National Diagnostic Protocol

Phytoptus avellanae Nalepa

Hazelnut big bud mite

NDP 39 V1

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- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
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Document status

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Further information

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1 INTRODUCTION

The Hazelnut big bud mite is a gall inducing eriophyoid mite. Gall formation by the mite causes distinctive 'big bud' symptoms, infested buds are swollen and deformed resulting in reduced yield as infested buds do not produce healthy shoots, flowers or nuts. Hazelnut big bud mite was originally described as *Phytoptus avellanae* by Nalepa (1889), but has since had a number of synonyms (see Section 2).

A number of other eriophyoid mite species have been reported from hazelnuts and other *Corylus* species (Appendix 1), this includes *Cecidophyopsis vermiformis*, which inhabits big buds of hazelnut and is thought to contribute to their development. This protocol has been prepared to distinguish *Phytoptus avellanae* from these other eriophyoid mite species. Australia has some eriophyoids that infest hazelnuts but *P. avellanae* appears to be restricted to Tasmania and *C. vermiformis* is not known to occur. Consequently, this protocol has been designed to be as simple to use as possible on this difficult group and includes a number of images plus published illustrations to aid identification. The protocol is based on adult females because nymphal descriptions for most eriophyoids are inadequate for identification.

1.1 Hosts

Corylus maxima (Filbert)
Corylus avellana (Common hazel)
Corylus sp.

1.2 Effect on hosts

Phytoptus avellanae causes the interior parts of the bud to become swollen, fleshy and deformed ('big buds') (Jeppson et al. 1975). Infested vegetative buds develop weak and unhealthy shoots, damaged male catkins become stiff and brittle producing little pollen, and weakened female buds produce no nuts (Jeppson et al. 1975). Terminal buds are often favoured.

Hazelnut big bud mites are a serious pest in all areas where hazelnuts are distributed (eg Europe, Asia, North America, Australasia) (Castagnoli & Oldfield 1996). Two eriophyoid mite species are frequently found in big buds, *Phytoptus avellanae* (Nalepa) (Acari: Phytoptidae) and *Cecidophyopsis vermiformis* (Nalepa) (Acari: Eriophyidae). For a long time *P. avellanae* was considered the sole causative agent of galls or big buds whereas *C. vermiformis* was thought to be a harmless inquiline (Castagnoli & Oldfield 1996). Although *P. avellanae* is considered the more harmful pest (Ozman & Toros 1997b), it is recognised that *C. vermiformis* can contribute to damage, particularly summer big buds (Krantz 1979; Ozman & Toros 1997a).

2 TAXONOMIC INFORMATION

Phylum	Arthropoda
Class	Arachnida
Subclass	Acari
Superorder	Acariformes
Order	Trombidiformes
Suborder	Prostigmata
Supercohort	Eupodides
Superfamily	Eriophyoidea
Family	Phytoptidae
Subfamily	Phytoptinae
Genus	*Phytoptus*
Species	*Phytoptus avellanae* Nalepa, 1889: 126.
Synonyms	*Eriophyes avellanae*.— Essig, 1926: 47; Evans, 1942: 142.
	Phytocoptella avellanae.— Jeppson et al., 1975: 397.
	Phytoptus avellanae.— Keifer, 1940: 112; Manson, 1984: 24; Amrine & Stasny, 1994: 263; Baker et al., 1996: 118; Halliday, 2013.
	Old synonyms of *Phytoptus avellanae*
	The following names for this species have been formally suppressed by the International Commission on Zoological Nomenclature, and should not be used (ICZN, 1989).
	Acarus pseudogallarum Vallot, 1836.
	Calycophthora avellanae Amerling, 1862.
	Phytoptus coryli Frauenfeld, 1865.
	Phytoptus coryligallarum Targioni-Tozzetti, 1885.
	Phytoptus pseudogallarum Targioni-Tozzetti, 1888.

Common names

- Filbert bud mite
- Filbert big bud mite
- Hazelnut gall mite
- Nut gall mite
- Hazelnut big bud mite

3 DETECTION

Like many eriophyoid mites *Phytoptus avellanae* is very small, ranging in length between 180-255 µm and width between 56-69 µm (Manson 1984), detection of these mites can therefore be difficult.

3.1 Symptoms

Phytoptus avellanae causes profound changes within the interior parts of the bud. Infested buds consist of nutritive tissue including scales and fleshy bracts which become swollen, fleshy and deformed ('big buds'); infested vegetative buds develop weak and unhealthy shoots, damaged male catkins become stiff and brittle producing little pollen, and weakened female buds produce no nuts (Jeppson et al. 1975). Detection is most efficient if buds displaying the distinctive big bud symptoms are targeted in winter or spring. Infested big buds become swollen and spherical with a diameter of about 10mm; this can be about twice the size of normal buds (**Figure 1**). Big buds can be dissected and examined using a dissecting microscope to reveal mite colonies towards the centre (**Figure 2**).

In mid to late spring, big buds can expand and become deformed and desiccate instead of growing into a flower or shoot, leading to a blasted appearance (

Figure 3). Not all big buds end up as blasted buds, buds with low levels of infestation can produce a short shoot with misshapen leaves or flowers (Castagnoli & Oldfield 1996) (eg the lowest bud in

Figure 3A). Mites can be seen emerging and dispersing as blasted buds desiccate (**Figure 4**).

Figure 1. Buds of *Corylus avellana*: (A) normal bud and (B) with big bud symptoms; Tasmania, Australia [Photos: J. Davies].

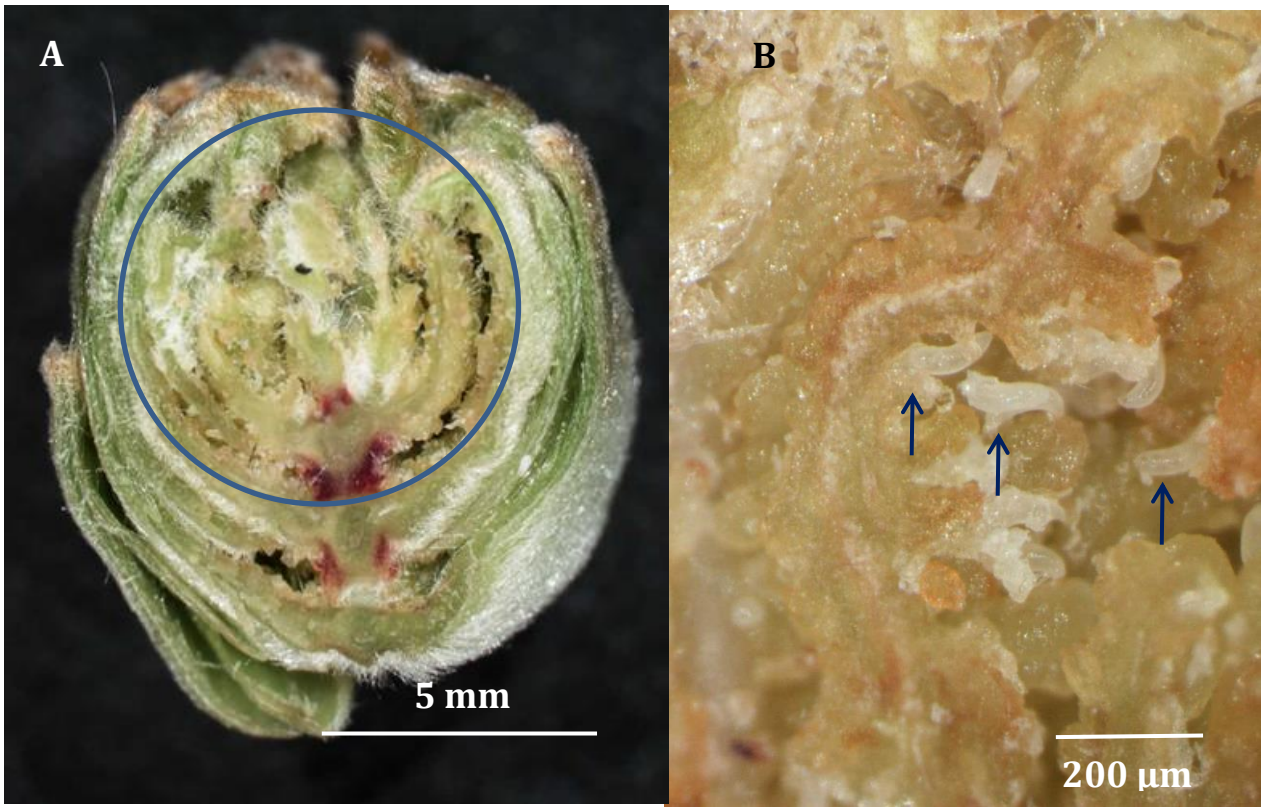

Figure 2. Dissected big bud of *Corylus avellana* (A) whole bud, circled area is where *Phytoptus avellanae* colonies are located and (B) close up of galled bud tissue and *Phytoptus avellanae* (in focus examples indicated by blue arrows); Tasmania, Australia [Photos: J. Davies].

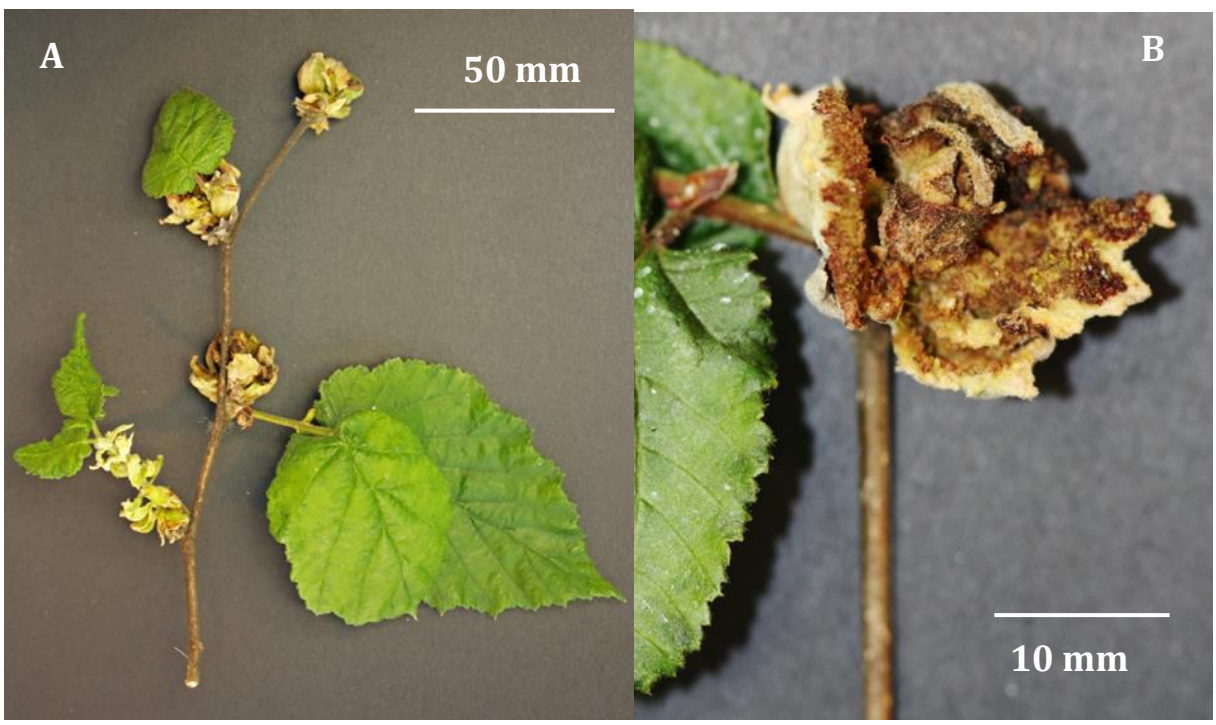

Figure 3. Blasted buds of *Corylus avellana* (A) distributed along stem and (B) close up showing internal bud damage; Tasmania, Australia [Photo: J. Davies].

Figure 4. Close up of *Phytoptus avellanae* on blasted bud (in focus examples indicated by blue arrows); Tasmania, Australia [Photo: J. Davies].

3.2 Sampling

3.2.1 Collecting and preserving plant samples

The best way to find eriophyoid mites is to collect the plant material on which they are present. In the case of *P. avellanae,* this is tissue displaying big bud symptoms as displayed in the preceding figures (1, 3). This material can be kept fresh for a short period of time until mites are collected. To extend the life of the plant material, place samples in a plastic bag and keep refrigerated. Absorbent paper in the bag can extend the useable life of the plant material, stems with little leaf material can be wrapped in slightly dampened paper or include some dry paper with leafy material to help reduce moisture build up. Alternatively, keep samples out of the fridge with lower ends of stems submerged in water like a bouquet of flowers.

Samples can be dried and kept as herbarium specimens. Good quality slide-mounted specimens can be readily made from dried specimens collected from well-prepared dried plant material.

Follow the advice of a good herbarium guide such as Bedford and James (1995) for preparing plant samples, in summary:

1. Place plant specimens as flat as possible between sheets of absorbent blotters or semi-absorbent paper such as newspaper.

2. Place these sheets with plant specimens between sheets of thick, preferably smooth-sided, centre-corrugated cardboard (such as used in cardboard carton sides), this will assist air circulation through the press.
3. Plant samples should be kept with enough separation to prevent cross contamination of mites (eg one set of plant samples between two sheets of paper, which are each between two pieces of corrugated cardboard).
4. Specimens are best pressed with moderate pressure, preferably in an arrangement that will permit as free a circulation of air as possible. This can be achieved by strapping the pile of pressings together in a press, i.e. between frames made, for example, from sheets of heavy (non-bending) cardboard, hardboard, plywood, pegboard or, best of all, a lattice of wood or weldmesh. The press frames should be the same size as or a little larger than the drying papers.
5. The press should be kept in a dry environment, preferably with some warmth and air movement. If this is difficult, periodiocally place in front of a fan heater with the press positioned so that warm air can flow through the cardboard corrugations. This will help the drying process.
6. The papers should be checked for dampness and changed when necessary.
7. Once dry, samples should be stored in a way to prevent cross contamination of mites across samples. A suitable method is to store small samples in a piece of A4 paper folded in half then at the sides, then fold the open end and secure with a large paperclip or a small foldback clip. This forms a small sealed pocket where samples can easily be accessed by fully unfolding the paper (unlike envelopes and paper bags where residues containing mites accumulates at the corners and is inaccessible). Each of these pockets can then be stored in 20cm x 30cm paper bags with all collection information on the outside of the bag.

3.2.2 Extracting eriophyoids from plant material

Handling, extracting and collecting eriophyoids is difficult due to their small size. This section summarises reviews on the topic (Amrine & Manson 1996; de Lillo et al. 2010; Monfreda et al. 2010; Monfreda et al. 2007), it is recommended that these publications are followed for anyone wanting to work with eriophyoid mites.

Eriophyoid mites can usually be located on plant samples using a stereo dissecting microscope. Finding and collecting eriophyoids can be very time consuming, particularly when mites are scarce on the plant sample or the plant organs are severely modified and architecturally intricate, especially when dried.

In some cases, mummified eriophyoid mites can be located on dried plant material using a stereomicroscope with the aid of ultraviolet light (Chetverikov 2016). In this study it was found that the detection of mummies was notably faster with the application of UV light at 365 nm than when trying to locate the same specimens under regular white light. This was due to the distinct autofluorescence of the exoskeleton which allowed the glowing mummified specimens to be clearly visible under the stereomicroscope.

A set of customised tools are useful for handling eriophyoids, they can be picked up using pin-like or other tools, even if the plant material is deformed. Tools can consist of modifications of the following:

1. A pair of size 3 insect pins in wooden dowels is useful for dissecting galls and unrolling leaf margins.

2. Size 00 minuten pins mounted on fine wooden dowel or similar for ''needling'' mites from solution to solution and slide to slide.
3. Eyelash tool, consisting of an eyelash adhered to a fine wooden dowel or similar.
4. Fine artists brush trimmed to a few fine hairs.

The moistening of the tip of the tool with water or other media (eg Hoyer's[1]) can help with the process of picking up mites.

Collecting can be greatly improved by concentrating the mites. In the case of dried material, mites can be recovered by soaking part of the sample overnight in a water solution with a few drops of surfactant and bleach at room temperature. The suspension is then stirred and sieved: the specimens can be more easily detected, because of their restored shape, then picked up from filter paper or from filtered sediment (through a 20–25 µm sieve) and poured into a Petri dish using water plus a small amount of a surfactant.

3.2.3 Preserving specimens

Eriophyoid mites are best either preserved with the plant host as dried samples (see 3.2.1) or slide mounted from fresh samples reasonably soon after collection (see 3.2.4) (de Lillo et al. 2010). Storing in 70% ethanol or AGA is not recommended as specimens become difficult to slide mount over time. If a mite sample requires storage in fluid (eg if posting specimens or keeping samples for future slide making) then one of the following methods is recommended (Amrine & Manson 1996; de Lillo et al. 2010):

1. Preparation of work slides: A very small droplet (eg approximately 2mm in diameter) of modified Berlese medium[1] (alternatively lactic acid can be used) is placed in the centre of a microscope slide, eriophyoid mites are collected and placed in the droplet. Ensure these work slides are covered when stored to keep free from dust and contamination with other eriophyoids.
2. Sorbitol fluid[1]: A small droplet is placed inside the lid of a polypropylene micro centrifuge tube. About 100 specimens can easily be collected in this droplet, and when closed, it can be safely transported and mailed. The droplet becomes very sticky, dries out over time and can be re-hydrated by breathing over it. Otherwise, the entire droplet, even when crystallized, can be added to clearing medium (see 3.2.4) prior to slide preparation. This method is suitable for storing for at least a few months but has not been tested over longer time periods.
3. For molecular studies specimens can be stored in 95-100% ethanol, preferably in a -80^{o}C freezer, for approximately a year or more. Specimens can be stored on a short term basis (eg up to a week) in a fridge in ATL buffer (a buffer containing edetic acid and sodium dodecyl sulphate) prior to DNA isolation.

3.2.4 Preparing eriophyoid mites for identification

Of the four life stages (egg, larva, nymph and adult) only adult females are identifiable to species using morphological features. Definitive diagnosis of *Phytoptus avellanae* requires microscopic examination (using phase contrast or DIC) of well-prepared slides of adult female specimens known to be collected from *Corylus*. A range of slide mounting techniques can be used for preparing slides of eriophyoid mites, these are reviewed in detail by de Lillo et al. (2010) and

[1] See Appendix 2 for methods of preparation.

Amrine and Manson (1996). The following procedure is an adapted summary of these references:

1. Collect samples; retain dried samples of damaged plant material containing mites (see 3.2.1).
2. Extract mites (see 3.2.2) and keep preserved samples if necessary (see 3.2.3)
3. Clear mites until internal body contents are transparent, this can be checked by passing a mounted minuten pin under mites when viewed with a dissecting microscope with sub-stage lighting. Suitable clearing media include Keifer's booster[1] (preferred), Kono's medium[1] and Nesbitt's medium[1]. Clearing is usually reasonably rapid and can be achieved within half an hour, especially if some gentle heating is applied during the process (eg 5-10 minutes in a 50°C oven). In some cases, clearing may not be required if mounting in modified Berlese medium[1] or Hoyer's[1], which have clearing properties and adequately clear some eriophyoids.
4. Mount specimens (3-6 mites per slide) in a small drop of modified Berlese medium[1] (preferred) or Hoyer's medium[1]. Mites can be orientated within the media using an eyelash tool or mounted minuten pin.
5. Add an 8-12 mm coverslip (size 0 or 1 depending on microscope) gently to media and let settle under its own weight. Adjust final position of mites on slide by gently moving the coverslip with fine pointed forceps under a dissecting microscope with sub-stage lighting. Aim for at least one female positioned dorso-ventral and one lateral per slide.
6. Dry in oven at approximately 50°C for approximately two weeks. If mites have been mounted straight into modified Berlese or Hoyer's, a period of heating is required to clear specimens (eg one to seven days in a 50°C oven or as little as 30 min if placed on the edge of a hotplate at 80–90°C).
7. Ring coverslip with a high quality insulating varnish (eg Glyptal, Isonel or MR8008) (MR8008 is available from http://australia.rs-online.com/, stock number 199-1480).

3.2.5 Preventing contamination

As eriophyoid mites are very small and are often in high numbers, cross contamination between samples, particularly on tools and containers, is a real risk. When handling and preparing eriophyoid mites as detailed in the preceding sections, it is very important to work in an organised and methodical fashion to prevent contamination. This includes ensuring labels are kept with samples at all times as they are being worked on and cleaning all tools and containers thoroughly after working on a sample.

4 IDENTIFICATION

The accurate diagnosis of *P. avellanae* can be made relatively rapidly using morphological methods as long as adult females are available. Therefore, most of this section focuses on morphological methods. Molecular methods are also briefly outlined to provide an alternative diagnostic method and potentially to detect cryptic speciation (Cvrković et al. 2016).

4.1 Morphological diagnosis

4.1.1 Characters used for identifying eriophyoid mites

Due to reduction and simplification of the eriophyoid body plan, the morphological structures used for general Acari systematics are relatively few on eriophyoid mites (Lindquist & Amrine 1996). However, despite the relatively simple body plan, the considerable diversity, evolving classification, difficulties in specimen preparation and their tiny size all contribute to making the diagnostics of the Eriophyoidea one of the more challenging groups of Acari.

The characters used to identify eriophyoid mites, including *P. avellanae*, are all present on the protogyne adult female and are reviewed in detail by Lindquist (1996), the main characters are illustrated in **Figure 1**, **Table 1** and subsequent figures.

Table 1. Abbreviations used in illustrations (refer to Figure 5), modified from Amrine et al. (2003) and Lindquist (1996).

Setal notation		Other abbreviations	
Prodorsal Shield		cx	coxa
vi	internal vertical seta	em	empodium
ve	external vertical seta	fm	femur
sc	scapular seta	ge	genu
Opisthosoma		T	tarsus
c1	setae *c1*	ti	tibia
c2	setae *c2*	tr	trochanter
d	setae *d*		
e	setae *e*		
f	setae *f*		
h1	setae *h1*		
h2	setae *h2*		
Palp			
ep	pedipalp coxal setae		
d	dorsal pedipalp genual setae		
v	subapical pedipalp tarsal setae		
Coxal plates			
1a	proximal setae on coxisternum I		
1b	anterolateral setae on coxisternum I		
2a	proximal setae on coxisternum II		
3a	proximal setae on coxisternum III (relocated to lateral margin of genital plate)		
Legs			
bv	basiventral femoral setae		
l''	antaxial genual setae		
l'	paraxial tibial setae		
ϕ	tibial solenidion (Phytopdidae)		
ft'	paraxial, fastigial, tarsal setae		
ft''	antaxial, fastigial, tarsal setae		
u'	paraxial, unguinal, tarsal setae		
em	tarsal empodium		
ω	tarsal solendion		

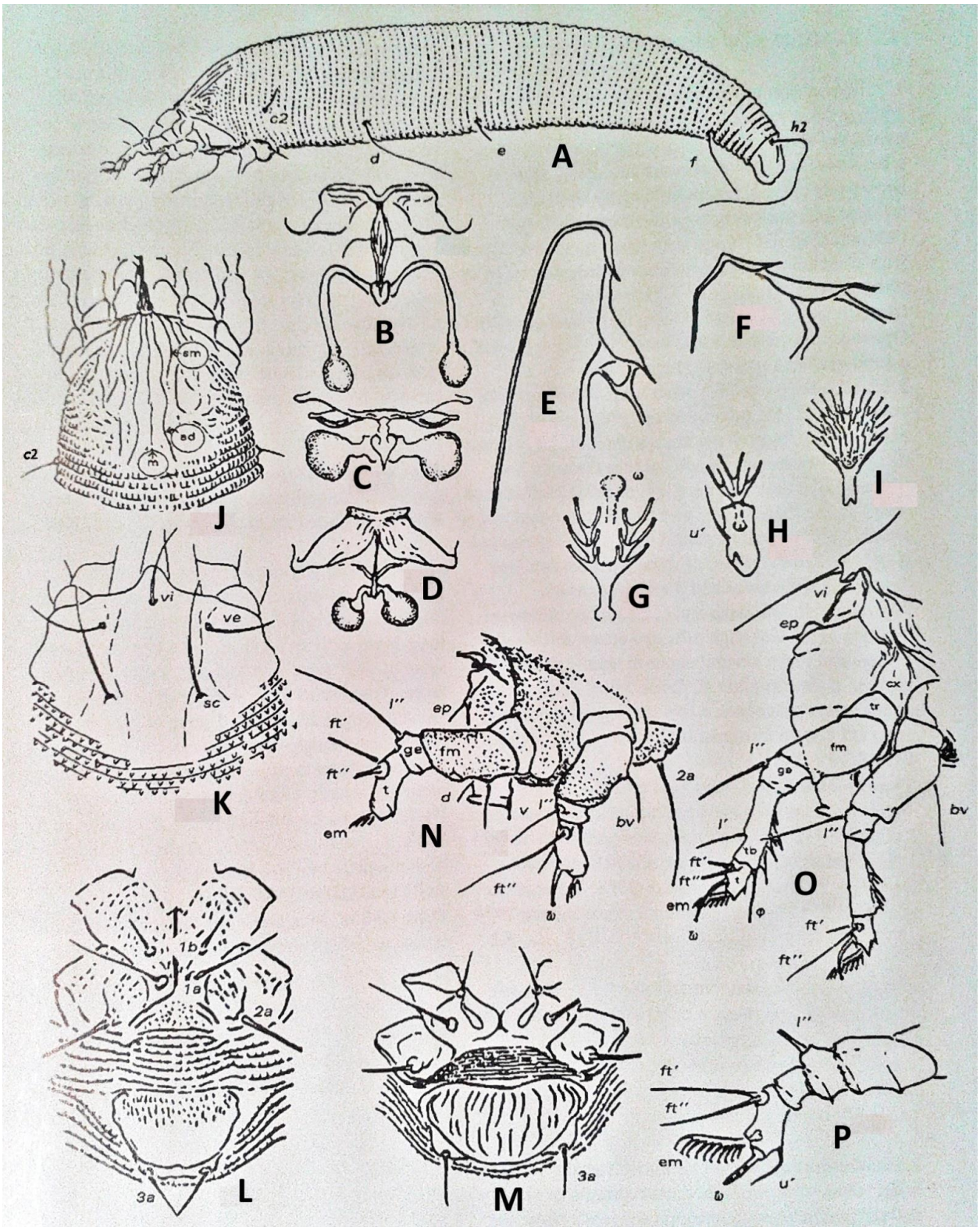

Figure 5. Eriophyoid characters, refer to Table 1 for setal notation.

Lateral view of entire mite *Cecidophyopsis vermiformis*. (B) Internal genitalia of *Nalepella tsugifoliae*. (C) Internal genitalia of *C. vermiformis*. (D) Internal genitalia of *Epitrimerus pyri*. (E) Long form oral stylet (labrum), Diptilomiopidae. (F) Short form oral stylet, Eriophyidae or Phytoptidae. (G) Divided empodium of *Acaphylla steinwedeni*. (H) Entire empodium of *Tetra concave*. (I) Modified empodium of *Cheiracus sulcatus*. (J) Prodorsal shield of *C. vermiformis*. (K) Prodorsal shield of *Pentasetacus araucariae*. (L) Coxigenital region of *C. sulcatus*. (M) Coxigenital region of *Cecidophyes collegiatus*. (N) Legs of *Cosella deleoni*. (O) Legs of *Nalepella tsugifoliae*. (P) Leg of *Catachela machaerii* with reversed empodium-solenidion. [Figures by H. H. Keifer and J. Schliesske, 1985; source: Amrine et al. (2003)].

4.1.2 Key to eriophyoid mite families recorded from Corylus,

Adapted from Amrine et al. (2003). Further details of characters specific to Phytoptus avellanae are marked with a letter in superscript and explained in section 4.1.3.

1. **(A)** Prodorsal shield with one to five setae, always with anterior setae present (paired or unpaired *vi* and/or *ve*) (Figure 5K)[A]. Gnathosoma of various sizes, often large, but with chelicerae straight or slightly and evenly curved; pedipalps usually short and truncate and enclosing the short-form oral stylet (labrum) (Figure 5F)[B]. Legs with usual setae and often with an apicolateral or apicoventral solenidion (ϕ) on tibiae of legs I (this is absent in *P. avellanae*); all empodia, as far as known, undivided or entire[C]. Opisthosoma with all usual setae; some species with a subdorsal seta pair (*c1*), accessory seta (*h1*) often long (Figure 5A)[D]. Female genital coverflap without ridges; anterior female apodeme always extending a moderate distance forward; spermathecal tubes usually long (three to five times or more longer than spermathecal tubes in the Eriophyidae and Diptilomiopidae), often extending diagonally forward then recurving caudad (Figure 5B)[E]. Dimorphism of the three types known (see *Trisetacus*, *Phytoptus* and *Sierraphytoptus*). Phytoptids do not make leaf erineum but otherwise create galls, leaf edge rolls and cause deformed foliage and buds; several occur in needle sheathes or are free-living on plant surfaces. Many occur on conifers and monocots; a few make galls on dicots; none are known to transmit viruses.

PHYTOPTIDAE – 1 species recorded from *Corylus*: *Phytoptus avellanae* Nalepa (see section 4.1.3 and Appendix 1).

(B) Prodorsal shield without anterior setae (*vi* or *ve*) (Figure 5A&J); scapular setae (*sc*) present or absent; never with tibial solenidion (ϕ) (Figure 5N); opisthosoma never with subdorsal seta (*c1*) (Figure 5A); female spermathecal tubes always short, either projecting laterally (Figure 5C) or diagonally caudad (Figure 5D); other characters variable. **2**

2. **(A)** Gnathosoma usually small in comparison to the body; when large, chelicerae straight or slightly curved; pedipalps with terminal segments short and truncate and enclosing the short form oral stylet (Figure 5E). Legs with usual segments and setae or with various reductions or modifications; some are divided or modified. Opisthosoma with standard setae or with various reductions; accessory setae small or often absent. Female genital coverflap usually with ridges (Fig 5M); genital apodeme usually of moderate anterior length (Figure 5C&D), but folded up and appearing as a transverse bar in ventral view in some forms. Many species occurring on dicots are dimorphic with diapausing or aestivating deutogynes. Mites of this family inhabit all available refugia on plants; many form galls, erinea, witches' brooms, leaf roll edges, enlarged buds and other unique sheltered habitats. Many species are serious plant pests and several transmit viral diseases.

ERIOPHYIDAE – 9 species recorded from *Corylus* (see Appendix 1).

(B) Gnathosoma large in comparison to body; chelicerae abruptly curved and bent down near base; pedipalps attenuate, enclosing the long form oral stylet (Figure 5E).Legs with standard setae or with various reductions. Empodia often large, either entire (Figure 5H) or divided (Figure 5G). Opisthosoma with standard setae or various reductions. Female coverflap usually smooth (Figure 5L), less often with ridges. Female genital apodeme of moderate length, often narrowed anteriorly. Many species are dimorphic. All known mites of this family are leaf vagrants, rarely causing injury to hosts; none are known to transmit viruses.

DIPTILOMIOPIDAE – 1 species recorded from *Corylus*: *Diptacus calicoryli* (see Appendix 1).

4.1.3 Diagnosis of Phytoptus avellanae

Phytoptus avellanae possesses the following characters, adapted from Keifer (1940), Manson (1984), Amrine et al. (2003) and Chetverikov (2014):

General characters:

- White to light yellow in life (Figure 4).
- Vermiform (wormlike) body form (Figure 6, 12 and 13).
- Adult female approximately 180-255 µm long and 45-69 µm wide (Figure 6).

Characters specified in family key, couplet 1A:

- [A] Paired *ve* approximately 5.5 µm long and paired *sc* approximately 10.5 µm long (Figures 6, 9, 12A&B and 13A&F).
- [B] Gnathosoma is between 18-24 µm long with a short form oral stylet (Figure 7).
- [C] ϕ is absent (Figures 8A, 12G&H and 13C), empodial featherclaw is 4-5 rayed (Figures 8B, 12D and 13D).
- [D] *c1* setae is approximately 36 µm long, accessory seta (*h1*) is not long (approximately 5-6 µm long) (Figures 6, 9, 12A and 13A).
- [E] Female genital coverflap is unornamented except for short basal lines (Figure 12I), prosternal apodeme (sternal line) enlarged posteriorly (Figure 10, 12F, 13E), anterior apodeme extended forward (Figures 10 and 11), spermathecae ovoid, sausage-like (Figures 10 and 11, 12F, 13E).

Characters to diagnose genus Phytoptus not already specified:

- Genitalia relatively close to coxae, separated by fewer or smaller annuli (Figure 10A).
- Setae *sc* pointing up if short, forward if long (Figures 6, 12A and 13A).
- Opisthosomal setal pair *c1* present (Figures 6, 12A and 13A).

Morphological variation:

- Considerable morphological variability is present between descriptions of *P. avellanae* in Keifer (1940) and Manson (1984). Keifer describes and illustrates distinct admedian lines and illustrates rounded microtubercles (although describing them as pointed) (Figure 12) whereas Manson describes and illustrates absent admedian lines and microtubercles rounded and elongate or triangular and almost toothlike (Figure 13). Similar differences have been observed in the Tasmanian populations (Figure 9).
- Morphological variation in the nymph of the vagrant form of *P. avellanae* has been reported. Keifer (1940) describes a nymph with a structure inconsistent with the adult consisting of a flattened nymph with annuli broader and fewer dorsally than ventrally, and projections spine-like laterally. Similarly, "*Tegonotus* like nymphs" are reported by Ozman and Toros (1997b). This variation has recently been documented with images in Cvrković et al. (2016).

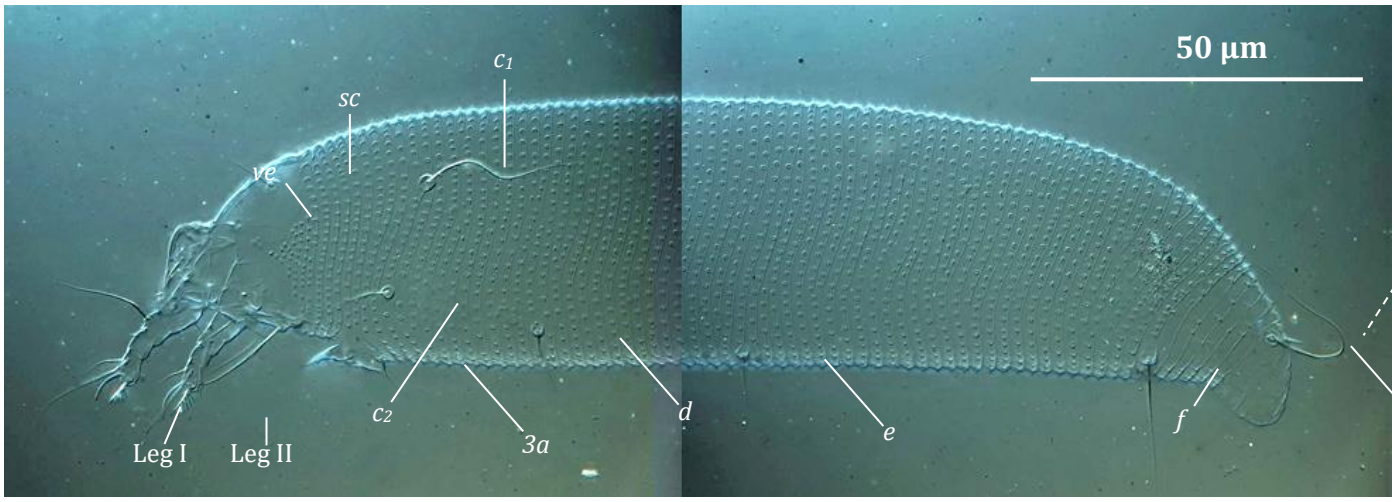

Figure 6. Lateral view of adult female *Phytoptus avellanae*; Tasmania, Australia [Photo: J. Davies].

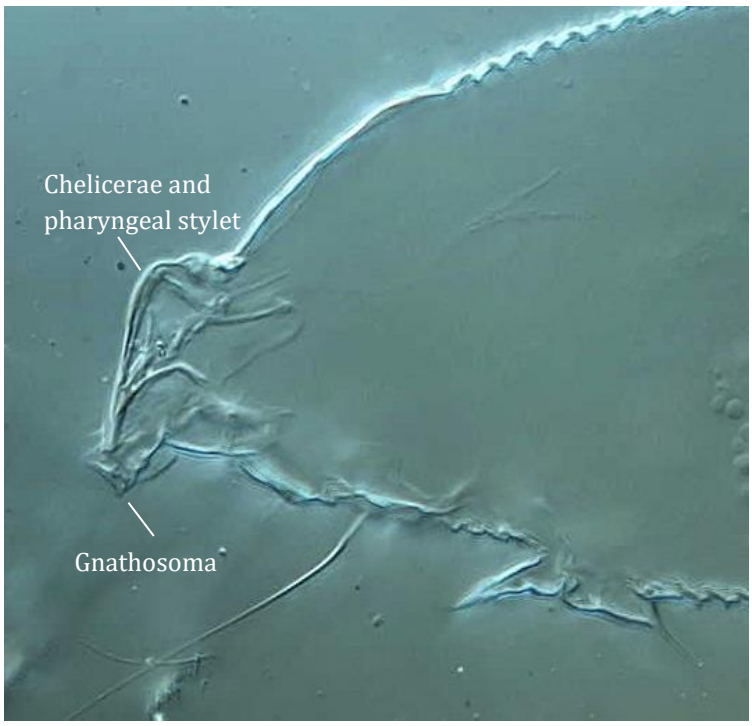

Figure 7. Lateral view of anterior region of adult female *Phytoptus avellanae*; showing gnathosoma details. Tasmania, Australia [Photo: J. Davies].

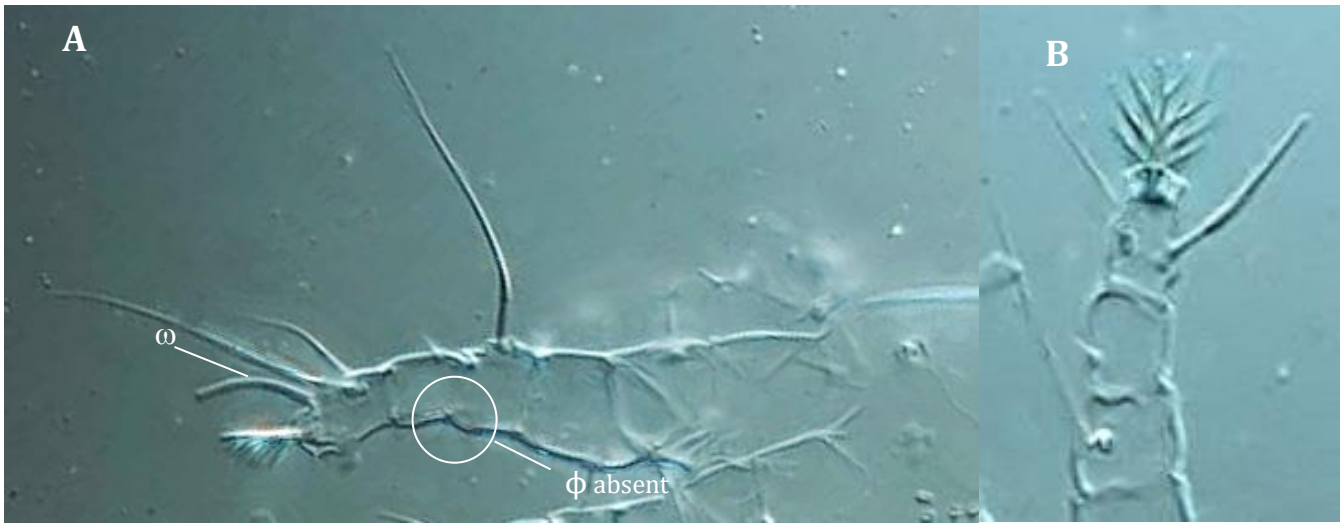

Figure 8. *Phytoptus avellanae*: (A) Lateral view of Leg I; (B) Dorsal view of femur, tibia, tarsus and empodial featherclaw.

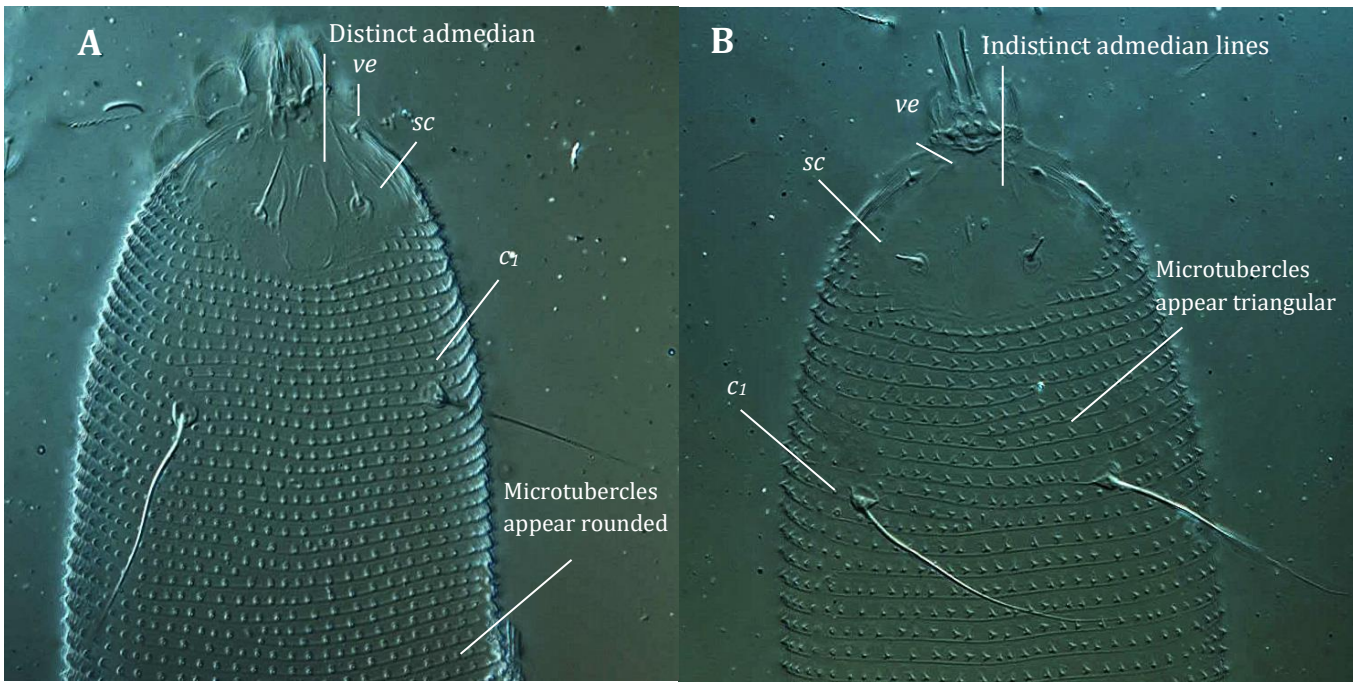

Figure 9. Anterior dorsal region of adult female *Phytoptus avellanae* showing variability. Setae *ve* and *sc* are broken in both specimens. (A) Specimen with distinct admedian lines and rounded microtuberculation, (B) specimen with indistinct admedian lines and triangular microtuberculation. Tasmania, Australia [Photo: J. Davies].

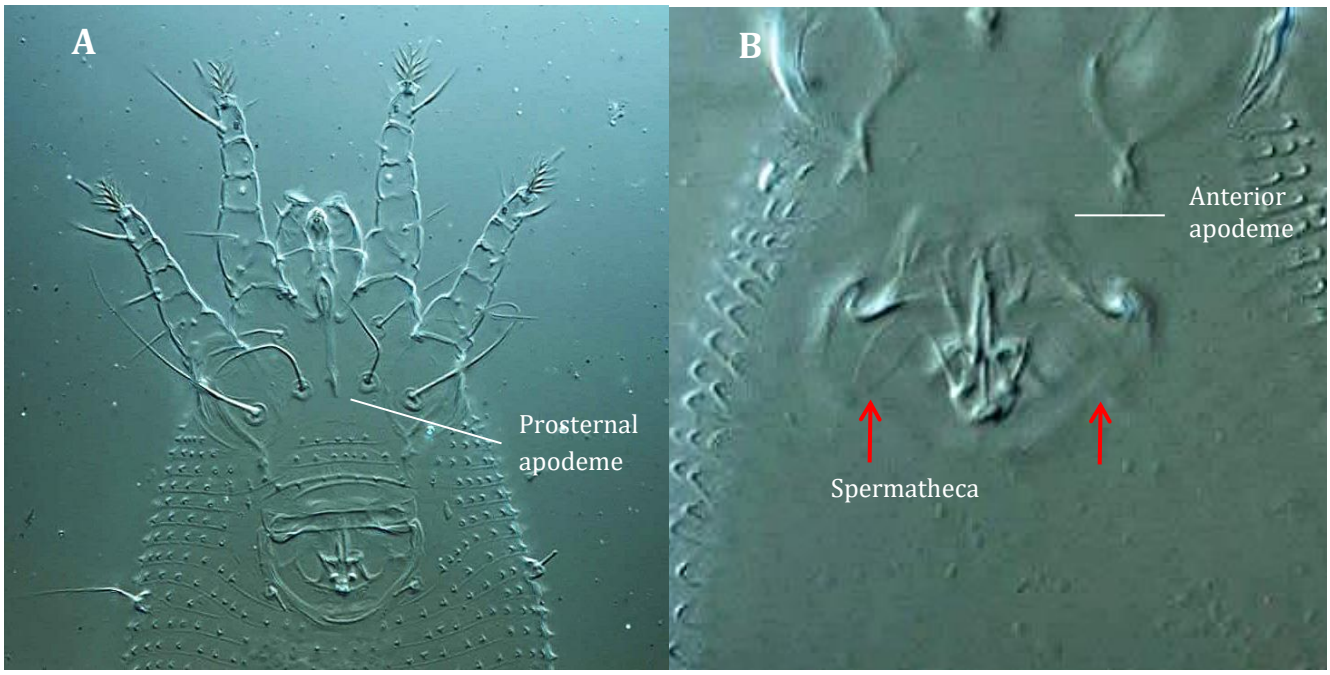

Figure 10. Anterior ventral region of adult female *Phytoptus avellanae*: (A) Ventral view of legs, coxae and female genitalia (B) Close up of female internal genitalia with faint outline of spermatheca (red arrows); Tasmania, Australia [Photo: J. Davies].

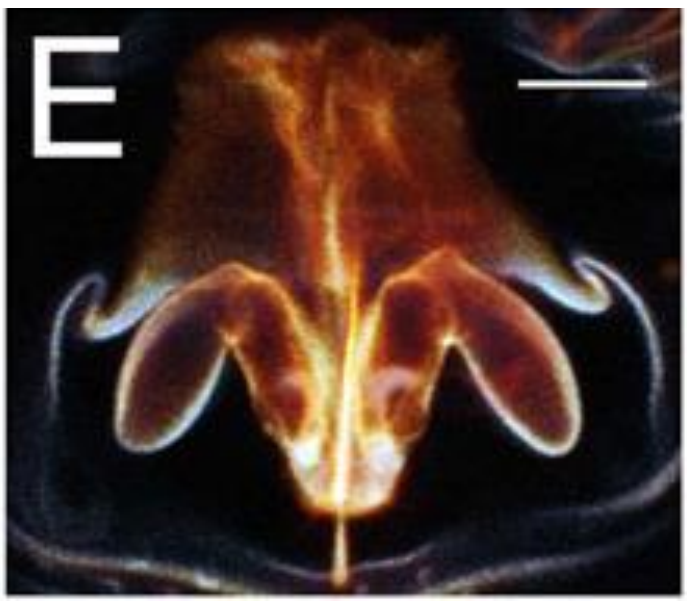

Figure 11. Confocal laser scanning microscopy (CLSM) image of female internal genitalia of *Phytoptus avellanae* (Scale bar = 5µm) [Source: Chetverikov (2014)].

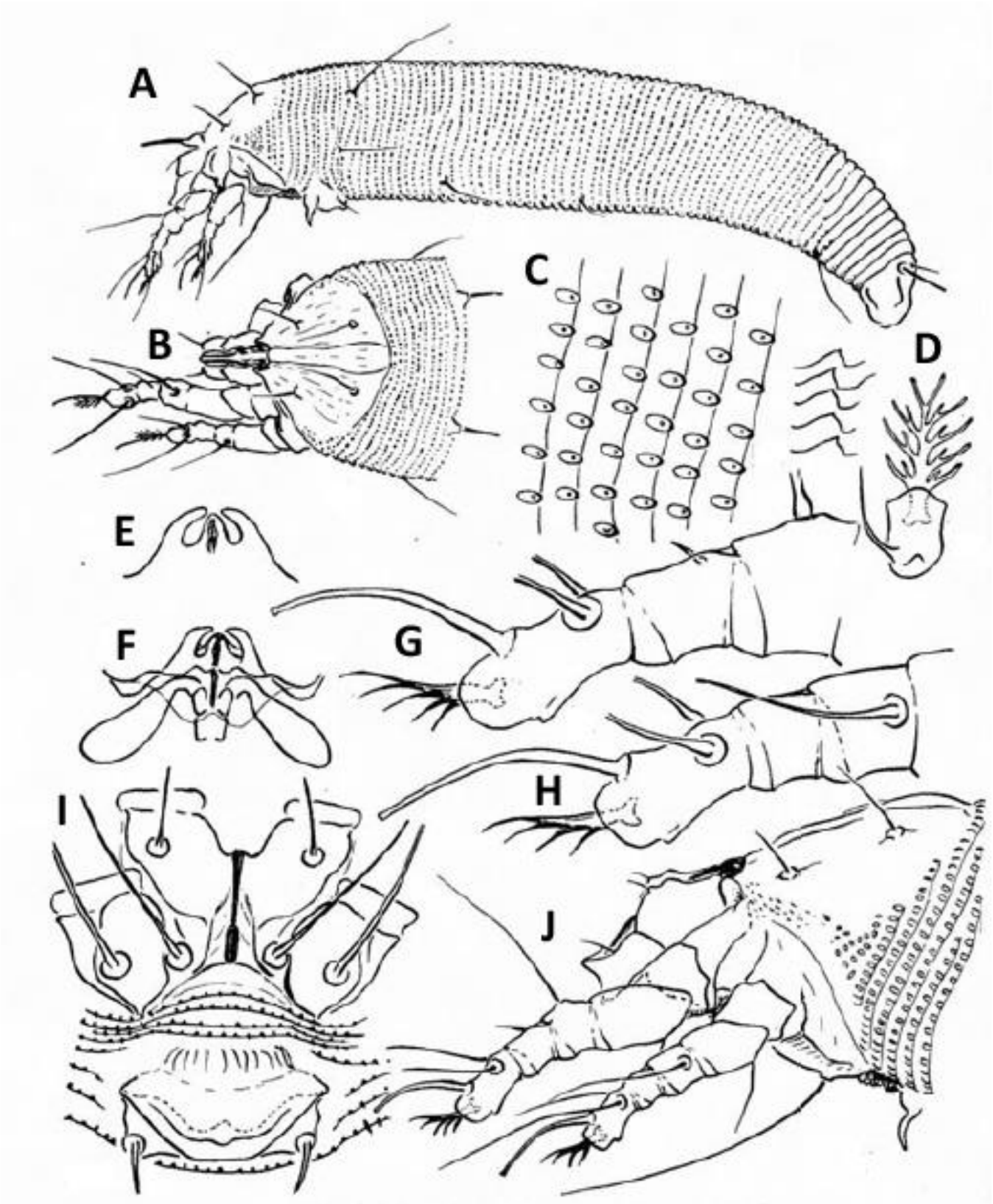

Figure 12. Keifer's illustrations of *Phytoptus avellanae*: (A) Lateral view of entire mite; (B) Prodorsal shield (note: distinct admedian lines); (C) Close up of microtuberculation of annuli in lateral view; (D) Empodium; (E) Anterior apodeme; (F) Internal genitalia; (G) Leg I; (H) Leg II; (I) Coxigenital region; (J) Lateral view of anterior region [Source: Keifer (1940)].

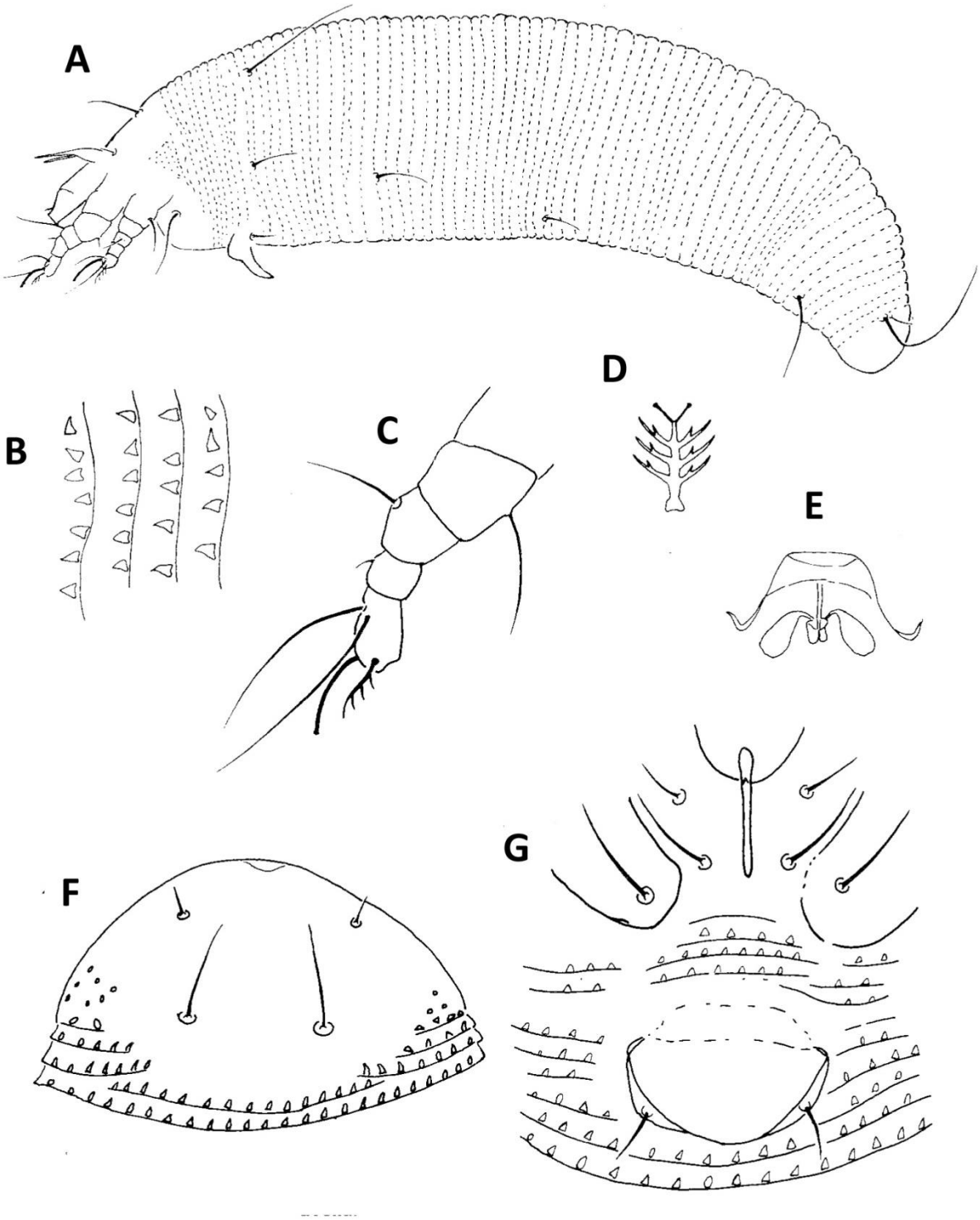

Figure 13. Manson's illustrations of *Phytoptus avellanae*: (A) Lateral view of entire mite, female; (B) Close up of microtuberculation of annuli in lateral view; (C) Leg I; (D) Empodium; (E) Internal genitalia; (F) Prodorsal shield (note: admedian lines are absent) (G) Coxigenital region. [Source: Manson (1984)].

4.2 Molecular diagnosis

Suitable methods for molecular diagnosis of eriophyoid mites are provided in Navajas and Navia (2010), Skoracka and Dabert (2009) and Cvrković et al. (2016). Non-destructive molecular diagnostic methods can be used if the procedures of Dabert et al. (2008) are followed. Mite exoskeletons are retained for morphological diagnosis following DNA extraction in this procedure.

The following procedure, adapted from the references above, was used to provide sequences from a Tasmanian population of *P. avellanae* as part of development of this protocol. Exoskeletons were not able to be suitably prepared for morphological diagnosis following DNA extraction due to damage sustained during handling.

Extract DNA from approximately 20 to 50 mites using the DNeasy® Blood and Tissue kit (Qiagen) following manufacturers' instructions (July 2006).

Perform PCR using the DNA barcoding primers LCO1490 (~5-GGTCAACAAATCATAAAGATATTGG) and HCO2198 (~5-TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994) that target a 710 bp (base pair) section of the cytochrome oxidase I mitochondrial gene (COI) at a final concentration of 0.5 µM. PCR conditions are as follows; 1 cycle of 95°C for 5 mins, 40 cycles for 94°C for 1 min, 45°C for 1 min, 72°C for 1.5 mins and a final extension at 72°C for 10 mins using the Qiagen HotStarTaq MasterMix according to the manufacturers' protocol. Other commercially available PCR taq can be used following the manufacturers' instructions.

Run a 5 µL aliquot of the PCR reactions on a 1-1.5% agarose gel to confirm successful amplification.

Sequences from a *P. avellanae* population collected in Sandy Bay, Tasmania as part of development of this protocol (Tasmanian Agricultural Invertebrate Collection enquiry number EN4726) are lodged on BOLD (ACK5909) (http://www.boldsystems.org/). Sequences from populations of *P. avellanae* originating from Russia, Serbia and the USA are lodged in GenBank (accession numbers KR149013 to KR149042) (Cvrković et al. 2016). Sequences from a New Zealand population of *P. avellanae* are available in Webber (2007).

5 CONTACTS FOR FURTHER INFORMATION

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The Tasmanian Agricultural Invertebrate Collection (DPIPWE Tasmania) and Agricultural Scientific Collections Unit (DPI NSW) have slide-mounted specimens of *P. avellanae* for comparison and have copies of the main taxonomic literature enabling positive identification of this species to be made.

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6 ACKNOWLEDGEMENTS

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Danuta Knihinicki provided advice and guidance on eriophyoid taxonomy and reviewed the protocol.

Alison Dann conducted sequencing and prepared section 4.2 (molecular diagnosis).

Bruce Halliday provided clarification for the history of nomenclatural classification in *Phytoptus avellanae*.

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8 APPENDICES

8.1 Appendix 1: Features of eriophyoid mites recorded from *Corylus* spp.

Species	**Life style, damage symptoms and seasonal location (W = Winter; Sp = Spring; Su = Summer, Au = Autumn)**	**Main diagnostic features of adult female**	**Main references with illustrations, description and/or keys**	**Comments**
PHYTOPTIDAE				
Phytoptus avellanae Nalepa (Phytoptidae: Napellinae) (hazelnut big bud mite)	Gall inducing (causes big bud symptoms) **W:** In big buds. **Sp:** In blasted buds. **Su:** In buds, possibly on leaves (vagrant form). **Au:** In buds.	**Body Shape:** Vermiform (wormlike). **Prodorsal shield:** 4 setae (paired *sc* and *ve*), admedian lines present or absent. **Ornamentation on female genital coverflap**: Unornamented except for short basal lines. **Number of rays on empodia**: 4-5. **Other features**: Forecoxae separated by a sternal line.	This protocol, Cvrković et al. (2016), Chetverikov (2014), Manson (1984), Baker et al. (1996), Keifer (1952), Keifer (1940), Nalepa (1889).	Serious pest when present in hazelnut growing regions (Castagnoli & Oldfield 1996), primary cause of big bud of hazelnut. Cryptic speciation may exist between gall forming and vagrant forms (Cvrković et al. 2016).
ERIOPHYIDAE				
Cecidophyopsis vermiformis (Nalepa) (Eriophyidae: Cecidophyinae) (syn. *Phytoptus vermiformis*)	Refuge seeking or gall inducing **W:** Living as inquilines in big buds caused by *P. avellanae.* **Su:** Produce summer big buds.	**Body Shape:** Vermiform (wormlike). **Prodorsal shield:** 0 *sc* setae, distinct median, admedian and submedian lines. **Ornamentation on female genital coverflap**: Heavily ribbed. **Number of rays on empodia**: 5.	Amrine et al. (2003), Baker et al. (1996), Keifer (1944), Nalepa (1889).	Not known to occur in Australia. Often occurs in buds with *Phytoptus avellanae*. Probably contributes to summer big bud formation.

Coptophylla lamimani (Keifer) (Eriophyidae: Cecidophyinae) (syn. *Phyllocoptes lamimani*)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Fusiform. **Prodorsal shield:** Smooth, 0 *sc* setae. **Ornamentation on female genital coverflap**: With 12-14 longitudinal irregular ridges. **Number of rays on empodia**: 5.	Amrine et al. (2003), de Lillo (1988), Baker et al. (1996), Keifer (1939a).	Not known to cause serious injury Castagnoli and Oldfield (1996). Found along leaf veins on leaf undersides.
Aceria biradiatus de lillo & Fontana (Eriophyidae: Eriophyinae)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Vermiform (wormlike). **Prodorsal shield:** Tubercles near margin, *sc* setae directed to rear. Anteriormedian lobe over gnathosoma base. **Ornamentation on female genital coverflap**: 6 longitudinal striae. **Number of rays on empodia**: 2. **Other features**: Posterior opisthosoma with annuli continuous and subequal dorsoventrally.	de Lillo and Fontana (1996).	
Aculus comatus (Nalepa) (Eriophyidae: Phyllocoptinae) (Filbert rust mite)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Fusiform. **Prodorsal shield:** 2 extremely long *sc* setae on anterior of dorsal shield (protogyne), or absent (deutogyne). **Ornamentation on female genital coverflap**: 4 basal transverse lines and about 11 short ridges orientated radially near margin (protogyne). **Number of rays on empodia**: 4. **Other features**: Annuli broader and fewer dorsally than ventrally, annuli often with few or no microtubercles dorsally. Light amber to dark brown in life.	Krantz (1973); Baker et al. (1996).	Has a deutogyne form. Can have particularly high populations in spring, leading to leaf browning or russeting and to edge-rolling.

Aculus tamalpais (Keifer) (Eriophyidae: Phyllocoptinae) (syn. *Phyllocoptes tamalpais*)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Fusiform. **Prodorsal shield:** 2 long *sc* setae. **Ornamentation on female genital coverflap**: 6 or 8 very faint ridges. **Ornamentation on female genital coverflap**: 6-8 faint longitudinal ridges. **Number of rays on empodia**: 4. **Other features**: Light amber to amber in life. Annuli broader and fewer in number dorsally than ventrally, annuli with few or no microtubercles dorsally.	Baker et al. (1996), Keifer (1939b).	Causes no visible damage. Vagrants of both leaf surfaces.
Anthocoptes loricatus (Nalepa) (Eriophyidae: Phyllocoptinae) (syn. *Phyllocoptes loricatus*)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Fusiform. **Prodorsal shield:** 2 *sc* setae. **Ornamentation on female genital coverflap**: **Number of rays on empodia**: **Other features**: Annuli broader and fewer in number dorsally than ventrally, annuli often with few or no microtubercles dorsally.	Nalepa (1889).	Not known to cause serious injury Castagnoli and Oldfield (1996). Can cause minor rusting of leaves.
Phyllocoptes coryli Liro (Eriophyidae: Phyllocoptinae)	**W:** Unknown **Sp:** Unknown **Su:** Unknown **Au:** Unknown	**Body Shape:** Fusiform. **Prodorsal shield:** 2 *sc* setae, anterior lobe over gnathosoma. **Ornamentation on female genital coverflap**: **Number of rays on empodia**: **Other features**: The original illustration indicates that this is a typical *Phyllocoptes* mite.	Liro and Roivainen (1951), Liro (1931) .	

Tegonotus depressus (Nalepa) (Eriophyidae: Phyllocoptinae) (syn. *Oxypleurites depressus*)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Fusiform. **Prodorsal shield:** Small *sc* setae. **Ornamentation on female genital coverflap**: With about 8 or 9 longitudinal ridges. **Number of rays on empodia**: 4. **Other features**: Annuli broader and fewer in number dorsally than ventrally forming thickened bands.	Baker et al. (1996), Keifer (1952), Keifer (1939b).	Not known to cause serious injury Castagnoli and Oldfield (1996).
Vittacus mandshurica Xue (Eriophyidae: Phyllocoptinae)	Vagrant on leaf surface **W:** Unknown, presumably inside **i**n protected areas (eg beneath axillary buds, under bud scales) **Sp:** Unknown, presumably on leaves **Su:** On leaves **Au:** Unknown, presumably on leaves	**Body Shape:** Fusiform. **Prodorsal shield:** 2 *sc* setae on posterior margin, admedian and submedian lines short and parallel. **Ornamentation on female genital coverflap**: With 12 longitudinal ridges. **Number of rays on empodia**: 4. **Other features**: Annuli broader and fewer in number dorsally than ventrally forming thickened bands.	Xue et al. (2013).	Recorded from China on *Corylus sieboldiana* var. *mandshurica.*
DIPTILOMIOPIDAE				
Diptacus calicoryIi (Keifer) (Diptilomiopidae: Diptilomiopinae) (syn. *Diptilomiopus calicoryli*)	Vagrant **W:** In protected areas (eg beneath axillary buds, under bud scales). **Sp:** On leaves. **Su:** On leaves. **Au:** On leaves.	**Body Shape:** Fusiform. **Prodorsal shield:** Faint pattern, *sc* anterior of rear margin, projecting forward. **Ornamentation on female genital coverflap**: Smooth with slight transverse basal microtubercular area. **Number of rays on empodia**: Split, 7 rayed. **Other features**: In life, light yellow and covered by dense white flocculent wax.	Baker et al. (1996), Keifer (1943).	Not known to cause serious injury.

8.2 Appendix 2: Media used to store, clear and slide mount eriophyoid mites.

Sorbitol fluid

Use: Specimen storage

Method:

Composed of 25% solution of propan-2-ol in water to D-sorbitol powder (e.g., add about 4 ml propan-2-ol diluted with 12 ml water to 30 g D-sorbitol powder) until forming a thin syrup with the consistency of heated honey, at most. When the liquid is added to the powder, the mixture is milky white and after a few hours it dissolves properly, becoming clear and slightly thick. At warm and humid environmental conditions, a very small amount of potassium iodide and an iodine crystal should be added to the mixture to prevent mould growth. The mixture should be kept in a sealed and well closed container, because it quickly becomes too thick and crystallizes when exposed to air.

Source: Modified from de Lillo et al. (2010)

Keifer's booster

Use: Clearing agent

Method:

- 3.0 g sorbitol
- 7.5 g chloral hydrate
- 1.0 g iodine crystals
- 15.0 cc distilled water
- 1.0 cc concentrated HCl

Source: Amrine and Manson (1996)

Kono's medium

Use: Clearing agent

Method:

- 100g chloral hydrate
- 10g glycerine
- 50mL distilled water
- 1mL conc. HCl

Source: Jeppson et al. (1975)

Nesbitt's fluid

Use: Clearing agent

Method:

- 40 g chloral hydrate
- 25 mL distilled water

- 2.5 mL concentrated hydrochloric acid

Hoyer's medium

Use: Medium for slide mounting with clearing properties

Method:

- 40cc distilled water
- 30g gum arabic
- 200g chloral hydrate
- 20g glycerine

Source: Amrine and Manson (1996)

Modified Berlese medium

Use: Medium for slide mounting with clearing properties

Method:

- 5.0 g sorbitol
- 1.0 cc glycerine
- 1.0 cc distilled water

Gently boil to dissolve then add:

- 3.0 g Benzophenone-3,3′,4,4′-tetracarboxylic dianhydride, 96% (BTDA) (Product number B9750 from Sigma Aldrich http://www.sigmaaldrich.com/australia)

Gently boil again to dissolve – solution becomes clear yellow – then add:

- 7.0 cc distilled water
- 4.0 cc glycerine
- 3.0 cc glacial acetic acid
- 70.0 g chloral hydrate

Stir on hot plate until dissolved and clear. Pour about 4 cc of medium into small snap cap vials. Place open vials on a hot plate (low setting) for several minutes until the medium becomes slightly thicker than honey. Add 6-8 drops of glacial acetic acid to each 4 cc of medium. Many eriophyoids can be mounted directly into this medium and cleared on a hot plate. Various stains may be added to this medium: I_2 chlorazol black E, lignin pink or toluidine blue. A small piece of metallic iodine and approximately 30 mg of KI can be added to each small (4 cc) vial of medium. The salt must be added to allow the metallic iodine to dissociate. The iodine enhances setae, microtubercles and sculpturing of the cuticular structures.

Source: Amrine and Manson (1996)