RESEARCH

Low Temperature–Scanning Electron Microscopy to Evaluate Morphology and Predation of *Scolothrips Sexmaculatus* Pergande (Thysanoptera: Thripidae) against Spider Mites (Acari: Tetranychidae: *Tetranychus* spp.)

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Abstract: This paper reports the potential usefulness of low temperature-scanning electron microscopy (LT–SEM) to evaluate morphology and predation behavior of the six-spotted thrips (*Scolothrips sexmaculatus* Pergande) against the two-spotted spider mite [*Tetranychus urticae* (Koch)]. Morphological features of stage II larva, pupa, and adult six-spotted thrips are imaged for the first time, including fine detail of (1) chemoreceptors on larval and adult antennae and (2) structural differences of the mesothoracic spiracle (or the stigmata). Aspects of predation by the six-spotted thrips on the two-spotted spider mites are seen for the first time using LT–SEM. Based on these images, two possible functions of the barbed setae on the six-spotted thrips may be (1) its potential to eliminate sticky exudates on webbing laid down by the mite, and (2) barbs may redirect web strands away from the body of the thrips as it approaches its prey.

Low temperature–scanning electron microscopy (LT–SEM) is an innovative technology for studying arthropod specimens in a natural state, using fast freeze or cryofixation with liquid nitrogen (Echlin et al. 1970, Echlin 1992). Cryofixation is a technique for fixating and stabilizing biological materials as the first step in specimen preparation for electron microscopy. The method involves ultra-rapid cooling of small samples to the temperature of liquid nitrogen (–196 °C), instantly stopping all motion and metabolic activity and preserving the internal structure by freezing all fluid phases into a solid state. The ultimate objective is to freeze the specimen so rapidly (at 10^4 to 10^6 K per second) that ice crystals are unable to form or are prevented from growing large enough to cause damage to the external ultrastructure of the specimen. Scanning electron micrographs that result from this specimen preparation consistently display images of the specimen in a state that are essentially unaltered or undistorted from its natural state. A variety of subject material has been studied with LT–SEM technology, including parasitic nematodes (Giblin-Davis et al. 2001), plant-feeding mites (Ochoa et al. 2011), western flower thrips, *Frankliniella occidentalis* Pergande (Vestergaard et al. 1999), tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Dickens et al. 1995), honey bee mites (Ochoa et al. 2005), fire ants (Renthal et al. 2003), corn earworm *Helicoverpa zea* (Boddie) (Raina et al. 2000), goldeneyed lacewing, *Chrysopa oculata* Say (Zhang et al. 2004), and even snow flakes (Wergin et al. 1996).

Recently, LT–SEM technology has been used to study mites (Acarina) of agricultural importance. Using different groups of mites as test subjects. Achor et al. (2001) compared four preparation techniques used in ambient temperature–scanning electron microscopy (AT–SEM) with those of LT–SEM. They found LT–SEM generally to be superior to AT–SEM. Using LT–SEM techniques to observe honey bee tracheal mite, Ochoa et al. (2005) described new methods of the mite's locomotion and provided new information on morphology that may have taxonomic implications in the family Tarsonemidae.

Reinforcing the work of Funk et al. (2005), Dowling et al. (2010) pointed out the importance of specimen vouchering as a critical aspect of systematics. They went on to discuss the use of a single specimen for LT–SEM and DNA extraction to provide full documentation of all external characteristics of an organism and ample whole genomic DNA extraction for DNA sequencing.

The two-spotted spider mite, *Tetranychus urticae* Koch, is considered one of the most economically important spider mites worldwide. This mite has been reported infesting more than 900 species of plants and trees, including many economically important species (Johnson and Lyon 1991, Bolland et al. 1998, Fasulo and Denmark 2010).

A notable behavioral adaptation of the two-spotted spider mite and other spider mites is the production of sticky webs usually deposited on the underside of leaves and on flowers on which the mites are feeding. This webbing serves in part to protect them from potential predators such as predatory mites and thrips (Tuttle and Baker 1968). Spider mites are commonly attacked by predator mites, five species of which are commercially available in the United States: *Phytoseiulus persimilis* Athias-Henriot, *Mesoseiulus longipes* (Evans),

Neoseiulus californicus (McGregor), *Galendromus occidentalis* (Nesbitt), and *Neoseiulus fallicus* (Garman) (Denmark and Evans 2011). They are also prey to several predaceous thrips, notably members of the genus *Scolothrips* Hinds.

In a recent review of the genus *Scolothrips*, Mound (2011) recognized 14 species, including 3 species found in the United States: *S. sexmaculatus* Pergande, *S. pallidus* (Beach), and *S. hoodi* Priesner. Mound (2011) suggested that *S. pallidus* and *S. hoodi* may be color variants of *S. sexmaculatus*, but because of the poor condition of available slide material to evaluate actual morphological characters, he deferred from synonymizing them. Gilstrap (1995) discussed the biology of *S. sexmaculatus* as predators of *Tetranychus* mites and related forms, and he reviewed their commercial availability as biological control agents under the name of "six-spotted thrips".

This paper is an attempt to reflect the work of Achor et al. (2001), who used mites to evaluate the value of LT–SEM in morphological and behavioral studies, by evaluating living *S. sexmaculatus* in association with *T. urticae*. To date, *S. sexmaculatus* has not been imaged with LT–SEM techniques; and during the course of our study, we encountered problems with these thrips not indicated in the those studies wherein mites were used as subject material. During the course of prey capture, thrips and mite were frequently dislodged from the substrate and lost in the freezing process. This study reveals several morphological features heretofore not seen under phase-contrast microscopy or AT–SEM. It also provides LT–SEM images of presumed behaviors in *S. sexmaculatus* that can be inferred, based on quick-freeze immobility of specimens caught in the act of interacting with mites and in a natural environment.

Materials and Methods

Specimens. A basic stock of six-spotted thrips (n = 3,000 specimens) and two-spotted spider mites (n = 5,000), along with rooted pinto bean (*Phaseolus vulgaris*) plants, were obtained from a commercial insectary (Valley Agri Services, Fresno, CA) that specializes in supplying biological agents for agricultural enterprises. Thrips and mites were maintained in separate containers; thrips were fed periodically with mites by excising the leaves most densely populated with mites and introducing them to the container housing the thrips. After 3 wk, the initial stock of mites was exhausted; and a local population of a second species of spider mite (*Tetranychus bimaculatus* Harvey) feeding on butterfly bush (*Buddleja davidii* Franch.) was then used to supplement the mite availability. LT–SEM images show interactions of thrips with both of these mite species.

Methods. Preparation of specimens. Leaves with spider mite webbing and having a density of 3–6 mites/cm^2 were cut with a razor to fit a 16 × 30 × 1 mm copper plate with a thin layer of Tissue Tek (OCT Compound, Ted Pella, Inc., Redding, CA), which acted as the cryo-adhesive upon freezing. Each cut sample included at least a portion of midrib, webbing, and mites. This process was replicated 10 times, and plates were placed close together in a Petri dish. About 100 thrips were then introduced into the Petri dish and allowed to disperse for 30 min. Interactions between thrips and mites were observed under a Wild W2 dissecting microscope. Whenever a thrips encountered a mite and began to feed, the plate on which it was interacting was lifted into a Styrofoam box housing a precooled (–196°C°) brass bar whose lower half was submerged in liquid nitrogen (LN_2); the plates were placed on this bar and instantly conductively frozen.

After 20–30 s, the holders containing the frozen samples were transferred to a LN_2 Dewar storage chamber for future use or cryo-transferred under vacuum to the cold stage in the prechamber of the cryotransfer system. Twenty such interactions were captured for observation in the LT–SEM microscope.

Because the nature of LT–SEM cryofixation imposes limits on what can be seen on any particular specimen, we arbitrarily selected those images that revealed morphological features with the greatest level of detail. The features we selected included mouthparts, mesothoracic spiracles, and antennal segments; representative images of these structures from larva II, pupa, and adult were presented for comparisons.

Preparation of specimens to observe predation. Leaves with mites underneath webbing were cut into sections, glued to stubs, and placed in Petri dishes for observation. After some initial locomotor

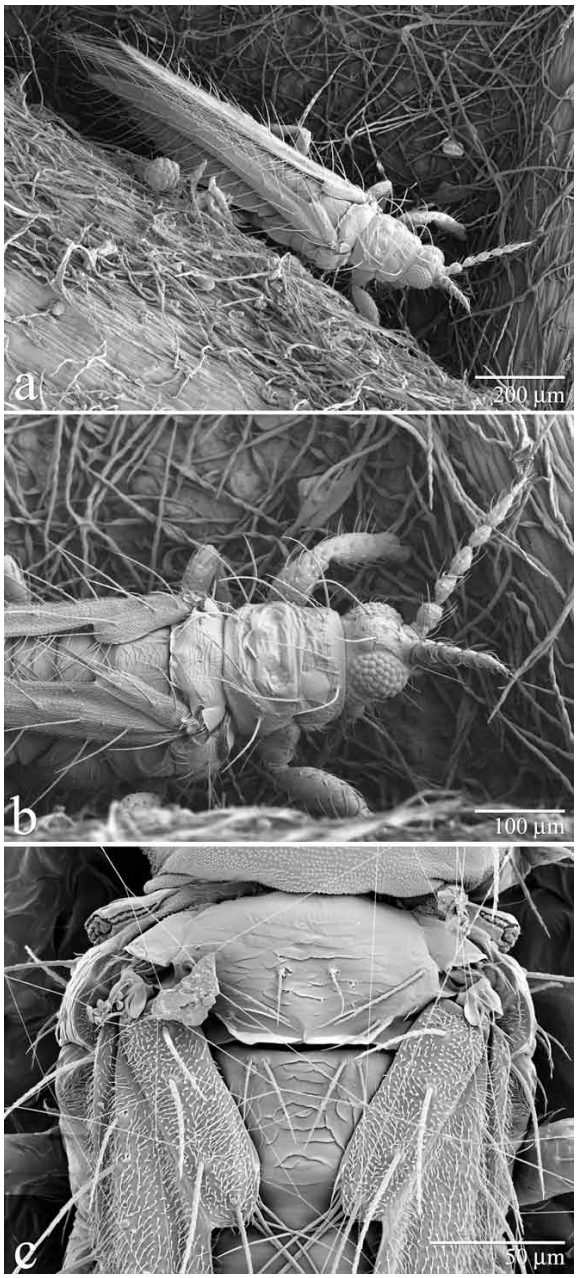

Fig. 1. LT–SEMs of morphological features of adult two-spotted thrips, *Scolothrips sexmaculatus*. (a) Habitus, specimen 1, right dorsolateral. On leaf of orange eye butterfly bush (*Buddleja davidii* Franch.). (b) Same specimen, in higher magnification, showing posture of wings, forelegs, antennae, and natural disposition of setae on the head pronotum and wing scale. (c) Mesonotum, metanotum, and forewing scales, dorsal view.

activity, the mites settled down after a few minutes and moved only occasionally. Thrips that had been deprived of food for 1–2 d were then introduced onto the mite-infested leaf samples. Samples were observed under a WildW2 microscope. When a thrips encountered a mite and appeared to be impaling the mite, the stub was moved to the cryofixing chamber and immediately frozen with liquid nitrogen.

LT–SEM observations were made using an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA) equipped with a Quorum CryoPrep PP2000 (Energy Bean Sciences, East Grandby, CT) cryo-transfer system. Samples were transferred to a LN2 Dewar chamber for future use or cryo-transferred under vacuum to a cold stage in the prechamber of the cryo-transfer system. Removal of any surface contamination (condensed water vapor) took place in the cryo-transfer system by etching the frozen specimens for 10–15 min by raising the temperature of the stage to –90 °C. Following etching, the temperature was lowered below –130 °C, and a magnetron sputter head equipped with a platinum target was used to coat the specimens with an ultrafine layer of platinum. The specimens were transferred to a precooled (–140 °C) cryostage in the SEM for observation. An accelerating voltage of 5 KV was used to view the specimens. Images were captured using a 4pi Analysis system (Durham, NC). Images were sized and placed together to produce a single figure using Adobe Photoshop 7.0 (at 600 psi).

Results

Morphology. The original intent of this project was to evaluate the efficacy of LT–SEM to resolve morphological incongruities and look for characters that could not be seen under lower resolutions and with distortions occurring in postmortem preservation of specimens.

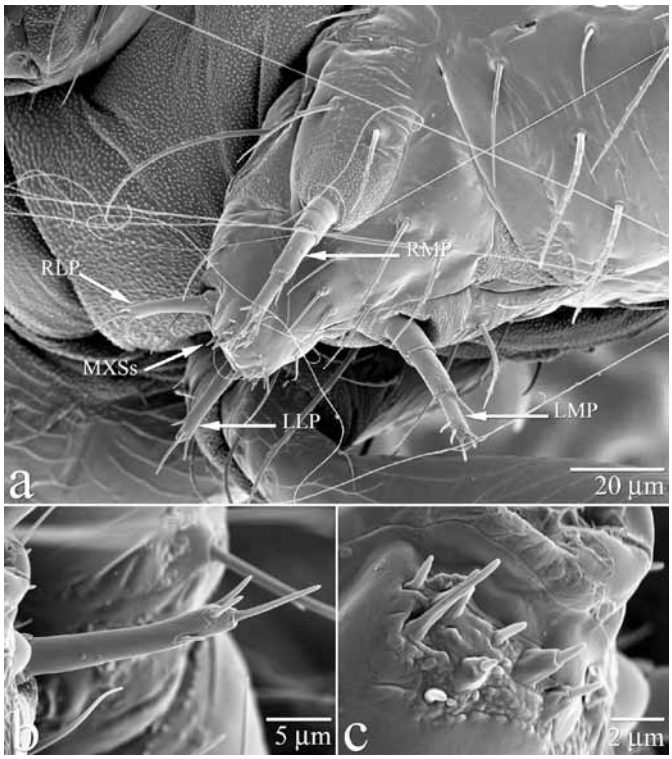

Fig. 2. Mouthparts of the two-spotted thrips. (a) Mouth cone, adult, specimen 2, right ventrolateral, showing right (RMP) and left (LMP) maxillary and right (RLP) and left (LLP) labial palps and clusters of sensory pegs (MXSs) at the base of paraglossa. (b) Stage II larva, left labial palp, medial view. (c) Same specimen, sensory pegs at the base of paraglossa.

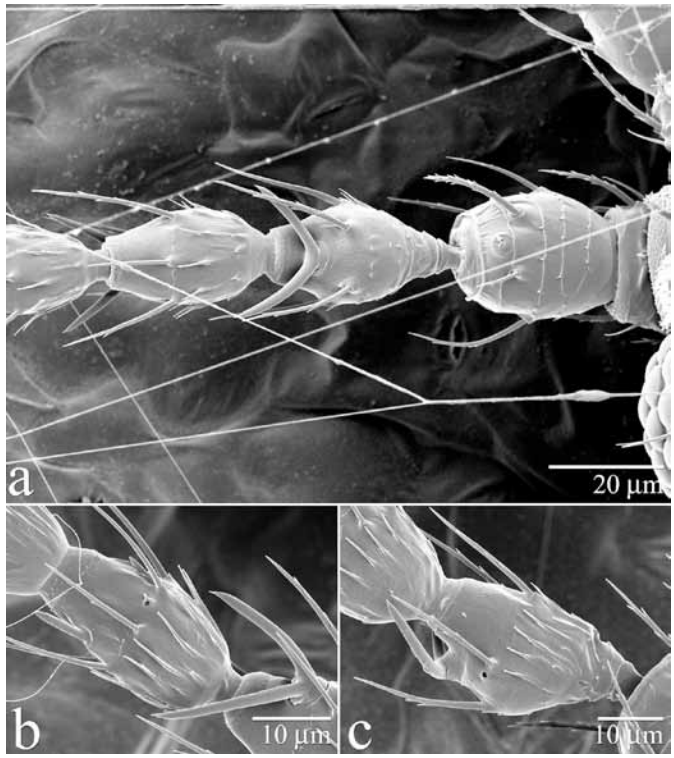

Fig. 3. Antennal segments of adult two-spotted thrips. (a) Left antennal segments I–V[in part], dorsal view, showing forked sense cones on III [on dorsal surface] and IV [on ventral surface, only apices of fork visible], and circular campaniform sensillum on segment II. (b) Right antennal segment V, ventral view, showing scolopidium and coeliconic sensilla, as well as forked sensilla of segment IV. (c) Right antennal segment III, ventrolateral view, showing forked sensilla arising from a-n extended base, and coeliconic sensilla.

A total of 180 images of *S. sexmaculatus* were captured as LT-SEMs. Most of the images were of high resolution (4,096 × 3,072 pixels/frame = 26 MB). Images included adult stage (n = 84 images, y = 10 specimens), pupa (n = 18, y = 4), and second-stage larva (n = 65, y = 6). From these images, 15 were selected to show features not seen to date on specimens of *Scolothrips*; several of the images of these insects are in natural settings and therefore somewhat novel views in contrast to those generated with ambient temperature SEMs (Nickle 2003, 2004, 2008, 2009), automontage photographs (Mound 2011), or standard line drawings generated from slide-mounted preparations (Stannard 1968).

Habitus views (Figs. 1, 5, 7). An adult six-spotted thrips is imaged sitting in the recessed border of the mid-vein of butterfly bush (Fig. 1a). The dorsal surface of this particular plant is covered with velveteen fibers, which appear as a coarse network of intertwining twisted cords. The eight-paired, elongated marginal setae on the pronotum (characteristic for all species of *Scolothrips*) in their natural state rise up vertically and gradually slope posteriorly, as do the long ocellar three setae (Fig. 1b). This configuration is usually lost in all slide-mounted material, wherein the setae are displaced and compressed at all angles because of the compressed cover slips and fluid mounting medium (e.g., Hoyer's or Balsam). This image shows natural body posture and configuration of setae, not seen on AT–SEMs or slide-mounted material. An active moving pupa (Fig. 5a, b) with somewhat balloonlike wing pads and bloated antennae and legs is seen on a pinto bean leaf. This is the first published image of the pupa of this species. Several views of the larva II (Fig. 7a, b, c), on

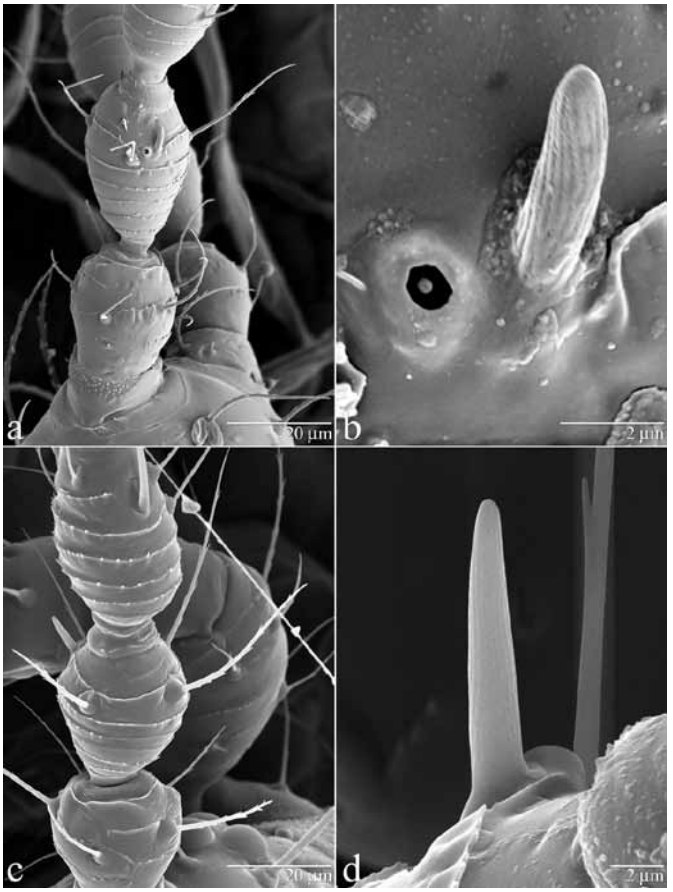

Fig. 4. Right lateral view of larva of two-spotted thrips. (a) Right antennal segment 3, specimen 2, ventrolateral aspect, showing solenidium and basiconic pit. (b) Higher detail of solenidium and basiconic pit. (c) Dorsomedial view of antennal segments II-IV. (d) Higher detail of solenidium on segment III.

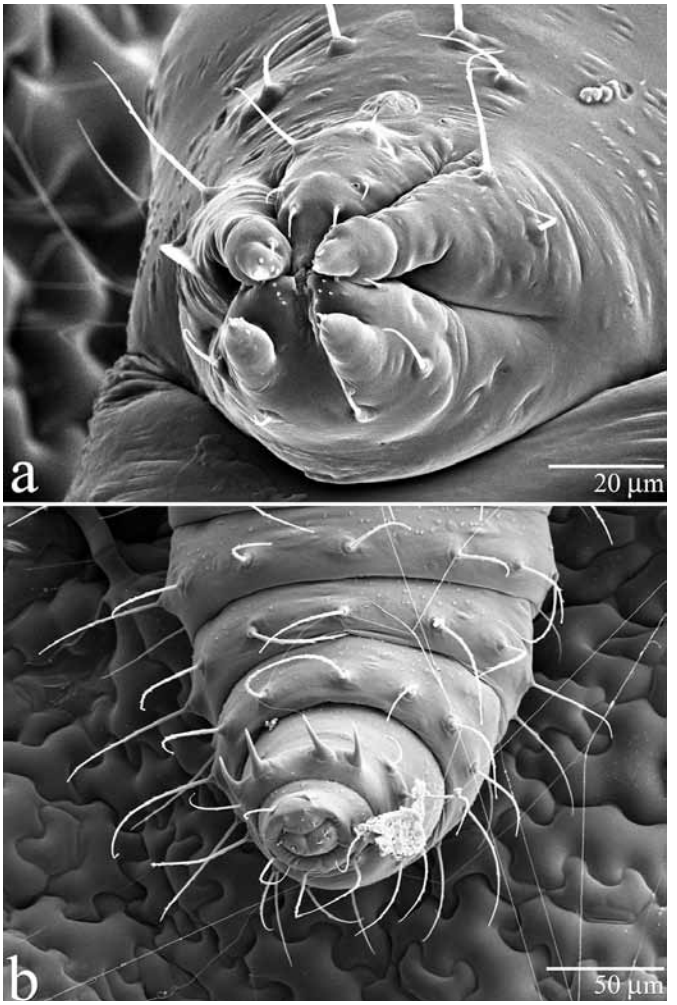

Fig. 6. Two-spotted thrips, pupa. (a) Mouth cone, ventrolateral view. (b) Tip of abdomen, right dorsolateral view.

a pinto bean leaf, are seen for the first time in a natural state, with six pairs of elongate tergal setae arranged around each body segment.

Mouthparts of adult (Fig. 2a) and pupa (Fig. 6a) are compared, as well as left lateral view of the larval maxillary palp (Fig. 2b) and clusters of sensory pegs at the base of the paraglossa of the larva (Fig. 2c). In the adult (Fig. 2a), the three-segmented maxillary palps (RMP, LMP), two-segmented labial palps (RLP, LLP), maxillary stipes (MS), prementum (PM), postmentum (PSM), and labrum (LM) are clearly visible and lack any distortion. In the pupa (Fig. 6a), mouthpart segments are not evident; the only distinctive features are the maxillary palps (MP) with some weak evidence of segmentation, and labial palps (LP). Dextral distortion characteristic in adult mouthparts is absent in the pupa. A clear image of all larval mouthparts was not available in this series, but eight sensory cones at the base of the paraglossa (Fig. 2c) are clearly imaged.

In traditional slide-mounted adults, sculpturing of the pronotum, mesonotum, and metanotum are often important diagnostic

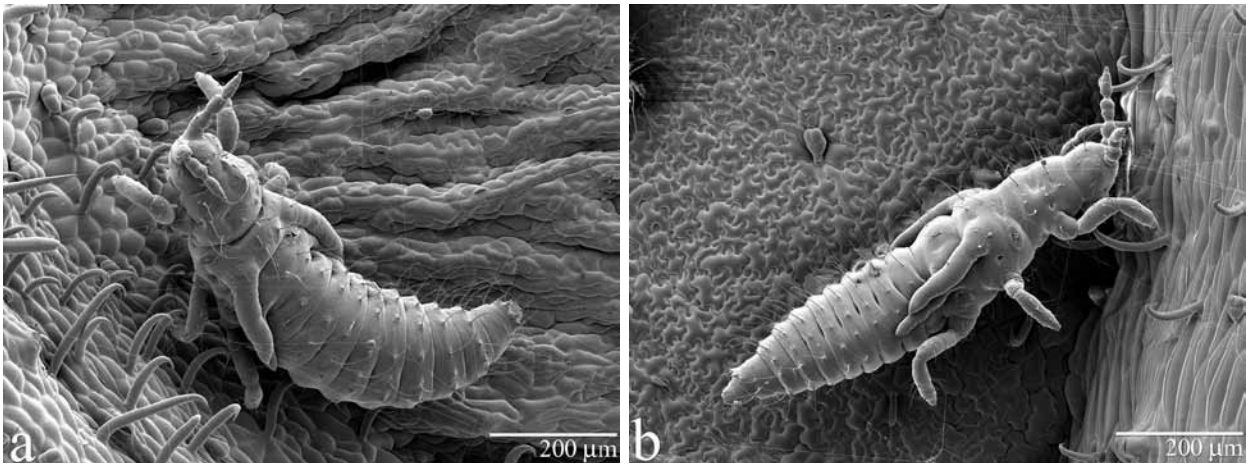

Fig. 5. Two-spotted thrips, pupa. (a) Specimen 1, dorsolateral view. (b) Specimen 2, right lateral view.

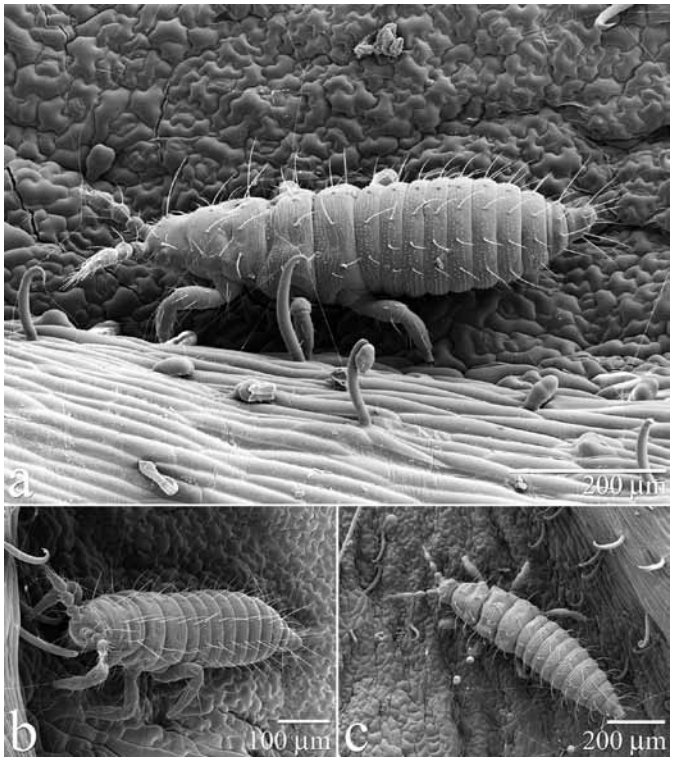

Fig. 7. Several views of stage II larvae of two-spotted thrips. (a) Left lateral view. (b) Dorsolateral view. (c) Dorsal view.

characters for separating species. In small soft-bodied thrips such as *Scolothrips* spp., however, these features are often obscured, even in well-preserved specimens. Fig. 1c shows meso- and metanotal sculpturing in detail and also the wing scale with three discal and three distal setae.

The ultrastructure of adult antennal segments I to V is shown in Fig. 3a. Five sensory structures were identified on segments III and IV (Fig. 3b, c). Structures indicated on antennal segment III include (Fig. 3b) the forked trichome; on segment IV, (Fig. 3c) three rows of tactile setae (the basal row of five very short hairs, the middle row of about 12 longer setae and the distal row of about 5 much longer barbed setae [2.5× longer than those in middle row]); two thin-walled chemoreceptors (only one totally visible in Fig. 3b, c), seen as a thick-bodied elongated cone; and one coeloconic chemoreceptor (seen as a minute peg emerging from a craterlike hole) (terminology of Slifer and Sekhon 1974) (also called sensillum campaniforme).

Antennae of the pupa are simple, with a reduced number of only five segments (Fig. 5). Antennal segment I to III on stage II larva is shown in Fig. 4a, c: segment III is barrel-shaped, with seven concentric bands; between bands 5 and 6 are four elongated barbed setae emerging from thick-walled bases; on the ventrolateral side is a single thin-walled chemoreceptor, seen here as a thick-bodied elongated cone. Under high magnification (4,000×), two specialized sensilla are seen on segment IV: the craterlike coeloconic chemoreceptor and the elongated, cone-shaped scolopidium (Fig. 4d).

One of the most complex structures on the thorax of thrips is the mesothoracic spiracle. This paired opening on the posterolateral margin of the mesothorax is crescent-shaped, with a complex infrastructure. In the center is the fleshy circular atrium. The ventral margin of the crescent is notched beneath the central atrium. On either side of the atrium is a network of 7–10 amorphous elongated plates (peritreme) connected to each other and to the margins of the crescent by a few very fine membranes. In *S, sexmaculatus*, this spiracle is similar in shape and complexity in the adult (Fig. 8a, b) and pupa (Fig. 9a, b), but somewhat less developed in the larva (Fig. 10a). An image of the spiracle on larval abdominal segment VIII is provided to indicate the greater complexity of the mesothoracic spiracle.

Predatory behavior. Our intention in using LT–SEM was to capture images of interaction of two-spotted spider mites with six-spotted thrips that would yield new information about predation behavior. This was difficult to accomplish. Although 24 encounters were observed under the stereo microscope and gently transferred to the cryofixing chamber, only 8 retained specimens attached to the plates for images.

Aspects of predation of the six-spotted thrips on spider mites were captured in Fig. 11–13. In Fig. 11a, *S. sexmaculatus* just before cryofixation was on top of the mite (*Tetranychus* sp.) but had stepped off by the time activity was frozen. The mite appears to have been pulled off its footing and held in place by the right foreleg of the thrips.

Both *S. sexmaculatus* and *T. urticae* have similar barbed setae covering their bodies (Fig. 11b). A possible function of the barbs on these setae on mites may be to facilitate movement around and beneath the webbing laid down on the leaf surface as a defense by

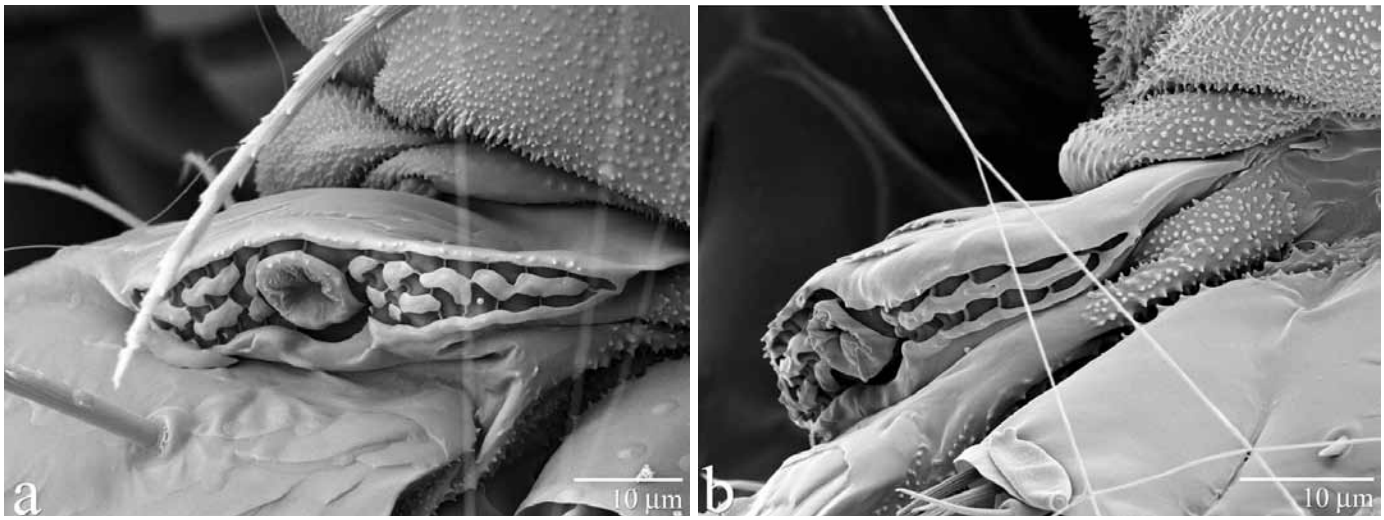

Fig. 8. Variation in morphology of mesothoracic spiracles (stigmata) on a single individual of two-spotted thrips, pupa. (a) Left spiracle, dorsal view. (b) Right spiracle, dorsal view.

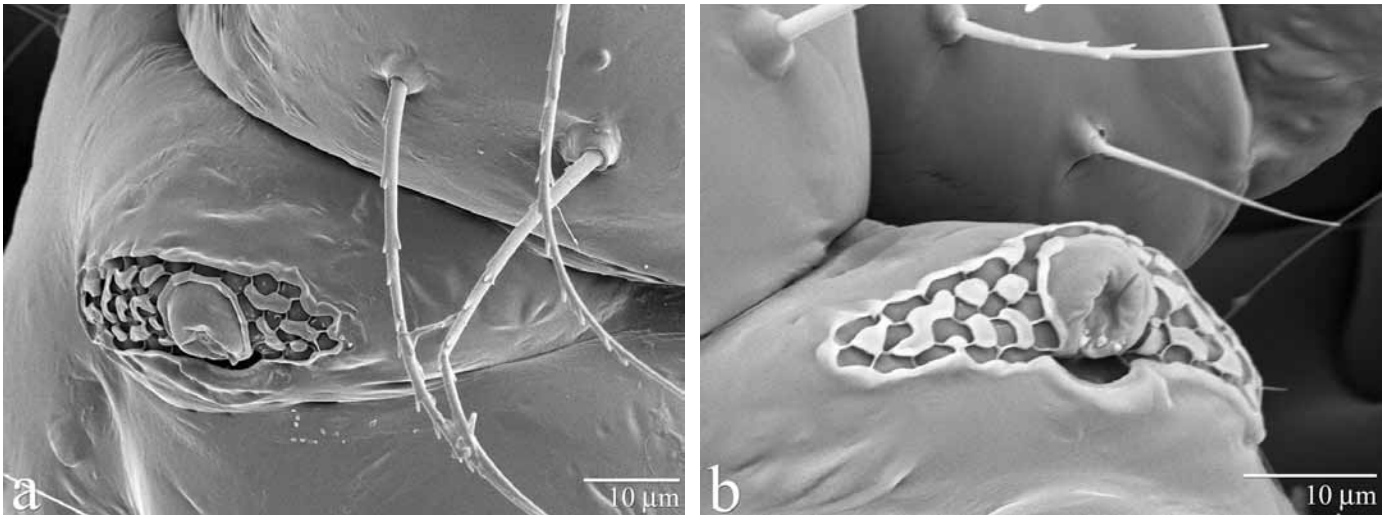

Fig. 9. Variation in morphology of mesothoracic spiracle (or stigmata) of 2 adult two-spotted thrips. (a) Specimen 1. (b) Specimen 2.

the mites. It is possible that the same kind of setae have evolved within *Scolothrips* as an adaptation to overcome the mite's defense mechanism. In Fig. 11b, the foreleg of this six-spotted thrips is seen pushing down on the body of the mite, and it appears as though the setae on the lateral margin of the leg of the thrips are displacing webbing aside to get access to the mite. It also suggests another function: The same web strand on which the mite is channeling its movement through the webbing is being used by the thrips to channel the mite's movement and detect the location of the mite.

Another function of the barbs on setae of *S. sexmaculatus* is suggested in Fig. 12a, b. Barbs on pronotal and mesothoracic setae (Fig. 12a) have caught a web strand and channeled it over the body of the thrips. On closer examination (Fig. 12b), the barbs appear to have an additional function: scraping the sticky proteinaceous material off the web strand as it passes through the barb, leaving a strand that, though still intact, may not retain the capacity to stick to intruders (potential predators, including, but not limited to, thrips). The leading strand (in forecenter of figure) bears several droplets of sticky material, which appear to have accumulated at base of several barbs; the trailing portion of the web strand (left of figure) appears to be clean and devoid of sticky material.

Fig. 13a depicts the midleg of a six-spotted thrips pushing four web strands away from the axis of the body of the thrips, seeming to clear the way for it to gain access to its prey. The mite prey (just out of the picture to the right of the leg) is also attached to these same four strands, and it is possible that the thrips is using vibrations or movements of these strands as a guide to direct nearby potential prey.

A successful encounter (Fig. 13b), in which a thrips (with damaged antennae)—underneath an unusually dense mat of webbing, feeding for some time on a mite and having sucked out most of its body contents—is seen with its head and mouthcone directed forward toward the carcass of the mite.

Discussion

In this study numerous morphological features of *Scolothrips sexmaculatus*, heretofore not observed, were revealed in high resolution by using LT–SEM technology. Also, for the first time, several aspects of predation were recorded: tapping onto the back of its prey, possible functions of barbs on the setae of the six-spotted thrips, and a first LT–SEM image of successful feeding by the six-spotted thrips on the two-spotted spider mite.

A problem was encountered, however. Many successful captures by the six-spotted thrips of the two-spotted spider mite were lost, when the plate on which this activity was being observed was transferred to the freezing platform. Two reasons for this loss are possible: (1) as the plate was moved to the super-cooled platform, the thrips may have become intimidated by the change in its environment and

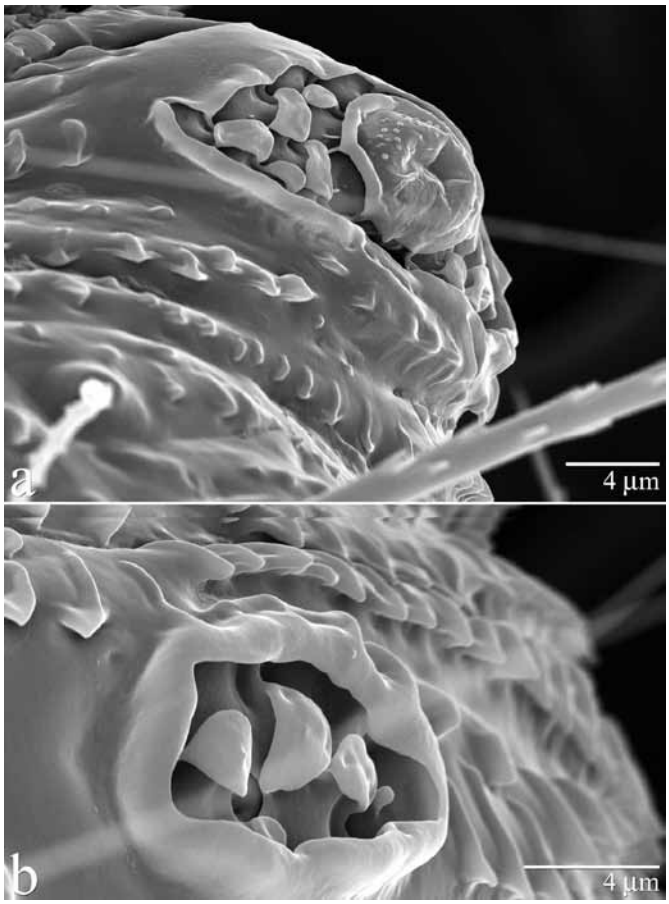

Fig. 10. Spiracles of larva of two-spotted thrips. (a) Right mesothoracic spiracle, dorsal view. (b) Right spiracle, eighth abdominal segment.

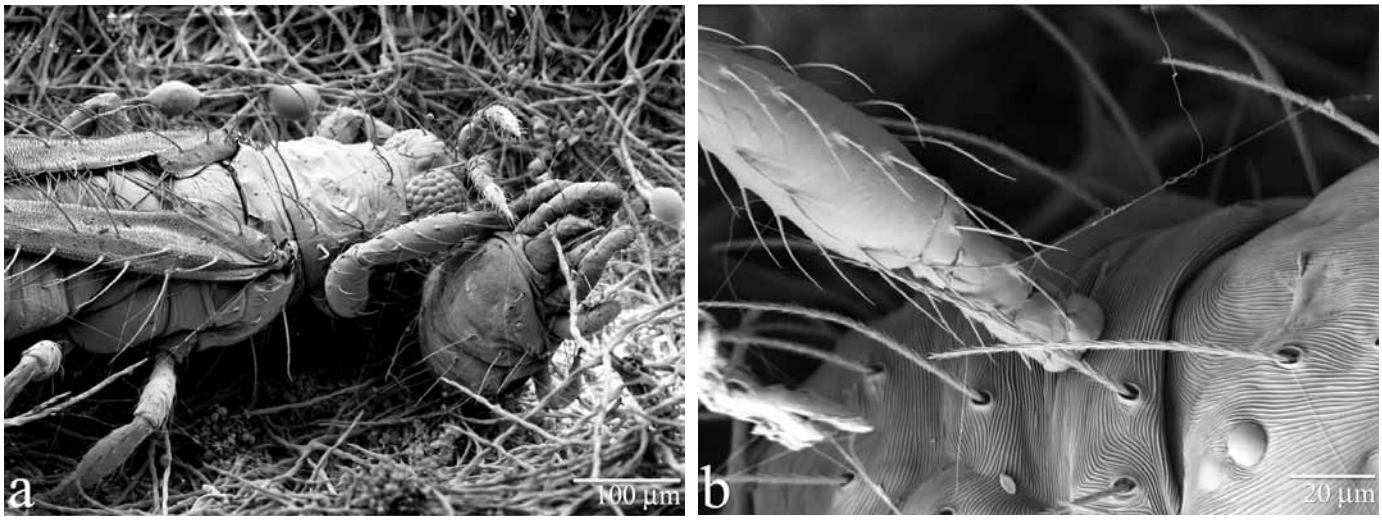

Fig. 11. Interactions between six-spotted thrips and two-spotted spider mite. (a) Thrips, on leaf of orange eye butterfly bush, pressing foreleg onto idiosoma of mite, which is actively moving away from contact. (b) A second encounter between different specimens, showing right foreleg of thrips pressed against idiosoma of mite. Webbing attached to the pilose setae on mite head is looped in several locations on the foreleg of the thrips, suggesting that the thrips may be using webbing to navigate to movement associated with mite activity beneath the webbing.

terminated its hold on its prey, or (2) because two of its six legs were not in contact with its base substrate, its hold on its substrate was not sufficiently strong to preserve it in position during the process of transferring the specimens to the stage of imaging the specimens. During the course of this process, in several arenas, specimens could be lost in any of the transfer arenas. Efforts are underway to reduce these losses and preserve these feeding behavior studies.

SEM technology offers several advantages over slide-mounted preparations of thrips: for example, external morphology can be seen with very high clarity and resolution, and the specimen can be viewed (with certain microscope stages) in all dimensions. Using four available techniques to prepare specimens for AT–SEM, Achor et al. (2001) demonstrated several failings: Shrinkage and distortions of soft tissue were common with all preservation techniques; this was especially true with studies of larval and pupal stages, wherein almost all body parts comprised soft tissue and little or no chitinous material was laid down in these developmental stages. Even in adult stages, some level of distortion was found to occur. Nickle (2003, 2008) used critical-point techniques; those using hexamethyldisilazane (HMDS) (Nation 1983) to dry soft tissues resulted in some distortions and wrinkling of chitinous areas. Furthermore, various body parts are often in unnatural positions because the insects usually were first killed in alcohol. Thus, legs, wings, antennae, setae, and other parts are in positions that do not reflect the actual posture of the insect in nature. Shrinkage and distortions do not occur with LT–SEM technology; specimens are quick frozen in their natural state; and legs, wings, setae, and other body parts also are not distorted. Plausible interpretations of the body movements of insects in LT–SEM images are possible, especially when they are combined with observations made under lower powers of magnification through a stereo microscope.

Two aspects of thrips morphology that are entirely lost to SEM technology are internal morphology of sclerotized structures and external color characters. SEM produces X-rays that are imaged, and therefore, all of the images are black and white. In cleared

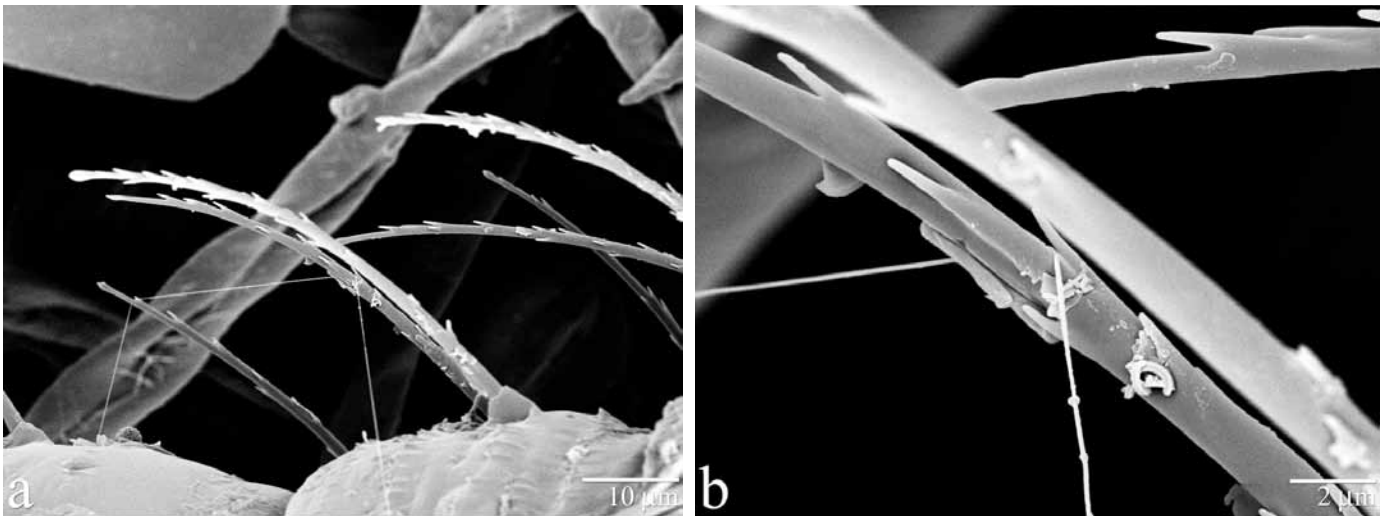

Fig. 12. More interactions between six-spotted thrips and webbing of the two-spotted spider mite, (a) showing a loop of webbing held above and away from body contact of the thrips with the aid of the barbs of several pilose setae. (b) Higher magnification of fore-loop of Fig. 12a, suggesting a second function of barbs: as webbing with defensive sticky material passes through the junction at base of barb, the material appears to be wiped clean (compare trailing portion of webbing with potion of webbing moving into barb area; also note accumulation of material at bases of several of the barbs).

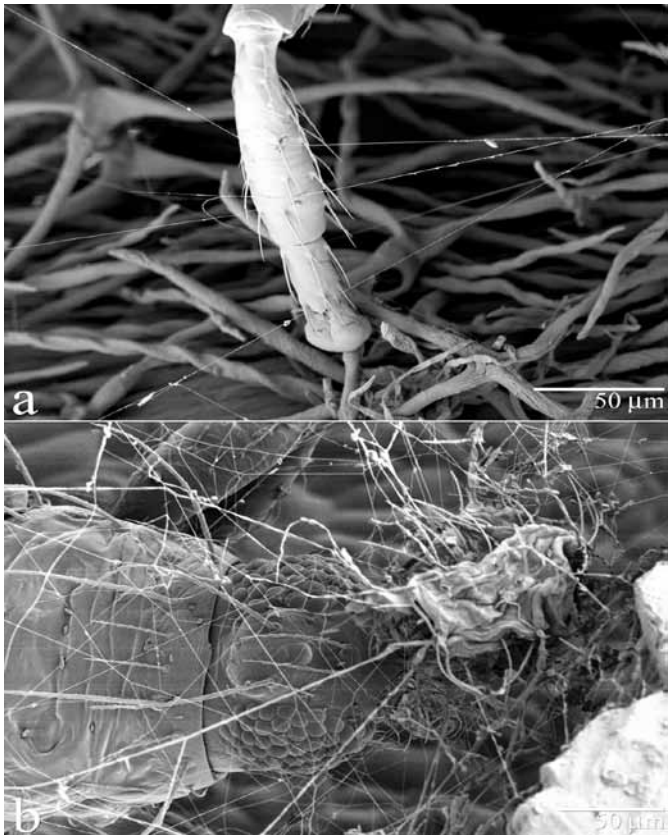

Fig. 13. (a) Midlegs of six-spotted thrips, showing three strings of webbing being redirected away from axis of body of thrips above and away from body by means of lateral pilose setae on the tibia and tarsus. Not in picture but to the right of image, these same strings of webbing are in contact with two-spotted spider mite. (b) A different specimen of six-spotted thrips in late stage of feeding on a two-spotted spider mite, desiccated from predation. Location of mite (and head and thorax of thrips) is beneath a particularly dense area of webbing). Head of thrips is directed forward toward its prey during feeding.

specimens prepared for slides, internal structures in the head, thorax, and abdomen, which are often used in taxonomic studies, are obliterated when the body is covered by the thin coating of platinum or other transmitting medium. Although external structures are seen in highest detail and clarity, coloration also is covered by the platinum coating. Mound (2011) used color dichotomies in 11 of 12 couplets to separate the 13 species in his key to the identification of *Scolothrips* spp. Only four morphological dichotomies were used: (1) presence or absence of ocellar setae I and II, (2) sculpturation of metanotum linear or reticulate, (3) pronotum without or with at least one pair of posteromedial discal setae, (4) male macropterous (either fully, hemi, or micropterous) or apterous. Mound (2011) indicated that reliance on color is based mainly on the fact that most museum specimens are poorly mounted and inadequately cleared. To overcome this problem when using specimens for SEM, it is advisable to photo-record the insect's color while observing it under a stereomicrocope. If the sample contains numerous specimens, some should be prepared as slides using standard improved techniques developed by Hoddle et al. (2008).

The two most significant advantages of LT–SEM over AT–SEM technology are its ability to examine soft-tissued organisms (such as *Scolothrips* larvae and pupae) and to evaluate aspects of behavior of living specimens in high definition. As more specimens of otherwise rare species of *Scolothrips* become available (Priesner 1950, Sakimura 1954, Han and Zhang 1982, zur Strassen 1999, Mound et al. 2010), improved slide-mounting techniques and specimens prepared for AT–SEM viewing are likely to reveal morphological characters for taxonomic studies. The use of AT–SEM technology has been used successfully in morphological studies on thrips for several years (Childers and Achor 1991, Kirk 1997, Moritz 1997) and recently for genus and species identification studies (Nickle 2003, 2004, 2008, 2009). Either AT–SEM or LT–SEM technology is certain to be useful to separate species because morphological characters will become more important than color to discriminate differences within species.

Future LT–SEM studies are anticipated: the quantification of images and DNA for voucher specimens to characterize new species of thrips (see Dowling et al. 2010); wing mechanics and take-off preparation (see Ellington 1980); and function and ultrastructure of the pretarsus of all stages of *Scolothrips* (see Heming 1972).

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