External mouthpart morphology in the Tenuipalpidae (Tetranychoidea): *Raoiella* a case study

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Abstract The use of low-temperature scanning electron microscopy (LTSEM) to study external mouthpart morphology in the Tenuipalpidae, in particular the genus *Raoiella*, has brought some aspects of the mechanics of feeding in this group into question. In addition, an LTSEM study on the specialized feeding behaviour of *Raoiella indica* Hirst (Tetranychoidea: Tenuipalpidae) revealed host plant use in this species could be affected by stomatal complex morphology.

Keywords External morphology · Functional morphology · Palmetto · *Sabal* ·
Spider mite · Stomata · Tetranychidae

Introduction

Wergin et al. (2000) and Achor et al. (2001) highlight several substantial advantages of low-temperature scanning electron microscopy (LTSEM) over the more traditional

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ambient-temperature SEM (ATSEM) for studying soft-bodied arthropods. The greatest advantage is the almost perfect preservation achievable with LTSEM. There is no shrinkage or collapsing of tissues, structures or specimens, and the turgidity of a live specimen is preserved and maintained. Because specimens are instantaneously frozen in situ, LTSEM not only offers an extraordinarily detailed glimpse of their micromorphology but also of their behaviour. The discovery, made possible by the use of LTSEM, that plant feeding flat mites in the genus *Raoiella* (Tetranychoidea: Tenuipalpidae) actively target the stomatal apertures of their hosts when feeding (Fig. 1) (Ochoa et al. 2011) provided a catalyst for further tenuipalpid feeding studies using LTSEM.

Phytophagous mites in the genus *Raoiella* Hirst (Tenuipalpidae) have attracted recent research interest due to the rapid spread of one pest species, red palm mite (RPM), *R. indica* Hirst. Most species in the genus *Raoiella* feed on plants in the family Myrtaceae (Ochoa et al. 2011; Dowling et al. 2012), but RPM feeds most commonly on species of palms (family Arecaceae). RPM has been devastating palm crops across the world and is currently spreading aggressively throughout the Americas (Flechtmann and Etienne 2004; Kane et al. 2012; Kane and Ochoa 2006; Rodrigues et al. 2007; Dowling et al. 2008). Since its arrival in the Caribbean and southeastern USA, RPM has been recorded on an ever increasing number of palm species in a broad range of genera including *Cocos*, *Phoenix*, *Chamaedorea* and *Veitchia* (Arecaceae), in addition to several other monocot hosts in different families including *Musa* (Musaceae) and *Heliconia* (Heliconiaceae) (Rodrigues et al. 2007; Welbourn 2008). Early observations on RPM indicated the largest populations were located on coconut palms (*Cocos nucifera* L.), ranging from 300 to more than 4,000 individuals per colony, usually with multiple colonies per leaflet and millions of mites per
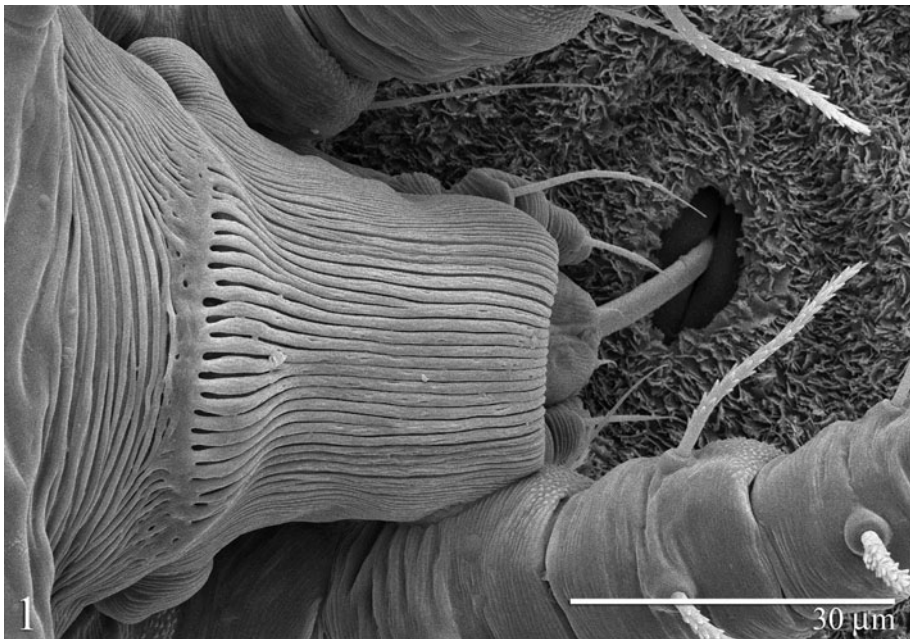

Fig. 1 *Raoiella* sp. nov. 3 (sensu Dowling et al. 2012), adult female—feeding via the stomata on host *Eucalytptus wandoo* Blakely. Note membranous ribbed collar is fully extended, obscuring most of the rostral gutter and palps

tree (Pons and Bliss 2007). Interestingly, RPM does not feed on palm species in the genus *Sabal*, commonly known as palmetto palms (*pers. obs.* Welbourn; Cocco and Hoy 2009), one of the most common and iconic genera of palms in the Caribbean basin. Triggered by the finding that all motile stages of several species of *Raoiella* feed via the stomata of their various dicot and monocot host plant species (Kane and Ochoa 2006; Ochoa et al. 2011), the current study of flat mite feeding was initiated to determine what prevents RPM from feeding on palmettos and enhance our understanding of flat mite feeding and mouthpart morphology. The mouthpart morphology of several species of *Raoiella* was studied and compared with a selection of other flat mite and tetranychoid species in a range of different genera (Table 1).

Methods and materials

Low-temperature scanning electron microscopy

Low-temperature SEM observations were made using an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA, USA) equipped with a Polaron Polar Prep 2000 (Energy Bean Sciences, East Grandby, CT, USA) cryotransfer system. Specimens of mites on leaves plus leaves were excised and placed on flat specimen holders consisting of 16 × 30 mm copper plates that contained a thin layer of Tissue Tek® (OCT Compound, Ted Pella, Redding, CA, USA), which acted as the cryo-adhesive upon freezing. The samples were frozen conductively, in a styrofoam box, by

Table 1 List of species examined for this study using LTSEM

Family	Genus	Species	Host	Origin
Tuckerellidae	*Tuckerella*	nr *knorri*	*Eucalyptus apodophylla*	Australia (N.T.)
Tetranychidae	*Oligonychus*	*coniferarum*	*Araucaria heterophylla*	USA (VA)
Tenuipalpidae	*Raoiella*	*indica*	various monocots	USA (FL), Caribbean
	Raoiella	sp. nov. 3	*E. wandoo*	Australia (W.A.)
	Raoiella	nr sp. nov. 3	*E. pleurocarpa*	Australia (W.A.)
	Raoiella	sp. nov. 4	*E. crebra*	Australia (QLD)
	Raoiella	sp. nov. 5	*Lophostemon lactifluus*	Australia (N.T.)
	Raoiella	sp. nov. 7	*Corymbia callophylla*	Australia (W.A.)
	Raoiella	sp. nov. nr *australica*	*Lophostemon confertus*	Australia (QLD)
	Aegyptobia	sp.	*Thuja* sp.	USA (D.C.)
	Australopalpus	*alphitoniae*	*Alphitonia excelsa*	Australia (QLD)
	Cenopalpus	*pulcher*	*Malus domesticus*	USA (OR)
	Larvacarus	sp. nov.	*Acacia caffra*	South Africa
	Lisaepalpus	*smileyae*	*Alphitonia excelsa*	Australia (QLD)
	Pentamerismus	sp.	*Thuja* sp.	USA (D.C.)
	Tenuipalpus	*pacificus*	*Dendrobium* sp.	USA (FL)
	Tenuipalpus	sp. nov.	*Syzygium australe*	Australia (QLD)
	Urigersonis	*bunyai*	*Araucaria bidwilli*	Australia (QLD)

All *Raoiella* species examined are new, except for *R. indica* (not present in Australia), and the associated species numbers refer to those listed in the molecular analysis by Dowling et al. (2012)

placing the plates on the surface of a pre-cooled (−196°C) brass bar whose lower half was submerged in liquid nitrogen (LN$_2$). After 20–30 s, the holders containing the frozen samples were transferred to a LN$_2$ Dewar for future use or cryotransferred under vacuum to the cold stage in the pre-chamber of the cryotransfer system. The specimens were etched inside the cryotransfer system to remove any surface contamination (condensed water vapour) by raising the temperature of the stage to −90°C for 10–15 min. Following etching, the temperature inside the chamber was lowered below −130°C, and the specimens were coated with a very fine layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled (−140°C) cryostage in the SEM for observation. An accelerating voltage of 10 kV was used to view the specimens. The scan rate of the electron beam was 80 s and images were captured at a resolution of 2,560 × 1,920 pixels. Images were sized and placed together to produce a single figure using Adobe® Photoshop 7.0.

Results and discussion

Tetranychoid feeding biology

All five families comprising the spider mite superfamily Tetranychoidea are strictly phytophagous on higher plants (Baker and Pritchard 1953; Jeppson et al. 1975). The gnathosoma of the Tetranychoidea is highly derived from the basic morphology of the Actinedida, as all major components are adapted for specialised feeding on vascular plants (Alberti and Crooker 1985). The few detailed anatomical studies available on the external morphology and functioning of the mouthparts of tetranychoid mites indicate that these adaptations are unique to the spider mite superfamily. Most research concerning feeding by tetranychoids has focused on the spider mites, Tetranychidae, with little known of the specifics of tenuipalpid feeding.

The movable digit of tetranychoid chelicerae has been modified to form an elongate stylet specialised for piercing plant tissues. When protracted during feeding, the cheliceral stylets lock together in a 'tongue and groove' arrangement to form a single hollow tube (Fig. 2) (Summers et al. 1973; Nuzzaci and de Lillo 1991; de Lillo et al. 2002), an adaptation characteristic of all tetranychoid families. The fusion of the cheliceral bases to form a stylophore (Fig. 3) that can be deeply retracted and extensively protracted, independently of the infracapitulum (previously referred to as the rostrum) is also unique to the superfamily Tetranychoidea (Lindquist 1985). The base of the stylophore is attached to a membranous structure which functions as a sheath that facilitates the protraction and retraction of the stylophore. This sheath or collar is reinforced by characteristic longitudinal ribbing in the families Tenuipalpidae and Linotetranidae and is developed to a greater extent in these families than in the Tetranychidae. It is particularly strongly developed in *Raoiella* (Fig. 1). In the Tetranychidae, the stylophore (Fig. 3) houses the stylets and fits into and slides along the rostral gutter, a dorsal longitudinal trough-like groove on the infracapitulum (Fig. 4). The stylophore of tenuipalpid species is not as well developed as it is in species of Tetranychidae, and subsequently the rostral gutter is reduced to a groove (Hislop and Jeppson 1976) with associated parallel longitudinal capitular lips (Fig. 4) (André and Remacle 1984; Nuzzaci and de Lillo 1989; de Lillo et al. 2002). The gutter does not function as a guide for the sliding stylophore in species of Tenuipalpidae (Hislop and Jeppson 1976), as these mites do not move their stylophore as extensively as do species of Tetranychidae. Even when the stylets are fully protracted the stylophore remains
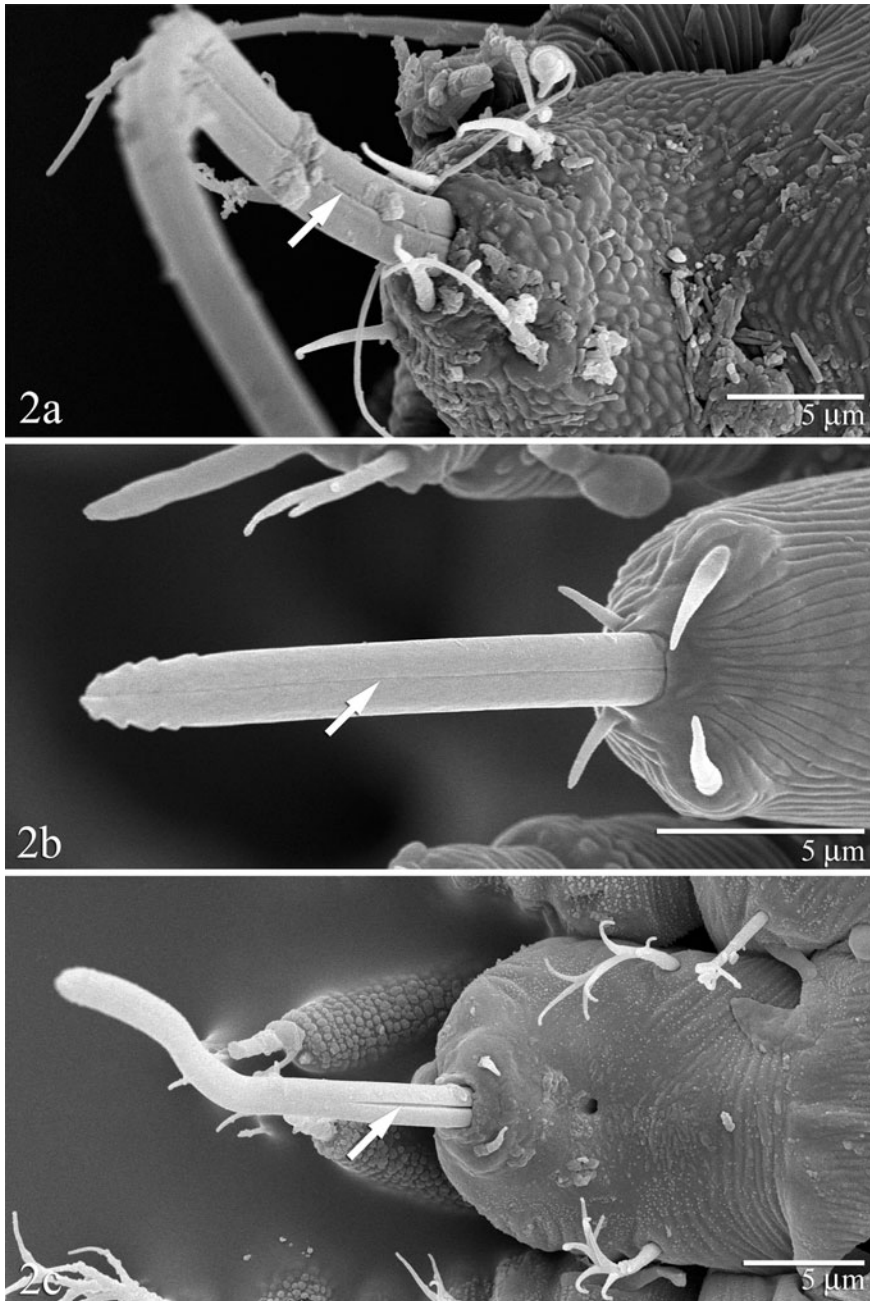
Fig. 2 Ventral infracapitulum, detail of protracted paired cheliceral stylets locked together in a "tongue and groove" fashion to form a single hollow tube; *arrow* indicates line of juncture—**a** *Raoiella* nr sp. nov. 3 (sensu Dowling et al. 2012) adult female, note three pairs of adoral setae; **b** *Larvacarus* adult female, note two pairs of adoral setae and oral flaps absent; **c** *Tenuipalpus* sp. nov. adult female, note single pair of adoral setae and clear view of the inferior oral commissure
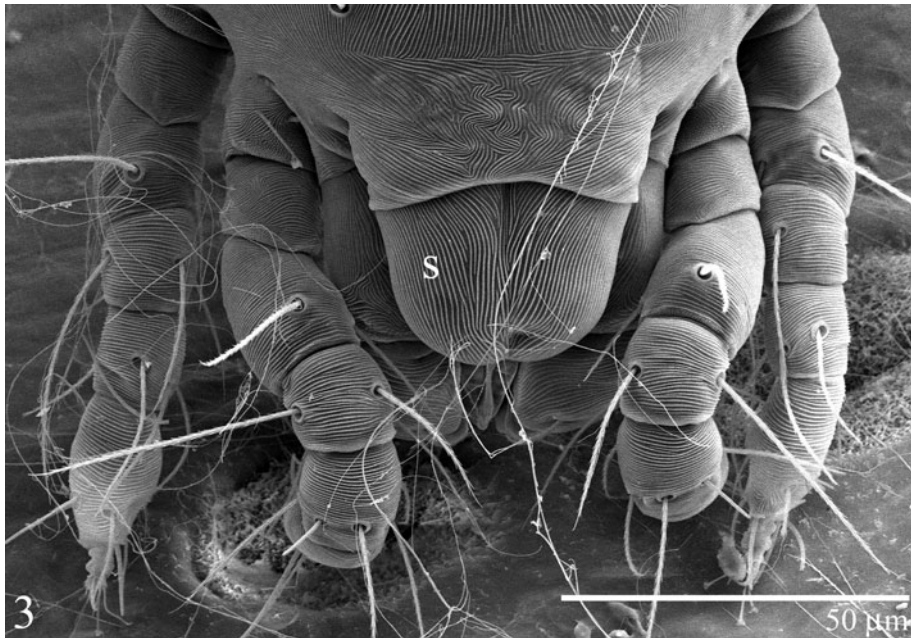
Fig. 3 *Oligonychus coniferarum* (McGregor) (Tetranychidae), deutonymph—stylophore (s) protruding over the infracapitulum, obscuring most of the rostral gutter

concealed within the ribbed collar (Fig. 5a) leaving the rostral gutter exposed (Figs. 4, 5a, b). Movement of the stylets appears to be independent of the stylophore. In addition to the employment of associated intrinsic muscle groups, the movement of the ribbed collar appears somehow connected with stylet movement. The ribbed collar is protracted when the stylets are protracted (Figs. 1, 5a) and retracted when the stylets are retracted (Fig. 5c), and during feeding, *Raoiella* mites repeatedly fully expand the ribbed collar out over the infracapitulum and retract it completely within the prodorsum (Plate 1).

The general tetranychoid infracapitulum is comprised of a broad base that extends into a rigid conical beak of various lengths, the apex of which bears the oral orifice (Fig. 6) that opens into the preoral cavity. The preoral cavity leads to the pharynx or food canal, thus complicating the location of an actual mouth in the Acari (Alberti and Coons 1999). This beak is particularly produced in the Tuckerellidae (Tetranychoidea) (Fig. 7). The infra-capitulum houses the pharynx and the inferior oral commissure (discussed later) and bears 1–3 pairs of apical adoral setae (*ad1-3*) that surround the oral orifice (three pairs in the genus *Raoiella*) (Figs. 2, 6). The adoral setae are thought to play a role in the fine-scale location of feeding sites (Nuzzaci and de Lillo 1989). A pair of stylet channels or cheliceral grooves (in which the stylets are sheathed and guided) and a median salivary duct, located deep within the rostral gutter, extend through the infracapitulum and terminate immedi-ately above the oral orifice (Summers et al. 1973; André and Remacle 1984; Nuzzaci and de Lillo 1991). Toward the apex of the infracapitulum, the walls of the rostral gutter curve upward to envelope the stylet channels. The walls of the rostral gutter (=lateral lips) close over and touch above the stylet channels without fusing together (Fig. 6a) (Summers et al. 1973), effectively sealing the stylets within a tunnel deep inside the infracapitulum, leaving only a dorsal longitudinal groove indicating where the walls of the gutter touch above the
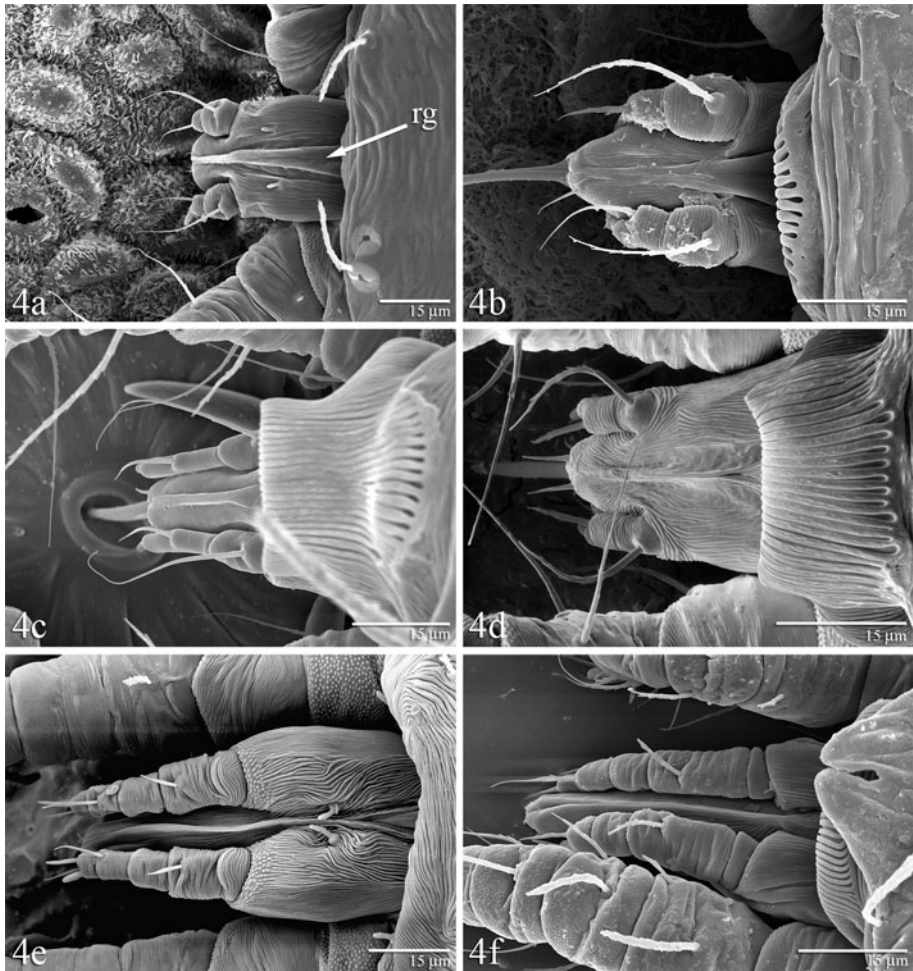
Fig. 4 The rostral gutter (rg—indicated in 4a) is visible as a longitudinal medial groove along the dorsal infracapitulum—**a** *Raoiella* nr sp. nov. 3 (sensu Dowling et al. 2012) adult female (ribbed collar fully retracted); **b** *Raoiella* sp. nov. 4 (sensu Dowling et al. 2012) adult female; **c** *Raoiella* sp. nov. nr *australica* adult female (ribbed collar partially extended); **d** *Raoiella indica* adult male; **e** *Larvacarus* sp. adult female; **f** *Aegyptobia* sp. adult female

stylets, forming the stylet or cheliceral groove (Figs. 4, 5, 6a, 8b). The physical act of forcing the stylets through this apical tunnel (distal section of the cheliceral groove) is thought to be the mechanism by which the stylets are forced to lock together to form the single tube during protraction prior to feeding. As the apical subcapitular tissue is strongly reinforced by annular sclerotisation (de Lillo et al. 2002), it would seem that this tunnel also offers some physical support to the stylet tube when piercing the leaf surface and during feeding. When retracted, the stylets of tetranychid mites are kept within the channels inside the rostral gutter (Summers et al. 1973). It is not known how tenuipalpid mites position their stylets while retracted, though slide preparations indicate that they are also held within the channels. In *Raoiella*, and other tenuipalpid genera, there is only one
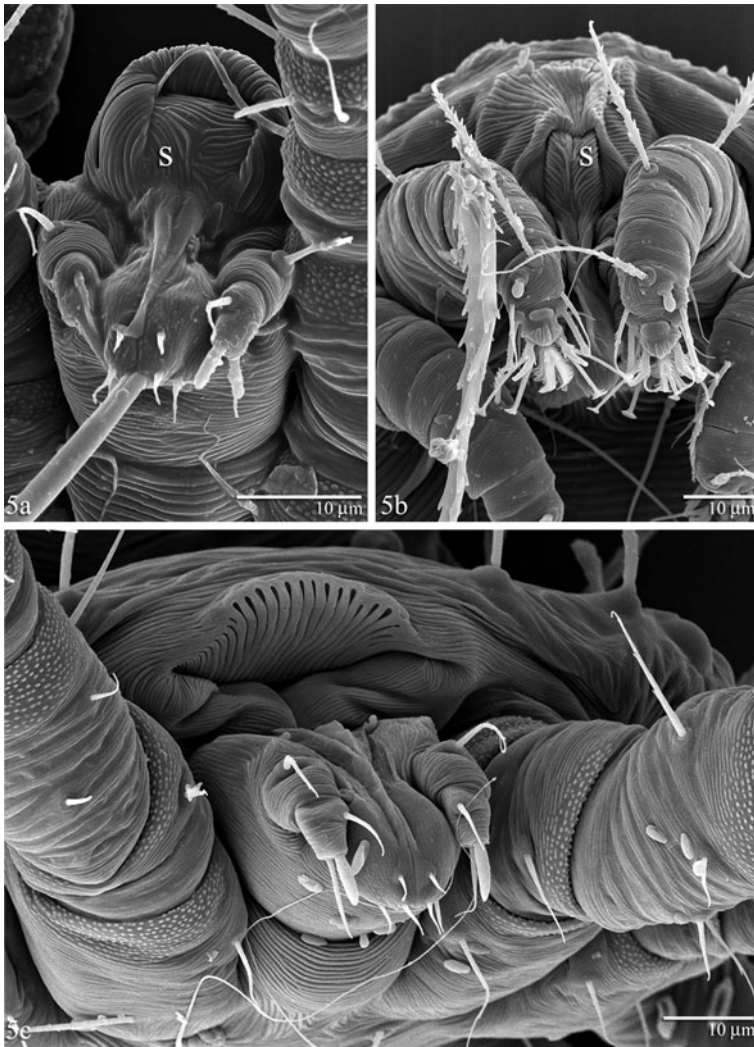
Fig. 5 Anterior view of tenuipalpid infracapitulum, indicating position of stylophore (s)—**a** *Raoiella* sp. nov. 7 (sensu Dowling et al. 2012) protonymph—stylophore (s) positioned within the extended ribbed collar, note protracted cheliceral stylets and fixed digits; **b** *Australopalpus alphitoniae* adult female, stylophore (s) positioned within the ribbed collar; **c** *Raoiella* sp. nov. 4 (sensu Dowling et al. 2012) adult female— ribbed collar and stylophore fully retracted within the propodosoma

opening at the apex of the infracapitulum, the oral orifice, through which the stylet tube is protracted and into which host fluid is sucked (Figs. 6, 8).

The inferior oral commissure, unique to tetranychoids, opens as a visible perforation (=rostral fossette, Summers et al. 1973) in the ventral cuticle of the infracapitulum anterior to the infracapitular setae, *m*, and links the pharynx to the exterior of the mite via a narrow sclerotised tubule (Nuzzaci and de Lillo 1991) (Figs. 2c, 6b, 7, 8b, d, 9). Although the absolute function of this structure remains unknown, it appears that the walls of the tubule, which are tricuspid-shaped in cross-section (Nuzzaci and de Lillo 1989), form a valve
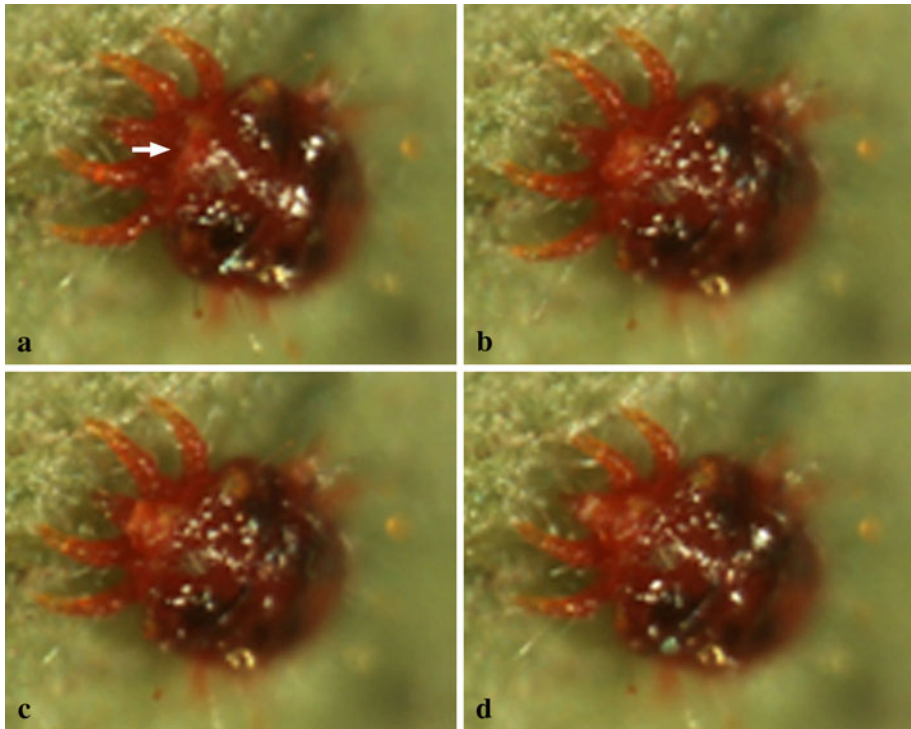
Plate 1 *Raoiella* sp. nov. 8 (sensu Dowling et al. 2012) adult female feeding on a species of *Eucalyptus* (Myrtaceae). Note the movement of the ribbed collar during feeding from **a** full retraction within the propodosoma (indicated by *arrow*) through to **d** full extension over the infracapitulum

system that closes during the decompression of the pharyngeal pump and opens during compression to allow a regular flow of plant fluid during ingestion (Nuzzaci and de Lillo 1991). The inferior oral commissure is presumed to eliminate any air bubbles that may be ingested with the food without disturbing the flow in the food channel (Baker and Connell 1963; Alberti and Crooker 1985; Nuzzaci and de Lillo 1989, 1991; Evans 1992). The opening and closing of the inferior oral commissure appears to be controlled by a perforated honeycomb-like valve located inside the external opening, as observed on *Raoiella* sp. nov. nr *australica* (Fig. 10). The commissure is often visible on slide-mounted preparations, though it is often obscured in SEM preparations, for example when fluid has been expelled from the opening during specimen preparation.

During feeding, spider mites protract and retract their stylophore several times, forcing the stylet tube through a single puncture site (Summers and Stocking 1972; Hislop and Jeppson 1976) resulting in very few puncture wounds relative to the number of palisade cells sucked empty by the mites (Summers and Stocking 1972). The tips of some tetranychoid stylets are armed with ornamentation that is assumed to assist with piercing and shredding the host tissue (Hislop and Jeppson 1976). For example, tiny evenly spaced projections have been observed on the apical antiaxial margins of the stylets of *Tetranychus evansi* Baker and Pritchard and *Brevipalpus obovatus* Donnadieu (see Fig. 2a in Hislop and Jeppson 1976). The tip of the stylets in *Raoiella* (Fig. 11) and several other genera, including *Tenuipalpus*, *Australopalpus*, *Lisaepalpus* and *Larvacarus* (Fig. 12), are
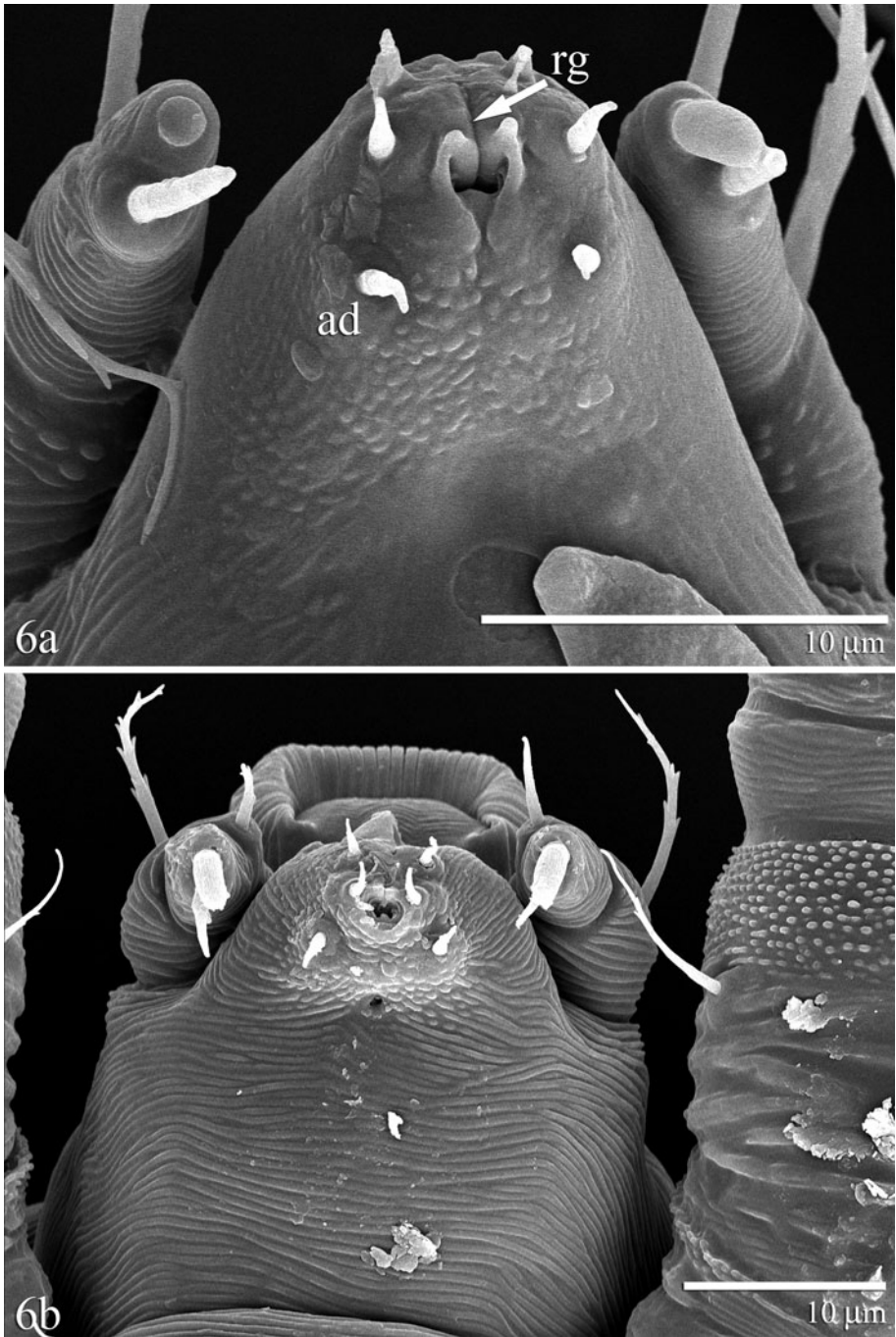
Fig. 6 Ventral infracapitulum with detail of single external oral orifice at apex, flanked by three pairs adoral setae (ad)—**a** *Raoiella* sp. nov. 5 (sensu Dowling et al. 2012) adult female—note rostral gutter (rg) joining oral orifice; **b** *Raoiella* sp. nov. 4 (sensu Dowling et al. 2012) deutonymph—note inferior oral commissure below oral orifice, stylophore concealed within ribbed collar
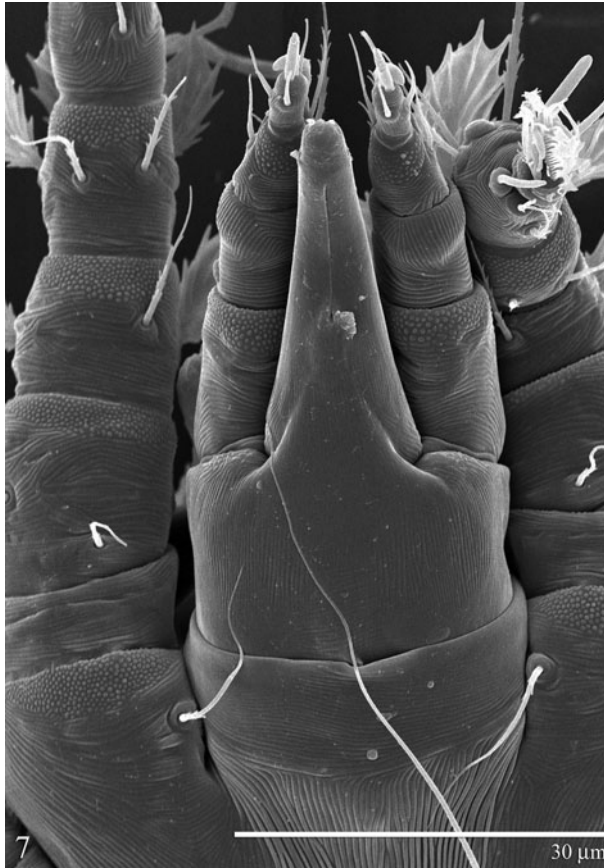
Fig. 7 *Tuckerella* sp. adult female ventral infracapitulum—produced into rigid conical beak. Note small inferior oral commissure centrally on cone (slightly obscured); infracapitular seta *m* absent; oral flaps absent

also ornamented with strong antiaxial serrations used for piercing host plant tissues. Interestingly, these serrations are also used by flat mites to anchor themselves to the leaf surface during moulting, as we have observed *Raoiella* anchored to the host leaf during moulting via the stylets inserted into a stomate (Fig. 13a). *Brevipalpus obovatus* [listed as *B. inornatus* (Banks)] (Morishita 1954) and *B. phoenicis* (Geijskes) (Haramoto 1969) have both been reported to anchor themselves during moulting by inserting their fully protracted stylets into the plant tissue, though the exact location of the insertion was not specified. Similarly, *Tenuipalpus pacificus* Baker mites anchor themselves to the leaf surface by inserting the stylets directly into the leaf epidermis (Fig. 13b).

Once a tetranychid mite has penetrated the host leaf surface with its stylets, the oral orifice is pressed against the plant surface by elevating the posterior half of its body (Baker and Connell 1963; Hislop and Jeppson 1976). At this moment, it is assumed that the mite retracts its stylets, pushes the labrum against the dorsal wall of the supralabral cavity to seal off the cheliceral groove from the food channel (André and Remacle 1984; Nuzzaci and de Lillo 1989), and tightly presses its oral orifice and oral flaps (see Fig. 1D *Tetranychus evansi*, 1E *Bryobia praetiosa* in Hislop and Jeppson 1976; Fig. 3c, e *Panonychus*
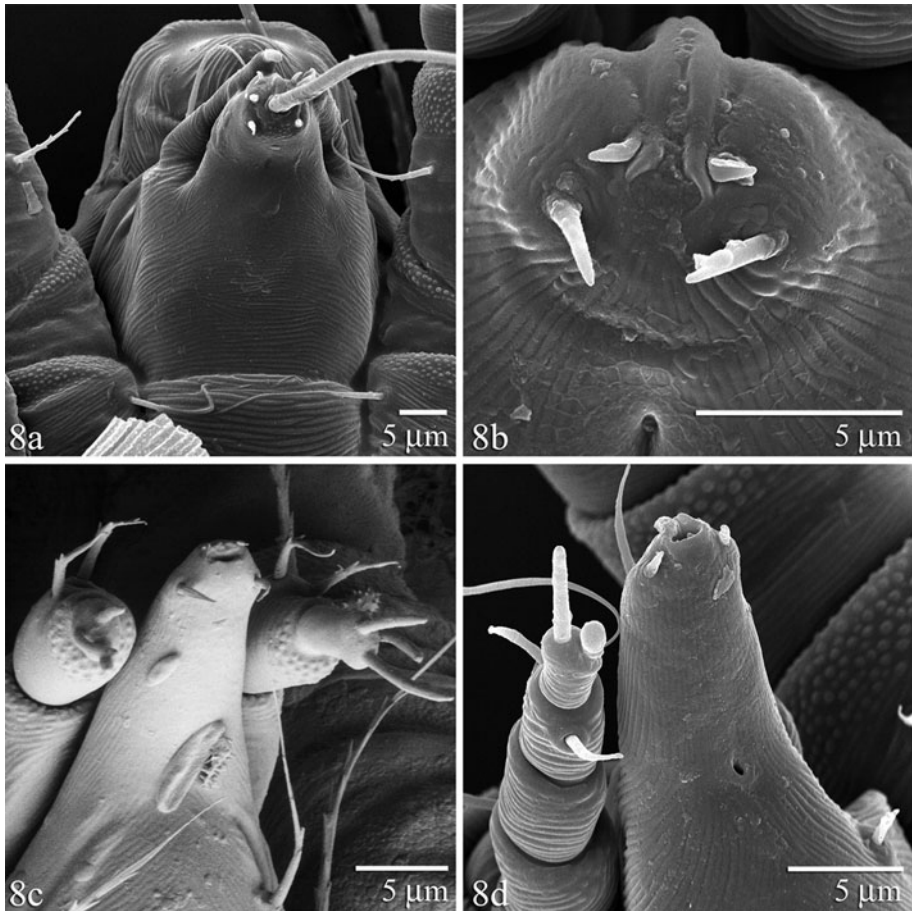
Fig. 8 A single oral orifice at infracapitulum apex, with oral flaps absent. **a** *Raoiella* sp. nov. nr *australica* adult female—stylets, stylophore and ribbed collar protracted; **b** *Urigersonis bunyai* adult female, detail of infracapitulum apex—two pairs adoral setae flanking oral orifice (actual orifice obscured by exuded fluid), note rostral gutter above oral orifice and inferior oral commissure below oral orifice; **c** *Cenopaplus pulcher* adult female; **d** *Australopalpus aphitoniae* adult female—note inferior oral commissure

citri in Razaq et al. 2000) to the fluid flowing from the puncture in an effort to create a vacuum under which the pharyngeal pump can function to suck plant fluids into the food channel or pharynx for ingestion (Hislop and Jeppson 1976; Alberti and Crooker 1985; Nuzzaci and de Lillo 1989). In a similar manner, the tenuipalpid mites *Tenuipalpus pacificus* (*pers. obs.*; Fig. 14a) and *Cenopalpus pulcher* (Canestrini and Fanzago) (Nuzzaci and de Lillo 1989, 1991) both press the apex of the infracapitulum to the plant surface during feeding. In addition, during feeding the food channel of *C. pulcher*, which is directly connected to the pharyngeal chamber, is hermetically sealed using specific anatomical features, ensuring a continuous flow of fluid occurs through the food channel towards the pharyngeal pump (Nuzzaci and de Lillo 1989, 1991). Despite these observations, the feeding method described for the Tetranychidae does not seem possible for all tetranychoid mites. As previously mentioned, mites in the genus *Raoiella* feed by inserting their stylets through stomatal openings in the leaf epidermis, not through the epidermis
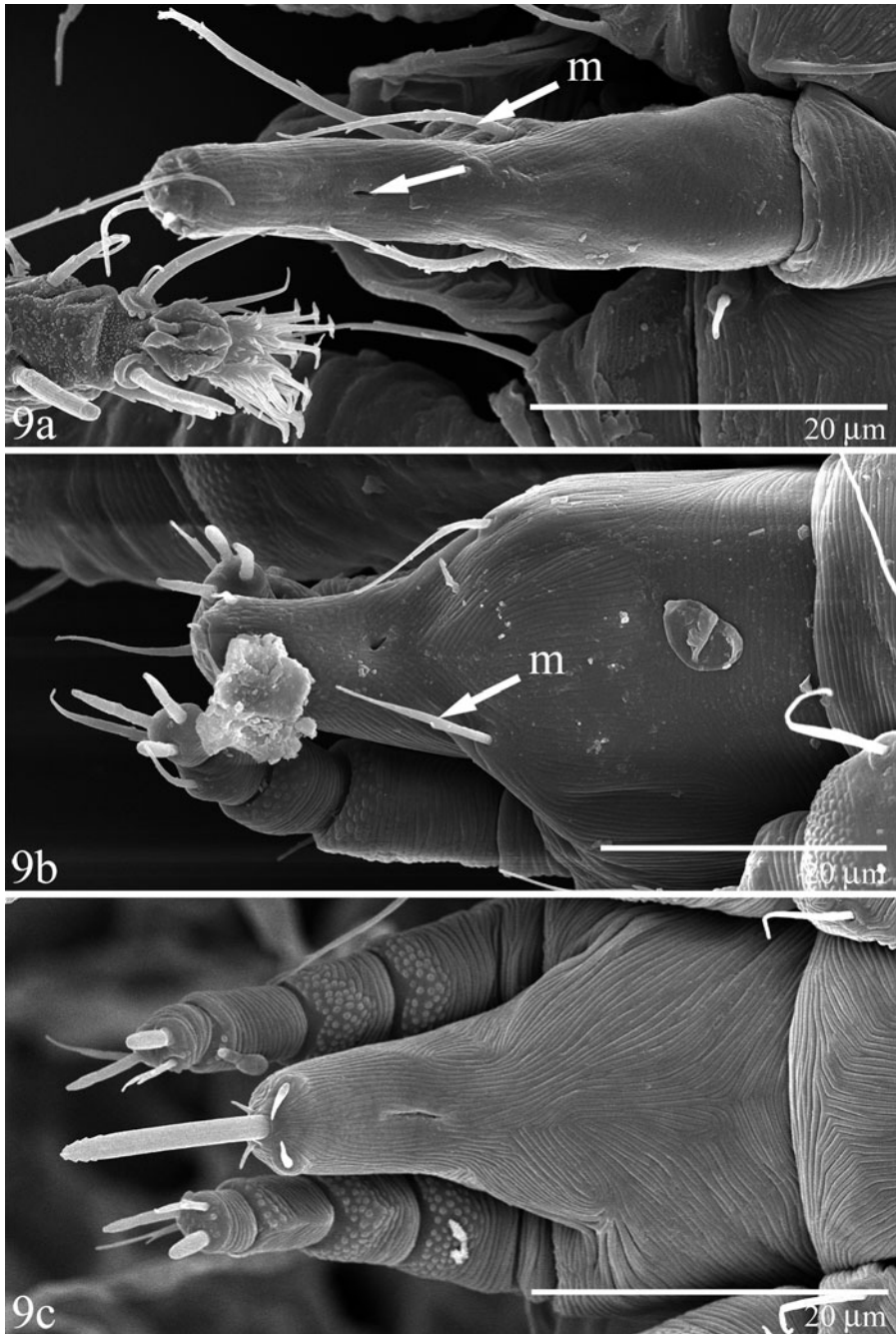
Fig. 9 The inferior oral commissure (=rostral fossette Summers et al. 1973) (indicated by *arrow* in 9a), unique to tetranychoids, is visible externally as a small perforation in the ventral cuticle of the infracapitulum, anterior to infracapitular setae *m*—**a** *Lisaepalpus smileyae* adult female, with an elongate infracapitulum, setae *m* inserted on lateral margins (indicated); **b** *Pentamerismus* sp. adult female, setae *m* indicated; **c** *Larvacarus* sp. adult female, note inferior oral commissure appears slit-like, setae *m* absent
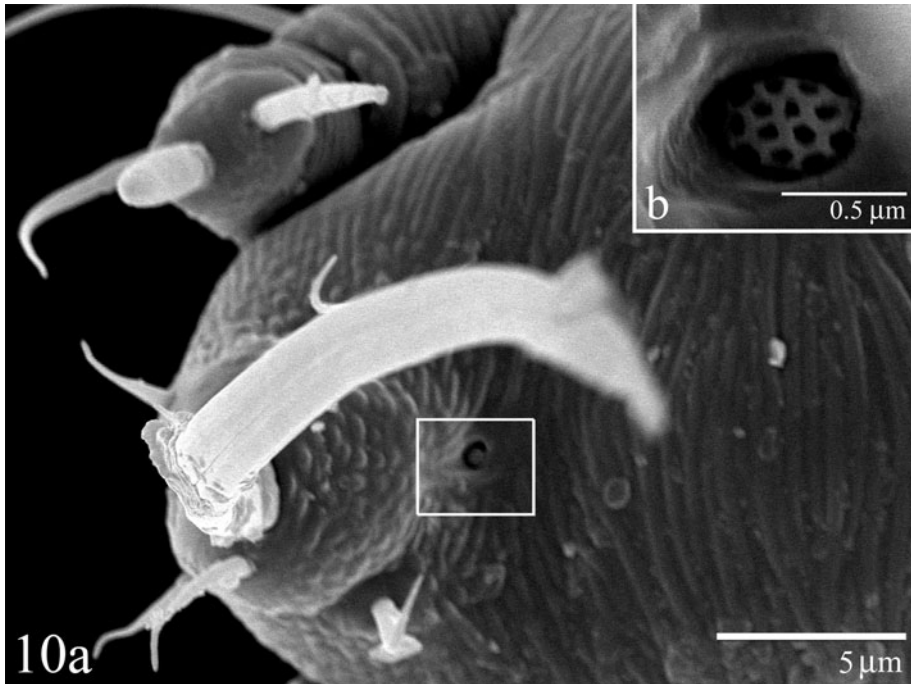
Fig. 10 *Raoiella* sp. nov. nr *australica* adult female—**a** Ventral infracapitulum indicating the inferior oral commissure; **b** Detail of the honeycomb-like valve visible inside the exterior opening of the inferior oral commissure, same specimen

itself (Ochoa et al. 2011; Figs. 1, 4c). The only way for their oral orifice to directly contact host plant cell fluids would be if the entire stomatal chamber filled with fluid and the mite inserted the apex of its infracapitulum inside the chamber to make contact with host fluids. *Raoiella* mites have been observed to touch their oral orifice to the surface of stomates (Fig. 14b), in addition to slowly rocking back and forth during apparent feeding bouts (*pers. obs.* JJ Beard and WC Welbourn), but they have never been observed to force the infracapitulum inside a stomatal chamber. If *Raoiella* mites feed by ingesting fluid directly through the oral orifice, the mites could be repeatedly penetrating cells inside the stomatal chamber in an effort to cause tremendous injury to the internal tissue and fill the chamber with fluid to facilitate feeding. Alternatively, the observed mites might not be feeding, but instead are simply probing the leaf surface in search of stomata.

   An alternative explanation of how tetranychoid mites ingest plant fluids lies in the functionality of the hollow tube formed by the interlocked cheliceral stylets. Summers et al. (1973) and André and Remacle (1984) believed that tetranychoid mites can actually suck plant material through the stylet tube. Hislop and Jeppson (1976) also tentatively proposed the possibility of using the stylet tube for feeding, because they observed that *B. obovatus* mites lack the oral flaps found in Tetranychidae that, in their opinion, are necessary to create a vacuum during feeding and concluded that host juices must be ingested in an alternate manner. The apex of the infracapitulum in the Tenuipalpidae and the Tuckerellidae has vestigial or no oral flaps (Figs. 2, 6a, 7, 8). Nuzzaci and de Lillo (1989) state that despite the lack of oral flaps, the infracapitulum of *C. pulcher* adheres well enough to the wounds on the leaf surface for the pharyngeal pump to operate. If oral flaps
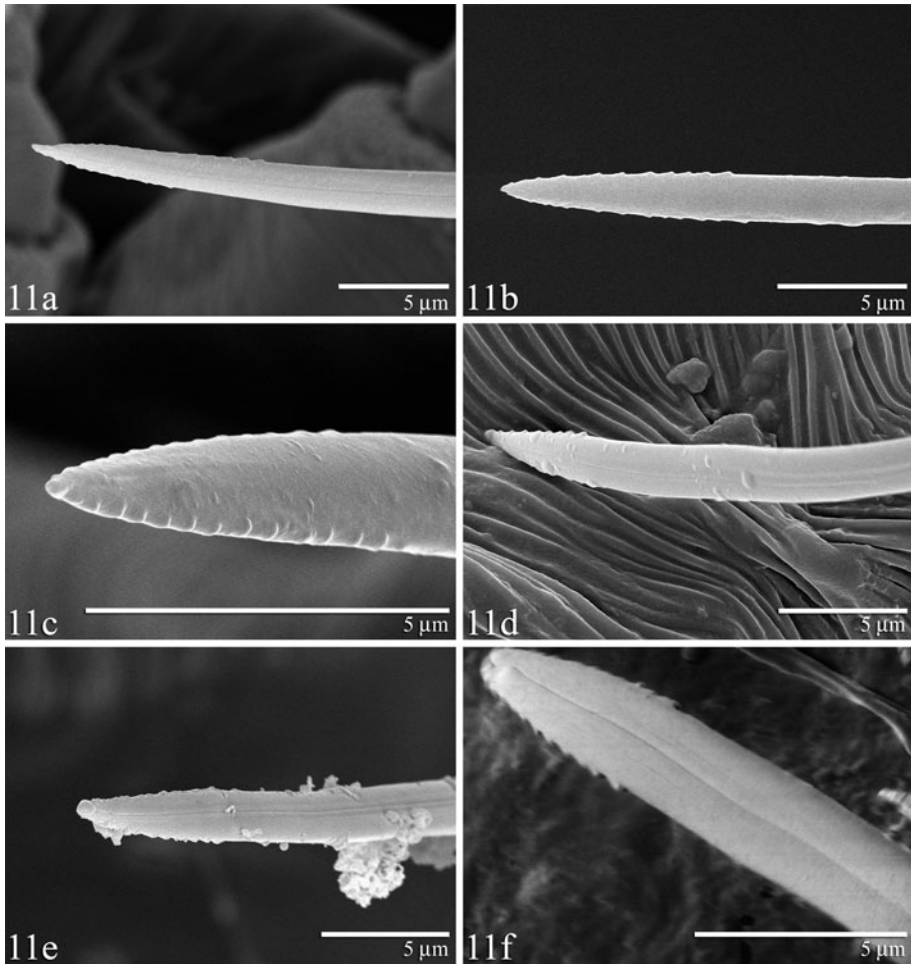
Fig. 11 Detail of ornamented tip of the stylets of adult female *Raoiella* spp.—**a** *Raoiella* sp. nov. 4 (sensu Dowling et al. 2012); **b** *Raoiella* sp. nov. nr *australica* (sensu Dowling et al. 2012); **c** *Raoiella* sp. nov. 5 (sensu Dowling et al. 2012); **d**. *Raoiella* sp. nov. 7 (sensu Dowling et al. 2012) protonymph; **e** *Raoiella* sp. nov. 3 (sensu Dowling et al. 2012); **f** *Raoiella indica*

are actually necessary to create a vacuum, the pharynx of these mites must operate in a manner different to that suggested for the Tetranychidae. If flat mites can feed via the stylet tube, then the implication is that the digestive system and stylet tube are directly connected; however, no such connection has ever been found. Though the stylet tube is physically distant to the food channel within the infracapitulum (see Alberti and Crooker, 1985:57; Nuzzaci and de Lillo 1991) and many anatomical studies indicate a definite lack of connection, further transmission electron microscope studies of multiple tenuipalpid genera, especially *Raoiella*, are required to completely eliminate the possibility that the stylets can convey food.

Because no direct connection has ever been found between the oral orifice and the stylet tube, it was proposed that the interlocked stylets, which protrude from in front of the median salivary duct, are used to convey expelled saliva to the feeding puncture (Summers
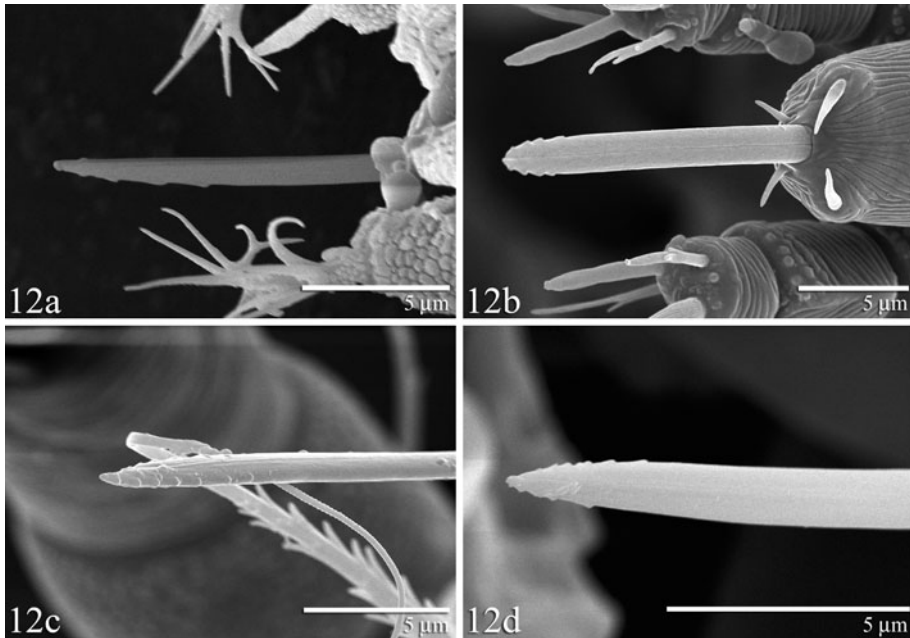
Fig. 12 Detail of ornamented tip of the stylets of various adult female tenuipalpids—**a** *Tenuipalpus* sp. nov.; **b** *Larvacarus* sp.; **c** *Australopalpus alphitoniae*; **d** *Lisaepalpus smileyae*

et al. 1973; Hislop and Jeppson 1976; Alberti and Crooker 1985) rather than for conveying plant fluids into the mite. The description of a 'salivary pump' mechanism for controlling the flow of saliva along the stylet channel supports the hypothesis that the channel functions as salivary and not a food channel (Nuzzaci and de Lillo 1991). Stylets may be involved in both piercing plant tissues and injecting saliva into plant cells to allow preoral digestion (de Lillo et al. 2002). In support of pre-oral digestion, Mothes and Seitz (1982) point out that the gut contents of *Tetranychus* is filled with granal thylakoids, starch grana and cytoplasmic portions indicating that the decomposition of nuclei, chloroplast stroma and other organelles must have occurred prior to ingestion by the mite. If the stylet tube can convey saliva into the injured leaf, then it follows that it could also convey fluid out of the leaf (Mothes and Seitz 1982).

Tetranychoid feeding damage to plants

The depth reached by stylets of tetranychids ranges from 70 to 120 μm, depending on the characteristics of the plant and the length of the stylets (Avery and Briggs 1968; Summers and Stocking 1972; Sances et al. 1979). The morphology of the pharyngeal pump indicates that a considerable force can be generated, sufficient to evacuate fluids from deep lying palisade leaf cells (Summers et al. 1973). Studies on the physical damage that feeding by spider mites causes to the leaves of their host plants indicate that the primary damage occurs in the parenchyma layer (Summers and Stocking 1972; Tanigoshi and Davis 1978). Once a cell is punctured, it plays no further role in plant metabolism. A spider mite such as *T. urticae* is capable of injecting the stylets 18–22 times per minute, exhausting the contents of 100 cells every five minutes (Liesering 1960). As with *R. indica* (Ochoa et al.
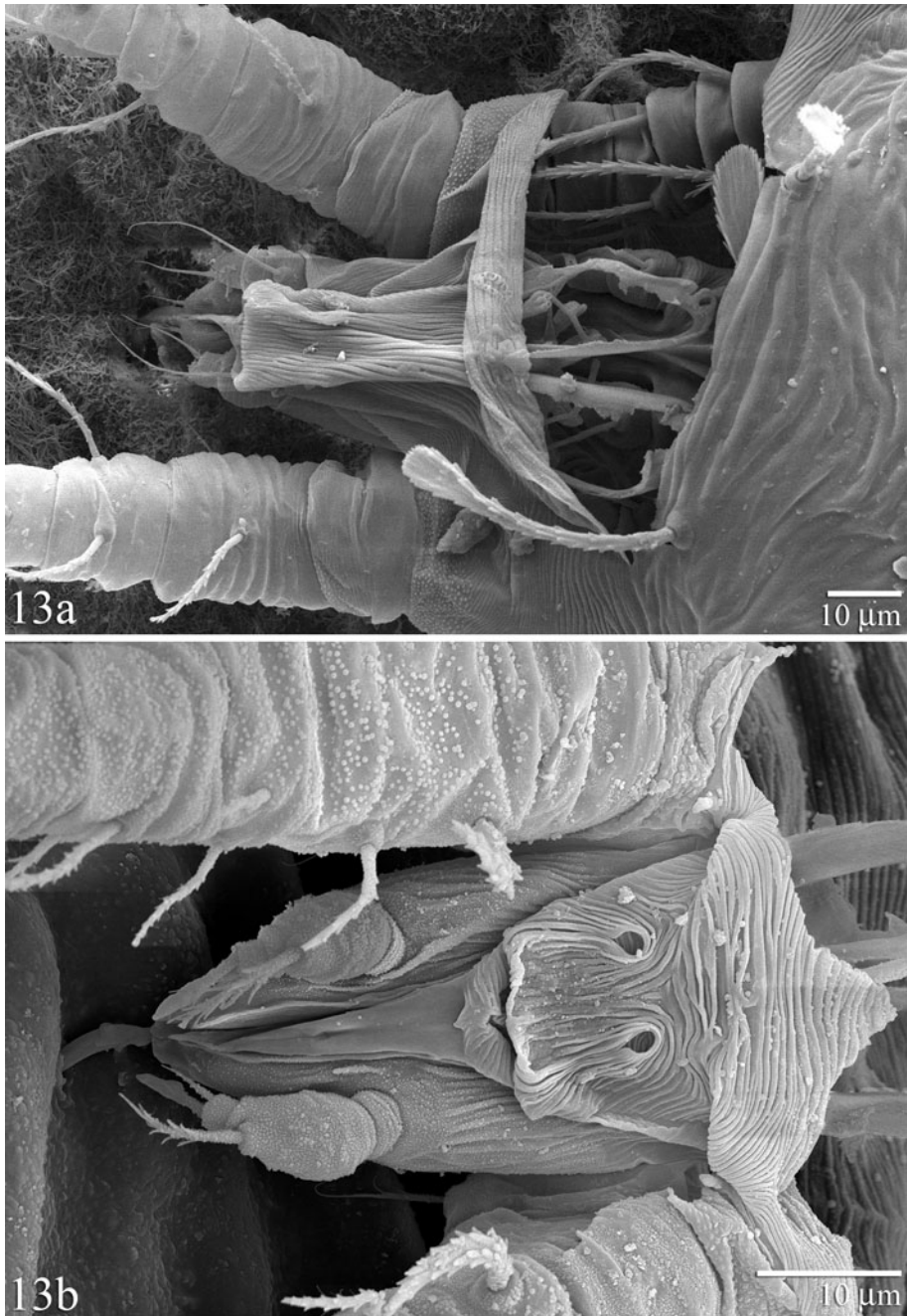
Fig. 13 Moulting flat mites anchor themselves by inserting their stylets into host plant tissue—**a** *Raoiella* nr sp. nov. 3 (sensu Dowling et al. 2012), moulting deutonymph—stylets inserted within the guard cells of a stomatal complex of *Eucalyptus pleurocarpa* Schauer. (Myrtaceae); **b** exuviae of *Tenuipalpus pacificus* with stylets still inserted directly into the leaf tissue via the epidermis
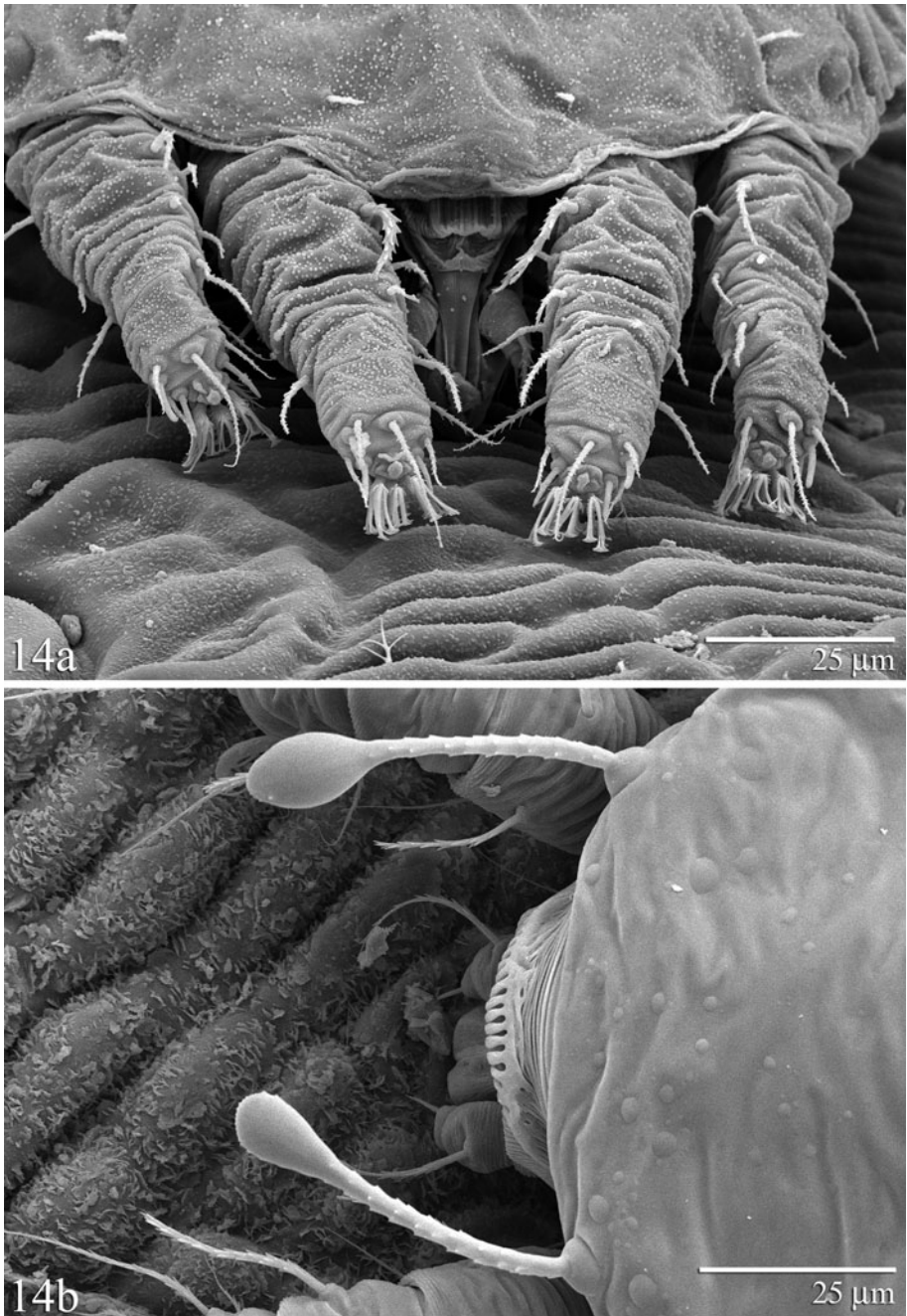
Fig. 14 Flat mites touch their oral orifice to surface of host plant leaf during apparent feeding bouts—
a *Tenuipalpus pacificus* nymph; **b** *Raoiella* sp. nov. 4 (sensu Dowling et al. 2012) adult female

2011), when *T. urticae* is present in huge numbers, it is capable of causing serious damage to its host.

The epidermis represents one of many mechanical defences developed by plants in response to herbivory (Lucas et al. 2000; Reina-Pinto and Yephremov 2009). The epidermis plays a critical role in plant survival and defence by protecting against excessive water loss and entry of harmful substances and pathogens (Reina-Pinto and Yephremov 2009). In many Myrtaceae, the cuticle, or outer covering of the epidermal cells, is raised into mounds or ridges of various shapes producing a particular pattern (Fig. 15). The cuticle in *Eucalyptus*, for example, can be thick enough to support the development of permanent folds, mounds or ridges that often form over the subsidiary cells associated with a stomatal opening and may be as thick as 30 µm (Carr and Carr 1987). The cuticle of a eucalypt leaf also extends over the surface of the guard cells of the stomata and the mesophyll cells that line walls of the stomatal chamber (Carr and Carr 1978), though it is often much thinner at the line of closure of the guard cells (Carr and Carr 1979). The epidermis and hypodermis of palms, which together form the surface layer, are tightly bonded and function as a unit, forming a kind of rind over the leaf (Horn et al. 2009). As an indication of how strong the bond between these two layers is, the epidermis of palm leaves cannot be removed by peeling but must be scraped away (Tomlinson 1990). Although normal epidermal cells in stomata-bearing regions vary a great deal in wall thickness, the cell wall exposed on the leaf surface is usually thick and heavily cutinised (Tomlinson 1990). For added protection, the cells of the hypodermis, below the epidermis, are usually larger and distinctly thicker-walled than the epidermal cells (Tomlinson 1990).
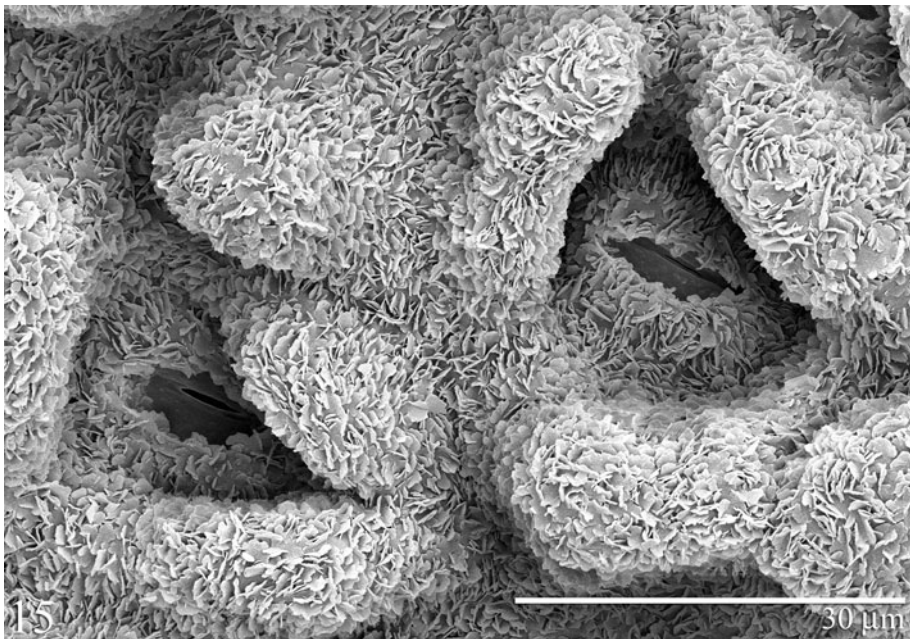

Fig. 15 Leaf surface of Karri, *Corymbia calophylla* (Lindl.) K.D. Hill and L.A.S. Johnson (Myrtaceae), host plant of *Raoiella* sp. nov. 7 (sensu Dowling et al. 2012)—detail of cuticle raised into mounds and ridges of wax crystals surrounding the stomata

It has always been assumed that tetranychoid mites must first penetrate the epidermis with their stylets before feeding can occur, but it has never been clear how this is achieved (Tomczyk and Kropczyńska 1985). While Avery and Briggs (1968) suggest *Panonychus ulmi* (Tetranychidae) feed by passing their stylets between epidermal cells, as no punctures in the epidermal cell walls were evident, others feel that both *T. urticae* (Tanigoshi and Davis 1978) and *Bryobia rubrioculus* (Summers and Stocking 1972) are actually able to pierce through the walls of epidermal cells. We now know that not all tetranychoid mites feed by directly piercing the epidermis. *Raoiella* mites by-pass the mechanical defences developed by their host plants by actively targeting the stomata for feeding (Fig. 1; Ochoa et al. 2011).

Grasses, palms and other monocots show a high degree of organisation in the development of their stomata, which tend to be arranged in rows parallel with the long axis of leaves (Esau 1977). Stomata in many hosts of RPM are restricted to or tend to be most abundant on the abaxial or lower leaf surface (hypostomatous), including *Cocos*, *Chamaedorea* and *Heliconia* (Figs. 16, 17). Some RPM host genera have isobilateral leaves on which stomata are present in more or less equal numbers on both surfaces (amphistomatous), examples include *Phoenix* (Fig. 18) and *Musa* (Fig. 19), as well as the non-host genus *Sabal* (Fig. 21).

Stomata are not just an obvious site for direct access to internal tissue but they can also have hidden structural weaknesses in their architecture that *Raoiella* mites seem to exploit. For example, in palms there is always a break in the thick-walled hypodermal cells under each stomatal chamber, and the walls of cells within the stomatal chamber of palms are thinner than those on the epidermis (Tomlinson 1990). Despite the inherent structural weaknesses, RPM do not feed on all palm genera. For example, RPM do not (Cocco and Hoy 2009) or can not feed on palmettos, i.e. palms in the genus *Sabal*. During feeding experiments by Cocco and Hoy (2009), no RPM were observed feeding on discs of cabbage palm *Sabal palmetto* (Walter) Lodd. ex. Schult. and dwarf palmetto *Sabal minor* (Jacq.) Pers. or the related saw palmetto *Serenoa repens* (Bartr.) Small.

We examined the leaf surface, including stomata, of six common host plants of RPM, *Cocos nucifera* L., *Chamaedorea seifrizii* Burret, *Heliconia aurantiaca* Ghiesbr. ex. Lem., *Musa acuminata* Colla and *Phoenix dactylifera* L., *P. sylvestris* (L.) Roxb., and of a non-host palmetto, *Sabal minor* (including a synonym *Sa. louisiana* (Darby) Bomhard), to determine whether there are any morphological characteristics present that could restrict host plant use by the mites. Our observations indicate that there is a broad range of cuticle development across RPM host plants. *Cocos nucifera*, *Ch. seifrizii* and *H. aurantiaca* have a smooth leaf surface with no built-up areas of wax or cutinous material accumulated on the leaf surface or around the stomatal openings (Figs. 16, 17). In contrast, the dorsal and ventral leaf surface of both *P. dactylifera* (Fig. 18e, f) and *P. sylvestris* (Fig. 18a–d) develop a thick layer of wax, often accumulating into thick ridges, with an obvious ring of clumped, vertically oriented wax crystals or cutinous material directly surrounding each stomatal opening (Fig. 18). The waxes on the cuticle of *Musa acuminata* epidermis are variously developed from patches of fine, often hair-like crystals (Fig. 19a–d) to a complex array of thin, fragile vertically oriented crystals that are easily broken and scattered, often accumulating in clumps on the leaf surface (Figs. 19e, f, 20) and the mites themselves (Fig. 20).

The leaf surface of RPM non-host *Sa. minor* is remarkably smooth, except for obvious accumulations of fine cutinous material immediately surrounding and covering each stomatal opening (Fig. 21). As the stomata of *Sabal* palmettos are sunken below the leaf surface to depths of 8–17 μm on the adaxial surface (upper) and 7–18 μm on the abaxial (lower), the accumulated material is not merely superficial but is actually a plug that fills
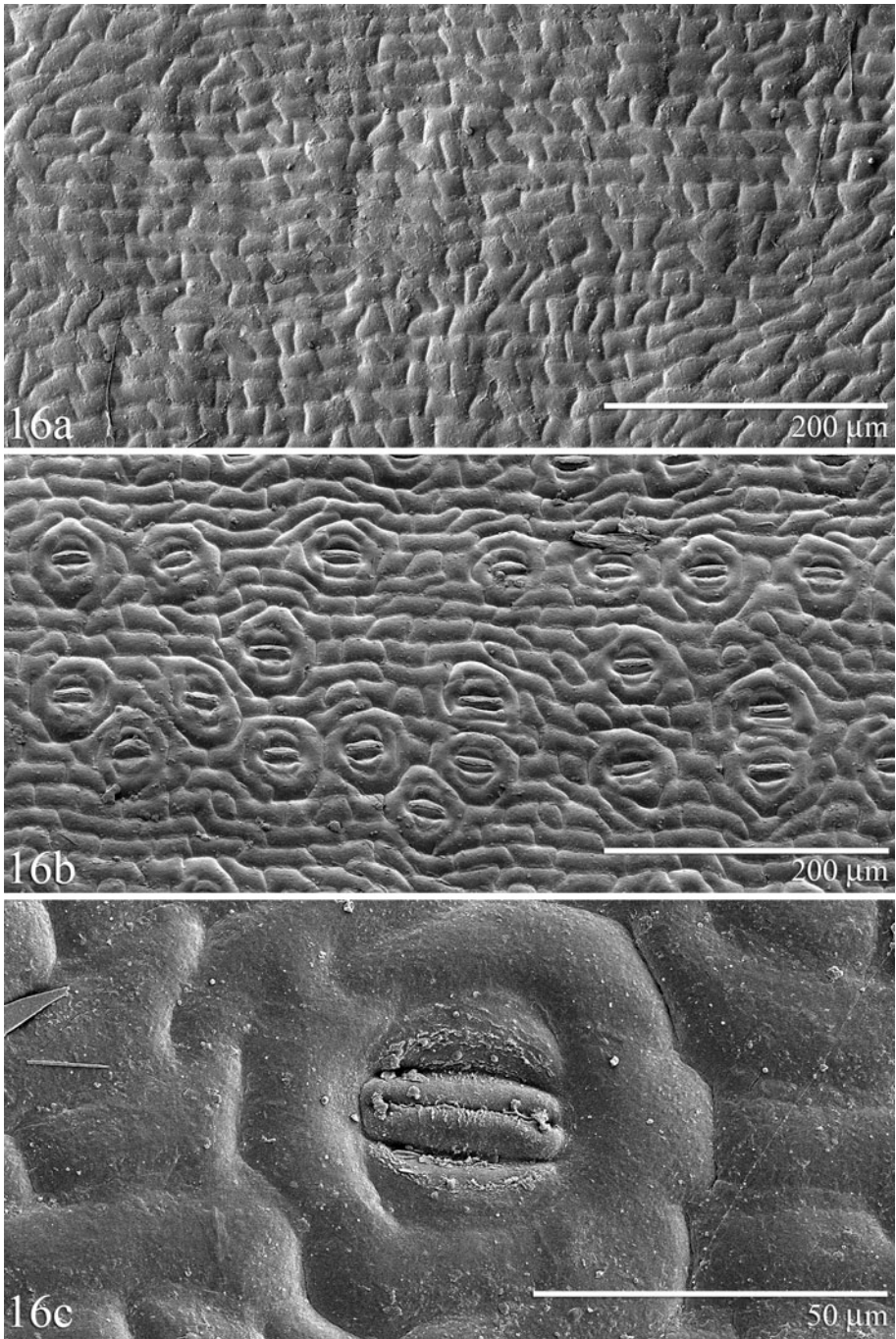
Fig. 16 Surface of coconut leaves, *Cocos nucifera* L., main host of red palm mite (RPM), *Raoiella indica*—**a** Dorsal surface of leaf (adaxial) without stomata; **b** Ventral surface of leaf (abaxial) with stomata arranged in *rows*; **c** Detail of ventral stomatal complex
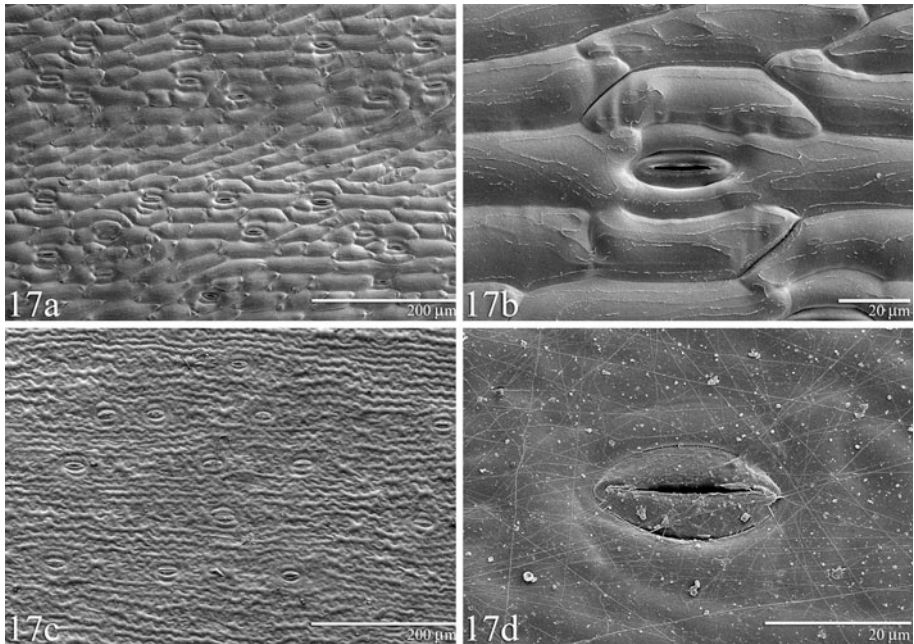
Fig. 17 Red palm mite host plant, bamboo palm *Chamaedorea seifrizii* Burrett—**a** Ventral leaf surface; **b** Detail of a ventral stomatal complex. RPM host plant, heliconia *Heliconia aurantiaca* Ghiesbr. ex. Lem.— **c** Ventral leaf surface; **d** Detail of a ventral stomatal complex

the entire stomatal pit (Zona 1990) (Fig. 21c). Though mites in the genus *Raoiella* are able to feed on a broad range of host plants with variously developed surface cuticle, this cutinous plug is enough to prevent RPM from accessing the stomatal aperture and therefore prevents RPM from feeding. Further examination of leaf sections will determine the extent of the plug and what other factors are involved.

It follows that the morphology and behaviour of stomata and their guard cells can profoundly affect the feeding by *Raoiella* mites. For example, if leaf surface and stomatal morphology and behaviour are not considered, a lack of feeding on a particular host could easily lead to that host being misinterpreted as nutritionally unacceptable. Therefore, it is of utmost importance to consider the specialized feeding behaviour found in the genus *Raoiella* when designing rearing methods or experimental protocols. For example, Cocco and Hoy (2009) found that RPM could not be reared in the laboratory using either potted banana plants or leaf discs of different varieties, though banana is a known host in the field. This led Cocco and Hoy to suggest that failure to establish adults and immatures on banana leaf discs could be due to differences in the characteristics of the cuticle, quantity of wax on the abaxial surface between varieties or differences in secondary plant compounds between mature and juvenile leaves. However, they did not consider the effect of their treatments on the behaviour of the stomata. The physical damage caused by cutting leaves into discs or by removing all but one leaf on an individual plant, as were both done in their experiments, creates tremendous water stress on a plant to which it responds by closing its stomata. This response by the host plant will completely disrupt feeding by *Raoiella*. Furthermore, in preparation for use in the experiments, the leaf discs and the intact leaves were cleaned with a brush to remove any predators or hand washed and allowed to dry.
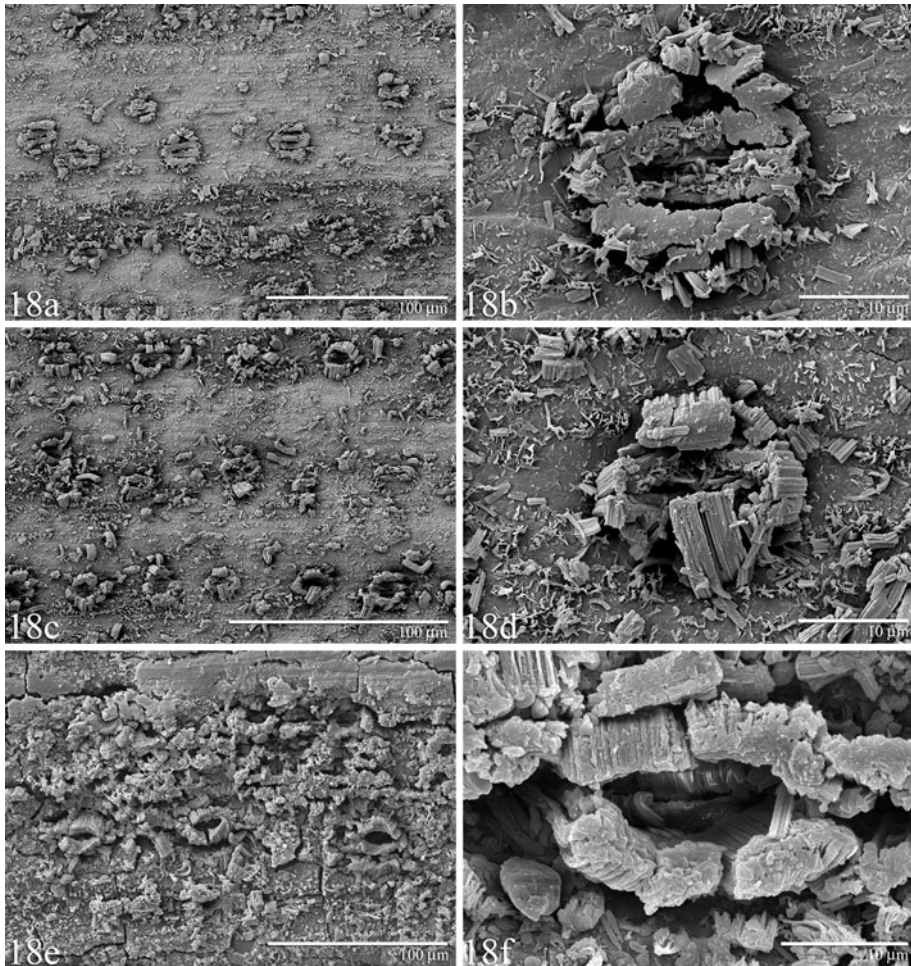
Fig. 18 Red palm mite host plant, silver date palm *Phoenix sylvestris* (L.)—**a** Dorsal leaf surface; **b** Detail of dorsal stomatal complex; **c** Ventral leaf surface; **d** Detail of ventral stomatal complex. RPM host plant, date palm *Phoenix dactylifera* L.—**e** Ventral leaf surface; **f** Detail of ventral stomatal complex

These simple acts could scour and break up the surface waxes found on banana leaves and would result in the stomatal openings being covered or filled in with loose wax and cutinous material, which are easily dislodged (Figs. 19e, f, 20). *Raoiella* mites cannot feed on a host with closed or covered stomatal openings. Interestingly, though the populations do not reach levels that match those found in the field, RPM can be reared on coconut leaf discs in the laboratory despite the plants undergoing the same treatments as the *Musa* plants. This indicates that either coconut stomata behave differently to banana stomata and can remain open despite the leaflet being cut apart or because the leaf surface is not covered in loose wax crystals (Fig. 16) as in banana, brushing the leaf surface does not disrupt access to the stomata for the mites.

Needle palm *Rhapidophyllum hystrix* (Pursh), native to Florida, is considered to be a palatable host for RPM because females were observed feeding and reproducing on leaf discs (Cocco and Hoy 2009). Though feeding differences between needle palm and
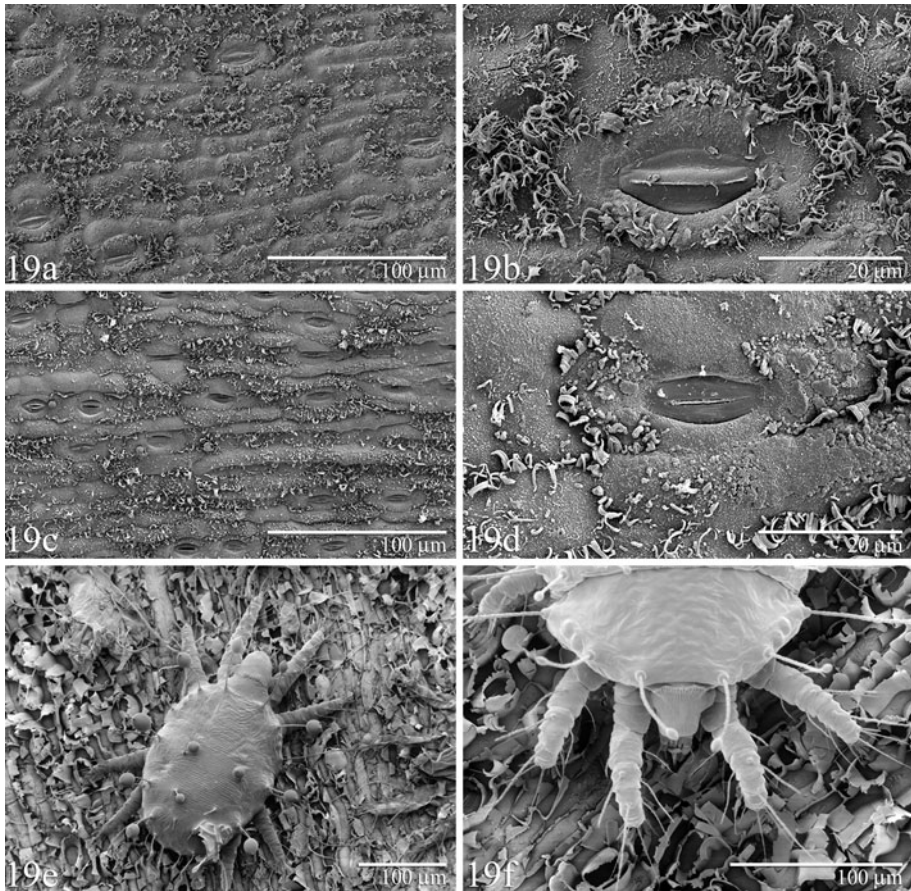
Fig. 19 Red palm mite host plant, banana *Musa acuminata* Colla—**a** Dorsal leaf surface; **b** Detail of dorsal stomatal complex; **c** Ventral leaf surface; **d**. Detail of ventral stomatal complex; **e** *Raoiella indica* protonymph feeding on ventral surface of banana leaf; **f** Detail of thin, fragile wax crystals on leaf surface of banana, with *R. indica* adult female feeding

coconut leaf discs were not significant after 2 days, the mites were observed feeding significantly less frequently on needle palm than on coconut leaf discs after 8 days. This led Cocco and Hoy to suggest that longer observations are required for reliable determination of a host plant's palatability. However, the impact of aging and consequent desiccation on the behaviour of the stomata was not considered. Before the palatability or nutritional value of a host can be assessed, access to feeding sites must be assessed and ensured.

Direct observations of *Raoiella* in the field indicate that females, the dispersal stage, move to new flushes of leaves when they become available, and the population dramatically increases thereafter (*pers. obs.* JJ Beard, R Ochoa and WC Welbourn). Though it has been suggested that differences in levels of secondary plant compounds between juvenile and adult leaves could have a negative affect on feeding by *Raoiella* mites (Cocco and Hoy 2009), physical differences can also have a major affect. For example, the stomata of juvenile leaves of many species of *Eucalyptus* (a common host genus for *Raoiella*) are

Fig. 20 The cuticle over the leaves of banana develops into thin, fragile vertically-oriented wax crystals that are easily broken and moved around—note the dislodged wax crystals accumulated over the infracapitulum, leg I and dorsum of an adult female *R. indica* feeding on banana, in addition to the accumulated material on the leaf surface

superficial and not sunken beneath the leaf surface as can occur in adult leaves (Carr and Carr 1979) making feeding on juvenile leaves preferable to feeding on adult leaves. In addition, the morphology and structure of juvenile leaves can be very different to adult leaves in *Eucalyptus*. The distribution of stomata on *Eu. globulus* ssp. *globulus* juveniles vs. adults is hypostomatous vs. amphistomatous; stomatal density on the abaxial surface is approximately 614 versus 183/mm^2; overall leaf thickness is 181 versus 363 μm; mesophyll thickness is 139 versus 299 μm (James et al. 1999). It should be noted, however, that juvenile leaves are not present year round on species of *Eucalyptus*, indicating that the mites are able to feed and reproduce on adult leaves during times of the year when juvenile leaves are absent.

The discovery that mites in the genus *Raoiella* feed via stomata of different host plants is a major breakthrough in our understanding of the impact and importance of flat mite-host plant associations. It also highlights the possibility that other flat mite genera also feed via the stomata and that what we know about the biology and ecology of the family Tenuipalpidae should be reassessed. It is still uncertain if *Raoiella* actively kill the guard cells during feeding; however, when guard cells no longer function and stomata remain permanently open to the external atmosphere, not only does a plant lose its ability to regulate gas exchange and water loss, but its internal tissue will be directly exposed to attack by fungi and plant pathogens. For example, massive RPM populations on banana could be a factor in aiding the spread of ascomycete fungi such as *Mycosphaerella fijiensis* Morelet and *M. musicola* Mulder, causal agents of the devastating banana diseases Black Sigatoka and Yellow Sigatoka, respectively, which are thought to enter the host via the stomata
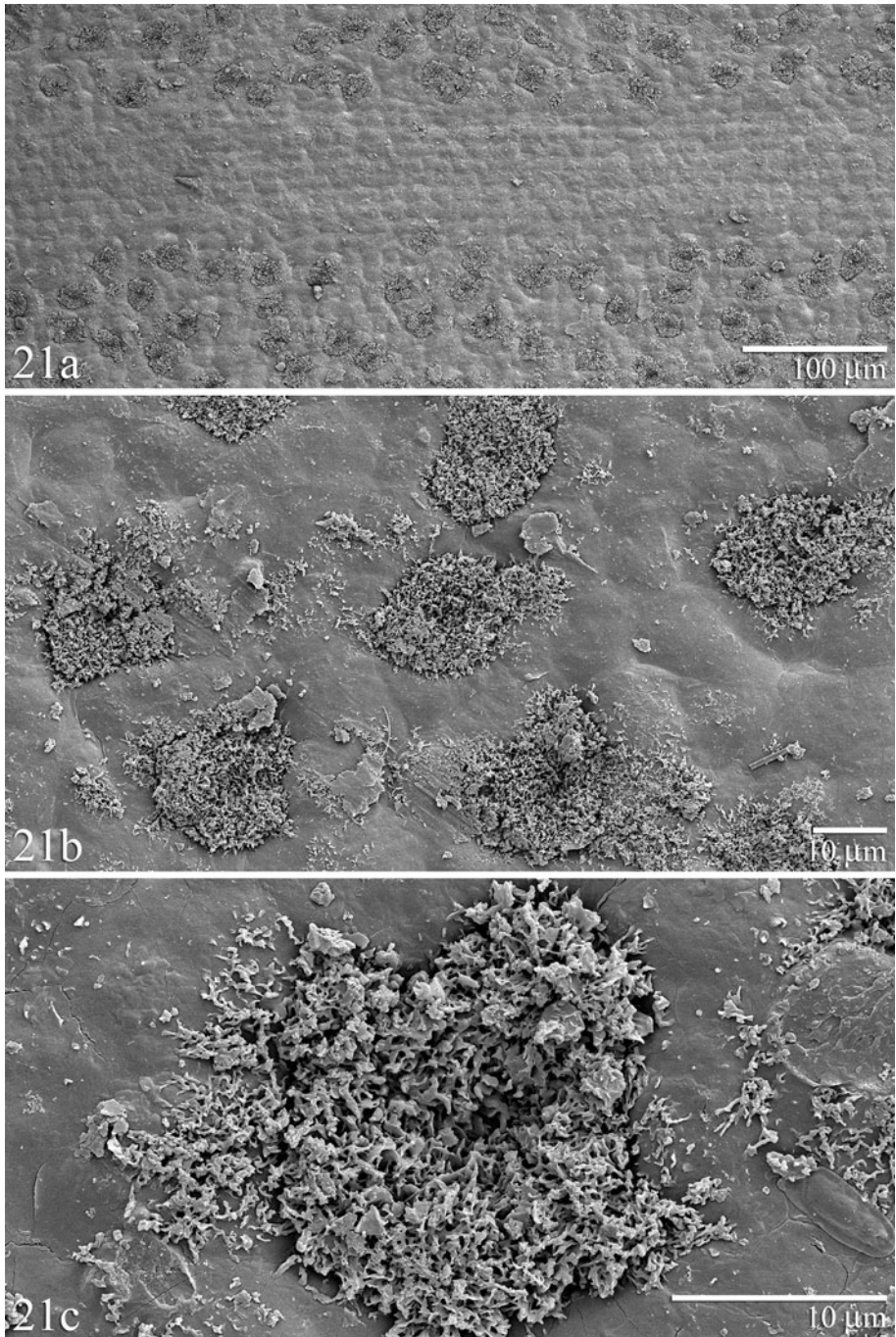
Fig. 21 Dwarf palmetto, *Sabal minor* (Jacq.) Pers.—**a** Dorsal leaf surface; **b** Ventral leaf surface; **c** Detail of cutinous plug filling each stomatal pit

(Vasquez et al. 1990). Additionally, several flat mite species often carry fungal spores and bacteria on their body (Ochoa et al. 1994).

This study has brought into question several aspects of our previous understanding of the mechanics of feeding in the family Tenuipalpidae. For example, how is the extension and retraction of the ribbed collar related to movement of the stylets and stylophore during feeding? How does the honeycomb valve inside the inferior oral commissure function? How do flat mites ingest fluids without the oral flaps apparently necessary to create the required vacuum? Feeding via the stomata and the absence of oral flaps, for example, are important not only for understanding the mechanics of tenuipalpid feeding but could also be critical to our understanding of the ability of some flat mites, such as species of *Brevipalpus* (Childers et al. 2003), to transmit plant viruses.

In light of the feeding behaviour found in *Raoiella*, the feeding behaviour of mites in the genera *Brevipalpus*, *Cenopalpus* and *Tenuipalpus* are in need of closer scrutiny as they have been observed feeding in close proximity to stomatal openings on their host plants. The possibility of separating genera based on their feeding behaviour could have an important impact on alternatives for management and control.

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