DOI: 10.1111/epp.12854

EPPO STANDARD ON DIAGNOSTICS

PM 7/116 (2) *Tetranychus evansi*

Specific scope: This Standard provides guidance for the identification of *Tetranychus evansi*.[1] It should be used in conjunction with PM 7/76 *Use of EPPO Diagnostic Protocols*.
Authors and contributors are given in the Acknowledgement section.
Specific approval and amendment: First approved in 2013–09. Revision approved in 2022–03.

1 | INTRODUCTION

Tetranychus evansi is a polyphagous spider mite, which is primarily a pest of Solanaceous crops, although it can also infest a variety of other hosts (Bolland et al., 1998). It originates from South America (Boubou et al., 2011) but has spread since (EPPO, 2022a; Migeon & Dorkeld, 2021). In EPPO countries, it is found in the Mediterranean area: Morocco, Algeria, Tunisia, Israel, Jordan, Greece (Crete), Italy, France, Spain (including Canary Islands and Balearic Islands), Portugal (including Madeira), Turkey and Serbia. Updated information on geographical distribution can be retrieved in the EPPO Global Database (EPPO, 2022a). The first damage was observed in Brazil (Silva, 1954), Argentina (Rossi Simons, 1961), Mauritius (Baker & Pritchard, 1960; Moutia, 1958) and the United States (Wene, 1956). *Tetranychus evansi* is currently not considered as a pest in Southern America but can be a highly destructive plant pest (e.g. on tomato) in Africa (Duverney & Ngueye-Ndiaye, 2005; Fiaboe et al., 2006; Knapp et al., 2003), Spain (Ferragut & Escudero, 2002) and France (Migeon et al., 2009). Northward dispersion capabilities are limited by winter temperatures for this tropical and non-diapausing species (Migeon et al., 2009) but such a species could occur in glasshouses as is the case for many tropical pests (e.g. *Eotetranychus lewisi*, EPPO, 2022b). The spider mite family (Tetranychidae), at present, includes 1321 valid species (Migeon & Dorkeld, 2021) belonging to more than 70 genera. The genus *Tetranychus*, contains 154 species, and is one of the largest genera in the family.

2 | IDENTITY

Name: *Tetranychus evansi* Baker & Pritchard, 1960.
Synonyms: *Tetranychus marianae nec* McGregor, 1950 *pro parte* sensu Silva, 1954; misidentification, corrected by Moraes et al., 1987. *Tetranychus takafujii* Ehara & Ohashi, 2002, synonymy by Gotoh et al., 2009.
Taxonomic position: Arachnida: Acarida: Prostigmata: Tetranychidae.
EPPO Code: TETREV.
Phytosanitary categorization: EPPO A2 list no. 349.

3 | DETECTION

Due to their minute size (about 0.5–0.6 mm in length for an average female adult), typical of many species of Acari, spider mites usually remain undetected until major plant damage occurs.

FIGURE 1 *Tetranychus evansi*, female; from Kenya (det. Dr. J. Ostoja-Starzewski, FERA, GB) (courtesy IO Kamayev)

[1]Use of names of chemicals or equipment in these EPPO Standards implies no approval of them as others may also be suitable.

Spider mites live on both sides of the leaves but prefer the underside areas close to the main veins. Unlike other injurious *Tetranychus* species, such as *Tetranychus urticae*, this species has a relatively gregarious behaviour. The feeding activity of the mites causes white spots of dead parenchyma cells to appear on both leaf surfaces, resulting in chlorosis of the foliage. Extensive silk webbing is also produced that can 'mummify' the host plant. In severe infestations, both web weaving and persistent feeding activity eventually leads to leaf fall and death of the host plant.

FIGURE 2 *Tetranychus evansi*, male at the top, female at the bottom, courtesy of Alain Migeon, INRAE Montpellier, FR

While high densities are easily detected, low densities and early infestation (i.e. the appearance of small and inconspicuous white spots) may remain unnoticed or be mistakenly attributed to deficiencies, viral or fungal diseases. In the field, mites can be detected with the naked eye and are best observed with a magnifying glass or with a stereomicroscope in laboratory. However, detection can be more difficult on host plants with very hairy leaves, such as aubergines.

Mixed populations of *T. evansi* and other spider mite species (e.g. common two-spotted spider mite *Tetranychus urticae* Koch) can occur.

Photos are provided in the EPPO gallery (https://gd.eppo.int/taxon/TETREV/photos).

4 | IDENTIFICATION

Specific identification requires examination of slide mounted specimens of both an adult male and a female (males should be in lateral position), details on the preparation of slides are given in Appendix 1.

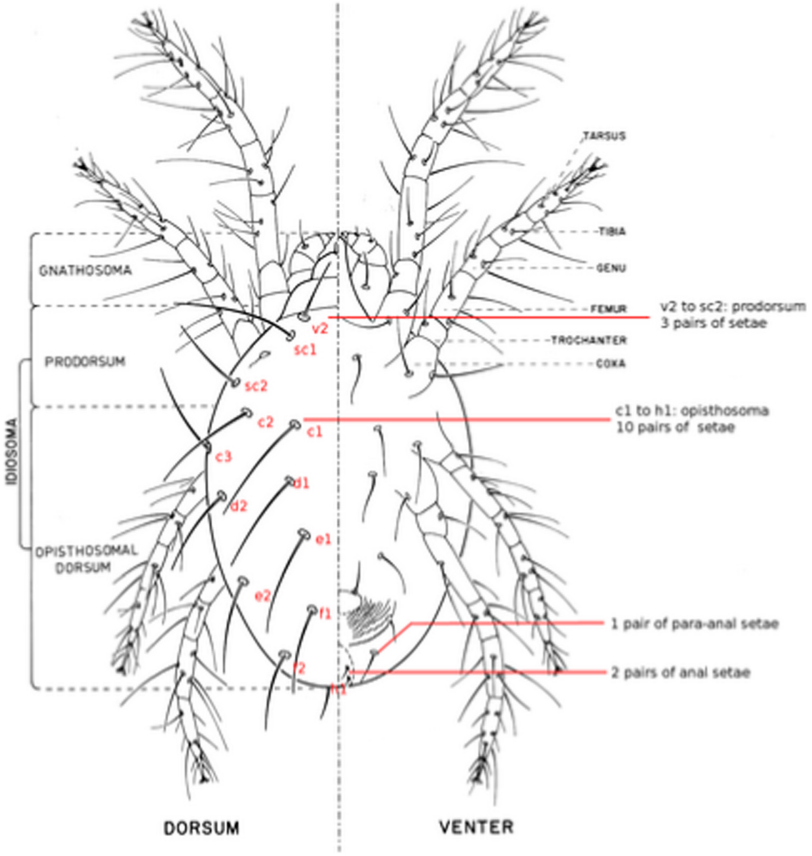

FIGURE 3 *Tetranychus* sp.: Dorsoventral aspect of the female showing the body setae. The characteristics of the genus are indicated in red (modified from Gutierrez, 1985b)

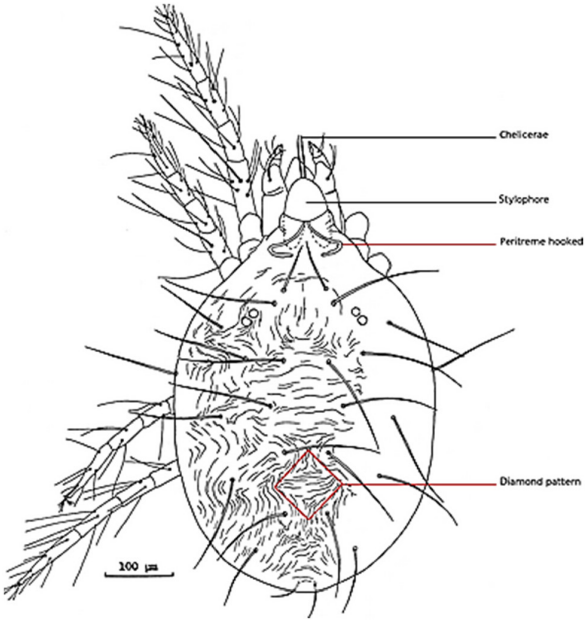

FIGURE 4 *Tetranychus evansi*: Dorsal aspect of the female – From Japan. Characteristics of the species are indicated in red (modified from Ehara & Ohashi, 2002)

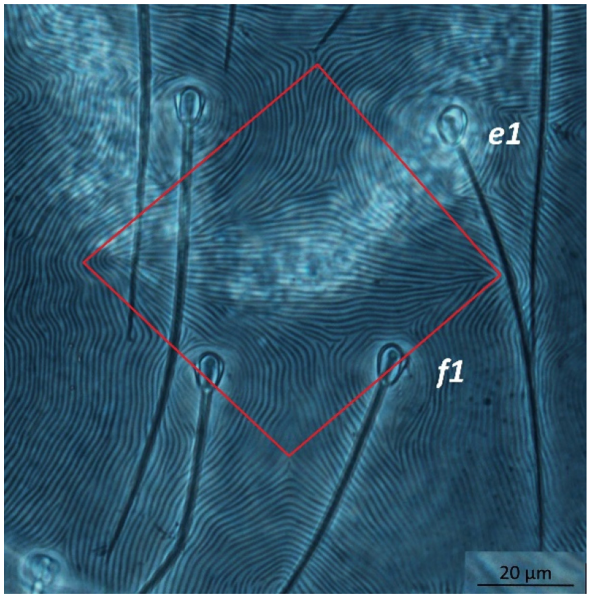

FIGURE 5 *Tetranychus* s. str.: Dorsal view of the posterior idiosoma showing the diamond-shaped pattern. (courtesy IO Kamayev) 200×. Note that e1 and f1 setae are indicated in Figure 3

Spider mite identification can be difficult and is reliant on a good knowledge of the group and previous experience. The species has been misidentified on several occasions (Gotoh et al., 2009), therefore it is recommended (at least for a first identification or in case of doubt) that a specialist is consulted for confirmatory diagnosis or a complementary method should be performed (e.g. sequencing).

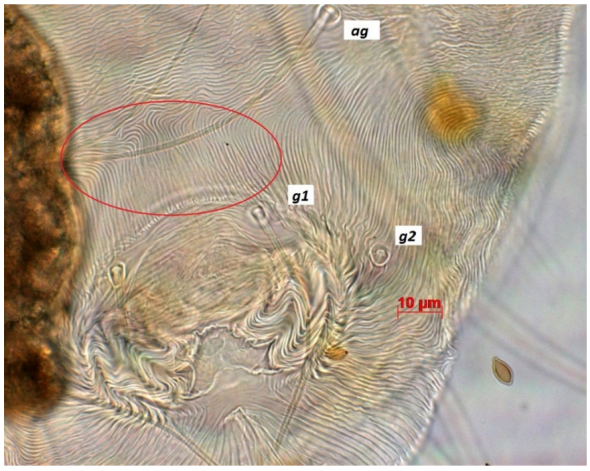

FIGURE 6 Pregenital area (indicated with a red circle) of a *Tetranychus evansi* female (ventral aspect), from Kenya (det. Dr. J. Ostoja-Starzewski, FERA). g1, g2: Genital setae 1 and 2; ag: Aggenital seta (courtesy IO Kamayev)

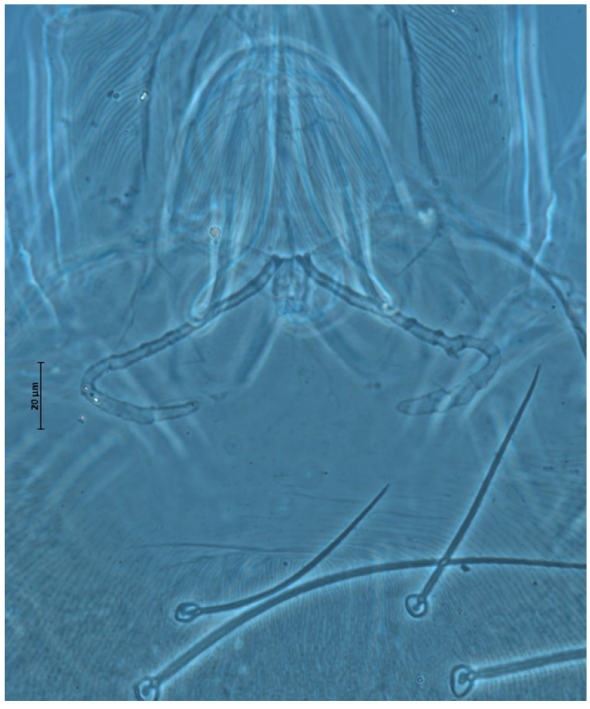

FIGURE 7 *Tetranychus evansi*: Dorsal view of the anterior region of the idiosoma (courtesy IO Kamayev) 200×

4.1 | Morphological characterization

4.1.1 | Family Tetranychidae

Colour when alive varies from green to yellow, orange and red.

The gnathosoma has a capsule-like structure, the stylophore, eversible with long slender whiplike chelicerae used for piercing parenchyma cells. Peritremes

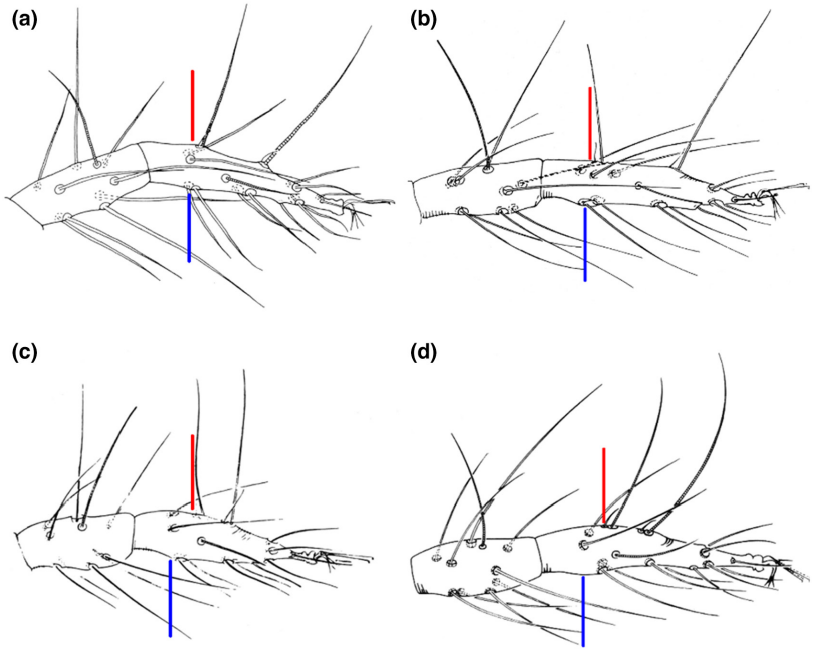

FIGURE 8 *Tetranychus* spp. female tibia and tarsus I: Variation of setae pattern on tarsus I. (a) *T. evansi* from Japan; (b) *T. evansi* from Mauritius; (c) *T. marianae*; (d) *T. piercei*. Red line indicates proximal duplex setae, blue line indicates proximal tactile setae. (a) and (b) All proximal 4 tactile setae in line with proximal pair of duplex setae. (c) and (d) proximal pair of duplex setae located distad of proximal tactile setae [(a) from Ehara & Ohashi, 2002; (b) from Baker & Pritchard, 1960; (c) from Pritchard & Baker, 1955; (d) from Gutierrez et al., 1979]

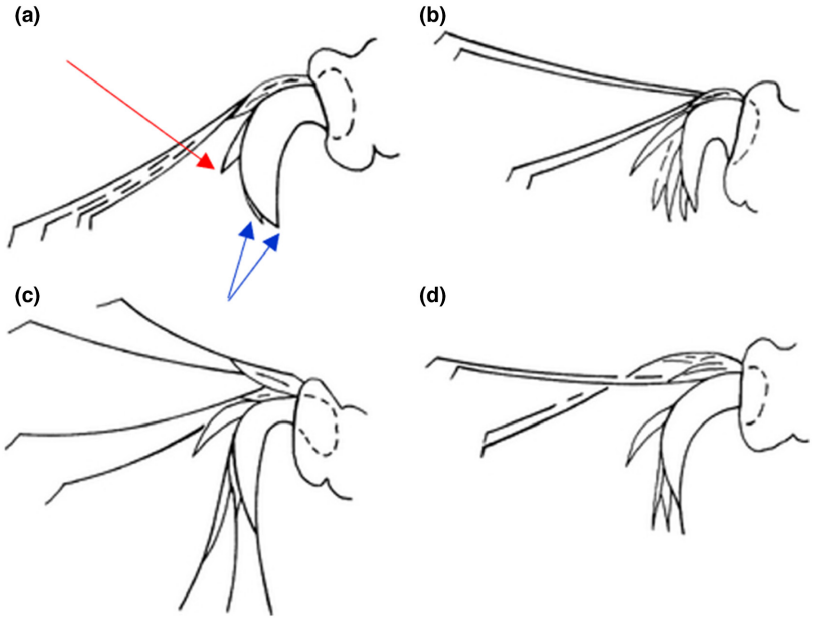

FIGURE 9 *Tetranychus evansi*: (a–d) possible variation of male empodium II (from Gotoh et al., 2009). Drawing (a) also represents a typical male empodium I; Drawing (c) represents a typical female empodium I; Red arrow: mediodorsal claw; blue arrows: proximoventral claw.

are simple or anastomosing distally, arising from a pair of stigmata near the base of the stylophore. The palps are five segmented. Tarsus I and II usually have duplex setae. The ambulacrum has tenent hairs; the tarsal claws and empodia are either padlike or clawlike; the palpal tibia forms a clawlike complex with the palpal tarsus.

Family descriptions, terms explanations and key to genera can be found in major works (Baker & Tuttle, 1994; Bolland et al., 1998; Gutierrez, 1985b; Jeppson et al., 1975; Meyer, 1987; Pritchard & Baker, 1955).

Tetranychid mites develop through five stages: egg, larva, protonymph, deutonymph and adult. Active

stages alternate with quiescent ones: protochrysalis, deutochrysalis and teleiochrysalis.

4.1.2 | Genus *Tetranychus*

Genus identification can be made using Bolland et al. (1998) by the examination of the empodium and setae pattern.

- The empodium does not bear tenent hairs and is split distally in three pairs of proximoventral hairs;
- Duplex setae of tarsus I well separated
- The idiosoma bears 13 pairs of dorsal setae (prodorsum 3 and opisthosoma 10);
- One pair of para-anal setae (*h*) and two pairs of anal setae are present (Figure 3).
- The peritreme is recurved distally and always bears a long, four or five chambered hook at the end.
- The aedeagus bends sharply dorsally (also called dorsad in Pritchard & Baker, 1955)

Tetranychus evansi belongs to the sub-genus *Tetranychus* s. str. and to the *desertorum* group that is characterized by tarsus I having all four proximal tactile setae in line with proximal pair of duplex setae. Within this group, the shape of the male aedeagus is the most important character used to discriminate species. Meyer (1987) is the most useful and relevant work on the spider mites of Africa, as it contains many of the tropical species encountered on Solanaceae, but difficult to use, particularly for the non-specialist. *Tetranychus evansi* is difficult to distinguish from other morphologically similar species and has been misidentified in the past as *T. marianae* (in particular in Brazil, Argentina and USA), or as *Tetranychus piercei* in Taiwan.

Illustration of the characters mentioned in this paragraph are presented in section 4.1.3.

4.1.3 | Species *Tetranychus evansi*

Identification to species level and separation from closely related species requires examination of both female and male specimens. Examination of the female should be made first, to confirm that the specimen belongs to the *desertorum* group. The following characters should be seen, diamond pattern (characteristic of *Tetranychus* s. str), tarsus I aligned setae (*desertorum* group), see descriptions below. Lateral examination of the male aedeagus is then performed and the characters should exactly match the drawings.

4.1.3.1 | *Egg*

The eggs of *T. evansi* are rounded, orange hyaline to whitish, becoming grey before hatching.

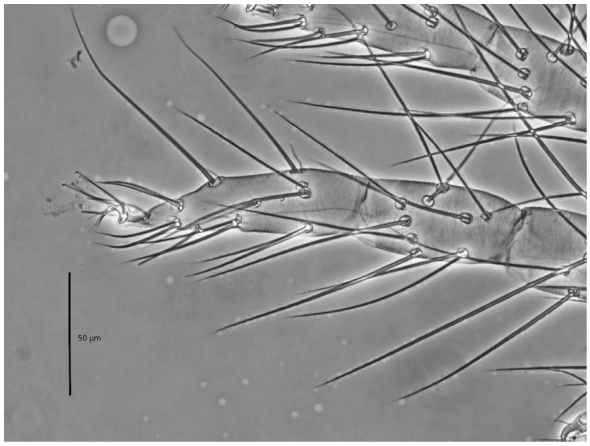

FIGURE 10 *Tetranychus evansi*. Female leg 1400× (Leica DMLB phase contrast microscope) (courtesy P AUGER INRAE Montpellier, FR)

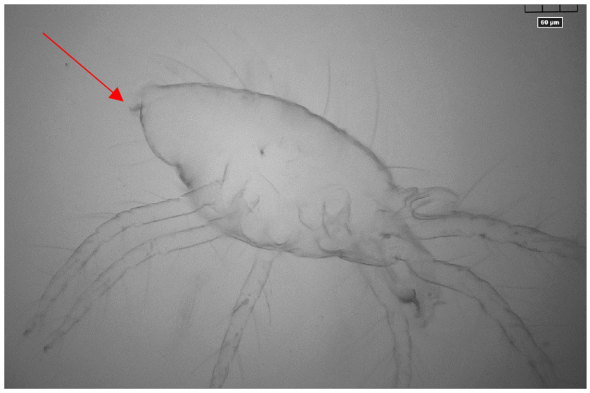

FIGURE 11 *Tetranychus evansi*, male in lateral position courtesy Recht E. PPIS, IL. Aedeagus indicated by the red arrow

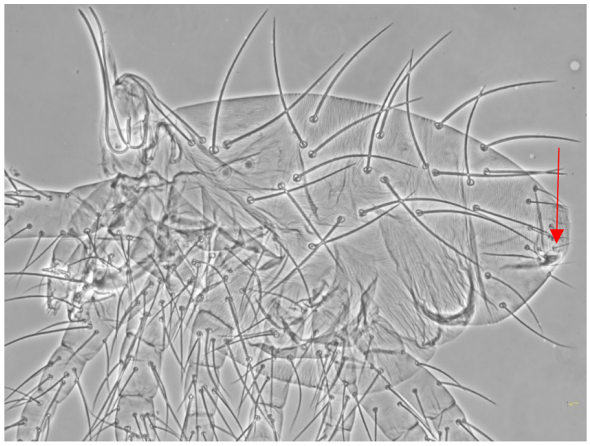

FIGURE 12 *Tetranychus evansi* male body 200× (courtesy P AUGER INRAE Montpellier, FR). Aedeagus indicated by the red arrow

4.1.3.2 | *Larvae*

Larval stage is hexapodal i.e., has three pairs of legs. Larvae are orange.

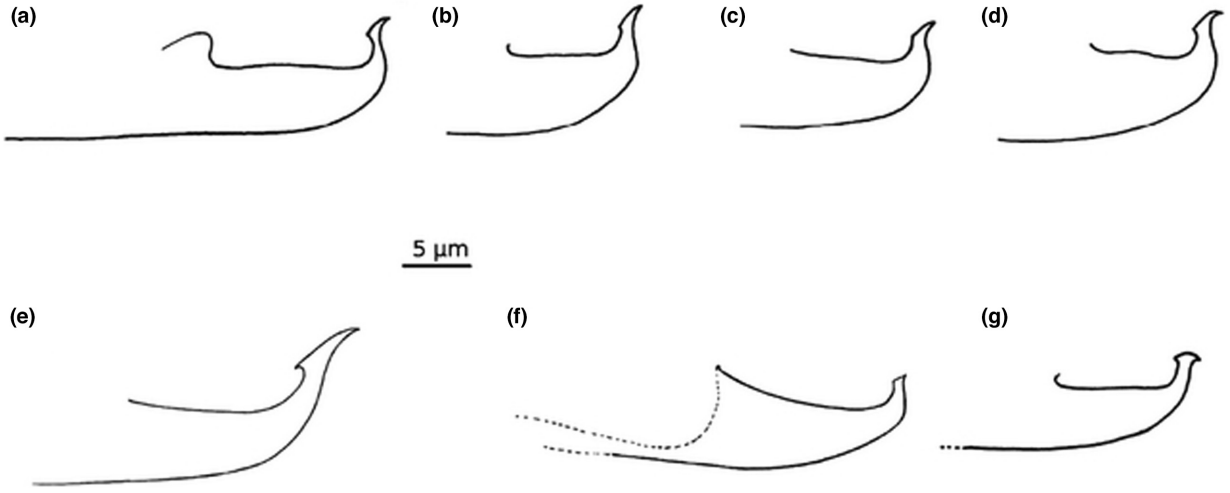

FIGURE 13 *Tetranychus* spp., males aedeagus. (a–d) Variation of the shape of the aedeagus of *T. evansi*; (a) from Mauritius, (b–d) from Japan; (e) *T. marianae*; (f) *T. piercei*; (g) *T. urticae* [(a) from Baker & Pritchard, 1960; (b–d) from Ehara & Ohashi, 2002; (e–g) from Pritchard & Baker, 1955]

4.1.3.3 | *Nymphs*

The two nymphal instars i.e., the protonymph and deutonymph like the adults have four pairs of legs. Nymphal instars can be paler than or the same colour as the adults i.e., varying from orange to brick-red or dark red.

4.1.3.4 | *Adults*

There are no fully comprehensive keys to all the known species of the genus *Tetranychus*. Some regional works can be useful: Baker and Tuttle (1994) for North America, Meyer (1987) for Africa and Seeman and Beard (2005, 2011) for Australia (the key include naturalized Australian species of *Tetranychus* and exotic species of quarantine concern to Australia). Flechtmann and Knihinicki (2002) give a key to major groups of the genus based on females.

4.1.3.5 | *Morphological characters of* T. evansi

4.1.3.5.1 | *Female (Figures 1–10)*. Body (Figure 1) 500–600 µm long and 280–360 µm wide. From orange to brick-red or dark red (Figure 2); legs pale orange. Idiosoma with 13 pairs of dorsal setae (prodorsum 3 and opisthosoma 10) (Figure 3), venter with 1 pair of para-anal setae and 2 pairs of anal setae (Figure 3). Dorsohysterosomal striae longitudinal between setae e1 and setae f1; forming a diamond shaped pattern between these two pairs of setae (Figures 4 and 5). Pregenital striae longitudinal, sometimes sparse and slightly broken medially (Figure 6). Peritremes hooked (Figures 4 and 7). Tibia I with 9 tactile setae and 1 sensory seta (Figures 8 and 10). Tarsus I with all proximal 4 tactile setae in line with proximal pair of duplex setae (Figures 8a,b and 10). All empodia with 3 pairs of proximoventral hairs; empodium I with minute dorsal claw (Figure 9c).

4.1.3.5.2 | *Male*. Body 400–470 µm long and 220–290 µm wide (Figures 11 and 12). From yellowish, pale orange to orange; legs pale orange. Tibia I with nine tactile setae and four sensory setae. Tarsus I with two proximal sensory setae and four tactile setae just proximal to first pair of duplex setae. Empodium I with mediodorsal claw and pair of proximoventral claws (Figure 9a). Empodium II is variable (Figure 9a–d). Aedeagus as in Figures 13a–d and 14. The aedeagus and the shape of the terminal knob of the aedeagus of *T. evansi*, as represented in Figures 13–15, are the main differential features to distinguish from other species within *Tetranychus* spp. and *desertorum* group encountered in Europe but should not be used alone in identification.

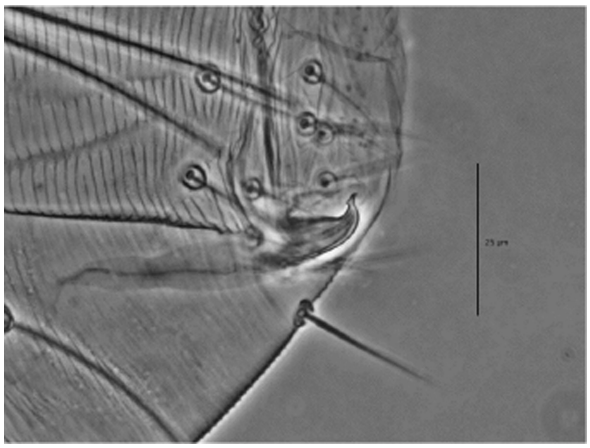

FIGURE 14 Aedeagus of *Tetranychus evansi* 1000× (courtesy P AUGER INRAE Montpellier, FR)

4.2 | Molecular identification

4.2.1 | DNA barcoding

COI sequences of *Tetranychus evansi* are available in EPPO Q-bank.

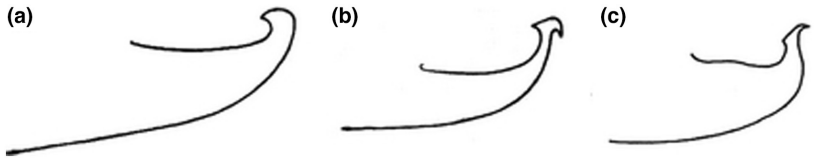

FIGURE 15 Aedeagus of the *desertorum* group species found in Europe. (a) *Tetranychus ludeni*; (b) *Tetranychus desertorum*; (c) *Tetranychus evansi* [(a–b) from Pritchard & Baker, 1955; (c) from Ehara & Ohashi, 2002]

(https://qbank.eppo.int/arthropods/taxon/TETREV/). These sequences have been generated according to Gotoh et al. (2009).

5 | REFERENCE MATERIAL

Specimens can be obtained from

CBGP – INRAE, Campus International de Baillarguet, Avenue du Campus Agropolis, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France.

All-Russian Plant Quarantine Center VNIIKR, 32 Bykovo, Russian Federation.

6 | REPORTING AND DOCUMENTATION

Guidance on reporting and documentation is given in EPPO Standard PM 7/77 (1) *Documentation and reporting on a diagnosis.*

7 | PERFORMANCE CHARACTERISTICS

When performance criteria are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8 | FURTHER INFORMATION

Further information on this organism can be queried from:

A. Migeon, CBGP – INRAE, Campus International de Baillarguet, Avenue du Campus Agropolis, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France. alain.migeon@inrae.fr.

9 | FEEDBACK ON THIS DIAGNOSTIC PROTOCOL

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share, please contact diagnostics@eppo.int.

10 | PROTOCOL REVISION

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website. When errata and corrigenda are in press, this will also be marked on the website.

ACKNOWLEDGEMENTS

This protocol was originally drafted by: Migeon A, CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier, France. The revision was prepared with the contribution of Kamayev IO, All-Russian Plant Quarantine Center (VNIIKR) RU. The protocol was reviewed by the Panel on Diagnostics in Entomology.

REFERENCES

Baker EW & Pritchard AE (1960) The tetranychoid mites of Africa. *Hilgardia* 29, 455–574.

Baker EW & Tuttle DM (1994) *A Guide to the Spider Mites (Tetranychidae) of the United States*. Indira Publishing House, West Bloomfield (US), pp. 347.

Bolland HR, Gutierrez J & Flechtmann CHW (1998) *World Catalogue of the Spider Mite Family (Acari: Tetranychidae)*. Brill Academic Publishers, Leiden (NL), pp. 392.

Boubou A, Migeon A, Roderick G & Navajas M (2011) Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions. *Biological Invasions* 13, 81–92.

Duverney C & Ngueye-Ndiaye A (2005) *Essais préliminaires pour limiter les dégâts de Tetranychidae sur les cultures maraîchères dans le Sine-Saloum (Sénégal)*. Deuxième colloque international sur les acariens des cultures, Montpellier (FR) (in French).

Ehara S & Ohashi K (2002) A new species of *Tetranychus* (Acari: Tetranychidae) from the Kinki District, Japan. *Acta Arachnologica* 51, 19–22.

EPPO (2022a) EPPO Global Database (available online). https://gd.eppo.int
EPPO (2022b) *Eotetranychus lewisi*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int
Ferragut F & Escudero LA (2002) La araña roja del tomate *Tetranychus evansi* (Acari, Tetranychidae) en España: distribuciòn, biología y control. *Phytoma España* 132, 111–113. (in Spanish)
Fiaboe KKM, Fonseca RL, de Moraes GJ, Ogol CKPO & Knapp M (2006) Identification of priority areas in South America for exploration of natural enemies for classical biological control of *Tetranychus evansi* (Acari: Tetranychidae) in Africa. *Biological Control* 38, 373–379.
Flechtmann CHW & Knihinicki DK (2002) New species and new record of *Tetranychus* Dufour from Australia, with a key to the major groups in this genus based on females (Acari: Prostigmata: Tetranychidae). *Australian Journal of Entomology* 41, 118–127.
Gotoh T, Araki R, Boubou A, Migeon A, Ferragut F & Navajas M (2009) Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. *International Journal of Acarology* 35, 485–501.
Gutierrez J, Helle W & Bolland HR (1979) Etude d'une souche de *Tetranychus piercei* (Acariens: Tetranychidae), d'Indonésie: redescription, caryotype et reproduction. *Entomologische Berichten, Amsterdam*, 39: 88–94. Available at https://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_5/b_fdi_08-09/09800.pdf
Gutierrez J (1985a) Mounting techniques. In *Spider Mites their Biology, Natural Enemies and Control*, Vol. 1A, (eds Helle W & Sabelis MW), pp. 351–353. Elsevier Science Publisher, Amsterdam (NL).
Gutierrez J (1985b) Systematics. In *Spider Mites their Biology, Natural Enemies and Control*, Vol. 1A, (eds Helle W & Sabelis MW), pp. 75–90. Elsevier Science Publisher, Amsterdam (NL).
Jeppson LR, Keifer HH & Baker EW (1975) *Mites Injurious to Economic Plants*, pp. xxiv + 614. University of California Press, Berkeley (US).
Knapp M, Saunyama IGM, Sarr I & de Moraes GJ (2003) *Tetranychus evansi in Africa -Status, Distribution, Damage and Control Options*. Deutcher Tropentag International Research on Food Security, Natural Resource Management and Rural Development, Göttingen (DE).
Krantz GW (1978) *A Manual of Acarology*. Oregon State University Press: Corvallis. 509 pp.
Krantz GW & Walter DE (Eds) (2009) *A manual of Acarology*. Third edition. Texas Tech University Press, 807 pp.
Meyer MKPS (1987) *African Tetranychidae (Acari: Prostigmata) - with Reference to the World Genera*. Entomology Memoir, Department of Agriculture and Water Supply, Republic of South Africa 69, 1–175.
Migeon A & Dorkeld F (2021) Spider Mites Web: a comprehensive database for the Tetranychidae. http://www1.montpellier.inrae.fr/CBGP/spmweb/index.php [accessed 18 January 2021].
Migeon A, Ferragut F, Escudero-Colomar L, Fiaboe KKM, Knapp M, de Moraes GJ *et al.* (2009) Modelling the potential distribution of the invasive tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology* 48, 199–212.
Moutia LA (1958) Contribution to the study of some phytophagous Acarina and their predators in Mauritius. *Bulletin of Entomological Research* 49, 59–75.
Pritchard AE & Baker EW (1955) A revision of the spider mite family Tetranychidae. *Memoirs Series, San Francisco, Pacific Coast Entomological Society*, 2: 472 p.
Rossi Simons NH (1961) Lista de las especies de Tetranychidae (Acari) de la Republica Argentina. *Idia* 163, 9–13 (in Spanish).
Seeman O & Beard J (2005) National diagnostic standards for *Tetranychus* spider mites. Plant Health Australia. 128 p.
Seeman O & Beard J (2011) Identification of exotic pests and Australian native and naturalised species of *Tetranychus* (Acari: Tetranychidae). *Zootaxa*, 2961: 1–72.
Silva P (1954) Um novo ácaro nocivo ao tomateiro na Bahia. *Boletim do Instituto Biologica da Bahia* 1, 1–20 (in Portuguese).
Wene GP (1956) *Tetranychus marianae* McG., a new pest of tomatoes. *Journal of Economic Entomology* 49, 712.

APPENDIX 1 - PREPARATION OF SPECIMENS

Specific identification requires examination of slide mounted adult males and females. Tetranychid mites are not hardly sclerotized and need to be mounted in aqueous media. Pritchard & Baker, 1955 (Hoyer's media), Krantz (1978), Gutierrez (1985a) and Krantz and Walter (2009) provide detailed descriptions of the mounting of specimens. A review of mounting media and technics is available in Krantz and Walter (2009).

An outline is presented below. Generally (not required), mites are cleared for 24 h in lactic acid bath (50% lactic acid/50% water) at room temperature in excavated glass block (staining block) (4 cm^3) with flat bottom and covered with a glass slide. Slide mounting with Hoyer's medium (see Table 1) is traditionally used but it contains toxic chloral-hydrate. Drying of slides (approx. 40–50°C) for about 1 week to 10 days followed by sealing with Euparal allows the preparation to be kept for 2 or 3 years or longer. Other aqueous media based on polyvinyl-alcohol (PVA; Table 2), with or without toxic substances like chloral-hydrate or phenol added can also be used. Both are commercially available.

Use of clearing and mounting directly for non-permanent slides in lactic acid is convenient for a rapid identification. Clearing should last 24 h at room temperature or few hours at approximately 40°C. Using a slide with a cavity as described by Gutierrez (1985a) is easier but a normal slide can also be used. The cavity is made with a small tungsten carbide grindstone mounted on a drill and should be about 3 mm diameter. The use of such slides allows the orientation of the specimen in any direction inside the cavity, by sliding the cover glass back and forth. Females are examined dorso-ventrally and males laterally to observe the shape of the aedeagus.

Different recipes are available for Hoyer's medium one is provided below (Pritchard and Baker (1955), Krantz (1978) and Krantz and Walter (2009).

TABLE 1 Hoyer's medium

Distilled H_2O	50 mL
Arabic gum (acacia)	30 g
Chloral hydrate	200 g
Glycerol	16 mL

Note: Dissolve Arabic gum completely in the distilled H_2O. Then, and only then, completely dissolve in Chloral hydrate, then add glycerol and mix well. Medium may be diluted when needed with small amounts of distilled H_2O. Before using Hoyer's medium, let it stand undisturbed for several days in order to clarify.

TABLE 2 PVA medium after Krantz and Walter (2009)

Polyvinyl alcohol	10 g
Chloral hydrate	100 g
Glycerol	10 g
Distilled H_2O	60 mL
85–92% lactic acid	35 mL

Note: Add water to PVA powder, stirring constantly in a water bath at just below boiling. Add lactic acid and stir for a few minutes. Add glycerol and stir until smooth. Cool until only lukewarm. Dissolve chloral hydrate in mixture. Stir thoroughly, pass through filter paper in a suction funnel (or centrifuge). Store in a brown bottle.