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New record and redescription of invasive cassava green mite, *Mononychellus mcgregori* (Flechtmann & Baker) in India on cassava, *Manihot esculenta* Crantz

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Original research

ABSTRACT

The unnoticed invasion of mites is attributed to the absence of consistent monitoring over time. This recent investigation identified the presence of the cassava green mite, *Mononychellus mcgregori* (Flechtmann & Baker 1970), on Cassava (*Manihot esculenta* Crantz) for the first time in the Indian subcontinent. The study involves a comprehensive re-description of the invasive mite, *Mononychellus mcgregori* (Flechtmann & Baker), employing an integrative approach. The report also highlights the potential future threat posed by the invasive mite to crop diversity in India.

Keywords alien mites; molecular confirmation; morphological identification; spider mite

Introduction

Cassava (*Manihot esculenta* Crantz), commonly known as Tapioca, continues to be a crop of food security for millions of people, especially in developing countries across the globe. It is an essential alternate energy source to meet the increasing population's demands. Originating in tropical America, cassava was introduced to southern peninsular India in the 17th century by the Portuguese, primarily in Kerala, before spreading to neighbouring states like Tamil Nadu, Andhra Pradesh, and Karnataka. Currently, India cultivates cassava across 172 thousand hectares, yielding 6.257 million tonnes. Tamil Nadu and Kerala lead in production, contributing 3.4 million tonnes and 2.5 million tonnes, respectively, primarily supporting employment through starch extraction and sago export (India Stat 2023). In recent times Cassava pest complexes have emerged and pose a threat to Cassava production in Asian countries. The major arthropod complex is increasing too significantly in coevolved specific pests of cassava. Among the pests, the cassava mealybug (*Phenacoccus manihoti*) and cassava green mites (*Mononychellus* spp.) complexes stand first (Bellotti *et al.* 2012; Graziosi *et al.* 2016). *Mononychellus* mite complex includes *M. tanajoa* (Bondar, 1938), *M .mcgregori* (Flechtmann & Baker, 1970) and *M. caribbeanae* (McGregor, 1950) where these mites are highly host-specific and invasive threats to cassava growing areas. The genus *Mononychellus* is represented by 31 phytophagous mite species. Including the genus *Mononychellus*, 57 species in 12 genera of Tetranychidae have been recorded on *M. esculenta* and other *Manihot* species so far (Byrne 1983; Migeon and Dorkeld 2023; IPPC 2023). Thirty-one species are known under

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Mononychellus, four of which viz., *M. tanajoa*, *M. caribbeanae*, *M. progresivus* Doreste, 1981 and *M. mcgregori* attained pest status in the cultivation of cassava worldwide (Parsa *et al.* 2015; Flechtmann and De Queiroz 2015).

The present study reports *Mononychellus mcgregori* (Flechtmann & Baker) for the first time from India and as the original descriptions are not very well detailed, we here provide a complementary description of the species. Further, the present study uses integrative taxonomy, both morphological and molecular studies to confirm the identity of species.

Material and Methods

Morphological identification

Mite infested cassava leaves were collected in polybags and brought to the laboratory for detailed observations under a Carl Zeiss® (Stemi 305) binocular stereo zoom microscope. Adult male and female mites were used for micro slide preparation using Hoyer's medium and cleared on a Hotplate at 70 °C for three days. The specimens were examined and photographed using a Zeiss® AxioScope.A1 phase-contrast and DIC microscope attached with a Nikon® DL7500 camera and the illustrations were edited with Adobe Photoshop CS 2020®. Species identification was done by using taxonomic keys (Flechtmann and de Queiroz 2015) followed by confirming morphological characters using original descriptions (Pritchard and Baker 1955; Flechtmann and Baker 1970) and supplementary descriptions (Gutierrez 1987).

In the redescription, the morphometric data are presented with the mean followed by the ranges in parenthesis, while the leg setal formula is presented with the number of tactile setae followed by the solenidia, eupathidia and duplex setae in parentheses. All the measurements are in micrometers.

All microscopic slide mounts are deposited at the mite repository of AINPAA (All India Network Project on Agriculture Acarology), UAS (University of Agricultural Sciences), GKVK (Gandhi Krishi Vignan Kendra), Bengaluru, Karnataka, India.

DNA analysis

Isoline cultures of *Mononychellus mcgregori*

Mites were maintained in the laboratory on Cassava leaves for study. Mites were freshly taken from the culture for DNA isolation.

DNA extraction and amplification

Total DNA was extracted from three to five individual mites using the modified CTAB method (Doyle and Doyle 1987). The mitochondrial cytochrome c oxidase subunit I gene (COI) barcode region was amplified using primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Polymerase chain reactions (PCR) were conducted using compositions of Taq buffer 1x (Tris with 15 mM MgCl2), 2.5 mM of each dNTP, 1 µM of each primer (Sigma-Aldrich®), 0.5 U Taq DNA polymerase (Genei, Bengaluru, India) in a 25 µL final volume.

Amplification of DNA was done in a Bio-Rad Master cycler (Bio-Rad, USA) with the following PCR conditions: initial denaturation at 94 °C 2 minutes, 94 °C 1 minute, annealing at 47 °C for 1 minute, extension and final extension at 72 °C for one minute followed by 10 minutes then holding at 4 °C. PCR amplicons were visualized on a 2% agarose gel using a Bio-Rad gel documentation unit (AINP, Acarology Lab, GKVK, Bengaluru). The sequence was obtained through Sanger sequencing and then edited using BioEdit v7.2.5 and aligned using the default parameters. All the sequences were verified for stop codons and insertions/deletions. BLASTs of nucleotide sequences were carried out on the NCBI database to determine the species identity by match percentage levels.

Results

Family Tetranychidae Donnadieu, 1875

Subfamily Tetranychinae Berlese, 1913

Tribe Tetranychini Reck, 1950

Genus *Mononychellus* Wainstein, 1971

Type species: *Tetranychus planki* McGregori, 1950

Eotetranychus planki (McGregori, 1950) New combination in Pritchard and Baker, 1955 Fig.108–110
Mononychus mcgregori Flechtmann & Baker, 1970. Description
Mononychellus mcgregori (Flechtmann & Baker 1970). New combination by Flechtmann and Baker (1975) (Fig: 109 in Pritchard and Baker (1955) is *Mononychellus mcgregori* (Flechtmann & Baker)

The name *Mononychellus* was proposed as a new combination for the genus *Mononychus* Wainstein, 1960 (with the type species *Tetranychus planki* McGregor, 1950). This change was necessary because the genus name *Mononychus* was already assigned to *Mononychus* Schueppel, 1824 (Insecta: Coleoptera) and *Mononychus* Agassiz, 1846 (Insecta: Hemiptera) (Wainstein 1970). Tuttle *et al.* (1976) characterised the genus *Mononychellus* Wainstein, 1960 by the following features: duplex setae on tarsus of leg I distal and adjacent; empodium split near the middle into three proximal hairs and tenent hairs on pad like claw (Bolland *et al.* 1998); dorsal striae of opisthosoma with different patterns; dorsal idiosomal setae usually strongly serrate and sometimes borne on small tubercles; two pairs of anal setae (Wainstein 1960, 1971; Meyer 1974; Tuttle *et al.* 1976).

Mononychellus mcgregori (Flechtmann & Baker, 1970)

Figs. 1–7

Diagnosis

Female — Body round to oval, dorsal idiosomal striation more or less transverse, dotted irregular striae around bases of setae. Longidutinal to transverse striae pattern between sc_1 and c_1, looks like a round anastomous pattern with semi-circular lobes on striations (fig. 1C). Dorsal setae serrated and enlarged distally, set on small tubercles (Tuttle *et al.* 1976; Gutierrez 1987; Flechtmann and De Queiroz 2015; IPPC 2023); bulbous peritreme at gnathosomal region. Terminal senisillum (spinneret) on palptarus wider than long. Opisthosomal setae c_1, d_1, e_1 and f_1 longer or equal to distance between consecutive setae. Setae v_2, c_3, and h_1 shorter than other setae. Ventral striation at anal region semi-circular lobe like (figs. 1E, 7D) along the striae with regular gaps. Leg tarsus I with four tactile setae and one solenidion proximal to proximal duplex setae. Tibia I with 9 tactile setae and one solenidion.

Male — Body smaller than female and narrow towards the caudal end. Terminal sensillum reduced to a minute-thick triangle on palpus. Dorsal striations strongly lobed with long, serrated and tapering setae on tubercles. Legs longer than idiosoma, elongate and end with a bifid claw. Aedeagus elongated, long shaft with a slight bent ventrad and with distal triangular knob. Anterior knob projection extended forward more sharply than the posterior projection.

Redescription

Female (n=5)

Dorsum (Figs. 1, 2) — Length of idiosoma (excluding gnathosoma) 340 (317–360), width (at c_3 level) 252 (228–290). Body more round to oval. Dorsal body striae mostly transverse except in propodosoma (longitudinal to irregular), dorsal striations dotted or lobed (depending

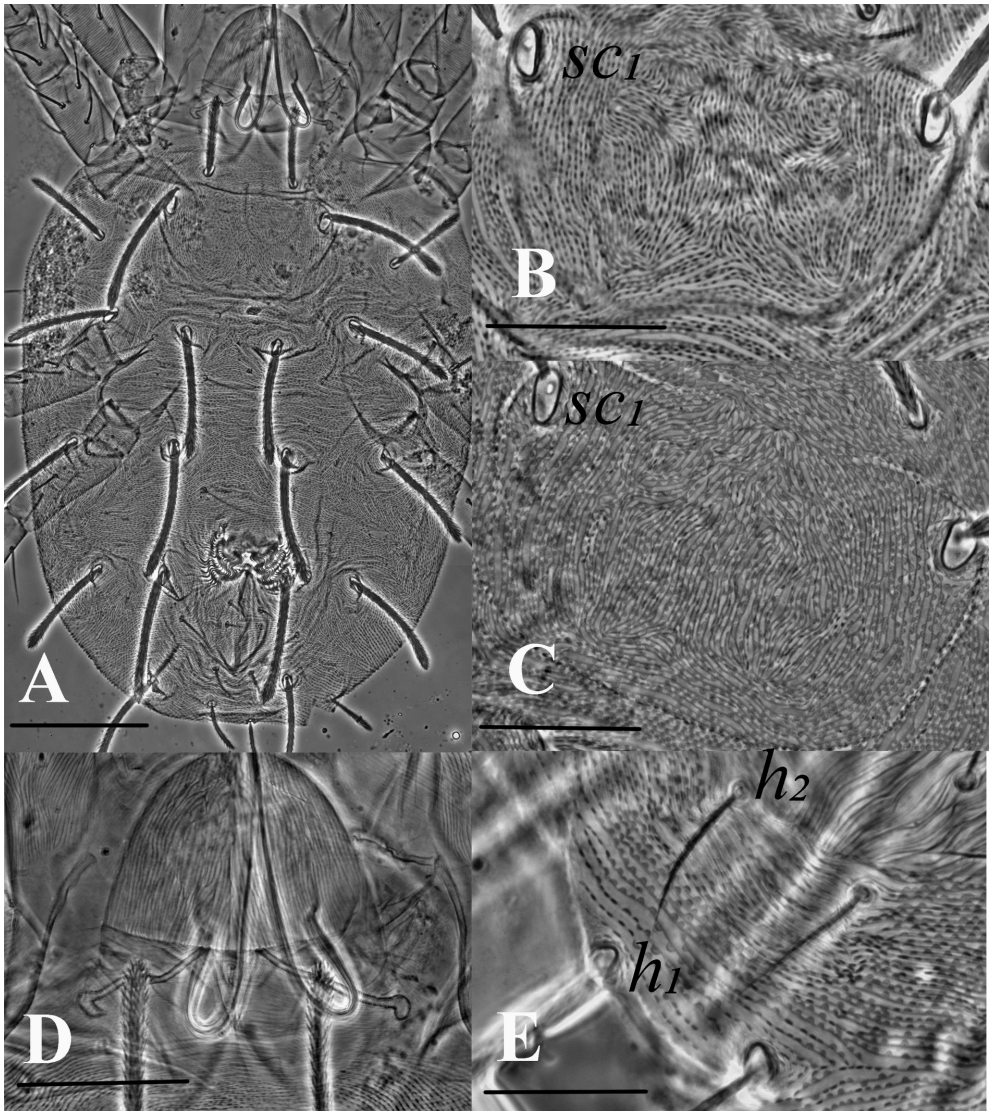

Figure 1 Adult female *Mononychellus mcgregori* A – dorsal; B – prodorsal striations with dotted lines; C – prodorsal striations with well developped lobes; D – peritreme; E – postanal striations. (Scale bars A, 60µm; B, C, D, 35µm; E, 25µm).

on morphotype and specimens), median prodorsal striations denser, more dotted or lobed, circling the base of the setae (figs. 1B, 1C, 2A). Dorsal setae spatulate and serrate towards the tip, sub equal in length (fig. 1A), sc_1 and dorsocentral setae c_1, d_1 and e_1 the longest, setae v_2, c_3, and h_1 the shortest, d_2 and e_2 of almost equal lenghths, f_1 twice as long as f_2. Prodorsal setae sc_1 and hysterosomal setae inserted on quite large tubercles excepted c_3, f_2 and h_1. Setal lengths: sc_1 75 (68–83), sc_2 48 (44–51), c_1 68 (62–78), c_2 65 (57–71), c_3 38 (35–41), d_1 76 (69–84), d_2 68 (57–78), e_1 76 (66–85), e_2 68 (55–80), f_1 62 (55–68), f_2 30 (26–35), h_1 28 (25–32), h_2 20 (15–22) and h_3 20 (15–22). Distances between setal bases: v_2–v_2 44 (41–48), sc_1–sc_1 78 (70–82) and sc_2- sc_2 155 (144–170); c_1–c_1 41 (33–45), c_2–c_2 130 (122–144), c_3–c_3 230 (201–255), d_1–d_1 55 (50–57), d_2–d_2 166 (147–184), e_1–e_1 60 (48–75), e_2–e_2 136 (119–158), f_1–f_1 69 (64–71), f_2–f_2 101 (93–114), h_1–h_1 26 (22–30); Distance between consecutive setae: c_1–d_1 67 (50–82), d_1–e_1 75 (62–91); c_3, f_2 and h_1 marginal.

Venter (Figs. 1E, 2B, 7D) — Ventral setae smooth and slender. Striations transverse except between *3a* and *4a*, slightly irregular to longitudinal; genital flap and area immediately

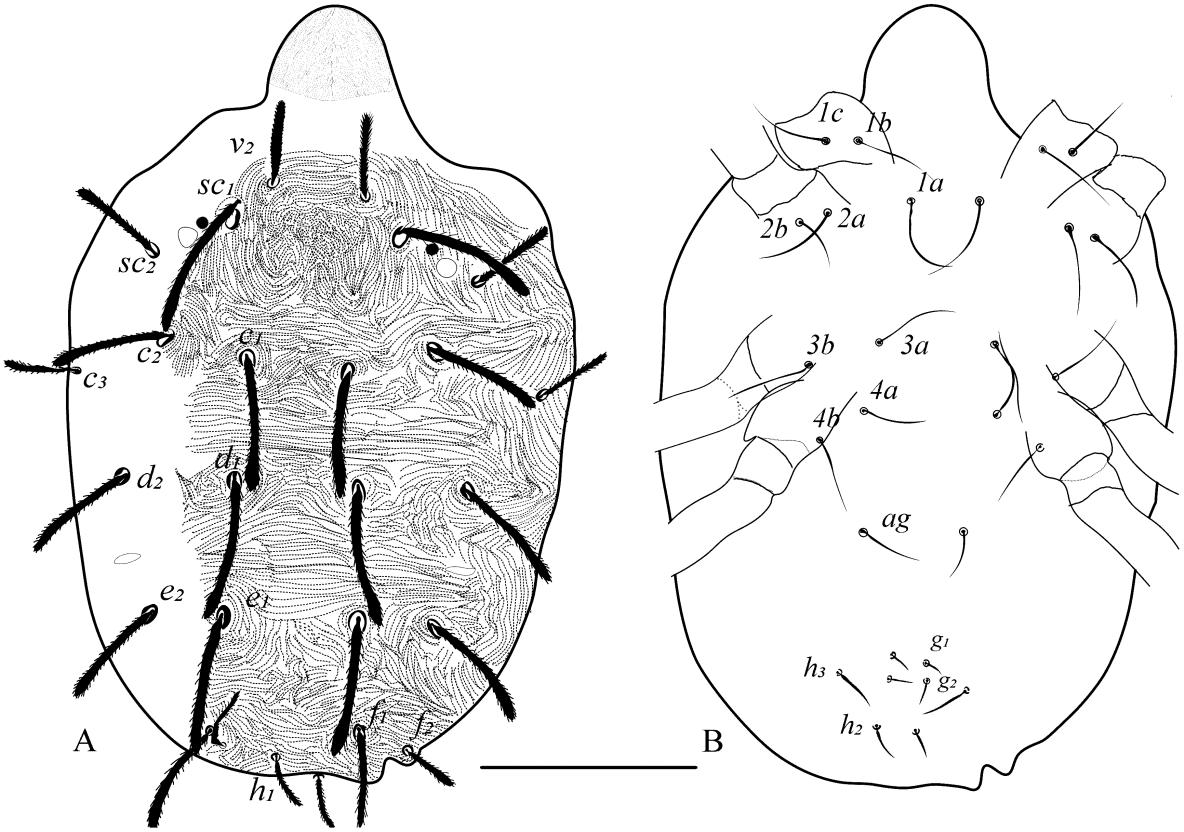

Figure 2 Adult female *Mononychellus mcgregori* A – dorsal; B – venter. (Scale bars A, B 100μm).

anterior to it with transverse striae, pregenital setae (*ag*) 40 (30–50) long and *ag–ag* 48 (45–50). Genital setae (g_1–g_2) and pseudanal setae ($ps_{1\text{-}2}$) short, tapering. Length of other ventral setae: *m* 28 (27–30), *1a* 45 (40–52), *3a* 45 (40–52), *4a* 45 (40–50). Distances between setae: *m-m* 28 (27–30), *1a–1a* 29 (27–31), *3a–3a* 55 (51–60), *4a–4a* 64 (60–72), *ag–ag* 48 (45–50). Ventrocaudal setae h_2 and h_3 tapering, slightly serrated, h_2–h_2 20 (18–22), h_3–h_3 65 (57–80). Pregenital striae transverse with two pairs of genital setae (g_1, g_2) and one pair of *ag* setae. Shape of lobes on ventral striae at h_1–h_2, semi-circular without extending to the dorsum (figs. 1E, 7D).

Gnathosoma (Figs. 1D, 7A, 7C) — Stylophore longer than wide. Peritreme enlarged distally like a bulb to a slight hook; out of five specimens, four were asymmetric (one side bulbed and the other side slightly hooked) (figs. 1D, 7C). Palp tibia with thumb claw and two setae. Palptarsus with three tactile setae (*a*, *b*, *c*), a well-developed spinneret, *suζ* 4 (4–5) width 3 (3–4), two eupathidia, *ul'ζ* 7 (6–7), *ul"ζ* 5 (5–6) and a solenidion, *ω* 3 (3–4).

Legs (Figs. 3, 7E) — Length of legs I–IV (femur to the bent portion of empodium): 244 (230-260), 174 (160-181), 190 (175–212), 210 (201–223), respectively; Leg I with four tactile setae and one solenidion proximal to proximal duplex setae. Tibia with 9 tactile setae and one solenidion. Empodium of leg I–IV split near the middle into three proximal hairs (fig. 7E). Leg setal count as follows: coxae 2–2–1–1; trochanters 1–1–1–1; femurs 10–7–4–3; genua 5–5–4–4; tibiae 9(1sol)–7–6–6; tarsi 11(1sol+2eup+2dup)–10(1sol+2eup+1dup)–8(1sol+2eup)–8(1sol+2eup).

Male (n=5)

Dorsum (Figs. 4, 5) — Length of idiosoma 230 (220–235) (excluding gnathosoma), width 147 (138–154) at c_3 setae. Prodorsum with 3 pairs (v_2, sc_1, sc_2) of setae. Striae between sc_1–c_1

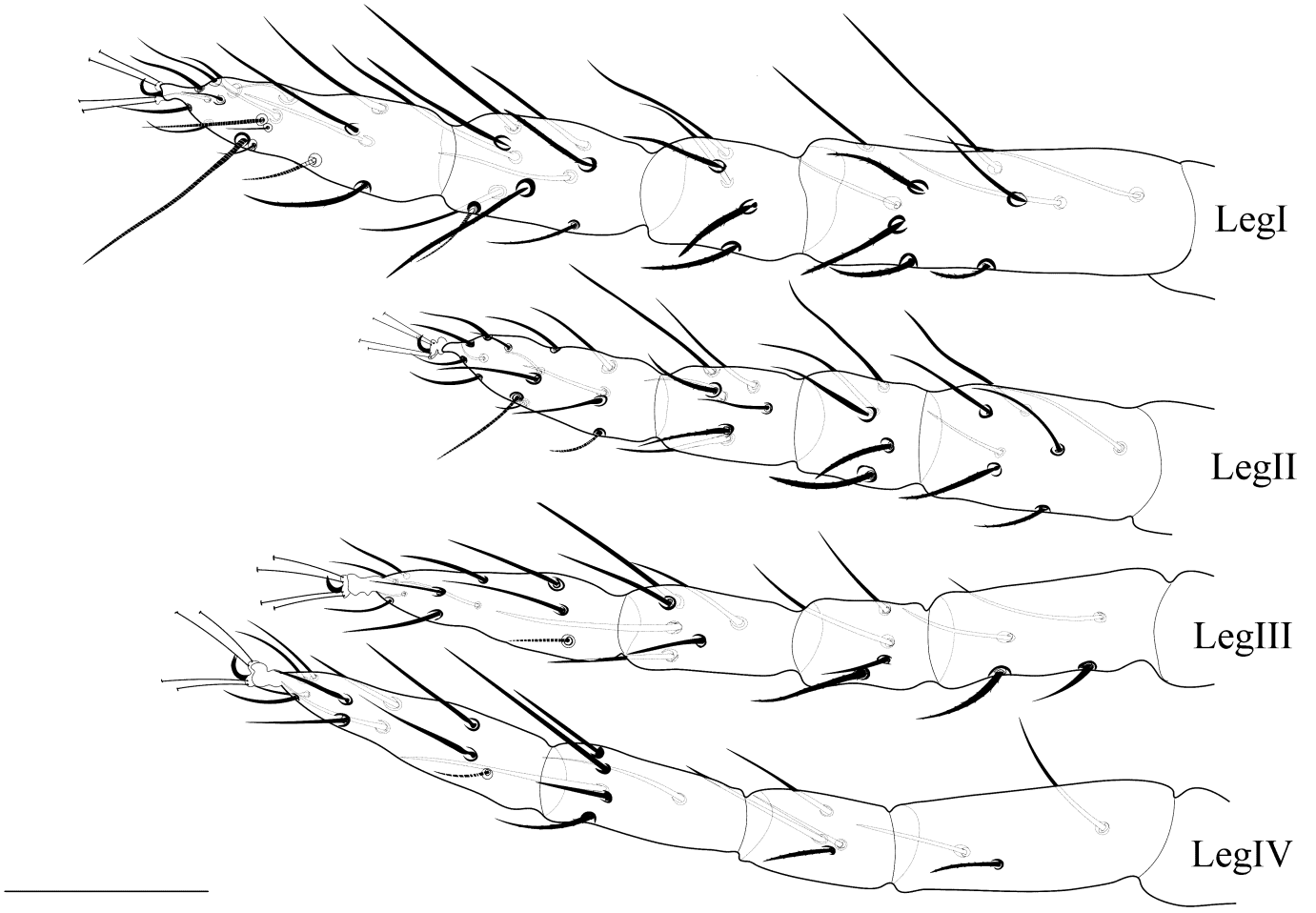

Figure 3 Adult female *Mononychellus mcgregori* leg I-IV. (Scale bars 40μm).

forming a U-shaped pattern (figs. 4A, 5A). All dorsal setae serrate, tapering, sc_1, c_2, d_2 and e_2 the longest. Setal lengths: v_2 35 (30-40) sc_1 65 (63–69), sc_2 41 (38–45), c_1 29 (25–37), c_2 52 (45–65), c_3 35 (30-40), d_1 30 (25–33), d_2 57 (46–66), e_1 29 (27–35), e_2 54 (43–65), f_1 33 (30-37), f_2 19 (19), h_1 16 (14–18). Distances between setal bases: v_2–v_2 34 (32–36), sc_1–sc_1 50 (35–60), sc_2–sc_2 98 (95–100), c_1–c_1 26 (25–28), c_2–c_2 75 (67–80), c_3–c_3 125 (115–130), d_1–d_1 31 (27–35), d_2–d_2 92 (86–95), e_1–e_1 19 (18–20), e_2–e_2 64 (60–66), f_1–f_1 29 (27–31), f_2–f_2 43 (42–45), h_1–h_1 10 (9–10), h_2–h_2 17, h_3–h_3 42 (40–45). Setae h_1–h_3 on dorsal side.

Venter (Fig. 4B) — Striations sparse and transverse. All ventral setae slender smooth, tapering, shorter than in females; *m* 18 (17–20), *1a* 31 (30–32), *3a* 27 (25–30), *4a* 25 (23–26), *ag* 13 (10-16). Distances between setae: *1a–1a* 18 (17–18), *3a–3a* 43 (40-45), *4a–4a* 36 (34–37), *ag–ag* 8 (8–8). Genital setae (g_1–g_2) and pseudanal setae ($ps_{1\text{-}2}$) short and tapering.

Gnathosoma (Fig. 7B) — All tactile setae on palp slender. Subcapitular setae *m* smooth, setaceous. Palptarsus with three tactile setae (*a*, *b*, *c*), with a small spinneret, *suζ* 1 (0.6–1), width 1, two eupathidia *ul'ζ* 5 (4–6), *ul''ζ* 4 (4–5) and one solenidion (*ω)* 3 (3–5); solenidion slightly thinner and longer than spinneret.

Legs (Figs. 6, 7E, 7F) — Length of legs I–IV (femur to tip of empodium): 271 (245–282), 173 (162–185), 185 (167–192), 214 (195–227) respectively; leg I with six tactile setae and one solenidion proximal to proximal duplex setae. Tibia with 10 tactile setae and two solenidia. Empodia I-II clawlike (Fig.7F), empodia III-IV as in female (Fig. 7E). Leg setal count as

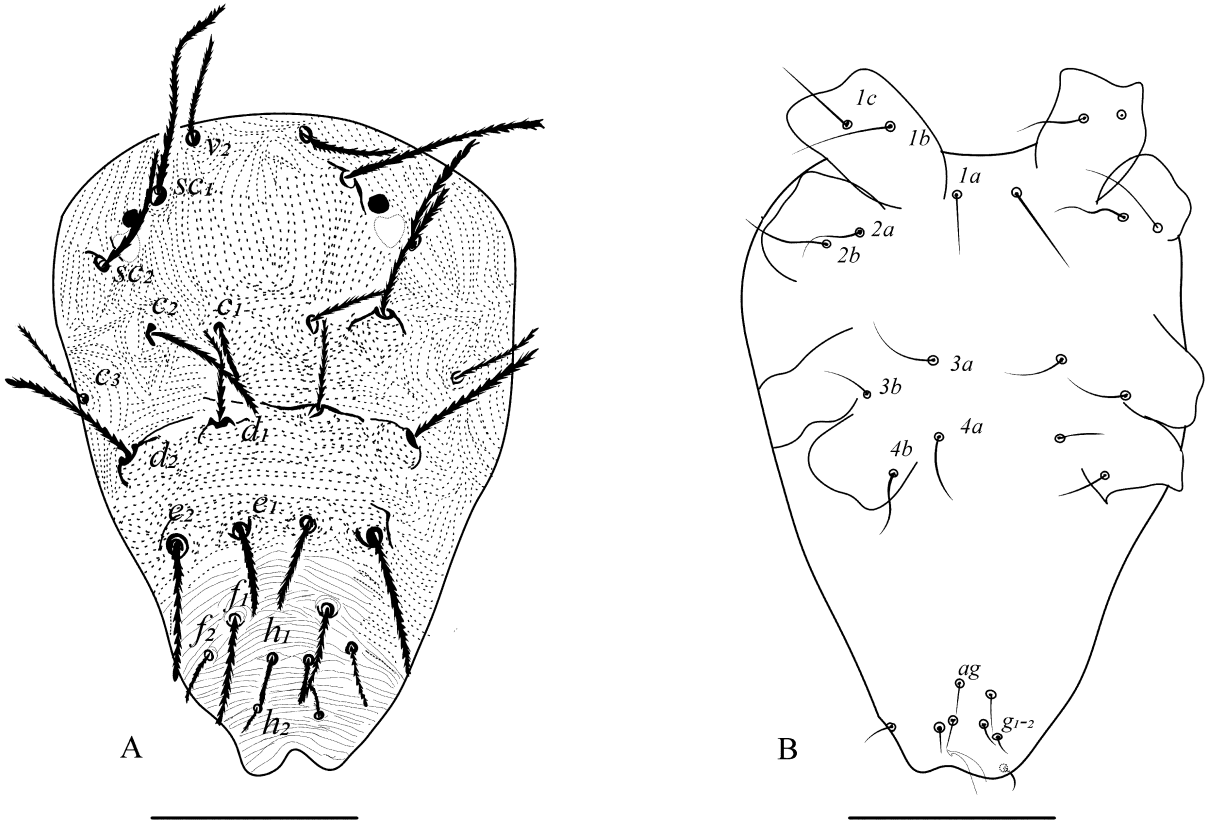

Figure 4 Adult male *Mononychellus mcgregori* A – dorsal; B – venter. (Scale bars A, B 55µm).

follows: coxae 2–2–1–1; trochanters 1–1–1–1; femora 10–7–4–3; genua 5–5–4–3; tibiae 11 (2sol)–7–6-6-); tarsi 12(1sol+2eup+2dup)–9 (1sol+2eup+1dup)–8(1sol+2eup)–8(1sol+2eup).

Aedeagus (Fig. 5B) — Aedeagus with a long shaft, slightly bent ventrally, featuring a distal triangular knob. Anterior projection of the knob acute, posterior rounded. Ventral margin of the knob with slight depression near the middle (variations can be seen in different focus).

Material examined

Nine males and 5 females on Cassava (*Manihot esculenta* Crantz) (Euphorbiaceae), Coffee Research Substation (CRSS), Chettalli (12°22′44.9″ N 75°50′17.2″ E, alt. 1002 m), Chettalli (Kodagu), India, 24 May 2023, coll. P. Dyamanagouda; 10 males, 10 females on Cassava (*M. esculenta*) (Euphorbiaceae), Bengaluru (13°04′36.0″ N 77°34′39.5″ E, alt. 924 m), GKVK, Bengaluru (Karnataka), India, 04 Sep. 2023, coll. P. Dyamanagouda.

Molecular identification

Mite samples collected at Chettalli (Specimen voucher UASB:3596 and NCBI accession number PP074174) and GKVK, Bengaluru (Specimen voucher UASB:3600 and NCBI accession number PP124899) showed similarities of 99.16% and 98.99% with the sequence of *M. mcgregori* (NCBI accession number MN913383) submitted by Ovalle *et al.* (2020) from Colombia, respectively.

Remarks

Mononychellus species identification was a bit complex earlier due to cryptic morphologies among the species. Pritchard and Baker (1955) introduced a new combination for the type

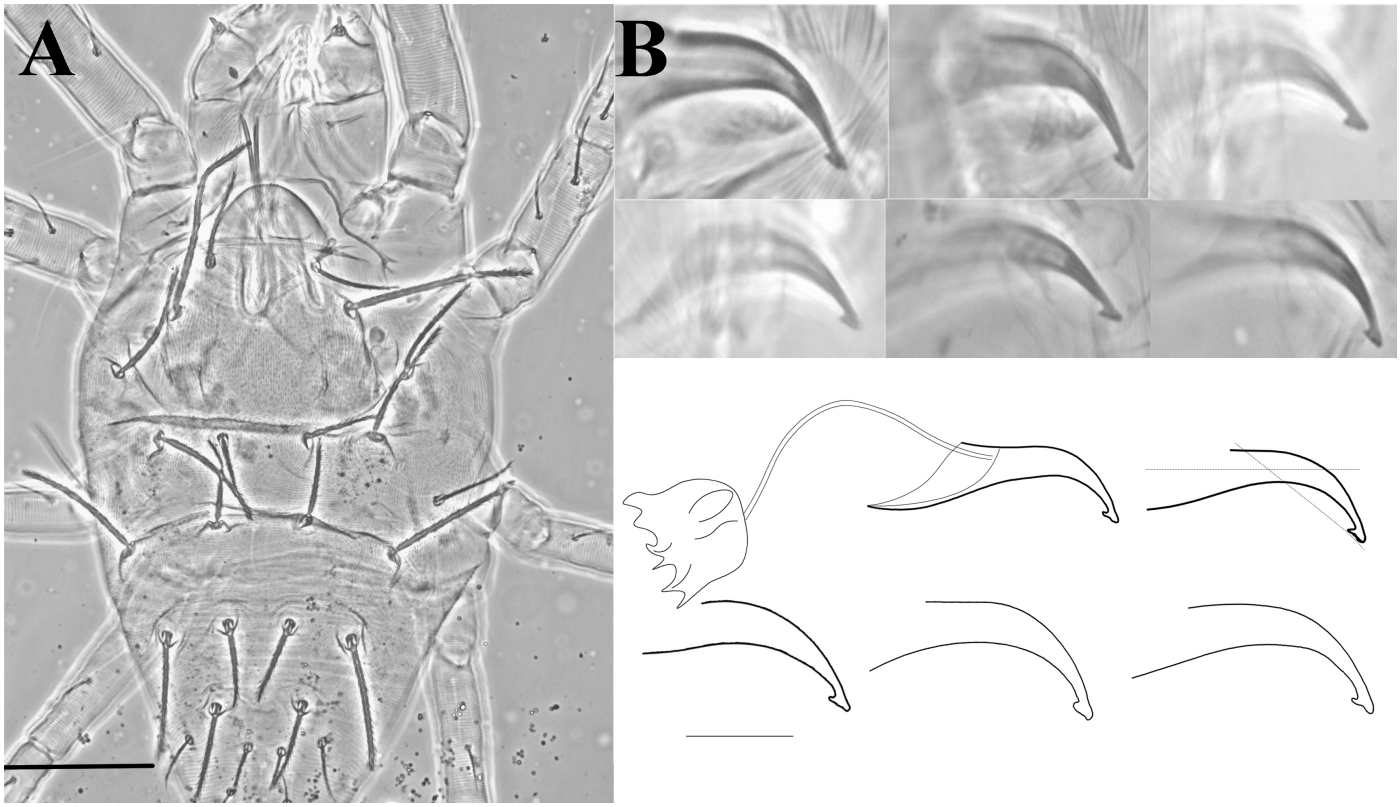

Figure 5 Adult male *Mononychellus mcgregori* A – dorsal; B – variations in aedeagus of males. (Scale bars A, 80μm; B, 8μm).

species *Tetranychus planki* McGregor, 1950, renaming it as *Eotetranychus planki*. They noted two morphotypes: one with reticulation at the bases of dorsal setae (Trinidad type) and the other without reticulations at the dorsal setae base (Argentina type). Later, *Eotetranychus planki* was reclassified as two separate species: *Mononychellus planki* sensu stricto (McGregor 1950) with reticulation and *M. mcgregori* without reticulation (McGregor 1950; Pritchard and Baker 1955; Flechtmann and Baker 1970; Gutierrez 1987).

Additionally, discrepancies in leg setal numbers, the presence of striations, and other descriptive characters among authors in subsequent publications have been observed (Gutierrez 1987; Flechtmann and De Queiroz 2015). To support this, our investigation observed variability in the lobe pattern present on dorsal striation between mites from two distinct locations. One morphotype (all females from Bengaluru) exhibited dorsal striations resembling dotted or dashed lines, while the other (found in two females from Chettalli) with semi-circular well developped lobes on the dorsal striations (fig. 1B for dotted and 1C for lobed). While, other three female mites from Chettalli are similar to the Bengaluru morphotypes. Despite this variation, all other characteristics remain consistent across both morphotypes in the present study. This variation in dorsal striation of *M. mcgregori* is in congruence with the observations of dotted lines by Pritchard and Baker (1955) (Fig. 109, p. 150) and lobed striations by Flechtmann and Baker (1970) on Argentina morphotypes.

Earlier research has suggested that variations in the dorsal striation pattern and shape could be attributed to several factors such as improperly mounted specimens, environmental conditions, and hybridization effects (Boudreaux and Dosse 1963; Monroe 1963; Mollet and Sevacherian 1984). This variation in present study might be due to mounting effect (as only two females observed) or due to genetic diversity due to haploidy nature and gene flow within population (Helle and Pieterse 1965; Dupont 1979). However, even among properly

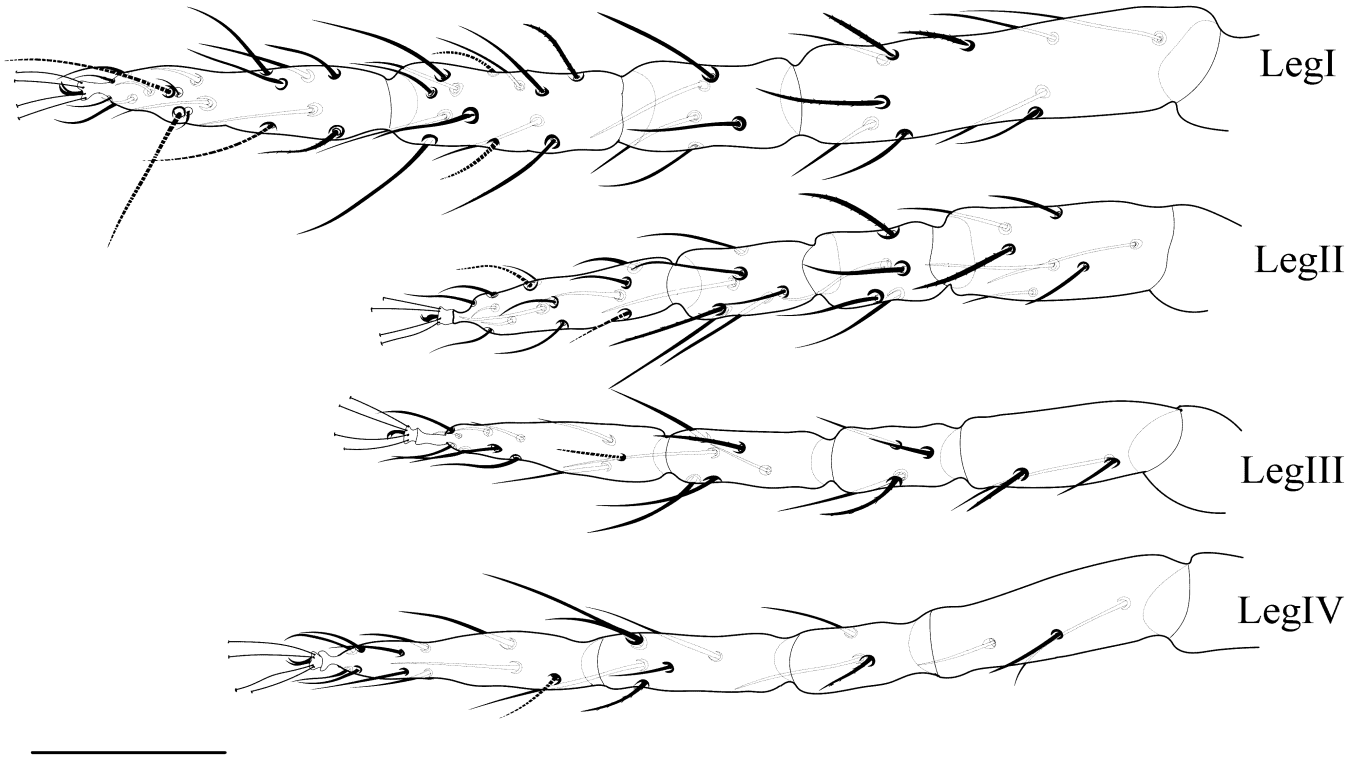

Figure 6 Adult male *Mononychellus mcgregori* leg I-IV. (Scale bar 40µm).

mounted specimens, variations are observed within populations (Boudreaux and Dosse 1963; Monroe 1963). Additionally, the dorsal striation shape and pattern of mites is not always a consistent characteristic for taxonomic identification as it is varies with each mite form within the population as it is dependent on above factors (Meyer 1974; Jordaan 1977; Auger *et al.* 2013).

Moreover, variations in setal counts in legs of *M. mcgregori* can be seen in earlier literature. Pritchard and Baker (1955) reported one sensory and nine tactile setae without specifying whether these mites were from Argentina or Trinidad. Later, Flechtmann and Baker (1970) provided a full description of *M. mcgregori* based on mites from Argentina, noting that tibia I had one sensory and eight tactile setae.

These kinds of conflicts in the description of *Mononychellus* clearly shows that the genus needs a deep revision. Firstly, descriptions are restricted to females in some sources (Beer and Lang 1958; Estebanes and Baker 1968). Secondly, the cryptic morphology of these mites complicate identification. Lastly, only a few mitochondrial nucleotide sequences (only for *M. tanajoa, M. mcgregori*, and *M. caribbeanae*) are present in the GeneBank (NCBI 2024) for comparative analysis.

Mononychellus mcgregori is closely related to *M. chemosetosus* (Paschoal) and *M. planki* (McGregor). *Mononychellus mcgregori* aedeagus is similar to those of *M. planki* and *M. chemosetosus* (IPPC 2023). However, it can be differentiated from latter species by comparatively longer shaft curved ventrad with anterior acute and posterior round knob. Additionally, reticulations on the prodorsum and the base of each of the opisthosomal setae are absent in *M. mcgregori*.

Mononychellus mcgregori is very similar to *M. manihoti* Doreste, 1981 in general morphology and host range but can be differentiated using several characters: 1) the aedeagus of *M. mcgregori* has a long shaft curved ventrad with the anterior projection of the knob acute and

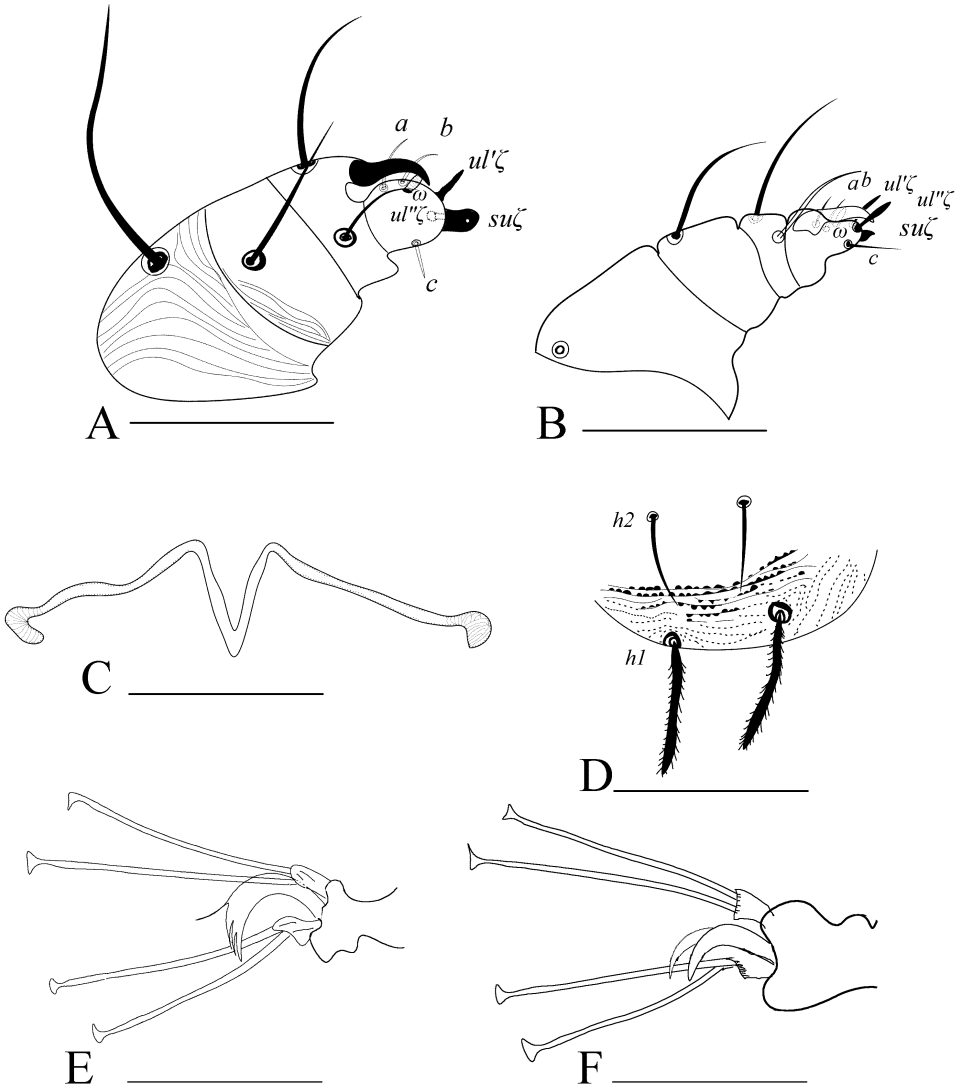

Figure 7 *Mononychellus mcgregori* A – female pedipalp; B – male pedipalp; C – female peritremes; D – post anal striae and lobes of female; E – female empodium I; F – male empodium I. (Scale bars A, B, C, 30µm; D, 50µm; E, F 15µm).

the posterior rounded, whereas in *M. manihoti*, the aedeagus has a straight shaft without bent and both anterior and posterior projections of the knob are acute; 2) the female tibia I bears 9 tactile setae and one solenidion in *M. mcgregori*, while *M. manihoti* has only 8 tactile setae and one solenidion; 3) the male tibia I bears eleven tactile setae and two solenidia in *M. mcgregori*, whereas *M. manihoti* has nine tactile and three solenidia (Flechtmann and Baker 1970; Doreste 1981).

Mononychellus mcgregori shows a high invasive probability to subtropical conditions according to climate models suitability (Lu *et al.* 2012; Rêgo *et al.* 2013; Parsa *et al.* 2015). This situation is particularly important in southern Indian states, notably Kerala, Tamil Nadu, and Karnataka, where cassava is cultivated as a major crop. At the same time, far North-eastern states of India also have a high alert of spread and colonization. Currently, determining the route of entry is challenging and requires further study and intensive surveys. Finally, the ability to feed and establish on multiple hosts is considerable. So, these facts support a significant threat to crop diversity and biodiverse hotspots of India in future.

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