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INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

ISPM 27 DIAGNOSTIC PROTOCOLS

DP 1: *Thrips palmi* Karny (2010)

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1. Pest Information

Thrips palmi Karny (Thysanoptera: Thripidae) is a polyphagous plant pest, especially of species in the Cucurbitaceae and Solanaceae. It appears to have originated in Southern Asia and to have spread from there during the latter part of the twentieth century. It has been recorded throughout Asia and is widespread throughout the Pacific and the Caribbean. It has been recorded locally in North, Central and South America and Africa. For more general information about *T. palmi*, see EPPO/CABI (1997) or Murai (2002); online pest data sheets are also available from the Pests and Diseases Image Library (PaDIL, 2007) and EPPO (EPPO, 2008).

The species causes economic damage to plant crops both as a direct result of its feeding activity and from its ability to vector tospoviruses such as *Groundnut bud necrosis virus*, *Melon yellow spot virus* and *Watermelon silver mottle virus*. It is extremely polyphagous, and has been recorded from more than 36 plant families. It is an outdoor pest of, amongst others, *Benincasa hispida*, *Capsicum annuum*, *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita* spp., *Glycine max*, *Gossypium* spp., *Helianthus annuus*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Pisum sativum*, *Sesamum indicum*, *Solanum melongena*, *Solanum tuberosum* and *Vigna unguiculata*. In glasshouses, economically important hosts are *Capsicum annuum*, *Chrysanthemum* spp., *Cucumis sativus*, *Cyclamen* spp., *Ficus* spp., Orchidaceae and *Solanum melongena*. The thrips may be carried on plants for planting, cut flowers and fruits of host species, as well as on or associated with packing material, and in soil.

Thrips palmi is almost entirely yellow in coloration (Figure 1–3), and its identification is hampered by both its small size (1.0–1.3 mm) and its great similarity to certain other yellow or predominantly yellow species of *Thrips*.

Figure 1: *Thrips palmi*, female (left) and male (photo: A. J. M. Loomans, PPS, Wageningen, the Netherlands; scale bar = 500 µm = 0.5 mm)

Figure 2: *Thrips palmi*, female
Figure 3: *Thrips palmi*, male
(Photos: W. Zijlstra, PPS, Wageningen, the Netherlands; scale bars = 200 µm)

2. Taxonomic Information

Name: *Thrips palmi* Karny, 1922

Synonyms: *Thrips alatus* Moulton, 1928
Thrips leucadophilus Priesner, 1936
Thrips graminicola Ramakrishna & Margabandhu, 1939
Chloethrips aureus Ananthakrishnan & Jagadish, 1967
Thrips gracilis Ananthakrishnan & Jagadish, 1968

Taxonomic position: Insecta, Thysanoptera, Terebrantia, Thripidae

Common name: melon thrips

3. Detection

Thrips palmi may be found in different locations depending on the life stages present.

eggs	in the leaf, flower and fruit tissue
larva I	on the leaves, flowers and fruits
larva II	on the leaves, flowers and fruits
pupa I	in the soil, packing cases and growing medium
pupa II	in the soil, packing cases and growing medium
adult	on the leaves, flowers and fruits

On plant material, *T. palmi* may potentially be found on most above-ground parts of the plant; the parts of the plant infested can differ according to variables such as the host and the characteristics of each separate *T. palmi* population.

During visual examination of plant material for the presence of *T. palmi*, attention must be paid to silvery feeding scars on the leaf surfaces of host plants, especially alongside the midrib and the veins. Heavily infested plants are often characterized by a silvered or bronzed appearance of the leaves, stunted leaves and terminals, or scarred and deformed fruits. Detection may be hampered in circumstances such as:

- low-level infestation, which may produce little or no detectable symptoms
- the presence of the eggs within the plant tissue only (for example after external treatment which may have removed visible life stages).

Specimens for morphological examination are best collected in a fluid called AGA, which is a mixture of 10 parts of 60% ethanol with 1 part of glycerine and 1 part of acetic acid. If the specimens are to be stored, they should be transferred to 60% ethanol and kept in the dark, preferably in a freezer to prevent loss of colour. However, several laboratories have reported that AGA may act to denature the DNA of the thrips thereby hindering any subsequent molecular work. An alternative is to use 80–95% ethanol as the collecting fluid as any unmounted specimens may then be used for molecular studies. However, in this case specimens must be stored in the freezer until used, or they may prove difficult to slide mount.

Several methods can be used to collect thrips specimens (Mantel and Vierbergen, 1995, modified):

- Thrips may be individually removed from the plant (leaves, flowers or fruit), and transferred into microtubes containing AGA, using a moist, fine brush.
- Thrips may be beaten from plant parts onto a small plastic tray (e.g. a white tray for dark-coloured specimens or a black tray for light-coloured specimens). In cooler conditions, the thrips usually start walking across the tray rather than flying off, allowing time for the thrips to be picked off with a moist fine brush. Whereas in warmer conditions collection has to be done more rapidly as the thrips are likely to fly off much more quickly. The thrips are easily seen on the tray using just a hand lens, but an experienced observer can also see them easily with the naked eye.
- Plant parts may be sealed in a plastic bag for 24 hours, with a piece of filter paper enclosed to absorb condensation. Most thrips will leave the plant parts and can then be collected from the inside of the bag.
- A Berlese funnel can be used to process plant material such as bulbs, flowers, turf, leaf litter, moss and even dead branches of trees. The funnel contains a sieve on which the plant material is deposited. Beneath the sieve, the bottom of the funnel leads into a receptacle containing 70–96% ethanol. An alternative is to use 10% ethanol plus wetting agent as some workers find that this makes the preparation of good quality microscope slide mounts easier. The funnel is placed under an electric lamp (25 W), and the heat and light will drive most of the thrips present in the plants downwards the receptacle. After an appropriate period (e.g. 8 hours for cut flowers), the contents of the receptacle can then be checked under a stereomicroscope.
- Thrips may be monitored (winged adults only) using coloured sticky traps or other appropriate methods. The ability of a colour to attract thrips varies for different thrips species, but blue or white traps are good for *T. palmi*, though yellow traps will also work. For microscope slide preparation and identification, the thrips will have to be removed from the traps using glue-removing fluids such as those based on citrus oils, dichloromethane or a turpentine substitute.

There are no recognized methods for extracting thrips pupae from the soil in a quarantine context.

4. Identification

Identification of thrips species by morphological examination is restricted to adult specimens because there are no adequate keys for the identification of eggs, larvae or pupae. However, the presence of larvae in samples can give important additional information such as confirming their development on the host plants. The primary method of identification of adult material is from morphological

characters. In order to achieve species identification, these must be examined using a high-power microscope (e.g. x400). Using this protocol with good-quality slide preparations should allow adult *T. palmi* to be identified with certainty by morphological examination alone.

Molecular assays can be applied to all life stages including the immature stages for which morphological identification to species is not possible. Additionally, in cases where adult specimens are atypical or damaged, molecular assays may provide further relevant information about their identity. However specificity of molecular assays is limited as they have been developed for specific purposes and evaluated against a restricted number of species, using samples from different geographic regions; therefore, such information needs to be carefully interpreted.

4.1 Morphological identification of the adult thrips

4.1.1 Preparation of thrips for microscopic examination

For high-power microscopic examination, adult thrips must be mounted on microscope slides. Specimens to be kept in a reference collection are best macerated, dehydrated and mounted in Canada balsam; Mound and Kibby (1998) provide a full description of this process. However, the full slide preparation protocol for archival mounts takes 3 days to complete.

For routine identifications, a water-soluble mountant such as Hoyer's medium (50 ml water, 30 g gum arabic, 200 g chloral hydrate, 20 ml glycerine) is more rapid and relatively inexpensive. One popular method of routine slide preparation is given by Mound and Kibby (1998) and described below (different laboratories may find that other variants work equally well):

Transfer the specimens from the collecting fluid into clean 70% ethanol; if the specimens are reasonably flexible, attempt to spread the legs, wings and antennae using micropins; transfer a single thrips, ventral side uppermost, to a drop of Hoyer's medium on a 13 mm diameter cover slip and use micropins to rearrange the thrips if necessary; gently lower a microscope slide onto the mountant so that the cover slip and mountant adhere to the middle of the slide; invert the slide as soon as the mountant has spread to the edges of the cover slip; label the slide with details including locality, date of collection and host plant; place the slide, cover slip up, into a drying oven at 35–40 °C and leave for 6 hours before attempting study; leave in the oven for approximately 3 weeks to dry the mountant, before sealing the cover slip with resin or nail varnish.

4.1.2 Identification of the family Thripidae

Thrips palmi belongs to the family Thripidae, which includes more than 2000 species in 276 genera. Species share the characteristics outlined in Table 1.

Table 1: Family Thripidae – shared characteristics

Body part	Characteristic
Antennae	seven or eight segments (occasionally six or nine)
	segments III–IV have emergent sense cones (sensoria)
Forewings (if fully developed)	usually slender, with two longitudinal veins each bearing a series of setae
Abdomen – female	with a serrated ovipositor, which is turned downwards at the apex
Median sternites – male	with or without glandular areas

4.1.3 Identification of the genus *Thrips*

The genus *Thrips* contains more than 280 species from all parts of the world, though the genus is primarily from the Holarctic region and the Old World tropics. Members of the genus share the characteristics outlined in Table 2.

Table 2: Genus *Thrips* – shared characteristics, adult specimens

Body part	Characteristic
Body form (female)	macropterous or micropterous
Antennae	seven or eight segments
	segments III–IV with forked emergent sense cones
Ocellar setae	only two pairs present (pair I absent)
	pair II shorter (at least no longer) than pair III
Pronotum	two pairs (rarely one or none) of major posteroangular setae
	usually three, sometimes four, pairs of posteromarginal setae
Prosternal basantra	no setae present
Forewings	the first vein with variably spaced setal row, second vein with complete setal row
	clavus with five veinal setae (rarely six)
Metascutum	median pair of setae at or behind the anterior margin
	striate or reticulate sculpturing
	campaniform sensilla (metanotal pores) present or absent
Metasternal furca	without a spinula
Fore tibia	apical claw absent
Tarsi	two-segmented
Abdominal tergites and sternites	without posteromarginal crass[illegible]da (flanges)
Abdominal tergites	tergites VII–VIII with [illegible] ctenidia laterally (combs – each comprising a submarginal row of microtrichia) (occasionally also on IV)
	[illegible] VIII: [illegible]nidia posteromesad to the spiracles
Abdominal sternites and pleurotergites	with or without discal (accessory) setae
Abdominal sternites (ma[illegible]	abdominal sterna III–VII, or less, each with a glandular area

(A simplified summary of the main characteristics is given in Table 4 and is accompanied by illustrative line drawings and photomicrographs (Figures 4 to 5.12).)

Identification of the adults can be carried out with keys. Mound and Kibby (1998) provided a key to 14 *Thrips* species of economic importance including *T. palmi*. In addition, a CD-ROM identification aid for thrips is available which includes an identification system to 100 pest species from around the world based on photomicrographs (Moritz *et al.*, 2004).

More comprehensive keys to the genus are available, produced on a regional basis (no such key has been produced for the Afrotropical region):

Asia: Bhatti (1980) and Palmer (1992) provide keys for the identification of species of *Thrips* occurring in the Asian tropics. Mound & Azidah (2009) provide a key to the species of Peninsular Malaysia.

Europe: zur Strassen (2003) has produced the most recent comprehensive key to the species of Europe including *Thrips* (in German).

North, Central and South America: Nakahara (1994) provides a key for *Thrips* species from the New World. A key to the species of *Thrips* found in Central and South America is given by Mound and Marullo (1996) though only one of these species is native to the region.

Oceania: Mound and Masumoto (2005) provide a key to the *Thrips* species of Oceania. (The authors of the paper are aware of the error inadvertently introduced on p. 42 in the section "Relationships" whereby a characteristic of *T. flavus* Schrank – ocellar setae III close together behind the first ocellus – is attributed to *T. palmi*. The correct information is provided in the *T. palmi* species description immediately above and is illustrated in Figure 72.)

4.1.4 Identification of *Thrips palmi*

4.1.4.1 Morphological characteristics of *Thrips palmi*

Bhatti (1980), Bournier (1983), Sakimura *et al.* (1986), zur Strassen (1989), Nakahara (1994) and Mound and Masumoto (2005) all provide detailed descriptions of *T. palmi*. Sakimura *et al.* (1986) gave a list of major diagnostic characters to distinguish *T. palmi* from the other known species of the genus *Thrips*; a modified version is presented in Table 3.

Thrips palmi can be reliably separated from all other species of the genus *Thrips* by the possession of all the characters listed in Table 3. Nevertheless, thrips morphology is subject to variation even within a single species and some characters listed here may be subject to occasional slight variation. For instance antennal coloration or the number of distal setae on the forewing can vary from the most commonly observed states. If the specimen differs with respect to one or more of these character states, then the identification should be checked by reference to the appropriate regional key such as those listed in section 4.1.3.

Table 3: A list of morphological characteristics that collectively distinguish *Thrips palmi* from other species in the genus *Thrips*

	Morphological character
1.	A clear yellow body with no dark areas on the head, thorax or abdomen (slightly thickened blackish body setae); antennal segments I and II pale, III yellow with apex shaded, IV to VII brown but usually with base of IV–V yellow; forewing uniformly slightly shaded, prominent setae dark
2.	Antennae always seven-segmented
3.	Postocular setae I and II much smaller than remaining setae
4.	Ocellar setae III standing either just outside of the ocellar triangle, or touching the tangent lines connecting the anterior ocellus and each of the posterior ocelli
5.	Metascutum with sculpture converging posteriorly; median pair of setae behind anterior margin; paired campaniform sensilla present
6.	Forewing first vein with three (occasionally two) distal setae
7.	Abdominal tergite II with four lateral marginal setae
8.	Abdominal tergites III to IV with setae S2 dark and subequal to S3
9.	Abdominal tergite VIII with posteromarginal comb in female complete, in male broadly developed posteriorly
10.	Abdominal tergite IX usually with two pairs of campaniform sensilla (pores)
11.	Abdominal sternites without discal setae or ciliate microtrichia
12.	Abdominal pleurotergites without discal setae
13.	Male: sternites III–VII each with a narrow transverse glandular area

A simplified summary of the main characteristics is given in Table 4 and is accompanied by illustrative line drawings and photomicrographs (Figures 4 to 5.12).

4.1.4.2 Comparison with similar species (species that are yellow without darker body markings, or predominantly yellow, or sometimes yellow)

For each species listed here, the main character differences by which they may be separated from *Thrips palmi* are given. If in any doubt, refer to an appropriate regional key such as those listed in section 4.1.3. These also give details of other *Thrips* species that are not listed below.

Two Indian species (*T. alatus* Bhatti and *T. pallidulus* Bagnall) are very similar to *T. palmi*, although little is known about their biology.

Thrips alatus

- antennal segment V uniformly brown
- abdominal tergites III and IV with setae S2 paler and much weaker than S3 in both sexes
- the striate sculpture on the metascutum usually not converging posteriorly
- distribution: India, Malaysia, Nepal.

Thrips pallidulus

- antennal segment IV pale
- sculpture on the metascutum medially reticulate, not striate
- distribution: India.

Three common Palearctic species (but also with wider distribution) that may be confused with *T. palmi* are *T. flavus*, *T. nigropilosus* Uzel and *T. tabaci* Lindeman.

Thrips flavus

- ocellar setae pair III inside the ocellar triangle, just behind the anterior ocellus
- length of antennal segment VI, 54–60 µm (42–48 µm in *T. palmi*)
- lines of sculpture on the metascutum not converging posteriorly
- distribution: common flower thrips throughout Asia, Europe.

Thrips nigropilosus

- usually with dark markings on the thorax and abdomen
- metascutum with irregular reticulations medially (longitudinal striae in *T. palmi*) and no campaniform sensilla
- abdominal tergite II with three lateral marginal setae
- abdominal tergites III–V with median pair of setae (S1) more than 0.5 times as long as the median length of their tergites (less than 0.3 times in *T. palmi*)
- distribution: common pollen-feeding species, sometimes a pest of plants in the family Compositae; Asia, East Africa, Europe, North America, Oceania.

Thrips tabaci

- highly variable in coloration, but usually with more or less brown or greyish markings
- all postocular setae subequal in length
- metascutum with irregular longitudinal reticulations, usually with small internal wrinkles medially, and no campaniform sensilla
- forewing first vein usually with four (occasionally between two or six) distal setae
- abdominal tergite II with three lateral marginal setae
- abdominal tergite IX with posterior pair of campaniform sensilla only
- abdominal pleurotergites with numerous ciliate microtrichia arising from lines of sculpture
- male: narrow transverse glandular area on abdominal sternites III–V only
- distribution: polyphagous pest with a worldwide distribution.

Two further species, one Palearctic (*T. alni* Uzel) and one European (*T. urticae* Fabricius), are less commonly encountered but may be confused with *T. palmi*. Females of *T. alni* are particularly similar in morphology to those of *T. palmi*.

Thrips alni

- antennal segment V uniformly brown
- abdominal tergites II–V with setae S2 pale
- abdominal tergite V with seta S2 much weaker than seta S3 (these setae are subequal in *T. palmi*)
- abdominal tergite VIII with seta S1 subequal to seta S2 (S1 is much weaker than S2 in *T. palmi*)
- male: abdominal sternites III–VI each with a small oval glandular area
- distribution: restricted to the leaves of *Alnus*, *Betula*, *Salix*; Europe, Siberia, Mongolia.

Thrips urticae

- pronotum with a pair of setae on the anterior margin almost twice as long as any of the discal setae (usually more than 30 µm; not so in *T. palmi*, all less than 25 µm)
- metascutum with longitudinal reticulations medially
- abdominal tergites usually with a grey area medially
- abdominal tergite IX with posterior pair of campaniform sensilla absent
- distribution: restricted to *Urtica dioica*; Europe.

Table 4: Simplified checklists of the diagnostic features for quick recognition: (a) the genus *Thrips*; (b) *Thrips palmi* (See Figure 4 for the location of the various features.)

(a) Specimens can be recognized as *Thrips* by the following combination of characters		
Antenna	with seven or eight distinct segments; segments III and IV with forked sense cones	Figs 5.1, 5.2
Head	with two pairs of ocellar setae (II and III); pair I missing, pair II shorter than pair III	Fig. 5.3
Forewing	1st vein – setal row on the first vein continuous or interrupted	Fig. 5.5
Abdominal tergites V to VII	with paired discal setae	Fig. 5.6
Abdominal tergite VII	with ctenidia posteromesad to the spiracles	Fig. 5.6
(b) Specimens can be identified as *Thrips palmi* by the presence of the following characters		
Body colour	pale yellow body with no dark areas on the head, thorax or abdomen; antennal segments I and II are pale	Figs 1–3
Antennal segment V	usually yellowish in basal ⅓ to ½	Fig. 5.1
Antennal segment VI	length = 42–48 µm	Fig. 5.1
Head: ocellar setae pair III	with their bases sited outside of the ocellar triangle or touching the tangent lines connecting the anterior ocellus to each of the posterior ocelli	Fig. 5.3
Pronotum	with two pairs of major posteroangular setae	Fig. 5.4
Forewing: 1st vein	with three (occasionally two) distal setae	Fig. 5.5
Metascutum	with median pair of setae behind the anterior margin and a pair of campaniform sensilla; with striate sculpture converging posteriorly	Fig. 5.7
Abdominal pleurotergites	discal setae absent; lines of sculpture without ciliate microtrichia	Fig. 5.8
Abdominal tergite II	with four lateral marginal setae	Fig. 5.9
Abdominal tergites III and IV	S2 almost equal to S3	Fig. 5.10

Table 4 continued

Abdominal tergite VIII	female with complete posteromarginal comb; male with posteromarginal comb broadly developed medially	Fig. 5.6
Abdominal tergite IX	with anterior and posterior pairs of campaniform sensilla (pores)	Fig. 5.11
Male: sternites	transverse glandular areas on sternites III to VII	Fig. 5.12

Figure 4. Location of general characters of *Thrips* (♀ – dorsal view)

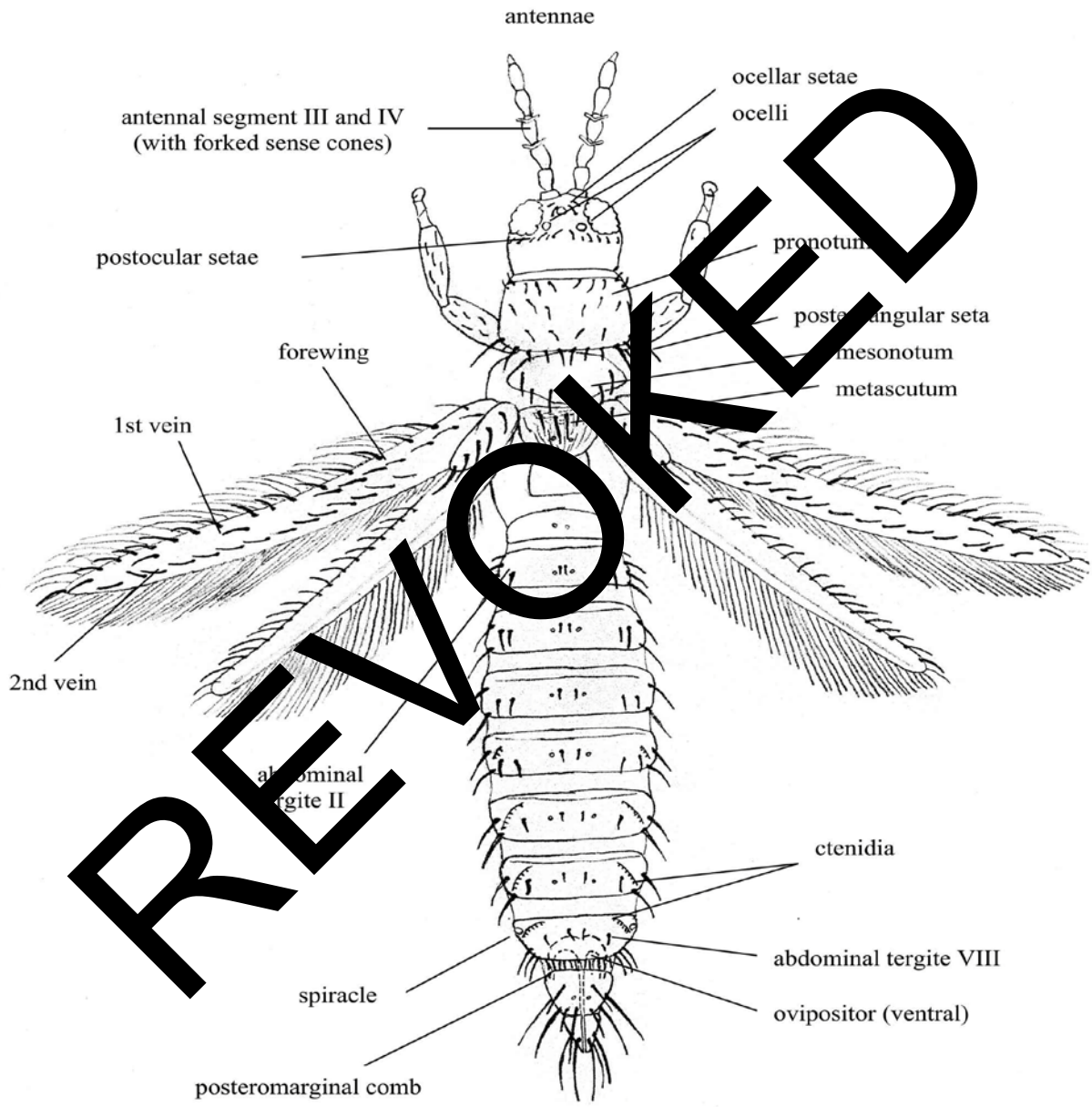
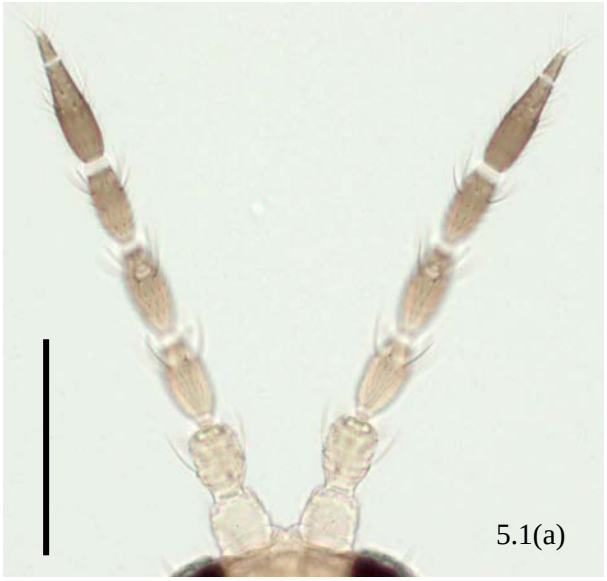


Figure 5 (Figs 5.1 to 5.12): Characters of *Thrips palmi* (photos: G. Vierbergen, PPS, Netherlands; figures drawn by S. Kobro, Norwegian Crop Protection Institute, Norway)



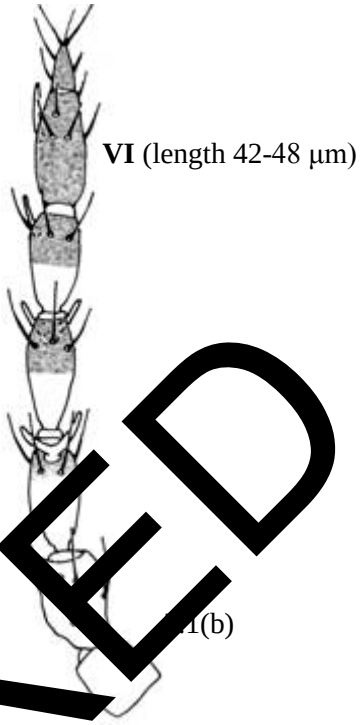
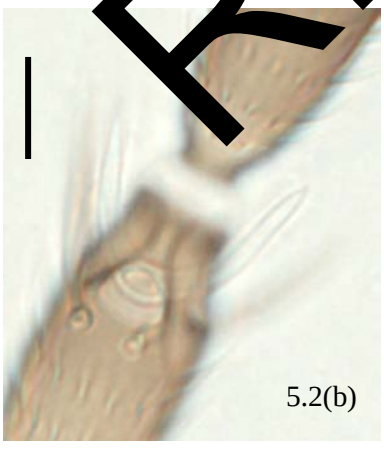


Fig. 5.1(a), (b): Antenna: seven segments (scale bar: 100 µm)



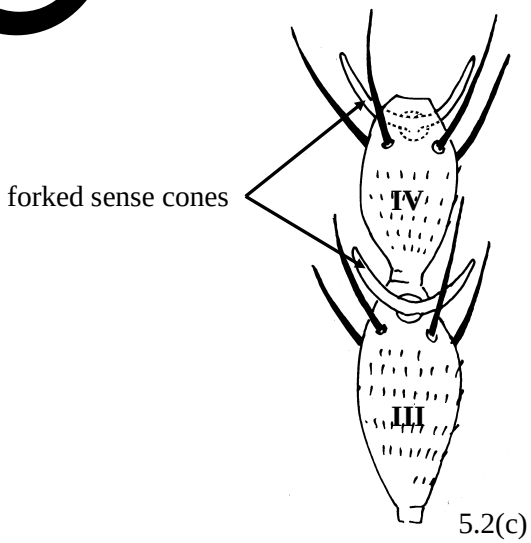


Fig. 5.2(a)–(c): Antenna, forked sense cones; (a) segment III, dorsal; (b) segment IV, ventral; (c) segment III and IV, dorsal (scale bars: 10 µm)

Fig. 5 continued.

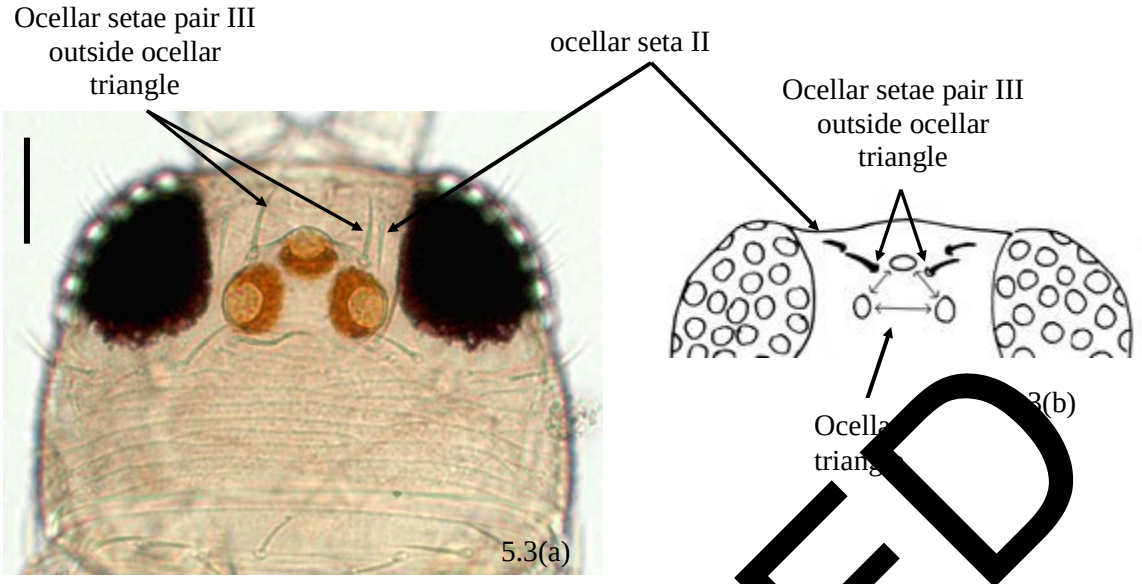


Fig. 5.3(a), (b): Head: with two pairs of ocellar setae (pair I missing). Ocellar setae pair III situated outside of ocellar triangle (scale bar: 30 µm)

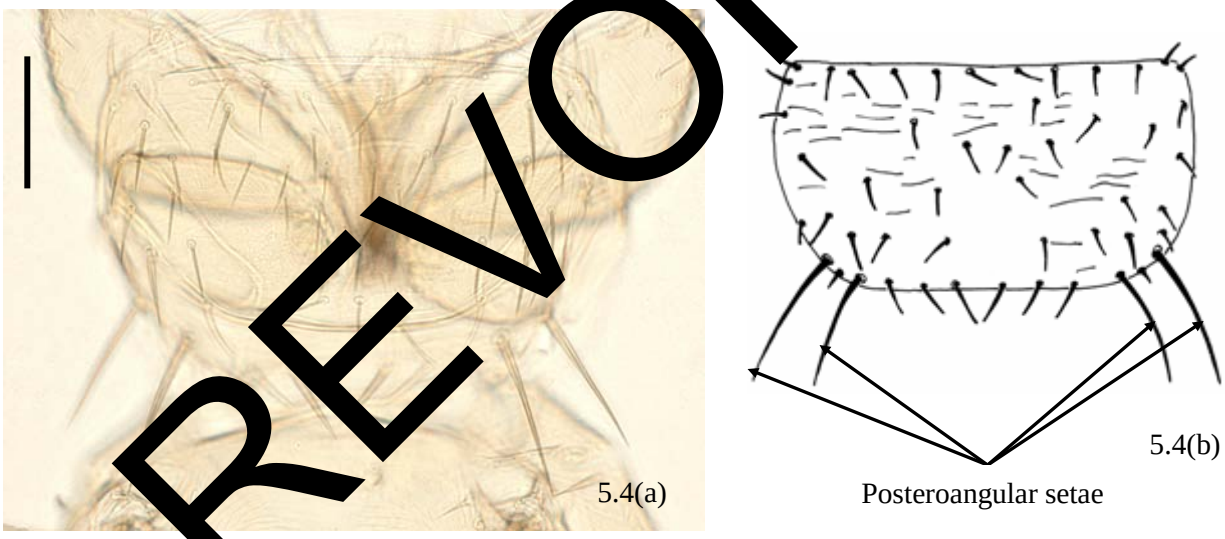


Fig. 5.4(a), (b): Pronotum, two pairs of major posteroangular setae (scale bar = 50 µm)

Fig. 5 continued

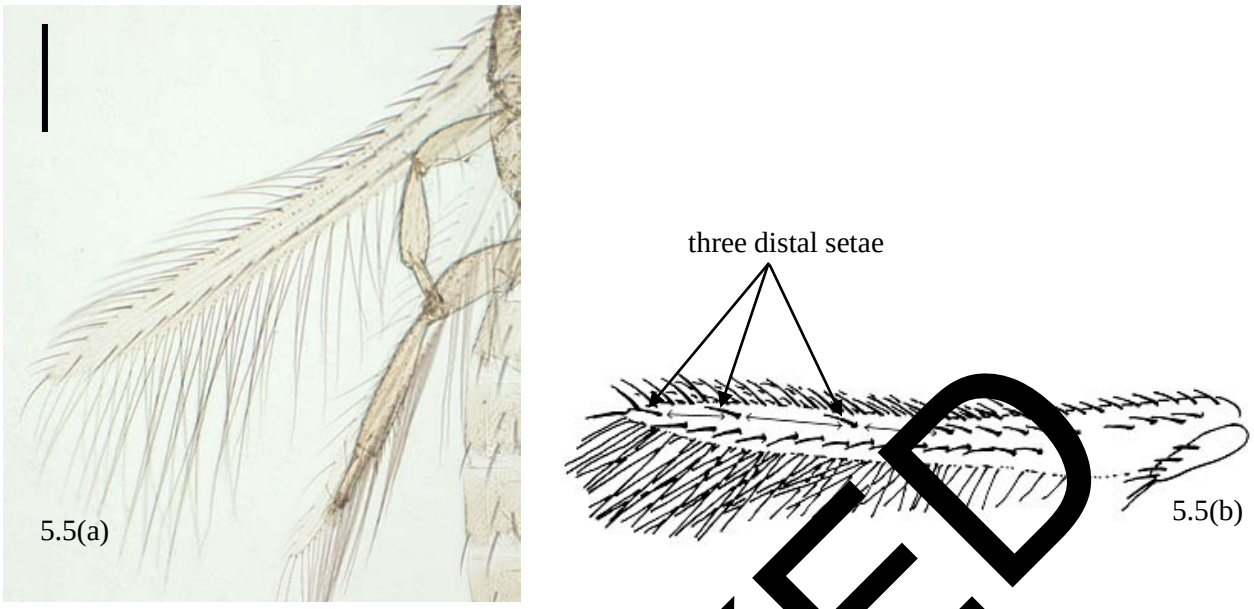
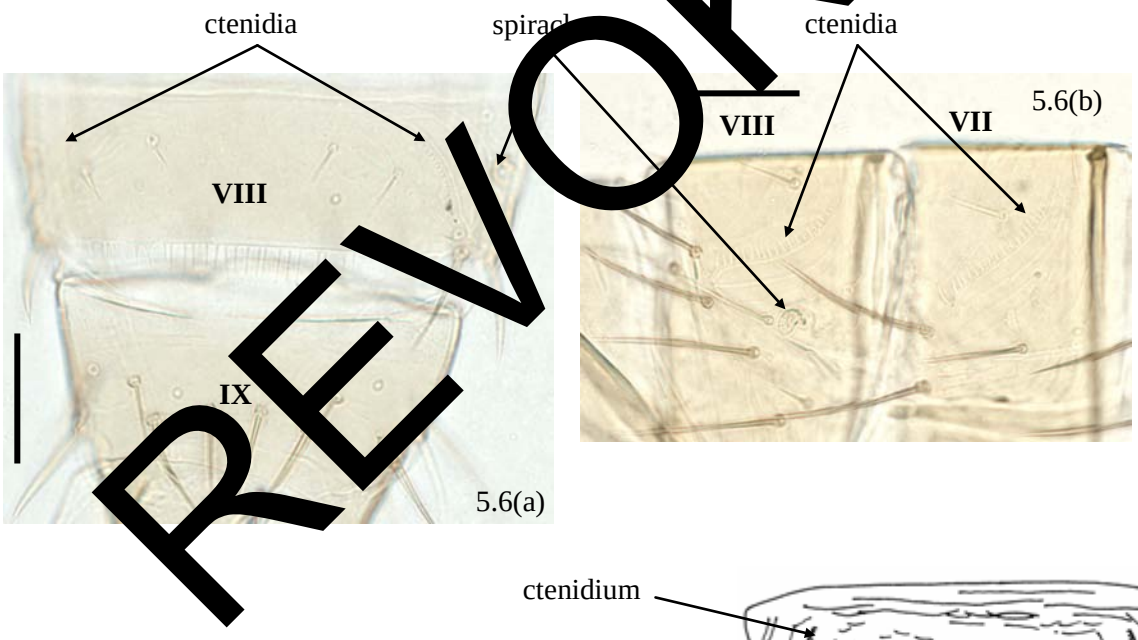


Fig. 5.5(a), (b): Forewing, first vein – three setae with gaps in dist half (scale bar: 1 µm)



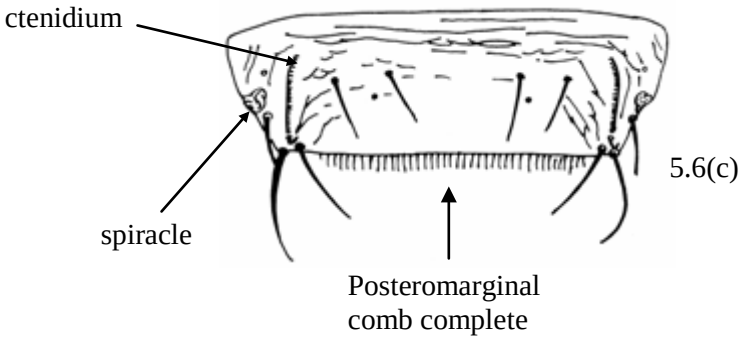
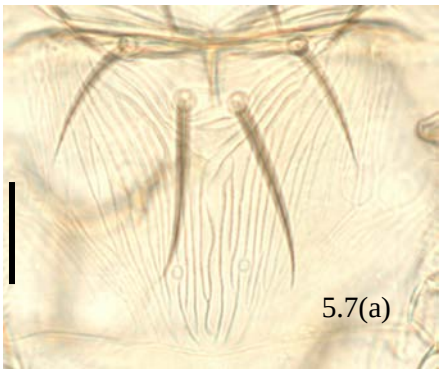
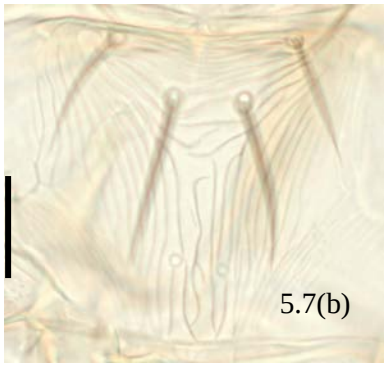
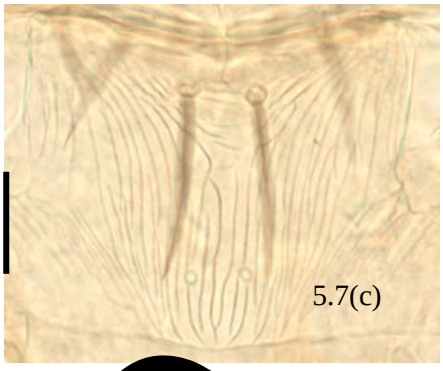


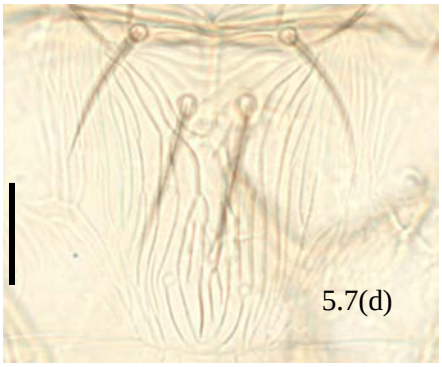
Fig. 5.6(a)–(c): Abdominal tergite VIII: ctenidia posteromesad to the spiracle; posteromarginal comb complete; **(a)** male, tergite VIII and IX, dorsal, comb complete medially; **(b)** female, tergite VII and VIII, lateral; **(c)** female, tergite VIII, dorsal, comb complete (scale bars: 30 µm)

Fig. 5 continued.









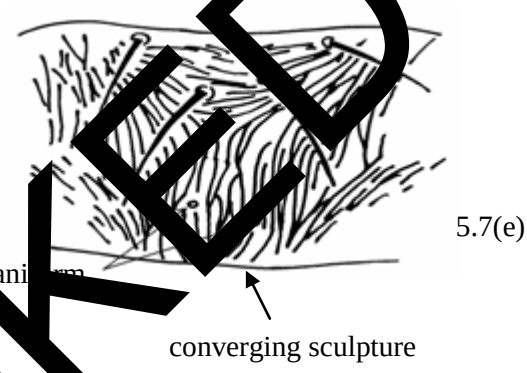
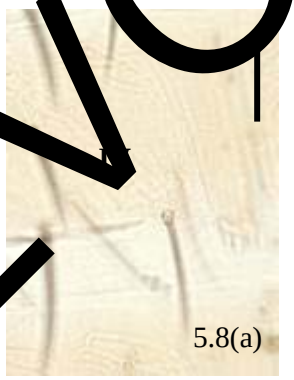
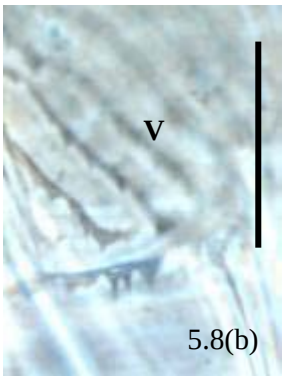


Fig. 5.7(a)–(e): Metascutum, variation in sculpture; campaniform sensilla (scale bars: 20 µm)





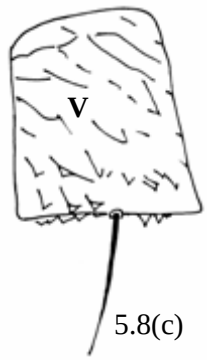


Fig. 5.8(a)–(c): Abdominal pleurotergites IV and V ciliation, microtrichia and discal setae absent; **(a)** bright-field; **(b)** phase contrast; **(c)** complete tergite (scale bars: 20 µm)

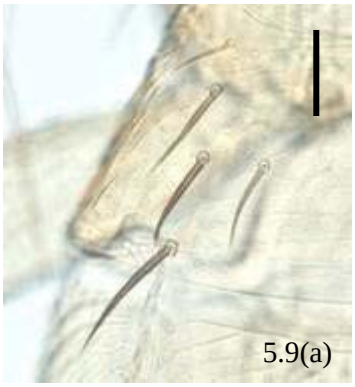


Fig. 5.9(a), (b): Abdominal tergite II, four lateral marginal setae (scale bar: 20 µm)

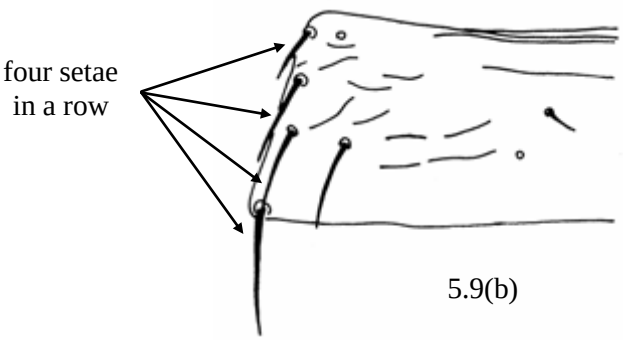
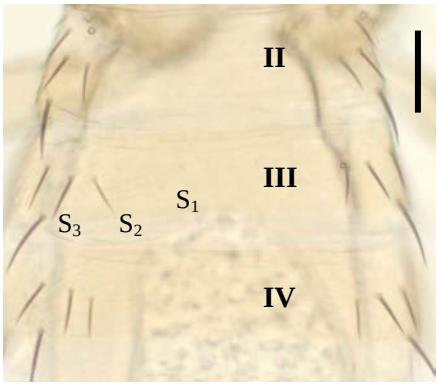
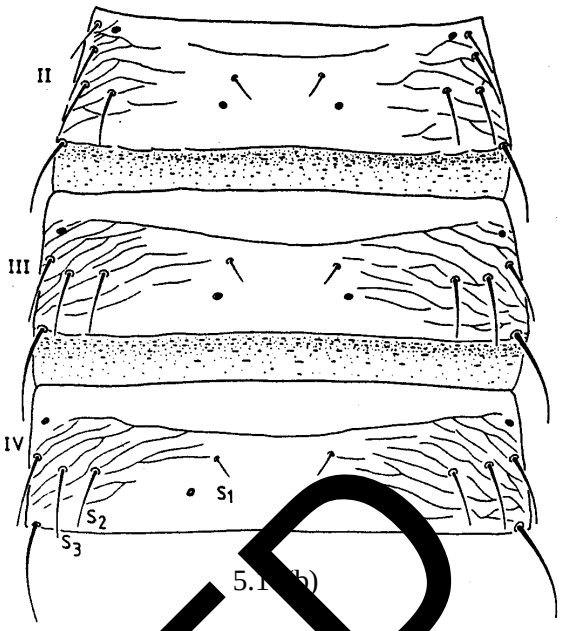


Fig. 5 continued.

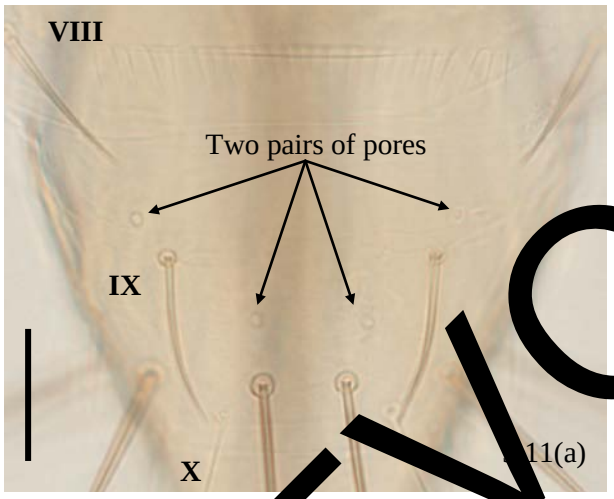


5.10(a)

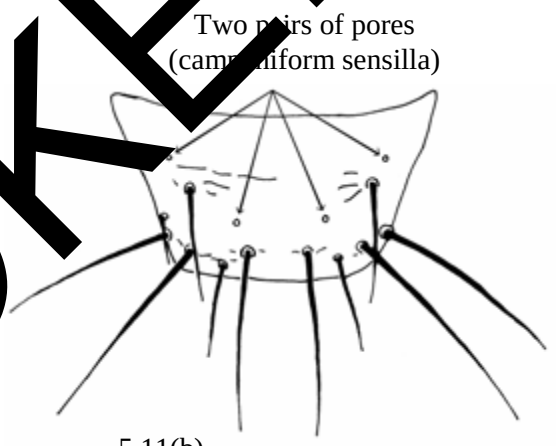


5.10(b)

Fig. 5.10(a), (b): Tergites II–IV, female, setae S2 about same size as setae S3 (5.10b from zur Strassen, 1989) (scale bar: 50 µm)

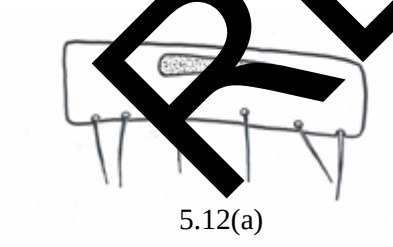


5.11(a)

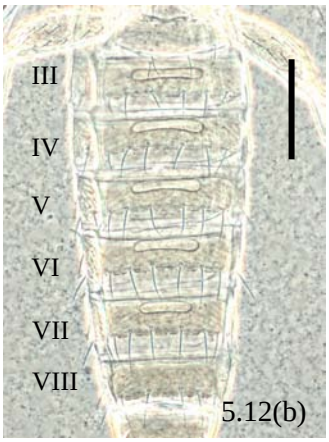


5.11(b)

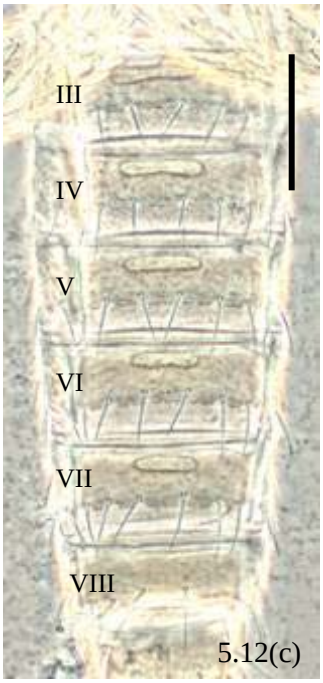
Fig. 5.11(a), (b): Abdominal tergite IX (dorsal), two pairs of campaniform sensilla (scale bar: 30 µm)



5.12(a)



5.12(b)



5.12(c)

Fig. 5.12(a)–(c): Male glandular areas (showing variation); **(a)** sternite V; **(b)-(c)** sternites III–VIII, phase contrast (scale bars: 100 µm)

4.2 Molecular assays for identifying *Thrips palmi*

Four molecular assays have been published that can be used to support a morphological identification of *T. palmi* and these are described below. The specificity of each assay is also described. This indicates the thrips species against which each assay was evaluated and the original use for which the assay was designed. A CD-ROM identification system is also available that includes molecular data for thrips species (Moritz *et al.*, 2004). Considering the specific limitations of molecular methods a negative molecular test result does not exclude the possibility of positive identification by morphological methods.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved.

Requirements for controls

With all molecular methods the use of appropriate controls is essential; a validated *T. palmi*-positive extract must be included as an additional sample to ensure that amplification has been successful. PCR amplification, either for real-time PCR or PCR-RFLP, must also be performed on a sample with no DNA. This negative control indicates possible reagent contamination and false positives.

DNA extraction

DNA may be extracted from single eggs, adults, pupae or larvae. For each of the assays described below refer to the source paper for the original specific DNA extraction technique used. Laboratories may find that alternative extraction techniques work equally well; DNA may be extracted using any DNA extraction methods suitable for insects. For example:

- The thrips may be ground in a lysis buffer in a microtube using a micropestle, and the homogenate taken through a proteinase-K based DNA extraction kit according to the appropriate manufacturer's instructions.
- Alternatively, a thrips may be ground in 50 µl nuclease-free water before the addition of 50 µl of a 1:1 (volume to volume) slurry of Chelex 100 resin, and nuclease-free water, heated to 95 ºC for 5 min and centrifuged at 11 000 *g* for 5 min. The supernatant is transferred to a new microtube and stored at −20 ºC.

Several recent papers have described non-destructive techniques for extracting DNA from thrips, which have the advantage that after DNA extraction has been completed a cleared specimen remains available for slide mounting (e.g., Rugman-Jones *et al.*, 2006; Mound and Morris, 2007).

4.2.1 SCAR marker-generated sequence-based real-time PCR assay for *Thrips palmi*

This assay of Walsh *et al.* (2005) was designed as a species-specific assay against *T. palmi* for use by the phytosanitary authorities in England and Wales. It was evaluated by screening it against 21 other species of Thysanoptera, including ten belonging to the genus *Thrips* (*T. flavus*, *T. major* Uzel, *T. minutissimus* L., *T. nigropilosus*, *T. sambuci* Heeger, *T. tabaci*, *T. trehernei* Priesner or *T. physapus* L., *T. urticae*, *T. validus* Uzel, *T. vulgatissimus* Haliday). These were predominantly, but not exclusively, European species.

Methodology

The *T. palmi*-specific PCR primers and TaqMan probe used in this assay were as follows:

PCR primer: P4E8-362F (5′-CCGACAAAATCGGTCTCATGA-3′)

PCR primer: P4E8-439R (5′-GAAAAGTCTCAGGTACAACCCAGTTC-3′)

TaqMan probe: P4E8-385T (FAM 5′-AGACGGATTGACTTAGACGGGAACGGTT-3′ TAMRA).

Real-time PCR reactions were set up using the TaqMan PCR core reagent kit (Applied Biosystems)[1], with 1 µl (10–20 ng) of DNA extract, 7.5 pmol of each primer and 2.5 pmol probe in a total volume of 25 µl. Plates were cycled at generic system conditions (10 min at 95 °C and 40 cycles of 1 min at 60 °C, 15 s at 95 °C) on either of the ABI Prism 7700 or ABI 7900HT Sequence Detection Systems (Applied Biosystems)[2], using real-time data collection. Ct values lower than 40 indicated the presence of *T. palmi* DNA.

4.2.2 COI sequence-based real-time PCR assay for *Thrips palmi*

This assay of Kox *et al.* (2005) was designed as a species-specific assay against *T. palmi* for use by the phytosanitary authorities in the Netherlands. It was evaluated by screening the assay against 23 other species of thrips, including 11 belonging to the genus *Thrips* (*T. alliorum* (Priesner), *T. alni*, *T. angusticeps* Uzel, *T. fuscipennis* Haliday, *T. latiareus* Vierbergen, *T. major*, *T. minutissimus*, *T. parvispinus* (Karny), *T. tabaci*, *T. urticae*, *T. vulgatissimus*). These were predominantly, but not exclusively, European species.

Methodology

The *Thrips palmi*-specific PCR primers and TaqMan probe used in this assay are as follows:

PCR primer: Tpalmi 139F* (5′-TCA TGC TGG AAT TTC AGT AGA TTT ATC-3′)

PCR primer: Tpalmi 286R* (5′-TCA CAC RAA TAA TCT TAG TTT TTC TCT TG-3′)

TaqMan probe: TpP (6-FAM 5′-TAG CTG GGG TAT CCT CAT 3′ MGB).

* Primers have been adjusted for greater sensitivity since original publication.

(COI sequences that mismatch with the TaqMan probe in this assay have been deposited on GenBank from a number of specimens from India identified as *T. palmi* on the basis of their morphology (Asokan *et al.*, 2007). These sequences would not produce a positive result using this assay. The taxonomic or phylogenetic significance of this sequence differentiation currently remains unclear.)

The 25 µl reaction mixture contained 12.5 µl of 2× TaqMan Universal Master Mix (Applied Biosystems)[3], 0.9 µM each primer, 0.2 µM TaqMan probe, 1.0 µl DNA. The real-time PCR was performed on either of the ABI Prism 7700 or ABI 7900HT Sequence Detection Systems (Applied Biosystems)[4] using the following conditions: 10 min at 95 °C; then 40 cycles of 1 min at 60 °C and 15 s at 94 °C. Ct values lower than 40 indicate the presence of *T. palmi* DNA.

4.2.3 ITS2 sequence-based PCR-RFLP assay for nine species of thrips including *Thrips palmi*

This assay (Toda and Komazaki, 2002) was designed to separate nine species of thrips, including *T. palmi*, that are found in fruit trees in Japan: *Frankliniella occidentalis* (Pergande), *F. intonsa* (Trybom), *F. hawaiiensis* Morgan, *T. coloratus* Schmutz, *T. flavus*, *T. tabaci*, *T. palmi*, *T. setosus* Moulton, *Scirtothrips dorsalis* Hood.

[1, 2] The use of the brand Applied Biosystems for the TaqMan PCR core reagent kit and the ABI Prism 7700 or ABI 7900HT Sequence Detection Systems in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.

[3, 4] The use the brand Applied Biosystems for the TaqMan Universal Master Mix and ABI Prism 7700 or ABI 7900HT Sequence Detection Systems in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.

Methodology

The PCR primers (located in the 5.8 S and 28 S regions flanking the ITS2 region of ribosomal DNA) used in this assay were as follows:

5′-TGTGAACTGCAGGACACATGA-3′

5′-GGTAATCTCACCTGAACTGAGGTC-3′.

T. palmi generated a 588-base-pair (bp) PCR product (longer or shorter fragments were produced from the other species). The 20 µl reaction mixture was composed as follows: 1 µM each primer, 250 µM dNTPs, 1 Unit of AmpliTaq Gold DNA polymerase (Applied Biosystems)[5], 2 µl 10x reaction buffer [with 25 mM $MgCl_2$], 0.5 µl DNA. The PCR was performed in a 9600 DNA thermocycler (Applied Biosytems)[6], with the following conditions: 9 min at 95 °C, 35 cycles of 1 min at 94 °C, 30 s at 50 °C, and 1 min at 72 °C, followed by a final extension for 7 min at 72oC and quickly cooled to room temperature. The PCR products were analysed by agarose gel electrophoresis.

5 µl of PCR product (without purification) was digested with the enzyme *Rsa*I according to the manufacturer's instructions. Digested PCR products were separated by 2.0% agarose gel electrophoresis.

Restriction fragment sizes produced by *T. palmi* when the ITS2 fragment is digested with *Rsa*I were as follows: 371, 98, 61 and 58 bp.

4.2.4 COI sequence-based PCR-RFLP assay for ten species of thrips including *Thrips palmi*

This assay of Brunner *et al.* (2002) was designed to separate ten species of thrips, including *T. palmi*, which are mostly, but not exclusively, pest species found in Europe: *Anaphothrips obscurus* (Müller), *Echinothrips americanus* Morgan, *Frankliniella occidentalis*, *Heliothrips haemorrhoidalis* (Bouché), *Hercinothrips femoralis* (Reuter), *Parthenothrips dracaenae* (Heeger), *Taeniothrips picipes* (Zetterstedt), *Thrips angusticeps* Uzel, *T. palmi*, *T. tabaci*.

Methodology

The PCR primers (located in the mitochondrial COI gene sequence) used in this assay are as follows:

mtD-7.2F (5′-ATTAGGAGCHGTWGAYATAGCATT-3′)

mtD9.2R (5′-CAAGGCAAGATTAAAATATAAACTTCTG-3′).

These primers amplified a 433-bp fragment in all the species separated by this assay. The 50 µl reaction mixtures were composed as follows: 0.76 µM each primer, 200 µM dNTPs, 1 Unit Taq DNA polymerase, 5 µl 10X reaction buffer [with 15 mM $MgCl_2$], 1 µl DNA. The PCR was performed in a standard thermocycler with the following conditions: 1 min 94 °C, 40 cycles of 15 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, followed by a final extension for 10 min at 72 °C and quickly cooled to room temperature. To gauge the fragment size produced after amplification, 5 µl of the PCR products were analysed by 1.0–2.0% agarose gel electrophoresis.

5 µl of PCR product (without purification) was digested with the enzymes *Alu*I and *Sau*3AI in separate reactions according to the manufacturer's instructions. Digested PCR products were separated by agarose gel electrophoresis.

[5, 6] The use of the brand Applied Biosystems AmpliTaq Gold DNA polymerase and 9600 DNA thermocycler in this diagnostic protocol implies no approval of them to the exclusion of others that may be suitable. This information is given for the convenience of users of this protocol and does not constitute and endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.

Restriction fragment sizes produced by *T. palmi* when the COI fragment is digested with *Alu*I and *Sau*3AI are as follows:

*Alu*I:	291 and 194 bp
*Sau*3AI:	293, 104, 70 and 18 bp.

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27:2006.

In cases where other contracting parties may be adversely affected by the diagnosis, the records and evidence (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.

6. Contact points for further information

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2009-05 SC recalled draft text to TPDP
2009-11 SC approved revised draft for MC
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