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DP 33: *Mononychellus tanajoa*

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ISPM 27

Diagnostic protocols for regulated pests

DP 33: *Mononychellus tanajoa*

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1. Pest information

The cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), is one of the major pests of cassava *Manihot esculenta* (Euphorbiaceae) (Byrne *et al.*, 1982; Byrne, Belloti and Guerrero, 1983; Veiga, 1985) – a staple crop for more than 11 percent of the world’s population (FAO, 2013). It prefers to feed on the underside of young leaves of growing shoots of the cassava plant. Immature and adult mites feed by piercing plant tissues and sucking out the contents of cells, leading to leaf distortion and chlorotic mottling (Figure 1A to Figure 1C). Severe mite damage can lead to defoliation of the upper parts of shoots, producing a “candlestick” appearance (Figure 1D) and resulting in 50–80% storage-root yield loss (Shukla, 1976; Byrne *et al.*, 1982; Byrne, Belloti and Guerrero, 1983; Veiga, 1985; CABI, 2023).

Mononychellus tanajoa (Figure 2A) is a tropical and subtropical species. It was first described on *Manihot* spp. in Brazil in 1938 and is now widely distributed in South and Central America (Machi *et al.*, 2014; Vásquez-Ordóñez and Parsa, 2014; Migeon and Dorkeld, 2021; EPPO, 2022; CABI, 2023). In Africa, *M. tanajoa* was first reported in Uganda in 1971 (Lyon, 1973). From there, it rapidly expanded its distribution throughout the cassava-growing regions of the continent and is now established in over 30 countries (Byrne, Belloti and Guerrero, 1983; Gutierrez *et al.*, 1988; Yaninek, 1988; Yaninek, Moraes and Markham, 1989; Bolland, Gutierrez and Flechtmann, 1998; Vásquez-Ordóñez and Parsa, 2014; Migeon and Dorkeld, 2021; EPPO, 2022; CABI, 2023).

This mite is mainly a pest of cultivated *M. esculenta*, although it has been recorded on other *Manihot* species (Bondar, 1938; Flechtmann and Baker, 1970). It also occurs on several species in other plant families, including *Erythrina* sp., *Gliricidia maculata*, *Gliricidia sepium*, *Phaseolus vulgaris* and *Senna occidentalis* (Fabaceae) (Rossi Simons, 1961; Baker and Pritchard, 1962; Estebanes-Gonzalez and Baker, 1968; Andrews and Poe, 1980; Mendonça *et al.*, 2011); *Passiflora cincinnata* and *Passiflora edulis* (Passifloraceae) (Moraes, Moreira and Delalibera, 1995; Mendonça *et al.*, 2011); and *Typha domingensis* (Typhaceae) (Moraes, Moreira and Delalibera, 1995; Aguilar and Murillo, 2008; Migeon and Dorkeld, 2021).

The life cycle of *M. tanajoa* consists of the egg and four active stages: six-legged larva, eight-legged protonymph, deutonymph and adult. An inactive (quiescent) stage is present between the active stages, during which moulting occurs. This species overwinters as eggs or adult females. It completes a generation in 24.7 days on *M. esculenta* at 24 ± 2 °C, $65 \pm 10\%$ relative humidity (Moraes, Moreira and Delalibera, 1995). The developmental time can decrease substantially with increasing temperature; *M. tanajoa* needs only eight days to develop from egg to adult at 31 °C. An adult female can lay about 58 eggs during her lifetime (Yaninek, Moraes and Markham, 1989). The highest population density of *M. tanajoa* occurs during the first half of the dry season (Yaninek, Moraes and Markham, 1989). In Brazil, severe damage is only observed in the dry areas of the northeast region, although the mite is widely distributed in the country (Moraes and Flechtmann, 2008).

Mites in the family Phytoseiidae (Figure 2B) are the main natural enemies of spider mites (Tetranychidae). Among the natural enemies of *M. tanajoa*, more than 30 species of Phytoseiidae are found on *Manihot* spp. (Zannou *et al.*, 2005; Mutisya *et al.*, 2017; Demite *et al.*, 2021). Apart from the phytoseiid mites, the acaropathogenic fungus *Neozygites tanajoa*e (Entomophthorales: Neozygitaceae) (Figure 2C) is a widespread host-specific pathogen of *M. tanajoa* in Brazil (Delalibera, Hajek and Humber, 2004). It was introduced into Benin in West Africa, where it is now widespread (Agboton, Hanna and von Tiedmann, 2011) alongside the much less virulent *Neozygites floridana*, which has a much broader host range among tetranychid mites (Lopes Ribeiro *et al.*, 2009).

2. Taxonomic information

Name: *Mononychellus tanajoa* (Bondar, 1938)

Synonyms and other scientific names:

Tetranychus tanajoa Bondar, 1938

Mononychus tanajoa (Bondar, 1938)

Eotetranychus estradai Baker and Pritchard, 1962

Taxonomic position: Acari, Trombidiformes, Prostigmata, Tetranychidae

Common names: cassava green mite, cassava mite

3. Detection

Mononychellus tanajoa may be first found on the underside of young leaves of the upper third of the cassava canopy (Yaninek, Moraes and Markham, 1989). With continuous feeding by a growing population of *M. tanajoa*, damage to the leaf expands to impact all interveinal leaf surfaces. Heavily damaged leaves eventually become desiccated and fall. As the mite prefers to feed on young leaves in the upper part of the cassava plant, severe damage can lead to shoot defoliation, giving the appearance of a “candlestick” (Figure 1D).

In addition to being found on plant foliage, *M. tanajoa* may also be found on cassava stem cuttings, packaging, farm machinery, vehicles and tools, farm waste, workers’ clothing, soil, and nearby plants that are in contact with the host. Because of the small size of *M. tanajoa*, it is extremely difficult to detect during the early stages of an infestation.

While host symptoms are seen with the naked eye, all life stages of *M. tanajoa* require magnification with a hand lens or stereomicroscope. The uppermost, recently unfolded, mature leaves are the best to look at for detecting and monitoring *M. tanajoa* infestations. A hand (or head) lens of a minimum of 5× magnification (preferably 10×) allows detection of *M. tanajoa* on leaf surfaces. Sampling programmes based on actual counts, classes of densities and binomial (presence/absence) sequential sampling have been developed (Yaninek, Moraes and Markham, 1989; Nachman *et al.*, 1993; Onzo *et al.*, 2005).

Other mites co-infest cassava in the Americas (principally several species in the *Mononychellus* genus) and in Africa (principally *Oligonychus gossypii* and rarely *Tetranychus urticae*). In Africa, *O. gossypii* is easily distinguishable from *M. tanajoa* by its larger size, reddish appearance – hence the common name “red spider mite” – and colonization of older leaves.

Various techniques can be used to collect spider mites from plants. The collected mites can be prepared immediately for microscopic examination (see section 4.1) or stored in small tubes containing 70% ethanol or Oudemans’ fluid (5 parts glycerine, 87 parts 70% ethanol, 8 parts glacial acetic acid). A concentration of 95–100% ethanol is needed for mites intended for molecular analyses (Walter and Krantz, 2009). Both female and male adults are needed for identification with dichotomous keys (Flechtmann and de Queiroz, 2015).

Mites can be collected individually from leaves using a forceps or fine brush (size 0) and with the aid of a hand lens (Walter and Krantz, 2009)

Beating the host plant is one of the most effective methods for collecting spider mites. A stick is used to vigorously beat the leaves and branches over a white tray from which the mites are picked up with a fine brush.

The washing and sieving method can also be used to collect spider mites. First, leaves or branches are dipped in 0.2–0.3% household detergent or 50–70% ethanol in a large container and stirred for a few minutes to dislodge the mites. Next, the suspension is poured into a stack of three stainless-steel sieves:

1 680 µm, 600 µm and 44 µm aperture for the top, middle and bottom sieves, respectively (modified from de Lillo, 2009). Finally, mites on the bottom sieve are backwashed with 70% ethanol into a Petri dish and picked up with a fine brush. An alternative method is to immerse plant material in 50–70% ethanol, allowing the mites to sink to the bottom of the container, from where they can be collected with a pipette.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4. Identification

Identification of spider mites in the *Mononychellus* genus has traditionally been based on microscopic morphological characters. Adult female and male specimens must be mounted on slides and examined using a high-power microscope (e.g. $\times 400$ – 1000). Morphological characters are best observed with a compound microscope using either differential interference contrast or phase contrast. Features of the adult body are illustrated and labelled in Figure 3 to Figure 8. Keys for the morphological species identification of immature stages of *Mononychellus* are not available.

DNA sequencing of a barcoding fragment of the cytochrome c oxidase subunit I (*COI*) can support identification but cannot provide confirmatory diagnosis, as only limited sequences of *M. tanajoa* and of some closely related cassava *Mononychellus* species (*M. caribbeanae*, *M. progresivus* and *M. mcgregori*) are available in the GenBank public database (a National Center for Biotechnology Information database) (Ovalle *et al.*, 2020).

4.1 Preparation of specimens for microscopic examination

Mites need to be properly prepared for morphological examination. Maceration dissolves internal tissues, increasing transparency and softening the cuticle. This clearing can be achieved by submerging the specimen in 60–95% lactic acid (suitable for recently collected specimens) or Nesbitt's fluid (chloral hydrate 40 g, concentrated HCl (12 M) 2.5 mL, distilled water 25 mL, suitable for old alcohol-preserved specimens). The clearing process varies from specimen to specimen. It is advisable to check occasionally until the specimens become translucent. Gentle heating in an oven or hot plate at 45 °C can accelerate maceration. Specimens are mounted in Hoyer's medium (chloral hydrate 200 g, crystalline gum arabic 30 g, glycerol 20 mL, distilled water 50 mL) or in Heinze-PVA medium (chloral hydrate 100 g, glycerol 10 mL, polyvinyl alcohol 10 g, distilled water 60 mL, 85–92% lactic acid 35 mL). Adult females are mounted dorsoventrally, but adult males should be mounted laterally to display the taxonomically informative characters of the aedeagus (male genitalia). The male specimens can be mounted as described by Henderson (2001) or repositioned laterally by gently pushing the coverslip to one side. Slides are labelled with the collection data (i.e. an accession number, locality, host, collector and collection date) and then put on a hot plate at 70 °C for 20 minutes before identification. If immersion objectives are to be used, a longer heating time (24 h) on the hot plate is required to ensure the slides are completely stable. If the specimens are to be retained following identification (see section 5), the identified specimens are placed in an oven at 45–50 °C for a few weeks until the medium is dry. For long-term storage, specimens mounted in Hoyer's medium on microscope slides should be sealed with a sealant (e.g. Glyptal® electrical insulating sealant or Euparal) to prevent water from entering or leaving the mount.¹ Nail polish can be used for short-term storage but is susceptible to cracking and may dissolve in substances used to clean slides. Detailed methods for mite specimen preparation and mounting are available in Walter and Krantz (2009).

¹ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.

4.2 Morphological characters of the family Tetranychidae

The spider mite family Tetranychidae includes more than 1 300 species in 86 genera (Migeon and Dorkeld, 2021). Various keys to separate Tetranychidae from other families are available in general acarological references (e.g. Walter *et al.*, 2009). Species of this family share the following characteristics:

- dorsal propodosoma without bothridia (Figure 3A);
- hysterosoma with four or fewer pairs of setae in the *c* series (Figure 3A);
- chelicerae basally fused, forming a retractable stylophore (Figure 3A);
- stylophore without a ribbed collar basally (Figure 3A, Figure 4 and Figure 5A);
- movable cheliceral digits greatly elongate, whip-like (Figure 4A and Figure 4C);
- palp with a thumb-claw structure (Figure 5B);
- tarsi I and II usually with one to two sets of duplex setae (Figure 6A and Figure 6B);
- solenidia on tarsi I and II setiform (Figure 6A and Figure 6B); and
- tarsal claws usually with tenent hairs (Figure 7).

4.3 Dichotomous key to genera of Tetranychidae on *Manihot* spp.

In addition to *M. tanajoa*, 56 species in 12 genera of Tetranychidae have been recorded on *M. esculenta* and other *Manihot* species so far (Migeon and Dorkeld, 2021): *Allonychus* (3 species), *Aponychus* (1 species), *Atrichoproctus* (1 species), *Eotetranychus* (1 species), *Eutetranychus* (5 species), *Mononychellus* (7 species), *Neotetranychus* (1 species), *Oligonychus* (8 species), *Panonychus* (1 species), *Petrobia* (1 species), *Schizotetranychus* (1 species) and *Tetranychus* (26 species). These genera can be distinguished by morphological traits.

This key can be used to distinguish the Tetranychidae genera encountered on *Manihot* spp. (modified from Meyer (1987) and Bolland, Gutierrez and Flechtmann (1998)).

1. Empodium absent or without tenent hairs (Figure 7B to Figure 7I); female with one or two pairs of pseudanal (*ps*) setae (Figure 3B); male with four pairs of setae (*g* and *ps*) on genito-anal valves (Figure 8A).....**2**
 - Empodium with tenent hairs (Figure 7A); female with three pairs of pseudanal setae; male with five pairs of setae on genito-anal valves***Petrobia* Murray**
2. Tarsus I without duplex setae or with one set of duplex setae; empodia I–IV in female and III–IV in male absent (Figure 7B) or vestigial.....**3**
 - Tarsus I with two sets of duplex setae (Figure 6A); empodia I–IV in female and III–IV in male uncinat (claw-like) (Figure 7C) or split into three pairs of proximoventral hairs (Figure 7D)**4**
3. Female with one pair of pseudanal setae; hysterosomal setae *f*₁ inserted in marginal area, lateral to dorsocentral setae *c*₁, *d*₁ and *e*₁ (Figure 8C)***Aponychus* Rimando**
 - Female with two pairs of pseudanal setae; hysterosomal setae *f*₁ inserted in median area, approximately aligned with dorsocentral setae *c*₁, *d*₁ and *e*₁ (Figure 8D) ***Eutetranychus* Banks**
4. Female with two pairs of *h* setae (*h*₁ missing) and one or two pairs of pseudanal setae**5**
 - Female with three pairs of *h* setae and two pairs of pseudanal setae (Figure 3B)**7**
5. Female with two pairs of pseudanal setae; tarsal duplex setae close to each other in the distal half of segment, or well separated from each other with one in the distal half and the other in the basal half; empodium variable**6**

- Female with one pair of pseudanal setae; tarsal duplex setae close to each other in distal half of segment; empodium uncinata.....*Atrichoproctus* Flechtmann
- 6. Duplex setae on tarsus I close to each other, in distal half; empodium claw-like, strong, not shorter than proximoventral hairs (Figure 7E); male with claw I bifid.....*Oligonychus* Berlese
 - Duplex setae on tarsus I relatively distant from each other, one in distal half and other in basal half of segment; empodium variable (Figure 7D), if claw-like, much shorter than proximoventral hairs; male with claw I not bifid *Tetranychus* Dufour
- 7. Empodium claw-like (Figure 7C and Figure 7F).....**8**
 - Empodium not claw-like, ending in a tuft of hairs (Figure 7D).....**10**
- 8. Empodial claw ending in a single tip (Figure 7C and Figure 7E).....**9**
 - Empodial claw bifurcate distally (Figure 7F)*Schizotetranychus* Trägårdh
- 9. Dorsal idiosomal setae not on tubercles; empodial claw shorter than proximoventral hairs (Figure 7I)*Allonychus* Pritchard and Baker
 - Dorsal idiosomal setae on strong tubercles; empodial claw longer than or as long as proximoventral hairs (Figure 7E).....*Panonychus* Yokoyama
- 10. Hysterosomal striae forming a basket-weave pattern (Figure 8B) or varying from longitudinal to irregular between the two e_I setae; dorsal idiosomal setae usually on tubercles (Figure 8B).....**11**
 - Hysterosomal striae transverse; dorsal idiosomal setae not on tubercles.....*Eotetranychus* Oudemans
- 11. Hysterosomal striae not forming a basket-weave (grouped granulation) pattern (Figure 3)*Mononychellus* Wainstein
 - Hysterosomal striae forming a basket-weave pattern (Figure 8B).....*Neotetranychus* Trägårdh

4.4 Morphological characters of the genus *Mononychellus* Wainstein

The genus includes 31 species worldwide, which can be recognized by the following characteristics.

For adult females, the characteristics are:

- prodorsum mostly striated (Figure 3A, Figure 9A, Figure 9B, Figure 9D and Figure 9F) or medially finely reticulate and dotted (Figure 9C and Figure 9E);
- dorsal idiosomal setae linear or slightly expanded apically or proximally, usually on tubercles (Figure 10C to Figure 10E) (with exceptions, e.g. in *M. tanajoa*);
- three pairs of h setae and two pairs of pseudanal (ps) setae present (Figure 3B);
- striae between the two e_I setae variable, from oblique to longitudinal (Figure 10);
- pregenital striae varying from procurved to transverse (Figure 11);
- palpal terminal eupathidium (spinneret) varying from as long as wide to 2.5× as long as wide (Figure 5B and Figure 12);
- tarsus I with two sets of distal duplex setae adjacent to each other in the distal part of the segment, and four (rarely five) tactile setae and one solenidion (rarely none or three) in proximal part (Figure 6A);
- tibia I with eight or nine (rarely 12) tactile setae and one solenidion (rarely four) (Figure 6A);
- tarsus II with three tactile setae and one solenidion in proximal part (Figure 6B); and
- tibia II with seven (rarely six) tactile setae (Figure 6B).

For adult males, the characteristics are:

- genito-anal valves with four pairs of setae (*g* and *ps*) (Figure 8A);
- aedeagus with shaft mostly straight, slender, bending upward or downward apically, forming a narrow neck, with a small, usually subtriangular knob (Figure 13 and Figure 14);
- palp with terminal eupathidium slightly tapered;
- tarsus I with three or four tactile setae and two to four solenidia in proximal part;
- tibia I with eight or nine (rarely 12) tactile setae and three or four solenidia;
- tarsus II with three tactile setae and one solenidium in proximal part; and
- tibia II with seven tactile setae.

4.5 Dichotomous key to species of *Mononychellus* on *Manihot* spp. based on females

Eight species of *Mononychellus* have been recorded from *Manihot* spp. (Flechtmann and de Queiroz, 2015; Migeon and Dorkeld, 2021) and can be differentiated by using the following key.

1. Prodorsum finely reticulate medially (Figure 9C and Figure 9E); most hysterosomal setae (c_1 , c_2 , d_1 , d_2 , e_1 , e_2 , f_1 , f_2 , h_1) with a finely reticulated area around the setal base (Figure 10C and Figure 10E)**2**
 - Prodorsum mostly striated (Figure 9A, Figure 9B, Figure 9D and Figure 9F); area surrounding the base of each hysterosomal seta merely striated, not reticulate (Figure 10A, Figure 10B, Figure 10D and Figure 10F).....**3**
2. Tarsus I with three solenidia and four tactile setae in proximal part; seta d_1 not reaching base of e_1 (Figure 10C); reticulated areas around the hysterosomal setae small and well isolated from each other (Figure 10C).....***M. chemosetosus*** (Paschoal, 1970)
 - Tarsus I with one solenidium and four tactile setae in proximal part; seta d_1 extending beyond base of e_1 (Figure 10E); reticulated areas around the hysterosomal setae large and close to each other (Figure 10E) ***M. planki*** (McGregor, 1950)
3. Hysterosomal setae c_1 , d_1 and e_1 oblanceolate and obviously shorter than distances between their bases (Figure 10B and Figure 10F); sc_1 shorter than or nearly as long as distance sc_1-sc_2 (Figure 3A and Figure 15C); c_2 shorter than c_3 (Figure 3A).....**4**
 - Hysterosomal setae c_1 , d_1 and e_1 linear or only slightly expanded apically, at least one of them longer than or nearly as long as distances between their bases (Figure 10A and Figure 10D); sc_1 longer than distance sc_1-sc_2 ; c_2 longer than c_3**5**
4. Area posterior to f_2 reticulated (Figure 10B); f_1 less than 1.5× as long as c_1 or d_1 (Figure 10B); palp with terminal eupathidium more than twice as long as wide (Figure 12B)***M. caribbeanae*** (McGregor, 1950)
 - Area posterior to f_2 simply striated (Figure 3B); f_1 more than twice as long as c_1 or d_1 (Figure 3A and Figure 10F); palp with terminal eupathidium no more than 1.5× as long as wide (Figure 12F)***M. tanajoa*** (Bondar, 1938)
5. Hysterosomal setae c_1 , d_1 and e_1 extending beyond or almost reaching bases of setae in next row (Figure 10A); c_1 , d_1 and e_1 longer than distances c_1-c_1 , d_1-d_1 and e_1-e_1 , respectively (Figure 10A)**6**
 - Hysterosomal setae c_1 , d_1 and e_1 far from reaching bases of setae in next row; c_1 , d_1 and e_1 much shorter than distances c_1-c_1 , d_1-d_1 and e_1-e_1 , respectively.....***M. progresivus*** Doreste, 1981
6. Seta sc_1 no more than 1.3× as long as sc_2 ; c_1 and d_1 as long as or slightly shorter than c_2 and d_2 , respectively**7**

- Seta sc_1 about twice as long as sc_2 ; c_1 and d_1 obviously longer than c_2 and d_2 , respectively *M. mcgregori* (Flechtmann and Baker, 1970)
- 7. Palp with terminal eupathidium about as long as wide; aedeagal knob about 3× as wide as neck; tarsus I with four (rarely five) tactile setae and one solenidion (rarely none) in proximal part
..... *M. manihoti* Doreste, 1981
- Palp with terminal eupathidium about 1.5× as long as wide (Figure 12A); aedeagal knob about twice as wide as neck; tarsus I with five tactile setae and one solenidion in proximal part.....
..... *M. bondari* (Paschoal, 1970)

4.6 Identification of *Mononychellus tanajoa*

4.6.1 Morphological identification

Adult female

Idiosoma: oval, 330–480 µm long and 187–217 µm wide, from greenish to yellowish in colour when alive (Figure 2A). Dorsal idiosomal setae stout, oblanceolate (Figure 3A and Figure 15); lobes of dorsal striae strong and rounded; prodorsum striated (Figure 3A, Figure 15A, Figure 15B and Figure 15C); seta sc_1 not reaching base of sc_2 ; hysterosomal setae c_1 , d_1 and e_1 short, not reaching half of the distances to bases of setae in next row (Figure 3A and Figure 10F); c_1 shorter than half the distance between the two c_1 setae, d_1 about one-third the distance between the two d_1 setae, e_1 shorter than half the distance between the two e_1 setae; marginal setae c_2 , d_2 and e_2 about 1.4–1.8× as long as c_1 , d_1 and e_1 , respectively; f_1 more than twice as long as c_1 or d_1 (Figure 3A and Figure 10F); striae between e_1 and e_1 varying from oblique to longitudinal (Figure 15D, Figure 15E and Figure 15F), area posterior to f_2 simply striated (Figure 3).

Gnathosoma: palp with terminal eupathidium about 1.5× as long as wide (Figure 5B and Figure 12F). Peritreme usually distally straight, ending in a small bulb or sometimes a tiny hook (Figure 4F and Figure 5A).

Legs: tarsus I (Figure 6A) with five tactile setae and one solenidion in proximal part; tibia I with nine tactile setae and one solenidion; tarsus II (Figure 6B) with three tactile setae and one solenidion in proximal part; tibia II with seven tactile setae; tarsi III (Figure 6C) and IV (Figure 6D) each with ten tactile setae and one solenidion; tibiae III and IV each with six tactile setae.

Adult male

Idiosoma: tapered posteriorly (Figure 3A), 275–308 µm long and 167–178 µm wide, paler than adult female when alive. Aedeagus (Figure 13) with main shaft nearly straight, slightly curving ventrally, progressively tapering and forming a narrow neck before reaching aedeagal knob; knob with two sharp projections (Figure 13).

Gnathosoma: palp with terminal eupathidium apically tapered, slightly longer than wide.

Legs: tarsus I with five tactile setae and three solenidia in proximal part; tibia I with nine tactile setae and three solenidia; tarsus II with three tactile setae and one solenidion in proximal part; tibia II with seven tactile setae.

4.6.2 Molecular identification

Mitochondrial DNA sequencing of the *COI* gene can be used to support identification of *M. tanajoa*, irrespective of the developmental stage.

4.6.2.1 Extraction of nucleic acid

Genomic DNA should be extracted from a single specimen of any developmental stage, since infestation by more than one species on the same host plant (mixed infestation) is typical for spider mites. There are many different methods available for DNA extraction, such as the modified cetyltrimethylammonium bromide method (potassium acetate 2.5 M, pH 5.5) (Ovalle *et al.*, 2020), the

DNeasy® Blood and Tissue Kit (QIAGEN) (de Mendonça *et al.*, 2011; Li *et al.*, 2015) and the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific) (Matsuda *et al.*, 2013).¹

4.6.2.2 Conventional PCR and sequencing

The DNA extracted can be used for polymerase chain reaction (PCR) amplification of the DNA barcoding region as chosen by the Consortium for the Barcode of Life with the following primers presented by Folmer *et al.* (1994):

LCO1490 (forward): 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'

HCO2198 (reverse): 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'

Ovalle *et al.* (2020) provide an example of the master mix composition and cycling parameters for the amplification of *COI* using the primers presented by Folmer *et al.* (1994) (Table 1).

Table 1. Master mix composition, cycling parameters and amplicons for PCR to distinguish *Mononychellus tanajoa* from *Mononychellus caribbeanae*, *Mononychellus mcgregori* and *Tetranychus urticae*

Reagents	Final concentration
PCR-grade water	_†
PCR buffer	1×
MgCl ₂	2.5 mM
dNTPs	2.5 mM of each
Primer (forward)	5 µM
Primer (reverse)	5 µM
DNA polymerase	0.25 U
DNA sample	20 ng
Cycling parameters	
Number of cycles	5
- Denaturation	94 °C for 40 s
- Annealing	45 °C for 40 s
- Elongation	72 °C for 60 s
Number of cycles	35
- Denaturation	94 °C for 40 s
- Annealing	51 °C for 40 s
- Elongation	72 °C for 60 s
Final elongation	72 °C for 10 min
Expected amplicons	
Size	709 bp (the amplicon size varies for species and individuals)

Notes:† For a final reaction volume of 20 µL.

bp, base pairs; PCR, polymerase chain reaction.

Source: Ovalle, T.M., Vásquez-Ordóñez, A.A., Jimenez, J., Parsa, S., Cuellar, W.J. & Lopez-Lavalle, L.A.B. 2020. A simple PCR-based method for the rapid and accurate identification of spider mites (Tetranychidae) on cassava. *Scientific Reports*, 10: 19496. <https://doi.org/10.1038/s41598-020-75743-w>

4.6.2.3 Controls for molecular tests

For the test result to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid extractions and PCR amplifications of the target pest. As a minimum, a positive nucleic acid control and a negative amplification control (no template control) should be used.

Positive nucleic acid control. This control is used to monitor the efficiency of the method used for the test (apart from the extraction). Pre-prepared (stored) genomic DNA may be used.

Negative amplification control (no template control). This control is necessary to rule out false positives resulting from contamination with other genetic material during the preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added in place of template DNA.

Negative extraction control. This control is used to monitor contamination during nucleic acid extraction. This requires extraction blanks to be processed alongside the samples to be tested.

4.6.2.4 Sequence editing and analysis

The sequences are edited using specific software (e.g. open-source Staden Package, BioEdit). The quality of the sequences should be checked. A consensus sequence should be obtained using the sequence-editing software by overlapping the DNA template's forward and reverse sequences. The edited sequences are compared with those available in the public DNA database GenBank using the Basic Local Alignment Search Tool (BLAST), available at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). Further guidance on sequence editing and analysis may be found in Appendices 7 and 8 of EPPO (2021). An identity with *M. tanajoa* equal to or higher than 99% based on a query cover higher than 90% is required to support species-level identification (Smith, Fisher and Hebert, 2005; Porter and Hajibabaei, 2020). One sequence of a 597 bp of the *COI* fragment is available in GenBank for an *M. tanajoa* haplotype (accession number MN913384.1) and for the closely related species *M. mcgregori* (MN913383) and *M. caribbeanae* (MN913382.1).

Additional work on the diagnostics of mites in the family Tetranychidae (Navajas *et al.*, 1992, 1998, 2000; Ros and Breeuwer, 2007; de Mendonça, *et al.*, 2011; Matsuda *et al.*, 2013; Li *et al.*, 2015; Zélé, Weill and Magalhães, 2018) has not yet been adapted to the identification of *M. tanajoa* and consequently not included in this protocol.

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be adversely affected by the diagnosis, the records and evidence of the results of the diagnosis (in particular, preserved or slide-mounted specimens, photographs of distinctive taxonomic structures, DNA extracts and photographs of gels, as appropriate) should be kept for at least one year in a manner that ensures traceability.

6. Contact points for further information

Further information on this protocol can be obtained from:

Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, P.O. Box 2095, Auckland 1072, New Zealand (Qing-Hai Fan; email: qinghai.fan@mpi.govt.nz).

Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), Centre de Biologie pour la Gestion des Populations (UMR CBGP), CS 30016, 34988 Montferrier-sur-Lez cedex, France (Denise Navia; email: denise.navia@inrae.fr).

Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, 90095, CA, United States of America (Rachid Hanna; email: rahanna@ucla.edu or rachidhanna01@gmail.com).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), who will forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

The first draft of this protocol was written by Qing-Hai Fan (Plant Health & Environment Laboratory, New Zealand (see preceding section)), Denise Navia (Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France (see preceding section)) and Rachid Hanna (Centre for Tropical Research, United States of America (see preceding section)).

In addition, the following experts were significantly involved in the development of this protocol: Frederic Beaulieu (Agriculture & Agri-Food Canada), Jurgen Otto (Department of Agriculture, Fisheries and Forestry, Australia), Hasan Rahmani (AgriBio, the Centre for AgriBioscience, Australia) and Karen McLachlan-Hamilton (entomology–diagnostic biologist, Canada).

The following experts provided some references and specimens: Carlos Holger Wenzel Flechtmann, Gilberto José de Moraes and Raphael de Campos Castilho (Departamento de Entomologia e Acarologia, Universidade de São Paulo, Brazil), and Renata Santos de Mendonça (Universidade de Brasília, Federal District, Brazil). Mite reference collections used for this protocol include those of the Department of Entomology and Acarology, Escola Superior de Agricultura Luiz de Queiroz/Universidade de São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil and Embrapa Recursos Genéticos e Biotecnologia, Brasília, Federal District, Brazil.

8. References

The present annex may refer to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at <https://www.ippc.int/core-activities/standards-setting/ispms>.

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9. Figures

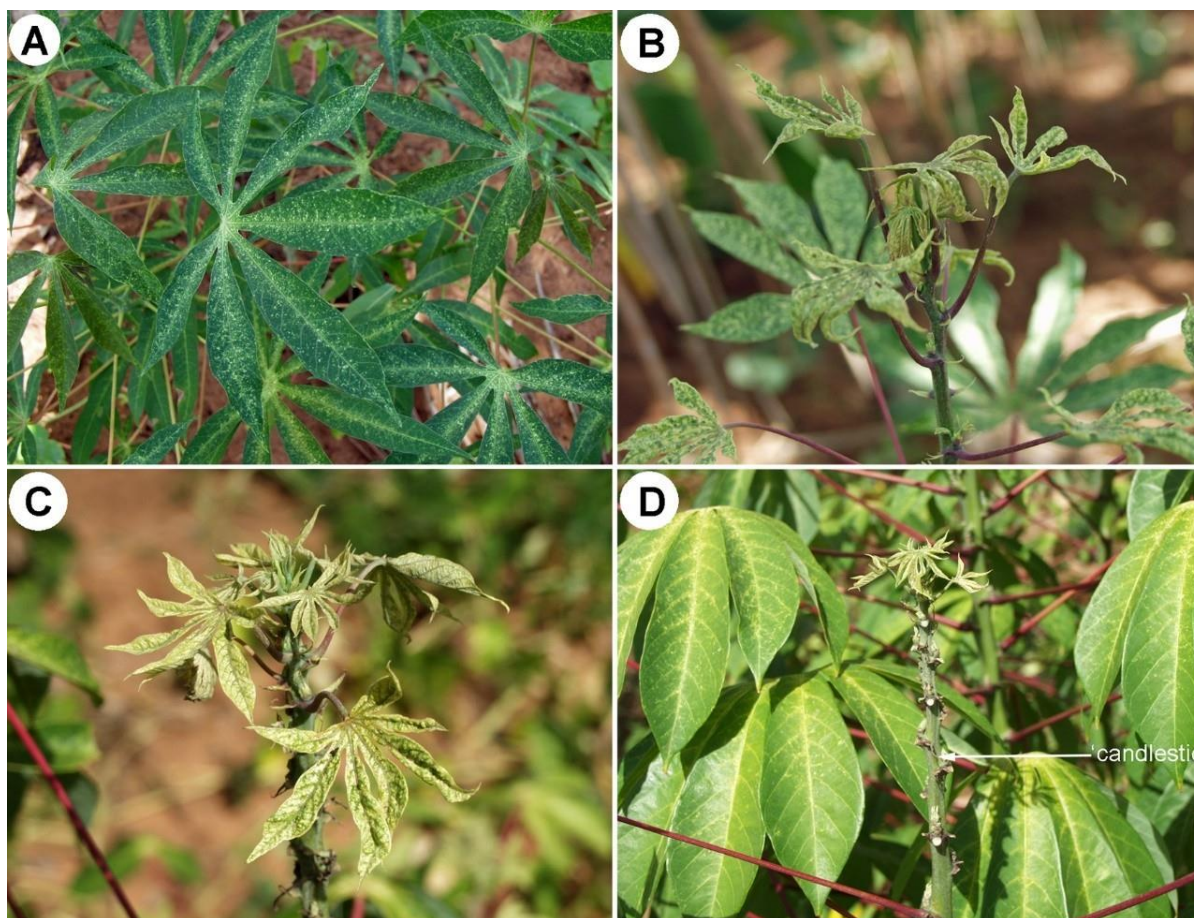


Figure 1. Progression of *Mononychellus tanajoa* damage to cassava plant: (A) stippled leaves; (B) distorted leaves; (C) chlorotic, mottled leaves with considerable reduction in leaf size; (D) death and fall of upper shoot leaves giving a “candlestick” appearance.

Source: R. Hanna, Center for Tropical Research, University of California, Los Angeles, United States of America.

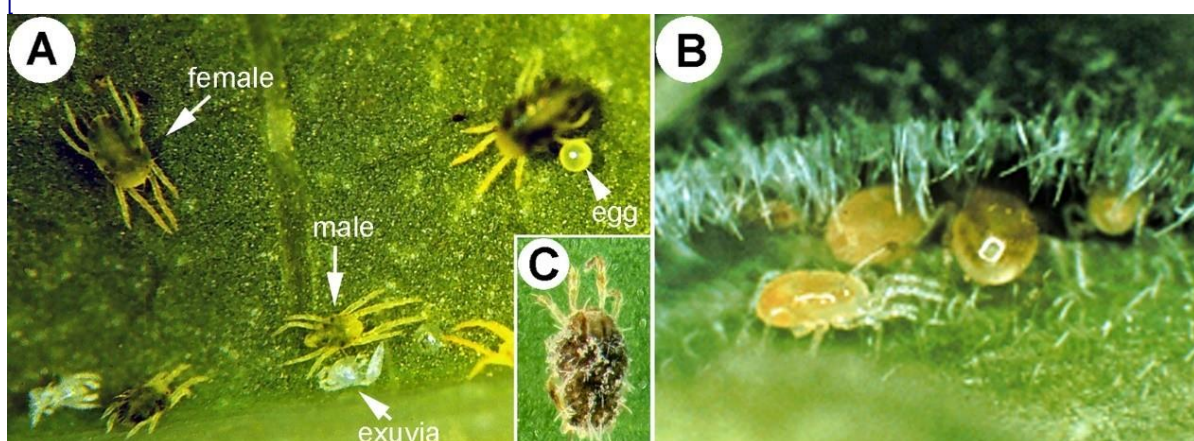


Figure 2. *Mononychellus tanajoa* and its principal biocontrol agents: (A) adult and immature stages of *M. tanajoa*; (B) predatory mite *Typhlodromalus aripo*; (C) *Neozygites tanajaoe* infected *M. tanajoa*.

Source: (A) J.S. Yaninek, (B & C) G. Goergen, International Institute of Tropical Agriculture, Nigeria.

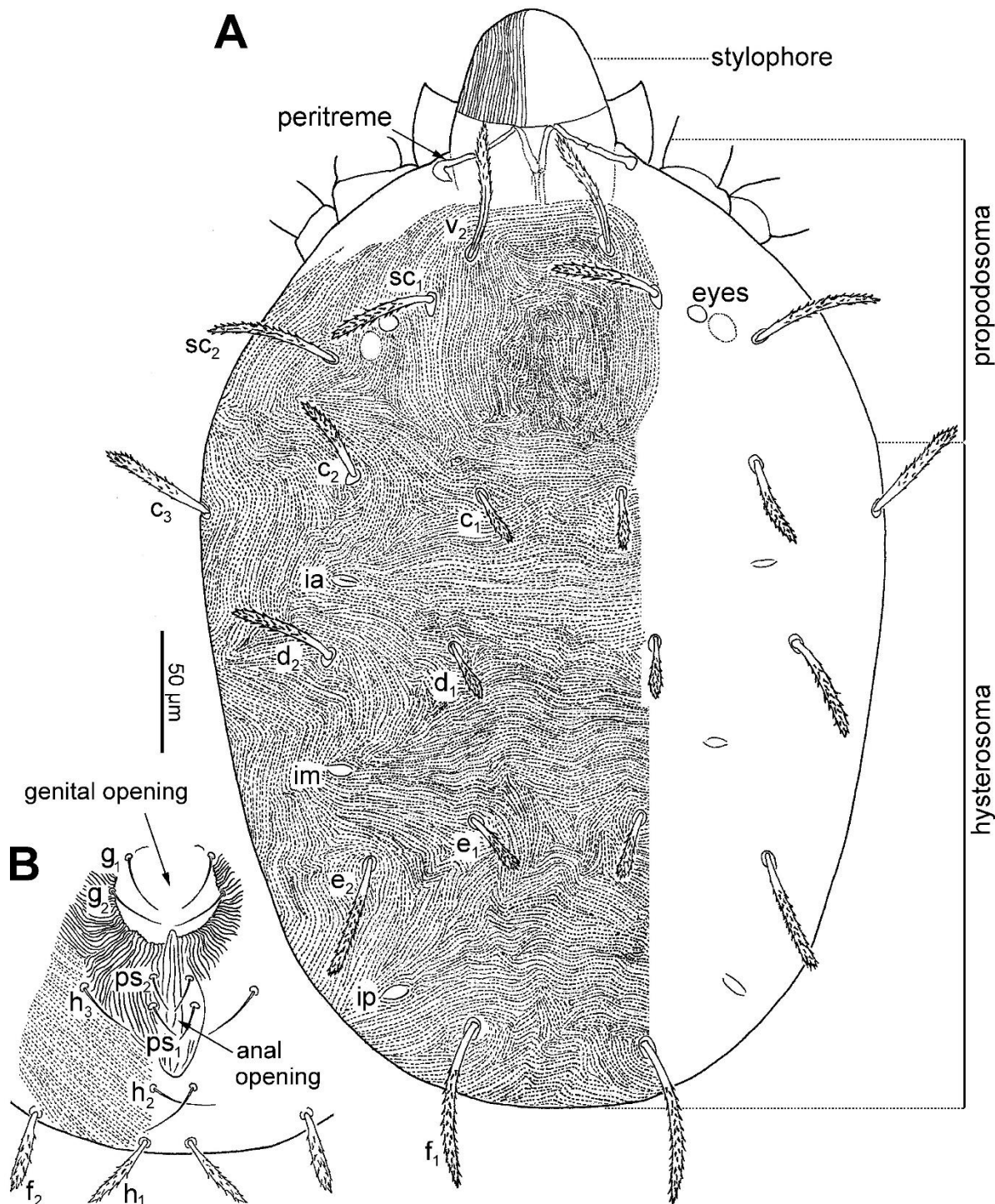


Figure 3. *Mononychellus tanajoa*, adult female: (A) dorsal view of idiosoma; (B) genital and anal region.

Note: For notation of structures, see Lindquist, E.E. 1985. Chapter 1.1 Anatomy, phylogeny and systematics. 1.1.1 External anatomy. In: W. Helle & M.W. Sabelis, eds. *Spider mites – Their biology, natural enemies and control, Volume 1A*, pp. 3–28. Amsterdam, Elsevier.

Source: Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand and D. Navia, Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement, France.

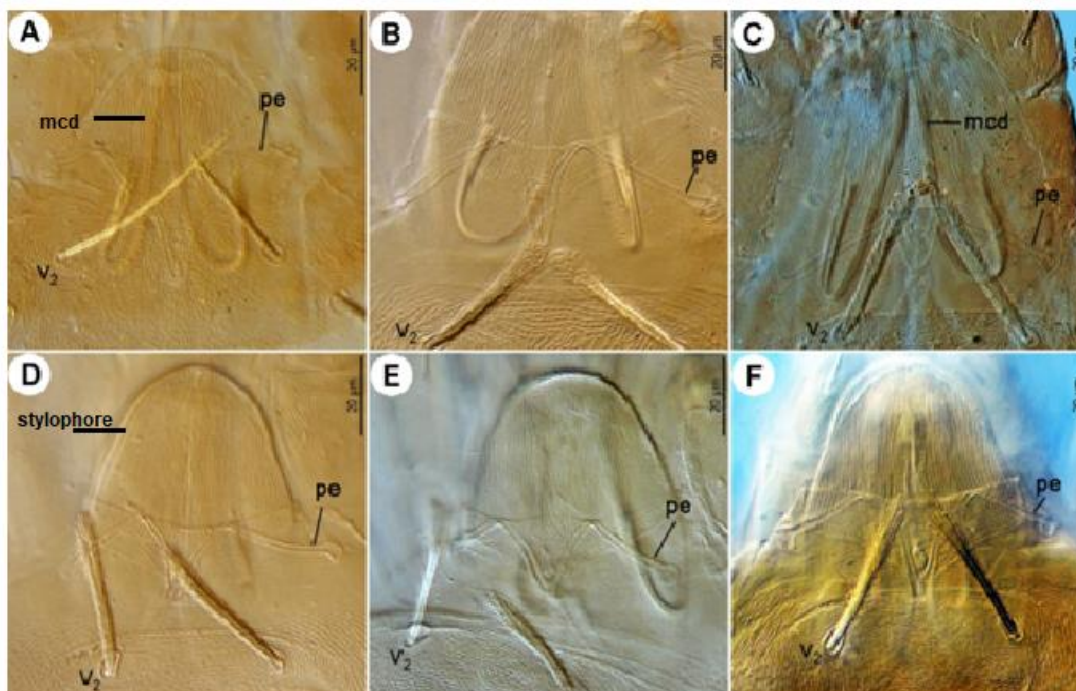


Figure 4. Stylophores and peritremes of adult females of *Mononychellus* spp. (A) *M. bondari*; (B) *M. caribbeanae*; (C) *M. chemosetosus*; (D) *M. mcgregori*; (E) *M. planki*; (F) *M. tanajoa*.

Notes: mcd, movable cheliceral digit; pe, peritremes.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

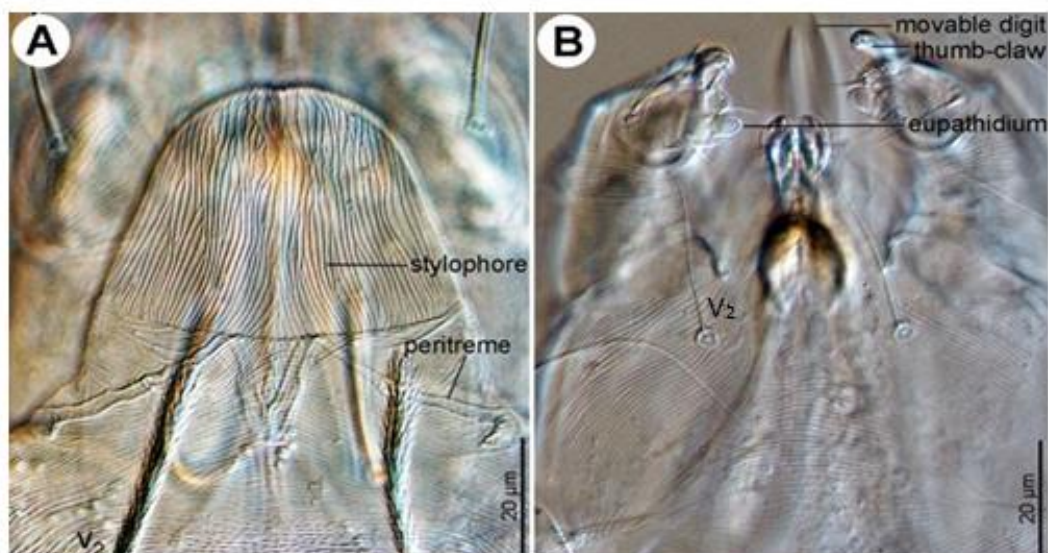


Figure 5. *Mononychellus tanajoa*, adult female: (A) dorsal view of stylophore and peritremes; (B) infracapitulum and palp.

Notes: For notation of structures, see Lindquist, E.E. 1985. Chapter 1.1 Anatomy, phylogeny and systematics. 1.1.1 External anatomy. In: W. Helle, W. & M.W. Sabelis, eds. *Spider mites – Their biology, natural enemies and control, Volume 1A*, pp. 3–28. Amsterdam, Elsevier; v_2 , seta.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

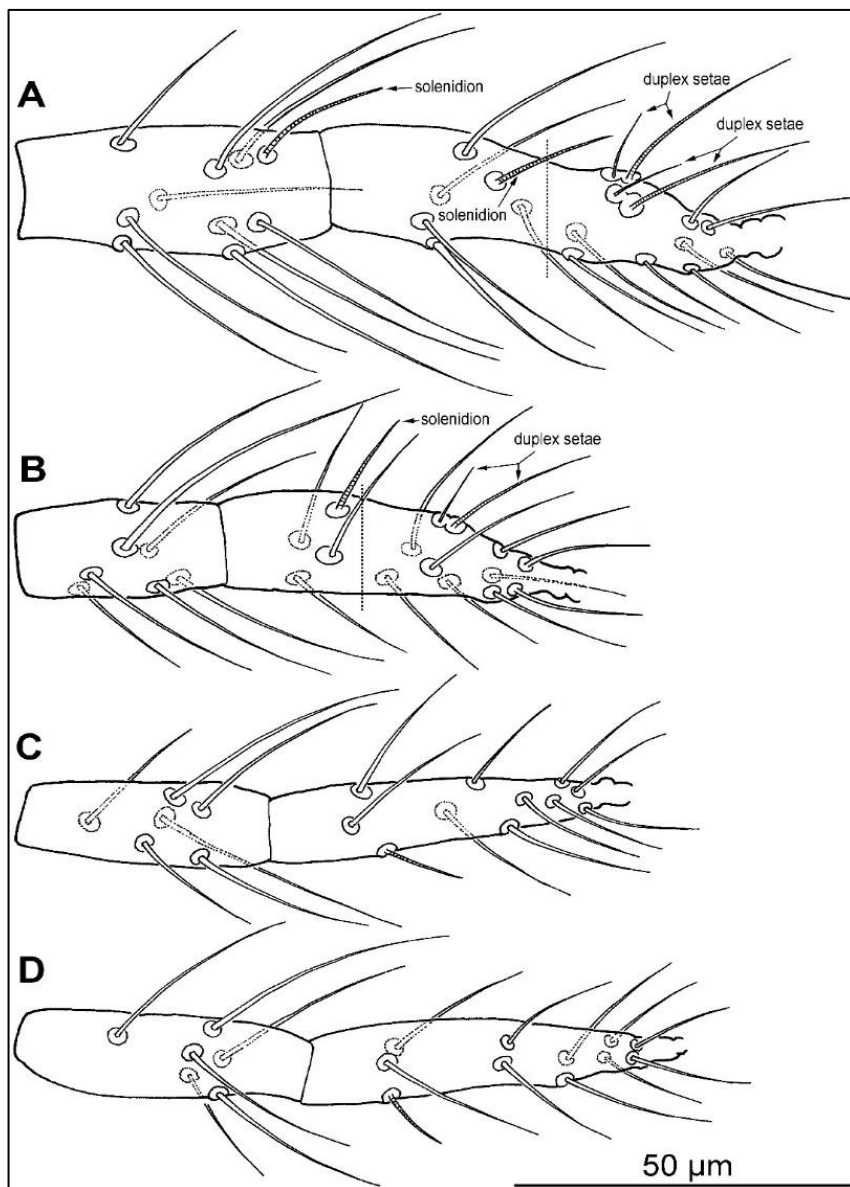


Figure 6. *Mononychellus tanajoa*, adult female: (A) tibia and tarsus I; (B) tibia and tarsus II; (C) tibia and tarsus III; (D) tibia and tarsus IV.

Note: Vertical dotted line in (A) and (B) marks the division between the proximal and distal parts of the tarsus.

Source: Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand and D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France.

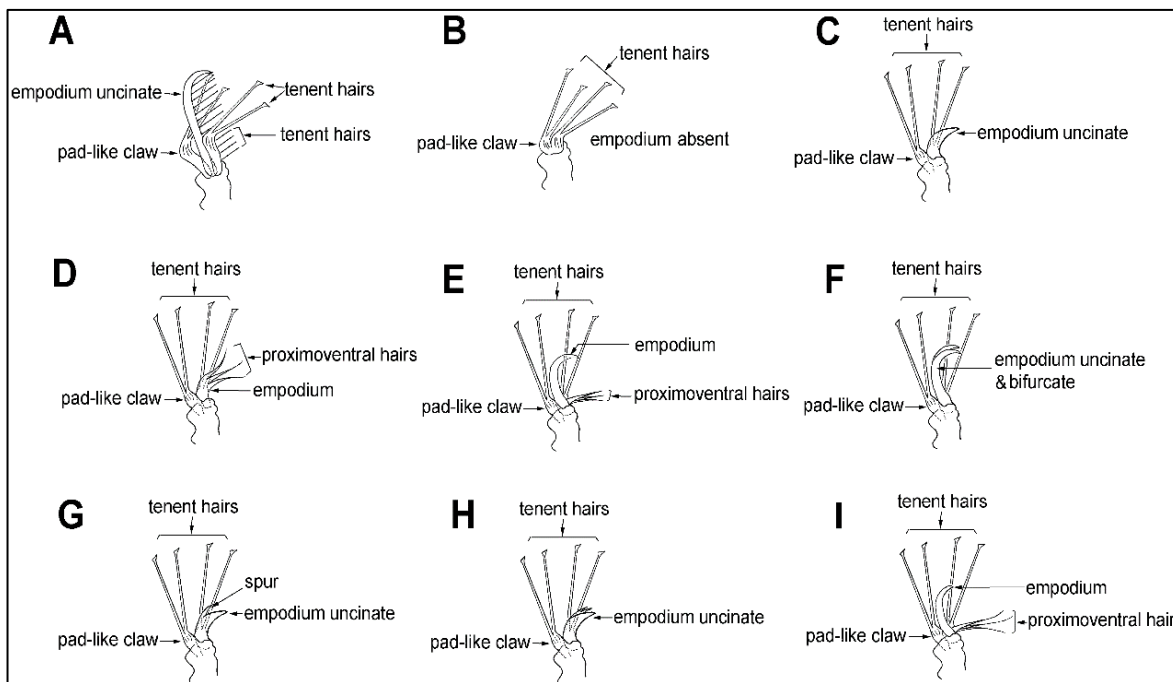


Figure 7. Types of pretarsi (II) in Tetranychidae on *Manihot* spp.

Source: Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand and D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France.

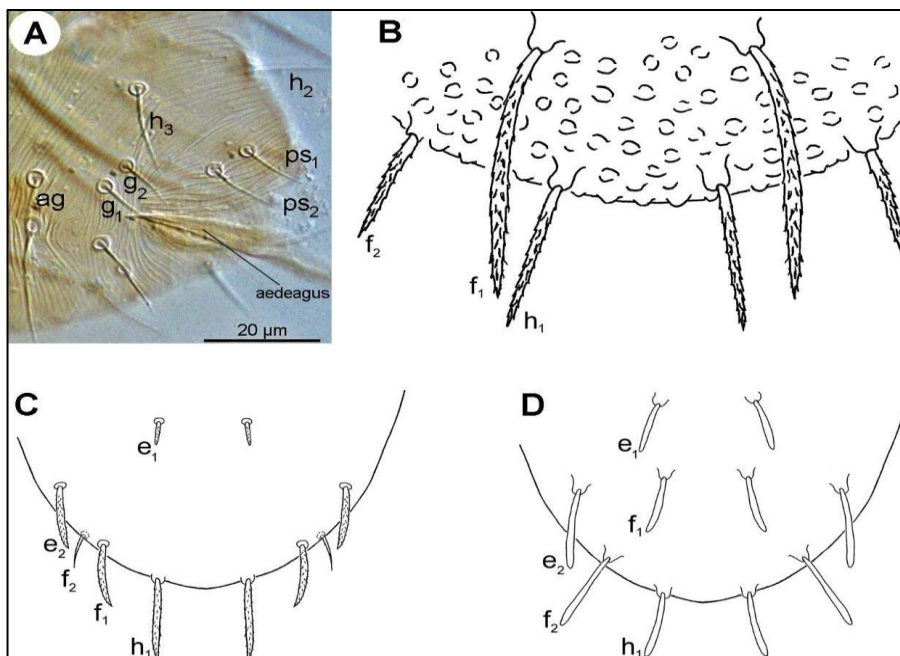


Figure 8. Hysterosoma: (A) male *Mononychellus*; (B) female *Neotetranychus*; (C) female *Aponychus*; (D) female *Eutetranychus*.

Source: Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand and D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France.

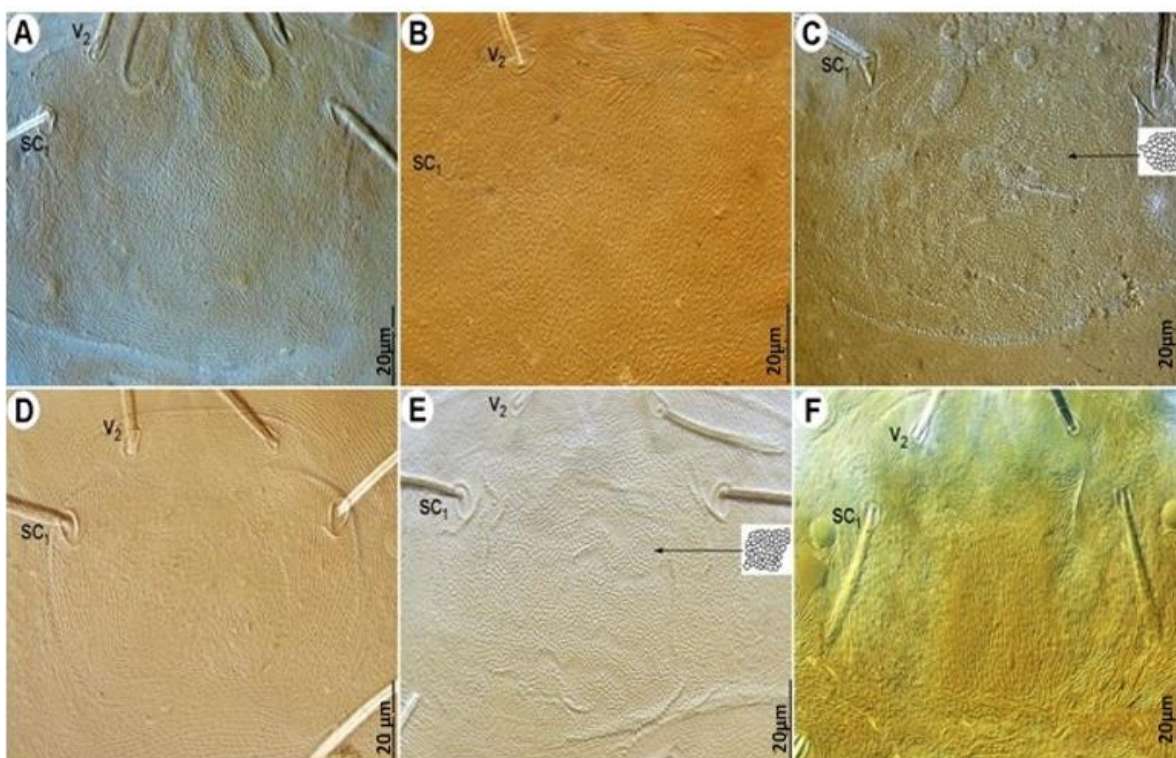


Figure 9. Pro dorsum of adult females of *Mononychellus* spp.: (A) *M. bondari*; (B) *M. caribbeanae*; (C) *M. chemosetosus*; (D) *M. mcgregori*; (E) *M. planki*; (F) *M. tanajoa*.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

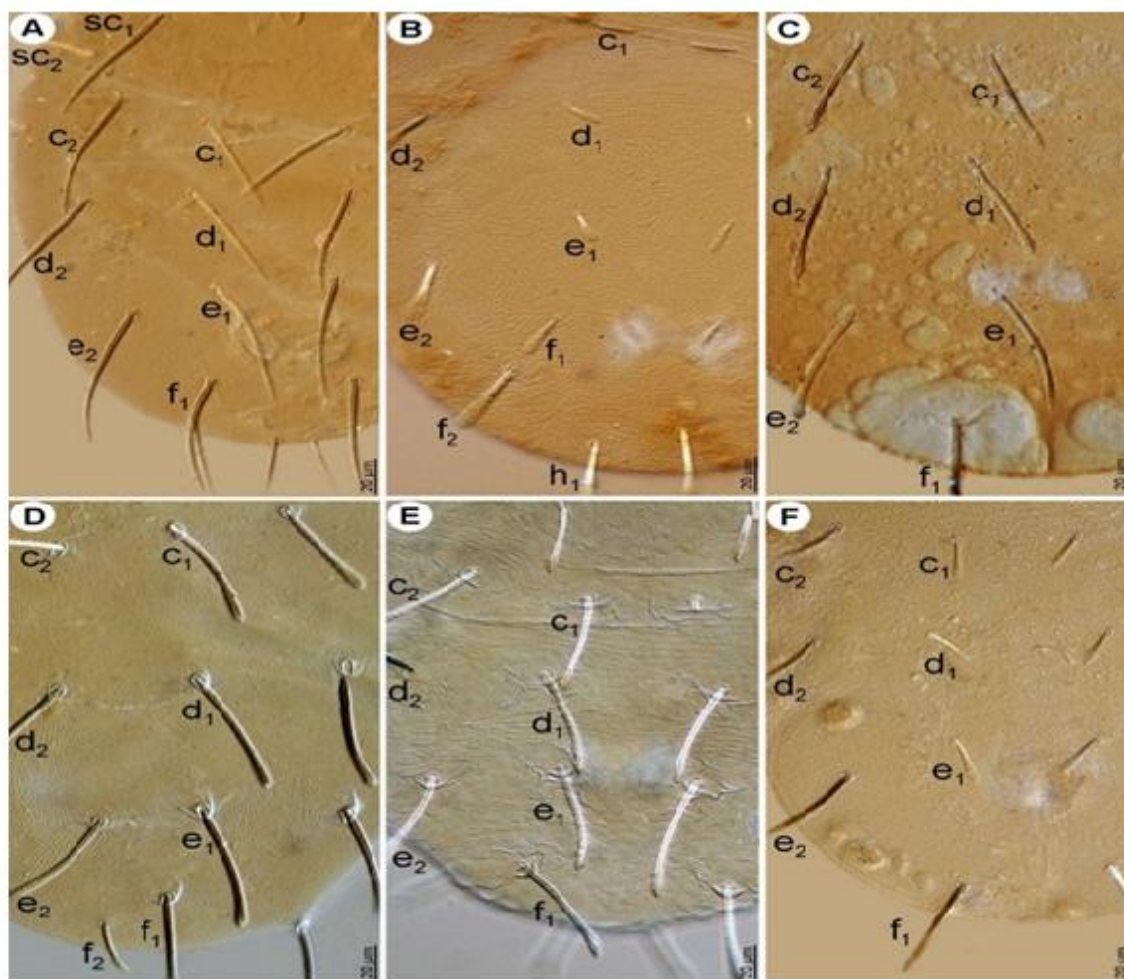


Figure 10. Hysterosoma of adult females of *Mononychellus* spp.: (A) *M. bondari*; (B) *M. caribbeanae*; (C) *M. chemosetosus*; (D) *M. mcgregori*; (E) *M. planki*; (F) *M. tanajoa*.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

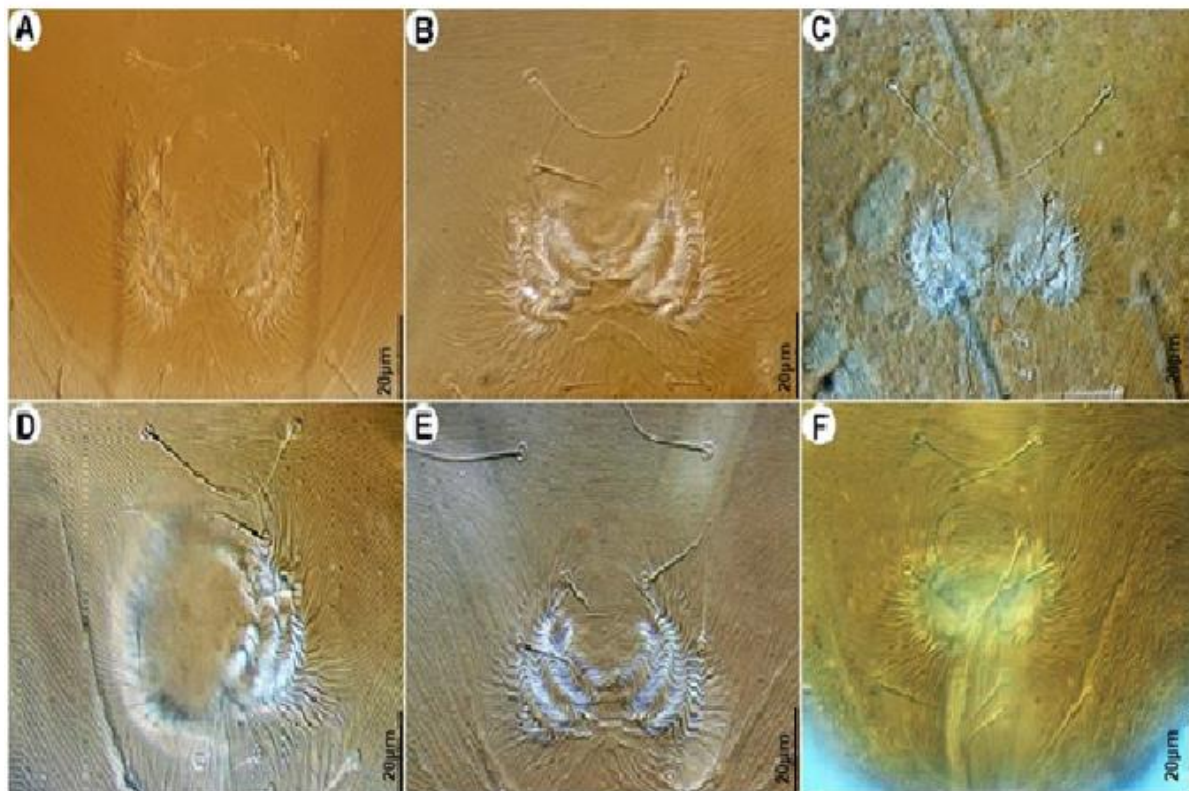


Figure 11. Genital and anal regions of adult females of *Mononychellus* spp.: (A) *M. bondari*; (B) *M. caribbeanae*; (C) *M. chemosetosus*; (D) *M. mcgregori*; (E) *M. planki*; (F) *M. tanajoa*.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

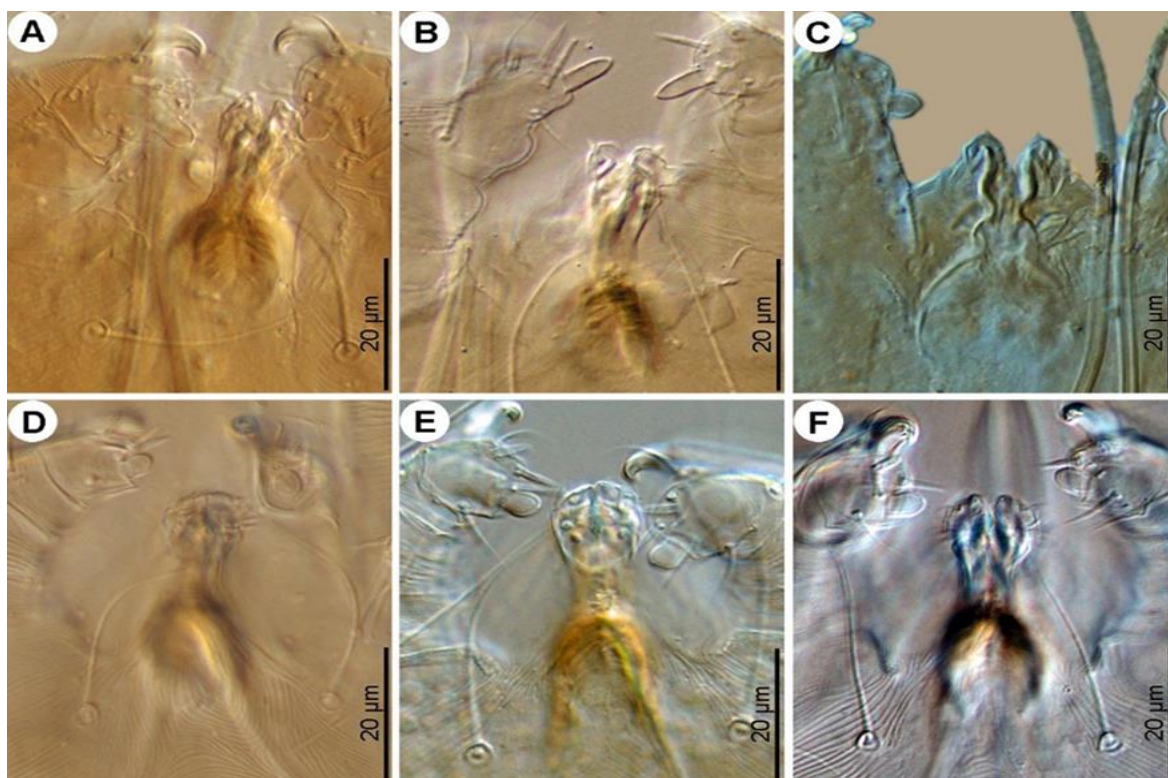


Figure 12. Infracapitulum and palps of adult females of *Mononychellus* spp.: (A) *M. bondari*; (B) *M. caribbeanae*; (C) *M. chemosetosus*; (D) *M. mcgregori*; (E) *M. planki*; (F) *M. tanajoa*.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

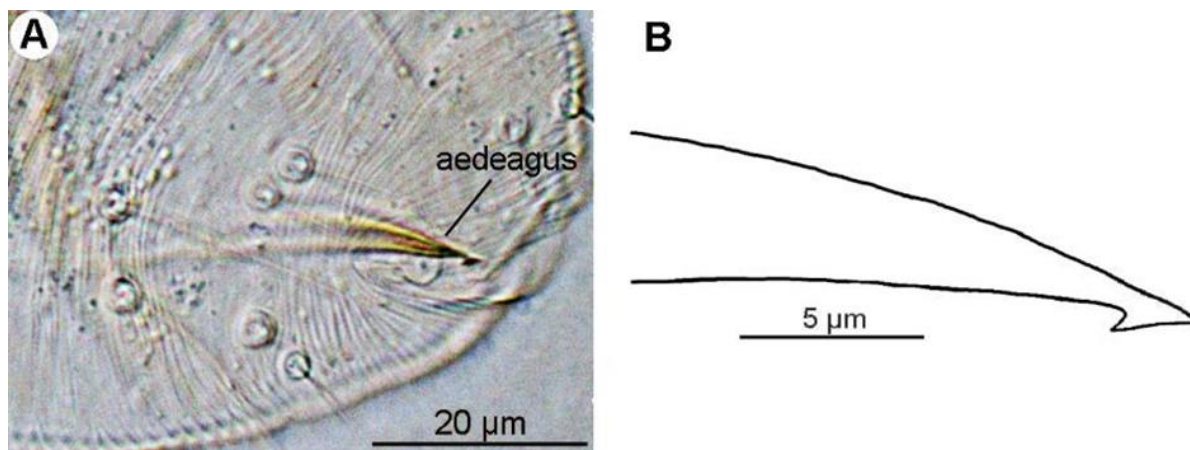


Figure 13. *Mononychellus tanajoa*, lateral view of aedeagus: (A) photograph; (B) line drawing.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

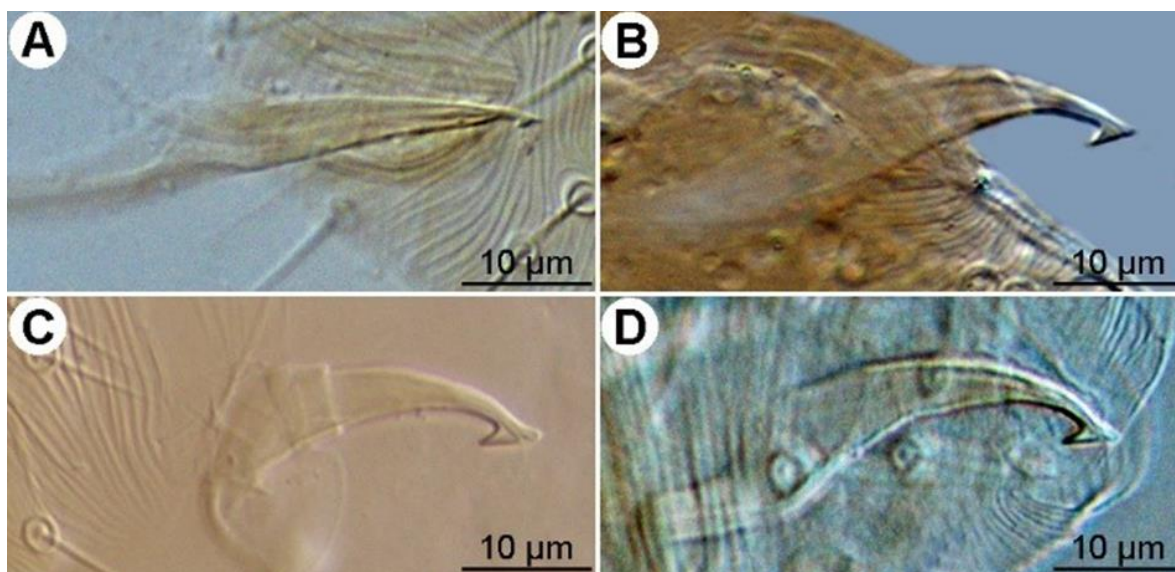


Figure 14. Aedeagi of adult males of *Mononychellus* spp. (A) *M. bondari*; (B) *M. chemosetosus*; (C) *M. mcgregori*; (D) *M. planki*.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

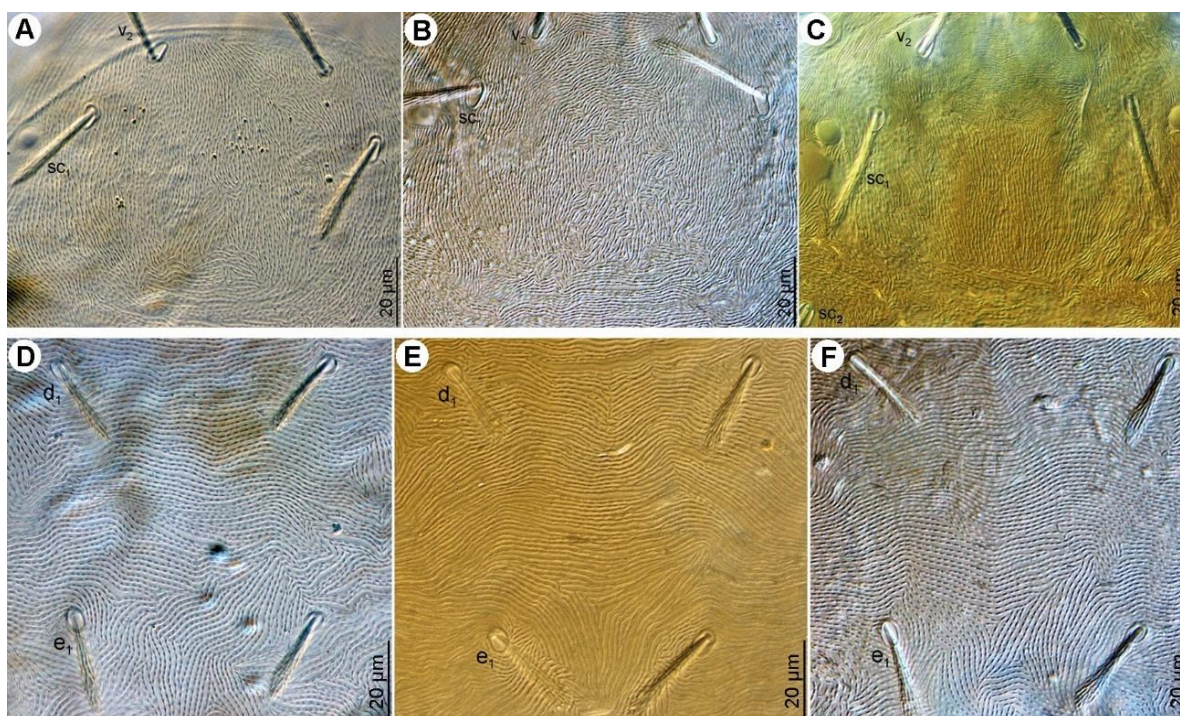


Figure 15. *Mononychellus tanajoa*: (A–C) variation of prodorsal striation in adult female; (D–F) hysterosoma.

Source: D. Navia, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, France and Q.-H. Fan, Plant Health & Environment Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, New Zealand.

Publication history

This is not an official part of the standard

2018-11 Standards Committee (SC) added subject *Mononychellus tanajoa* (2018-006) to work programme.

2021-03 Expert consultation.

2022-04 Technical Panel on Diagnostic Protocols (TPDP) recommended draft to SC to be approved for consultation.

2022-06 SC approved draft DP for consultation.

2022-07 Consultation.

2022-10 TPDP revised.

2023-02 DP drafting group revised.

2023-03 TPDP revised and recommended to SC for adoption.

2023-06 SC approved draft to be submitted to the 45-day DP notification period (2023_eSC_Nov_05).

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2023-08 SC adopted DP on behalf of CPM.

ISPM 27. Annex 33. *Mononychellus tanajoa*. Rome, IPPC Secretariat, FAO.

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IPPC

The International Plant Protection Convention (IPPC) is an international plant health agreement that aims to protect global plant resources and facilitate safe trade.

The IPPC vision is that all countries have the capacity to implement harmonized measures to prevent pest introductions and spread, and minimize the impacts of pests on food security, trade, economic growth, and the environment.

Organization

- ◆ There are over 180 IPPC contracting parties.
- ◆ Each contracting party has a national plant protection organization (NPPO) and an Official IPPC contact point.
- ◆ 10 regional plant protection organizations (RPPOs) have been established to coordinate NPPOs in various regions of the world.
- ◆ The IPPC Secretariat liaises with relevant international organizations to help build regional and national capacities.
- ◆ The Secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).

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