

National Diagnostic Protocol

Sugarcane Woolly Aphid *Ceratovacuna lanigera*



NDP 43 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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Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs, unless it is shown that the NDP has improved procedures for Australian conditions. NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

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Process

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NDPs are living documents. They are updated every 5 years or before this time if required (i.e. when new techniques become available).

Document status

This version of the National Diagnostic Protocol (NDP) for Sugarcane woolly aphid *Ceratovacuna lanigera* is current as at the date contained in the version control box below.

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1 INTRODUCTION

The genus *Ceratovacuna* Zehntner (1897) has 24 species listed in the aphid species file (aphid.specidfile.org accessed 3/6/2021). The type species is *Ceratovacuna lanigera* Zehntner, by original monotypy.

1.1 Host range

The primary host is sugarcane (*Saccharum officinarum*).

Alternative hosts include *Bambusa* sp., *Coix lacryma-jobi*, *Cynodon dactylon*, *Grassum* sp., *Miscanthus* sp. including *japonicas*, *sinensis* (elephant grass, maiden grass), *Oplismenus* sp., *Pseudechinolaena*, *Saccharum spontaneum* and *S. sinense*, *Sorghum halepense*, *Themeda* sp., *Xylosma longifolia* (Joshi & Viraktamath, 2004; Holman, 2009; Blackman and Eastop, 2006).

1.2 Transmission

The movement of sugarcane with leaf material remaining i.e. for seed or for crushing or using the cane tops as stock feed, promotes rapid movement to new areas. Infection by movement of cut cane is rare, as long as the green leaf sheath has been removed from the cane.

Local transmission of *C. lanigera* is mainly accomplished by dispersion of the alate (winged adults) from field to other close by fields. *C. lanigera* will move within the crop by active dispersal of young nymphs by crawling or by congregating on leaf tips and using air currents to “balloon” to adjacent plants.

1.3 Life history

Anholocyclic (no males) on Poaceae, no host alternation has been demonstrated.

The optimal temperature for the development of *C. lanigera* was found to be 20-23°C. It was not as active below 15°C or above 28°C. The nymphal stage of the apterous form lasts about 23-32 days, but that of the alate form lasts 32-40 days. The alate adult, which is capable of migrating from one cane field to another, lives for around 8 days and gives birth to an average of 10 offspring, whereas the apterous adult, stationed on the under-surface of a leaf, survives for 36 days and produces an average of 60 offspring per female (Takano, 1941).

2 TAXONOMIC INFORMATION

Order: Hemiptera

Family: Aphididae

Subfamily Hormaphidinae

Tribe Cerataphidini

Genus: *Ceratovacuna*

Species: *lanigera*

Ceratovacuna lanigera, Zehntner (1897) is the type species of the genus.

Synonyms: *Oregma lanigera* van Deventer (1906) and *Cerataphis saccharivora* Matsumura (1917) (Favret, 2015).

Strains: None identifiable by morphology.

Common names: Sugarcane woolly aphid, White sugarcane aphid.

3 DETECTION

3.1 Symptoms

Ceratovacuna lanigera aphids develop into large colonies on the under surface of sugarcane leaves, these are of a fluffy white appearance due to the waxy coats of the aphid (Figure 1). These colonies then exude honeydew which drips onto the leaves below, causing infections of sooty moulds on these leaves, which darken them (Figure 2). This dark coating can affect photosynthesis and lead to stunting of the plant. Most sugarcane varieties have been shown to be susceptible to the aphid.

These aphids can also be attended by ants. Five species of ants have previously been documented attending to *C. lanigera* populations around the world, with *Polyrhachis dives* the most abundant species that attends and protects the aphid (Rueda and Calilung, 1974). This ant species has been recorded in Australia, but is so far only found on the north western coastal region of the Northern Territory (Cardale, 1985).



Figure 1 Underside of sugarcane leaf, showing typical *Ceratovacuna lanigera* infestation (India, ©A. Boulton NSW DPI).



Figure 2 Sugarcane leaf, showing sooty mould resulting from *Ceratovacuna* infestation (India ©A. Boulton NSW DPI).

3.2 Sampling procedures

Collection for identification will require either a section of plant material hosting the aphids or preferably just the aphids themselves collected into a sample bottle containing alcohol (>70% ethanol). A large sample is preferred to increase the chances of collecting adult specimens needed for accurate identification.

3.2.1 Preparation of aphids for microscopic identification

For accurate species level identification, aphids need to be mounted on a microscope slide and viewed under a compound microscope.

Slide preparation for identification follows the protocol described by Blackman and Eastop (2000):

- Gently heat the specimens in 95% alcohol for 1-2 minutes.
- Pipette off alcohol, and add 10% Potassium hydroxide (KOH) solution.
- Gently make a small incision behind the rear leg and heat for 3-5 minutes.
- Pipette off the KOH solution and wash the specimens free of all KOH using 5-6 changes of distilled water, leaving them to soak for at least 5 minutes each time.
- Remove all distilled water and add glacial acetic acid and leave for 2-3 minutes.
- Pipette off and repeat with fresh glacial acetic acid. Pipette off.
- Add clove oil as a clearing agent (specimens will float). Leave for 10-20 minutes until specimens have cleared.

- Transfer 1-2 aphids to a drop of thin Canada Balsam on a clean microslide and arrange them with body untwisted, dorsal side up, and appendages spread out.
- Lower a clean coverslip onto the specimens so as to spread the mountant evenly and not trap any air bubbles.
- Dry the slide horizontally until the mountant has properly dried (as per usual laboratory practice eg. in an oven at 50°C for about 1-3 weeks)

A water based Hoyer's medium can be used as an easier alternative (it possesses a limited clearing ability making transfer of aphid directly from 70% alcohol into the mounting medium possible), but it should *only* be used to create temporary slides, not for long term storage and collection. During preparation, a dry block heater is recommended for any heating steps.

The specimen can be examined (with care) under a compound microscope once the Canada balsam has started to cure (1-2 days), however after examination the slide should be returned to complete the drying.

4 IDENTIFICATION

4.1 Morphological methods

C. lanigera apterae (wingless adults, Figure 3) are small to medium sized aphids (1.4-2.3 mm), pale green or brownish covered with white woolly wax, forming dense colonies on the lower sides of leaves of host plants, often attended by ants (Blackman and Eastop, 2000). The apterae are also easily recognised by the pair of horns projecting forward from the front of the head (eg: Figure 7).

Alatae (winged adults, Figure 4) are slightly larger (2.0-2.5 mm) with a brown to black head and thorax, and dusky transverse bands on dorsal abdomen (Blackman and Eastop, 2000).



Figure 3. Apterae (wingless) form of *Ceratovacuna lanigera*



Figure 4. Alate (winged) form of *Ceratovacuna lanigera*

(<http://www.nbair.res.in/Aphids/Ceratovacuna-lanigera.php>)

The following descriptions are taken from Ghosh (1988).

Apterous viviparous female: (Figures 5, 6, 7, 8). Body 2.1-2.7mm long with 1.1-1.6mm as maximum width. Head and prothorax fused, pale brown to pale; dorsal cephalic hairs fine, acute at apices, less than 10, anterior to the level of eyes, 0.033-0.066mm long, longer than the frontal horns, and 1.0-1.8x as long as the basal diameter of antennal segment III. Frontal horns acute, divergent, bearing 6-10 very short hairs up to 0.080mm long, and 1.2-1.6x (in specimens with 4-segmented antennae) or 1.4-1.9x (in specimens with 5-segmented antennae) as long as antennal segment II. Antennae 4-5-segmented, 0.12-0.16x as long as the body, usually pale brown with apical segments darker; flagellum gradually more distinctly imbricated, imbrications often spinulose apical; hairs on the flagellum sparse, longest hair on segment 1110.020-0.043mm long, 0.90-1.40x as long as the basal diameter of antennal segment III; terminal process 0.33-0.56x as long as the base of last antennal segment. Wax glands when present on cephalothoracic region composed of 3-5 cells and located just below base of antennae and on the posterolateral margin of pronotum. Rostrum reaches forecoxae, ultimate rostral segment 0.45-0.55x as long as second segment of hind tarsus. Abdominal dorsum pale bearing some fine

irregular sculpturing; hairs on the dorsum of abdomen fine, 0.033-0.056mm long, 1.0-1.8x as long as the basal diameter of antennal segment III; 7th and 8th tergites, each with 7 spinal hairs, 0.50-0.073mm and 0.033-0.046mm long and 1.6-2.5x and 1.0-1.4x as long as the mentioned diameter, respectively. Wax glands may be present laterally on meso- and meta-notum and almost always present on 1st-7th tergites, these glands when fully developed composed of 2-6, round or irregular-shaped cells; 8th tergite with a median wax gland on pale brown spino-pleural area, composed of 9-27 round or irregular-shaped cells. Siphunculi on slightly elevated cones. Cauda transversely oval bearing 11-12 hairs including two long and thick hairs. Subanal plate bilobed. Legs smooth, pale brown, bearing many long fine hairs, longest one on hind tibiae 0.053-0.063mm long, 1.1-1.3x as long as the diameter at the middle of hind tibia; first tarsal segments with 4, 3, 2 or 3, 3, 2 hairs, dorso-apical hairs on second tarsal segments expanded at apices.

Colour: Pale green/brownish yellow in life.

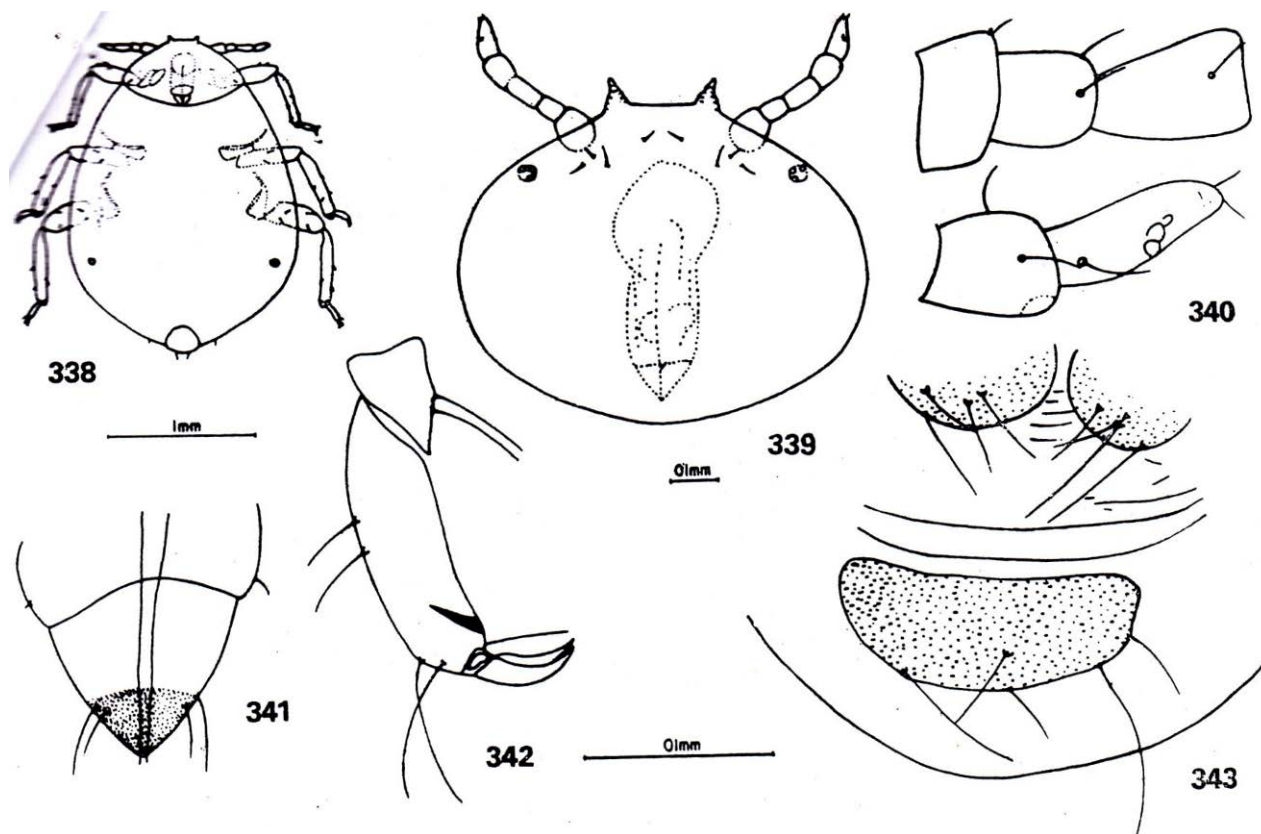


Figure 5 *Ceratovacuna lanigera*. Apterous viviparous female: 338, whole body 339, head; 340, antenna; 341, ultimate rostral segment; 342, hind tarsus 343, caudal region (Ghosh, 1988).



Figure 6 *Ceratovacuna lanigera* adult apterae (A. Boulton NSW DPI).



Figure 7 *Ceratovacuna lanigera* adult apterae head detail (A. Boulton NSW DPI).



Figure 8 *Ceratovacuna lanigera* adult apterae detail of cauda, anal and genital plates (A. Boulton NSW DPI).

Alate viviparous female: (Figure 9, 10) Body 2.3-2.6mm long with 0.90-1.28mm as maximum width. Head brownish black on anterior portion and darker on posterior portion; dorsal cephalic hairs fine, 0.03-0.04mm long, longest one 2.6-2.7x as long as the basal diameter of antennal segment III. Frontal horns short, much reduced. Antennae 5-segmented, 0.24-0.28x as long as the body, with spinulose imbrications on segment III; hairs on flagellum fine, sparse; segment III with 21-22 and IV and V each with 4-8 and 4-5 annular secondary rhinaria. Blackman and Eastop (2006) list a greater range of 16-25 on ANTIII, 5-10 on IV and 2-9 on base of V; terminal process 0.19-0.21x as long as base of last segment. Rostrum reaches little beyond fore-coxae, ultimate rostral segment 0.50x as long as second segment of hind tarsus. Abdominal dorsum pale bearing transverse sclerotic bands on 6th, 7th and 8th tergites; 7th tergite with 2 long fine spinal hairs, 8th tergite with 6 (sometimes more) hairs, longest one being 2.6-2.7x as long as the basal diameter of antennal segment III. Siphunculi pore-like. Cauda semi oval. Legs with femora pale to brown near base, rest dark brown. Wings with venation normal; media once branched; pterostigma- short.

Colour: Black brown in life

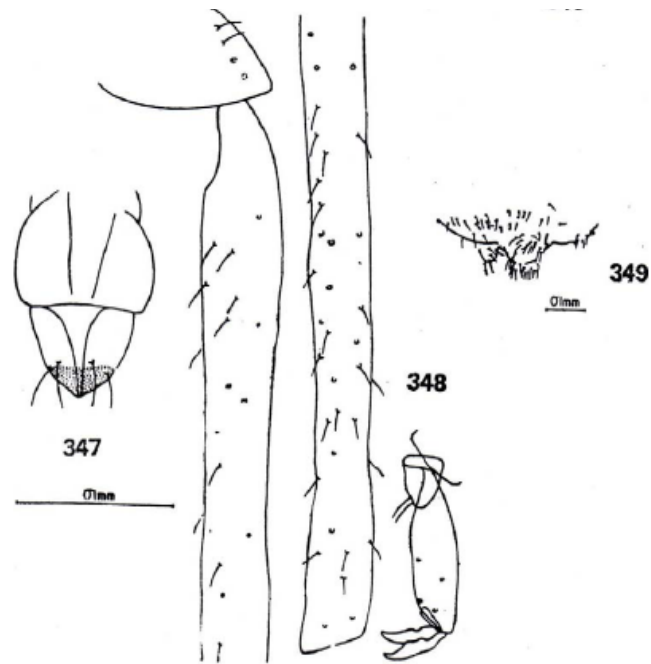


Figure 9 *Ceratovacuna lanigera*. Alate viviparous female: 347, ultimate rostral segment; 348, hind leg; 349, caudal region. (Ghosh 1998)



Figure 10 *Ceratovacuna lanigera* adult alate antennae (K. Walker <http://www.padil.gov.au/pests-and-diseases/pest/main/136157#>).

Nymph (Early stage: apterous Figure 11): Body 0.96mm long with 0.45mm as maximum width. Head fused with prothorax, dorsal cephalic hairs fine, up to 0.050mm long, nearly twice as long as basal diameter of antennal segment III, 1.5x as long as antenna segment II. Frontal horns divergent, acute at apex, up to 0.130mm long, 4.0x as long as antennal segment II, becoming much smaller (0.071mm) at later stage with body size 1.10mm. Antennae 4-segmented, little less than 0.16x as long as the body, segment I, II and IV each with single fine hair, segment III with 2 similar hairs, up to 0.033mm long; processus terminalis little over half as long as the base of last antennal segment. Abdominal dorsum pale; dorsal hairs fine, acute, 0.035-0.060mm long, always longer on posterior tergites; 8th tergite with 2-3 spinal hairs up to 0.060mm long. Siphunculi pore-like with thick rims. Cauda with 2 long hairs. Legs pale bearing many fine hairs on femora and tibiae, on hind tibiae 0.030-0.050mm long, 2.0x as long as the diameter at the middle of tibia; first tarsal segments with 3, 3, 2 hairs, which are fine and 0.056-0.066mm long; dorso-apical hairs on second tarsal segments distinctly expanded at apices.



Figure 11 *Ceratovacuna lanigera* nymph, note very conspicuous hornlike projections on head (A. Boulton NSW DPI).

4.1.1 Identification keys

A key to cover the entire species range is available online (<http://www.aphidsonworldsplants.info>. Accessed 2. 6. 2021). This key is extremely extensive, and covers all known 250 species of grass feeding aphids, hence is not replicated here.

Distinguishing features/characteristics that will prevent misidentification.

Ceratovacuna lanigera aphids should only be found on sugarcane. Other aphid species present in Australia that have been reported on sugarcane are:

- *Rhopalosiphum maidis* (Corn leaf aphid)
- *Sitobion miscanthi*
- *Hysteroneura setariae* (Rusty plum aphid)
- *Melanaphis sacchari*

C. lanigera is distinct from these species, as it is the only species of the above group found on sugarcane that has the combination of horns projecting off the front head, a woolly wax covering and the siphunculus on the fifth dorsal segment that are reduced to circular openings.

Key for the identification of aphids in sugarcane (Blackman and Eastop 2000).

Ten species are included in this key to aphids on sugarcane:

<i>Ceratovacuna lanigera</i>	<i>Rhopalosiphum maidis</i>
<i>Forda orientalis</i>	<i>Sipha flava</i>
<i>Geoica lucifuga</i>	<i>Sitobion miscanthi</i>
<i>Hysteroneura setariae</i>	<i>Tetraneura javensis, T. nigriabdominalis</i>
<i>Melanaphis sacchari</i>	

A number of other species are known to feed on *Saccharum officinarum* that are not included here, including:

- *Anoecia corni*;
- *Aphis aurantii, gossypii*;
- *Brachycaudus helichrysi*;
- *Ceratovacuna perglandulosa, sylvestrii*;
- *Melanaphis indosacchari, sorghi*;
- *Sipha maydis*;
- *Tetraneura fusiformis*

1. Front of head with a pair of horns projecting forward (Fig. 12a) *Ceratovacuna lanigera*
 Front of head without horns 2
2. Terminal process shorter than base of last antennal segment (Figs 12b, c) 3
 Terminal process longer than base of last antennal segment 6
3. Siphunculi completely absent. Tarsi 2-segmented 4
 Siphunculi present as small dark cones (Fig. 12d). Tarsi 1-segmented (Fig. 12e) 5
4. Anal plate displaced dorsally (Figs 12f,g). Dorsal abdominal hairs mainly with spatulate or fan-shaped apices *Geoica lucifuga*
 Anal plate in normal position. Dorsal abdominal hairs all small and pointed..... *Forda orientalis*
5. Abdominal wax pore-plates typically with one large cell incompletely surrounded by many smaller cells (Fig. 12h) *Tetraneura javensis*
 Abdominal wax pore-plates small, typically composed of one cell or a few similar-sized cells (Fig. 12i)..... *Tetraneura nigriabdominalis*
6. Dorsal body hairs long and spine-like. Cauda with a constriction and a knob-like apex (Fig. 10j). Siphunculi very short, truncate cones (Fig. 12k)..... *Sipha flava*
 Dorsal body hairs small. Cauda tapering, tongue-shaped. Siphunculi tubular 7
7. Cauda and siphunculi short and dark, neither more than 0.1 of body length 8
 Cauda pale and siphunculi dark, both, more than 0.1 of body length 9
8. Body rather elongate. Siphunculi (Fig. 12m) a little longer than cauda. Terminal process less than 2.5 times longer than base of last antennal segment *Rhopalosiphum maidis*
 Body ovate. Siphunculi (Fig. 12l) a little shorter than cauda. Terminal process more than 3 times longer than base of last antennal segment *Melanaphis sacchari*
9. Body broadly spindle-shaped. Siphunculi with a subapical zone of polygonal reticulation (Figs. 12 n,o) *Sitobion miscanthi*
 Body broadly ovate. Siphunculi without polygonal reticulation (Fig. 12p) *Hysteroneura setariae*

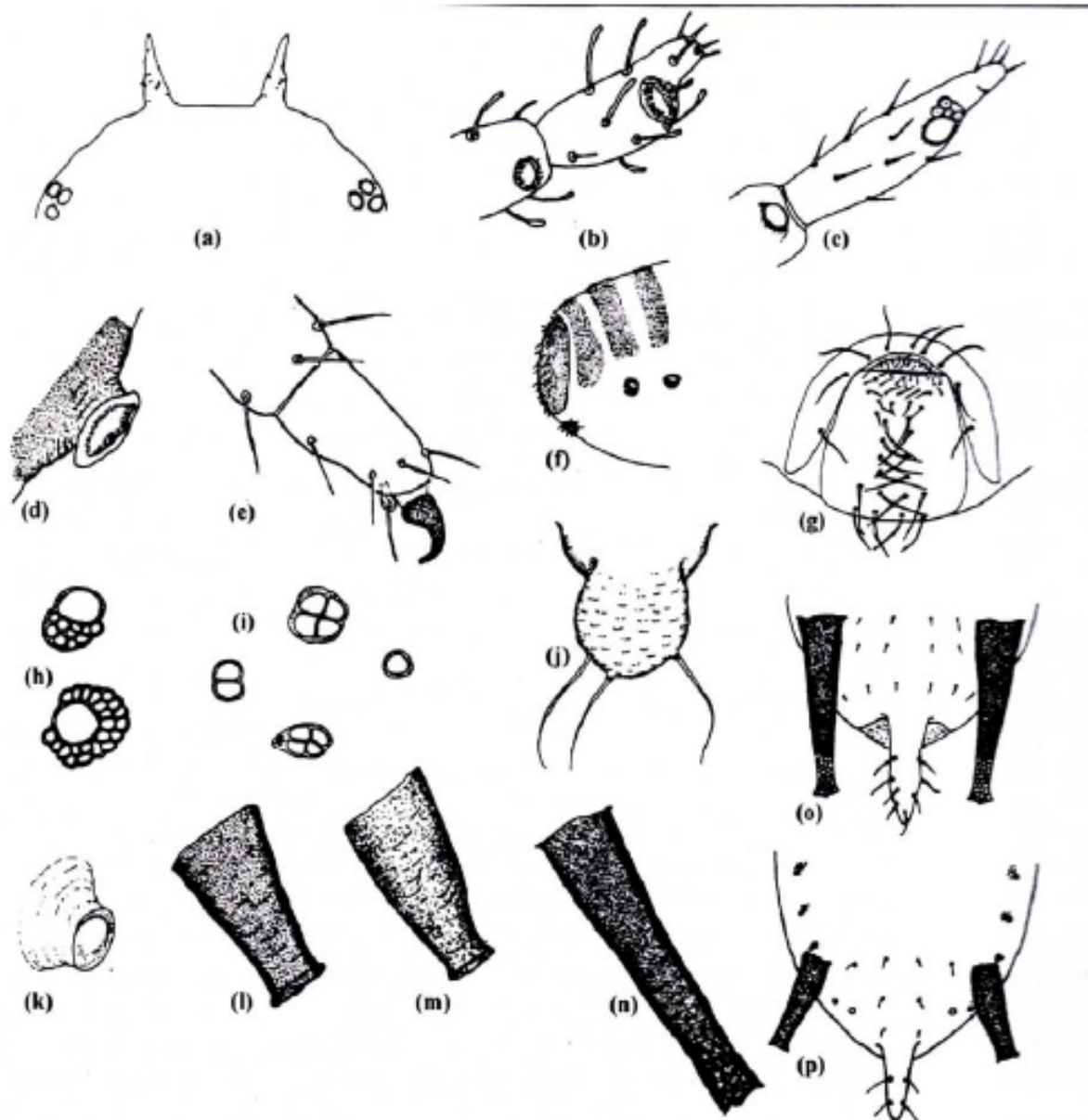


Figure 1. Aphids on sugarcane. (a) Front of head of *Ceratovacuna lanigera*; (b) last antenna segment of *Geoica lucifuga*; (c) same of *Forda* sp.; (d) siphunculus of *Tetraneura*, sp.; (e) hind tarsus of *Tetraneura* sp.; (f) end of abdomen *Geoica* sp. in lateral view; (g) posterior view of anal plate of *Geoica lucifuga*; (h) dorsal abdominal wax glands of *Tetraneura javensis*; (i) same of *T. nigriabdominalis*; (j) cauda of *Sipha flava*; (k)–(n) siphunculi of (k) *Sipha flava*, (l) *Melanaphis sacchari*, (m) *Rhopalosiphum maidis*, (n) *Sitobion* sp.; (o) end of abdomen of *Sitobion miscanthi*; (p) same of *Hysteroneura setariae*. (Blackman & Easop 2000).

4.2 Molecular methods

4.2.1 DNA Barcoding

The current worldwide standard for insect identification is sequences based the mDNA retrieved from the *cytochrome c oxidase* subunit I (COI or *cox1*).

COI barcoding is used in the project Barcode of Life (BOL). The International Barcode of Life (iBOL) is a global collaboration that aims to build DNA barcode libraries for each animal group including insects (ibol.org). The barcode sequences are placed in the Barcode of Life Data Systems (BOLD) database.

Currently the BOLD system has 31 published records which have been mined from Genbank and other sources ([Bold Systems v4](#)).

Barcoding methods for aphids can be found in many published papers (eg Rebijith *et al* 2013) and primers can be found on the BOLD database.

These methods have not been verified as part of this protocol.

5 CONTACTS FOR FURTHER INFORMATION

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The protocol was review by Cameron Brumley, DPIRD

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7.1 Resources

Barcode of Life Data Systems (BOLD) <http://www.boldsystems.org>

Blackman RL, Eastop VF, Kibby GG. (1998). *TAXAKEY Aphids on the World's Crops*. CAB International.