

A new species of *Acalitus* (Acari: Eriophyoidea) on *Viguiera dentata* and the first record of *Acalitus comptus* Flechtmann on *Sphagneticola trilobata* from Mexico

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Abstract

A new species of eriophyoid mite in the genus *Acalitus* is described and illustrated. *Acalitus viguierae* **sp. nov.** infests *Viguiera dentata* (Cav.) Spreng. and causes erineum on the lower surface of the leaves. Compared with other *Acalitus* species described from different host plants within the supertribe Helianthodae, it differs morphologically. The previously known species *Acalitus comptus*, originally described on *Sphagneticola trilobata* (L.) Pruski from Guadeloupe, is also recorded on the same host plant in Mexico. DNA fingerprinting for both species is reported and compared with available sequences of *Acalitus simplex* and *Acalitus phloeocoptes*. Pairwise comparison of mitochondrial *COI* gene sequences revealed 16.3% genetic divergence between *Acalitus viguierae* **sp. nov.** and *Acalitus comptus*, while divergence values between the sequences obtained in this study and previously published congeneric sequences ranged from 21.1% to 28.1%. Maximum Likelihood phylogenetic analysis showed that the four analysed species from the genus *Acalitus* clustered into a single, well-supported clade, with *A. comptus* and *A. viguierae* **sp. nov.** clearly placed within the genus.

Key words: Eriophyidae, Helianthodae, taxonomy, *mtCOI*

Introduction

The plant genera *Sphagneticola* O. Hoffm. and *Viguiera* Kunth belong to the family Asteraceae, subfamily Asteroideae (Cass.) Lindl., supertribe Helianthodae H. Rob., and tribe Heliantheae Cass. The tribe Heliantheae is one of 15 tribes within the supertribe Helianthodae. Within the tribe Heliantheae, 14 subtribes are recognized, with the genus *Sphagneticola* belonging to the subtribe Ecliptinae Less., and the genus *Viguiera* to the subtribe Helianthinae Dumort. (CWG 2025; Robinson 2004).

Sphagneticola trilobata (L.) Pruski is a tropical species native to tropical America, ranging from Mexico to Brazil and throughout the Caribbean. It has spread rapidly into new areas following its introduction as an ornamental plant, threatening native flora. The International Union for Conservation of Nature (IUCN) has included *S. trilobata* in its list of the 100 worst invasive alien species in the world. Although *S. trilobata* is the accepted name, *Wedelia trilobata* (L.) Hitch. is still commonly used in the Pacific (CABI 2013; HEAR 2008; Lowe *et al.* 2000)

Viguiera dentata (Cav.) Spreng. is native to Central America and Mexico. It is listed as an invasive species only in Cuba, where it grows as an invasive weed on cultivated land and in ruderal areas, as it does in its native region (CABI 2015; Oviedo Prieto *et al.* 2012).

Eriophyoid mites, arthropods with a minute body size (between 80 and 500 µm), are obligate phytophagous that can cause conspicuous symptoms through direct feeding and also transmit plant viruses. To date, approximately 4,750 species have been described, including significant plant pests, while some are considered potential agents of biological weed control (Lindquist *et al.* 1996; Marini *et al.* 2021; Smith *et al.* 2010; Sullivan & Ozman-Sullivan 2021). Most eriophyoid mites (about 80–95%) are specialized to colonies a single host plant or a few plant species within the same genus (Skoracka *et al.* 2010). They exhibit high host specificity, along with other attributes such as high reproductive rates, short generation times, wind dispersal, and significant impact on target plants, which favour them as potential biological control agents of weeds (Cullen & Briese 1998; Lindquist *et al.* 1996; Smith *et al.* 2010). In addition to the traditional description of eriophyoid mites based on their morphological characteristics, DNA-based methods are increasingly used and have become an indispensable part of taxonomic research. Among the available molecular markers, *mtCOI* (mitochondrial cytochrome c oxidase subunit I) has become a standard DNA barcode, providing a robust and widely used tool for precise species identification (Chetverikov *et al.* 2015; Hebert *et al.* 2003; Navajas & Navia 2010).

According to the available literature, eight species of eriophyid mites of the genus *Acalitus* have so far been described from different host plants belonging to various tribes and subtribes within the supertribe Helianthodae (family Asteraceae).

On three host plants of the tribe Eupatorieae Cass., four species of eriophyid mites have been described: *A. osmia* (Cromroy 1958) and *A. odoratus* Keifer, 1970, found on *Chromolaena* (*Eupatorium*) *odorata* (L.) R.M. King & H. Rob.; *A. inulaefolii* Keifer, 1970, on *Austroeupatorium* (*Eupatorium*) *inulaefolium* (Kunth) R.M. King & H. Rob.; and *A. mikaniae* Keifer, 1974, found on *Mikania scandens* (L.) Willd.

One species of eriophyid mite has been described for each host plant: one on a plant of the tribe Inuleae Cass. and one on a plant of the tribe Neurolaeneae Rydb. *A. purpurascensis* (Cromroy 1958) was described on *Pluchea odorata* (L.) Cass (*Pluchea purpurascens* (Sw.) DC.) of the tribe Inuleae, and *A. maracai* (Boczek & Nuzzaci 1988) was described on *Neurolaena lobata* (L.) R.Br. ex Cass. (*Pluchea symphytifolia*) of the tribe Neurolaeneae.

Two species have been described on host plants of the tribe Heliantheae Cass., but these host plants belong to different subtribes. *A. heliopsis* Keifer, 1975, was discovered on *Heliopsis buphthalmoides* (Jacq.) Dunal from the subtribe Zinniinae Benth. & Hook.f., and *A. comptus* Flechtmann, 1999, was discovered on *Sphagneticola* (*Wedelia*) *trilobata* (L.) Pruski from the subtribe Ecliptinae Less.

Of the eight *Acalitus* species described so far, four cause erineum on the underside of the leaves (*A. osmia*, *A. odoratus*, *A. inulaefolii*, and *A. heliopsis*), and two cause galls on the lower leaf surface (*A. purpurascensis* and *A. maracai*). The relationship to the host for *A. mikaniae* is unstated but possibly as a bud mite, while for *A. comptus*, the symptoms are not listed in the original description. These species have been described from the Caribbean islands (Puerto Rico, Guadeloupe, Trinidad), Colombia, Venezuela, and Florida (Amrine & Stasny 1994; Boczek & Nuzzaci 1988; Cromroy 1958; Flechtmann *et al.* (1999) [2000]; Keifer 1970, 1974, 1975).

There are no records of eriophyid mites on plants of the genus *Viguiera*, which belongs to the subtribe Helianthinae within the tribe Heliantheae, in the current literature.

The aim of this study is to describe the new eriophyid mite species *Acalitus viguierae* **sp. nov.** from *Viguiera dentata* and to provide a supplementary description of *Acalitus comptus* Flechtmann from *Sphagneticola trilobata*, recorded at a new locality, using traditional morphological methods.

The study also supplements the morphological characterization of both species with *COI* sequence data to facilitate DNA-based identification of future specimens.

Material and methods

Collection and Light Microscopy Morphological Study

Plant samples of *S. trilobata* and *V. dentata* collected in Mexico were placed in vials containing ethanol. Two samples of *S. trilobata* and one sample of *V. dentata* were collected (Tab. 1). The plant material was then sent to Serbia, where mites were removed from the leaf samples in the laboratory by direct examination under a stereomicroscope or by extraction methods (de Lillo 2001; Monfreda *et al.* 2007).

For morphological analysis, mite specimens were mounted in Keifer's F medium (Amrine & Manson 1996). Structures relevant to taxonomic identification were examined using an Olympus BX53 research microscope with phase contrast and measured with the cellSens Entry 2 (CS-EN-V2) software package on the same microscope. The morphology and nomenclature follow Lindquist (1996); the genus classification is based on Amrine *et al.* (2003), and the nomenclature of the internal female genitalia follows Chetverikov (2014). Measurements and illustrations are based on Amrine & Manson (1996) and de Lillo *et al.* (2010). All measurements are given in micrometers (μm) and indicate the length of the structure unless otherwise stated. Each measurement of the holotype is preceded by the corresponding range for the paratypes (indicated in brackets). Specimens were illustrated using a camera lucida on a Leica DMLS research microscope with phase contrast. Plant names are consistent with the online database The Plant List (2013).

The holotype and paratype slides are deposited in the collections of the Acarology Laboratory, Department of Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia.

TABLE 1. Collection data for *Acalitus* spp. inhabiting *Sphagneticola trilobata* and *Viguiera dentata*.

Species of mites	Sample code	Host plant	Locality	Date	Geographical coordinates	Accession numbers <i>COI</i>
<i>A. comptus</i>	ST1	<i>S. trilobata</i>	Mexico Veracruz Lagos Puente Moreno Sur	27/12/2024	19.10138889N 96.15138889W	PX640558
<i>A. comptus</i>	ST2	<i>S. trilobata</i>	Mexico Veracruz Lagos Puente Moreno	01/2025	19.10833333N 96.15833333W	PX640559
<i>A. viguierae</i> sp. nov.	VD	<i>V. dentata</i>	Mexico Felipe Carrillo Puerto	02/ 2025	19.6066670N 87.9866670W	PX640560

DNA extraction, PCR amplification and sequencing

Prior to DNA extraction, mite specimens were removed by direct examination under a stereomicroscope from leaves of *S. trilobata* and *V. dentata*, preserved in 96% ethanol, and stored at 4 °C. Total genomic DNA was extracted from a single specimen using the QIAGEN DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions with modifications based on Dabert *et al.* (2008). The isolated DNA is archived in the Laboratory for Molecular Diagnostics, Department of Plant Pests, Institute for Plant Protection and Environment.

The barcode region of the mitochondrial cytochrome oxidase subunit I gene (*COI*) was amplified by polymerase chain reaction (PCR) using primers LCO1490Hem/HCO2198Hem

(Germain *et al.* 2013). PCR amplification was performed in a 25 µL final reaction volume, following the procedure and reaction conditions specified by Chetverikov *et al.* (2015). Amplified products of the *mtCOI* gene were sent to Eurofins sequencing service (Eurofins Genomics-MWG, Ebersberg, Germany) for cleaning and sequencing in both directions.

Alignment and molecular analyses

The obtained sequences were edited with Finch TV 1.4.0 (www.geospiza.com) and aligned with ClustalW integrated in MEGA11 software (Tamura *et al.* 2021). Prior to analysis the sequences were translated into amino acids to confirm the absence of stop codons. The DNA sequences obtained in this study were deposited in GenBank under accession numbers PX640558-PX640560 (Tab.1).

Comparison with sequences from GenBank was carried out using the BLAST analysis (Basic Local Alignment Search Tool; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). In addition, sequences were compared with BOLD (Barcode of Life Data Systems) database, as well.

Molecular analyses were conducted using *mtCOI* sequences of the two species examined in this study together with available congeneric sequences of comparable length retrieved from GenBank (*Acalitus simplex*; OR351224) and *Acalitus phloeocoptes*; MZ482143-MZ482146). Phylogenetic relationships were reconstructed using Maximum likelihood (ML) phylogeny, implemented in MEGA11. Five hundred bootstrap replicates were performed to assess branch support in the resulting tree topology. The best-fitting model of sequence evolution was determined with Modeltest 3.7 (Posada and Crandall 1998), based on Akaike information criterion (AIC) and Bayesian information criterion (BIC). The HKY+G model was chosen as the best model for the maximum likelihood analysis (ML) based on BIC. The *mtCOI* tree was rooted using two species from the same tribe Aceriini: *Aceria tulipae* (Nalepa) and *Tetrameracarus taiwanensis* Huang as outgroups (GenBank acc. numbers EF409416 and MZ483733, respectively).

Results

Taxonomy

Acalitus comptus Flechmann (Fig. 1 & 2)

Family Eriophyidae Nalepa, 1898.

Subfamily Eriophyinae Nalepa, 1898.

Tribe Aceriini Amrine and Stasny, 1994.

Genus *Acalitus* Keifer, 1965

Supplementary description. FEMALE (n=10). Body worm like 185–210, 39–43 wide. **Gnathosoma** 12–15, cheliceral stylets 9–10. **Prodorsal shield** 18–20, 22–24 wide, ornamented. The median line is incomplete and present only on the rear half of the shield; the admedian lines are complete. Between the median and admedian lines, there are two short oblique lines on the rear edge of the shield. Between the admedian lines, two incomplete lines extend from the front edge of the shield. The first pair of submedian lines is present on the anterior half but is incomplete; the second pair is also incomplete, extending from the anterior margin and ending at the posterior quarter of the shield; the third submedian lines are complete. A small line and several smaller dashes are visible between the second and third submedian lines in the posterior half of the shield. In some specimen slides, this small line may merge with the third submedian line, making the third submedian line appear forked, with short dashes visible between the branches. Dorsal tubercles *sc* on rear shield

margin 12–16 apart, setae *sc* 14–17 directed posteriorly. **Legs I** without tibial I and femoral I setae. Leg I 17–20; femur 7–9, setae *bv* missing; genu 2, setae *l''* 10–11; tibia 3, setae *l'* missing; tarsus 4–5, setae *ft'* 5–6, setae *ft''* 12–14, setae *u'* 2; solenidia ω 5; empodium 4, and 4 rayed. **Legs II** 17–18; femur 7–8, setae *bv* 7; genu 2–3, setae *l''* 7–8; tibia 2–3, setae *l'* missing; tarsus 3–4, setae *ft'* 2–3, setae *ft''* 13–15, setae *u'* 2; solenidia ω 5–6; empodium 4, and 4 rayed. Coxa I fused to each other. **Coxigenital area** granulated; setae *lb* 6, *lb* tubercles 6 apart; setae *la* 8–12, *la* tubercles 7–8 apart; setae *2a* 23–26, *2a* tubercles 14–17 apart. **Genital coverflap** 8–10, 15–17 wide with 4–5 of coarse granules; setae *3a* 5–6 long, *3a* tubercles 11–13 apart. **Opisthosoma** with subequal annuli, 77–83 dorsal and 66–70 ventral annuli. Microtubercles slightly elongate with a minute distal spine. Last 12 dorsal annuli with smaller microtubercles. Setae *c2* 8–11, 34–40 apart, on annulus 7–9; setae *d* 23–30, 32–35 apart, on 20–22 annulus; setae *e* 11, 19–22 apart, on 38–41 annulus; setae *f* 11–12, 10–11 apart, on 61–65 annulus, setae *h2* 34–39, 7–9 apart, setae *h1* 2, 5–6 apart.



FIGURE 1. Symptoms of *Acalitus comptus* on *Sphagneticola trilobata*: erineum on the lower surface of the leaf.

Material and locality. Veracruz Lagos Puente Moreno Sur, Mexico (19.10138889N; 96.15138889W), 27 December 2024, 50 slides, coll. R. Segura; Veracruz Lagos Puente Moreno, Mexico (19.1083333N; 96.1583333W), 15 slides, January 2025, coll. Person J.C.

Host plant. *Sphagneticola trilobata* (L.) Pruski (Asteraceae).

Relation to host plant. The mites cause erineum on the lower surface of the leaves (Fig. 1).

GenBank accession numbers. PX640558 and PX640559

Remarks. When analyzing and comparing the quantitative morphological characteristics of the mite population from Mexico with the original description of *A. comptus* from Guadeloupe, only differences in the number of opisthosomal rings were observed; no other differences were found, and the measured characteristics of the Mexican population match those in the original description (Tab. 2). When analyzing the qualitative morphological characteristics, i.e. the pattern of drawing on the dorsal shield, between the mite population from Mexico and the original description of *A. comptus* from Guadeloupe, some differences are observed. The differences are as follows: in the Mexican population, on the rear edge of the shield, an incomplete median line and two short oblique lines

between the median and admedian lines are visible, which are not present in the original drawing of the description. In the population from Mexico, between the admedian lines, there are two incomplete lines extending from the front edge of the shield, whereas only one line is shown in the original description. Both populations have three submedial lines; the first and second are incomplete, and the third submedial line is incomplete in the original description but complete in the population from Mexico (Fig. 2). It should be emphasized that the original description did not provide a textual account of the shield's patterns, but only a drawing, in which the left and right sides of the shield do not have the same ornamentation.

TABLE 2. Comparison of morphological characters between the original description of *Acalitus comptus* and the population of *A. comptus* from Mexico.

Characteristics	<i>A. comptus</i> original description	<i>A. comptus</i> Mexico	Characteristics	<i>A. comptus</i> original description	<i>A. comptus</i> Mexico
Length of body	163–188	185–210	Length of setae <i>fi'</i>	4–5	2–3
Width of body	39–41	39–43	Length of setae <i>fi''</i>	13–14	13–15
Length of gnathosoma	13–15	12–15	Length of setae <i>u'</i>	3	2
Length of cheliceral stylets	11–12	9–10	Length of solenidion	5–6	5–6
Length of prodorsal shield	17–19	18–20	Length of empodium	4	4
Width of prodorsal shield	20–21	22–24	Length of setae <i>1b</i>	6–7	6
Length of setae <i>sc</i>	14–16	14–17	Apart of setae <i>1b</i>	6	6
Tubercles of <i>sc</i> apart	14–15	12–16	Length of setae <i>1a</i>	13–14	8–12
Length of leg I	18–20	17–20	Apart of setae <i>1a</i>	7	7–8
Length of femur I	7–8	7–9	Length of setae <i>2a</i>	23–26	23–26
Length of genu	2	2	Apart of setae <i>2a</i>	14–16	14–17
Length of seta <i>l''</i>	8–11	10–11	Number of dorsal annuli	64–68	77–83
Length of tibia	2–3	3	Number of ventral annuli	58–65	66–70
Length of tarsus	4–6	4–5	Length of female genitalia	8–9	8–9
Length of setae <i>fi'</i>	6–8	5–6	Width of female genitalia	15–17	15–17
Length of setae <i>fi''</i>	11–14	12–14	Length of setae <i>3a</i>	5–7	5–6
Length of setae <i>u'</i>	2–3	2	Apart of setae <i>3a</i>	/	11–13
Length of solenidion	5–6	5	Length of setae <i>c2</i>	9–11	8–11
Length of empodium	4–5	4	On annulus	2–4	7–9
Number of rays on tarsal empodium	4	4	Length of setae <i>d</i>	20–25	23–30
Length of leg II	17–18	17–18	On annulus	13–17	20–22
Length of femur I	5–7	7–8	Length of setae <i>e</i>	10–13	11
Length of setae <i>bv</i>	6–9	7	On annulus	30–34	38–41
Length of genu	2	2–3	Length of setae <i>f</i>	10–12	11–12
Length of setae <i>l''</i>	6–9	7–8	On annulus	53–60	61–65
Length of tibia	2	2–3	Length of setae <i>h1</i>	2	2
Length of tarsus	4	3–4	Length of setae <i>h2</i>	31–35	34–39

Considering that the observation of morphological features largely depends on the quality of the slides and the optical instruments used, we believe we were able to observe more details. A difference in the shield pattern alone, which could be due to the reasons mentioned above, is not a

sufficient or reliable morphological feature on which to base the claim that the populations from Mexico and Guadeloupe are different species. Given that eriophyid mites are highly specialised and closely associated with their hosts, we have concluded that the specimens of eriophyid mites collected from *Sphagneticola trilobata* in Mexico are the previously described species *A. comptus* from Guadeloupe.

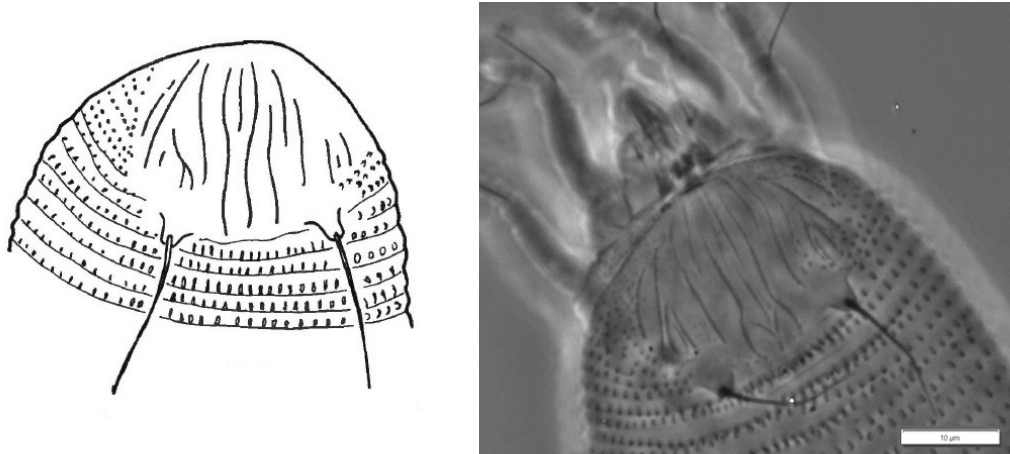


FIGURE 2. Shield pattern of *Acalitus comptus*: A—original description by Flechtmann, B—population from Mexico.

***Acalitus viguierae* sp. nov.** Vidović (Fig. 3 & 4)

Family Eriophyidae Nalepa, 1898.

Subfamily Eriophyinae Nalepa, 1898.

Tribe Aceriini Amrine and Stasny, 1994.

Genus *Acalitus* Keifer, 1965

Zoobank: <http://zoobank.org:act:9BA20F47-0B78-4662-BFD0-F661FD810C5E>

Description. FEMALE (Fig. 3) (n=5). Body worm like 188 (186–199), 34 (34–40) wide. **Gnathosoma** 12 (12–14), cheliceral stylets 10, pedipalp coxal setae (*ep*) 2, dorsal pedipalp genual setae (*d*) 2. **Prodorsal shield** 17 (16–18), 20 (20–23) wide, ornamented. Median line and admedian lines complete. I pair of submedian lines present on the anterior half, incomplete; II submedian lines incomplete, extending to three-quarters of the shield from the anterior margin; III submedian lines complete. Between the II and III submedian lines in the posterior half of the shield, one short diagonal line and multiple dots are visible. In some specimen slides, this short diagonal line may merge with the III submedian line, making the third submedian line appear forked, with multiple dots visible between the branches. Dorsal tubercles *sc* on rear shield margin 12 (11–13) apart, setae *sc* 17 (17–19) directed posteriorly. **Legs I** without tibial I and femoral I setae. Leg I 18 (17–18); femur 8 (7–8), setae *bv* missing; genu 2 (2–3), setae *l''* 10 (9–11); tibia 3, setae *l'* missing; tarsus 4, setae *ft'* 6 (5–6), setae *ft''* 14 (13–14), setae *u'* 2 long; solenidia ω 6 (5–6) long, empodium 3 (3–4), and 4 rayed. **Legs II** 15 (15–17); femur 7 (6–7), setae *bv* 5; genu 2 (2–3), setae *l''* 6 (4–6); tibia 2, setae *l'* missing; tarsus 3 (2–3), setae *ft'* 3 (2–3), setae *ft''* 14 (13–14), setae *u'* 2; solenidia ω 6 (5–6), empodium 4, and 4 rayed. Coxa I fused to each other. **Coxigenital area** granulated; setae *lb* 4 (4–5), *lb* tubercles 5 apart; setae *la* 11 (11–12) long, *la* tubercles 6 apart; setae *2a* 22 (22–25), *2a* tubercles 13 (13–15)

apart. **Genital coverflap** 8 (8–9), 14 wide with 4 transverse lines of coarse granules; setae *3a* 5 (4–5), *3a* tubercles 10 (10–12) apart. **Opisthosoma** with subequal annuli: 80 (75–80) dorsal and 71 (68–71) ventral annuli. Microtubercles slightly elongate with a minute distal spine. Setae *c*2 12 (10–12), 29 (29–38) apart, on annulus 6 (6–7); setae *d* 30 (30–32), 27 (27–31) apart, on 24 (20–24) annulus; setae *e* 13 (11–14), 18 (18–19) apart, on 42 (38–42) annulus; setae *f* 12, 10 apart, on 66 (61–66) annulus; setae *h*2 48 (43–48), 7 (7–8) apart, setae *h*1 2 long, 5 (5–6) apart.

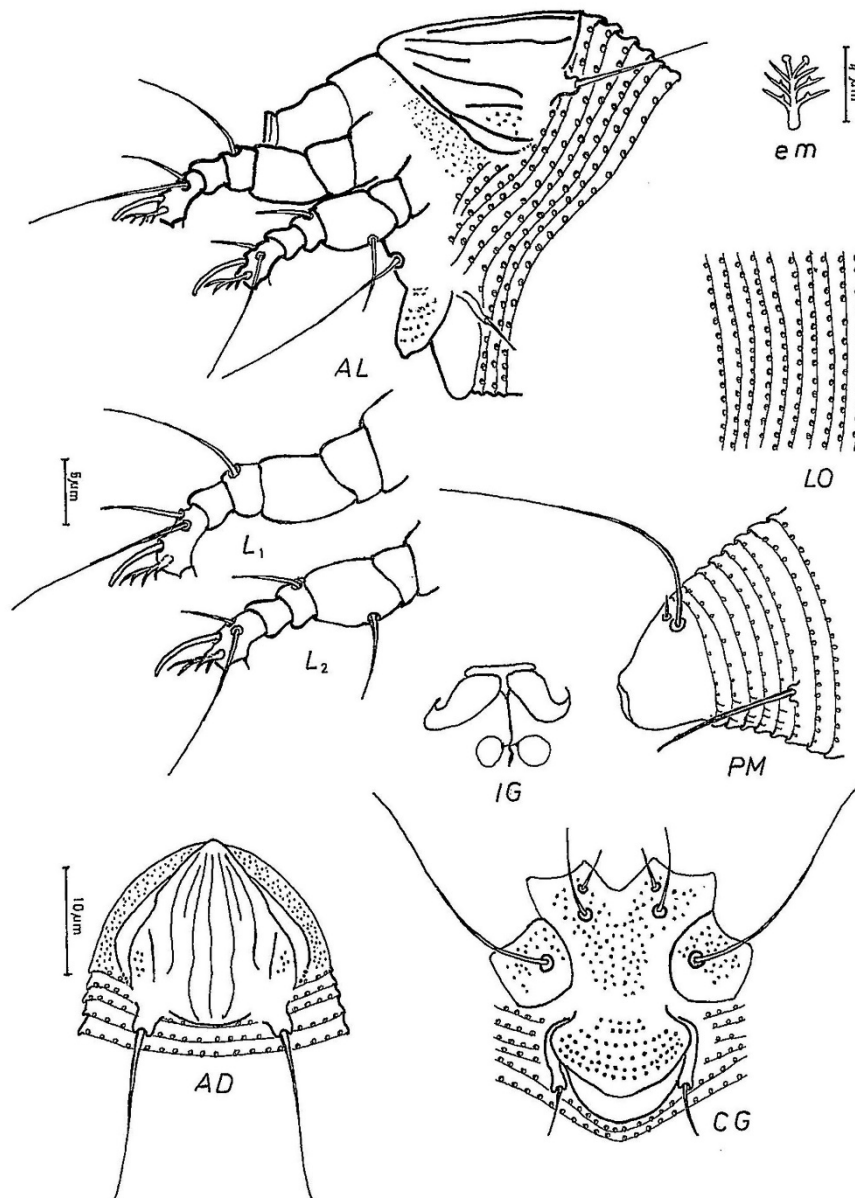


FIGURE 3. Line drawings of *Acalitus viguierae* sp. nov.: AD—prodorsal shield; AL—lateral view of anterior body region; CG—female coxigenital region; em—empodium; IG—internal female genitalia; LO—lateral view of annuli; L1—leg I; L2—leg II; PM—lateral view of posterior opisthosoma. Scale bar: 10 µm for AD, AL, CG, IG, PM; 5 µm for L1, L2; 4 µm for em.

Type material and locality. Female holotype and 4 paratypes. Mexico, Felipe Carrillo Puerto (19.6066670N; 87.9866670W), February 2025, coll. Callander. J.T.

Type of host plant: *Viguiera dentata* (Cav.) Spreng.

Etymology. The species designation *viguierae* is derived from the genus name of the host plant.

Relation to host plant. The mites cause erineum on the lower surface of the leaves (Fig. 4).

GenBank accession number – PX640560

Differential diagnosis and remarks. *Acalitus viguierae* **sp. nov.** differs from other *Acalitus* species described from host plants of the tribe Heliantheae by the pattern of the prodorsal shield, the presence of accessory setae *h1*, the appearance of the genital coverflap, and the length of the ventral setae. Based on shield patterns, *A. viguierae* **sp. nov.** can be distinguished from all eight previously described *Acalitus* species found on host plants in the tribe Heliantheae.

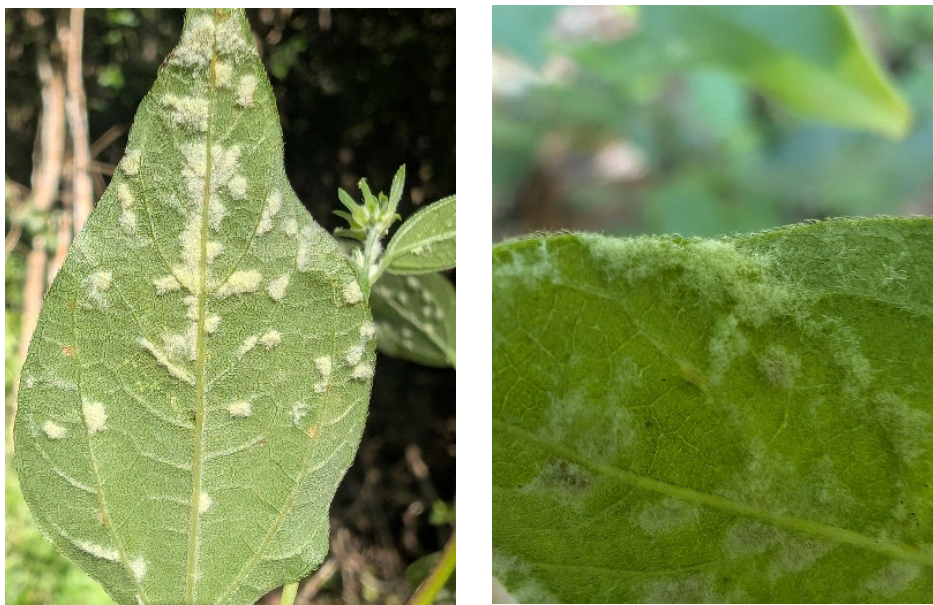


FIGURE 4. Symptoms of *Acalitus viguierae* **sp. nov.** on *Viguiera dentata*, erineum on the lower surface of the leaf.

Acalitus heliopsis and *A. odoratus* have the shield surface covered with numerous fine lines, so the median, admedian, and submedian lines are almost completely obscured. In contrast, in *A. viguierae* **sp. nov.**, the median, admedian, and submedian lines are clearly visible. Besides the different ornamentation of the shield, *A. viguierae* **sp. nov.** has accessory setae *h1*, which are absent in *A. heliopsis* and *A. odoratus*.

The shield of *A. mikaniae* has broken median and admedian lines, while *A. inulaefolii* has an interrupted median line, which is not present in *A. viguierae* **sp. nov.**, which has complete median and admedian lines. Additionally, *A. inulaefolii* lacks *h1* setae, unlike *A. viguierae* **sp. nov.**, which possesses them. *A. mikaniae* has longer first ventral setae (*d*) and shorter caudal setae (*h2*) compared to *A. viguierae* **sp. nov.** (*A. mikaniae*: setae *d* 40 μ m, setae *h2* 13 μ m; *A. viguierae* **sp. nov.**: setae *d* 30–32 μ m, setae *h2* 43–48 μ m).

Acalitus purpurascensis has a shield with four submedian lines, a genital coverflap with six longitudinal striae, and the third ventral setae longer than the second ventral seta, while *A. osmia* has a shield with several wavy submedian lines, a smooth genital coverflap, and lateral setae longer than the second ventral setae. This differs from *A. viguierae* **sp. nov.**, which has three submedian lines, a

genital coverflap with four transverse lines of coarse granules, and lateral, second, and third ventral setae of similar lengths.

On the prodorsal shield of *A. comptus*, between the median and admedian lines, there are two short oblique lines on the posterior edge of the shield and two incomplete lines extending from the anterior edge of the shield. These lines are not present on the shield of *A. viguierae* **sp. nov.**, and *A. comptus* has shorter setae *h*₂ compared with *A. viguierae* **sp. nov.** In addition to the shield pattern, *A. viguierae* **sp. nov.** differs from *A. maracai* in the length of the ventral setae, which are considerably longer in *A. maracai* (*A. viguierae* **sp. nov.**: setae *d* 30–32 µm, setae *e* 11–14 µm, setae *f* 12 µm; *A. maracai*: setae *d* 57 µm, setae *e* 30 µm, setae *f* 20 µm).

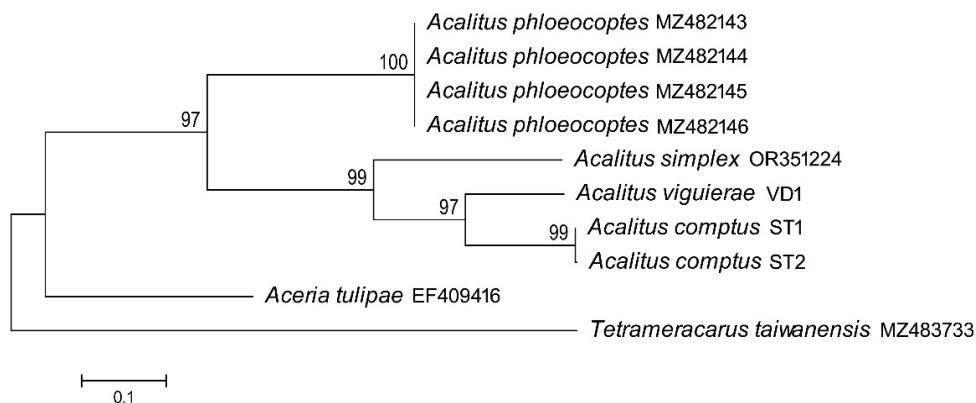


FIGURE 5. Maximum-likelihood (ML) tree performed with HKY+G model of the mitochondrial cytochrome *c* oxidase subunit I sequences. Maximum likelihood bootstrap values are above the branches. Scale bar indicates the number of substitutions per site (values lower than 70% are omitted). *Aceria tulipae* and *Tetrameracarus taiwanensis* are used as outgroups to root the tree.

Molecular Analyses

A 645 bp *COI* sequences were obtained for *A. comptus* and *A. viguierae* **sp. nov.** No insertions or deletions were detected between the *mtCOI* sequences. Translation of the nucleotide sequences into amino acids revealed a complete reading frame of 215 amino acid positions, with no stop codons or frameshift mutations, indicating the absence of pseudogene (NUMT) amplification.

Populations of *A. comptus* collected from *S. trilobata* at two localities in Mexico (Tab. 1) revealed two haplotypes (ST1 and ST2), which differ by a single SNP. The uncorrected pairwise genetic p-distance between *A. comptus* and *A. viguierae* **sp. nov.** was 16.3%. BLAST analyses identified *Acalitus simplex* (OR351224) as the closest available species in GenBank, showing 21.1% divergence from *A. viguierae* **sp. nov.**, while the divergence values between *A. simplex* and the ST1 and ST2 haplotypes of *A. comptus* were 22.5% and 22.6%, respectively. Higher levels of genetic divergence were observed between *Acalitus phloeocoptes* and *A. viguierae* **sp. nov.** (26%), as well as between *Acalitus phloeocoptes* and the ST1 and ST2 haplotypes of *A. comptus* (28.1% and 27.9%, respectively). The ML analyses showed that all analysed species belonging to the genus *Acalitus* clustered into a single, well-supported clade, indicating the monophyly of the genus (97% bootstrap support). The sequences of *A. comptus* and *A. viguierae* **sp. nov.** are fully integrated within the *Acalitus* clade, confirming their phylogenetic placement within the genus (Fig. 5). In contrast, *Aceria tulipae* and *Tetrameracarus taiwanensis*, although belonging to the same tribe (Acerinii), are clearly separated from the *Acalitus* clade and form distinct lineages outside it. Overall, the inferred tree topology supports the assignment of the studied taxa to the genus *Acalitus* and clearly delineates it from other genera within Acerinii.

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