the eriophyid mite *Phylocoptes parviflori* as a vector of BLMaV and clarified its taxonomic status as it was previously synonymized with *Phyllocoptes gracilis*. *P. parviflori* can efficiently transmit BLMaV. The virus was found to cause yellow vein disease symptoms on 'Ouachita' blackberry marking a paradigm shift as disease symptoms have always been associated with multiple virus infections. Therefore, we propose renaming the virus to blackberry leaf mottle virus. The occurrence of *P. parviflori* on wild and cultivated blackberries, as well as its ability to colonize other *Rubus* species, enhances its importance as a major contributor to the spread of yellow vein disease.

Key words: eriophyid, transmission, blackberry leaf mottle associated virus

1. Introduction

Eriophyids (Acariformes: Eriophyoidea) are phytophagous arthropods that feed on all plant parts except roots. Many species are economically significant as pests and virus vectors. Several viruses are transmitted by eriophyid mites, with the most extensively studied being members of the genera *Rymovirus* and *Tritimovirus* (family *Potyviridae*), *Allexivirus* (family *Alphaflexiviridae*) (de Lillo and Skoracka, 2010) and *Emaravirus* (family *Fimoviridae*, order *Bunyavirales*), the focus of this communication.

Two emaraviruses, blackberry leaf mottle-associated emaravirus (BLMaV) (*Emaravirus rubi*) and raspberry leaf blotch emaravirus (RLBV) (*Emaravirus idaeobati*), have been identified in *Rubus*, leading to significant losses in blackberry and raspberry production, respectively (Hassan et al., 2017; McGavin et al., 2012). BLMaV, along with other viruses cause blackberry yellow vein disease (BYVD), the most economically significant viral disease of blackberry in the southeastern United States. BYVD affects fruit quality and yield, resulting in yellowing of the main veins, mottling, oak leaf patterns, and chlorotic ringspots. The disease reduces yield and can render infected plants unproductive in as few as five years (Hassan et al., 2017; Martin et al., 2013a). Hassan et al. (2017) determined that the virus can be transmitted by unidentified eriophyid species, highlighting the importance of this finding for the management of BYVD.

RLBV was discovered in Europe in raspberry plants exhibiting symptoms of leaf blotch disorder. The disease was reported for the first time some 100 yearsago and attributed to feeding damage caused by *Phyllocoptes gracilis* (Massee, 1931). However, we now know that the disease is caused by RLBV and although there has not been conventional transmission experiments, observations suggest that *P. gracilis* is a vector (McGavin et al., 2012).

Phyllocoptes gracilis has been reported in Europe, North America, and Asia, and is considered as a common species across a wide range of hosts (Breakey, 1945; Kuang, 1995; Nalepa, 1890). Breakey (1945) initially observed this species on red raspberry in the United States, and this was confirmed by H.H. Keifer. However, a few years earlier Keifer (1939), described a closely related species named *P. parviflori*, which inhabits thimbleberry and salmonberry. In 1952, *P. parviflori* was synonymized with *P. gracilis* due to similarities in main morphological features and their shared habitat (Keifer, 1952). Later Jeppson et al. (1975) characterized *P. parviflori* as a variable species in terms of morphological features but no further research was conducted to clarify this conundrum.

We investigated the taxonomic status of *P. gracilis* and *P. parviflori* and determined that they are two distinct species. In addition, we assessed the competence of *P. parviflori* as a vector of BLMaV and estimated the transmission efficiencies through single and multiple mite transfers.

2. Material and methods

2.1. Sample collection and morphological analyses

Eriophyid-infested leaves samples were collected from raspberry (*Rubus idaeus* L.) in Poland (five locations) and the Czech Republic (one location; Supplemental Table 1). Material was examined for the presence of *P. gracilis* under a dissecting stereomicroscope. Similarly, eriophyid-infested leaves from blackberry (*Rubus* subg. *Rubus* Watson) were sampled and examined for *P. parviflori* and *P. calirubi*. The most important morphological traits commonly used in eriophyid species descriptions (Lewandowski et al., 2014; Valenzano et al., 2020) were compared using data from the original descriptions. All measurements were done on a Soft Imaging System Cell D connected to the camera and a phase-contrast Olympus BX51 microscope (Shinjuku, Tokyo, Japan). For mite transmission, *R. fruticosus* blackberry

(GenBank accessions OR001849-50) from Watsonville (California, USA), naturally infested with *P. parviflori* and BLMaV-positive, were used as the mite source. To verify the purity of the *P. parviflori* colony, 50 specimens were slide-mounted in a modified Berlese medium (Amrine and Manson, 1996) for phase-contrast microscopic examination. Moreover, ten mites from each plant were DNA-barcoded (Druciarek et al., 2019). All examined specimens are deposited as slide-mounted material in the Mite Collection of the Institute of Horticultural Sciences, Department of Plant Protection, Section of Applied Entomology, Warsaw University of Life Sciences, Warsaw, Poland. According to Liu et al., (2017), collections of the new eriophyid species, *P. parviflori* are named WULS, respectively.

2.2. Molecular data and phylogenetic analyses

Specimens from each population were also placed in 0.5 mL microcentrifuge tubes containing 5 µL of RNase-free water and used for molecular analyses. Transcripts of the cytochrome c oxidase subunit I (COI, mtDNA), D2 region in 28S and ITS1 of ribosomal DNA (D2, 28S and ITS1 rDNA) were amplified from single specimens using direct RT-PCR (Druciarek et al., 2019). Primers used for amplification and sequencing are shown in Supplementary table 2. Amplicons were Sanger-sequenced bidirectionally at Macrogen Inc. All sequences of *Phyllocoptes* have been deposited in GenBank (Supplemental Table 1).

Datasets containing 573 bp fragments for COI (mtDNA) and 604 bp for D2 (28S rDNA), were analyzed in Mega X (Kumar et al., 2018) unless otherwise stated. Sequences of *Leipothrix juniperensis* (GenBank MZ274920 and MZ289016), belonging to the Phyllocoptinae, Phyllocoptini were used as the outgroups for both datasets.

The COI and D2 sequences were aligned separately by ClustalW (Thompson et al., 1994) using default weighting parameters. Analyses of the pairwise genetic distances between and within nucleotide sequences, as well as the choice of the most appropriate evolutionary

models for estimation of inter- and intra-lineage genetic variation were performed using MEGA X (Kumar et al., 2018). The Tamura-Nei model with γ distribution (TN93 + G) (Tamura and Nei, 1993) was applied to the COI dataset, and Tamura 3-parameter model with a γ distribution (T92 + G) (Tamura, 1992) to the D2 dataset. Standard error estimates were obtained by a 1000 bootstrap pseudo-replicates.

Phylogenetic relationships for studied fragments were reconstructed using Maximum Likelihood (ML) and Bayesian Inference (BI). Models for both datasets were selected using jModelTest v.2.1.1 (Darriba et al., 2012) based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) likelihood scores for 88 different models. The GTR + I + G model (Tavaré, 1986) was applied to COI and HKY + G model (Hasegawa et al., 1985) to D2 datasets. To perform the Bayesian Inference (BI) combined analysis of COI, D2 fragments, the sequence files were concatenated in a single matrix in Mesquite v.3.0.4 (http://www.mesquiteproject.org). Bayesian analyses were performed in MrBayes v.3.2.6 (Ronquist et al., 2012). Two independent runs were executed for each dataset using four Markov chains (three heated and one cold) run for ten million generations, with trees sampled every 1000 generations. The analysis was performed until the average split deviation was below 0.01, and 25% of trees obtained at the beginning were excluded as burnin. Obtained trees were edited with FigTree v.1.4.4 (http://tree.bio.ed. ac.uk/software/figtree/).

2.3. Transmission

Mite-free black raspberry (*Rubus occidentalis*' Munger') and blackberry (*Rubus fruticosus* 'Ouachita') plants obtained from North American Plants, Inc nursery (USA) were used in two independent transmission experiments. Plants were pruned a week before mite transfer to force new growth and potted in 7.5 cm diameter pots. BLMaV-free status of the plants was verified prior to transmission studies (data not shown). Total nucleic acids were extracted

from plant tissues and reverse transcribed according to procedures described by Poudel et al. (2013). PCR targeting separately the NADH dehydrogenase β-subunit (an internal control ensuring the quality of nucleic acids) and the virus RNA 3 were performed (Hassan et al., 2017). Amplicons were Sanger sequenced bidirectionally at Macrogen Inc. Moreover, BLMaV-positive 'Ouachita' blackberry, obtained in transmission studies, was subjected to HTS, and analyzed in silico to verify its single infection status as described (Villamor et al., 2022). HTS revealed the presence of blackberry virus F, an intergrating badnavirus (Shahid et al., 2017) within the sample. The BVF presence was further investigated using primers designed to amplify both the reference genome (Shahid et al., 2017) and the HTS virus sequence (Supplemental Table 2). To determine whether BVF was present in the integrated or episomal form rolling circle amplification (RCA) and PCR/RT-PCR were performed. For PCR and RT-PCR ten plants positive for BLMaV were DNase-digested (4U of Turbo DNase, Thermo Fisher Scientific) and RNase-digested (4U of RNase T1, Thermo Fisher Scientific) separately for two hours before used as template for amplification (Supplemental Figure 3)

Adult mites were used in all transmission experiments. They were collected under a dissecting microscope with the use of an eyelash tool (human eyelash attached to a dissecting needle) and transferred onto test plants. In the first experiment, vector competence was checked using five viruliferous mites moved to each of the 20 black raspberry plants. In the second experiment, one or five viruliferous mites were moved to blackberry test plants, 20 plants in each combination. Plants were covered with plastic (PET) cylindrical cages as described (Druciarek et al., 2023) and arranged in a randomized complete block design. A month later plants were tested for BLMaV as described above.

3. Results

3.1. A resynonymisation of *Phyllocoptes parviflori* Keifer, 1939 (locus typicus: USA, California)

Several morphological characteristics distinguish eriophyid mites from the United States when compared to *P. gracilis* collected in Europe. The most important qualitative characters are prodorsal shield ornamentation and microtubercles on the dorsal annuli. Specimens from the US have a more complex pattern of lines on the prodorsal shield compared to *P. gracilis*, and oval microtubercles located near the margin of annuli. Mites from Europe also have pointed micro tubercles located on the rear margin of annuli (Fig. 1). Both characters are also helpful for the distinction from another eriophyid species occurring on blackberries in the US, *P. calirubi* (Fig. 1). From quantitative characters, the most distinct are the number of dorsal annuli, length of scapular setae (*sc*), *c2* and *c3* (Table 1). Supplementary description of *P. parviflori* is shown in supplementary file 1. The morphological distinction of *P. parviflori* from *P. gracilis* was also supported by analyses of two molecular markers, COI (mtDNA) and D2 (28S rDNA).

The final datasets consisted of 49 aligned sequences of COI (573 bp), and 50 sequences of the D2 region of the 28S rDNA (604 bp), representing three *Phyllocoptes* and one outgroup species. In the alignments, 182 (31.8%) sites were parsimony informative, 236 (41.2%) variable for COI; 55 (9.1 %) sites were parsimony informative, and 143 (23.7%) variable for D2. General topologies of the phylogenetic trees inferred by Bayesian inference (BI) and maximum likelihood (ML) analyses revealed similar structures, thus, only the BI trees are presented in Figure 2. The data strongly supports the monophyly of the genus *Phyllocoptes* (Fig 2 and supplementary Fig. 1 and 2) those of *P. gracilis* and *P. parviflori*. Both species were clustered separately, whereas *P. calirubi* and *P. gracilis* in the same sub-clade (Fig. 2). Similar tree topology, with stronger support for *P. calirubi* and *P. gracilis* sub-clade, were constructed based on the D2 dataset (Supplementary Fig. 1). However, for COI dataset, *P.*

gracilis and *P. parviflori* were clustered in one strongly supported clade (Supplementary Fig. 2). COI variation among these two populations was 28.0% (SE=3.0%), while D2 divergence between *P. parviflori* and *P. gracilis* was 5.9% (SE=1.1%), and *P. parviflori* and *P. calirubi* were 7.3 (SE=1.3%). A pairwise comparison of both markers' distances is provided in Table 2.

3.3. Transmission

One of the 20 black raspberry 'Munger' infested with five *P. parviflori* individuals tested positive for BLMaV. Efficiency was higher on 'Ouachita' blackberry; with single mite-transfers reaching 35% transmission. When increasing the number of mites to five individuals, efficiency increased to 70% (Table 3). Amplicons obtained from each plant were sequenced and were all found to be virus specific. HTS results were analysed using VirFind (Ho and Tzanetakis, 2014) and read mapping approaches and contig assembly using Geneious Prime 2023.1.1 (https://www.geneious.com) default parameters. Analysis showed 279,536 virus-specific reads for BLMaV that represented 99% of the virus genome and nine reads (76bp each) for Blackberry virus F (BVF) that mapped to five regions of the genome, with one region of 124 bp mapping five of the total reads. The nucleotide identity of these reads with the BVF reference genome in GenBank was between 80% to 91%. Previously designed detection primers (Shahid et al., 2017) failed to yield any amplicon; however, amplicons of the expected size were obtained with the primers designed in this study. RCA was unsuccessful possibly due to inhibitors, similar to what observed in the virus characterization study (Shahid et al., 2017). PCR using the RNase-digested samples yielded amplicons for BVF in all samples, whereas RT-PCR in DNase-digested samples yielded faint amplicons in three out of the ten samples (Supplemental Figure 3) with at least seven samples

that are not infected with the episomal form of the virus. The approach used provides evidence that BVF is integrated within the 'Ouachita' genome and symptoms can solely be caused by BLMaV andare typical of BYVD (Fig. 3; Martin et al., 2013b).

4. Discussion

Mites have been linked to several devastating diseases. However, only recently has the scientific community begun to realize the full extent of the species involved in transmission. Plant virus-transmitting mites mainly belong to the genus *Brevipalpus* (flat mites) and a limited genera of eriophyid mites, such as *Aceria*, *Cecidophyopsis*, *Phytoptus*, and *Phyllocoptes* (Bragard et al., 2013). This study extends the list of confirmed eriophyid vectors to *P. parviflori* and indicates that the virus can by itself cause BYVD symptoms on 'Ouachita', a paradigm shift as the disease has always been associated with multiple virus infections. For this reason, we propose that the virus is renamed to blackberry leaf mottle virus (BLMV).

It is important to note that based on the findings of Hassan et al. (2017), *P. parviflori* is not the only vector of this virus, as the authors used a different, unidentified species to successfully transmit the virus. Transmission of an emaravirus with multiple eriophyid species has recently been reported by Druciarek et al. (2023). Multiple vectors increase the risk of virus spread and further complicates the epidemiology of the disease. According to Hassan et al. (2017), BLMV vector may play a crucial role in BYVD epidemiology by transmitting the virus between cultivated and wild blackberries that act as natural reservoirs for the virus.

Our study demonstrates that individual *P. parviflori* mite could transmit BLMV. However, when multiple mites were used, transmission efficiency reached 70%, a high number when compared to all eriophyid transmission studies (Seifers et al., 1997; Kulkarni et

al., 2002; Di Bello et al., 2018; Druciarek et al., 2023). We observed low BLMV transmission efficiency on black raspberry (*R. occidentalis* 'Munger'), with one out of 20 plants infected in five mites-transfer experiment. Nevertheless, this result demonstrates that *P. parviflori* can move BLMV between different *Rubus* species. BLMV has been detected in numerous cultivated and wild blackberries (Hassan et al., 2017; Hassan and Tzanetakis, 2019). However, it is important to note that the virus is highly underreported, and there are no surveys to clearly demonstrate its true distribution. The successful graft transmission onto black raspberry 'Munger' plants, as confirmed by Hassan et al. (2017), aligns with our findings of raspberry as a suitable host, and also show that *P. parviflori* can feed on raspberry plants for a time sufficient to acquire and transmit the virus. The lower efficiency of transmission compared to blackberry 'Ouchita' plants may result from the specificity of a host or the adaptation costs. Currently, BLMV has only been reported in blackberries (Hassan et al., 2017; Hassan and Tzanetakis, 2019); whereas the vector of the virus was initially observed on *Rubus parviflorus* (*Rubus* subg. *Anoplobatus*), commonly known as thimbleberry (Keifer, 1939) and later identified on cultivated and wild blackberries during these studies.

Eriophyid mites, as highly specialized plant feeders, may incur adaptation costs when they inhabit a new plant species, as demonstrated for *A. tosichella* (Laska et al., 2021; Skoracka et al., 2022). Populations of *P. parviflori* used in our experiments originated from blackberries, and their transfer to black raspberry plants could potentially reduce the fitness of mites, ultimately lowering the efficiency of transmission. It should be emphasized that the presence of *P. parviflori* on wild and cultivated blackberries and their ability to colonize other *Rubus* species significantly increases the species' importance as a key factor responsible for the spread of BYVD. Other potential hosts, *e.g.*, thimbleberry and wild *Rubus* species should be screened for both, the vector and BLMV. Undoubtedly, there is an urgent need for further studies on BLMV-*P. parviflori* pathosystem, as disease management should include other

strategies (control of alternate hosts and targeting the virus vector) in addition to the use of virus-tested propagation material.

The considerable reduction and simplification in the body plan of eriophyid mites necessitate the use of structures from all parts of the body and appendages for their systematic classification. However, these structures are scarce (Lindquist and Amrine, 1996), which can lead to mistakes when describing a new species, as in the case of *P. parviflori*. The species has been noted only in the United States (Keifer, 1939). However, its occurrence and host range remains unclear due to its synonymization with *P. gracilis* (Keifer, 1952), a species commonly found in Europe on raspberry plants (Boczek, 1961; Denizhan et al., 2015; Nalepa, 1890; Roivainen, 1950, 1947), but also reported in the United States (Breakey, 1945). Phylogenetic analysis shows the unjustified nature of synonymization. The genetic distance in the COI marker ranged from 28.0% to 39.3%, and in the D2 marker ranged from 5.9% to 7.3%, confirmed that three *Phyllocoptes* species commonly found on blackberry plants are distinct . Similar or lower genetic distances using these markers have been observed in the differentiation of *Phyllocoptes* species on rose (Druciarek et al., 2021), cryptic speciation among lineages of *A. tosichella* (Skoracka et al., 2013) or *Trisetacus* species inhabiting coniferous plants (Lewandowski et al., 2014). The resynonymization is also supported by the examination of quantitative and qualitative morphological characters of *P. parviflori*, *P. gracilis*, as well as the third species occurring on blackberries, *P. calirubi*. One of the most useful quantitative features is the ornamentation of the prodorsal shield, which can be easily analyzed on slide-mounted specimens. This character is commonly employed for species identification and is considered one of the most important on the species level (de Lillo et al., 2010; Lindquist and Amrine, 1996). Identification based on quantitative characters can be further supported by comparing the number of dorsal annuli and measuring the length of *sc*,

c2, and *c3* setae. These characters are among 27 features commonly used in the identification of eriophyid species (Lewandowski et al., 2014; Skoracka et al., 2002).

Proper identification of species, especially those that are closely related or cryptic, holds significant importance not only in the field of taxonomy and systematics but also in translational research, particularly when dealing with economically important organisms such as *Phyllocoptes*- inhabiting crops. The presence of three morphologically similar *Phyllocoptes* species on *Rubus*, each with a different potential for BLMV transmission, could further complicate the management of BYVD. Accurate vector identification is crucial to control disease outbreaks and prevent spread to new areas. Furthermore, the findings of this study can serve as a valuable contribution to further research on the ability and efficiency of BLMV transmission and the phylogeny and systematics of *Phyllocoptes* as important virus vectors. These insights can aid in enhancing our understanding of the intricate interactions between vectors and viruses in plant disease dynamics.

5. Conclusions

In conclusion, our results demonstrate that *P. parviflori* serves as a vector for the spread of BLMaV among different *Rubus* species. Even a single individual of this mite vector could transmit BLMaV, but the efficiency of transmission increases to 70% when multiple mites are involved. This study expands the list of confirmed eriophyid vectors to include *P. parviflori* and suggests that the virus can independently cause BYVD symptoms on ‘Ouachita’, marking a paradigm shift as the disease has traditionally been associated with multiple virus infections. Therefore, we propose renaming the virus as blackberry leaf mottle virus (BLMV). Phylogenetic analysis supports the distinction between *P. parviflori* and *P. gracilis*, invalidating the previous synonymization. This study provides additional information describing *P. parviflori* as a vector for BLMV. The presence of the vector on wild and

cultivated blackberries, along with its ability to colonize other *Rubus* species, significantly amplifies its importance as a key factor responsible for the spread of BYVD.

Authorship contribution statement

Tobiasz Druciarek: Conceptualization, Methodology, Formal analysis, Experimentation, Writing – original draft, Writing – review & editing**. Andrea Sierra-Mejia:** Experimentation**. Stanislaw K. Zagrodzki:** Experimentation.. **Shivani Singh:** Experimentation. **Thien Ho:** Experimentation. **Mariusz Lewandowski:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Ioannis E. Tzanetakis:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

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Declaration of Competing Interest

The authors declare that they do not have any conflicts of interests associated with the research presented here.

Data availability

All sequences of BLMV and eriophyid mites were submitted and available in the GenBank Database (https://www.ncbi.nlm.nih.gov/nuccore).

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Table 1. Comparison of female morphological characters of *Phyllocoptes gracilis*, *P. parviflori*, and *P. calirubi*.

Taxonomical traits	*P. gracilis*		*P. parviflori*		*P. calirubi*	
	Nalepa, 1891	Polish populations	Keifer, 1939	USA populations	Keifer, 1938	USA populations
Length of body	120	174-257	170-180	152-249	140-155	187-240
Width of body	38	44-55	-	42-57	47-56	60-68
Length of chelicerae	-	12-16	-	12-16	-	13-15
Length of prod. shield	-	33-38	36	32-40	-	40-48
Width of prodorsal shield	-	33-42	-	32-43	-	46-54
Length of frontal lobe	-	3-4	-	3-5	-	4-6
Length of setae sc	-	17-20	14	12-15	11	9-12
Sc setae tubercle apart	-	17-22	17	16-21	20	18-21
No. of dorsal annuli	80	69-82	65-70	53-70	45-50	47-57
No. of ventral annuli	-	76-96	-	64-87	70-75	68-73
Length of setae c2	-	31-39	9	42-57	5	16-25
Length of setae d	-	45-56	24	33-48	23	41-51
Length of setae e	-	22-28	11	11-17	20	33-41
Length of setae f	-	25-29	22	15-23	24	23-28
Length of genitalia	-	9-12	8	8-12	12.5	11-13
Width of genitalia	-	19-24	18	18-22	20	21-25
Length of setae 3a	-	26-33	14	12-17	9	19-25
Ridges on genital coverflap	-	8-13	10	10-12	10-12	11-13
Length of leg I	-	29-32	30	26-31	28	31-34
Length of tibia I	-	5-6	7	6-8	6.5-7.5	6-8
Length of tarsus I	-	6-8	7	6-7	6.0-6.5	6-8
Rays in empodia I	-	5	5	5	5	5
Length of leg II	-	27-31	27	25-31	27	28-32
Length of tibia II	-	5-6	5	4-6	4.5	4-6
Length of tarsus II	-	7-8	7	6-8	6.5	7-8
Rays in empodia II	-	5	-	5	-	5
Type host	*Rubus idaeus*		*Rubus fruticosus*		*Rubus fruticosus*	

Table 2. Estimates of average evolutionary divergence (standard error in parentheses) for the cytochrome c oxidase subunit 1 (COI, mtDNA) and D2 subunit (28S rDNA) sequence pairs within and between *Phyllocoptes* species from *Rubus* spp. and the outgroup species.

COI (mtDNA)	*P. parviflori*	*P. gracilis*	*P. calirubi*	outgroup
P. parviflori	**0.020 (0.000)**			
P. gracilis	0.280 (0.030)	**0.030 (0.010)**		
P. calirubi	0.393 (0.034)	0.354 (0.042)	**0.000 (0.000)**	
outgroup	0.528 (0.062)	0.537 (0.060)	0.430 (0.049)	**n/c**
D2 (28S rDNA)				
P. parviflori	**0.004 (0.002)**			
P. gracilis	0.059 (0.011)	**0.001 (0.001)**		
P. calirubi	0.073 (0.013)	0.070 (0.013)	**0.000 (0.000)**	
outgroup	0.295 (0.038)	0.300 (0.038)	0.328 (0.042)	**n/c**

Table 3. BLMaV transmission efficiency by *Phyllocoptes parviflori*. Plants infected/no. of plants used (% transmission)

Host plant	Single-mite transfer	5-mite transfer
Rubus occidentalis 'Munger'	-	1/20 (5)
Rubus fruticosus 'Ouachita'	7/20 (35)	14/20 (70)

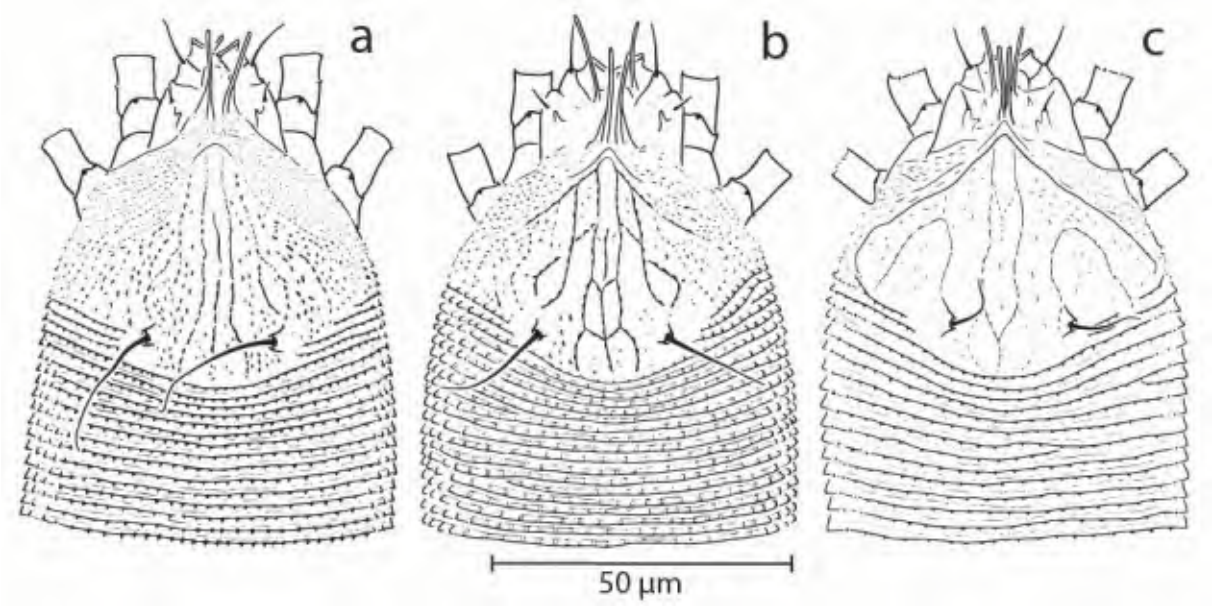


Figure 1. *Phyllocoptes* from *Rubus*, antero-dorsal females: a – *P. gracilis*; b *P. – parviflori*; c – *P. calirubi*

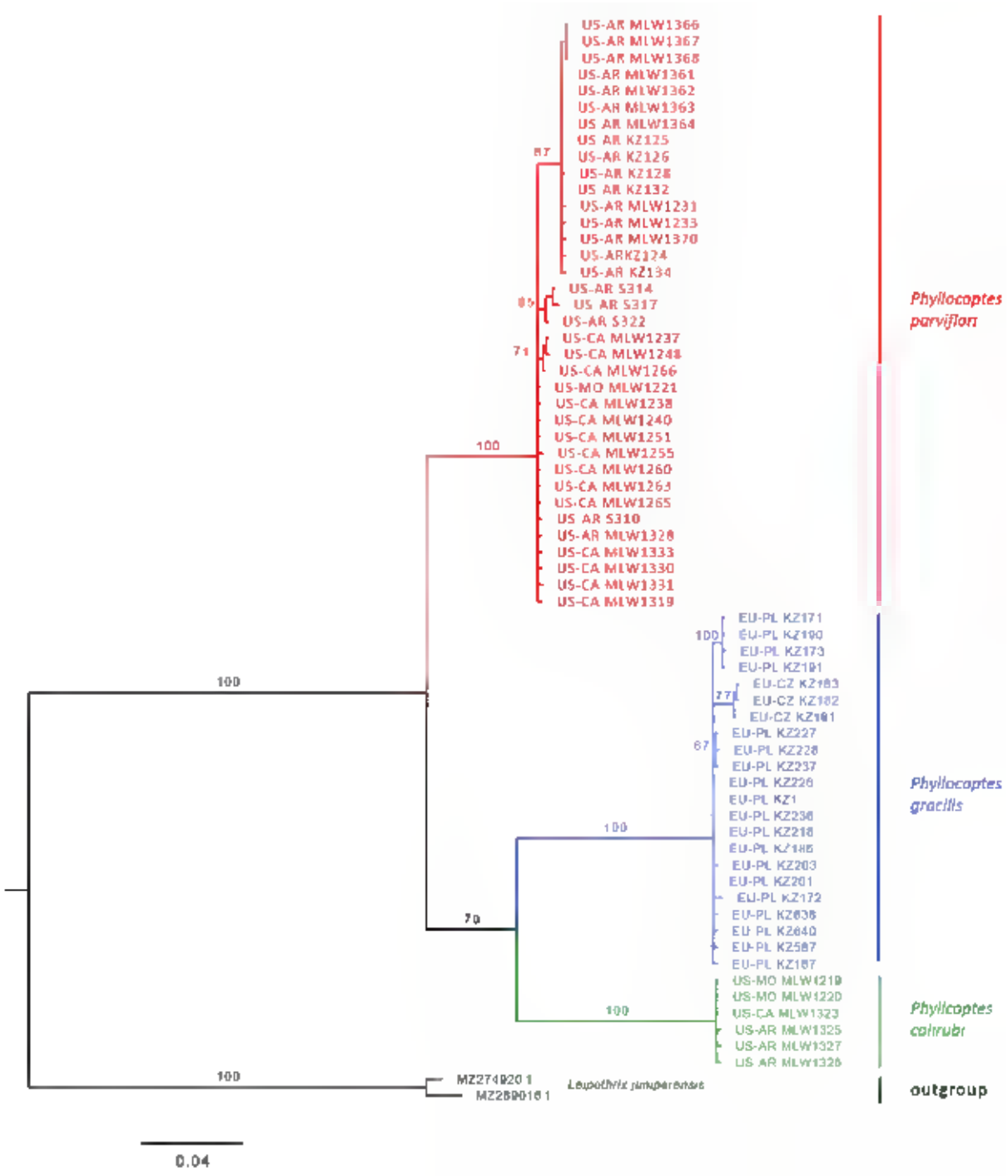


Figure 2. Combined Bayesian inference (BI) analysis tree constructed from the concatenated cytochrome c oxidase subunit I sequences (COI) and 28S r-RNA subunit D2 sequences of *Phyllocoptes* mites collected from *Rubus* plants and the outgroup species (*Leipothrix juniperensis* obtained from GenBank).

A.

B.

Figure 3. Blackberry leaf mottle virus symptoms that are typical of blackberry yellow vein disease. A) Ringspot symptoms B) Vein yellowing and chlorotic feathering pattern.

Supplemental Table 1. Collection data for *Phyllocoptes* spp. from *Rubus* plants located in the USA and Europe

Taxon name	Locality	Geographical coordinates	Host plant taxon	Date of collection	Sample code	Accession numbers	
						COI	D2
Phyllocoptes parviflori	US-AR: Rogers	36°20'50"N 94°06'35"W	*Rubus fruticosus*	15.05.2019	KZ125	OQ869677	OQ888660
					KZ126	OQ869678	OQ888661
					KZ128	OQ869679	OQ888662
					KZ132	OQ869680	OQ888663
					KZ124		OQ888659
					KZ134		OQ888664
	US-AR: Clarksville	35°31'52"N, 93°24'10"W	*Rubus fruticosus*	19.07.2017	s310	OQ869674	
			Rubus sp. (wild blackberry)	19.07.2017	s314	OQ869673	
					s317	OQ869676	
					s322	OQ869675	
	US-MO: Ironton	37°34'57"N, 90°37'42"W	*Rubus* sp. (wild blackberry)	23.04.2022	MLW1221	OQ869655	OQ888636
	US-AR: Rogers	36°20'50"N 94°06'35"W	*Rubus fruticosus*	27.04.2022	MLW1231	OQ869681	
					MLW1233	OQ869682	
	UA-AR: Huntsville	36°07'00"N 93°45'04"W	*Rubus* sp. (wild blackberry)	01.05.2022	MLW1328		OQ888641
	US-AR: Fayetteville	36°02'41"N 94°10'15"W	*Rubus* sp. (wild blackberry)	08.05.2022	MLW1361	OQ869656	OQ888638
					MLW1362	OQ869657	OQ888639
					MLW1363	OQ869658	OQ888640
					MLW1364	OQ869659	
					MLW1370		OQ888637
	US-CA: Watsonville	36°53'22"N 121°46'03"W	*Rubus fruticosus*	17.05.2022	MLW1237	OQ869660	OQ888642
					MLW1238	OQ869661	OQ888643
					MLW1240	OQ869662	OQ888644
					MLW1251	OQ869663	OQ888645
				21.05.2022	MLW1255	OQ869664	OQ888646
					MLW1260	OQ869665	OQ888647
					MLW1333		OQ888648
					MLW1330		OQ888649

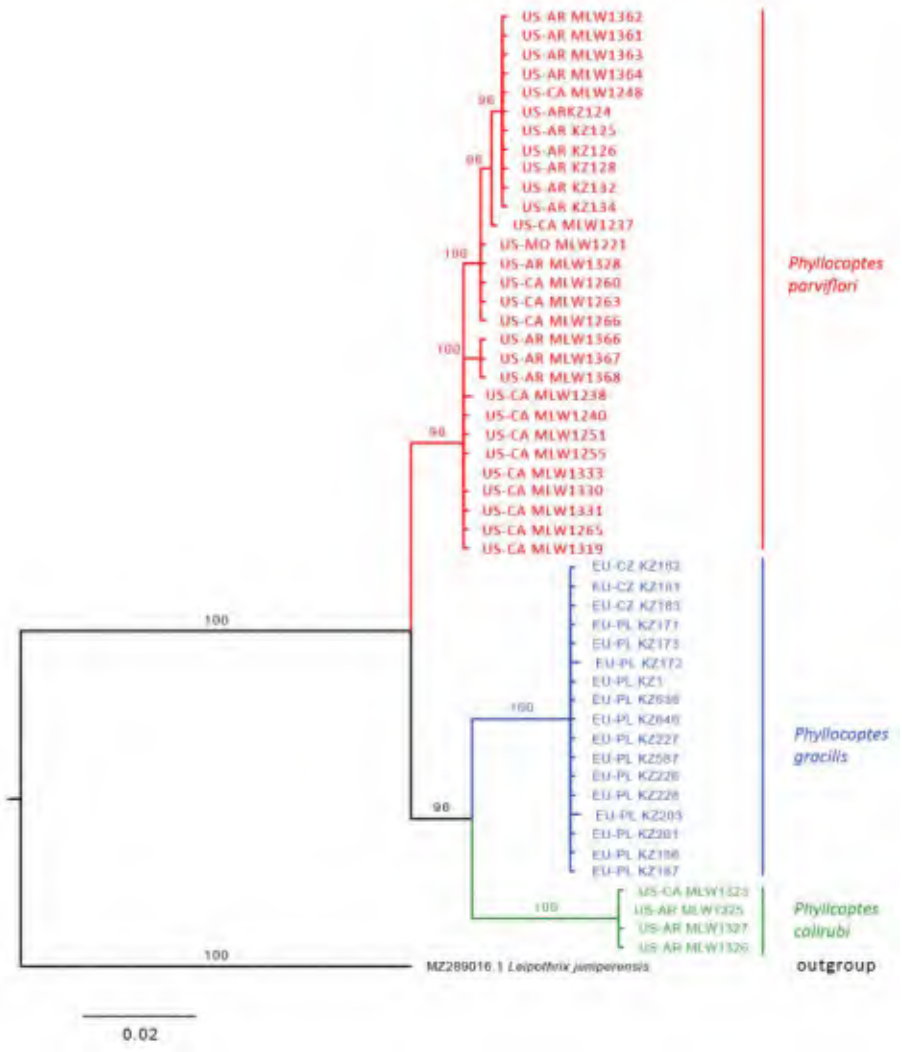
					MLW1331		OQ888650
				23.05.2022	MLW1263	OQ869670	OQ888654
					MLW1265	OQ869672	OQ888656
					MLW1266	OQ869671	OQ888655
					MLW1319		OQ888658
	US-AR: Winslow	35°49'01"N 94°11'24"W	*Rubus* (wild blackberry)	22.05.2022	MLW1248	OQ869666	OQ888651
					MLW1366	OQ869667	OQ888652
					MLW1367	OQ869669	OQ888653
					MLW1368	OQ869668	OQ888657
Phyllocoptes calirubi	US-MO: Boone Township	38°08'09"N 91°16'52"W	*Rubus* sp. (wild blackberry)	23.04.2022	MLW1219	OQ869683	
					MLW1220	OQ869684	
	UA-AR: Huntsville	36°07'00"N 93°45'04"W	*Rubus* sp. (wild blackberry)	01.05.2022	MLW1325		OQ888666
					MLW1327		OQ888667
					MLW1326		OQ888668
	US-CA: Watsonville	36°53'22"N 121°46'03"W	*Rubus fruticosus*	23.05.2022	MLW1323	OQ869685	OQ888665
Phylocoptes gracilis	EU/CZ: Smrek	50°53'33"N 15°16'01"E	*Rubus idaeus* (wild raspberry)	07.07.2020	KZ181	OQ869687	OQ888670
					KZ182	OQ869688	OQ888669
					KZ183	OQ869686	OQ888671
	EU/PL: Polesie	52°45'31"N 20°19'23"E	*Rubus idaeus* (wild raspberry)	01.08.2020	KZ226	OQ869691	OQ888680
					KZ227	OQ869689	OQ888678
					KZ228	OQ869690	OQ888681
				05.10.2020	KZ587		OQ888679
	EU/PL: Michrów	51°56'31"N 20°47'35"E	*Rubus idaeus*	10.10.2018	KZ1	OQ869692	OQ888675
				01.08.2019	KZ236	OQ869693	
					KZ237	OQ869694	
				10.07.2020	KZ638		OQ888676
					KZ640		OQ888677
	EU/PL: Kaleń	52°00'41"N 20°42'24"E	*Rubus idaeus*	18.06.2019	KZ171	OQ869695	OQ888672
					KZ172		OQ888674
					KZ173	OQ869697	OQ888673
					KZ190	OQ869696	

					KZ191	OQ869698	
	EU/PL: Dąbrowice	51°55'15"N 20°06'14"E	*Rubus idaeus* (wild raspberry)	20.07.2019	KZ201	OQ869702	OQ888683
					KZ203	OQ869701	OQ888682
	EU/PL: Stóg Izerski	50°53'28"N 15°18'11"E	*Rubus idaeus* (wild raspberry)	02.07.2020	KZ186	OQ869700	OQ888684
					KZ187		OQ888685
					KZ218	OQ869699	
						ITS1	
Phyllocoptes parviflori	US-CA: Watsonville	36°53'22"N 121°46'03"W	*Rubus fruticosus*	17.05.2022	MLW1309	OR050588	
					MLW1310	OR050589	
					MLW1311	OR050590	
					MLW1312	OR050591	

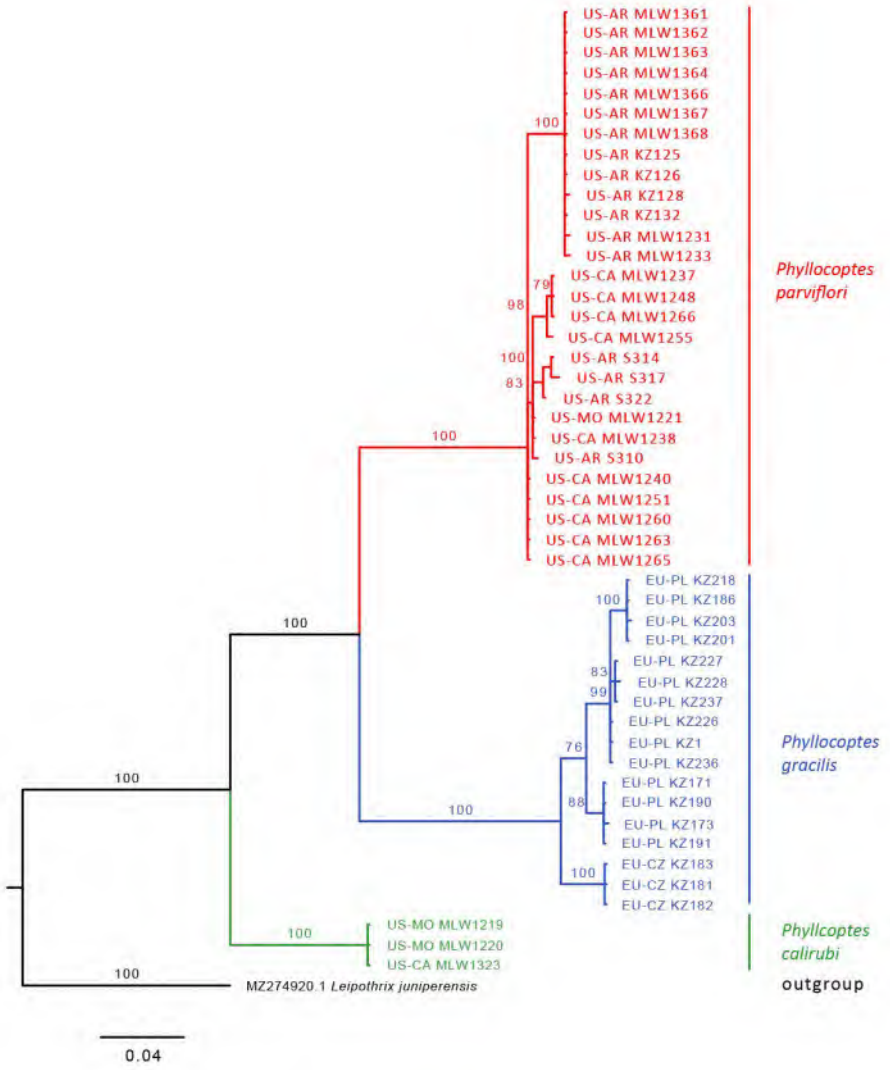
Supplemental Table 2. Primers designed for the detection of BVF based on the reference genome and the sequences obtained in high throughput sequencing

Primer name [a]	**Sequence**
BVF_HTS_tail_4814F	5'-CATCGGAGCAACTACCTGCTGTG-3'
BVF_HTS_tail_5355R	5'- GCAGTCGGCTGAATATCTGGATTGATG-3'
BVF_HTS_tail_6461F	5'- GAGTCGGCTGATTCAAAATCTACTACC-3'
BVF_HTS_tail_7457R	5'-ATTGGGCCCCATCGTCACTC-3'

[a] primers were designed to align to both the reference BVF genome and the HTS sequence. Due to high diversity 5' adaptors were added to increase the primer length and melting temperature.

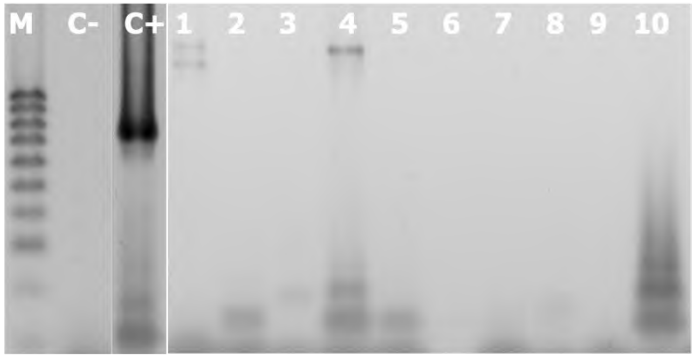


Supplementary Figure 1. Bayesian inference (BI) analysis tree constructed using HKY + G model for the 28S rRNA subunit D2 data sequences of *Phyllocoptes* mites collected from *Rubus* plants and the outgroup species (*Leipothrix juniperensis* obtained from GenBank).

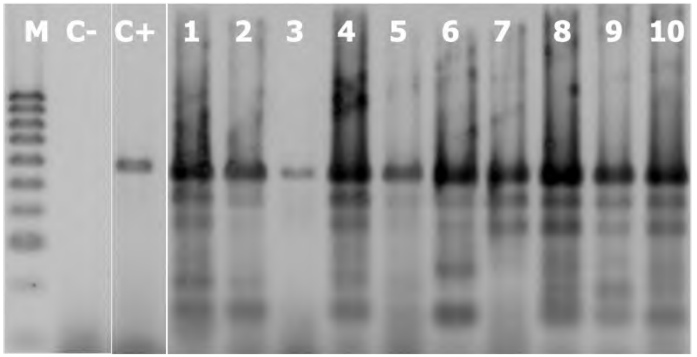


Supplemental Figure 2. Bayesian inference (BI) analysis tree constructed using GTR + I + G model for the cytochrome c oxidase subunit 1 (COI) sequences of *Phyllocoptes* mites collected from *Rubus* plants and the outgroup species (*Leipothrix juniperensis* obtained from GenBank).

A.


M C- C+ 1 2 3 4 5 6 7 8 9 10

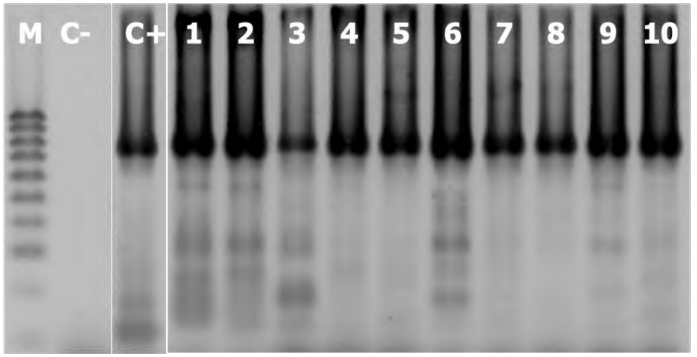
B.


M C- C+ 1 2 3 4 5 6 7 8 9 10

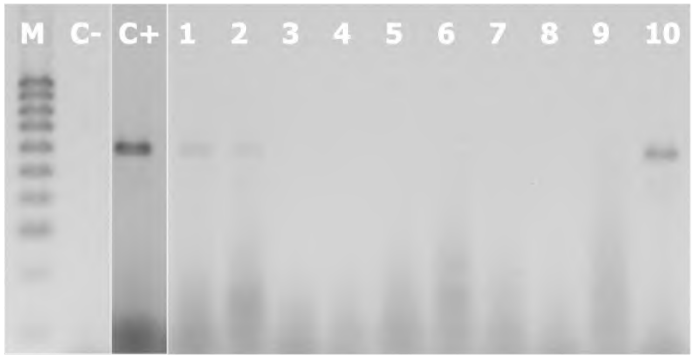
C.


M C- C+ 1 2 3 4 5 6 7 8 9 10

D.


M C- C+ 1 2 3 4 5 6 7 8 9 10

E.


M C- C+ 1 2 3 4 5 6 7 8 9 10

Supplemental Figure 3. Polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (RT-PCR) of NADH dehydrogenase β-subunit (NADH-β, expected size ~1300bp (genomic copy), 721bp (transcript)), and blackberry virus F (BVF) using primer set BVF_HTS_tail_4814F and BVF_HTS_tail_5355R (expected size 541 bp).

A) NADH-β PCR M: 100 bp ladder, C-:water control, C+: cDNA BLMaV HTS sample, 1-10: RNase-digested BLMV positive samples; the absence of a 721 bp amplicon suggests only DNA is being amplified.

B) BVF PCR M: 100 bp ladder, C-:water control, C+: cDNA BLMaV HTS sample, 1-10: RNase-digested BLMV positive samples.

C) NADH-β PCR M: 100 bp ladder, C-:water control, , C+: cDNA BLMaV HTS sample ,1-10: DNase-digested BLMV positive samples; the absence of amplification suggests complete DNA digestion.

D) NADH-β RT-PCR M: 100 bp ladder, C-:water control, C+: cDNA BLMaV HTS sample, 1-10: DNase-digested BLMV positive samples; the presence of a 721 bp amplicon validates RNA integrity.

E) BVF RT-PCR M: 100 bp ladder, C-:water control, C+: cDNA BLMaV HTS sample, 1-10: DNase digested BLMV positive samples.

Supplementary file 1. Supplementary description of *Phyllocoptes parviflori* Keifer, 1939 resyn. from *Phyllocoptes gracilis* (Nalepa, 1890)

Description methodology

Mite specimens used for morphometric analyses were mounted on slides using a modified Berlese medium (Amrine and Manson, 1996). The morphological terminology, systematic classification and abbreviation used in the figures follow that of Amrine et al. (2003) and Lindquist (1996). All measurements are in micrometers and unless specified otherwise, are lengths. Mite measurements were made according to Amrine and Manson (1996) and de Lillo et al. (2010). Leg length was measured from the posterior margin of trochanter to the tip of the tarsus. Positions of leg setae were measured from the proximal margin of the seta-bearing segment. Locations of ventral setae *c2*, *d*, *e*, and *f* on ventral annuli were measured from the first entire annulus after posterior margin of *coxae II*. All measurements were done on a Soft Imaging System Cell D connected to the camera and a phase-contrast Olympus BX51 microscope (Shinjuku, Tokyo, Japan). Drawings were made according to (De Lillo et al., (2010), using a multimedia LED projector connected to the measurement system. Initial drafts of drawings were digitized, and final plates were produced using the Adobe Photoshop Elements 8 and Illustrator CS6 software. All examined specimens are deposited as slide-mounted material in the Mite Collection of the Institute of Horticultural Sciences, Department of Plant Protection, Section of Applied Entomology, Warsaw University of Life Sciences, Warsaw, Poland. According to Liu et al. (2017), collections of the new eriophyoid species, *P. parviflori* are named WULS, respectively.

Supplementary description of *P. parviflori*

FEMALE (38 paratypes): Body fusiform, light amber in colour, 152–249; width 42–57. **Gnathosoma** 11–17, curved downward, dorsal pedipalpal genual setae *d* 3–4, setae *ep* 2–3, pedipalp tarsal setae *v* 1–2, cheliceral stylets 12–16. **Prodorsal shield** 32–40, 32–43 wide, subtriangular with triangular frontal lobe, 2–5 over the gnathosomal base. Shield pattern: median line only on the rear part of the shield; at about half and ¾ length of the shield, the line joined with admedian lines, forming V-shaped marks. Admedian lines are parallel from the anterior margin of the shield to about half of its length, where they are joined by V shape transverse line; from that place, admedian lines gradually diverge to ¾ of its length and are joined with next V shape traverse line; the remaining parts of these lines semi-circular. Submedian lines I slightly divergent and curved at the ends, running from the anterior part of the shield to the basis of scapular setae tubercles. Submedian lines II are short and curved, visible in the middle part of the shield. All lines compose with dashes. The lateral and rear part of the shield with short lines and granules. Scapular setae *sc* tubercles 1–3, located ahead of rear shield margin, 16–21

apart; *sc* 12–15 directed upwards. **Legs** with all usual segments and setae present. Leg I 26–31; femur 8–10, basiventral femoral seta (*bv*) 6–10, position of *bv* 2–4; genu 4–5, antaxial genual seta (*l″*) 14–25, position of *l″* 2–3; tibia 6–8, paraxial tibial seta (*l′*) 4–7, position of *l′* 2–3; tarsus 6–7, antaxial, fastigial, tarsal seta (*ft″*) 17–23, paraxial, fastigial, tarsal seta (*ft′*) 12–17, paraxial, unguinal, tarsal seta (*u′*) 2–5; tibial solenidion (*ω*) 7–8; empodium simple 6–7, bilaterally symmetrical, with 5 paired rays. Leg II 25–31; femur 8–10, seta (*bv*) 5–10, position of *bv* 2–4; genu 3–5, seta (*l″*) 4–9, position of *l″* 2–3; tibia 4–6; tarsus 6–8, seta (*ft″*) 16–22, paraxial, fastigial, tarsal seta (*ft′*) 4–6, seta (*u′*) 2–4; tibial solenidion (*ω*) 7–9; empodium simple 6–7, bilaterally symmetrical, with 5 paired rays. **Coxisternal** plates with short lines and granules. Anterolateral setae on coxisternum I (*1b*) 5–8, 9–11 apart; proximal setae on coxisternum I (*1a*) 13–19, 6–9 apart; proximal setae on coxisternum II (*2a*) 29–44, 18–26 apart; distance between setae *1b* and *1a* 7–9, distance between setae *1a* and *2a* 6–9. **External genitalia** 8–12, 18–22 wide, genital coverflap with 10–12 longitudinal ridges; proximal setae on coxisternum III (*3a*) 12–17, 12–16 apart. **Opisthosoma** with 53–70 dorsal and 64–87 ventral annuli, 5–7 coxigenital annuli. Annuli with rounded microtubercles, placed on rear part of annuli., on posterior annuli microtubercles elongated. Setae: *c2* 16–29, 42–57 apart, on 10–14 ventral annulus; *d* 33–48, 30–40 apart, on 23–31 ventral annulus; *e* 11–17, 13–21 apart, on 40–56 ventral annulus; *f* 15–23, 15–20 apart, on 58–82 ventral annulus, 5–6 annulus from rear. Setae *h1* 3–5, 5–7 apart; setae *h2* 38–58, 7–11 apart; distance between *h1* and *h2* 2–3.

MALE (Figures ? and ?) 16 specimens: Body fusiform, light amber in colour, 133–191; width 36–52. **Gnathosoma** 11–16, curved downward, dorsal pedipalpal genual setae *d* 3–4, setae *ep* 2–3, pedipalp tarsal setae *v* 1–2, cheliceral stylets 13–15. **Prodorsal shield** 29–38, 31–40 wide, subtriangular with triangular frontal lobe, apically rounded, 2–4 over the gnathosomal base. Shield pattern similar to that of females. Scapular setae *sc* tubercles 1–2, located ahead of rear shield margin, 15–20 apart; *sc* 11–13 directed upwards. **Legs** with all usual segments and setae present. Leg I 25–28; femur 7–9, seta (*bv*) 6–9, position of *bv* 2–3; genu 4–5, seta (*l″*) 14–22, position of *l″* 2–3; tibia 6–8, seta (*l′*) 4–7, position of *l′* 2–3; tarsus 6–8, seta (*ft″*) 14–23, seta (*ft′*) 10–16, seta (*u′*) 3–4; tibial solenidion (*ω*) 7–8; empodium simple 5–7, bilaterally symmetrical, with 5 paired rays. Leg II 24–28; femur 7–9, seta (*bv*) 6–9, position of *bv* 2–3; genu 3–4, seta (*l″*) 5–8, position of *l″* 2–3; tibia 4–7; tarsus 6–7, seta (*ft″*) 14–21, seta (*ft′*) 4–6, seta (*u′*) 3–4; tibial solenidion (*ω*) 7–9; empodium simple 5–6, bilaterally symmetrical, with 5 paired rays. **Coxisternal** plates with short lines and granules. Anterolateral setae on coxisternum I (*1b*) 5–7, 7–10 apart; proximal setae on surface near the genital opening with conical, pointed microtubercles coxisternum I (*1a*) 11–18, 6–8 apart; proximal setae on coxisternum II (*2a*) 21–37, 18–25 apart; distance between setae *1b* and *1a* 7–11, distance between setae *1a* and *2a* 6–9. **External genitalia** 11–20, 11–18 wide, surface below eugenital setae with granules; proximal

setae on coxisternum III (*3a*) 8–14, 12–15 apart. **Opisthosoma** with 47–61 dorsal and 56–76 ventral annuli, 5–7 coxigenital annuli. Annuli with rounded microtubercles, placed on rear part of annuli, on posterior annuli microtubercles elongated. Setae: *c2* 16–25, 36–52 apart, on 9–13 ventral annulus; *d* 23–39, 26–35 apart, on 19–27 ventral annulus; *e* 12–18, 13–18 apart, on 34–49 ventral annulus; *f* 16–24, 15–19 apart, on 51–70 ventral annulus, 5–6 annulus from rear. Setae *h1* 2–5, 4–6 apart; setae *h2* 38–52, 7–10 apart; distance between *h1* and *h2* 1–2.

Examined material: 38 females and 16 males collected in Fayetteville (US-AR), Rogers (US-AR) and Watsonville (US-CA) (for details see Table 1)
Type host: *Rubus parviflorus* Nutt
Type locality: Stinson Beach, Martin County, California, USA; August 13, 1938.
Relation to the host plant: All stages of *P. parviflori* were leaf vagrants, mainly on the lower leaf surfaces.

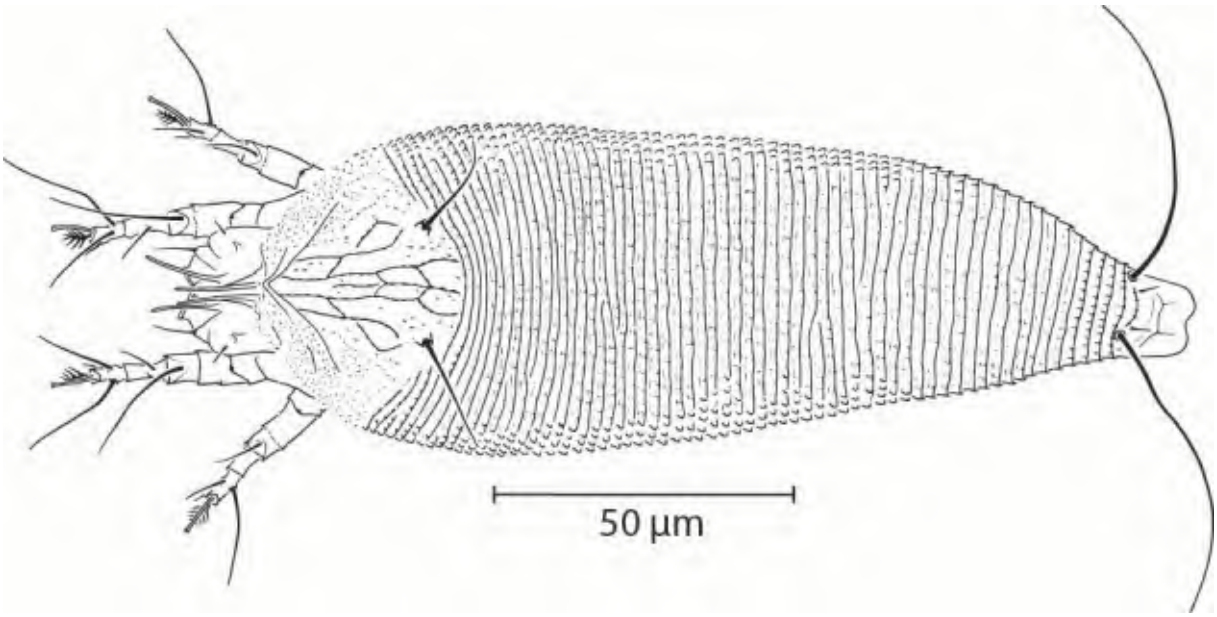


Figure 1. *Phyllocoptes parviflori* female; D – dorsal mite

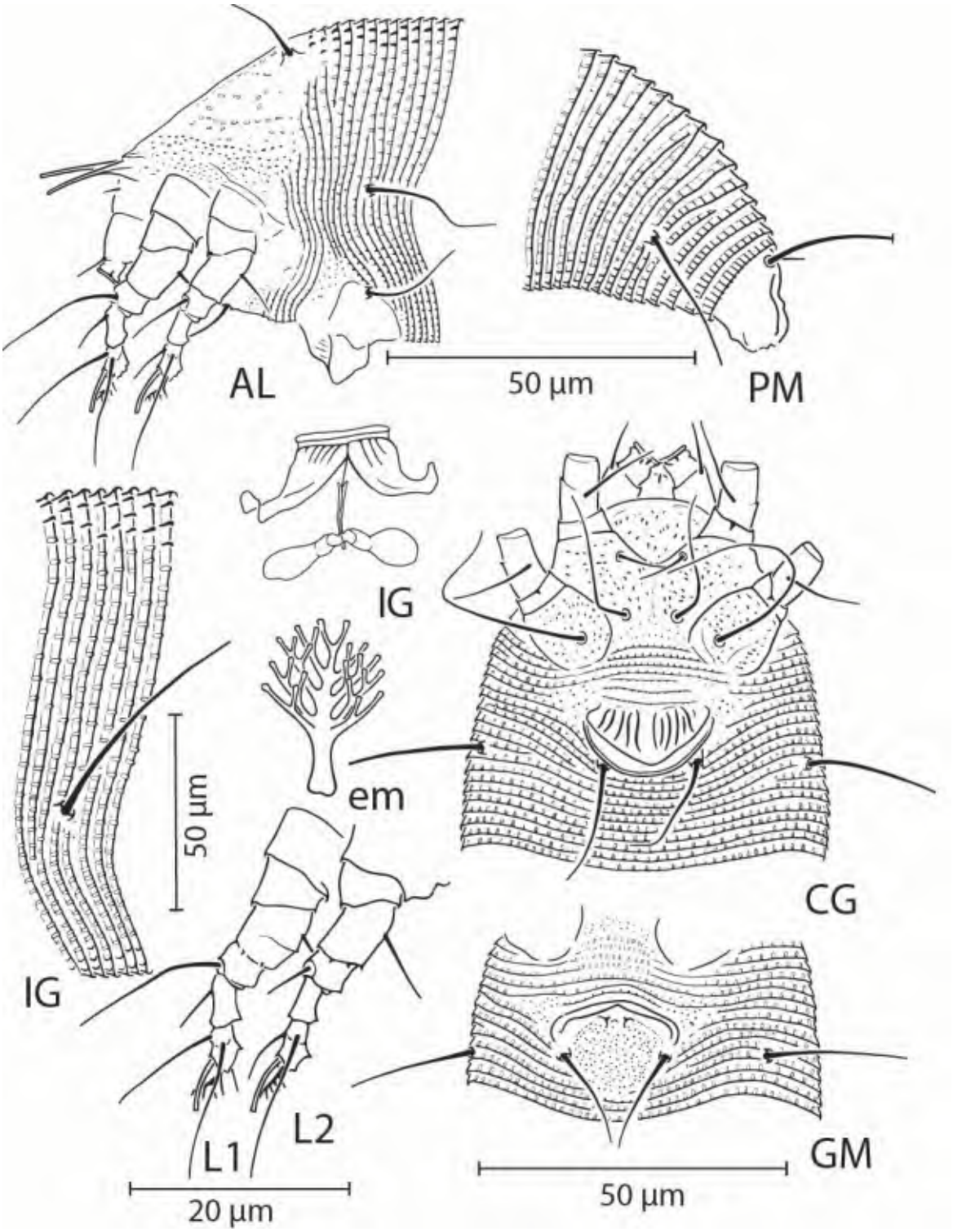


Figure 2. *Phyllocoptes parviflori* female: AL – antero-lateral mite; PM – postero-lateral mite; LO – lateral opisthosoma; CG – coxigenital region; L1, L2 – legs; IG – internal genitalia; em – empodium. Male: GM – genital region

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