Revised description of the blueberry bud mite, *Acalitus vaccinii* (Acari: Trombidiformes: Eriophyidae), and a key to all Eriophyoidea on *Vaccinium*

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INTRODUCTION

Blueberries, *Vaccinium* spp. (Ericaceae), have become a rapidly expanding commercial crop in South Africa since the 1980s. Most commercial blueberry plantations are in the Western Cape province, where the longer winters contribute to better berry yield (Meyer and Prinsloo 2003). South African blueberry plantations have been relatively free of pests until 2012 when *Acalitus vaccinii* (Keifer 1939) (Trombidiformes: Eriophyidae), the blueberry bud mite, was discovered for the first time in South Africa on a farm in the Mpumalanga province. It was identified as such based on the original and subsequent species descriptions, and by comparison to other eriophyoids known on *Vaccinium* spp., and to other *Acalitus* spp. known from Africa. The mite caused substantial damage that resulted in an estimated 80% decrease in yield within only two years of its detection. Symptoms included red blistering on buds, production of small leaves and fruit, as well as malformed flowers (Craemer 2018). Further surveys by the South African Department of Agriculture, Land Reform and Rural Development (DALRRD) also confirmed blueberry bud mite infestations in other locations within the Mpumalanga and North West provinces (Ngubane-Ndhlovu et al. 2018). *Acalitus vaccinii* is part of the superfamily Eriophyoidea, casually referred to as eriophyoid mites. Eriophyoidea contains three families, namely Eriophyidae, Diptilomiopidae and Phytophtidae. Eriophyoid mites are highly specialised, plant-feeding and are typically host-specific. These mites are minute, between 100 and 300 µm long with worm-like bodies and two pairs of legs. Many species are of commercial interest as they can cause malformation of buds, can form galls, or cause rust-like symptoms on leaves and fruit.

The lifecycle of *A. vaccinii* is typical for eriophyoid mites and includes eggs, larvae, nymphs, and adult males and females. Two female forms can be present, a deutogyne hibernating winter form and protogyne sexually active summer form. In *A. vaccinii*, the presence of a deutogyne has been noted in the colder areas of its native range in North America (Manson and Oldfield 1996; Cromroy and Kuiter 2001). The identification of both forms is important for assigning species identity to eriophyoid mites, where misidentification of the deutogyne is frequent (Zhao 2000; Smith et al. 2010; Guo et al. 2015). As is the case with many eriophyoids, accurate identification of *A. vaccinii* is hampered by incomplete species descriptions, inaccurate description of some characters and life stages in original descriptions and a lack of identification keys. For example, no comprehensive key to the >90 *Acalitus* species worldwide or to the nine eriophyoids on *Vaccinium* spp. (one Diptilomiopidae and eight Eriophyidae) exist.

*Acalitus vaccinii* was first described by Keifer (1939) as *Eriophyes vaccinii*, but later moved to *Aceria* (Keifer 1946) and thereafter to *Acalitus* (Baker and Neunzig 1970). In this paper we used modern methods to examine *A. vaccinii* and revise its description, including originally missed characters and all developmental stages. For enhanced clarity, specimens are examined using two imaging techniques, namely phase-contrast light microscopy (PCLM) and low-temperature scanning electron microscopy (LT-SEM) to scrutinise characters on slide-mounted and in situ mites. We also aim to provide diagnostic DNA barcoding sequences including nuclear (28S) and mitochondrial...
(COI) regions of *A. vaccinii* to accompany morphological descriptions and to increase accuracy of future identifications of this important pest. Additionally, we provide an identification key to eriophyoid species on *Vaccinium* worldwide.

**METHODS**

**Mite collection**

Plant material showing symptoms of *A. vaccinii* infestation was collected from farms near three different towns in the Mpumalanga province in South Africa (Table 1). On each farm at each sampling occasion, 30 samples of 30 cm long shoots were taken at random per cultivar and per block and placed in resealable plastic bags. These shoot samples were kept at 4 °C until examination for the presence of eriophyoids using a stereomicroscope. For traditional microscopic examination using a compound microscope, eriophyoids were collected into a drop of sorbitol and isopropyl-alcohol solution until mounting (de Lillo et al. 2010). For scanning electron microscopy (SEM), mites were kept in situ until preparation. For molecular analysis, eriophyoids were placed into a drop of distilled water on a glass slide and processed immediately.

**Morphological examination**

**Phase contrast light microscopy (PCLM)**

Collected eriophyoids were mounted on glass slides using F-medium following published protocols (Keifer 1975; de Lillo et al. 2010). Specimens were examined at 1 000× magnification using a Zeiss Axioskop Imager M2 microscope (Zeiss, Germany), equipped with a drawing tube and Zeiss AxioCam Cc5 digital camera. ZEN 2012 software was used for line drawings and capturing of images. Seventy-five characters for females, 69 for males and 68 for immatures were measured using a Leica DM 2500 microscope (Leica Wetzlar, Germany) connected to a Leica digital camera and Leica application suite v 3.1.0 software.

**Principal component analysis (PCA)**

To determine if distinctive clusters of characters were present, we performed a principal component analysis using morphological characters of 11 females collected from different seasons (six in summer and five in winter) (Table S1). From the initial 75 morphological characters measured, only independent and non-repeated characters which showed a standard deviation greater than one were used, resulting in 28 characters used in the PCA. PCA was performed in Rstudio v.1.1.447 running R statistical analysis v.3.5.0 (R Core Team 2020; R Studio Team 2020). To visualise the results, the packages ggfortify (Tang et al 2016; Horikoshi and Tang 2018) and ggplot2 (Wickham 2016) were used. Slide-mounted voucher material of all stages was deposited in the mite collection of DALRRD, Plant Quarantine Station in Stellenbosch, South Africa, and in the National Collection of Arachnida – Acari of the Agricultural Research Council – Plant Health and Protection, in Pretoria, South Africa.

**Scanning electron microscopy (SEM)**

A modified version of the cryo-fixation technique described by Echlin et al. (1970) was used for preparing specimens and studying them with a conventional JEOL JSM 840 SEM with a cryo-stage. Fourteen females, two males, two nymphs and two larvae were mounted on double-sided carbon tape. The tape was attached in a specimen holder with silver paint, which was plunged-frozen in liquid nitrogen slush and then transferred via the pre-chamber of the cryo-system to the pre-cooled cryo-stage in the chamber of the SEM (ca. –170 °C). Here the specimen was etched for ca. 30 minutes by increasing the temperature to ca. –80 °C to remove ice crystals. The specimen holder was then transferred back to the pre-chamber and sputter-coated with gold, then returned to the cryo-stage for observation of specimens at an accelerating voltage of 5 kV or 2 kV (to prolong viewing time). Digital images were captured using a frame grabber controlled by Orion 6.6.

**Revised description of Acalitus vaccinii**

Identification was confirmed based on species-specific microscopic characters according to Keifer (1939, 1946) and Baker and Neunzig (1970). A revised description of *A. vaccinii* is presented following the recommendations of Amrine and Manson (1996) and De Lillo et al. (2010). All measurements are given in micrometers (μm), rounded off to the nearest integer, as a range (minimum to maximum). Measurements refer to the length (not width) of the morphological character unless specified otherwise. Terminology follows that of Lindquist (1996).

**Identification key**

An identification key to all Eriophyoidae species known from *Vaccinium* spp. worldwide was compiled. The key was adapted from published literature (Amrine et al. 2003; Lindquist and Amrine 1996) and original species descriptions (Keifer 1939, 1940, 1953, 1971; Roivainen 1947, 1951; Wei et al. 2009).

**Genetic analyses**

**DNA extraction**

Groups of four to eight live mites were crushed in a small drop of distilled water on a glass slide. Using a micropipette, the drop containing the mites was then transferred into a sterile microcentrifuge tube for DNA extraction. DNA extractions were performed using a Qiagen QIAamp DNA Micro Kit (Qiagen, California, USA) following the manufacturer’s instructions with the exception that all reaction volumes were halved to improve

### Table 1. Location and collection information of blueberry plantations from which *Acalitus vaccinii* was collected from November 2014 to November 2016

<table>
<thead>
<tr>
<th>Site* and collection times</th>
<th>GPS location of town*</th>
<th>Size of blueberry plantation</th>
<th>Blueberry species</th>
<th>Cultivars</th>
<th>Age of plantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dullstroom (Nov 2015; Dec 2016)</td>
<td>25.4184° S, 30.1041° E</td>
<td>4 ha</td>
<td><em>Vaccinium corymbosum</em></td>
<td>‘Elliott’</td>
<td>4 yrs</td>
</tr>
<tr>
<td>Lydenburg (Nov 2015; Dec 2015; Aug 2016; Dec 2016)</td>
<td>25.0816° S, 30.4473° E</td>
<td>3 ha</td>
<td><em>Vaccinium corymbosum</em></td>
<td>‘Elliott’</td>
<td>8 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium virgatum</em></td>
<td>‘Climax’</td>
<td>25 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium virgatum</em></td>
<td>‘Delite’</td>
<td>25 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium corymbosum</em></td>
<td>‘Bluecrop’</td>
<td>6 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium corymbosum</em></td>
<td>‘Berkley’</td>
<td>14 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium corymbosum</em></td>
<td>‘Elliott’</td>
<td>14 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium corymbosum</em></td>
<td>‘Spartan’</td>
<td>14 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Vaccinium virgatum</em></td>
<td>‘Centurion’</td>
<td>14 yrs</td>
</tr>
</tbody>
</table>

*Due to confidentiality of information, sites are named in this study according to the nearest town. GPS coordinates also refer to the nearest town.
DNA amplification and sequencing

PCR reactions were performed in 25 μl volumes on a Techne Prime Thermal Cycler (Staffordshire, UK). Amplification was performed using 6 μl of DNA extract with half volumes of Promega Corporation (Madison, WI) GoTaq DNA polymerase, following manufacturer’s instructions.

A 657 bp segment of the D2 domain of the nuclear 28S rDNA gene was amplified via nested PCR following the protocol of Chetverikov et al. (2019) (Table 2), with an additional 1.5 μl 25 mM MgCl₂ and 1.0 μl 10 mM dNTP mix to boost amplification (Table 2). PCR reactions for COI contained an additional 1.0 μl 25 mM MgCl₂ and 1.0 μl 10 mM dNTP mix to boost amplification while minimising mis-priming. Cycling conditions are provided in Table 2. All PCR products were viewed by electrophoresis on a 1.5% agarose gel. DNA sequencing was performed by Inqaba Biotech (Pretoria, South Africa). Trace files were checked, edited, and prepared for submission to GenBank using MEGA v.11.0.13 (www.megasoftware.net). Both sets of sequences were compared and prepared for submission to GenBank using blastn and blastx (for COI) algorithms (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Revised description

Superfamily: ERIOPHYOIDEA Nalepa, 1898
Family: Eriophyidae Nalepa, 1898
Subfamily: Eriophyinae Nalepa, 1898
Tribe: Aceriini Amrine and Stasny, 1994
Genus: Acalitus Keifer, 1965

Table 2. DNA markers, PCR primers and cycling conditions used for amplification and sequencing of Acalitus vaccinii specimens in this study. F/R indicates a forward (F) or reverse (R) primer. Accession numbers for sequences submitted to GenBank are included. Extractions are from the Dullstroom (D) and Lydenburg (E) populations.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>F/R</th>
<th>Primer sequence 5’-3’</th>
<th>Cycling conditions</th>
<th>Reference</th>
<th>GenBank accession</th>
<th>Isolate</th>
</tr>
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<tbody>
<tr>
<td>28S (step 1)</td>
<td>f1230</td>
<td>F</td>
<td>TGAAACTTAAAGGAATTGACG</td>
<td>95 °C for 3 m</td>
<td>Dabert et al. 2010</td>
<td>MW246114</td>
<td>D2</td>
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<tr>
<td></td>
<td>D1D2wv4_E</td>
<td>R</td>
<td>GTTAGACTCTTGGTGCGTG</td>
<td>95 °C for 30 s</td>
<td>Sonnenberg et al. 2007</td>
<td>MW246115</td>
<td>D3</td>
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<tr>
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<td>52 °C for 30 s x30</td>
<td></td>
<td>MW246116</td>
<td>D4</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>72 °C for 3 m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72 °C for 9 m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28S (step 2)</td>
<td>D1D2wv2_E</td>
<td>F</td>
<td>ACAAGTACCGTGGAGGAAAGTG</td>
<td>95 °C for 1 m</td>
<td>Chetverikov et al. 2019</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>28SR_990</td>
<td>R</td>
<td>CCTGGTCCGTGGTTCACAGAC</td>
<td>95 °C for 30 s</td>
<td>Mironov et al. 2012</td>
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<td>62 °C for 30 s x30</td>
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<td></td>
<td></td>
<td>72 °C for 3 m</td>
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<td></td>
<td>72 °C for 9 m</td>
<td></td>
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<tr>
<td>COI partial</td>
<td>C1-J-2183</td>
<td>F</td>
<td>CAACATTATTGTAGTTTGG</td>
<td>95 °C for 1 m</td>
<td>Simon et al. 1994</td>
<td>MW250771</td>
<td>D2</td>
</tr>
<tr>
<td>barcode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hedin and Maddison 2001</td>
<td>MW250772</td>
<td>D3</td>
</tr>
<tr>
<td>(region 1)</td>
<td>C1-N-2568</td>
<td>R</td>
<td>GCWACWACRTAATAKTATCATG</td>
<td>95 °C for 30 s</td>
<td></td>
<td>MW250773</td>
<td>D4</td>
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<tr>
<td></td>
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<td>50 °C for 45 s x33</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72 °C for 1 m</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72 °C for 10 m</td>
<td></td>
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</tr>
</tbody>
</table>

Acalitus vaccinii (Keifer 1939)

Eriophyes vaccinii Keifer 1939: 328–345, figure: plate LXIX (original designation)

Aceria vaccinii (Keifer) Keifer 1946: 568, (no images)
Keifer 1965:2, figure: plate 1


Female

(Tables 1–7) (n = 11)

Idiosoma: (Figure 1 and 2) Whitish, wormlike body 167–261 including pedipalp, 150–233 excluding gnathosoma, 48–63 wide (at the level of c2 setae). Opisthosoma dorsally arched with 65–88 dorsal and 57–72 ventral microtuberculate annuli (from first annulus posterior to coxae II). Dorsally and ventrally with round to oval microtubercules, ventrally gradually elongated towards the rear, dorsally becoming more elongated and vague (probably subsurface) towards the rear until spiny microtubercules protruding from the posterior annulus margins of the telosome. Opisthosomal seta c2 21–33 on ventral annulus 10–11, 46–57 apart; opisthosomal seta d 30–52 on ventral annulus 21–24, 35–49 apart; opisthosomal seta e 33–46 on ventral annulus 35–40, 24–31 apart; opisthosomal seta f 11–17, on annulus 5–6 from the rear, 15–18 apart, fine at apex. Opisthosomal setae h1 minute, less than 0.5. Opisthosomal setae h2 46–59, finely tapered.

Gnathosoma: (Figure 3) 17–23, directed forward and slightly downward, basal part covered by small, pointed frontal lobe, chelicerae 18–25, palp coxal seta ep 4–6, apico-ventral setae v 2, palp genual setae d absent.

Prodorsal shield: (Figure 4) oval, 23–28 long, 31–50 wide; frontal lobe small, thin, anteriorly pointed or slightly rounded. Prodorsal shield with pair of usually obscure admedian lines on posterior ¼ of shield between scapular sc setae, more or less curving outwards from rear, then curving inwards, few granules on the outer side of scapular tubercles, with eye-like structures on their outer side partly margined with single rounded, shallow
ridge, band of granules on outer margins of shield and on epicoxal area (sensu Chetverikov and Craemer 2015). Scapular setae sc 20–24, 22–25 apart, projecting posteriad.

Leg I: (Figure 5 and 6) all usual segments present, 20–25; trochanter 4–5, femur 3–7, basiventral femoral seta bv absent, genu 3, antaxial genual seta l′ 17–23; tibia 3–5, paraxial tibial seta u′ 1–3, paraxial fastigial tarsal seta ft′ 12–18, antaxial fastigial tarsal seta ft″ 5–9. Tarsal solenidion ω 5–7, slightly curved, sometimes straight and slightly knobbed, tarsal empodium em 4–6, simple, symmetrical, 6-rayed.

Leg II: (Figure 5 and 6) all usual segments present, 19–21; trochanter 3–5, femur 3–6, basiventral femoral seta bv 4–7, genu 2–4, antaxial genual seta l′ 19–21; tibia 3–6, tarsus 4–5, paraxial unguinal tarsal seta u′ 2–3, paraxial fastigial tarsal seta ft′ 14–20, antaxial fastigial tarsal seta ft″ 4–9. Tarsal solenidion ω 6–8, slightly curved, sometimes straight, and slightly knobbed. Empodium em 4–6, simple, symmetrical, 6-rayed.

Coxisternal area: (Figure 7) suboral plate rounded, with few granules and three slight longitudinal elevations medially (only visible with SEM). Coxisternal plates I and II ornamented with rounded to elongated granules, granules arranged in single row, parallel to and close to margin between coxisternal plates and leg trochanters. Anterolateral setae on coxisternal plate I 1b 5–7, 7–10 apart, proximal setae on coxisternal plate I 1a 20–25, 12–16 apart, proximal setae on coxisternal plate II 2a 23–36, 22–25 apart. Inverted Y-shaped prosternal apodeme. 0 complete and 0–3 incomplete microtuberculate annuli between external genitalia and coxae. Genital cover flap 11–14, 18–21 wide, with 8–12 longitudinal ridges on one rank. Pregenital plate (sensu Flechtmann et al. 2015) present, with elongated tubercles in about four transverse rows arranged in more or less two transverse areas with the basal two rows slightly rounded. Proximal setae of coxisternal plate III 3a 7–13, 13–18 apart. Internal genitalia (Figure 7C) extending moderate distance forward.

Female

Deutoynes: not observed during this study.

Male

(n = 2)

Morphology similar to female, including presence or absence of setae. Only measurements are given here. Features are not described unless they differ from those of the female.

Idiosoma: 172–191 including pedipalp, 152–176 excluding gnathosoma, 50–55 wide (at the level of c2 setae). Opisthosoma with 62–63 dorsal and 50–54 ventral microtuberculate annuli (from first annulus posterior to coxae II). Telosome dorsally with spiny microtubercles protruding from the posterior margin of the annuli. Opisthosomal setae c2 22–23 on ventral annulus 9, 48–51 apart; opisthosomal setae d 17–28 on ventral annulus 18–20, 37–40 apart; opisthosomal setae e 31–34 on ventral annulus 21–28, 24–25 apart; opisthosomal setae f 14–18, on annulus 4–5 from the rear, 16–17 apart, fine at apex. Opisthosomal setae h1, minute, less than 0.5. Opisthosomal setae h2 38–42, relatively long and finely tapered.

Gnathosoma: 21 long, chelicerae 17–19, pedipalp coxal setae ep 4–5, apico-ventral setae v 2–3.

Prodorsal shield: oval 23–24 long, 39–44 wide; scapular setae sc 18–21, 23 apart.

Leg I: 16–18, trochanter 3–4, femur 4, genu 3, antaxial genual setae l′ 15; tibia 2–3; tarsus 4–5, paraxial unguinal tarsal seta u′ 2, paraxial fastigial tarsal setae ft′ 13, antaxial fastigial tarsal

Figure 1. SEM image of the opisthosoma (body) of A. vaccinii protogyne female, showing the ventral (A) and dorsal (B) aspect. Annuli are the rings around the body, and microtubercles are the protrusions on these rings (more detail can be seen in Figure 2). SEM images were cropped to show the region of interest. For sizes of structures, refer to the measurements included in text.
setae ft 7–8. Tarsal solenidion ω 6, tarsal empodium em 4–6, simple, symmetrical, 6-rayed.

**Leg II:** 17–18, trochanter 3, femur 4–5, basiventral femoral seta bv 3–4; genu 3, antaxial genual setae l′ broken could not be measured; tibia 2.6, paraxial tibial setae l′ absent; tarsus 4–5, paraxial unguinal tarsal seta u′ 2, paraxial fastigial tarsal setae ft′ 15–17, antaxial, fastigial tarsal setae ft′ 3–4. Tarsal solenidion ω 7–7. Empodium em 4–5, simple, symmetrical, 6-rayed.

**Coxisternal area:** (Figure 8) anterolateral setae on coxisternal plate I lb 4, 7 apart, proximal setae on coxisternal plate I la 19–22, 11 apart, proximal setae on coxisternal plate II la 16–19, 20–21 apart. 2 complete and 2 incomplete microtuberculate annuli between external genitalia and coxae. External genitalia 11 long, 15–16 wide. Proximal setae on coxisternal plate III la 7 and 14–15 apart, with dense irregularly arranged granules posterior to 3a.

**Nymph**

(Figure 9) (n = 2)

**Idiosoma:** chunky and shorter than adults, translucent to whitish, wormlike body, 143–170 long including pedipalp, 47–52 wide (at the level of c2 setae). Opisthosoma dorsally arched with 50–52 dorsal and 44–45 ventral semiannuli. Ventrally, few, scattered oval to round microtubercles arranged medially in a band about the width of the distance between setae 3a, and approximately 10 µm on the inside of setae d, up to a short distance posterior to d, sometimes down to setae f. Dorsally, oval to round microtubercles spreading over a wider area compared to the ventral side, present medially in a band about a width of the distance between setae sc arranged in an hourglass shape. Opisthosomal setae c2 14–15, 43 apart on annulus 6–7, opisthosomal setae d27–28, 32 apart on annulus 16; opisthosomal setae e25–26, 19 apart on annulus 25; opisthosomal setae f9–10, 15–16 apart on annulus 41, or on annulus 4–5 from the rear. Seta h1 minute, seta h2 37–43.

**Gnathosoma:** 18–23, directed forward and slightly downward, chelicerae 17, pedipalp coxal seta ep 2–3, apico-ventral setae v 1–2, pedipalp genual setae l′ absent.

**Prodorsal shield:** 25–26 long, 39–43 wide, unlike adult, granules are not visible, faint admedian lines. Scapular setae sc 16–17, 22 apart, projecting posteriorly.

**Leg I:** All usual segments present, 14–15; trochanter 3, femur 4, basiventral femoral setae bv absent, genu 2–3, antaxial genual setae l′ 13; tibia 2–3, paraxial tibial setae l′ absent; tarsus 4.6, paraxial unguinal tarsal seta u′ 1.4, paraxial fastigial tarsal setae ft′ 3, antaxial fastigial tarsal setae ft′ 10. Tarsal solenidion ω 4, slightly curved, blunt to slightly knobbed. Empodium em 3.5 simple, 5-rayed.

**Leg II:** All usual segments present, 13; trochanter 2, femur 3.5, basiventral femoral bv setae 2, genu 2, antaxial genual setae l″ 10–16; tibia 2, paraxial tibial setae l″ absent; tarsus 4, paraxial unguinal tarsal seta u′ 1–2, paraxial fastigial tarsal setae ft′ 3,
Figure 4. The prodorsal shield in dorsal (A and B) and lateral (C and D) view, as viewed by SEM (A and C) and PCLM (B and D). Setae sc, admedian lines and the frontal lobe are labelled. The eye-like area can be seen in lateral view with a band of granules on the outer margin. SEM images were cropped to show the region of interest. PCLM images were taken with 100x objective. For sizes of structures, refer to the measurements included in the text.

Figure 5. Schematic line drawings of Legs I and II showing the segments and setae names.


Coxisternal area: suboral plate rounded, sometimes with faint curved lines, fewer granules than female adult. Prosternal apodeme not visible. Coxisternal plates I and II ornamented with very few granules. Anterolateral setae on coxisternal plate I 1b 3, 7–8 apart, proximal setae on coxisternal plate I 1a 11–12, 10 apart, proximal setae on coxisternal plate II 2a 20–23, 19 apart. External genitalia absent. Proximal setae of coxisternal plate III 3a 3–4, 8–9 apart.

Larva
(Figure 10) (n = 2)

Idiosoma: transyluscent, wormlike body 112–128 (including pedipalp), 52–55 wide. Opisthosoma dorsally arched with 29–31 dorsal and 30 ventral annuli. Opisthosomal microtubercles varied between specimens, and could be absent or present on dorsal, ventral or both surfaces. In dorsal view, when present, irregular shaped to pointed microtubercles scattered towards the rear end. In ventral view, when present, few oval to rounded microtubercles between 3a and e setal-area. On both sides, the microtubercles were along setae 3a and on the dorsal rear end. Opisthosomal setae c 6, 50 apart on annulus 3 or 4, opisthosomal setae d 6, 28 apart, on annulus 10–11; setae e 3 long, 19 apart on annulus 16; setae f 8 long, 15–16 apart on annulus 26–27, or annulus 4 from the rear. Setae h2 23 long, Setae h1 minute.
Figure 6. Legs I and II (A, C and D) and empodia (B and E) viewed by SEM (A and B) and PCLM (C, D and E). Leg segments are labelled (A), as well as femoral II setae  bv which was previously described as missing. The six empodial rays are indicated by arrows (B). In PCLM images, features may only be visible at different focal points (C and D). SEM images were cropped to show the region of interest. PCLM images were taken with 100× oil objective. For sizes of structures, refer to the measurements included in the text.

Figure 7. The coxisternal area of the protogyne female, viewed by SEM (A) and PCLM (B and C). External features, including the genital coverflap with ridges in a single rank (row) and setae are labelled (A and B). C shows the shape of the internal genitalia, only visible with PCLM. SEM images were cropped to show the region of interest. PCLM images were taken with 100× oil objective. For sizes of structures, refer to the measurements included in the text.
Figure 8. The coxisternal area of the male, viewed by SEM (A) and PCLM (B). External features and setal arrangement are as for the female (Figure 6), except for the genitalia and genital cover flap, which is not present in the male. SEM images were cropped to show the region of interest. PCLM images were taken with 100x oil objective.

Figure 9. Nymph, viewed by SEM (A) and PCLM in dorsal (B) and ventral (C) view. Setal arrangement is as for the adult. Dorsally, microtubercles form an hourglass shape approximately the width of sc – sc (A and B). Ventrally, microtubercles are arranged in a band about the width of 3a – 3a (C). SEM images were cropped to show the region of interest. PCLM images were taken with 100x oil objective. For sizes of structures, refer to the measurements included in the text.

Figure 10. Larva, viewed by SEM (A) and PCLM (B and C), showing dorsal (A and B) and ventral (C) aspects. SEM images were cropped to show the region of interest. PCLM images were taken with 100x oil objective. For sizes of structures, refer to the measurements included in the text.

**Prodorsal shield:** prodorsal shield smooth, 19–21 long, 32–37 wide, admedian lines and granules not visible. Scapular setae sc 8–9, 20 apart, projecting posteriorly.

**Leg I:** all usual segments, 12–13; trochanter 3–4, femur 3–4, genu 3, antaxial genual setae f l 13; tibia 2–3, tibial setae l′ absent; tarsus 3, paraxial unguinal setae u′ 2, paraxial fastigial tarsal setae ft 5–6, antaxial fastigial tarsal setae ft 9–10. Tarsal solenidion ω 4, slightly curved, blunt to slightly knobbed. Empodium em 3–4, simple, 3-rayed.

**Leg II:** all usual segments, 11; trochanter 2-3, femur 3, basiventral femoral seta bv 3, genu 2, antaxial genual setae f 14; tibia 1, tarsus 3, paraxial unguinal tarsal seta u′ 1, paraxial fastigial tarsal setae ft 5–4, antaxial fastigial tarsal setae ft 9–11. Tarsal solenidion ω 5–6, slightly curved, blunt to slightly knobbed. Empodium em 3, simple, 4-rayed.

**Coxisternal area:** suboral plate rounded, sometimes with faint curved lines, fewer granules than female adult. Prosternal apodeme not visible. Coxisternal plates I and II ornamented with very few granules. Anterolateral setae on coxisternal plate 1 lb 2, 6–7 apart, proximal setae on coxisternal plate 1 Ia 4, 8–9 apart, proximal setae on coxisternal plate II 2a 8–9, 16–19 apart. External genitalia absent. Proximal setae of coxisternal plate III 3a 2, 6 apart.

**Material examined**

Specimens observed for qualitative features: 38 females, 16 males, 28 nymphs, 13 larvae, Dullstroom farm, MP, SA March 2015 – May 2016; 24 females, 10 males, 18 nymphs, 8 larvae, Lydenberg farm, MP, SA March 2015 – May 2016; 425 females, 74 males, 163 nymphs, 60 larvae, Amsterdam farm, MP, SA November 2014 – May 2016. See Table 2 for further details.

**Specimens for measurements**

All specimens were collected from Amsterdam farm in Mpumalanga, South Africa.

- 6 females (slides 47803 & 47806) collected in March 2015.
- 3 females (slides T1S12, T2S19, T2S20) collected in July 2015.
- 2 males (slides T1S3; T1S11) collected in August 2015.
- 2 nymphs (slides T1S5, T1S6) collected in March 2015, and
- 2 larvae (slides T2S14, T2S16) collected in November 2014.

**Principal component analysis (PCA)**

The PCA revealed no clear clustering of individuals, nor a strong influence from any single component or character (Figure S1 in Supplementary Material). PCA1 and PCA2 explained 51.5% of the total variation (32.94% and 18.53%, respectively) (Table S2 in Supplementary Material). PCA1 was strongly influenced by setal lengths, with leg I setae f′, coxal seta δ and ventral seta e lengths being the most influential (Table S2 in Supplementary Material).

**Remarks**

Female measurements included individuals collected in winter and summer seasons. On average, females collected in winter appeared to be a bit longer and larger than females collected in summer. There was no clear distinction in measurements of individual characters between individuals of different seasons (Table S1 in Supplementary Material).

**Identification key to Eriophyoidea known on Vaccinium species worldwide**

This key is based on morphological features visible on slide-mounted specimens viewed under PCLM. Note that this is not a dichotomous key, and some points have more than two options.

1. Gnathosoma large in comparison to body. Cheliceral stylets relatively long, abruptly bent down near base. Empodia often large, entire or divided. Female genital coverflap usually smooth, female genital apodeme of moderate length, often narrowed anteriorly ......Diptilomipidae Keifer 1944 (1 species)

Prodorsal shield wide with ridges, complete median and admedian lines, submedian lines incomplete, four cells on each side of anterior shield, horned projection present near median shield rear margin. Empodium 5-rayed and divided. Tarsal solenidion knobbed, bv absent. Genital coverflap with basal granules and 14 distal ridges. Short dorsal median ridge, smooth dorsal annuli, ventral annuli with rounded microtubercles. Sternal line, coxal area sculpted with granules, prosternal apodeme present. Vagrant on the underside of leaves of *Vaccinium bracteatum* ..........................................................2

2. Vermiform mites, annuli subequal dorsiventrally. Frontal lobe typically absent, or with a light projection over gnathosoma base. If frontal lobe present, then it is narrow, basally flexible, and combined with narrow annuli. Genital apodeme usually of moderate anterior length ................................. .....Eriophyinae Nalepa 1898a (1 species) (Nalepa 1898).

- Gnathosoma usually small in comparison to body, with short straight or slightly curved chelicerae, pedipalps with terminal segments short and truncate, enclosing the short-form oral stylet. Empodia usually simple. Genital coverflap usually with ridges. Eriophyidae Nalepa 1898 (species on *Vaccinium*) .................................2

- No opisthosomal ridges. Leg I with basiventral femoral seta and paraxial tibial setae absent. Forecoxae often confluent. Coxal setae 2a, Ia and Ib present. Genital coverflap with 8–10 ridges in a single rank. Empodium 6-rayed. Tarsal solenidion slightly knobbed. Prodorsal shield without strong central lines. Inverted Y-shaped prosternal apodeme, rounded, granulate suboral plate. In buds of *Gaylussacia baccata* and *Vaccinium* species ..................................... Acallitus vaccinii (Keifer 1939)

- Fusiform mites, annuli typically dorsiventrally differentiated (broad dorsal annuli and narrow ventral annuli). Frontal lobe typically present, broad-based and rigid. Female genital apodeme usually extending moderate distance forward. Female genitalia usually not appressed to coxae and, in lateral view, lying on level with venter. Genital coverflap variably ornamented, ridges typically occur in one (rarely 2) ranks. Phyllocoptinae Nalepa 1892b (7 species) .................................................................3

3. Scapular setal tubercles usually set ahead of prodorsal shield rear margin, directing setae sc anteriorly, dorsally or convergently. Opisthosoma with a single middorsal ridge or with 3 or more longitudinal ridges with prominent middorsal ridge. Middorsal ridge ending in a broad furrow before termination of suboral ridges. Opisthosomal dorsum flattened in cross section. Prodorsal shield without frontal lobe. Caleptitrimerus Keifer, 1938 (3 species) ...................4

- Scapular setal tubercles set ahead or near prodorsal shield rear margin, directing setae sc forward or up, medially or convergently posteriad. Opisthosoma evenly arched, round in cross section, and less sharply tapered posteriorly. Opisthosomal shape variable: broad dorsal
semi-annuli and narrow ventral semi-annuli, or with little dorsoventral differentiation. Prodorsal shield with frontal lobe. *Phylloloeptes* Nalepa 1887 (4 species) .....................5

4. Wax stripes along the ridges. Prodorsal shield with a central ridge extending back and ending just beyond the dorsal tubercles setting. Broad and blunt frontal lobe, setae sc projecting up and ahead, setae h1 absent. Empodium 3-rayed. Smooth annuli. Genital cover flap with 8–9 ridges and weak horizontal markings at the top. Around the lateral buds of fresh succulent twigs of Vaccinium ovatum .................................................................Calepitirimurus gibsoni Keifer 1953

- Prodorsal shield pattern obscure or virtually absent. Frontal lobe with spines. sc setae projecting up and forward. Genital cover flap with 6–8 ridges, prosternal apodeme moderately long. Setae h1 present. Empodium 6-rayed. Vagrants on both sides of leaves of Vaccinium atrooccum .................Calepitirimurus darrowi Keifer, 1940

- Prosordal shield with lateral lines and granules, median line absent, admedian lines curving back, submedian lines curving back from side of anterior shield lobe and joining with sc tubercles. Annuli with fine and elongate microtubercles. Weak middorsal opisthosomal ridge extends back to 25th–30th dorsal annuli. Coxae ornamented with curved lines and granules, prosternal apodeme divided and short. Genital cover flap with two ranks of faint parallel markings at the top and 8 weak longitudinal ridges at the bottom. Vagrants on both sides of leaves of Vaccinium parvifolium .................................................................Calepitirimurus olympisci Keifer, 1971

5. Flattened wedge-shaped body. Empodium on leg I 4-rayed, leg II 6-rayed. Genital cover flap with 6 ridges. Vagrant on upper leaf surface of Vaccinium amoenum .................................................................Phylloloeptes vandinei Keifer, 1940

- Empodium 4- or 5-rayed, same number on leg I and II. Genital cover flap with 8–10 ridges .....................................................6


- Empodium 4-rayed. Genital cover flap with 10 ridges. h1 setae minute .................................................................7


- Body shape narrow and long, 42–46 µm wide, 185–220 µm long. Genital cover flap with 10 ridges. Empodium 4-rayed. Vagrant on leaves of Myrtilus uliginosus, M. nigra and Vaccinium myrtillus .................................................................Phylloloeptes vaccinii (Flögel & Goosmann, 1933)

**DISCUSSION**

*Acillus vaccini* occurs on wild and cultivated blueberry, causing significant economic damage on susceptible varieties in its native distribution. The damage seen due to *A. vaccini* on South African blueberry was significant, with yield losses ranging from 30–90% (Craemer 2018). When *A. vaccini* was first identified in South Africa it was noted that the description of this mite needed revision (Craemer 2018). Available descriptions did not include all life stages, and important morphological features had not been noted or were inadequately described. No comprehensive key to *Acillus* species nor eriophyoid species on blueberry was available. Here we rectified these omissions by providing accurate details of key features of multiple life stages.

In the original description of *A. vaccini* by Keifer (1939), some key morphological features were omitted in both the drawing and text description. Most importantly these included the *h1* (accessory) setae, leg I & II u’ (mesal) setae and leg II bv (femoral setae) that are considered taxonomically important as the presence or absence of setae may be an indication of a different species (Amrine and Manson 1996; De Lillo 2010). Keifer measured 33 female and 5 male (without description and drawing) characteristics, as compared to the 75 characters measured for females, 69 for males and 68 for immatures in this study.

Many of the features not included in the original description are minute and may have been missed in original observations. For example, the observation of the setae mentioned above in the current study may largely be due to advancements in microscopy since the original description. The Scanning Electron Microscopy (SEM) technique, specifically Low-temperature SEM (LT-SEM), used here was able to substantially increase visual detail of *A. vaccini* including these minute structures. LT-SEM also eliminated uncertainties in the shape of structures, especially when viewing the *h1* setae, empodium and other subtle features such as the frontal lobe and shape of microtubercles.

In addition to Keifer (1939), Baker and Neunzing (1970) described the immatures of *A. vaccini*. Differences observed between the former and this study is in the presence and arrangement of the opisthosomal microtubercles in immatures. The original description presented the larva without microtubercles and the nymph with microtubercles covering the entire opisthosoma. In the present study, on nymphs, the ventral microtubercles were arranged medially about the width of 3a – 3a setae and the dorsal microtubercles were arranged in an hourglass shape medially about the width of sc – sc setae and were more widely spaced than those on the ventral side. On larvae, microtubercles were variously present or absent on either the dorsal, ventral or both surfaces, with variable non-uniform arrangements. These observations may be a result of intraspecific variations due to a limited number of studied specimens in previous studies and advances in microscopy encouraging qualitative and quantitative analysis. Many measurements that are standard for modern descriptions were not presented by Baker and Neunzing (1970) for the immature
The presence of all life stages (females, males, immatures and eggs) of *A. vaccinii* on cultivated blueberries confirmed that the crop is an obligate host. Specimens were collected and studied throughout the year to capture variation and in attempt to detect the presence of a deutogyne, should one exist. A deutogyne is a winter form of eriophyoid mite and was detected in North America for *A. vaccinii* (Baker et al. 1996; Manson and Oldfield 1996; Cromroy and Kuitert 2001). It is important to establish whether both forms of a species occur in a particular area to avoid future misidentification of the deutogyne as a separate species (or even genus) because of morphological differences (Zhao 2000; Smith et al. 2010; Guo et al. 2015). Although females collected in winter appeared, on average, larger than the summer specimens, this was not uniform and did not form a separate cluster when analysed by PCA (Figure S1 in Supplementary Material). Other characters did not differ between winter and summer specimens. Further, immature life stages and males were collected in all seasons. Thus, morphological differences and biological evidence do not prove without doubt the presence of a deutogyne in South Africa. The absence of deutogyne in SA might be explained by the mild winter conditions of Mpumalanga (8–19 °C) (South African Weather Service, 2018), in comparison to the mite’s native range (−1 to −7 °C) (www.usclimatedata.com/climate/united–states/us). The lack of deutogyne and the viability of all life stages through the winter season might have contributed to the increased population size and significant crop injury at the Mpumalanga farm. This also suggests that the mite is likely to be a more serious pest in warmer regions of blueberry production. In addition to the enhanced morphological descriptions added here, sequence information for two DNA regions commonly used in mite species identifications were made available on GenBank to aid future identification. Partial sequences of the COI gene are routinely used for identification of many animal species including mites, and the D2 region of 28S rDNA has shown differences between eriophyid species within a genus (Skoracka and Dabert 2010). In conjunction, these regions have potential for identification of *A. vaccinii* and other eriophyid species. It will be of great benefit if more sequences were generated and deposited in GenBank to increase the pool of sequences for molecular identification of eriophyid mites.

This study supplemented and enhanced the previous descriptions of *A. vaccinii* to enable more accurate identification and ease of comparison when conducting taxonomic analyses on this important group. Importantly, additional characters (including two DNA barcodes), morphological measurements and some life stages that were not included in previous descriptions are here presented in detail. The use of complementary morphological and molecular techniques greatly enhanced our ability to see and image minute characters and provide additional information and it is recommended that future workers on this group do the same.

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SUPPLEMENTARY MATERIAL

There is supplementary material available with this article.

REFERENCES


