Acarologia
Open Science in Acarology

Diptacus rubuscolum Trinidad, Duarte & Navia (Eriophyoidea: Diptilomiopidae) on blackberry (*Rubus ulmifolius* Schott) in Mexico: occurrence, complementary description, and biology

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* *The author died before the publication of this paper. This represent his last scientific contribution.*

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Original research

ABSTRACT

Blackberry (*Rubus ulmifolius* Scott) is an economically important crop in Mexico. Eriophyoidea mites are among the most important arthropod pests affecting production of this crop worldwide. Despite this, reports on species diversity of Eriophyoidea on blackberry in Mexico are scarce. We sampled six orchards from two municipalities in the state of Michoacan, the most important region for blackberry production. Sampling was carried out monthly between May and November 2018. Only one species of eriophyoid mite was found, *Diptacus rubuscolum* Trinidad, Duarte and Navia. We conducted a detailed morphological study of this mite and a complementary description is presented. In addition, we obtained partial sequences of the mitochondrial cytochrome oxidase subunit I (mt *COI*), the 18S and 28S of the rRNA. The duration of each developmental stage was determined under controlled conditions (25 °C, 60% RH and 12:12 light regime). We confirmed the identity of *D. rubuscolum* by comparing partial sequences of the D2 region within the 28S rRNA from our samples and sequences retrieved from GenBank. Overall, morphological characteristics were in line with the type specimens. However, some differences on body shape and empodium structure were pointed out. The developmental stages of *D. rubuscolum* were egg, larva, nymphochrysalis, nymph, imagochrysalis and adult, with a mean duration of 75 (±10), 56 (±9), 21 (±7), 41 (±8), 19 (±6) and 114 (±12) hours respectively. The information produced provides insight and knowledge on the occurrence of this species on blackberry in Mexico as well as on original biological aspects.

Keywords Rosaceae; eriophyid mite; North America; taxonomy; life cycle

Received 14 May 2022
Accepted 06 June 2023
Published 27 June 2023

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Academic editor
Navia, Denise

https://doi.org/10.24349/j7im-cara

ISSN 0044-586X (print)
ISSN 2107-7207 (electronic)

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How to cite this article González-Domínguez S. G. *et al.* (2023), *Diptacus rubuscolum* Trinidad, Duarte & Navia (Eriophyoidea: Diptilomiopidae) on blackberry (*Rubus ulmifolius* Schott) in Mexico: occurrence, complementary description, and biology. **Acarologia 63(3): 793-806.** https://doi.org/10.24349/j7im-cara

Introduction

Blackberries (*Rubus ulmifolius* Scott) have become increasingly popular with consumers due to their high levels of antioxidants (Schulz *et al.* 2019). Currently, Mexico is the main producer of blackberries worldwide (Stupková 2016) and it is among the most important fruit crops in Mexico; the state of Michoacan produces 60% of the Mexican blackberry crop (Strik *et al.* 2007; Servicio de Información Agroalimentaria y Pesquera [SIAP] 2020).

Mites from the family Tetranychidae and the superfamily Eriophyoidea (Eriophyidae, Phytoptidae and Diptilomiopidae) are among the most important arthropod pests affecting blackberry production (de Lillo and Duso 1996; Marchetti and Ferla 2011), and some Eriophyidae species have greater negative impact on cultivated plants because they transmit plant viruses (de Lillo *et al.* 2016; de Lillo *et al.* 2018). Despite the economic importance of this crop, there are few studies reporting species diversity and biology of associated eriophyoids in Mexico. The following eriophyoid mite species have been reported associated with *Rubus* spp., *Acalitus essigi* Hassan, *A. orthomera* Keifer, *Chakrabartiella* sp. and *Diptacus rubuscolum* Trinidad, Duarte & Navia in Brazil (Marchetti and Ferla 2011; Trinidad et al. 2018); *Diptacus glaber* (Huang and Wang 2009), *Diptacus gigantorubra* (Xin and Dong, 1983), *Diptacus chihouensis* (Wang *et al.* 2009) and *Diptacus rubi* (Kuang 2001) in China and Taiwan; and *Rhynacus abronius* (Keifer) (Cham. & Schltdl.), *Apodiptacus rubi* (Kuang) and *Diptacus caeseius* in Germany and Austria (Domes 2000).

To provide more information on species diversity of eriophyoid mites in Mexican blackberry crops, a field survey was carried out between April and November 2018, in six orchards from two municipalities in the state of Michoacán. We conducted both morphological and molecular identification, only *D. rubuscolum* was found and a complementary description of this species is provided. In adition, biological information on the duration of the different developmental stages of this mite species is presented.

Material and methods

Collection and identification of species

Monthly samples were taken between May and November 2018 from six blackberry (*R. ulmifolius* var. Tupy) orchards in the municipalities of Los Reyes de Salgado and Peribán, Michoacán. In each orchard 40 sampling points were randomly selected but covered the entire orchard. At each sampling point buds, flowers, leaves and fruits infested with mites were collected in plastic bags and transported to the Acarology Laboratory, Colegio de Postgraduados, Mexico state in a cool box. Mites were washed off the plant samples and collected after filtration through sieves with mesh numbers 200, 325 and 500, representing 74, 44 and 25 µm openings respectively.

Identification of mites was done using phase contrast microscopy (PCM), scanning electronic microscopy (SEM) and using sequence information from the D2 region of the 28S rRNA. For PCM evaluation, specimens were mounted in modified Keifer mounting liquid on glass slides (Amrine and Manson 1996). Slides were maintained at 40° C for 15 days before observations were made. All specimens were evaluated using an Olympus microscope (model BX41, Olympus Corporation of the Americas, PA, USA) with the 40X and 100X objectives. Photographs were taken using a digital camera (Olympus) attached to the microscope. For genus identification, we used the information reported by Amrine *et al.* (2003) and the genitalia anatomy reported by Lindquist (1996). We measured all the structures suggested for identification of eriophyid mites (De Lillo et al. 2010). All measurements (µM) were made using the software Image J v 1.5 (Abràmoff *et al.* 2004).

For SEM analysis, two females and one male, previously stored in 70% ethanol, were used. The specimens were freeze dried, to ensure all ethanol was removed, and then coated with colloidal gold by ionization (1 mA) for 100 seconds in a sputter coater (Fine-Coat Ion

Sputter JFC-1100, JEOL Ltd., Tokyo, Japan). They were then observed and photographed in the vacuum chamber of a scanning electron microscope (JOEL JSM-6390, JEOL Ltd., Tokyo, Japan).

Molecular studies

DNA extraction, amplification, and sequencing

For molecular identification and characterization, partial sequences of the *COI* gene, 18S and two regions of the 28S of the rRNA were obtained from three specimens and deposited in GenBank. DNA was extracted from individual mites using the ZR Tissue and Insect DNA MicroPrep™ kit (ZYMO Research Corporation, Irving, CA, USA), following the manufacturer's instructions. The concentration of DNA in samples was estimated using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the DNA then stored at -20 °C until required. *COI* sequences were obtained using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994). The 18S region of the rRNA was amplified with the primers fw390 and rev960 (Dabert *et al.* 2010). Within the 28S rRNA gene, two regions were amplified, the D2-5 region using two pair of primers, ND2f /D2R (Campbell *et al.* 1994) and 28S3F/28S3R (Whiting *et al.* 1997), and the D9-10 region using the primers 28S9F and 28S9R (Hillis and Dixon 1991). All PCRs were conducted in a 30µL reaction volume containing 3 µL of 10X PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 15mM $MgCl_2$; pH 8.7), 0.3 µM of each primer, 0.2 mM of dNTPs, 0.5U of Taq DNA polymerase (QIAGEN, GmbH, Hilden, Germany), 1.2 µL of $MgCl_2$ (25 mM), three µL of DNA (approx. 10 ng/µL) and 20.3 µL of 6% sterile trehalose. Amplifications were done in a MyCycler™ thermal cycler (BIORAD Laboratories Inc., Hercules, CA, USA) using the following conditions: for the *COI* primers, one cycle of 60 s at 94 °C; five cycles of 60 s at 94 °C, 90 s at 45 °C and 90 s at 72 °C, followed by 35 cycles of 60 s at 94 °C, 90 s at 60 °C, 60 s at 72 °C and a final extension at 72 °C for 5 min. For the 18S and the D9-10 region (28S rRNA) primers, one cycle of 5 min at 96 °C, followed by 30 cycles of 30 s at 95 °C, 60 s at 50 °C, 60 s at 72 °C and a final extension at 72 °C for 5 min. For the other two pairs of primers, all conditions were the same except the annealing temperatures which were 52 and 55 °C for the ND2f/D2R and 28S3F/28S3R combinations, respectively. PCR products were visualized on 1.5% agarose gels in 1X TAE, stained with ethidium bromide (10 mg mL^{-1}), and photographed to confirm amplifications. All amplicons obtained were sent to Macrogen Inc. (Geumchen-gu, Seoul, Korea) for direct Sanger sequencing.

Sequences edition, genetic distances and phylogenetic analyses

Resulting sequences were edited using BioEdit v.7.1.9 (Hall, 1999). Multiple alignments were made using Clustal W (Thompson *et al.* 1994) implemented in BioEdit. For analyses, we retrieved sequences from the families Diptilomiopidae that had been reported by Druciarek *et al.* (2019) and Li *et al.* (2014), including sequences of *D. rubuscolum.* Genetic distances and phylogenetic analyses were carried out using only sequences for the D2 region of the 28S rRNA, as they were the only ones available in GenBank. Analyses of the pairwise genetic distances between and within nucleotide sequences amongst samples studied, as well as the choice of the most appropriate evolutionary models for estimation of inter- and intra-lineage genetic variation were performed in Molecular Evolutionary Genetic Analysis (MEGA) v.7 (Tamura *et al.* 2016) using the Kimura 2-Parameters (K2P) model (Kimura 1980). Standard error estimates were obtained by a bootstrap procedure with 1000 replicates. Phylogenetic analysis was done using maximum likelihood in MEGA ver. 7, with the Close-Neighbour-Interchange algorithm. The robustness of branches was estimated by bootstrap analysis with 1000 repeated samplings of the data (Felsenstein, 1985).

Biological studies

For this study , adult *D. rubuscolum* mites were collected from blackberry plants at one sampling site selected randomly, and the development time of their progeny determined on leaf discs in Petri dish arenas. Leaf discs were cut from leaves harvested from four-month-old blackberry plants grown under glasshouse conditions in 20 L pots of compost (1:1:1 mix of peat moss: coconut fibre: vermiculite). Leaf discs (0.5 cm diameter) were cut from leaves avoiding leaf ribs, washed and rinsed with distilled water, and dried with a clean paper towel. Single leaf discs were placed in the base of 5 cm diameter Petri dishes containing damp sterile cotton wool. For ventilation the lid of the Petri dish had a 3 cm diameter hole covered with printing mesh. An individual adult female mite was placed, using a fine brush (size 15/0), into each arena (n=24) and incubated at 25 °C, 60% RH in a 12:12 light:dark regime for 10 hours during which time she laid eggs. The females and any additional eggs were then removed leaving only one egg in each arena. The cotton wool in each arena was moistened every 24 h with 3 mL of sterile distilled water and leaf discs replaced every four days. Observations were made every 12 h using a stereomicroscope (Nikon SMZ 1500, Nikon Instruments, Inc. Melville, NY) to determine when the larva hatched out of the egg and how many and how long each life stage was until the adult stage was reached. The duration of each developmental stage was estimated using the equations described by Perring *et al.* (1984). The duration of the egg stage was estimated using the following equation:

$$TE = \frac{Te_1 + Te_2}{2} - T0$$

Where: Te_1 = time before egg hatch, Te_2 = time after egg hatch, $T0$ = time when oviposition took place, and TE = time to complete egg stage. The larval stage was considered to last until an individual became inactive (nymphochrysalis). The total larval developmental time was estimated using the equation:

$$TL = \frac{Tl_1 + Tl_2}{2} - TE$$

Where: Tl_1 = time before the larva became quiescence (nymphochrysalis), Tl_2 = time spent as a nymphochrysalis, TE = time spent as an egg, and TL = time spent as a larva. All other developmental stages were estimated using the same equation.

Results

All 800 specimens mounted were identified as belonging to one species, *D. rubuscolum.* The morphological characteristics obtained matched the description of *D. rubuscolum* made by Trinidad *et al.* (2018); however, few differences and details on morphological structures observed through SEM are pointed out. Average measurements obtained from Mexican specimens are shown in Table 1, alongside data from Brazilian specimens provided by Trinidad *et al.* (2018) for comparison.

Complementary description

The adult female body is fusiform (Fig. 1A, B), with gnathosoma projecting downwards and strongly curved at the base; prodorsal shield with ornamentation net-like and scapular seta *sc* projecting forward ahead of rear shield margin (Fig. 2A); coxal plates with short lines and coxal plate I separated (Fig. 2B). In leg I, the tarsal empodium (*em*) is divided, each half of the empodium with five rays, terminal pair of simple rays (without subrays), and four basal pairs of complex rays (each ray with one additional medial subray); all rays were terminal with a tiny knob (Fig. 2C) and femoral setae *bv* absent (Fig. 3). In leg II, tarsal empodium (*em*) divided, each half of the empodium with five rays, terminal pair of simple rays (without subrays), and four basal pairs of complex rays (each ray with one additional medial subray);

all rays were terminal with a tiny knob and femoral setae *bv* absent (Fig. 3); external genitalia had a coverflap with short irregular lines at the base (Fig. 2B); internal genitalia with anterior genital apodeme trapezoidal, the oblique apodeme under the anterior genital apodeme and a pre-spermathecal tube joined to the longitudinal bridge, spermathecal tube shorts, and a globose

Table 1 Measurements of *Diptacus rubuscolum* mites from Mexico and Brazil (Modified from Trinidad *et al.*, 2019) (L—length, W—width). = this characteristic was evident in only one specimen. – = Not comparable, different body regions were considered for measurements.

Character	*Diptacus rubuscolum* (Mexico)								*Diptacus rubuscolum* (Brazil)					
	Female (n=6)		Male (n=4)		Larva (n=5)		Nymph (n=6)		Female		Male		Larva	Nymph
	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-max	Mean	Min-Max	Mean	Min-Max		
Body L	258.4	238-277	245.4	237.4-256.7	165.1	153.8-177.7	192.7	185.6-202.3	--	--	--	--	--	--
Body W	89	81.0-94.4	71.6	64.0-76.9	64.4	58.9-69.4	78.8	67.2-81.3	--	--	--	--	--	--
Prodorsal shield L	37.3	34.8-39.4	34.9	30.1-38.2	30	26.3-36	31.8	30.5-32.9	37.8±1.8	35-40	28.3±1.5	27-30	28	29-30
Prodosal shield W	56.5	46.3-63.5	53.1	47.5-57.8	44.6	42-47.5	49	46.6-51.2	61.8±4	57-67	51.3±4.7	46-55	50	50
Scapular seta (sc) L	2.4	1.27-2.8	1.72	1.5-2.0	1.2	1.1-1.6	1.46	1.1-1.8	3±0.0	3	3.0±0.0	3	1	1-2
Gnatosoma L	56.9	51.3-61.5	43	36.1-49.9	47.4	47-48	47.7	44.8-49.1	42.9±2.9	40-47	42.9±2.9	40-47	27	30-32
Cheliceral stylets L	58.4	49.7-64.5	46.9	44.6-53.7	45.3	44.2-46.9	45.9	42.4-48.3	62.4±7.2	50-69	62.4±7.2	50-69	47	47-49
Auxiliary stylet L.	53.6	46.0-56.8	46.6	42.7-50.6	42.3	40.8-44.3	42.4	40.2-44.5	51.1±5.2	44-57	51.1±5.2	44-57	40	40
Oral stylet L	43	36.3-51.1	35.9	34.8-36.9	28.3	28.0-28.7	29.9	25.6-34.0	11.2±0.8	10-12	11.2±0.8	10-12	12	10
Leg I L	44.18	41.9-46.9	42.79	37.85-52.74	25.1	23.5-26.3	31.1	25.5-35.1	47.4±2.1	43-49	47.4±2.1	43-49	28	30-32
Femur I L	15.06	13.7-15.7	13.3	11.96-13.51	10.3	9.1-11.3	9.5	8.6-11.9	10.8±0.6	10-12	10.8±0.6	10-12	10	7
Genu I L	6.2	5.6-7.4	5.73	5.06-6.48	4.1	3.6-4.8	4.3	3.5-5.2	6.9±0.6	6-8	6.9±0.6	6-8	4	4
Paraxial tibial (l')	8.7	5.9-10.9	7.61	6.73-8.26	5.2	4.3-5.7	5.5	5.3-5.9						
Antaxial genual I(l")	45.9	44.1-47.6	37.27	36.32-38.26	27.7	22.8-30.1	29.1	25.3-31.2	45.6±1.1	44-47	45.6±1.1	44-47	29	26
Tibia I L	13	12.5-13.4	9.9	7.14-11.69	5.4	5.1-5.9	6.6	5.2-7.2	15.6±0.5	14-15	14.6±0.5	14-15	5	7-8
Tarsus I L	8.5	7.6-9.3	7.51	6.61-8.22	5.3	5.1-5.6	5.6	4.9-6.2	10.3±1.1	9-12	9.0±0.0	9	6	5-6
Paraxial fastigial tarsal seta (ft´) L.	20.9	16.0-24.74	20.96	18.44-22.83	17.2	16.5-17.9	15.6	13.8-16.7	26±2.4	23-40	19.7±0.6	19-20	17	11-15
Antaxial fastigial tarsal seta (ft") L.	24.9	22.4-30.2	23.05	20.72-24.23	17.36	17.30-17.41	19.2	17.7-21.5	30.6±1.3	30-34	22.0±2.0	20-24	20	20
Paraxial unguinal (u´) L.	5.38	4.9-6.0	4.48	4.22-4.92	4.1	3.7-4.4	4.4	3.4-5.5	5.7±0.7	5-7	5.0±0.0	5	3	5
Tarsal solenidion I L.	7	6.6-7.6	6.55	5.97-7.12	5.7	5.1-6.3	6.0	4.0-6.7	8.0±0.0	8	6.0±0.0	6	6	7
Leg II L.	44.4	41.4-48.6	37.5	35.54-41.20	23.8	23.5-24.1	28.4	27.3-30	44.7±2.2	40-47	34.7±0.6	34-36	29	28
Femur II L.	14.2	12.9-15.7	13.8	12.58-14.89	9.8	9.0-10.6	9.2	7.4-10.4	10.7±0.8	9-12	9.0±1.0	8-10	6	7-8
Genu II L.	6.2	5.1-7.2	5.13	4.93-5.67	3.6	3.1-4.3	4	3-4.5	6.1±0.6	5-7	5.0±0.0	5	4	4
Antaxial genual II (l")	11.5	8.6-13.1	10.63	10.34-10.90	6	5.7-6.6	6.6	5.7-7.5	12.3±1.3	10-14	10.0±1.0	9-11	5	5
Tibia II L.	12.2	10.4-13.6	9.71	8.85-10.97	4.5	4.1-5.1	5.7	5.1-6	12.9±0.9	11-14	8.0±0.0	8	5	6
Tarsus II L.	8.4	8.0-8.7	7.26	6.63-8.33	6.1	5.3-6.7	5.7	5-6.7	8.3±0.7	7-9	7.3±0.6	7-8	5	6
Paraxial fastigial tarsal (*ft´*)	16.8	15.6-18.3	21.38	19.90-22.85	10.7	9.5-12.8	14.2	12.9-16.9	6.4±1.0	5-7	4.3±0.6	4-5	4	4-5
Antiaxial fastigial tarsal (*ft"*)	28.8	23.01-35.2	23.85	22.45-25.22	17.2	16.7-18	19.2	18.6-20.3	31.1±1.4	28-33	21.7±1.5	20-23	19	18-19
Paraxial unguinal II (*u´*) L.	5	3.7-6.0	3.91	3.32-4.27	4.26	4-5	3.8	3-4.8	5.6±0.5	5-6	4.7±0.6	4-5	3	3-4
Tarsal solenidion II L.	6.5	4.5-7.7	7.14	6.38-8.29	6.2	5.3-6.9	6	4.0-6.7	8.1±0.3	8-9	6.3±0.6	6-7	6	6
Empodium II L.	7.3	6.9-8.5	5.75	5.29-5.93	4.1	3.5-5.1	5.14	3.7-6	7.0±0.0	7	5.3±0.6	5-6	5	5
Coxal seta I (1b) L.	12.2	10.6-15.0	9.4	6.3-11.8	7	6.4-7.6	8.8	*	16.7±1.2	15-18	12.0±1.0	11-13	8	8
Seta I (la) L.	17.2	14.5-22.0	15.3	11.4-18.9	13.1	9.8-17.3	7.8	5.8-12.6	25.6±4	19-33	20.0±3.6	17-24	15	15-17
Seda II (2a) L.	50.6	40.4-65.1	44.2	43.2-45.2	16.6	10.1-23	17.7	12.6-23	62.4±4.7	55-70	41.0±10.4	29-48	32	32
Genitalia L	22.4	21.8-26.1	--	--	--	--	--	--	22.6±2.0	20-25	--	--	--	--
Genitalia W.	29.5	28.4-30.3	--	--	--	--	--	--	31.0±0.9	30-33	22.0±0.0	22		
Seta (3a) L.	9.5	8.0-11.6	7.6	6.2-9.2	9.3	6.9-12.32	7.6	5.5-9.7	11.9±1.0	10-13	8.0±0.0	8	6	5-6
Seta (c2) L.	41	33.7-45.8	31.94	27.78-37.33	13	9.5-15	15	11.3-18.1	53.5±3.8	49-60	33.3±4.2	30-38	23	23-24
Ventral seta I (*d*) L.	65.2	45.5-86.8	49.1	43.86-56.23	38.31	27.3-49.1	42	33.3-49.5	87.8±2.8	83-92	54.3±4.0	50-58	52	50-53
Ventral seta (e) L	52.1	39.2-73.8	48.5	42.1-52.4	38	36-40.8	31.7	25.5-39.6	--	--	--	--	--	--
Ventral seta (*f*) L.	37.4	35-39	28.74	23-31.6	20.8	19.1-23.3	21.9	19-23.5	41.7±3.3	35-45	33.3±2.9	30-35	24	25-28
No. of dorsal annuli	72.5	68-77	62	56-68	57	55-59	60	58-66	69.4 ±2.5	65-72	64.0±1-7	63-66	52	55-57
No. of ventral annuli	70.5	68-73	60.5	57.64	52	48-55	55	50-57	69.4±1.9	68-73	62.3±4.2	59-67	53	52
Seta (*h2*) L	70.6	51.8-93	57.9	43.4-66.7	25.6	23.2-27.1	36.8	26.7-54.2	81.0±9.5	60-89	58.7±14.6	42-69	38	46-52
Seta (*h1*) L	1	1	0.85	0.81-1					1.0±0.0	1	1.0±0.0	1		

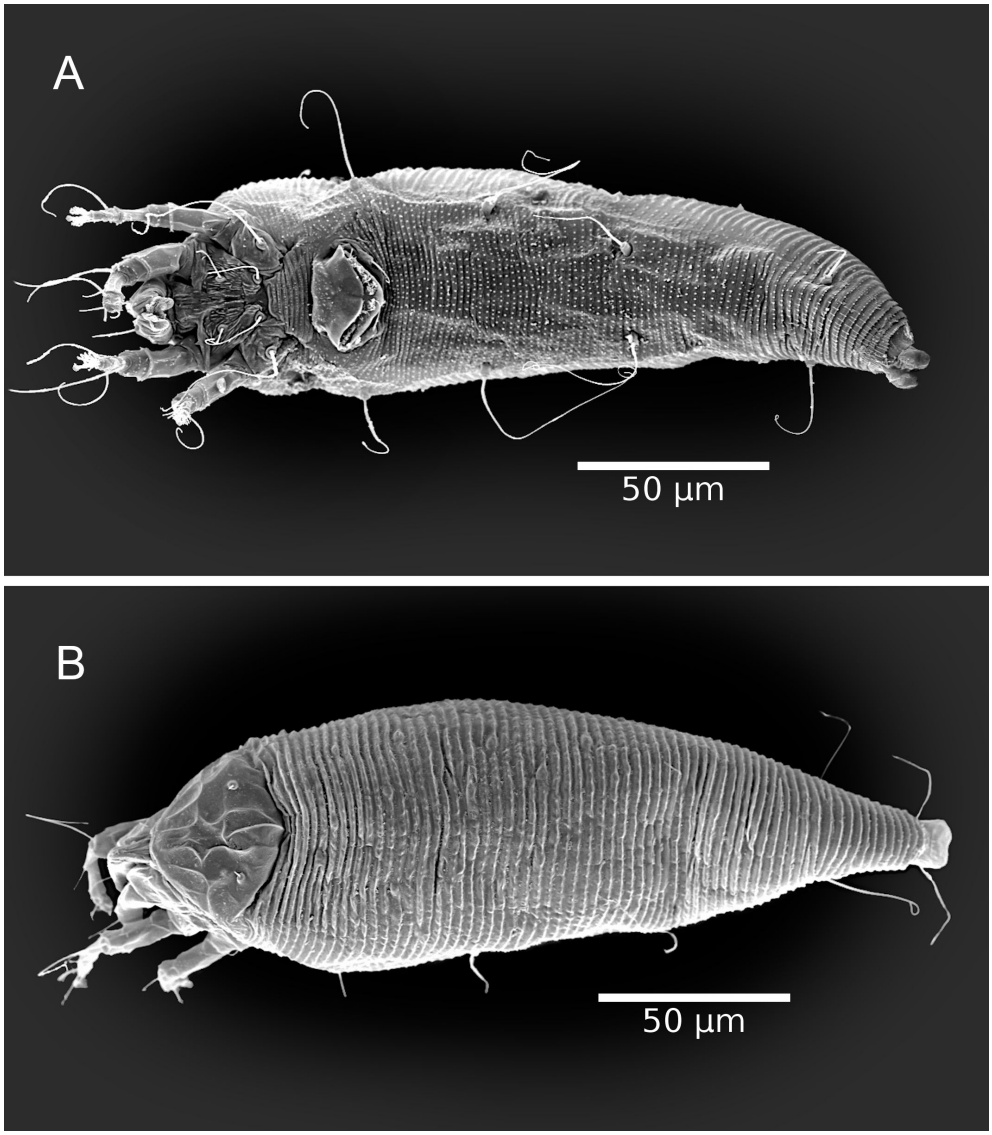


Figure 1 Dorsal (**A**) and ventral (**B**) view of *Diptacus rubosculum* adult female.

shaped spermatheca (Fig. 3); the opisthosoma with 68–77 subequal dorsoventral smooth annuli, and 68–73 ventral annuli with round microtubercles on rear annulus margin (Fig. 1A).

The adult male body is fusiform (Fig 4); gnathosoma projecting downwards and strongly curved at base; prodorsal shield with ornamentation net-like; coxal plates with short lines and coxal plate I separated; in leg I, tarsal solenidion slightly curved and knobbed, tarsal empodium (*em*) divided, each half of the empodium with five rays, terminal pair of simple rays (without subrays), and four basal pairs of complex rays (each ray with one additional medial subray); all rays were terminal with a tiny knob, and femoral setae *bv* absent; in leg II, tarsal solenidion slightly curved and knobbed and tarsal empodium (*em*) divided, each half of the empodium with five rays, terminal pair of simple rays (without subrays), and four basal pairs of complex rays (each ray with one additional medial subray); all rays terminal with tiny knob, and femoral setae *bv* absent; the opisthosoma with 56–68 dorsal subequal smooth annuli, and 57–64 ventral annuli with round microtubercles on rear annulus margin.

Nymphs with vermiform body; gnathosoma projecting downwards and strongly curved at base; prodorsal shield with ornamentation net-like and projecting forward ahead of rear shield

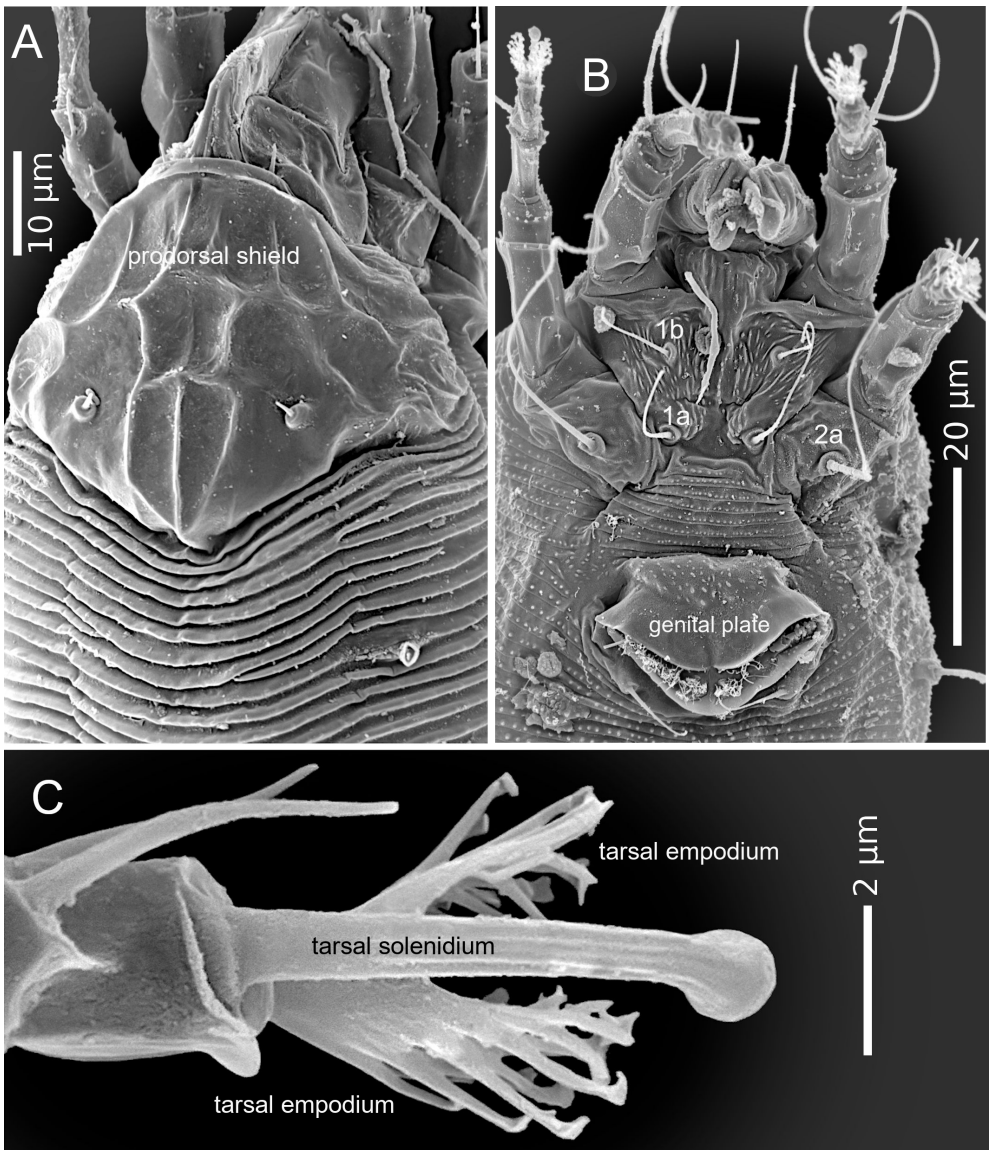


Figure 2 Adult female of *Diptacus rubuscolum* showing, **A** – prodorsal shield and scapular seta (*sc*), **B** – genital plate, anterolateral setae on coxisternum I (*1b*), proximal setae on coxisternum I (*1a*) and proximal setae on coxisternum II (*2a*), and **C** – tarsal solenidion and tarsal empodium divided.

margin; in leg I, tarsal solenidion slightly curved and knobbed, and tarsal empodium (*em*) divided with femoral setae *bv* absent; in leg II, tarsal empodium divided and tarsal solenidion slightly curved and knobbed; the opisthosoma with 58–66 dorsal annuli and 55–57 ventral annuli; both with round microtubercles on rear annulus margin.

Larva with vermiform body; gnathosoma projecting downwards and strongly curved at base; prodorsal shield with ornamentation net-like and scapular tubercles in the rear shield margin; in both legs the empodium is divided, the solenidion is slightly curved and knobbed and seta *bv* absent on both legs; the opisthosoma with 55–59 dorsal annuli, 48–55 ventral annuli, both with round microtubercles on rear annulus margin.

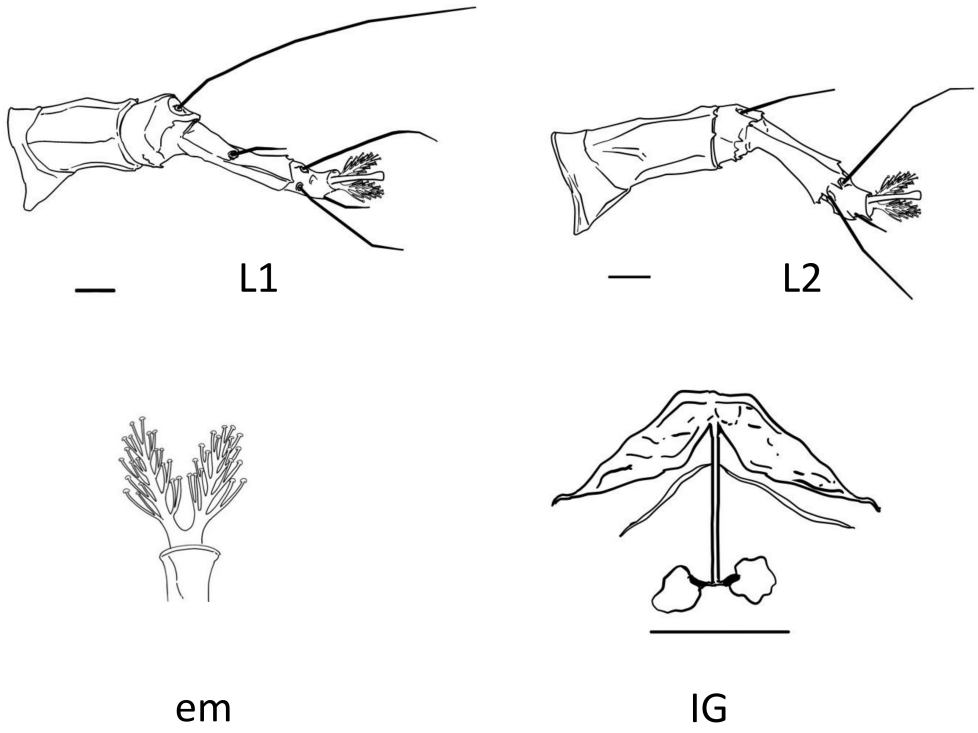


Figure 3 Empodium (em), internal female genitalia (IG), leg I (L1) and leg 2 (L2) of female adult *Diptacus rubuscolum*. Scale bar: 10µm for IG; 5µm for L1 and L2.

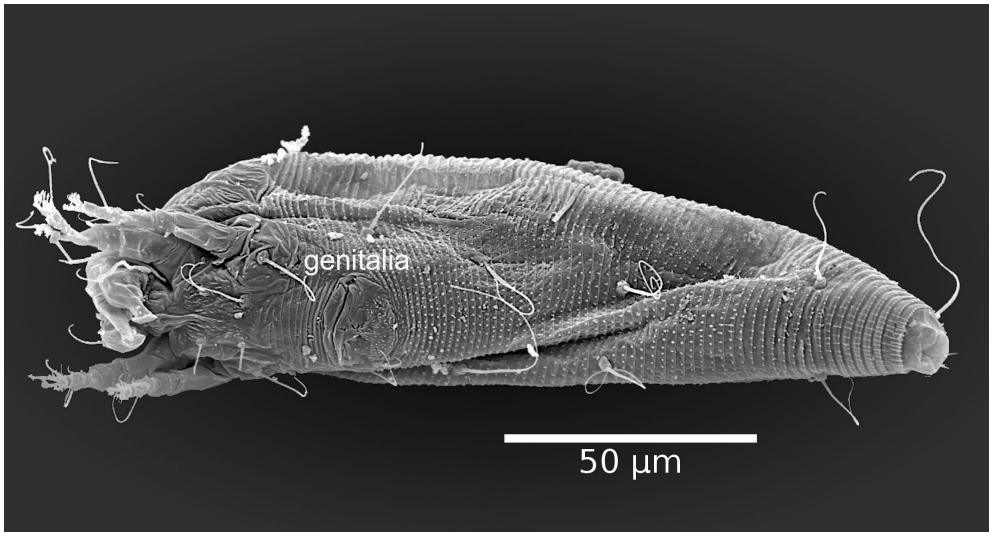


Figure 4 Ventral view and genitalia of male *Diptacus rubuscolum*.

Molecular characterization

The final length of the mt*COI* sequences were 652 bp and available from GenBank with the accession numbers MZ519880 and MZ519881; for the 18S they were 792 bp (MZ518817-MZ518818); for the D2-5 and D9-10 regions of the 28S rRNA they were 945 bp (MZ518781-

MZ518782) and 739 bp (MZ518794-MZ518795), respectively.

Sequence diversity

The sequence data from the D2 region of the 28S rDNA we used, comprised 11 aligned sequences of 950 bps, two obtained in this study from *D. rubuscolum* and nine retrieved from GenBank including one outgroup species. In the alignment, 128 sites were parsimony informative, and 268 sites were variable. The average mean divergence over all sequence pairs (including the outgroup taxa) was 25% (SE=2%) and ranged from 0.2 to 62.0%. The average mean divergence over the *Diptacus* spp. sequences was 15.0% (SE =1%) and ranged from 0.2 to 25% (Table 2). The average mean divergence between the *D. rubuscolum* sequence we obtained and that obtained from Genbank was 0.2 % (SE = 0.2%) confirming species identity (Table 2).

Phylogenetic analysis

The phylogenetic tree inferred from the maximum likelihood analysis of the D2 region of the sequences studied, consisted of two well-supported clades, one containing *D. rubuscolum* and *D. mengdaensis* sequences; all *D. rubuscolum* samples were grouped with 99% probability but separated from *D. mengdaensis* with 89% probability. The other clade contained all other *Diptacus* species clustered separately with probabilities ranging from 96 to 99% (Fig. 5). This analysis successfully supported identification of *D. rubuscolum* in our samples.

Biology of *Diptacus rubuscolum*

Diptacus rubuscolum has a simple life cycle with the same developmental stages typical of the Eriophyioidea superfamily: egg, larva, nymph and adult. They had two quiescent stages; the first was between the larval stage and the nymphal stage (nymphochrysalis), and the second was between the nymphal stage and the adult stage (imagochrysalis). The crystalline colour of the egg became yellow and translucent, and it took on a semi-spherical shape. Fertilized females oviposited on leaf surfaces, particularly along the secondary ribs. The mean duration of the egg stage was 56.5±2 h. Larvae were crystalline in colour and became translucent before the first quiescent stage; the mean duration of this stage was 56.5±2 h. The first quiescent stage, or nymphochrysalis, was white in colour with a mean duration of 21.8±1.5 h. There was only one nymphal stage, white in colour, which showed greater mobility and feeding activity than the larva; the mean duration of this stage was 41.3±1.9 h. The second quiescent stage, or imagochrysalis, was white in colour, and occurred just before the adult stage and had a mean duration of 19.7±1.3 h. The adult was white in colour and the most active stage with a mean duration of 114.5±2.7 h.

Table 2 Percentage of divergence with standard error (SE) for D2 region of the 28S rDNA sequences pairs within and between Diptacus species and other members of Diptilomiopidae, Phytoptidae and Eriophyidae. Samples labelled as 1A and 1C are *D. rubuscolum* from Mexico. All other sequences were retrieved from GenBank and accession numbers are shown.

	1	2	3	4	5	6	7	8	9	10	11
1- 1A	N/C										
2- 1C	0.2 (0.2)										
3. *Diptacus rubuscolum* MH626119	0.2 (0.2)	0.4 (0.2)									
4. *D. rubuscolum* MH626120	0.2 (0.2)	0.4 (0.2)	0.0 (0.0)								
5. *D. mengdaensis* KF782554	7.7 (1.3)	7.9 (1.3)	7.5 (1.3)	7.5 (1.3)							
6. *D. persicae* F782576	23.0 (2.5)	22.7 (2.5)	23.0 (2.5)	23.0 (2.5)	21.6 (2.3)						
7. *Dialox* sp. KF782552	23.3 (2.6)	23.0 (2.5)	23.3 (2.6)	23.3 (2.6)	21.3 (2.3)	3.3 (0.8)					
8. *Diptacus* sp. KF782555	24.5 (2.6)	24.2 (2.5)	24.5 (2.6)	24.5 (2.6)	21.3 (2.3)	6.6 (1.2)	6.3 (1.2)				
9. *D. aceris* KF782553	25.7 (2.7)	25.4 (2.7)	25.7 (2.7)	25.7 (2.7)	26.0 (2.7)	11.9 (1.6)	12.1 (1.6)	16.9 (2.5)			
10. *Diptilomiopus ligustri* KF782551	28.1 (3.0)	28.1 (3.0)	28.1 (3.0)	28.1 (3.0)	26.4 (2.8)	21.1 (2.4)	21.2 (2.5)	24.4 (2.7)	22.7 (2.6)		
11. *Trisetacus juniperinus* KF782470	54.5 (4.8)	55.0 (4.8)	54.5 (4.8)	54.5 (4.8)	52.0 (4.6)	62.1 (5.7)	61.6 (5.6)	60.0 (5.3)	65.0 (5.9)	62.0 (5.6)	N/C

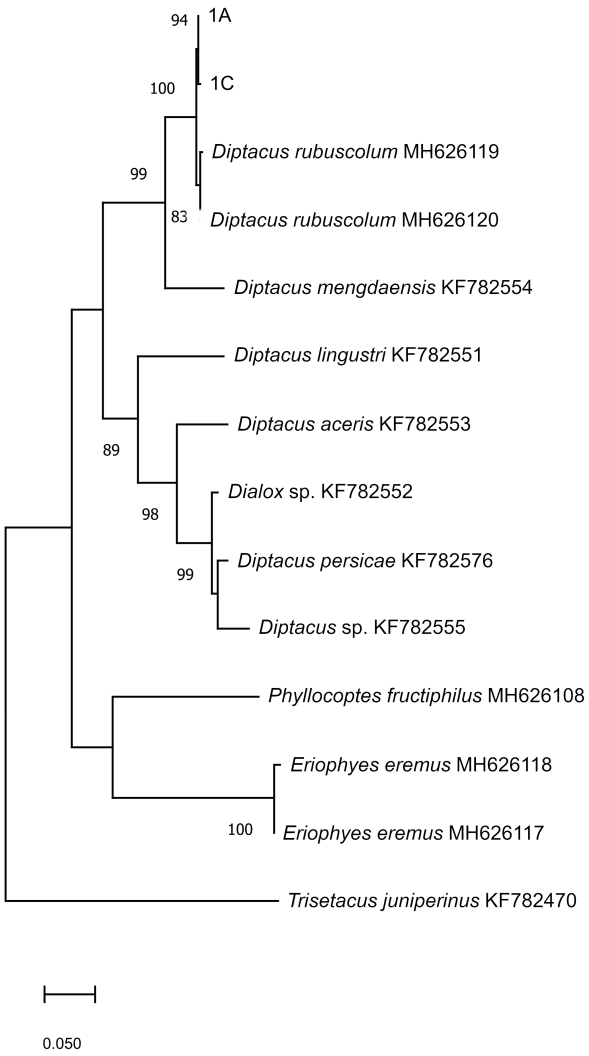


Figure 5 Dendogram inferred from maximum likelihood (ML) analysis of the D2 region within the 28S rRNA data of mites. Sequences used as a reference are labelled according to their GenBank accession numbers. The sequence of *Trisectacus juniperinus* was used as an outgroup. Only bootstrap values above 80% are shown.

Discussion

Morphological characteristics, genetic diversity and phylogenetic analyses confirmed the identity of this mite species in our samples. The small size of eriophoid mites, as well as the existence of potential cryptic species, make it difficult to rely only on morphological methods to identify these mite species. Therefore, a combination of morphological and molecular methods is considered the most accurate strategy for identification of eriophyoid mites (Duarte *et al.* 2019). Partial sequences of the D2 region have been reported to show low variation amongst species, compared with *COI* or ITS, sometimes being very similar amongst closely related species (Lee and Ó Foighil 2004). However, our results after analysing partial sequences of the D2 region confirmed a clear separation between *D. rubuscolum* and other *Diptacus* species, with genetic variations of 7–25%. Analyses of this region from other Eriophyidae species revealed variation of 2–19% amongst *Abacarus* species (Duarte *et al.* 2019), 30–36% amongst *Aculops* species (Duarte *et al.* 2023), 2% between *Aceria tosichella* Keifer and *A. eximia* Sukhareva (Skoracka *et al.* 2012), and 4% between *Eriocaenus ramosissimi* Petanović

& Amrine and *E. equiseti* Farkas (Petanović *et al.* 2015).

In all our surveys and despite sampling all plant structures and on six different occasions, we did not find *Acalitus essigi* or *A. orthomera*, which have been reported previously on blackberry, *R. ulmifolius* from Michoacán, Mexico (Ayala-Ortega *et al.* 2019). Previously, *A. essigi*, has been reported causing uneven ripening of blackberry fruits in Australia (Scott *et al.* 2008), a symptom previously called the 'redberry disease' (de Lillo and Cuso 1996). In our surveys, we did observe some blackberry fruits with uneven ripening, but the fact that we did not find *A. essigi* suggest more research is needed to determine more conclusively the relationship between *A. essigi* and the redberry disease (Low *et al.* 2020).

The morphological description we have provided, in addition to the existing description of the type material (Trinidad *et al.* 2018), represents an important contribution towards overall knowledge about this species. All morphological traits were in line with what has been reported previously for the type specimen; all our measurements fell within the range reported for the type species (Trinidad *et al.*, 2018). However, we found some differences; the adult type specimen was considered to have a vermiform body, while we consider that the specimens observed in our study had fusiform bodies (Fig. 1). Having a fusiform body is considered an important and unique characteristic of members of the subfamily Diptilomiopinae (Hong and Shang 1997), which includes the genus *Diptacus.* Moreover, in fusiform mites, the dorsal tergites are morphologically differentiated from the ventral esternites (Linquist 1996), which is the case for the specimens we evaluated (Fig. 1). A more detailed observation through SEM photography, revealed the existence of a divided empodium, and in each half, a terminal pair of simple rays with four basal pair, and each one with an additional subray (Fig. 2C, 3).

Protogyne females of *D. rubuscolum* deposited their eggs close to the secondary leaf ribs, presumably for better access to nutrients (Royalty and Perring 1996). The average duration from egg to the end of the adult stage was 13 days, with two quiescent stages between larva and nymph and between nymph and adult, known as the nymphochrysalis and imagochrysalis, respectively (Sternlicht and Goldenberg 1971). Information regarding life cycle and longevity on Eriophydae are very scarce; some publications on longevity of mites in this family report mean values of 24 days for *Rhyncaphytoptus ficifoliae* Keifer (Trombidiformes; Diptilomiopidae) (Bahirai *et al.* 2019) and 19 days for *Aculops guajavae* (Abou-Awad *et al.* 2016), considered a synonym of *Tegolophus guavae* Boczek (Elhalawany 2018).

None of the active stages of *D. rubuscolum* caused visible damage when feeding on blackberry plants, as also reported for *D. caseius* on *Rubus caeseius* plants in Germany (Domes 2000); or any symptom of virus infection, potentially transmitted by *D. rubuscolum*. It has been reported that the effect of eriophyid mites, in most cases, is non-symptomatic where mechanical damage caused by feeding activity is normally insignificant (Lindquist and Oldfield 1996), but in high populations they may cause phytotoxemia (de Lillo *et al.* 2018). Potential explanations for the lack of visible damage to plant tissues are that either chloroplast particles or cell contents are not fully extracted during feeding, or that the cell wall repairs itself after the mite´s mouthparts are removed (Lindquist and Oldfield 1996). It has also been suggested that the lack of visible damage is because of low population densities of the mite, making damage to the plants negligible (Petanović and Kielkiewicz 2010). However, a recent study carried out on Mexican blackberries, showed larger populations of *D. rubuscolum* than *T. urticae* (González-Domínguez *et al.* 2023). Here we studied only one plant cultivar (Tupy), and damage caused by eriophyoid mites may be different depending on the plant cultivar, or even on the eriophyoid genotype, in addition to population density and environmental conditions (Petanović and Kielkiewicz 2010). Overall, more research is required to determine whether any damage is caused by *D. rubuscolum* when feeding on blackberry plants.

In conclusion, *D. rubuscolum* was the only eriophyoid mite found on blackberry plants in the studied areas in México. We have provided more information about the morphology of this species, which in combination with partial sequences of mt*COI*, the 18S and 28S of the rRNA, will facilitate accurate identification of this species in future studies. This is the first report

about the biology and duration of the different life stages of this species, contributing to the overall knowledge of Eriophyoidea.

Acknowledgements

SGGD received a scholarship from CONACYT for her PhD. This study was funded by Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA) within the project entitled 'Dinámica espacio-temporal de ácaros plaga en el cultivo de zarzamora en Michoacán', grant number PM-18-4017. The authors are grateful to the growers that allowed the first author to take samples from their blackberry crops. We thank Dr P.E. Chetverikov for his valuable help with the morphology of the internal genitalia and empodium.

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