Subcommittee on Plant Health Diagnostics

National Diagnostic Protocol

Exotic leafhopper *Xylella* vectors and related species (Cicadellinae)

NDP 55 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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Process

NDPs are facilitated and endorsed by the Subcommittee on Plant Health Diagnostics (SPHD). SPHD reports to Plant Health Committee and is Australia's peak technical and policy forum for plant health diagnostics.

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Document status

This version of the National Diagnostic Protocol (NDP) for exotic leafhopper *Xylella* vectors and related species (*Cicadellinae*)– NDP55 V1 is current as at the date contained in the version control box below.

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Further information

Inquiries regarding technical matters relating to this project should be sent to: sphd@aff.gov.au

Endorsement and publication details

The NDP was endorsed by the Subcommittee on Plant Health Diagnostic (SPHD) in 2025.

The NDP has been reviewed and verified by subject matter experts within Australia. The NDP has been verified only for the morphological identification methods. The molecular identification methods were not verified due to the limited reference sequence available for the species covered in the NDP.

Review and update: First approved in 2025.

Specific notes on the revision: None

Contents

1 INTRODUCTION

Cicadellinae leafhoppers, commonly known as sharpshooters, are xylem–feeders (Wilson *et al*. 2020). One of the main diagnostic features of this group is the strongly inflated frons and clypeus which contain well developed (cibarial) muscles used for feeding on the xylem tissue of plants under negative pressure.

There are at least 350 genera and 3100 species known in the subfamily Cicadellinae worldwide (Dietrich 2006). This includes several serious economic pests, which vector plant diseases. Cicadellinae species are important vectors of *Xylella fastidiosa*, a xylem–limited bacterium that causes disease in crops such as "Pierce's Disease" in grapes, "Phony Peach Disease" and "Plum leaf scald" in stone fruit, "Citrus Variegated Chlorosis (CVC)" in citrus, "Coffee Leaf Scorch (CLS)" in coffee and "Almond Leaf Scorch" in almonds (Paradell *et al.* 2012, SPHD 2013). The bacterium is also responsible for olive quick decline syndrome (OQDS).

This protocol focusses on 7 exotic sharpshooter species: [Proconiini] *Acrogonia terminalis* Young, 1968, *Oncometopia facialis* (Signoret, 1854); [Cicadellini] *Cicadella viridis* (Linnaeus, 1758), *Dilobopterus costalimai* Young, 1977, *Draeculacephala minerva* Ball, 1927, *Graphocephala atropunctata* (Signoret, 1854), and *Xyphon fulgidum* (Nottingham, 1932). These species are considered to be highly to moderately invasive and have a wide host range or are widely distributed in their current distribution range (Redak *et al*. 2004). All of these species (except for *C. viridis*) are known to vector *Xylella* in a variety of crops including grapevine, citrus, coffee and almond. A general distribution of these species can be found in Appendix 8.1.

Cicadella viridis (Cicadellini), is included in this protocol, although not known to be a vector of *X. fastidiosa* in its natural environment. However, it is a common species found in humid meadows and grasslands across Europe, the Middle East and Asia. Studies have shown that the acquisition of *Xylella* by *C. viridis* is low (Bodino *et al*. 2022), but like other xylem feeders, it has the potential to act as a vector of *X. fastidiosa* (Wilson & Turner 2021). It is considered a "relevant vector candidate" on grapevine (EFSA Panel on Plant Health (PLH) *et al*. 2019, Hasbroucq *et al*. 2020, Bodino *et al*. 2022).

Graphocephala atropunctata is one of the principle and most efficient natural vectors of *X. fastidiosa*, causing Pierce's Disease in grapevines. It is a highly invasive threat due to its abundance where it does occur and is found in diverse ecosystems (Hill & Purcell 1994, Redak *et al*. 2004, EFSA 2015, EFSA 2019, Robinson 2022). *Draeculacephala minerva* and *X. fulgidum* are also important vectors of Pierce's Disease, and *D. minerva*, is of high concern as an invasive pest, having a wide plant host range. *Xyphon fulgidum* is only a moderate invasive risk vector as it feeds mostly on grasses (Redak *et al*. 2004). These three species are known to cause Almond leaf scorch and Alfalfa Dwarf Disease through transmission of *X. fastidiosa* (Redak *et al*. 2004, Wilson *et al*. 2021).

Acrogonia terminalis, *D. costalimai* and *O. facialis* are associated with citrus orchards and are recorded as vectors of *X. fastidiosa* causing CVC (Roberto *et al*. 1996, Garcia *et al*. 1997, Gould & Lashomb, 2007). A biological study of the latter two species is found in Paiva *et al*. (2001) (in Portuguese) and both are vectors of CVC and CLS (Redak *et al*. 2004; Wilson & Turner, 2021).

There is little recent literature on *A. terminalis* as a vector of *Xylella*. Instead, two other *Acrogonia* species, *A*. *citrina* Marrucci & Cavichioli, 2002 and *A. virescens* (Metcalf, 1949), are presented as important vectors (Redak *et al*. 2004, Wilson & Turner 2021). *Acrogonia citrina* is illustrated in this protocol (Figs. 6C,F and 7A–C) and is also highly invasive (Redak *et al*. 2004).

At least 37 other Cicadellinae are known to be *Xylella* vectors (Redak *et al*. 2004, EFSA 2019), but these species are not covered in this protocol, in part as they are either low to medium level invasives, have limited distribution or are considered to be less efficient vectors. This includes species such as *Bucephalogonia xanthophis* (Berg, 1879), *Macugonalia leucomelas* (Walker, 1851) and *Oncometopia nigricans* (Walker, 1851).

Kolla paulula (Walker, 1858) and *Bothrogonia ferruginea* (Fabricius, 1787), both found in Asia, are potential vector candidates in vineyards in Taiwan where *X. fastidiosa* has been introduced. In laboratory studies, these species were shown to have a low rate of transmission (Tuan *et al*. 2016) compared with the other species presented in this protocol.

Further information about host plants and diseases vectored by the sharpshooter species covered in this protocol can be found in Table 6 (Appendix 8.2).

National Diagnostic Protocols of two other significant exotic *Xylella fastidiosa* vector species, including 1) the Glassy–winged sharpshooter (GWSS), (SPHD (2013) Glassy–winged sharpshooter, *Homalodisca vitripennis* (Germar) NDP 23, (currently being revised)) and 2) the meadow spittle bug (SPHD (2025) , Meadow Spittlebug (*Philaenus spumarius* – NDP 54), can be referred to alongside this protocol. Only the morphological identification methods included in this protocol have been verified. Currently there are limited reference sequences available (Sections 4.2 and 8.3) for the species covered in this NDP. The protocol will be reviewed and updated when verification of the molecular methods is undertaken, once the appropriate reference sequence becomes available.

2 TAXONOMIC INFORMATION

Taxonomic Position

Synonyms and basionyms as listed in Wilson *et al*. (2020); Dmitriev *et al*. (2017).

***Acrogonia terminalis* Young, 1968**

Class:	Insecta Linnaeus, 1758
Order:	Hemiptera Linnaeus, 1758
Suborder:	Auchenorrhyncha Duméril, 1806
Superfamily:	Membracoidea Rafinesque, 1815
Family:	Cicadellidae Latreille, 1825
Subfamily:	Cicadellinae Latreille, 1825
Tribe:	Proconiini Stål, 1869
Genus:	*Acrogonia* Stål, 1869
Species:	*Acrogonia terminalis* Young, 1968

Synonyms or alternate names
None

Common names
None

Taxonomic Position
***Cicadella viridis* (Linnaeus, 1758)**

Class:	Insecta Linnaeus, 1758
Order:	Hemiptera Linnaeus, 1758
Suborder:	Auchenorrhyncha Duméril, 1806
Superfamily:	Membracoidea Rafinesque, 1815
Family:	Cicadellidae Latreille, 1825
Subfamily:	Cicadellinae Latreille, 1825
Tribe:	Cicadellini Latreille, 1825
Genus:	*Cicadella* Latreille, 1817
Species:	*Cicadella viridis* (Linnaeus, 1758)

Synonyms or alternative names
Acopsis viridis (Linnaeus, 1758)
Amblycephalus viridis (Linnaeus, 1758)
Amblycephalus viridis viridis (Linnaeus, 1758)
Cercopis viridis (Linnaeus, 1758)
Cicada viridis Linnaeus, 1758, basionym
Cicadella viridis concolor (Haupt, 1912)
Cicadella arundinis (Germar, 1821)
Cicadella riridis Linnaeus, 1758

Cicadella viridis subsp. *arundinis* Germar, 1821
Cicadella viridis subsp. *concolor* (Haupt, 1912)
Neokolla viridis (Linnaeus, 1758)
Iassus viridis (Linnaeus, 1758)
Tettigella arundinis (Germar, 1821)
Tettigella viridis (Linnaeus, 1758)
Tettigella viridis subsp. *suffusa* Salmon, 1954
Tettigonella concolor Haupt, 1912
Tettigonia arundinis Germar, 1821
Tettigonia concolor Haupt, 1912
Tettigonia flavicatella DeGraaf, 1854
Tettigonia melanchloa Walker, 1851
Tettigonia virescens Linnaeus, 1758
Tettigonia viridis (Linnaeus, 1758)
Tettigonia viridis concolor Haupt, 1912
Tettigoniella virides (Linnaeus, 1758)
Tettigoniella viridis (Linnaeus, 1758)

Common names
Green Leafhopper

***Dilobopterus costalimai* Young, 1977**

Class:	Insecta Linnaeus, 1758
Order:	Hemiptera Linnaeus, 1758
Suborder:	Auchenorrhyncha Duméril, 1806
Superfamily:	Membracoidea Rafinesque, 1815
Family:	Cicadellidae Latreille, 1825
Subfamily:	Cicadellinae Latreille, 1825
Tribe:	Cicadellini Latreille, 1825
Genus:	*Dilobopterus* Signoret, 1850
Species:	*Dilobopterus costalimai* Young, 1977

Synonyms or alternate names
None.

Common names
None.

Taxonomic Position
***Draeculacephala minerva* Ball, 1927**

Class:	Insecta Linnaeus, 1758
Order:	Hemiptera Linnaeus, 1758
Suborder:	Auchenorrhyncha Duméril, 1806

Superfamily: Membracoidea Rafinesque, 1815
Family: Cicadellidae Latreille, 1825
Subfamily: Cicadellinae Latreille, 1825
Tribe: Cicadellini Latreille, 1825
Genus: *Draeculacephala* Ball, 1901
Species: *Draeculacephala minerva* Ball, 1927

Synonyms or alternate names
Acopsis minerva (Ball, 1927)

Common names
Green Sharpshooter

Taxonomic Position
***Graphocephala atropunctata* (Signoret, 1854)**
Class: Insecta Linnaeus, 1758
Order: Hemiptera Linnaeus, 1758
Suborder: Auchenorrhyncha Duméril, 1806
Superfamily: Membracoidea Rafinesque, 1815
Family: Cicadellidae Latreille, 1825
Subfamily: Cicadellinae Latreille, 1825
Tribe: Cicadellini Latreille, 1825
Genus: *Graphocephala* Van Duzee, 1916
Species: *Graphocephala atropunctata* Signoret, 1854

Synonyms or alternate names
Cicadella atropunctata (Signoret, 1854)
Cicadella circellata (Baker, 1898)
Cicadella circulata (Baker, 1898)
Hordnia atropunctata (Baker, 1898)
Hordnia circellata (Baker, 1898)
Neokolla circellata (Baker, 1898)
Tettigonia atropunctata Signoret, 1854, basionym
Tettigonia circellata Baker, 1898
Tettigonia circillata (Baker, 1898)

Common names
Blue–green sharpshooter

Taxonomic Position
***Oncometopia facialis* (Signoret, 1854)**
Class: Insecta Linnaeus, 1758
Order: Hemiptera Linnaeus, 1758
Suborder: Auchenorrhyncha Duméril, 1806

Superfamily:	Membracoidea Rafinesque, 1815
Family:	Cicadellidae Latreille, 1825
Subfamily:	Cicadellinae Latreille, 1825
Tribe:	Proconiini Stål, 1869
Genus:	*Oncometopia* Stål, 1869
Species:	*Oncometopia facialis* (Signoret, 1854)

Synonyms or alternate names

Oncometopia facialis (Signoret, 1854)
Proconia facialis (Signoret, 1854)
Tettigonia facialis Signoret, 1854

Common names

None

Taxonomic Position

***Xyphon fulgidum* (Nottingham, 1932)**

Class:	Insecta Linnaeus, 1758
Order:	Hemiptera Linnaeus, 1758
Suborder:	Auchenorrhyncha Duméril, 1806
Superfamily:	Membracoidea Rafinesque, 1815
Family:	Cicadellidae Latreille, 1825
Subfamily:	Cicadellinae Latreille, 1825
Tribe:	Cicadellini Latreille, 1825
Genus:	*Xyphon* Hamilton, 1985
Species:	*Xyphon fulgidum* (Nottingham, 1932)

Synonyms or alternate names

Carneocephala fulgida Nottingham, 1932
Xyphon fulgida (Nottingham, 1932): Hamilton 1985

Common names

Red–headed sharpshooter

3 DETECTION

3.1 Symptoms

Sharpshooter feeding may not cause any visible damage to the plant. However, xylem feeding leafhoppers excrete a watery substance which when dry, appears as traces of a white powder (composed of brochosomes) on the leaves, fruit and stem (Figure 1A) (SPHD 2013). Other traces which may indicate the presence of sharpshooters, include scarring on leaves due to deposition of eggs in plant tissue or a mass of eggs laid in row, usually on the underside of leaves (Figure 1B). However, some species lay their eggs individually or in a cluster underneath the leaf tissue and are not obvious.

Figure 1. A) Citrus fruit covered with Glassy–winged sharpshooter (GWSS) excrement; B) GWSS eggs laid in a row underneath *Pittosporum* leaf (source of image: A, SPHD (2013), © Regents, University of California 1999; B, CABI 2022, © P.A. Phillips).

Host plant symptoms caused by *X. fastidiosa* can express different symptoms in a variety of crops (see Figures 2A–F as an example). Further symptoms are described and illustrated more specifically in Robinson (2022), SPHD (2013) and SPHD (2018). Symptoms in grapevines include "leaf scorching, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing" (SPHD 2018).

Figure 2. *Xylella fastidiosa* symptoms, A–B) Pierce's Disease (PD) in Grapevine (red varieties); C) PD on 'Pinot noir' grape showing marginal leaf scorching; D) leaf tip desiccation in Olive trees, Salento, Italy;

E) Citrus variegated chlorosis in sweet orange from Brazil; F) outer fruits showing advanced variegated chlorosis compared with normal fruits in middle, in sweet orange (source of image: A–B, SPHD 2013, © Regents, University of California 1999; C, E–F, Robinson 2022, © A.H. Purcell; D, Scortichini *et al*. 2023).

3.2 Sampling

3.2.1 Number and Preferred Life Stage

Ideally all suspect specimens should be collected, including both male and female specimens if possible. The morphological identification of leafhoppers is based on the genitalia structures of male specimens, so it is important to have a male representative in the collection where possible. Suspect nymphs, which can be recognised by the presence of wing buds (where wings are not yet fully developed), and eggs (Figure 1B and see images online, CABI 2022), can also be collected but can only be identified by molecular testing methods (where available).

3.2.2 Collecting Methods

Methods for collecting Auchenorrhyncha (including leafhoppers and planthoppers) are described in Löcker *et al*. (2018). Similar methods are also presented in SPHD (2013) Glassy–winged sharpshooter NDP 23, SPHD (unpublished) *Hyalesthes obsoletus* NDP (methods described in detail), SPHD (2025) Meadow Spittlebug NDP 54 and SPHD (unpublished) Maize Leafhopper NDP.

Hand collecting in the field is a good opportunity to observe the biology, host and habitat of the leafhoppers collected. In the field, check the trunk, stems, fruit or leaves of the plant for adults and nymphs. Specimens can be collected directly into a specimen tube, but as both adults and nymphs can jump suddenly, this can require some patience and skill. Sweeping is the preferred method for collecting *Xylella* vectors (IPPC 2018). Specimens can be collected from the net using an aspirator, but Cicadellinae are relatively large specimens (between 5–15 mm), and might be damaged during the process, so care is required when aspirating.

Beating, pit fall traps, vacuum/ suction traps, malaise traps and sticky traps can also be used for collecting Cicadellidae (and other Auchenorrhyncha). These methods are described in detail in SPHD (unpublished) *H. obsoletus* NDP, section 3.2.2. The latter three methods could be useful for wider scale surveillance as they can collect a large number of specimens, and sticky traps and malaise traps can be left in the field over a period of a few weeks. However, this may result in a large amount of non–Auchenorrhyncha bycatch. Additional information about the collecting methods specifically for *Xylella* vectors are provided in Appendix 9.2 Sampling for surveillance.

Eggs are laid in a batch or singularly within the leaf tissue or may be visible on the underside of the leaf in a cluster (see Figure 1B), depending on the species. Leaves with suspect eggs can be individually collected and placed into a plastic zip lock bag or solid plastic container (see below for preservation details).

3.2.3 How to kill, preserve and transport specimen samples in the field

Specimens/ samples collected in the field need to be appropriately stored and preserved until they are ready to be further processed for testing. It is best for field officers to **discuss preferred sampling and preservation methods** with the diagnosticians receiving the samples as each method has positives and negatives. Below is a summary of options for preserving and storage of specimens collected by hand, sweeping and beating (points 1–3) and by sticky trap (point 4) before they are sent to a diagnostic laboratory:

1) **Live**: Plant material (leaves and stems) collected for the detection of eggs or nymphs, should be placed in a plastic bag. A slightly damp piece of paper towel should be added to the plastic bag or container with plant material, to keep the sample fresh. Double bagging is always a good idea when transporting any live material.

Adults or nymphs collected into specimen tubes and kept alive or plant samples (leaves or stems) with suspect nymphs or eggs, need to be kept in a cool environment (preferably a refrigerator or otherwise an esky) until ready to transport to a diagnostic laboratory. Keeping the specimens alive means that the diagnostician can decide how to deal with the specimens for testing and also means specimens will not incur damage or breaking in case of over–drying. However, in general, sending of live pest specimens should be avoided to prevent the risk of suspect exotic specimens escaping during transportation (e.g. if containers break and samples are not well sealed). Also, it is not advisable to send live specimens through the post.

In addition, refer to the current version of the PLANTPLAN (PLANTPLAN | Plant Health Australia) for requirements on the transport of any live specimens before initiating this process.

2) **Dry**: for morphological identification, specimens can be preserved dry. Specimens can be killed in a jar containing scrunched up tissue paper at the bottom and adding a few drops of ethyl acetate. Take care to use a glass or plastic container, such as polyethylene, that does not react with ethyl acetate. Specimens usually die within 5–10 minutes but can be left in the “killing jar” for a longer time if needed. Specimens are then transferred to a separate dry container. To further help keep specimens dry, add scrunched up paper towel to the bottom of the container. Another technique which is used for processing bulk samples collected from the same site is to add wood chips or paper towel to the bottom of the container, with a few drops of ethyl acetate, and place specimens between layers of tissue paper. For both of these methods the absorbing agent (e.g. paper towel) needs to be changed every couple of days until the specimens are dry to avoid specimens becoming mouldy.

3) **Wet**: Alternatively, specimens can be killed directly in 70–80% ethanol although this is not the preferred preservation method, as while the specimens can be used for morphological examination, the ethanol can cause discolouration and the wings to become wrinkled when card mounting; 70–80% ethanol is also not optimal for molecular testing. Adults, nymphs and eggs placed directly into a vial containing **95–100% ethanol** is the best method to preserve **specimens for molecular testing**. Adults can later (in the diagnostic laboratory) be removed from the ethanol and pinned for morphological study (see section 4.1.1), but high ethanol concentrations may cause the specimens to become very brittle and also causes discolouration and wrinkling of wings.

4) **Sticky traps**: sticky traps should be placed in a plastic sheet protector, placed in a zip lock bag and retained in a cool environment (such as a refrigerator or esky).

All samples (whether live, dry, in ethanol or on sticky traps) should be placed in a refrigerator, if the sample cannot be delivered to a diagnostic laboratory on the same day as collection. Collection details including the method of preservation should be provided along with the sample. If transporting live specimens, this should be clearly marked on the container, and the samples need to be well sealed in a strong plastic container which is doubled bagged.

Transport by express post or courier is preferred and samples in ethanol should conform to UN requirements for transport of dangerous goods (https://www.unece.org/trans/danger/danger.htmL). If transporting by air, follow the CASA guidelines for transport of specimens in ethanol under special provision A180 (https://www.casa.gov.au/standard-page/special-provision-a180). This means vials are placed into a plastic bag, with some absorbent material (e.g. paper towel or tissue), which is then heat–sealed. The heat–sealed bag is then placed inside a second heat–sealed plastic bag. The package is then placed into a sturdy box with good padding inside. Each vial cannot contain more than 30 mL of ethanol and any parcel posted not more than a total of 1 litre of ethanol. The wording “Special Provision A180” should be added to the outside of the parcel.

For further information about methods of preservation and transporting samples see also SPHD (2013) Glassy–winged sharpshooter, *Homalodisca vitripennis* (Germar) NDP 23, SPHD (unpublished) *H. obsoletus* NDP, SPHD (unpublished) Maize Leafhopper NDP and SPHD (2025) Meadow Spittlebug NDP 54.

4 IDENTIFICATION

The limitations of the keys and descriptions in this protocol are that they can only be used to positively identify a small number (7 species) of highly invasive and effective or potential *Xylella* vectors. However, there are at least 350 genera (only 3 known in Australia) and over 3100 species (only 13 known in Australia) of Cicadellinae worldwide (Dietrich 2006), and some genera and species can appear very similar externally. Furthermore, the taxonomy of certain Cicadellinae genera still requires serious revision. It is strongly recommended that a DNA barcoding sequence is acquired for any suspect specimens and a specialist is contacted for advice or confirmation of the identification, if uncertain.

A list of Australian collections which hold reference specimens of the exotic *Xylella* vector species treated in this protocol, can be found in Table 8 (Appendix 8.4).

Steps for Identification

1. Follow the preservation and preparation techniques as in section 4.1.1 to start examination of specimens. Go to **step 2.**

2. Identify the life stage and determine whether the specimen is a male or female. Nymphs have wing pads (only partially developed wings) while adults have fully developed wings. Males are slightly smaller than females with a pair of subgenital plates ventrally (below pygofer) (Figure 4C,D, 9A,C) while females bear a distinct ovipositor ventrally (Figure 4F, 9M). FOR adults go to **step 3**. FOR eggs or nymphs go to **step 6**.

3. Use the morphological keys and descriptions (in section 4.1.4 and 4.1.5), to determine whether the specimen is a Cicadellinae and to which tribe it belongs, noting that all Proconiini are exotic to Australia and require urgent attention. If **POSITIVE** for Cicadellinae and go to **step 4**.

4. Use the tools (keys, diagnostic characters and images) in section 4.1.6 for initial triage to determine whether the specimen could be one of the 7 exotic species treated in this protocol OR belongs to one of the 3 endemic Australian Cicadellinae genera. The key can be used for both male and female specimens, although is best to use with male representatives if available. If suspected **POSITIVE** for any one of the target exotic genera/ species, OR *Ishidaella* or *Cofana** and if male go to **step 5**; if female go to **step 6**. If specimen doesn't match any genus or exotic species covered in this protocol or the identity is uncertain go to **step 6**. IF **POSITIVE** for Australian endemic genus *Conoguinula* then no further steps may be necessary.

5. Dissection of male genitalia is required to confirm identifications (following instructions in section 4.1.1, **Dissection**). Compare the description of the genitalia structures and images provided in section 4.1.6. If the match is uncertain or is a first time detection, go to **step 6**. If **POSITIVE** for exotic species, go to **step 7**. If **POSITIVE** for Australian endemic species of *Cofana* or *Ishidaella* no further steps may be necessary.

6. Any suspect exotic eggs, nymphs, females or males that cannot be identified with certainty or require further confirmation, should be tested using a molecular DNA barcoding test (useful where sequence data is available). If **POSITIVE** for any exotic Cicadellinae (genus/ species) or if results are uncertain or do not match any sequences go to **step 7**.

7. If specimens are identified as **POSITIVE** for any exotic Cicadellinae (genus/ species) or if the identification is doubtful, contact one of the Australian Auchenorrhyncha specialists (see Contacts in section 5) for a second confirmation. All exotic Cicadellinae should be reported to the state Chief Plant Health Officer (CPHO), Chief Plant Protection Officer (CPPO) or equivalent Biosecurity authority in the respective jurisdiction.

*It is recommended that specimens matching *Cofana*, should be identified further to species (using Young 1986) to determine whether it is one of the three endemic species or one of 23 exotic *Cofana* species. This requires dissection of the male genitalia.

! Note: Any exotic species detected needs to be reported through the relevant jurisdictions, even if they do not match one of the species covered in this protocol. **All species** in the tribe PROCONIINI are **exotic to Australia** and are potential vectors of *Xylella*.

4.1 Morphological identification.

4.1.1 Specimen preparation and preservation in the laboratory

The following is adapted from Löcker *et al.* (2018) Auchenorrhyncha workshop manual, section on "Preservation and preparation of Auchenorrhyncha for identification". Further details about preparation of Auchenorrhyncha specimens can be found in resources such as Fletcher (2009 and updates), SPHD (2013) Glassy–winged sharpshooter NDP 23, SPHD (unpublished) *H. obsoletus* NDP, SPHD (unpublished) Maize Leafhopper NDP and SPHD (2025) Meadow Spittlebug NDP 54.

Once specimens are received in the diagnostic laboratory, they require further processing for long term preservation. It is important to retain voucher or reference specimens either as pinned or card mounted material or as specimens placed in ethanol. This also allows ease of handling specimens for examination under a microscope. Below is a summary of leafhopper preparation and preservation techniques recommended before identification can commence.

Methods to kill specimens

Live specimens can be euthanised by placing specimens in the freezer at minus 10 °C (or colder) for at least 24 hours or placing them in a "killing jar" using a few drops of ethyl acetate (see section 3.2.3 for more details about the killing jar). As specimens can hop quite suddenly, care needs to be taken to prevent any specimens escaping from the containers.

Methods to process and preserve specimens

Pinning and card mounting (Dry):

[Method suitable for morphological examination and molecular testing (of freshly mounted specimens only.)]

Leafhopper adults are preferably preserved as dry pinned/ card mounted specimens for morphological examination. This works best for specimens that have been frozen for at least 24 hours, or that have been killed with ethyl acetate or removed from ethanol and dried. It is also possible to mount specimens removed from sticky traps if care is taken during the removal process (see section on "Removing specimens from sticky traps" below).

Mounting procedures generally follow Upton & Mantle (2010). Before pinning or card mounting, if the specimens are too dry and brittle, they need to be relaxed in a humid chamber before handling. This can be achieved by placing specimens in a container with a damp sponge or paper towel at the bottom. Chlorocresol crystals can be placed in a small glass petri dish at the bottom of the container to prevent mould growth. Some workers use apple cider vinegar on a sponge or tissue instead of water to relax specimens. Leave specimens in the closed container for a few hours or overnight. This will soften the specimens so they can be handled without damage. However, this step is not necessary for freshly killed specimens, which should be soft enough to pin/ card mount directly.

Specimens up to 10 mm can be card mounted with the right side of the body glued to a card point (Figure 3F). Card points can be purchased or punched using a triangular or tear–drop shaped card punch and acid free paper or cardboard such as Goatskin parchment paper.

Alternatively, specimens larger than 10 mm and less than 15 mm may be double mounted with a micropin (Number B2) inserted through the scutellum. The micropin is then passed through a length of polystyrene pith with a Number 3 insect pin passed through the other end of the pith (Figure 3E).

Ideally, for male specimens, prior to pinning/ card mounting, the entire abdomen or apex of abdomen should be removed and kept in a genitalia vial (either dry or in glycerine) for further processing (see section on "Examination of leafhopper genitalia" and "Dissection"). The genitalia vial should be attached to the pin of the corresponding specimen. As above, if the specimens are too dry, they should be softened first before removing the abdomen.

Relaxing specimens in a damp environment may degenerate their DNA and impact on suitability for molecular testing. Therefore, if specimens will be used for molecular as well as morphological examination, it is advisable to remove a front or mid leg for molecular testing prior to any relaxing/softening.

Specimens that were collected in the field into 70–100% ethanol can be pinned or card mounted, by removing specimens from container and placing them on paper towel to dry for a few minutes before mounting. The ethanol or any other liquid such as water, causes the wings to become wrinkled when drying, making wing venation characters difficult to study. However, specimens can be placed with wings flattened on cardboard squares to prevent wrinkling while drying.

Specimens in ethanol (Wet):

[Method suitable for morphological examination and molecular testing (in 95–100% ethanol only.)]

Adults can be preserved in 70–80% ethanol (for morphological examination), particularly recommended if they have been collected in water traps. But the ethanol causes discoloration of the body and specimens are less easily handled under the microscope (than pinned/ card mounted specimens) as they require manipulation with forceps and could be damaged. Also, specimens stored in 70–80% ethanol are not optimal for molecular testing, particularly in the long term. Genitalia dissected from specimens stored in ethanol should, be returned to the tube with the remaining body once examined.

Adult specimens to be preserved for DNA testing should be stored in 95–100% ethanol in a freezer at minus 20 °C. As nymphs and eggs can only be identified through molecular testing and are prone to shrivelling and drying, these life stages should also be placed into 95–100 % ethanol.

However, preserving insect specimens in high concentrations of ethanol (90% and above) causes the exoskeleton to become brittle over time (Marquina *et al*. 2021).

Removing specimens from Sticky Traps

Specimens need to be free from the sticky trap glue and should be preserved in a reasonable condition so that they can be handled to examine diagnostic characters (e.g. features of the head, and thorax, wing venation, spines on hind legs etc.) and allow for genitalia dissections. This can be done using De–Solv–it (goo & stain remover with citrus base), mineral turpentine, kerosene or a similar solvent. Several washes are needed to remove glue and oils.

In some studies, the whole sticky mat is soaked in orange oil (Marshall *et al*. 2010), but it is preferable to remove targeted individuals from sticky traps, following procedures in Miller *et al*. (1993). In order to do this, place a drop of the solvent on the specimen on the sticky trap, let it soak in for a few minutes, then gently remove the specimen and place it in a test tube with the same solvent and shake it gently (personal communication Michael Maixner, November 2019). Alternatively, cut out the piece of sticky trap with suspect specimen, place it in a watch glass filled with the solvent and leave for several minutes (may take up to 20 minutes) gently stirring it with a pin from time to time. Once removed, to wash off solvent, specimens can either be washed in warm to hot water (around 80 °C) to remove further glue and oil from the specimens or otherwise, transferred into a new watch glass or test tube filled with a 50:50 detergent–water (room temperature) solution. Leave in there for 10 minutes. If the specimen still floats on top of the solution after 10 minutes repeat this wash–step in a new watch glass or test tube. Finally rinse the specimen in water. Specimens can either be card mounted or retained in 70–80% (for permanent storage) or 95–100% ethanol (for DNA testing) and are ready for further identification. Note that if specimens are pinned/ card mounted, the wings may become wrinkled from the washing process. Follow instructions as in "Pinning and card mounting" section to keep wings flat.

! Note: As with all chemicals used for specimen preparation, safety data sheet guidelines should be referred to before use.

Labelling

Reference specimens require clear labels which should include at least 1) the collection locality, preferably with GPS generated latitude and longitude, 2) the date of collection, 3) the name of the collector(s) and 4) further details such as host plant or collection method, e.g. malaise trap. Labels may be printed (using a laser printer) or handwritten (with a good quality waterproof pen) on acid free (archival quality) paper. It is important to attach the labels to specimens soon after they are collected.

Examination of leafhopper genitalia

The male genitalia structures are the most distinctive and reliable characters to identify leafhopper, planthopper and spittlebug genera and species. Leafhopper male and female structures are encased within the pygofer (the ninth abdominal segment). To examine the leafhopper genitalia structures the pygofer requires maceration (described in further detail in step 3 below). The females can be recognised by the presence of a ventrally positioned ovipositor (Figure 4F) and the pygofer (in Cicadellinae females) is often elongate and tapered posteriorly (as seen in ventral view) (Figures 13C, 17N, 18F).

Males have a pair of subgenital plates (eg. Figures 4C–D, 9C, 11H, 13A–B) and the pygofer is variously shaped, for example in lateral view they can be elongate (Figure 7D), moderately elongate but tapering towards posterior margin (Figures 9A–B, 11H), or shorter and rounded/ truncated posteriorly (Figure 14F).

Dissection techniques are outlined below and adopted from Fletcher (2009 and updates). Similar techniques are described in Oman (1949), Knight (1965), Davis (1975), Young (1977), Webb (1983) and Hamilton (1983).

Dissection

1. If the abdomen has already been previously removed but not cleared, go straight to step 3. Otherwise, the abdomen needs to be detached from the male specimens to examine their genitalia. Prior to removing the abdomen from dry pinned specimens, they should be softened overnight in a humid chamber (see further details under section on "Pinning and card mounting"). Specimens that have been freshly killed or that have been removed from ethanol can be dissected directly (without relaxing) as they should be soft enough to handle already.

2. It is easiest to remove the entire male abdomen using the point of an entomological pin (Number 3) which is wedged between the first abdominal segment and thorax (Figure 3A), ventrally or laterally depending on access to the abdomen. Pressure is applied to the pin to lever the abdomen away from the thorax (Knight 1965). Using a pair of fine forceps remove the entire abdomen (Figure 3B). Care should be taken not to damage the first and second abdominal segments which contain the apodemes in leafhoppers, the shape of which is sometimes useful for helping to identify a species.

3. Transfer the abdomen into a test tube or glass cavity dish (with glass lid) containing a few drops of 10% Potassium Hydroxide (KOH). Heat on a small hotplate or heat block (Figure 3C) at approximately 50–60 °C and check specimens regularly as leaving specimens in KOH for too long causes over clearing and some weakly sclerotised internal structures may become almost invisible. Alternatively, the abdomen can be kept in cold 10% KOH overnight. Maceration time varies between species depending on the size and amount of sclerotisation of the abdomen and the temperature at which they are cleared. For example, a lightly sclerotised pygofer (such as that in *Cofana* species) would require around 4 hours with concentrate KOH at room temperature (Young 1979).

! Note: Always refer to the SDS for safe handling of chemicals in the laboratory. KOH, at 10% is corrosive and will cause burns if it contacts your skin (Fletcher 2009 and updates).

4. Once internal tissues are sufficiently cleared (check under a stereo microscope if necessary), the abdomen is removed from the test tube (or cavity block) with great care. The abdomen is rinsed twice in water, dehydrated in 70% ethanol for at least 10 minutes and then placed into glycerol (Figure 4D).

5. Examination of the genitalia requires a stereomicroscope (with at least 40x magnification). The genitalia is placed in a cavity block with glycerol. Using micropins the ninth abdominal segment (along with the anal tube and subgenital plates) can be removed from the rest of the abdomen to better position the pygofer for examination. If required, particularly for small specimens (less than 4 mm) the genitalia can be examined under a higher-powered stereomicroscope (up to 160x) or can be placed onto a microscope slide with a cavity and a few drops of glycerol to be examined under a compound microscope (up to 1000x).

6. Following dissection, clearing and examination, the abdomen is placed in glycerol in a polyethylene insect genitalia vial with a rubber stopper through which the pin, of the corresponding mounted specimen, is passed (following Upton & Mantle 2010). Ensure that all original labels are reattached.

Additional note: In a partial non–destructive DNA extraction method, maceration (clearing) of the abdomen can be achieved through soaking overnight in extraction kit buffers. This method has the benefit of acquiring both, DNA for sequencing as well as providing a cleared abdomen for examination of internal morphological features that can be kept with the original source specimen. However, these buffers could potentially cause the exoskeleton to become more fragile and brittle than using 10% KOH, and as the method has only more recently been used, the long-term effect on specimens is still unknown. While the female genitalia is not commonly used for identification at the species level, some features are useful, and in some leafhopper studies, the female ovipositor has been characterised (eg. Hummel *et al.* 2006, Mühlethaler 2008). The female genitalia may be dissected to examine the shape, armature and ornamentation of the ovipositor valvulae. Dissection and maceration are carried out following the same procedures as for male specimens.

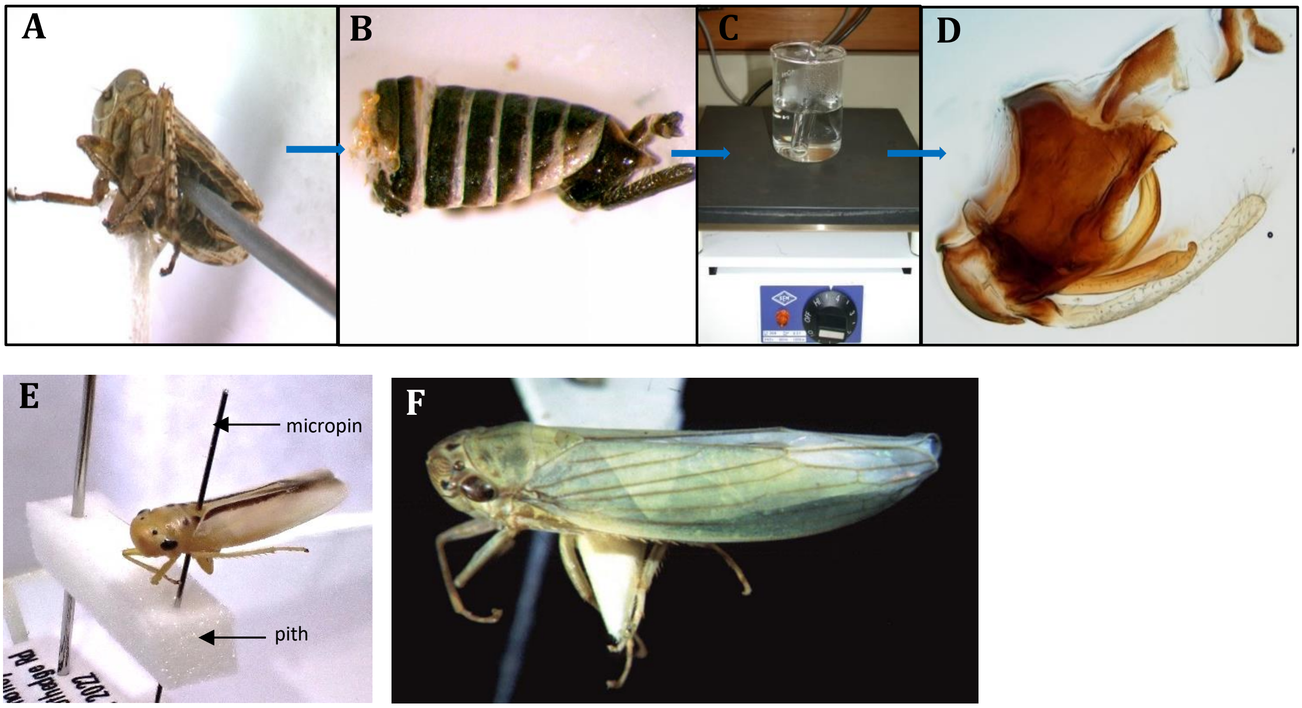


Figure 3. Dissection process of male genitalia. A) removal of abdomen using an entomological pin; B) abdomen removed from specimen prior to macerating; C) macerate abdomen in 10% KOH by heating on hot plate; D) male pygofer (ninth abdominal segment) removed from the abdomen following maceration, to be placed in glycerol in a genitalia vial; E) specimen micro pinned on a pith; F) specimen card mounted on triangle point. (Source of images: A–B, D, Agriculture Victoria; E, Jérôme Constant, Royal Belgian Institute of Natural Sciences (RBINS); F, Auchenorrhyncha keys, Fletcher (2009 and updates).

4.1.2 Diagnostic characters of Auchenorrhyncha

The Cicadellidae belong to the suborder Auchenorrhyncha, which includes the Cercopoidea, Cicadoidea, Membracoidea, Fulgoroidea (Dietrich 2009, Dietrich *et al*. 2017) and Delphacoidea (Deng *et al*. 2024).

The characteristics of Auchenorrhyncha are summarised in Carver *et al*. (1991), Dietrich (2009) and Biedermann & Niedringhaus (2009) and are recognised by the following diagnostic characters:

1) antennae with two basal segments and a terminal filiform flagellum or bristle–like arista (Figure 4B, 5A–B);
2) rostrum arising on the posteroventral part of the head (Figures 5C–D, I–K);
3) wings when at rest, usually tectiform (= roof–like)* over body (Figures 5G, 8A–C);
4) venation of fore and hind wings well developed (Figure 4A);

(*Note: in some Fulgoroidea families, the wings may be held flat or weakly tectiform as in Achilidae or held almost vertically such as in some Tropiduchidae and Derbidae.)

4.1.3 Identification of Cicadellidae

General features to identify the Cicadellidae include: 1) the general wedge–shaped body, normally broadest across the head and tapered posteriorly; 2) wings held tectiform over the abdomen (not flat); 3) antennae with setae–like sensilla and 4) relatively long hind tibiae, usually with rows of spines.

Key to identify Cicadellidae from other Auchenorrhyncha in Australia

An illustrated online key in Fletcher (2009 and updates) allows the identification of the superfamilies and families of the Auchenorrhyncha. The key below, can be used to identify Cicadellidae (Figure 4) from other Auchenorrhyncha and is adapted from Fletcher (2009 and updates), Carver *et al*. (1991) and Dietrich (2005).

1. – Tegulae present on mesothorax (mesonotum) (Figure 5H); pedicel bulbous, with wart-like sensilla (Figure 5B)…………………………………………………………………… Fulgoroidea and Delphacoidea

– Tegulae absent (Figure 5F); pedicel not bulbous and about the same width as the scape, without sensilla (Figure 5A)………2

2. – Head with three ocelli on crown (Figure 5E)……………….………………………………………Cicadoidea

– Head usually with two ocelli (Figures 4B, 5F) or with ocelli absent…………………………………………3

3. – Hind tibia short, cylindrical, with 1–2 enlarged lateral spines, with pecten (=apical spines) along flared apex (Figure 5L); ocelli always on crown of head; body and wings with fine setae (Figure 5Q) ………………………………..Cercopoidea (also see SPHD (2025), Meadow Spittlebug NDP54)

– Hind tibia elongate, usually with longitudinal rows of enlarged spines and setae, or if bearing only a few or no spines or setae, then apex of tibia not flared (Figure 5M–O); ocelli variously positioned, on face, margin of vertex or dorsally on crown; without conspicuous fine setae on body and wings………………………………………………………………………………………..Membracoidea (go to 4)

4. – Pronotum extended posteriorly, covering scutellum; usually with one horn or a pair of horns on pronotum (Figure 5G); hind tibia with few or small and inconspicuous setae or spines or setae (Figure 5O)..………Membracidae

–* Pronotum rarely extending over scutellar suture, usually pronotum without horns (except occasionally in few groups which have a single pronotal horn); hind tibia usually with rows of longitudinal rows of (usually) conspicuous setae (Figures 4A, 5M–N) ……...............................**Cicadellidae (go to section 4.1.4)**

(Note: *In Dietrich's key (2005) this branch leads to another couplet which separates Myerslopiidae from Cicadellidae, but as the former is not known from Australia (only New Zealand and Chile) the key is resolved between Cicadellidae and Membracidae).

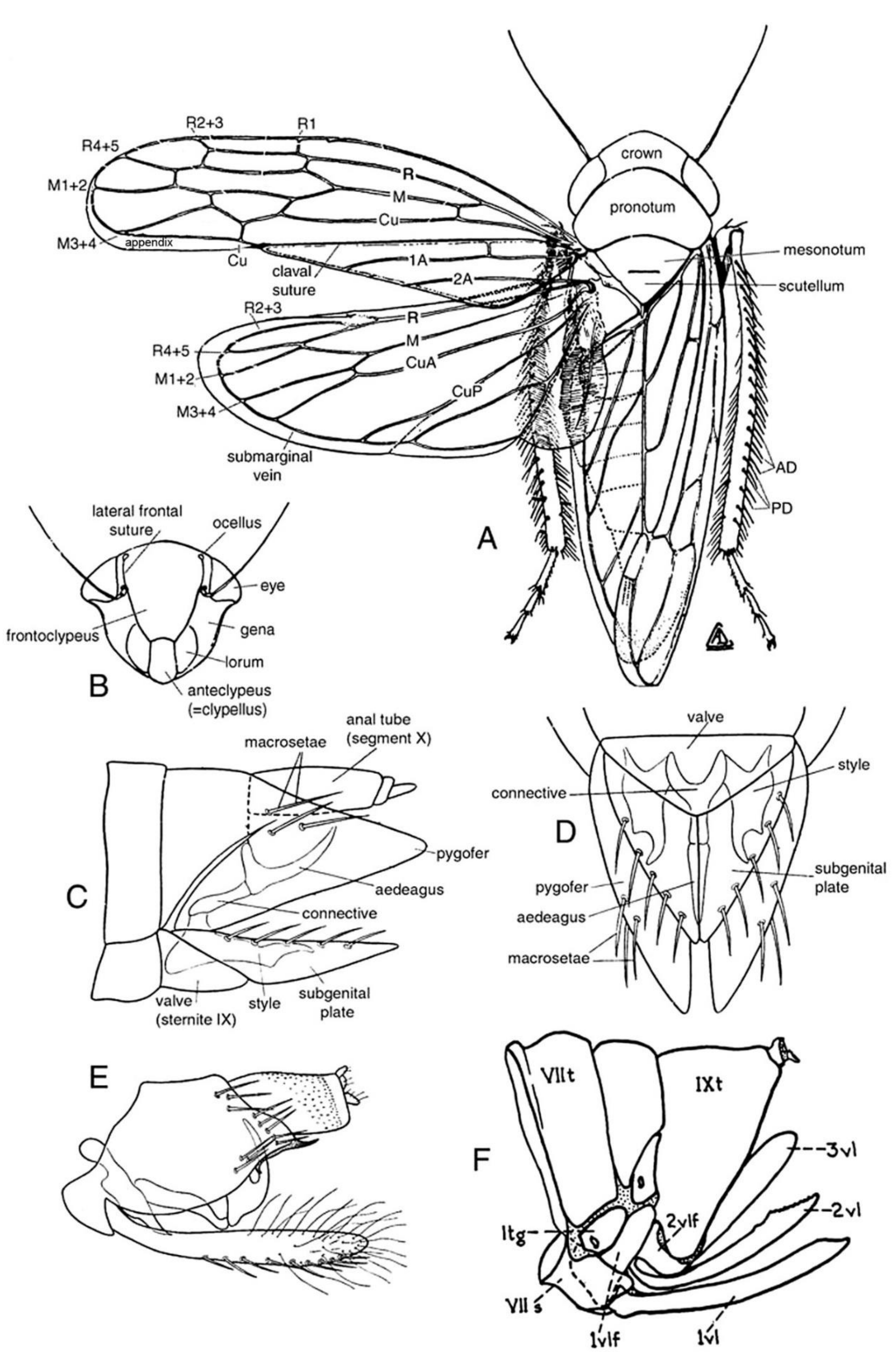


Figure 4. Morphology of leafhopper. A) dorsal habitus; B) face; C) Deltocephalinae male pygofer, lateral view; D) Deltocephalinae subgenital plates ventral view; E) Typhlocybinae, male pygofer, lateral view; F) female pygofer, lateral view. (Source of images A–F: adapted from Dietrich 2005).

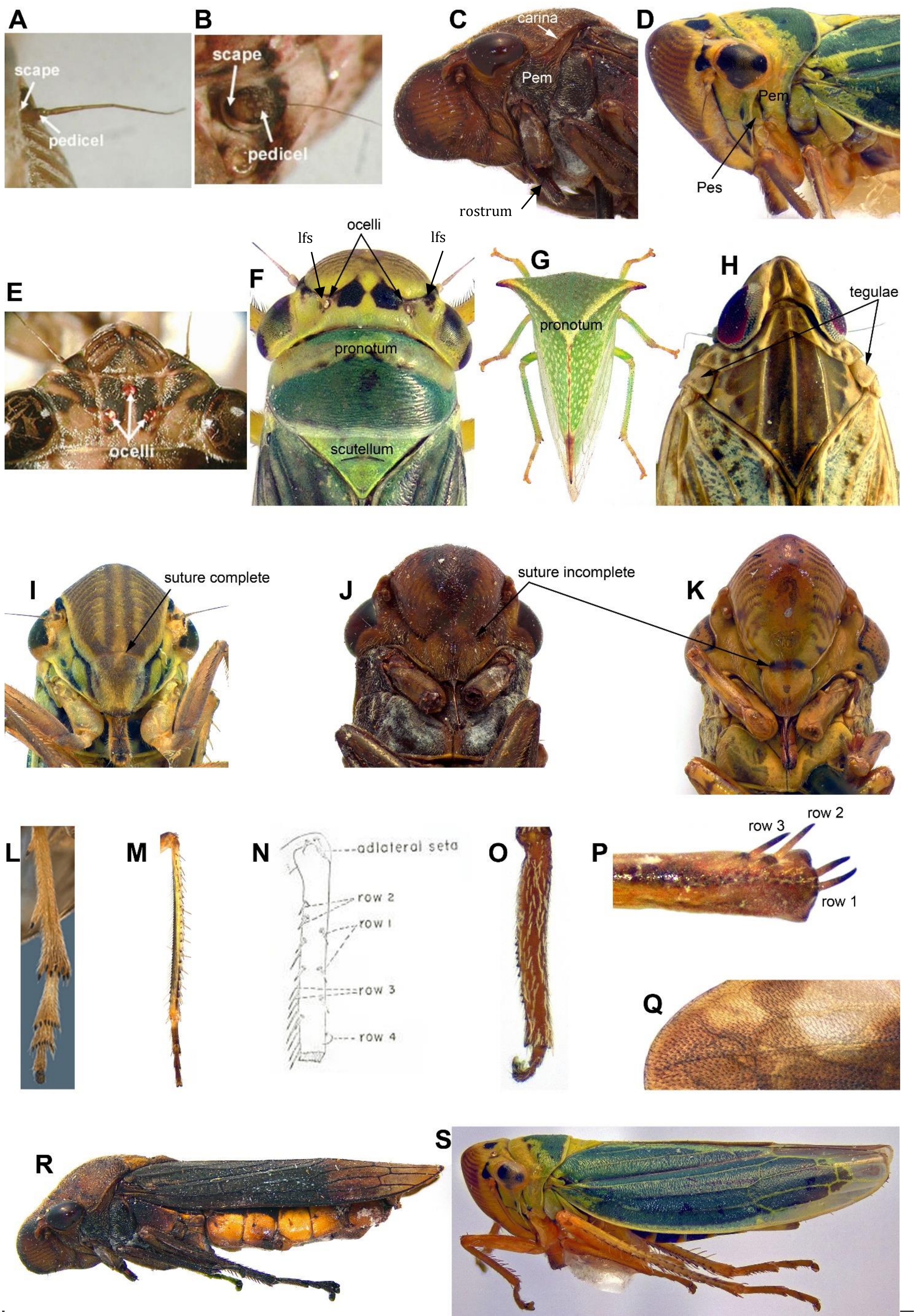

A
scape
pedicel
B
scape
pedicel
C
carina
Pem
rostrum
D
Pem
Pes
E
ocelli
F
lfs
ocelli
lfs
pronotum
scutellum
G
pronotum
H
tegulae
I
suture complete
J
suture incomplete
K
L
M
N
adlateral seta
row 2
row 1
row 3
row 4
O
P
row 3
row 2
row 1
Q
R
S

Figure 5. Key characters. A) antenna, Membracoidea; B) antenna, Fulgoroidea; C) head and thorax, lateral view, *pem* = proepimeron, *Oncometopia venosula* Distant, 1908, (Proconiini); D) head and thorax, lateral view, *pem* = proepimeron; pes = proepisternum, *Cicadella viridis* Linnaeus, 1758 (Cicadellini); E) head with 3 ocelli, Cicadoidea; F) head with 2 ocelli and lateral frontal sutures (lfs) extended dorsally on crown, *Cicadella viridis* (Cicadellini); G) dorsal habitus, *Stictocephala bisonia* Kopp & Yonke, 1977, (Membracidae); H) head and thorax, dorsal view, showing tegula in Achilidae,; I) face, ventral view, *Cicadella viridis* (Cicadellini); J) face, ventral view, *Oncometopia venosula* (Proconiini); K) face, ventral view, *Homalodisca cornuta* Young, 1968 (Proconiini); L) hindtibia and tarsus, *Philaenus spumarius* (Aphrophoridae); M) hind tibia and tarsi, Cicadellidae; N) illustration of hind tibia showing four rows of spines, Cicadellidae; O) hind tibia and tarsi, Membracidae; P) hind femur, showing femoral macrosetal formula (2:1:1); Q) tegmen showing small setae, Aphrophoridae; R) lateral habitus, *Oncometopia venosula* (Proconiini); S) lateral habitus, *Cicadella viridis* (Cicadellini).

(Source of images: A–B, D, Fletcher 2009 and updates, Auchenorrhyncha keys; C–D, F–K, M, O–S, Jérôme Constant (RBINS); L, by © Pia Scanlon, Western Australian Agricultural Authority 2020, SPHD (2025) Meadow Spittlebug NDP 54); N, Young 1968, page 11).

4.1.4 Identification of the subfamily Cicadellinae

Diagnostic features of Cicadellinae

The Cicadellinae can be readily identified from other Cicadellidae subfamilies in Australia by their relatively large and elongate bodies (Figures 16A–C, 18A), up to 15 mm, the frons and clypeus being distinctively swollen (Figures 18B, D), the crown which is weakly or strongly projected, being rounded or subquadrate anteriorly and by the pair of ocelli on the crown (dorsal aspect) (Figures 18C, D).

Key characteristics of Cicadellinae are also illustrated in the online Auchenorrhyncha key (Fletcher 2009 and updates) which can be used to identify the Australian endemic Cicadellini (Cicadellinae) and the exotic tribe Proconiini (Cicadellinae) from all other groups of leafhoppers known to occur in Australia (see key features below). The online key in Fletcher (2009 and updates) also includes characters to identify the exotic GWSS, *H. vitripennis*, (Proconiini, Cicadellinae) from Australian leafhopper species.

Key characters of the subfamily Cicadellinae

(Following Young 1968 and Dietrich 2005, 2006):

1) Medium to large (up to 15 mm), cylindrical leafhoppers, may be colourful (as in many Proconiini);
2) Head produced (Figures 5F, 6A);
3) Frons inflated (Figures 5C–D);
4) Ocelli dorsal on crown, usually closer to posterior margin than to apex (Figure 5F);
5) Lateral frontal sutures extended to ocelli (Figures 4B, 5F);
6) Proepisternum exposed (Figures 6D);
7) Hind femur macrosetal formula usually 2:1:0 or 2:1:1 (but can be 2:0:0, 2:1:1:1) (Figure 5P).
[Note: The first digit in the formula represents the number of setae in the first row along the apex of the femur; the second digit represents the number of setae (or seta) in the second row behind (preapical to) the apex of the femur; the third digit is the number in the third row and so on];
8) Hind tibia with macrosetae in 4 regular rows (Figures 4A, 5M–N,S);
9) Male subgenital plates usually triangular or parallel sided (Figures 9C, 13A).

Identification of the Australian Cicadellinae is also possible following the key characteristics in Fletcher (2009 and updates).

If the identification is **POSITIVE FOR CICADELLINAE** proceed to section 4.1.5.

4.1.5 Identification of Cicadellinae tribes

There are two main tribes recognised in the Cicadellinae subfamily – the **Proconiini** and the **Cicadellini** – following the classification as in Dietrich (2005), Mejdalani (1998), Wilson *et al*. (2020), and Young (1968, 1977). Knowing the key features of the two tribes can help to distinguish the Australian Cicadellinae from some of the exotic species.

Cicadellini is the only tribe, known to occur in Australia. There are **no Proconiini** known to occur in the Australasian region. Therefore, any specimens in Australia that are identified as Tribe: Proconiini, are likely representatives of an exotic species.

Cicadellini can be separated from the Proconiini following the descriptions and key below.

A key to cicadellid subfamilies and tribes can be found in Dietrich (2005). The following section provides diagnostic characters of each tribe following Dietrich (2005) and Young (1968). Table 1 summarises those main characters which distinguish between the Cicadellini and Proconiini. Also see the key in section 4.1.6 which separates the tribes and genera/ species of interest in this protocol.

IDENTIFICATION OF PROCONIINI following Young (1968):

1) Antennal ledges distinctly protuberant in dorsal aspect (Figures 6A–C, 14A–B);
2) Face almost always obviously pubescent with relatively long setae (Figures 5C,J);
3) Transclypeal suture incomplete – (this character needs careful examination as transverse markings present in this area may be misleading) (Figures 5J,K);
4) Prothorax (=prontoum) with distinct lateral carinae (Figure 5C);
5) *Hind femur when at rest, far from reaching the posterior margin of the proepimeron (Figures 5C,R);
6) Hind femur, with apical setal formula variable – 2:0:0, 2:1:0, 2:1:1, or 2:1:1:1;
7) Part of the lateral abdominal margins exposed, abdomen not completely covered by tegmen (Figure 5R);
8) Male subgenital plates and pygofer mainly with small microsetae evenly distributed (Figures 7D, 14F).

Proconiini can be externally differentiated from **Australian Cicadellini** in having a more produced, often triangular or sometimes rounded vertex of the head (crown only slightly to moderately produced in Australian Cicadellini); frontoclypeus strongly swollen (moderately swollen in Australian Cicadellini); the tegmina often transparent or translucent in part (opaque, black, yellow or whitish in Australian Cicadellini); tegmina narrow and lateral margins of the abdomen exposed (tegmina largely covering lateral margins of abdomen in Australian Cicadellini); the anal veins may be joined medially (e.g. GWSS) (anal veins always separate in Australian Cicadellini).

IDENTIFICATION OF CICADELLINI following Young (1977):

1) Antennal ledges not protuberant in dorsal view (Figures 5F, 11D, 12H);
2) Face if pubescent, only weakly so with short setae (Figures 5I, 11E–F, 12G);
3) *Hind femur at rest reaching near posterior margin of the proepimeral ledges (at least closer than in Proconiini);
4) Hind femur, with apical setal formula variable – 2:0:0, 2:1:0, 2:1:1, or 2:1:1:1;
5) Lateral abdominal margins mostly covered by tegmen (Figures 11C, 12D–E);
6) Subgenital plates and pygofer having distinct macrosetae (Figures 9B, 10F & 13D).

Differential diagnosis

The Australian Cicadellinae all belong to the tribe Cicadellini and share the same tribal characteristics (see also Table 1 below to differentiate Cicadellini from Proconiini). A detailed description of the old world Cicadellini (including genera and species known from Australia) can be found in the taxonomic study by Young (1986). The tegmina cover the abdomen laterally (abdomen laterally exposed in Proconiini) and anal veins of the tegmen are always separate throughout (not joined medially as in some Proconiini). The male pygofer and subgenital plates usually bear macrosetae in the Australian species (while macrosetae are lacking in males of Proconiini). Some of the Australian Cicadellini (*Cofana* and *Ishidaella*) have the vertex appearing shorter, more rounded and less prominent anteriorly compared with some of the exotic Cicadellini. Other features which might separate Australian from exotic Cicadellini include the lack of male paraphyses (these are present in a number of exotic genera, including those presented in this protocol) and the dorsopleural carina (carina on lateral pronotum) is incomplete (the carina may be lacking in many Cicadellini genera while it is prominent and complete in many Proconiini). See further descriptions in section 4.1.6. for each of the three Cicadellini genera represented in Australia.

Key differences between the tribes:

Table 1. Based on features in Dietrich (2005) and Young (1968, 1977).

	Cicadellini	**Proconiini**
Facial pubescence	not present or weakly present as very short setae	usually distinctly present
Antennal ledges	not distinctly protuberant from dorsal view	distinctly protuberant from dorsal view
Pronotal carina	may be absent or present but incomplete	usually present and distinct
*Hind femur (at rest)	reaching near posterior margin of proepimeron	far from reaching posterior margin of proepimeron

Anal veins on tegmen	separate throughout	can be separate throughout or medially joined (e.g. *Homalodisca*, *Oncometopia*)
Tegmen	mostly covers abdomen laterally	does not fully cover abdomen laterally (parts of abdominal segments visible in lateral view)
Subgenital plates and pygofer	macrosetae usually present, macro and microsetae not evenly dispersed	microsetae evenly dispersed and only occasionally a few macrosetae

*The position of the hind femur at rest is highlighted in Young (1968) as a key character separating the Proconiini and Cicadellini and for this reason is included in the diagnostic characters above and Table 1. However, this feature may not be useful when studying pinned specimens as often, the legs are not preserved in their natural position (as in live specimens). Furthermore, this feature seems to be variable within the Cicadellini where some genera (from New Guinea) do not share this feature, and the posterior femur does not reach the proepimeron (Young 1986). Therefore, the utility of this character is questionable.

4.1.6 Identification of exotic target genera and species

In this section a key and summary of diagnostic characters is provided to identify the targeted exotic genera and species. There are further references provided for each genus and diagnostic features of each species with illustrations.

Other useful online resources for identifying these genera and species include the 3i database (Dmitriev *et al.* 2017) which automatically generates descriptions, references and checklists from existing resources. Takiya & Dmitriev (2004) also provides an online interactive key to the genera of the Proconiini (http://dmitriev.speciesfile.org/key.asp?key=Erythroneura&lng=En&i=1&keyN=18).

In this protocol, it is recommended that male genitalia structures (when available) should be examined to assist in verifying species identification. However, for some sharpshooter species the male genitalia illustrations are not available or are incomplete and while the male structures are normally used to confirm identifications of leafhopper species, Young (1986) did not consider them to be very useful for some genera such as in the genus *Ishidaella*. The female genitalia of selected species known from Argentina, including species covered in this protocol, were studied in Dellapé (2015) and these characters were found to have some value in separating genera and species.

Key to genera and species

The following morphological key includes the seven species of concern in this protocol (*A. terminalis*, *C. viridis*, *D. costalimai, D. minerva, G. atropunctata*, *O. facialis* and *X. fulgidum*) as well as a further significant *X. fastidiosa* vector, *Homalodisca vitripennis* (covered in SPHD (2013) Glassy–winged sharpshooter, NDP 23), and distinguishes these exotic genera and species from three endemic Cicadellinae genera known to occur in Australia (*Cofana, Conoguinula* and *Ishidaella*). The limitation of this key is that it is best used with male representatives, and it does not cover all of the many other exotic Cicadellinae genera or species, which are also potential *Xylella* vectors. However, the key is a guide to the diagnostic characteristics of the seven target exotic species and allows a possible match and distinguishes these from the endemic Cicadellinae genera. If there are any suspect exotic specimens under investigation or if there is any doubt about the identification, consult a taxonomist specialised in Auchenorrhyncha (see Section 5 Contact list).

Identification Key

1. **–** Antennal ledge prominent in dorsal view (Figures 6A, 14A); prothorax usually with a prominent lateral carina; lateral margin of abdomen partly exposed, not entirely covered by tegmen (Fig. 14A); male subgenital plates and pygofer with microsetae evenly dispersed (Figure 7D, 14F)……………………………………………………… **Proconiini** go to 2 (**EXOTIC to Australia**, could be *Acrogonia*, *Homalodisca* or *Oncometopia*)

– Antennal ledge not prominent in dorsal view (Figures 5F, 10A, 11E); prothorax with carina weak, incomplete or absent; lateral margin of abdomen mostly covered by tegmen; male subgenital plates and pygofer with macrosetae and some microsetae not evenly dispersed (Figure 9A,B, 10F, 13B,D, 16G,H)……**Cicadellini** go to 4 (may be **native** or **EXOTIC**, includes species of *Cicadella*, *Dilobopterus, Graphocephala* and *Xyphon*, and native Australian species of *Cofana, Conoguinula* and *Ishidaella*)

2. – Head strongly produced medially, distinctly longer medially than wide between eyes (Figures 6A–C); often with distinctive yellow and black reticulate pattern dorsally on head, pronotum and scutellum (Figures 6A–C), sometimes reticulation diffuse or not present on pronotum; in lateral view, apex of head curved slightly dorsally; angulate or carinate along margin between crown and face (Figures 6D–F); male pygofer distinctly elongate and produced past apex of tegmen (Figure 6A); female seventh sternite with rounded lateral lobes (deep v–shaped notch medially along posterior margin) (Figure 7B)……*Acrogonia terminalis*

– Head less strongly produced medially, either as long as wide between eyes or slightly shorter than wide between eyes (Figures 14A); may have some brown or black reticulate markings with yellow and black patterns on head and thorax, but then head not longer than width between eyes and not with other combination of characters as above; margin of crown rounded onto face, not angulate (Figures 14D,E); male pygofer not distinctly elongate or produced past apex of tegmen (Figures 14B–C,F); female seventh sternite not deeply notched, without rounded lobes or if divided not deeply notched medially and lobes not distinctly rounded as above (Figures 14H,I) ………………………………………………………………3

3. – Crown, pronotum and scutellum with a brown to dark brown reticulate pattern (SPHD 2013, figs. 5.2 and 6); head distinctly produced, about as long medially as wide between eyes; clypeus may be flattened or slightly depressed or convex, not granulate, may be shiny (Figure 5K); tegmen hyaline with red or brown veins (SPHD 2013, fig 5.1–5.2); male aedeagus with two basal processes (SPHD 2013, figs. 4E–G); female seventh sternite moderately emarginate medially (v–shaped), with lateral lobes appearing triangular, and each inner edge appearing slightly sinuous along inner margin (SPHD 2013, figs. 4H–J)………………………………………………………………………………………………*Homalodisca vitripennis*

– Colour of crown and thorax not as above but brownish/ purplish (Figures 14A–C); head slightly produced medially, shorter or about the same length medially as wide between eyes (Figure 14A); clypeus strongly swollen (Figures 5R,14D,E), surface granulate or at least, not shiny (Figure 5J); tegmen mostly opaque brown up to about ¾ length of tegmen from base and translucent brown on apical ¼, veins usually brown but may be yellow, with opaque yellow patches in between cells (Figures 14A–C); male pygofer short and broad (Figure 14F); aedeagus with processes, but usually asymmetrical or basal process not paired (Figure 14G); female seventh sternite not deeply emarginate, broadly shallowly concave, with two short lateral triangular projections (Figures 14H,I) ……*Oncometopia facialis*

4. – Head strongly or moderately produced medially, head triangular, anterior margin distinctly angulate medially in dorsal view (Figures 11A,B,D); tegmen apical ¼ with distinctly reticulate venation (Figures 11A,B, 15A,B) ……………………………………………………………………………………………………...5

– Head weakly or moderately produced medially, broadly rounded or parabolic but if triangularly produced, tegmen not as above (Figures 8A–C, 12H); tegmen apical ¼ without reticulate venation (Figures 12D,E) ……6

5. – Anterolateral margin between crown and face, carinate or angulate (Figure 11G); crown surface with median longitudinal sulcus around ¾ length of crown and transverse depression anterior to ocelli (Figures 11D, G); face of males with brown to black markings or entirely black (Figure 11E–G); male subgenital plates with macrosetae present (Figure 11H) ………………………………*Draeculacephala minerva*

– Anterolateral margin of crown rounded on to face (without carinate margin); crown lacking median sulcus (Figures 15A,B); males without brown or black markings on face; male subgenital plates without macrosetae….……………………………………………………………………………...............*Xyphon fulgidum*

6. – Body colour mostly yellow to dark yellow (Figures 18A,B); relatively large specimens between 10–15 mm; crown yellow, without black markings except for dark ocelli; crown strongly produced medially, laterally parallel–sided, broadly parabolic along anterior margin; posterior pronotum and scutellum may have a pair of black spots laterally (Figures 18A,C) or body may be entirely yellow with very fine lateral spots on scutellum only; tegmen may be, pale yellow with a black stripe along anal margin or olivaceous but apically colourless; male pygofer, elongate, strongly produced posteriorly, distinctly extending beyond of apex of subgenital plates, not distinctly tapering (Figure 18G)…..*Conoguinula coeruleopennis* (KNOWN FROM AUSTRALIA, PAPUA NEW GUINEA, PHILIPPINES)

– Combination of characters not as above; body colour green, yellowish, white or orange and black patterned; body length between 5–13 mm; crown normally with distinct black spots, stripes or other patterns; crown anterior margin variously shaped, broadly rounded or slightly produced triangularly but rounded medially; pronotum and scutellum with various black markings or with no distinct markings; tegmen colour various but not as above; male pygofer not so produced posteriorly, may be slightly tapering or rounded along posterior margin, normally, not much extended beyond apex of subgenital plates, if at all (Figures 9A, 13B)……………………………………………..……………………………..7

7 – Body colouration orange/ dark yellow and black or, sometimes entirely black; head, pronotum and scutellum with various distinct brown or black lines and patterns; tegmina opaque black with costal margin colourless or paler then rest of tegmen or entirely translucent……………………………………………8

– Body coloration and tegmina generally greenish, yellow or pale yellow/ white; head, distinct black spots or stripes; pronotum and scutellum may have distinct black patterns and markings or indistinct or no markings………………………………………………………………………………..…………………………..9

8. – Between 8.8–9.1 mm; head, pronotum and scutellum orange with fine black transverse sinuous patterns (Figures 10A–C); tegmen translucent with brown veins and slight black markings at base along

anal margin; male pygofer with macrosetae very numerous over entire disk, even on basal half (Figure 10F); male paraphyses present (Figure 13E)…………………………………………………*Dilobopterus costalimai*

– Between 5–8 mm; head, pronotum and scutellum with various distinctive black markings or pattern (Figures 17A–L); if transverse sinuous markings on head or pronotum, then tegmen opaque black, costal margin and subcostal area sometimes transparent or opaque white or yellow; male pygofer with macrosetae on posterior half of disk; male paraphyses absent……………………………………*Ishidaella* (9 SPECIES IN AUSTRALIA)

9. – Body colour greenish/ yellow; crown with paired black patches medially between ocelli or anterior to ocelli, pronotum and scutellum may have diffuse grey markings or multiple black spots; tegmen opaque yellowish green or grey/ black ……………………………………………………………………………………………10

– Body colour whitish/ yellow (Figures 16A–C); crown may have black spot at apex and paired spots laterally along anterior margin, dorsomedially with black tear or diamond–shaped marking (Figures 16A–C), or sometimes with a longitudinal black stripe along midline extending onto pronotum and/ or scutellum (Figure 16A); tegmen opaque, cream/ pale yellow coloured with brown veins (Figure 16A–C)………*Cofana* (3 SPECIES IN AUSTRALIA; 23 SPECIES EXOTIC TO AUSTRALIA)

10. – Crown with black almost pentagonal–shaped patches medially between ocelli, may have fine or diffuse brown stripes on apex of head on area of frontoclypeus which extends onto crown (Figures 8A–C); crown lacking transverse concavity before ocelli; pronotum and scutellum may have diffuse greyish/ black transverse lines or pattern (Figures 8A–C); tegmen normally opaque greenish blue in females, in males blackish/ blue or grey, apically translucent, colourless ; male genitalia with paraphyses symmetrical (Figures 9H,I); female seventh sternite posterior margin sinuate, with shallow v–shaped median emargination (slightly notched)…………………………………………………………………*Cicadella viridis*

– Crown with anterior black spot medially, pair of black spots anterior to ocelli (Figure 12A), or black stripes laterad of ocelli, area where frontoclypeus extends onto crown often outlined posteriorly with black curved lines (Figures 12B,C); crown with transverse slight depression anterior to ocelli; pronotum usually with a small black spot medially, and a pair of anterior and posterior submedial black spots or patches, may have pair of additional anterior medial and lateral black spot on pronotum (Figures 12A–C); tegmen opaque yellowish green/ greyish with brown veins; male paraphyses asymmetrical (Figure 13E); female seventh sternite distinctly produced medially along posterior margin, appearing as forked points (Figure 13C)……………………………………*Graphocephala atropunctata*

NOTE!: As there are a large number of exotic Cicadellinae genera (more than 350) and species (3100), some caution should be taken even once an identification is reached using the above key, as species and genera can appear outwardly similar. The key could lead to a false match with other genera and species, which are also exotic to Australia. For example, there are 23 species of *Cofana* exotic to Australia. Any species identified in this genus should be identified further by examining male genitalia features and/ or DNA barcoding to confirm whether the species identification. IF the specimen in question does not convincingly match any of the above species or genera covered in this key, or you have reached a doubtful identification, or if you have keyed your specimen to an exotic Cicadellinae, contact an Australian Auchenorrhyncha specialist immediately (see section 5. Contacts for further information) for further confirmation of the identification.

Generic and species descriptions

Acrogonia Stål, 1869 (Proconiini)

The genus was described by Stål (1869). At least 39 species are known in this genus with a Neotropical distribution (Dmitriev *et al*. 2017). A full description of the genus *Acrogonia*, can be found in Young (1968) (pages 257–271), including the description of new species and a key to the species, which includes *A. terminalis*.

Other more recent references with descriptions of new species of *Acrogonia* can be found in Marucci *et al*. (2002), and Da Silva *et al*. (2017, 2018), in which a total of 16 species are described from South America. Marucci *et al*. 2002 provides a key to species of Brazil including two species of *Acrogonia* (*A. citrina*, described in that paper and *A. virescens* (Metcalf, 1949)) which are also known to vector the *Xylella* bacterium in citrus, but the key does not include *A. terminalis*.

Acrogonia terminalis Young, 1968

(Figures 6–7)

References:

Young (1968): p. 261 [key to species and original description], p. 269 [illustrations of male genitalia, Figs. 259c,f].

Online ressources: Wilson *et al*. (2020); Dmitriev *et al*. (2017).

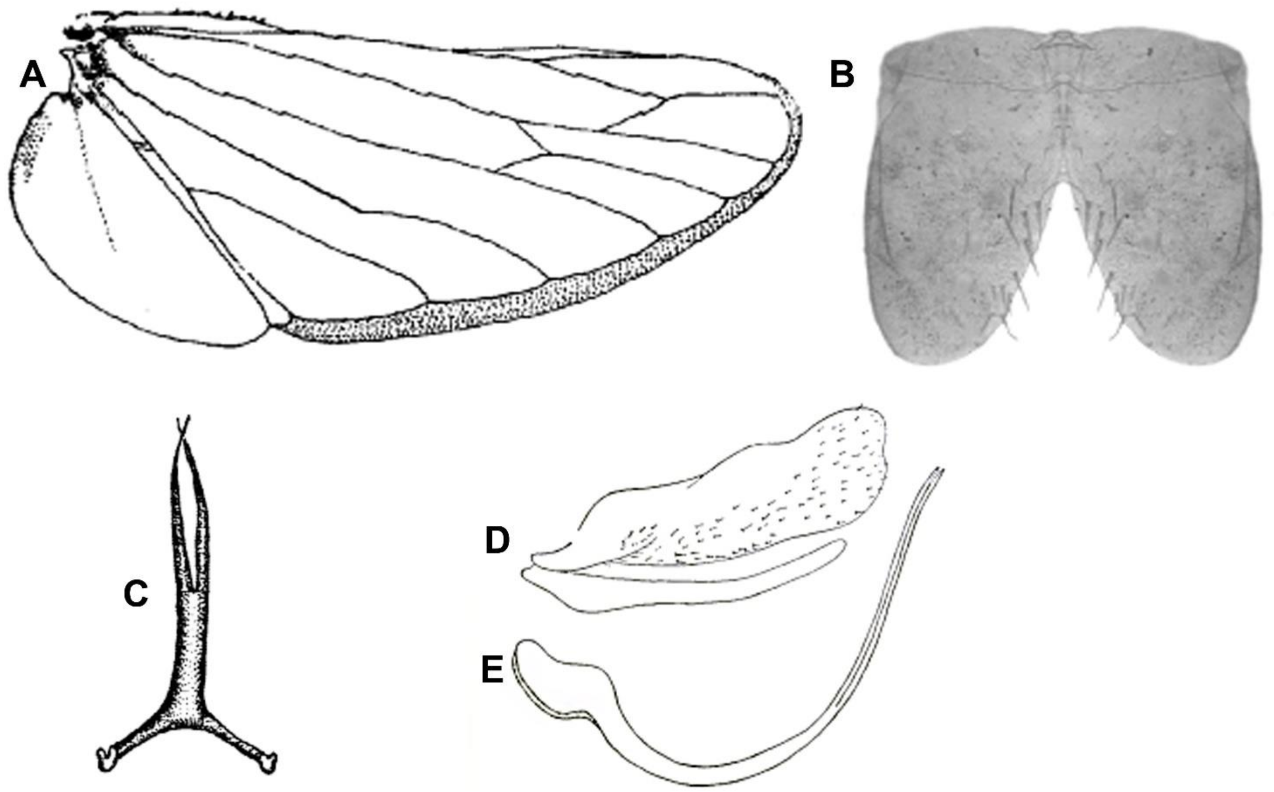
Distribution: Neotropical

Body length: Males 8.5 mm, Females 10 mm

Distinguished from Australian Cicadellinae by the following combination of characters:

1) Head strongly produced medially, longer medially than interocular width; in lateral view face is curved slightly dorsally at apex (Figure 6E);
2) distinctive black, reticulate pattern on head (Figures 6A–C);
3) anterior margin of head, between crown and face, carinate;
4) antennal ledges protuberant from dorsal view (feature of Proconiini);
5) pronotum narrower than transocular width, lateral margins parallel;
6) strongly swollen frons and clypeus (feature of Proconiini);
7) hind femur not reaching posterior margin of proepimeron (feature of Proconiini);
8) legs with first tarsomere length longer than 2nd and 3rd segments;
9) male pygofer extremely elongate posteriorly (visible beyond tegmen apex) (Figures 6A, 7D);
10) paramere narrow and elongate;
11) aedeagus forked at apex (Figure 7C);
12) female sternite VII produced laterally, bilobed (Figure 7B).

Figure 6. A)–B) *Acrogonia terminalis* males, dorsal habitus; C) *Acrogonia citrina* paratype, sex unknown, dorsal habitus; D) *A. terminalis*, sex unknown, lateral habitus; E) *Acrogonia* sp., male, head and pronotum, lateral view; F) *A. citrina* paratype, sex unknown, lateral habitus. (Source of images A–D, Wilson *et al*. 2020; E, Agriculture Victoria, specimen borrowed from Agricultural Scientific Collections Unit (ASCU); F, Wilson *et al*. 2020).



Figures 7. A) *Acrogonia citrina*, hind wing; B) *A. citrina,* female, seventh sternite, ventral view; C) *A. citrina* aedeagus, caudal view; D) *A. terminalis*, male pygofer, lateral view; E) *A. terminalis*, aedeagus, lateral view. (Source of images: A, Marucci *et al*. 2002; B, Dellapé 2015; C, adapted from Marucci *et al*. 2002; D–E, Young 1968, page 269).

Cicadella Latreille, 1817 (Cicadellini)

A redescription of this genus can be found in Young (1977; page 569). Dmitriev *et al*. (2017) considers that there are 10 valid species in this genus. However, Wilson *et al*. (2020) lists 45 species of *Cicadella* with many species of uncertain placement and a revision of the genus is recommended.

Cicadella viridis (Linnaeus, 1758)

(Figures 8–9)

References:

Linnaeus (1758): p. 438 [original description as *Cicada viridis*]; Young (1977): p. 570 [illustrations of head and male and female genitalia], p. 572 [list of synonyms of *C. viridis*]; Ossiannilsson (1981): p. 389 [key to the 2 species of *Cicadella* in Europe including *C. viridis*], p. 389–391 [description of species], p. 390 [illustrations of head and male genitalia]; Biedermann & Niedringhaus (2009): p. 221 [illustrated key to the two species of *Cicadella* in Europe]; Shah *et al*. 2019: p. 1–12 [diagnostic characteristics of *Cicadella viridis* from China including photographs and illustrations].

Online resources: Wilson *et al*. (2020); Wilson & Turner (2021); Dmitriev *et al*. (2017); Postle (2022).

Distribution: Widespread species, recorded from Europe, Middle East and Asia.

Body length: Males 5–7 mm and Females 7–9 mm.

Distinguished from Australian Cicadellinae by the following characters:

1) Colour generally greenish yellow, (females) to greenish blue/ grey (males);
2) head bearing a pair of black pentagonal–shaped patches dorsally between ocelli;
3) face often with a small black spot on lateral margin of clypeal suture;
4) pronotum is greenish/ yellow, sometimes with indistinct black transverse markings anteriorly;
5) tegmen opaque greenish yellow in females and blue/ grey in males (Figures 8A–C), apically membranous;
6) male pygofer moderately produced, apically narrowly convex, a number of small macrosetae along posteroventral margin (Figure 9A–B);
7) male with pair of paraphyses present, symetrical (Figures 9H–I);
8) male paramere apically hook–shaped, narrowed to a point (Figure 9D);
9) female abdominal sternite VII posterior margin with shallow notch medially (Figure 9M).

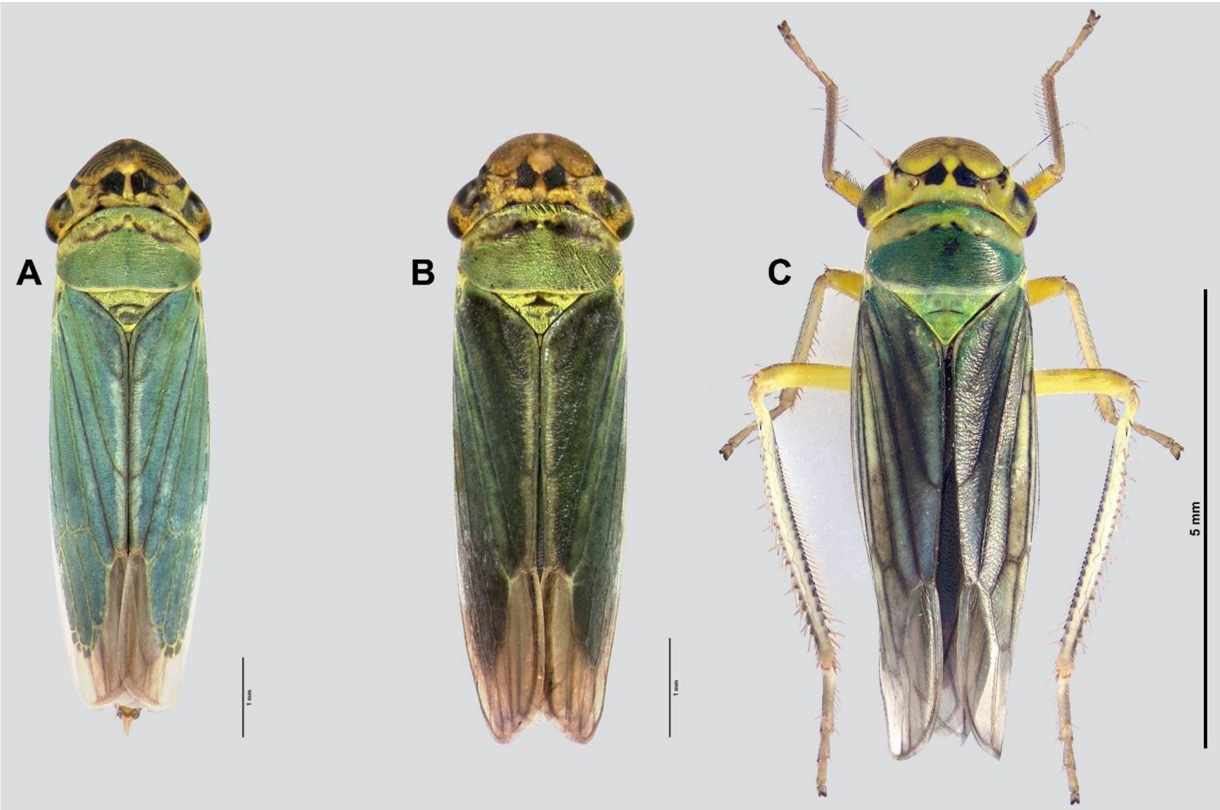


Figure 8. *Cicadella viridis*. A) female, dorsal habitus; B) male, dorsal habitus; C) male, dorsal habitus. (Source of images: A–B, Wilson *et al.* 2020; C, Jérôme Constant, RBINS).

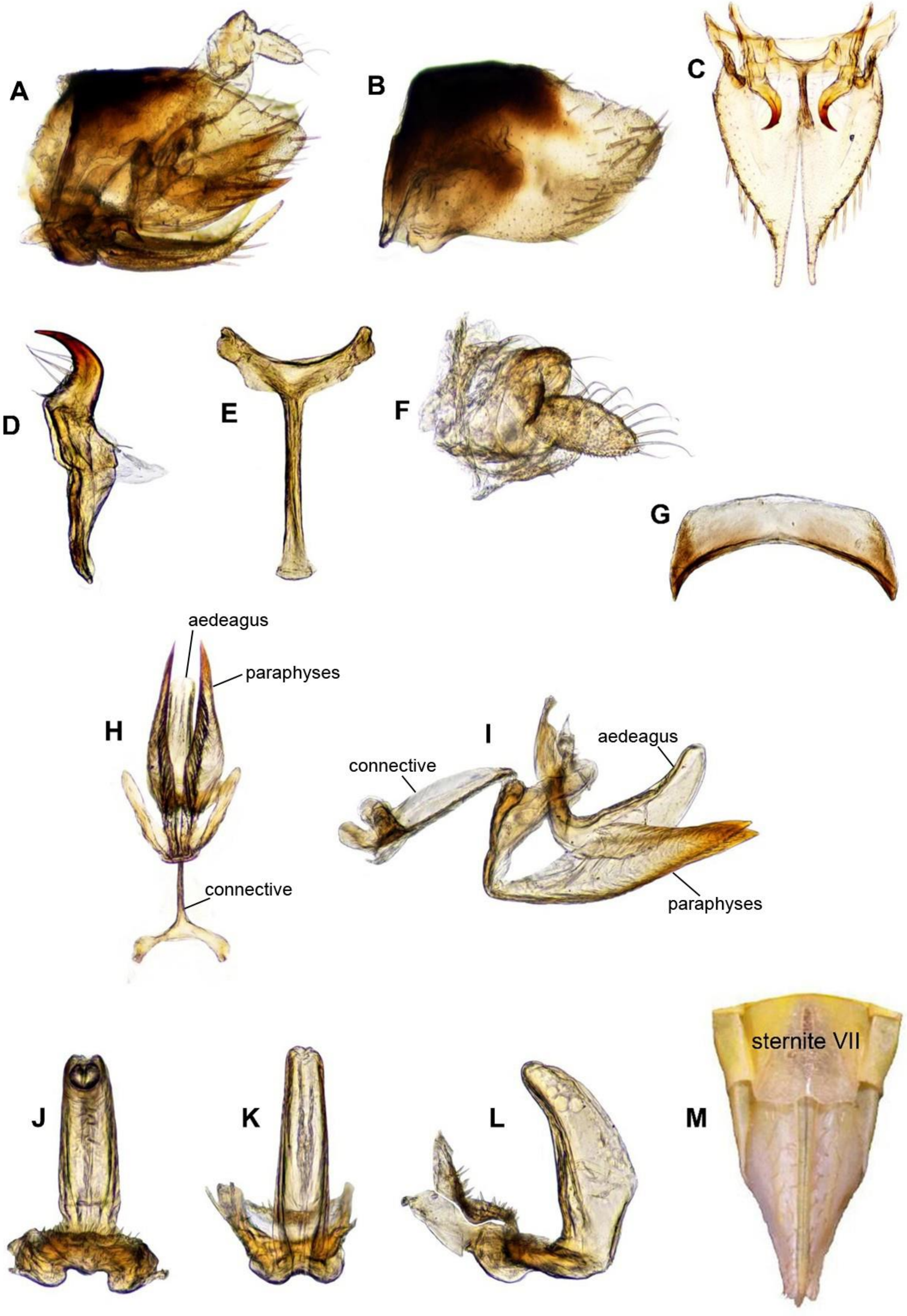

A
B
C
D
E
F
G
aedeagus
paraphyses
H
connective
I
aedeagus
connective
paraphyses
J
K
L
M
sternite VII

Figure 9. *Cicadella viridis*, male genitalia. A) lateral pygofer, undissected; B) lateral pygofer, aedegus and other structures removed; C) ventral subgenital plates and parameres; D) parameres lateral view; E) ventral connective; F) anal tube; G) valve; H) ventral aedeagus, paraphyses and ventral connective; I) lateral aedeagus, paraphyses; J) aedegus dorsal view; K) aedeagus, ventral view; L) aedeagus, lateral view; M) female seventh sternite and ovipositor, ventral view. (Source of images: A–M, Shah *et al.* 2019).

Dilobopterus Signoret, 1850 (Cicadellini)

A description of the genus and key to species can be found in Young (1977), page 104. Dmitriev *et al*. (2017) lists 52 species in the genus *Dilobopterus*, which have a Nearctic distribution.

Dilobopterus costalimai Young, 1977

(Figure 10)

References:

Young (1977): p. 136 [original description]; Marucci *et al*. 2002 : p. 151 [*D. costalimai* included in key to species] p. 157 [redescription, key characteristics], p. 158 [illustration of head and thorax, fig 9a; tegmen and hind wing, 9b–c; and male genitalia, figs. 9d–h].

Online resources: Wilson *et al*. (2020); Wilson & Turner (2021); Dmitriev *et al*. (2017).

Distribution: Brazil, Paraguay and Argentina

Body length: Males 8.8 mm and Females 8.8–9.1 mm

Distinguished from Australian Cicadellinae by the following characters:

1) Head, pronotum, mesonotum and scutellum orange or yellow with distinctive black markings;
2) pronotum with transverse black markings sinuous or almost w shaped and additional black transverse marking with median vertical line along posterior margin (Figures 10A–C);
3) tegmen with large membranous area medially;
4) claval veins indistinct;
5) hind wing enlarged jugal lobe, narrow apically and distinctly shorter than tegmen;
6) legs yellow/ orange with black tarsal claws;
7) first tarsomere with length much greater than combined length of second or third tarsomeres;
8) male pygofer with very numerous macrosetae on basal half (Figure 10F);
9) aedeagus in lateral view with distinct lobe above apex of shaft, directed posteriorly and no distinct separation between lobe and shaft.

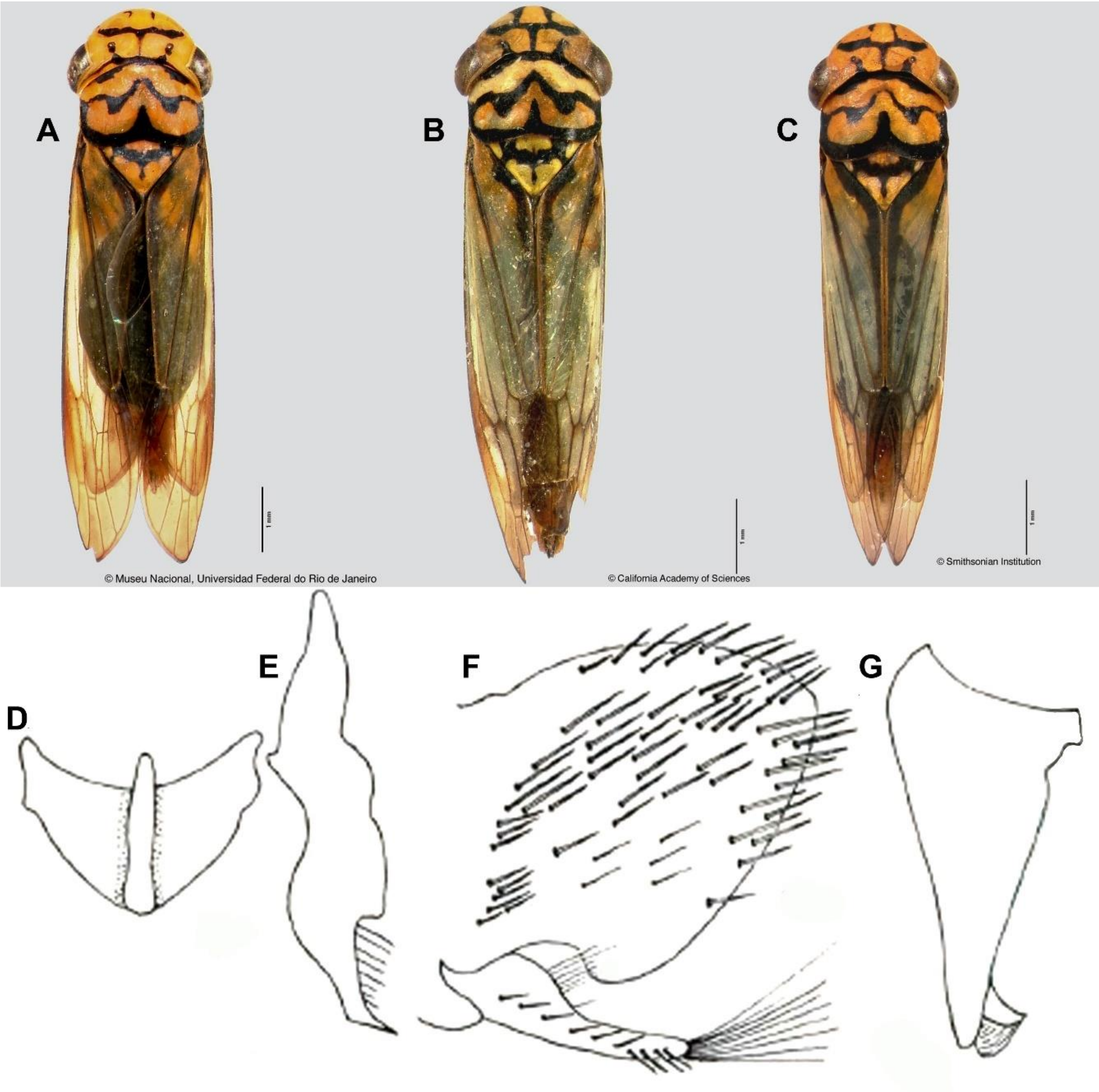


Figure 10. *Dilobopterus costalimai.* A)–B) females, dorsal habitus, showing pattern variation; C) male, dorsal habitus; D) aedeagus, dorsal view; E) paramere, dorsal view; F) male pygofer, lateral view; G) male pygofer, dorsal view, left side. (Source of images: A–C, Wilson *et al*. 2020; D–G, Young 1977, page 122).

Draeculacephala Ball, 1901 (Cicadellini)

Ball erected the genus *Draeculacephala* in 1901 (page 66) in his work on Tettigoniidae of North America, North of Mexico and included this genus in a key of the subfamily Tettigoniina (page 39). Young & Davidson (1959), reviewed the genus and included a key to the species (page 4). *Draeculacephala* is mentioned in the generic key in Young (1977), (page 14) and a redescription of the genus is found on page 580. Hamilton (1985) reviewed the genus and Dietrich (1994) further revised the genus, adding three new Neotropical species and providing a key to the species including *D. minerva*. Catanach (2009) discusses and compares morphological characteristics of *Draeculacephala* with the genus *Xyphon*.

Dmitriev *et al*. (2017) lists 27 species in *Draeculacephala* recorded from Neotropical and Neartic regions. A recent review of the New World *Draeculacephala* from Mexico was conducted by Blanco–Rodríguez & Pinedo–Escatel (2022) and described one new species.

Draeculacephala minerva Ball, 1927

(Figure 11)

References:

Ball (1927): p. 24 [original description of the species], p. 25 [illustrations of the head and genitalia, fig. 15]; DeLong & Severin, 1949: p.174–176 [description and illustrations of head and male genitalia, figs 3a–f]; Young & Davidson, 1959: p. 24–26 [species redescription and illustrations of head and male genitalia]; Hamilton, 1985: p. 91 [illustration of male subgenital plates], p. 92 [illustration of male second abdominal apodeme], p. 93 [specific characters discussed], p. 99 [in key to *producta* complex]; Dietrich, 1994: p. 91 [in key to species of the genus *Draeculacephala*], p. 96 [mentioned in annotated checklist], p. 109 [illustration of male head, fig. 3].

Online resources: Wilson *et al*. (2020); Wilson & Turner (2021); Dmitriev *et al*. (2017).

Distribution: North and central America

Body length: Males 5.2–6.5 mm and Females 6–7.8 mm

Distinguished from Australian Cicadellinae by the following characters based on the descriptions and keys in Ball (1927), Young & Davidson (1959), Dietrich (1994) and Wilson & Turner (2021):

1) Body generally greenish/ yellow;
2) female head with vertex often orange with a black tip, pronotum and scutellum pale green, male similar but more greenish yellow;
3) face may be dark brown/ black with yellow around margin or with some diffuse brown marking (Figures 11E–G);
4) head triangular, produced medially, vertex flat with definite edge between crown and face; medial length of crown equal to or less than interocular width; crown with median longitudinal sulcus not quite reaching apex, crown depressed anterior to ocelli (Figure 11D);
5) frontoclypeus convex or very slightly concave;
6) tegmen with apical veins reticulate;
7) tegmen veins yellowish green or blueish to white;
8) hind femur macrosetal formula 2+1+1;
9) male abdominal sterna mostly dark brown;
10) male pygofer moderately produced posteriorly, a few short macrosetae along posterodorsal margin (Figure 11H);
11) subgenital plates extending posteriorly almost reaching length of pygofer apex, with row of fairly stout macrosetae along lateral margin (Figure 11H);
12) paramere with preapical lobe, apically truncate;
13) male genitalia with paraphysis present, paired and symmetrical;
14) aedeagus with distinct triangular tooth on dorsal margin when viewed laterally.

See also Van Dyk (2020), Bug Guide https://bugguide.net/node/view/255829 for some further general information.

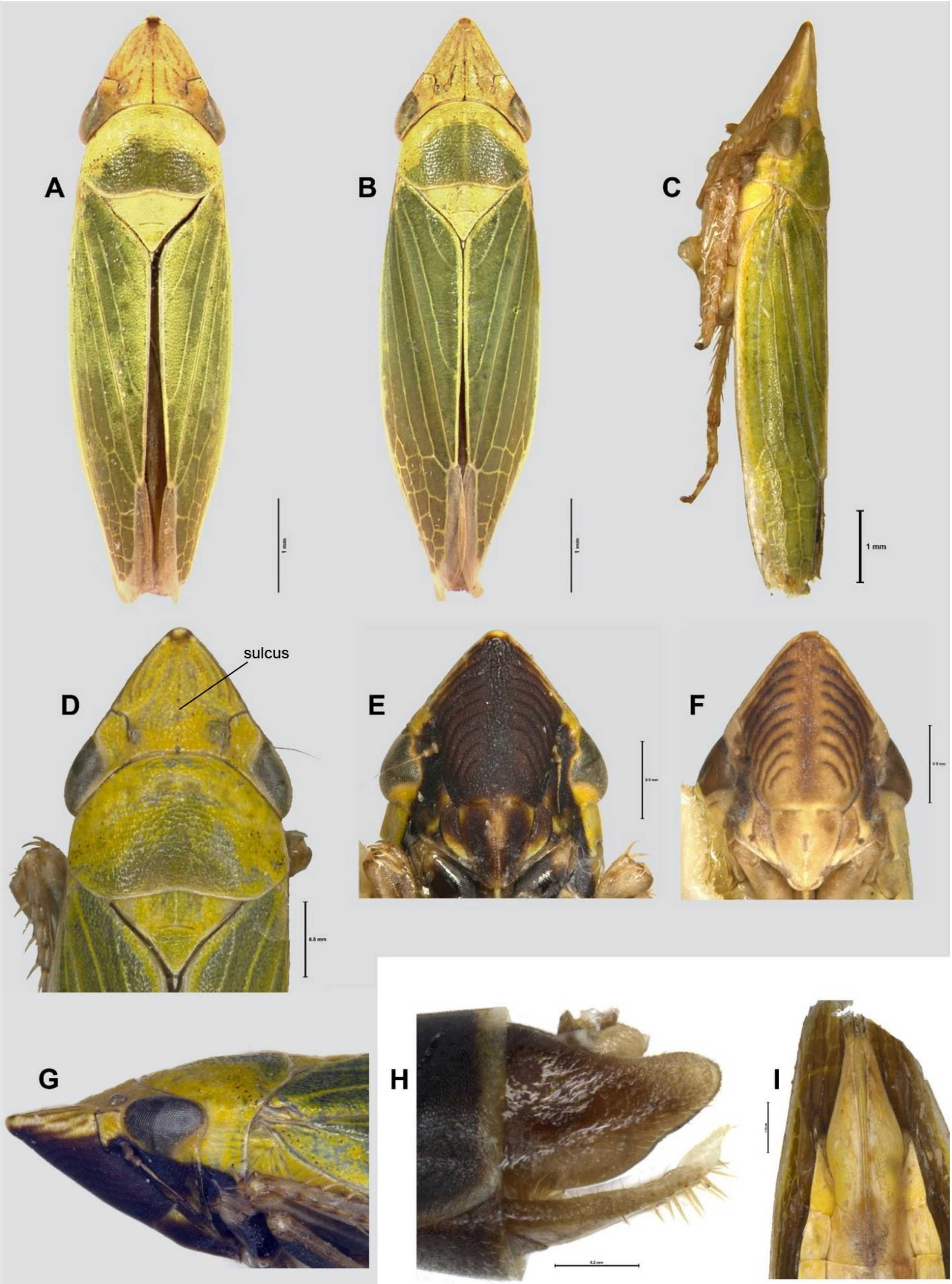

A
B
C
1 mm
D
sulcus
E
F
G
H
I

Figure 11. *Draeculacephala minerva*. A) female, dorsal habitus; B) male, dorsal habitus; C) female, lateral habitus; D) head and pronotum, dorsal view; E) male, face, ventral view; F) female, face, ventral view; G) head and pronotum, lateral view; H) male, pygofer, lateral view; I) female, seventh sternite and pygofer, ventral view. (Source of images: A–B, Wilson *et al*. 2020; C–I, Agriculture Victoria, specimens borrowed from ASCU).

Graphocephala Van Duzee, 1916 (Cicadellini)

There are currently over 61 species described in the genus according to Dmitriev *et al*. (2017). Further information about *Graphocephala* can be found in Young (1977), with a description of the genus (page 849) and key to species (page 864). Remarks about the taxonomy of this genus can be found on the Bug Guide website Bartlett (2022) (https://bugguide.net/node/view/332). There are no recent revisions of the genus, although one is likely required.

Graphocephala atropunctata (Signoret, 1854)

(Figures 12–13)

References:

Signoret (1854): p. 354 [original description under the name *Tettigonia atropunctata*]; DeLong & Severin 1949: p. 176 [description and illustrations as synonym *Neokolla circellata*, figs. 4a–d]; Young, 1977: p. 849 [listed as the type species of the genus *Hordnia* Oman, 1949 which was synonymised with *Graphocephala*], p. 860 [mentioned in checklist], p. 872 [in key to species of *Graphocephala*], p. 888 [illustrations of head and male and female genitalia].

Online resources: Wilson *et al*. (2020); Wilson & Turner (2021); Dmitriev *et al*. (2017).

Distribution: Nearctic

Body length: less than 7mm

Distinguished from Australian Cicadellinae by the following characters:

1) General body colour yellowish/ green to grey;
2) crown with tiny anterior medial black spot, pair of black spots anterior to ocelli (Figure 12A) or with black curved lines outlining posterior margin where frontoclypeus extends onto crown, may have black stripes laterad of ocelli (Figures 12A–C, H);
3) head with transverse slight depression near ocelli;
4) face with black markings, clypellus pale with median black spots (Figure 12G);
5) black/grey spots and markings on pronotum, may have paired spots anteriorly (submedially and/or laterally) and posteriorly (submedially), a black patch anteromedially and/or one fine central spot;
6) mesonotum + scutellum yellow with black triangles anterior laterally (may be partially covered by pronotum);
7) tegmen opaque yellowish green/ greyish with brown veins;
8) hindwing with R2+3 incomplete;
9) pygofer slightly to moderately produced, macrosetae on apical half of disc (Figure 13B, D);
10) aedeagal shaft short, without processes (Figure 13E);

11) paraphyses present, asymmetrical (Figure 13E).

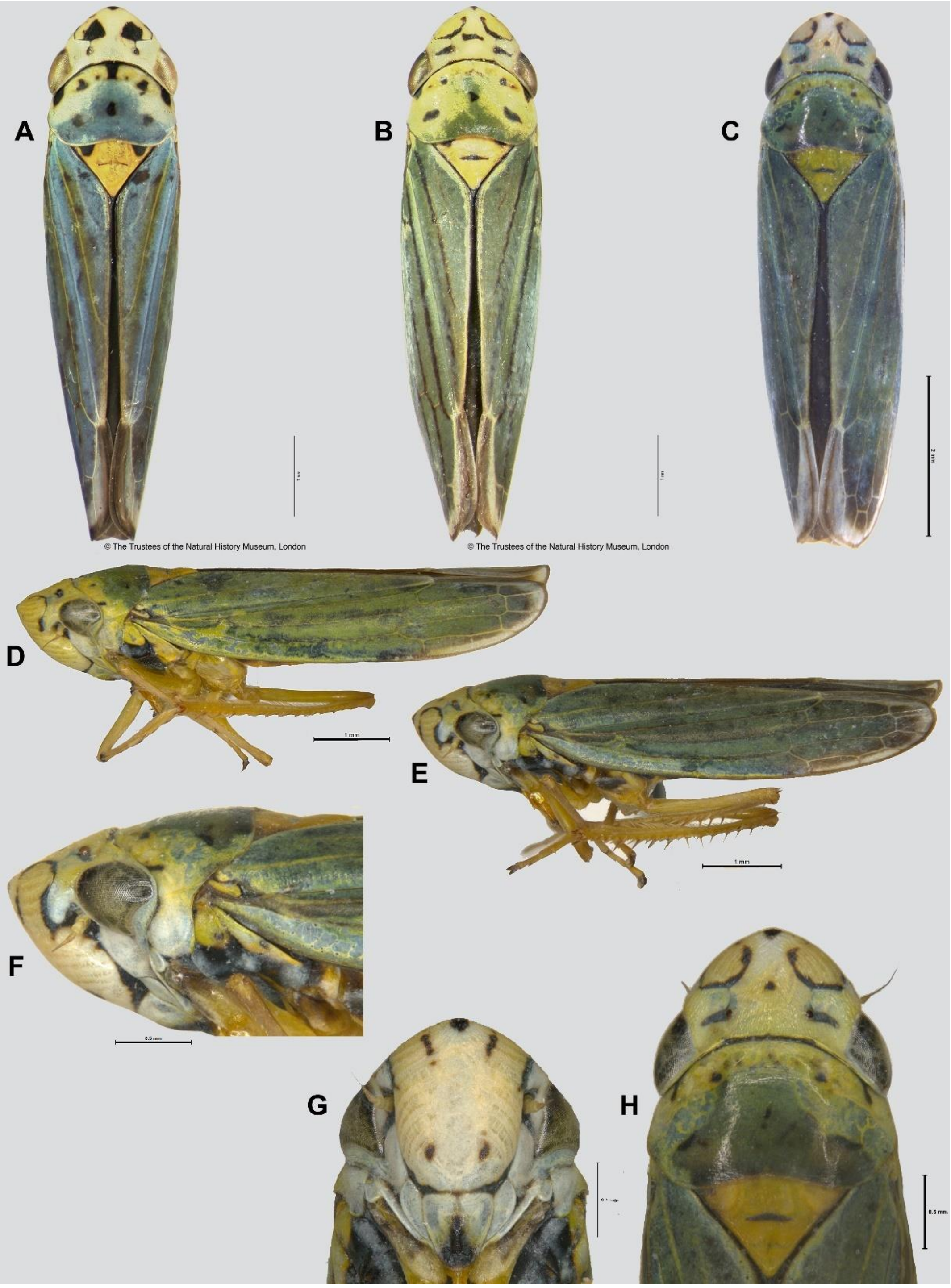


Figure 12. *Graphocephala atropunctata*. A)–B) females, dorsal habitus, showing pattern variation; C) male, dorsal habitus; D) female, lateral habitus; E) male, lateral habitus; F) head and pronotum, male, lateral view; G) face, male, ventral view; H) head and pronotum, male, dorsal view. (Source of images: A–B, Wilson *et al*. 2020; C–H, Agriculture Victoria, specimens borrowed from ASCU).

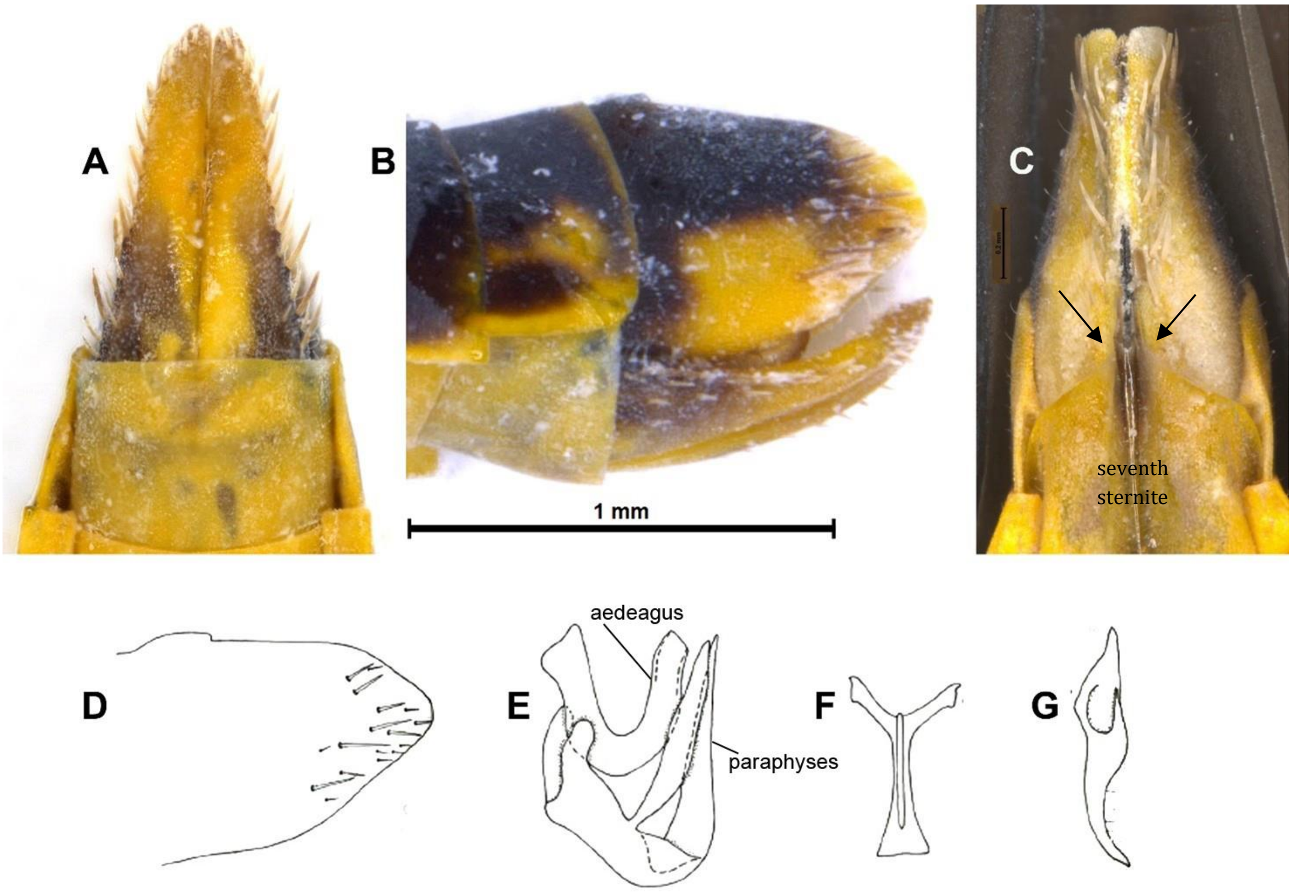


Figure 13. *Graphocephala atropunctata*. A) male, subgenital plates, ventral view; B) male, pygofer, lateral view; C) female, seventh sternite and pygofer, ventral view; D) male pygofer, lateral view; E) aedeagus and paraphyses, lateral view; F) ventral connective, dorsal view; G) paramere, dorsal view. (Source of images: A–C, Agriculture Victoria, specimens borrowed from ASCU; D–G, Young 1977, page 888).

Oncometopia Stål, 1869 (Proconiini)

A redescription of this genus can be found in Young (1968) *sensu lato*. page 220 (figs 207–219) and page 222, *sensu stricto* (figs 207–219). Emmrich (1975, 1984) published on *Oncometopia* including descriptions, records and information about the genus. At least 61 species are described in this genus distributed in the Nearctic and Neotropical regions (Dmitriev *et al*. 2017).

Oncometopia facialis (Signoret, 1854)

(Figure 14)

References:

Signoret (1854b): p. 489 [original description as *Tettigonia facialis*, in Latin and French]; Young 1968: p. 226–227 [listed as a species of *Oncometopia* sensu stricto]; Marucci *et al*. (2002)): p. 151 [included in key to species of Cicadellini in Citrus Orchards, Bebedouro, Brazil, in Portugese]

Online resources: Wilson *et al*. (2020); Wilson & Turner (2021); Dmitriev *et al*. (2017).

Distribution: USA and Central and South America (Argentina, Brazil, Bolivia, Colombia, Ecuador and Paraguay)

Body length: Males 10.5–11.5 mm and Females 11–12 mm

Distinguished from Australian Cicadellinae by the following characters:

1) Head, pronotum, mesonotum+ scutellum brown/ purplish (described as reddish/ yellow in Signoret 1854b);
2) frons, vertex and top of vertex, usually black;
3) frons and clypeus strongly convex, bulbous, anterior margin rounded, and crown rounded onto face (Figures 14D, E);
4) frons with granulations between the muscle impressions;
5) transocular width distinctly greater than pronotal width;
6) antennal ledge protuberant from dorsal view (feature of Proconiini);
7) metepimeron with shelf like projection;
8) pronotal disk punctate;
9) tegmen dark brown, opaque with pale veins (may have bright yellow veins and pattern in some specimens – see Fig. 14C), membranous at apex, surface often punctate;
10) hind wing with R2+3 absent;
11) hind femora not attaining posterior margin of epimeron (feature of Proconiini);
12) male pygofer in lateral view, short and broad, with a number of microsetae and occasionally macrosetae, processes arising on ventral margin (Figure 14F);
13) aedeagus asymmetrical, with a series of irregularly arranged processes preatrium elongate and articulated with connective (Figure 14G).

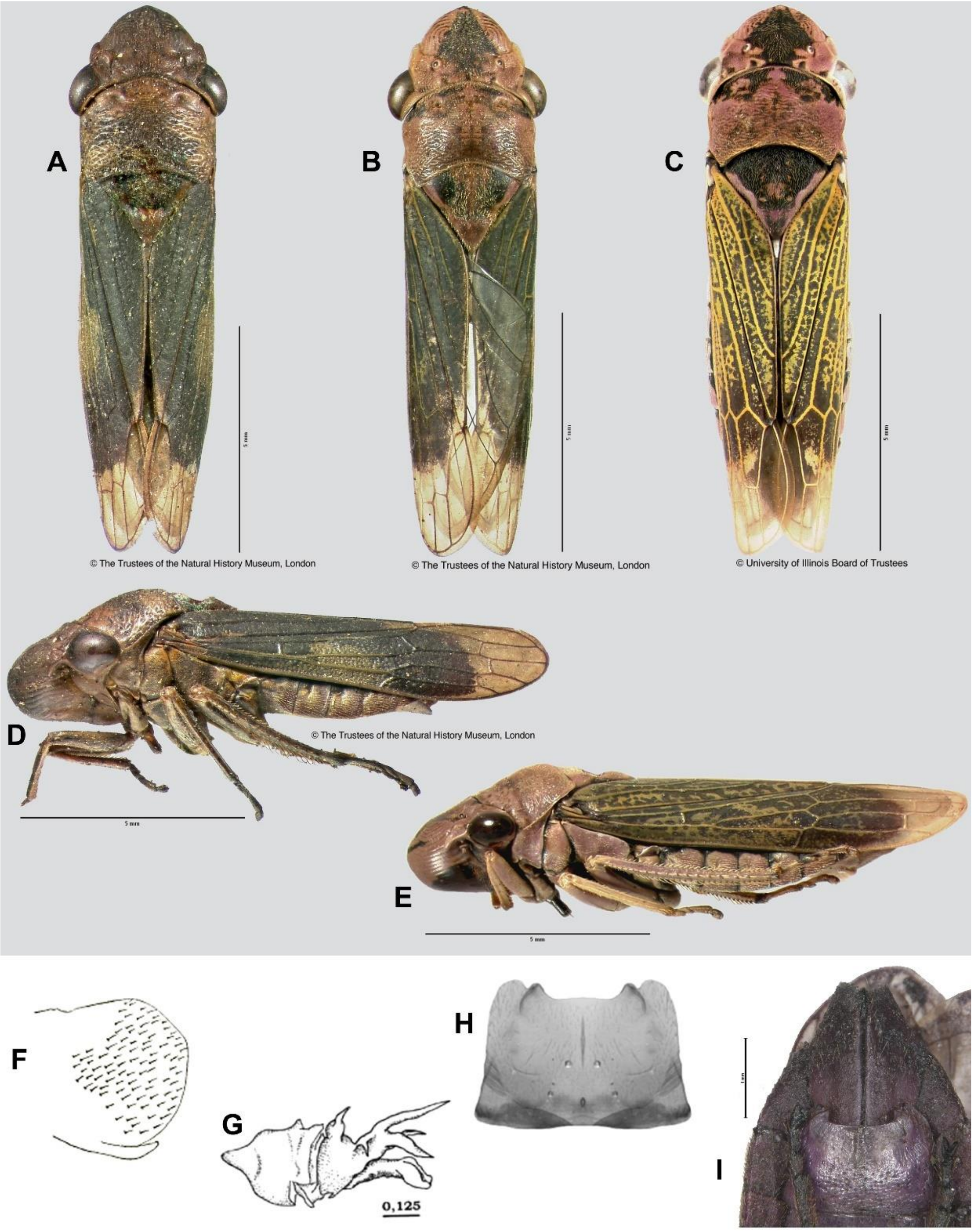


Figure 14. A) *Oncometopia facialis*, female, dorsal habitus; B)–C) *O. facialis*, males, dorsal habitus, showing colour variation; D) & E) *O. facialis*, females, lateral habitus; F) *Oncometopia orbana*, male pygofer, lateral view; G) *O. facialis*, aedeagus, lateral view; H) female seventh sternite; I) *O. tucumana*, female seventh sternite and ovipositor. (Source of images: A–E, Wilson *et al*. 2020; F, Young 1968; G, Marucci *et al*. 2002; H, Dellapé 2015; I, Agriculture Victoria, specimen borrowed from ASCU).

Xyphon Hamilton, 1985 (Cicadellini)

The description of this genus can be found in Hamilton (1985), Catanach (2009), Catanach *et al*. (2013). In Catanach *et al*. (2013) there is a key to species of this genus which includes *X. fulgidum*. Catanach (2019) also provides an interactive Key to species of the genus *Xyphon* including *X. fulgidum* and 5 other species. There are 7 species of *Xyphon* as listed in Dmitriev *et al*. (2017).

Xyphon fulgidum (Nottingham, 1932)

(Figure 15)

References:

Nottingham (1932): p. 99 [key to species of *Carneocephala*, including *C. fulgida*], p. 101 [original description as *Carneocephala fulgida*], p. 114–115 [illustrations of head Plate I, figs 6 a–c, and illustrations of male genitalia, Plate II, figs 6a–c); Nielson, 1968: p. 111–114 [under then name *Carneocephala fulgida*, descriptions, illustrations of male genitalia and biological information]; Young, 1977: p. 585 [in catalogue as a species under the genus *Carneocephalus*]; Catanach, 2009: p. 40 [redescription of species], p. 73 [photograph of species, dorsal habitus], p. 75 [characters used to differentiate *X. fulgidum* from other *Xyphon* species]; Catanach, *et al*. 2013 [key to species of *Xyphon*, including *X. fulgidum*].

Online resources: Wilson *et al*. (2020); Wilson & Turner (2021); Dmitriev *et al.* 2017.

Distribution: USA: California

Body length: Males 4.5–5.0 mm and Females 5.5–6.0 mm

Distinguished from Australian Cicadellinae by the following characters:

1) Body generally, straw coloured;
2) head lacking dark markings, crown often orangish in appearance, produced medially, apical margin appearing angular from dorsal view;
3) distance between ocelli more than 7 times width of ocellus; distance of ocellus to margin of head more than two times width of ocellus;
4) pronotum posterior margin slightly concave, sinuate;
5) tegmen with densely reticulate veins apically, venation mainly green (Figures 15A, B);
6) male pygofer with basolateral setae; subgenital plates without macrosetae, but finer setae present (Figure 15C);
7) aedeagal shaft dorsal processes, acute, not compressed (Figures 15E, H);
8) paraphyses present, short and stout, “U” shaped (Figures E, I).

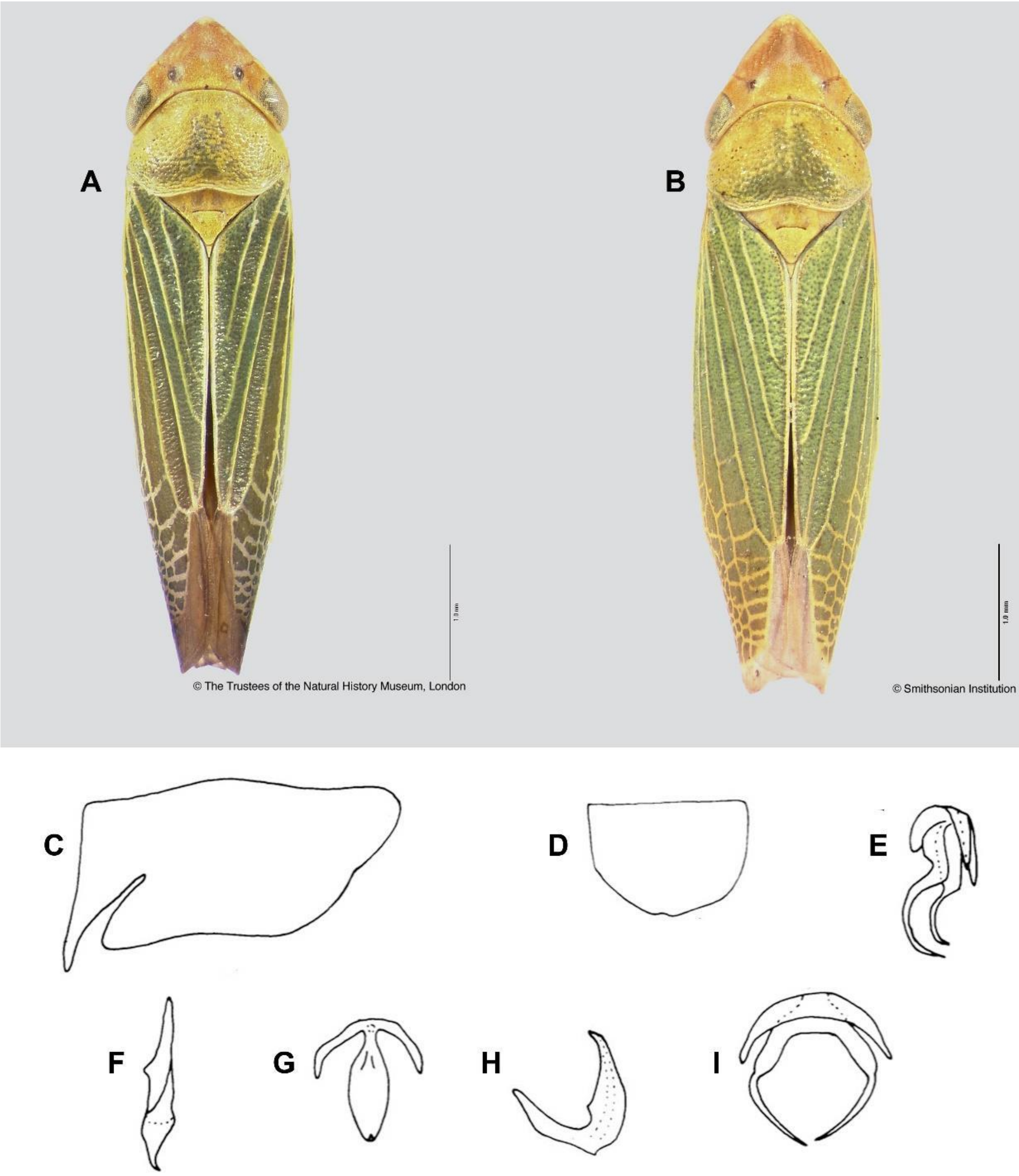


Figure 15. *Xyphon fulgidum*. A) male, dorsal habitus; B) female, dorsal habitus; C) male pygofer, lateral view; D) female seventh sternite, ventral view; E) aedeagus and paraphyses, lateral view; F) paramere; G) aedeagus, ventral view; H) aedeagus, lateral view; I) aedeagus and paraphyses, dorsal view. (Source of images: A–B, Wilson *et al*. 2020; C–I, Nielson 1968, page 112, figs 35a–f).

4.1.7. *Australian Cicadellinae which could be confused with exotic genera and species*

In Australia, there are currently three genera of Cicadellinae (Cicadellini) and at least 13 described species, including nine species of *Ishidaella*, the largest (in number of species) genus of Cicadellinae in Australia. Species of *Ishidaella* are described as "medium sized leafhoppers with blue–black tegmina and yellow heads with various patterns of black" (Fletcher 2009 and updates). There are also three species of *Cofana* and one species of *Conoguinula* (*C. coeruleopennis* Fabricius, 1803) found in Australia. There is some doubt around the presence of a fourth genus/ species, *Conogonia coerulescens* (Fabricius, 1803), in Australia, which was recorded from "New Holland" by Fabricius (1803) (see Fletcher 2009 and updates) and is not included in this protocol.

Also refer to SPHD (2013) for other Australian Cicadellidae species that could be confused with exotic Cicadellinae.

Cofana Melichar, 1926 (Cicadellini)

(Figure 16)

Young (1979) reviewed *Cofana* which included the three species known to occur in Australia (*C. perkinsi* (Kirkaldy, 1906), *C. spectra* (Distant, 1853), and *C. unimaculata* (Signoret, 1854)). In that publication there are illustrations of the head and thorax and male genitalia structures of *C. spectra* (page 4), a checklist of species (page 6) and key to 17 of the 19 species known at that time (page 9). In Young (1986), *Cofana* is included in a key to genera of Cicadellini of the Old World (page 10). There is also an updated key to the species (page 613), which can be used for male and female specimens. Dmitriev *et al.* (2017) lists 26 species in this genus.

Distribution: East and South East Asia, Afrotropical, Madagascar, New Guinea and Australasian regions (Wilson *et al*. 2020).

Body length: Males 5.3–11.8 mm and Females 5.3–13.0 mm

Diagnostic features of Australian species of *Cofana*:

Species in this genus are **greenish yellow to pale yellow, almost white**; the head is usually only moderately projected medially but may be triangular and more projected in some exotic species; the medial length is shorter than the inter–ocular width and the anterior margin more or less rounded. The **head** has either a **black spot medially** or a **black stripe**. The male pygofer is slightly produced at apex, the posterodorsal margin is convex, the posteroventral margin is obliquely angled, the pygofer processes are absent, the apex and posteroventral sections bear macrosetae, while the disc has no setae (Figure 16G). The subgenital plates are triangular and there are macrosetae along the margin from base to apex (Figure 16H). The parameres are short with a distinct lateral lobe on shank and the apex is truncate. The aedeagus has a well-developed, dorsal apodeme, processes are absent (Figure 16I). The paraphyses are absent. The female seventh sternite is slightly produced, but the posterior margin can vary in shape.

Compared with exotic species presented in this protocol, *Cofana* could mostly be confused with *G. atropunctata* or *C. viridis* but see below for differences.

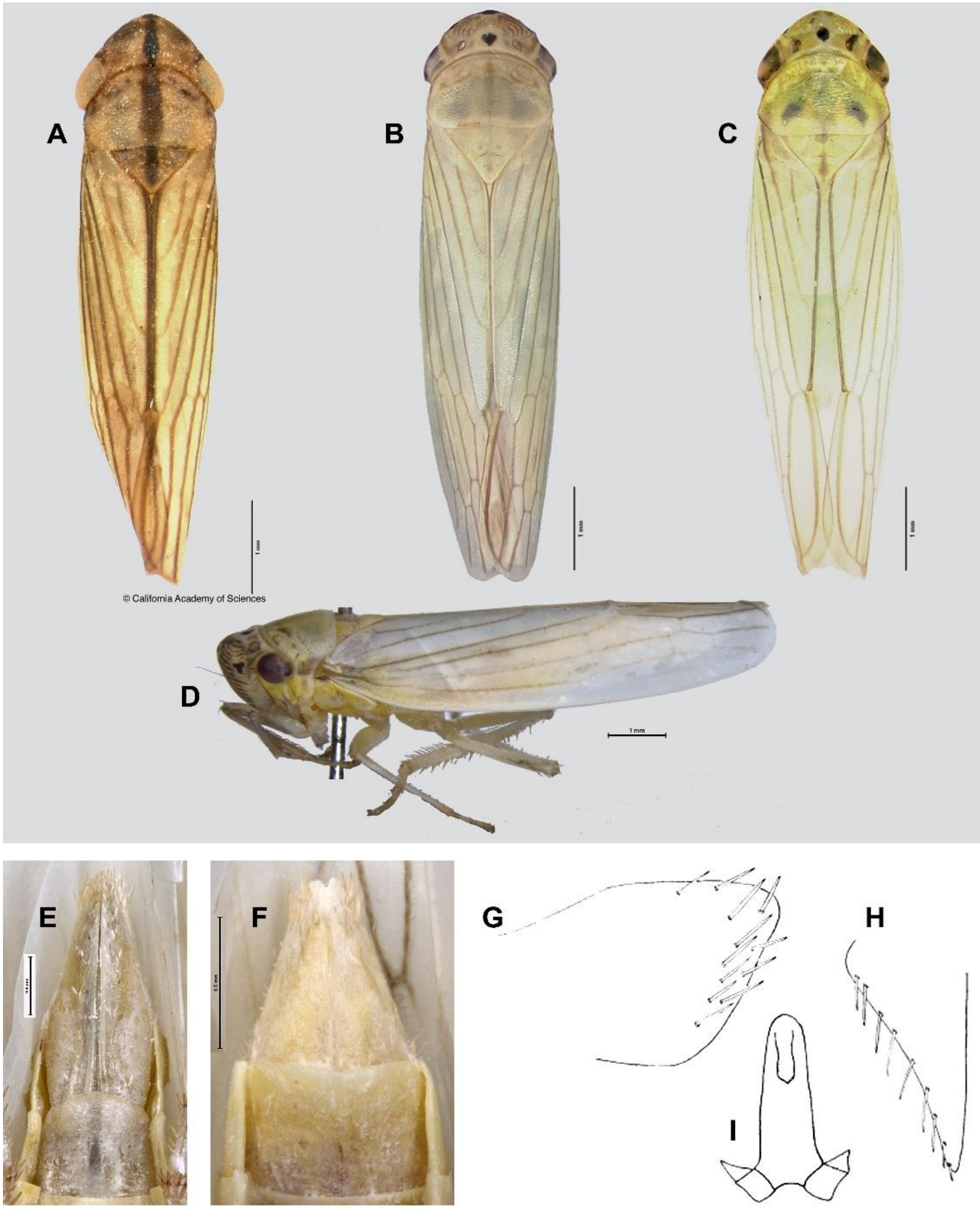


Figure 16. *Cofana species from Australia*. A) *C. perkinsi*, female, dorsal habitus; B) *C. spectra*, dorsal habitus; C) *C. unimaculata*, dorsal habitus; D) *C. spectra*, female, lateral habitus; E) female seventh sternite and pygofer, ventral view; F) male pregenital sternite and sub genital plates, ventral view; G) *C. spectra*, male pygofer, lateral view; H) *C. spectra*, subgenital plate, ventral view; I) *C. spectra*, aedeagus, caudoventral view, (Source of images: A–C, Wilson *et al*. (2020); D–F, Agriculture Victoria, specimen borrowed from ASCU; G–I, Young 1979, page 4).

***Cofana* distinct from exotic species of Cicadellini:**

1) Differs from *Draeculacephala* and *Xyphon* in having head more rounded anteriorly, medially distinctly shorter than inter–ocular width (*D. minerva* and *X. fulgidum* have arrow shaped heads which are almost equal to or slightly shorter medially than width between eyes); tegmen venation without multiple cross–veins apically, not reticulate (*D. minerva* and *X. fulgidum* with tegmen bearing distinctive reticulation apically).

2) Differs from *D. costalimai* and *G. atropunctata* in colouration. In *Cofana* species the head has a black spot or line medially but is not extensively patterned and may or may not have some indistinct brown or black markings on pronotum and scutellum (*D. costalimai* is generally orange/yellow and black and has extensive sinuous black wavy lines on the head and pronotum, while *G. atropunctata* is generally greenish and may have a distinct pair of black spots on the head and a few black spots or markings on the anterior of the pronotum and scutellum). Further differences can be found by examining the male genitalia where *Cofana* lacks paraphyses (but paraphyses are present in both *Graphocephala* and *Dilobopterus* species) and the pygofer has macrosetae just along posterior margin, while in *Dilobopterus* the stout macrosetae are scattered all over the disk.

3) *Cofana* species differ from *C. viridis* in coloration being pale yellow/ greenish, straw coloured or almost white with slightly opaque tegmen (the latter species has tegmen opaque green or grey/ black in males); in *Cofana* species, there is usually one black spot or line medially on the head while *C. viridis* has a pair of dorsal black spots medially between eyes.

4) Differs from *A. terminalis* and *O. facialis* in having less swollen frons and features that separate Cicadellini from Proconiini. Additionally, *Cofana* differs from *A. terminalis* in having less extensive black colour markings on the dorsal head and the medial length of the head is shorter than the inter–ocular width (*A. terminalis* has a black reticulate pattern on the head and the medial length is greater than the inter–ocular width).

Ishidaella Matsumura, 1912 (Cicadellini)

(Figure 17)

Ishidaella is included in the key to genera (for male specimens) of Cicadellini of the Old World (Young 1986) (page 11). He also provides a redescription of *Ishidaella* (page 123), a checklist, a key to species (for males) of this genus (page 128) and there are illustrations of the male genitalia for some species (figs. 85–91). There are currently nine species of *Ishidaella* described, which are mainly restricted to the Australasian and South East Asian regions. This includes *I. albomarginata* (Signoret, 1853), *I. anemolua* (Kirkaldy, 1906), *I. angustatus* (Evans, 1938), *I. latomarginata* (Distant, 1917), *I. naomiae* (Evans, 1938), *I. pettimolua* (Kirkaldy, 1906), *I. quadrata* (Walker, 1851), *I. richmondensis* (Distant, 1917) and *I. tumida* (Evans, 1966). These species are catalogued and images (habitus) are provided in Fletcher (2009 and updates) and Wilson *et al*. (2020) as illustrated in Figures 17A–J.

Distribution: Australia (Young 1986) – Note: Wilson *et al*. 2020 records this genus as present in parts of SE Asia and PNG but it is unclear which references were used to determine this distribution. Young (1986, page 622) tentatively places one of the Indonesian species *Kolla opalinula* Jacobi, 1941 in the genus *Ishidaella* but no further work is carried out to verify this proposed placement.

Body length: males & females 5.8–8.2 mm

Diagnostic features of Australian species of *Ishidaella*:

Species in this genus often have an orange head, pronotum and scutellum often with black markings (stripes, spots), pronotum and scutellum sometimes entirely black. The head is weakly produced. The tegmen is opaque black sometimes with orange markings and subcostal margin is translucent or opaque white. The general appearance and colouration of *Ishidaella* could possibly be confused with *D. costalimai* but see differences outlined below. The male pygofer is posteriorly moderately produced, convex, macrosetae on posterior half. The subgenital plate is triangular with uniseriate or multiseriate macrosetae along the margin; plates do not extend far beyond pygofer apex. The male paraphyses are absent; aedeagus with long preatrium, without suture or constriction between basal portion and shaft; connective relatively short, not reaching posteriorly as far as styles and subgenital plates not extended posteriorly beyond apex of pygofer. The female seventh sternite is short and may be slightly concave to slightly convex (Figure17M). **Note!**: Some genera in South East Asia (such as *Kolla* Distant) can appear, very similar to species of *Ishidaella*. The male genitalia features should be examined for more accurate identification of this genus.

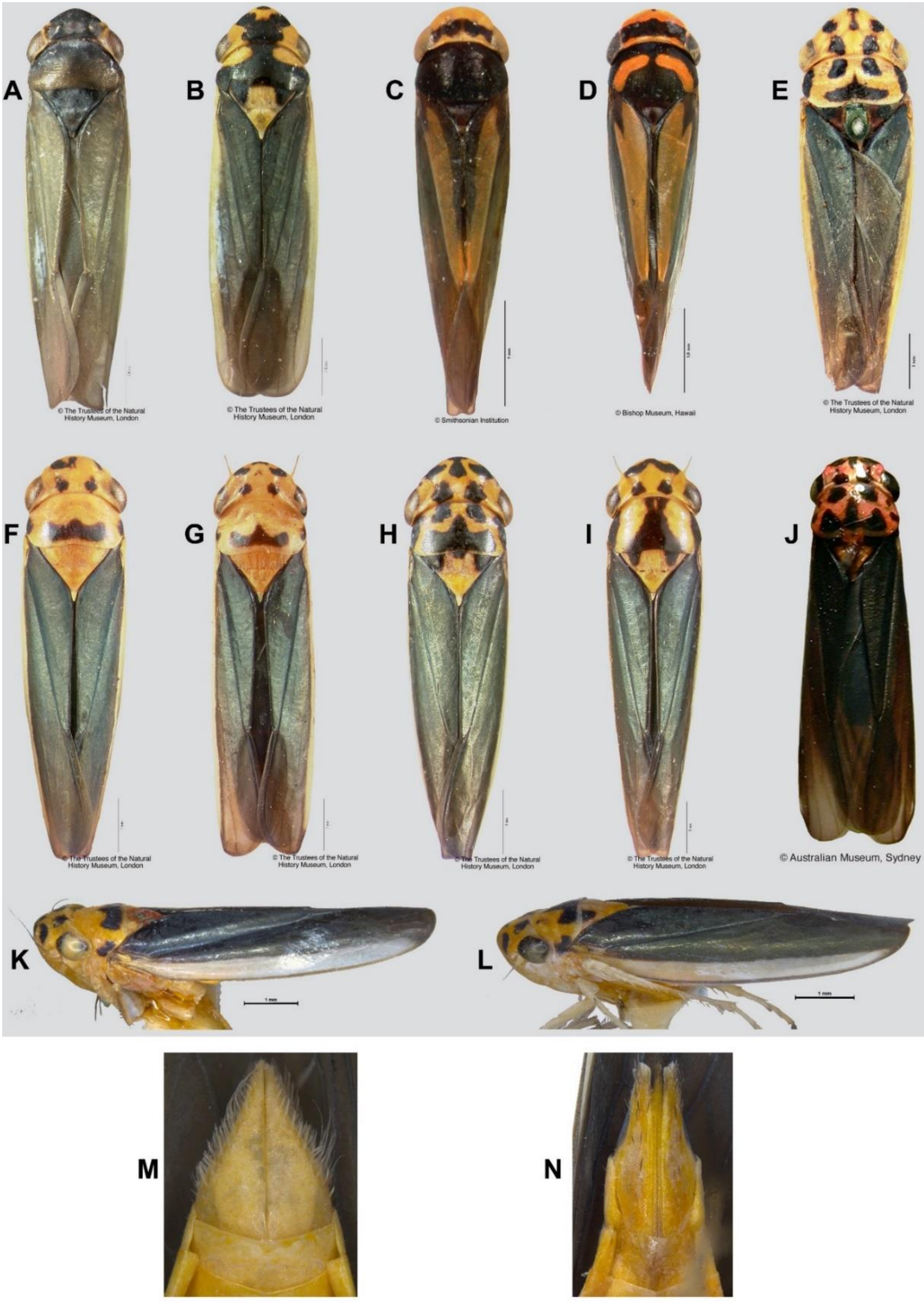


Figure 17. *Ishidaella species from Australia*. Dorsal habitus A–J. A) *I. albomarginata*, male; B) *I. albomarginata*, female; C) *I. anemolua*, female; D) *I. anemolua*, male; E) *I. angustatus*, female; F) *I. latomarginata*, female; G) *I. naomiae*, sex unknown; H) *I. pettimolua*, female; I) *I. richmonensis*, female; J) *I. tumida*, male; K) *I. latomarginata*, male, lateral habitus; L) *I. albomarginata,* female, lateral habitus; M) *I. latomarginata*, male subgenital plates, ventral view; N) *I. albomarginata*, female seventh sternite and pygofer, ventral view, (Source of images: A–J, Wilson *et al*. 2020; K–N, Agriculture Victoria, specimens borrowed from ASCU).

***Ishidaella* distinguished from exotic species of Cicadellinae:**

1) Differs from *Draeculacephala* and *Xyphon* in the general colouration being orange with black markings and black tegmen in *Ishidaella* (while *D. minerva* and *X. fulgidum* are green or yellow and without markings), shape of the head is more rounded along anterior margin, head medially shorter than inter–ocular width (*D. minerva* and *X. fulgidum* have arrow shaped heads which are almost equal to or slightly shorter medially than interocular width); tegmen venation without multiple cross–veins apically, not reticulate (*D. minerva* and *X. fulgidum* with tegmen bearing distinctive reticulation apically).

2) *Ishidaella* may be confused with *D. costalimai* because of the orange and black colouring but in *Ishidaella* the tegmen is usually black with or without some orange stripes and there is a translucent or opaque white subcostal margin (*D. costalimai* has a translucent orange/ brown or at most slightly opaque tegmen without a contrasting translucent costal margin).

3) Differs to *G. atropunctata* and *C. viridis* in colouration being orange and black and different black markings on head and pronotum (yellowish green in *G. atropunctata* and *C. viridis*). *Ishidaella* males are lacking paraphyses (this feature is present in *G. atropunctata* and *C. viridis*).

4) Differs from *A. terminalis* and *O. facialis* in having less swollen frons plus all of the features that separate Cicadellini from Proconiini. Additionally, *Ishidaella* differs from *A. terminalis* in having less extensive black colour markings on the crown of head and the medial length of the head is shorter than the inter–ocular width (*A. terminalis* has a black reticulate pattern on the head and the medial length is greater than the inter–ocular width).

Conoguinula Young, 1986 (Cicadellini)

(Figure 18)

Young (1986, page 532) described the monotypic genus *Conoguinula* which he separated from another similar genus C*onogonia*, also previously recorded in Australia, but the record is considered doubtful (Fletcher 2009 and updates). As a result, *Conogonia* is not included in this protocol. A key separating these two genera and *Conoguinula* from other Cicadellini genera can be found in Young (1986) (page 449). In Young (1986), a description is provided (page 532) and illustrations of the head and male genitalia of the only species known in the genus, *Conoguinula coeruleopennis* (Fabricius, 1803) (figs. 359, 360).

Distribution: Australia, Indonesia, PNG and the Philippines (Young 1986)

Body length: Males 10.4–13.9 mm and Females 12.5–15.3 mm

Diagnostic features of *Conoguinula coeruleopennis*:

Canary yellow, usually with a pair of black spots on pronotum and scutellum, ocelli on crown usually dark, sometimes area around ocelli dark. Shape of crown strongly produced medially, laterally parallel–sided, broadly parabolic along anterior margin. Tegmen opaque yellow, or dark brownish or olivaceous, apically hyaline, sometimes with black line along the anal margin. The male pygofer is elongate, strongly produced posteriorly, macrosetae are present along the ventral section of the plate. The aedeagus has a pair of basal processes (Figure 18J). *Conoguinula* differs to *Conogonia* in having the crown apex more blunt or broadly convex (more narrowly rounded or angular in *Conogonia*), and female abdominal tergite VI does not have an acute posterior projection (a projection is distinctly visible on the VI tergite in females of *Conogonia*).

Of the exotic species presented in this protocol, it could most readily be confused with *G. atropunctata* and *C. viridis*. See below for features to distinguish these species.

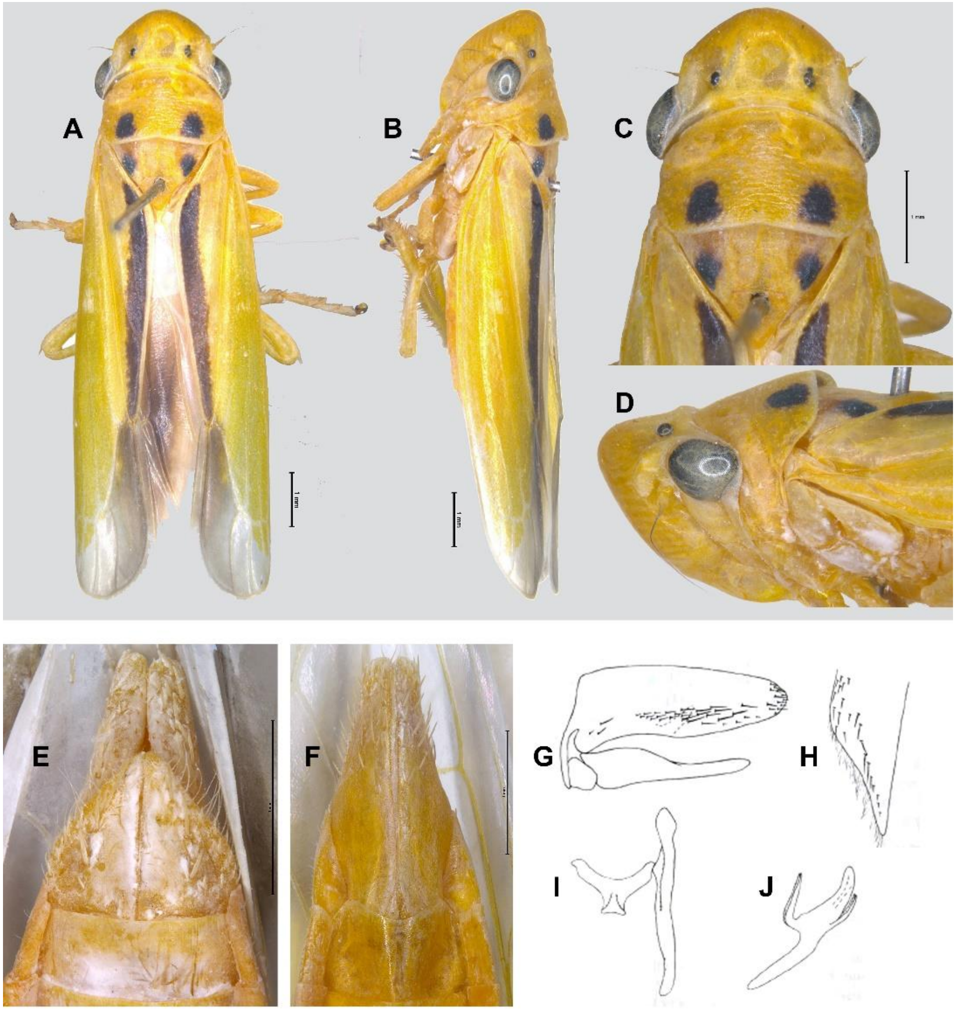


Figure 18. *Conoguinula coeruleopennis*. A) dorsal habitus; B) lateral habitus; C) head and pronotum, dorsal view; D) lateral head; E) male subgenital plates; F) female seventh sternite and pygofer, (source of image: Agriculture Victoria, specimen borrowed from ASCU); G) male pygofer, lateral view; H) male subgenital plate, ventral view; I) ventral connective and paramere, dorsal view; J) aedeagus, lateral view, (Source of images: A–F, Agriculture Victoria, specimens borrowed from ASCU; Young 1986, page 533).

Conoguinula coeruleopennis distinct from exotic species of Cicadellini:

1) Differs from *Draeculacephala* and *Xyphon* in having a more rounded anterior margin of head, medially shorter than wide between eyes (*D. minerva* and *X. fulgidum* have arrow shaped heads which are almost equal to or slightly shorter than interocular width); tegmen venation without multiple cross–veins apically, not reticulate (*D. minerva* and *X. fulgidum* with tegmen bearing distinctive reticulation apically).

2) Differs in size from *D. costalimai*, *G. atropunctata*, *C. viridis*. *Conoguinula coeruleopennis* is over 10 mm and up to 15 mm (*D. costalimai*, *G. atropunctata*, *C. viridis* are less than 10 mm in size), and in colour. It

is canary yellow, sometimes with black spots on pronotum and scutellum and black stripe along anal margin (*D. costalimai* is orange with sinuous black bands on head and pronotum, *G. atropunctata* and *C. viridis* are green with some black markings on head and some on pronotum but not pair of spots).

4) While the antennae are somewhat protuberant from dorsal view in *Conoguinula*, it differs from *A. terminalis* and *O. facialis* in having a less swollen frons and clypeus and other features that separate Cicadellini from Proconiini. It also differs in colouration in being mostly yellow with only a few black markings (in *A. terminalis* the head has a distinct black reticulated pattern, and *O. facialis* is purplish brown). The shape of the head is also very different in *Conoguinula* being slightly subquadrate, while in *A. terminalis* it is strongly produced medially and with a carina between crown and face.

4.2 Molecular identification

Eggs, nymphs, adult females and males of uncertain identification, suspected to be an exotic sharpshooter, should be tested using molecular methods. However, currently there are limited reference sequences available (see section 8.3) for the species covered in this NDP, and several species are part of multiple BINs (clusters of sequences produced by algorithms) in the Barcode of Life Data System (BOLD) which may indicate cryptic species or misidentifications, therefore molecular identifications should be interpreted with a degree of caution for most taxa. Due to this reason, molecular methods included in this protocol have not been verified in an Australian laboratory.

Specimens to be used for DNA extraction should be, preferably, preserved in 95–100% ethanol and stored in a freezer (minus 10 °C or colder) or be kept dry (card mounted adults) as a reference specimen (see also section 3.2.3 Preservation Methods).

A DNA barcoding test is the standard molecular method recommended to confirm species identification where barcode sequences are available online.

Techniques used for DNA extraction and amplification; PCR conditions and sequence analysis are similar across many invertebrate groups. The steps involved in DNA barcode identification are outlined in Löcker *et al.* (2018) Auchenorrhyncha Workshop manual and adopt the general guidelines of DNA barcoding as in Plant Health Australia (2011) the Australian Handbook for the identification of Fruit Flies, section 6.3.2. The procedure involves obtaining a DNA sequence for a specific region, such as a 648–bp region of the Cytochrome Oxidase I (CO1) gene (Hebert *et al*. 2003). First, DNA is extracted from a specimen, and a Polymerase Chain Reaction (PCR) test is conducted using the DNA extract. The PCR product is then sequenced. Sequences are then searched (BLASTed) against online databases such as the Barcode of Life Data System (BOLD) and NCBI GenBank, to reach a final species identification (where possible).

Further background and details about the DNA barcoding approach can be found in Ratnasingham & Hebert (2007).

DNA barcode sequence testing relies on the availability of sequence data. It is important to keep in mind that there are a large number of sharpshooter species worldwide (over 3100 species) and many of these do not have reference sequences available. However, 6 of the 7 species covered in this protocol have barcode sequence data available on the BOLD systems database or on GenBank.

Specimen preparation

Fresh, frozen, dry mounted and alcohol (95–100% EtOH) preserved specimens can be used for DNA extraction. Both, partially destructive and non–destructive methods may be used.

1) As it is important to retain a voucher specimen, the "partially destructive" method involves removal of one or two legs from one side of the body only (fore and/or mid leg should be used as these have fewer diagnostic characters). The legs are crushed and destroyed but the rest of the specimen is retained as a voucher specimen for examination.

2) Non–destructive methods which digest the abdomen overnight in Proteinase K, are considered equally as effective for DNA extraction (Gilbert *et al*. 2007, Gopurenko *et al*. 2013) and have the added benefit of clearing the abdomen for morphological examination of the male genitalia.

3) Commercial kits, such as Qiagen DNA extraction kits, for animal tissue can be used for steps 2. and 3.

4.2.1 DNA Extraction methods

Robotic DNA Extraction

Gopurenko *et al*. (2013), used a Qiagen DNA extraction kit. The abdomen, legs or whole specimen was placed in aliquots of 360 µL of tissue digestion buffer and 40 µL of proteinase K and left to digest overnight in an incubator at 55 °C. In that study, a Corbett Research 1820 X–tractor Gene robot was used to acquire the final DNA elution in 120 µL.

Non–destructive DNA extraction method with QIAGEN DNeasy Blood & Tissue kit

Alternatively, DNA extraction can be performed manually using a QIAGEN DNeasy ® Blood and Tissue extraction kit. The procedure follows steps in the "Protocol: Purification of Total DNA from Animal Blood or Cells (Spin–Column Protocol)" from the Qiagen DNEasy® Blood & Tissue handbook (2023) with the steps slightly modified (see steps in bold) for a non–destructive approach as below. If using a destructive approach, which involves crushing one or two legs with a pestle in extraction buffer or using microbeads in a homogenizer, all steps can be followed as in the handbook, except for step 7 where buffer volumes have been modified as outlined below. Use reagents as provided in the Qiagen DNEasy® Blood & Tissue kit.

Equipment

- Dry heating block
- Microcentrifuge tubes (sterile 1.5 mL)
- Freezer/Refrigerator
- Forceps (sterile)
- Micropestle (sterile) or microbeads and homogenizer – if necessary for partially destructive DNA extraction
- Microcentrifuge
- Pipettes and sterile tips (0–2 µL, 2–20 µL, 20–200 µL, and 200–1000 µL)
- Pins (sterile – for removing abdomen of specimens)

• Vortex

Procedure for non–destructive manual DNA extraction

Steps in Bold = modified from handbook for non–destructive method; steps not in bold = unchanged from handbook.

Step 1. Remove whole abdomen, or 1–2 legs (middle and/ or foreleg), using sterilised forceps or a pin, and place in a 1.5 mL microcentrifuge tube with 180 µL ATL Buffer.

Step 2. Add 20 µL Proteinase K to eppendorf tube. Vortex and incubate at 56 °C overnight. Incubation period can be less (minimum 1 hour) but clearing may not be sufficient in that time and DNA yield may be much lower.

Step 3. Remove from incubation and vortex for 15 seconds. Add 200 µL Buffer AL to the sample and mix thoroughly by vortexing. Then add 200 µL ethanol (96–100%) and mix again thoroughly by vortexing.

Step 4. Pipet the mixture from step 3 (including any precipitate) into the DNeasy Mini spin column placed in a 2 mL collection tube (provided). *Be careful not to pipette the abdomen or leg into the spin column as the insect body part should remain in the Eppendorf tube for later removal, rinsing and reattaching to the pinned specimen to which it belongs.

Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard flow–through and collection tube.

Step 5. Place the DNeasy Mini spin column in a new 2 mL collection tube (provided), add 500 µL Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard flow–through and collection tube.

Step 6. Place the DNeasy Mini spin column in a new 2 mL collection tube (provided), add 500 µL Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard flow–through and collection tube.

Step 7. Place the DNeasy Mini spin column in a clean 1.5 mL or 2 mL eppendorf tube (not provided), and pipet 100 µL Buffer AE (or less, such as 60 µL if using a leg) directly onto the DNeasy membrane. Incubate at room temperature for 1 min and then centrifuge for 1 min at ≥ 6000 x *g* (8000 rpm) to elute.

Elution with smaller quantities increases the final DNA concentration.

Step 8. For maximum DNA yield, repeat elution once as described in step 7.

Step 9. Store eluted DNA in a minus 20 °C freezer.

Note: The abdomen, leg or complete nymph should be immediately recovered from the column following extraction, using forceps and rinsed in water and then ethanol. Place the dissected abdomen in glycerol in a genitalia tube or glue the leg to a card point and attach to the corresponding specimen.

4.2.2 Polymerase Chain Reaction

The standard universal primer sets can be used to amplify the COI (barcoding) gene, (**LCO1490**–GGTCAACAAATCATAAAGATATTGG and **HCO2198**–TAAACTTCAGGGTGACCAAAAAATCA, designed by Folmer *et al.* 1994 and recommended by Hebert *et al*. 2003), for Cicadellidae.

However, Gopurenko *et al.* (2013) and Footit *et al.* (2014) developed or modified primers, as listed in Tables 2 & 4, and found that these were suitable for DNA barcoding of various Auchenorrhyncha families including Cicadellidae.

In Gopurenko *et al*. (2013), the full–length DNA barcode sequences were produced using a combination of the forward primer **BC1Fm** with reverse primers **BC3RDm** or **JerR2m**, resulting in a 672 and 646 basepair fragment respectively. Where the full fragment could not be amplified, smaller fragments of the barcode gene were amplified using a combination of **BC1Fm** with **Scar–2RDm** (5' end of the barcode gene producing a 328 basepair amplicon) and **Scar–3aFm** with **JerR2m** (3' end of the barcode gene producing a 406 basepair amplicon). The two amplicons can be joined together with an overlapping section of 88 basepairs. Gopurenko *et al*. (2013) utilised this barcode test for Auchenorrhyncha families including Caliscelidae, Cicadellidae, Cixiidae, Delphacidae, Eurybrachidae, Flatidae, Issidae, Meenoplidae, Membracidae, Nogodinidae, Tropiduchidae.

PCR procedure following Gopurenko (2013)

Table 2. PCR primers for DNA barcode (COI gene) sequencing of Cicadellidae and other Auchenorrhyncha. Primer sequences written with 5'-M13 tail italicised and are separated from taxon–specific primer sequences by a hyphen.

Locus	Direction	Primer Name	Primer Sequence	Original source
COI	Forward	BC1Fm	*GTAAAACGACGGCCAGT*-TCWACWAAYCAYAARGAYATYGG	Cho *et al*. (2008) modified from Folmer *et al*. (1994)
COI	Reverse	BC3RDm	*CAGGAAACAGCTATGAC*-GWARAATWARAATRTAWACYTCDGG	(Modified from Cho *et al*. 2008)
COI	Reverse	Scar–2RDm	*CAGGAAACAGCTATGAC*-GADARWGGDGGRTANACDGTTC	Gopurenko *et al*. 2013 (sequence reported incorrectly in that paper, correct sequence used in Semeraro 2014).
COI	Forward	Scar–3aFm	*GTAAAACGACGGCCAGT*-GCHCCHGAYATAGCNTTYCCNCG	Gopurenko *et al*. 2013 (sequence reported incorrectly in that paper but see Mitchell 2015)
COI	Reverse	JerR2m	*CAGGAAACAGCTATGAC*-CCAAARAATCARAAYARRTGTTG	Gopurenko *et al*. 2013 modified from "Jerry" Simon *et al*. 1994 (sequence reported incorrectly in that paper)

Reagents and concentrations recommended in Gopurenko *et al*. (2013) are provided in Table 3. A negative control should be included in the PCR test by adding purified water (instead of DNA) to a well with mastermix.

Some of these primers were redesigned (being slightly more degenerate) and renamed in Micthell (2015) (including AMbc0f1m based on BC1Fm, AMbc3f1m based on Scar-3aFm, AMbc5r1m based on Scar-2RDm and AMbc0r2m based on JerR2m). These could be used as alternative primers if required.

Table 3. PCR reagents.

Reagent	Concentration
PCR buffer	1 x
$MgCl_2$	3 mM
dNTP (each)	200 µM
F primer	2 pmol
R primer	2 pmol
Platinum Taq Polymerase	0.4 units
DNA Template	50 – 150 ng

In Gopurenko *et al.* (2013) the PCR was set up in a 15 µL mastermix containing 4µL of DNA extract and Invitrogen reagents. The PCR amplicons were prepared using a Corbett 1200 PCR robot. The PCR cycle followed an initial denaturation of 94 °C for 2 minutes; then 40x cycles of denaturation at 94 °C for 30 seconds; annealing at 50 °C for 60 seconds; 72 °C for 60 seconds; followed by a final extension at 72 °C for 5 minutes.

PCR procedure following Footit et al. (2014)

Table 4. PCR primers used for DNA barcode (COI gene) sequencing of Cicadellidae and other Auchenorrhyncha (Footit *et al*. 2014).

Locus	Primer Name	Primer sequence	Source
COI (5') Forward primer	LepF2–t1	AATCATAARGATATYGG	Modified from Hebert *et al*. (2004)
COI (5') Reverse primer	LepR1	TAAACTTCTGGATGTCCAAAAAATCA	Hebert *et al*. (2004)

The combination of primers LepF2–t1–LepR1 to amplify the barcode gene, resulted in a >600 basepair fragment. If the DNA could not be amplified in the first test, then further primer combinations, not included in this protocol, were tested (see Footit *et al*. 2014). These primers were tested for Cicadidae, Cercopidae, Aphrophoridae, Membracidae, Aetalionidae and Cicadellidae including species in genera which are covered in this protocol. PCR reagents and conditions are not described in Footit *et al*. 2014 but in that study, they followed procedures as in Hajibabaei *et al*. 2007.

Alternative PCR set–up

An alternative PCR set–up, adapted from the PCR reagents and cycling conditions in Plant Health Australia (2011) (see Table 5) could also be used but these have not been tested with the species covered in this protocol.

Table 5. PCR Reagents (manual PCR set–up)

Reagent	Concentration	1x reaction volume (25µl)
1x BSA	0.1 mg/ mL	15.3
PCR buffer	10x	2.5
$MgCl_2$	25 mM	0.5
dNTP (each)	2.5 mM	2.0
F primer	10 µM	1.25
R primer	10 µM	1.25
Taq Polymerase	5 units/ µl	0.2
DNA Template	50 – 150 ng	2

The PCR is performed in a 25 µL reaction. PCR thermocycling conditions require an initial denaturation of 94 ^{0}C for 2 minutes; 40 cycles of denaturation at 94 ^{0}C for 30 seconds; annealing at 52 ^{0}C for 30–45 seconds; and extension at 72 ^{0}C for 30–45 seconds; a final extension at 72 ^{0}C for 2 minutes.

The PCR product is visualised on a 1% agarose gel with 1% TBE buffer.

4.2.3 Sequence databases

Once the DNA barcode sequences have been obtained, they can be compared with sequences of identified Cicadellidae species. The two main public databases used are: 1) GenBank (https://www.ncbi.nlm.nih.gov/GenBank) – GenBank contains a large number of DNA sequence data deposited from a broad range of scientific research, including phylogenetic studies, and use a variety of genes (including full genomes). GenBank sequence data, do not have complete specimen collection data associated with them but sequences have some basic quality checks; 2) The BOLD systems database (Ratnasingham & Hebert 2007) is focused on the DNA barcoding region (primarily the 648–bp region of the 5' end of the Cytochrome Oxidase subunit I, COI, mitochondrial gene) and encourages submission of specimen images and retention of reference specimens. The BOLD systems database also records collection and identification information and the quality of sequences is assessed before being made public.

However, accurate identification using molecular data relies on the availability of a validated DNA reference sequence, from a morphologically correctly identified specimen. For further information about assessing sequence data in GenBank and BOLD, see Meiklejohn *et al*. (2019).

It is possible to perform a DNA barcoding test to positively confirm the following exotic species treated in this protocol: *A. terminalis*, *C. viridis*, *D. costalimai*, *D. minerva*, *G. atropunctata* and *O. facialis*. However, for *A. terminalis, D. costalimai* and *O. facialis*, there is currently only one COI gene sequence (in GenBank) available for comparison. A DNA barcoding test cannot be performed for *X. fulgidium* as there are no sequences available on the BOLD or GenBank databases. See summary of available sequences in Table 7 (Appendix 8.3). A brief comment about DNA barcode availability for each of the 7 exotic species and of the Cicadellinae genera known to occur in Australia, is provided.

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8 APPENDICES

8.1 Distribution of species

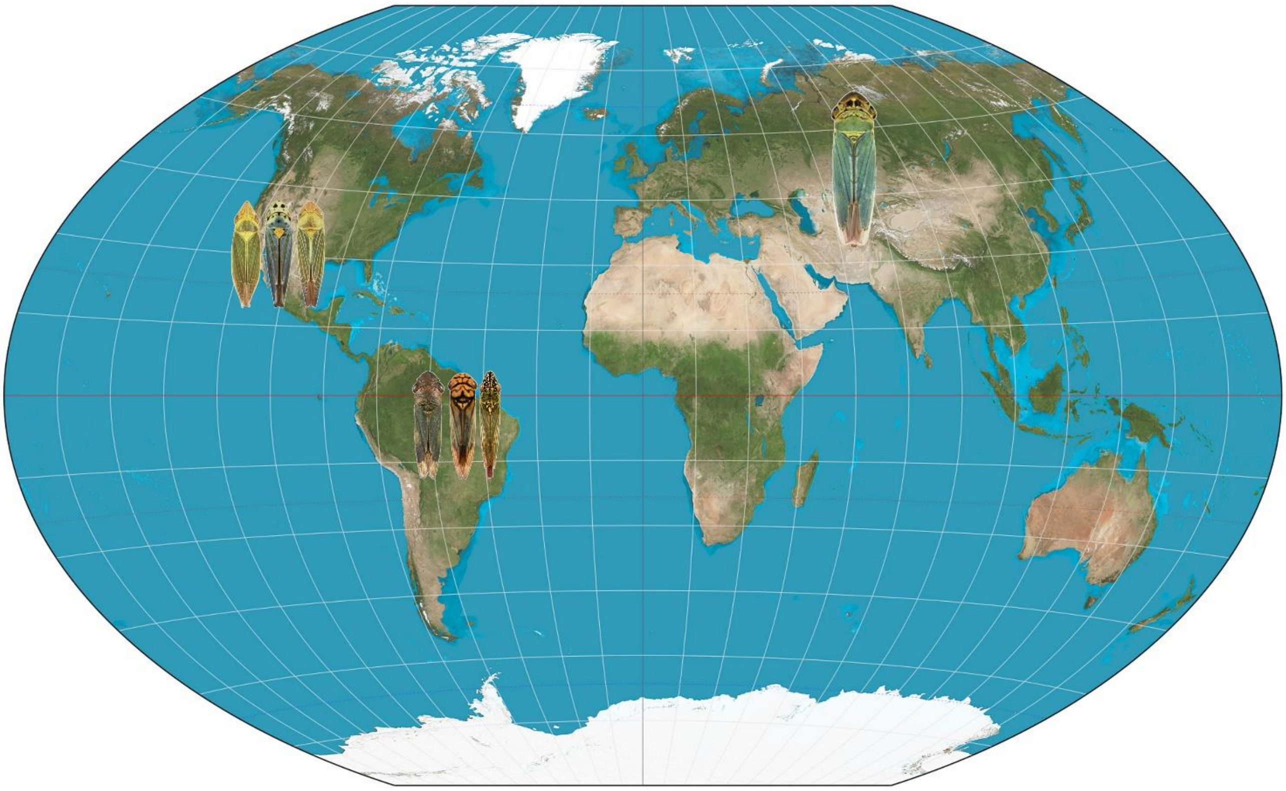

Figure 19. Distribution of the exotic sharpshooters presented in this protocol (species from left to right: *Draeculacephala minerva*, *Graphocephala atropunctata*, *Xyphon fulgidum*, *Oncometopia facialis*, *Dilobopterus costalimai*, *Acrogonia terminalis*, *Cicadella viridis*), per zoogeographical region (Source of image: prepared by Jérome Constant Royal Belgian Institute of Natural Sciences, 2020).

8.2 Host Plants of vector species

Table 6. Exotic vectors of *Xylella fastidiosa*, some of their main host plants and the diseases they vector.

Species name	Associated plants	Vector of:	Citation
Acrogonia terminalis	Citrus[1,2]; papaya[3] ; *Helianthus annus*, *Gossypium hirsutum*, *Coffea* sp.[8]	*Xylella fastidiosa* causing CVC[1,2]	1. Roberto *et al*. (1996); 2. Garcia *et al*. (1997); 3. Lozada & Arellano (2008); 8. Keil & Lozardo 2021
Cicadella viridis	*Juncus* and *Carex*[4,5]	Plant phytoplasma–16Sr group[5]; **potential** *Xylella* vector[5] Not currently known to vector *Xylella* in its natural environment.	4. Biedermann & Niedringhaus (2009); 5. Wilson & Turner (2021)
Dilobopterus costalimai	polyphagous including on ornamental plants, coffee, *Citrus* x *aurantium* var. *sinensis*, *Vernonia condensata*, *Aloysia virgata*[6,7]	*Xylella fastidiosa* causing CVC and CLS [6,7]	6. Redak *et al*. 2004; 7. EFSA 2015 & 2019
Draeculacephala minerva	Mainly grass feeding like other *Draeculacephala* species[9]; *Cynodon dactylon* (Bermuda grass)[10] is a main host; polyphagous including on ornamental plants, coffee, *Citrus, Vernonia condensata, Aloysia virgata, Medicago sativa, Oryza sativa, Zea mays, Juncus* sp., *Prunus* spp. *Catharanthus roseus, Prunus dulcis*, fruit trees and *Vitis vinifera*[6,7,10]	*Xylella fastidiosa* causing CVC and CLS [6,7]; PD[5,6,7,9]	5. Wilson & Turner (2021); 6. Redak *et al*. (2004); 7. EFSA (2015 & 2019); 9. Young & Davidson (1959); 10. EPPO (2025d)

Graphocephala atropunctata	polyphagous including on *Vitis*, blackberry, elderberry, mugwort, stinging nettle, snowberry and many others[7]	PD[7]	2. Garcia *et al*. (1997); 7. EFSA 2015 & 2019
Oncometopia facialis	*Citrus* x *aurantium* var. *sinensis*, (also associated with *Vernonia condensata, Aloysia virgata*)[7] ; coffee[5]; *Lantana camara* (lantana)[12.]	*Xylella fastidiosa* causing CVC; CLS[5,7]	5. Wilson & Turner (2021); 7. EFSA 2015 & 2019; 12. Milanez (2003).
Xyphon fulgidum	Mainly grasses – bermudagrass, saltgrass (*Distichlis stricta*, foxtail fescue (*Festuca megalura*), hairy crabgrass (*Digitaria sanguinalis* (L.) Scop.), redmaids (*Calandrinia ciliata* var. *menziesii*), common purslane (*Portulaca oleracea*), redstem filaree (*Erodium cicutarium* ft.), and puncturevine (*Tribulus terrestris*)[6]; Alfalfa, *Vitis*, *Cynodon dactylon*, *Chrysothammus* sp.[7]; vinegarweed (*Trichostema lanceolatum*)[8]; *Cynodon dactylon*, *Cyperus esculentus*, *Echinochloa crus–galli*, and *Sorghum halepense*; has also been found on *Vitis vinifera* if displaced[11.]	*Xylella fastidiosa* causing PD, ALS and AD[6,7]	6. Redak *et al*. 2004; 7. EFSA 2015 & 2019; 8. Catanach 2009; 11. EPPO (2025a).

The list of associated plants presented in Table 6 is not extensive for each species but provides some of the well documented plants which are considered to be a host or plants from which these species have been collected.

Legend: Alfalfa Dwarf Disease (AD); Almond Leaf Scorch (ALS); Citrus Variegated Chlorosis (CVC); Coffee Leaf Scorch (CLS); Pierce's Disease (PD)

8.3 Summary of DNA barcode sequences available by species

Table 7. Sequence data available on BOLD systems and NCBI GenBank online databases at the time of publication (data accessed 12/03/2025). In the third column, highlighted in green bold text are species where 5' COI gene data (barcode gene with sequences >500bp) are publicly available in the BOLD systems database. Black text denotes species where sequences are available in the Genbank database only or are not available (such as in *X. fulgidum*).

Genus	Species	COI sequence publicly available	BOLD – No. specimens with COI sequences (in brackets no. with COI sequence publicly available)	GenBank – No. of COI sequences publicly available
Acrogonia	*terminalis*	YES	0	1
Cicadella	*viridis*	**YES**	190 (139)	25
Dilobopterus	*costalimai*	YES	0	1
Draeculacephala	*minerva*	**YES**	14 (14)	5
Graphocephala	*atropunctata*	**YES**	30 (27)	22
Oncometopia	*facialis*	YES	0	1
Xyphon	*fulgidum*	NO	0	0

***Acrogonia terminalis* Young, 1968**

There is one COI gene sequence of *A. terminalis*, available on the NCBI GenBank database (Accession No MH618931).

No DNA barcode sequences of *A. terminalis* are available on the BOLD systems database. However, there are COI gene sequences of 2 congeneric species (*A. nigriceps* (Signoret, 1855) and *A. virescens* (Metcalf, 1949)) which are publicly available.

***Cicadella viridis* (Linnaeus, 1758)**

COI barcode sequences (>500 basepairs) of *C. viridis* are publicly available on the BOLD systems and NCBI GenBank databases for this species.

Six other genes and the whole mitochondrial genome have been sequenced for this species and sequences are also available on GenBank.

Additionally, there are COI sequences of another 3 *Cicadella* species available in BOLD, including *C. lasiocarpae* Ossiannilson, 1981 and 2 undetermined species.

***Dilobopterus costalimai* Young, 1977**

There is one COI sequence of *D. costalimai* available on the NCBI GenBank database (Accession No. MH618958) and 4 other genes sequenced (refer to work by Krishnankutty 2012).

No DNA barcode sequences of *D*. *costalimai* are recorded on the BOLD systems database but there are COI gene sequences of 2 congeneric species (*Dilobopterus demissus* (Fabricius, 1803) and *Dilobopterus exaltatus* (Fabricius, 1803) publicly available.

***Draeculacephala minerva* Ball, 1927**

Barcode sequences of *D. minerva* are publicly available on the BOLD systems and NCBI GenBank databases.

Additionally, there are COI sequences of a further 25 *Draeculacephala* species available on BOLD, including 4 undetermined species.

***Graphocephala atropunctata* Signoret, 1854**

Barcode sequences *G. atropunctata* are publicly available on the BOLD systems NCBI GenBank databases. References include Ballman *et al*. (2011) and Footit *et al*. (2014).

Additionally, there are COI sequences of another 22 *Graphocephala* species available on BOLD, including 4 undetermined species.

***Oncometopia facialis* Signoret, 1854**

There is one COI gene sequence of *O. facialis*, available on the NCBI GenBank (Accession No. MH618990) and also three other genes sequenced for this species.

No DNA barcode sequences of *O. facialis* are available on the BOLD systems database but there are COI gene sequences of 10 congeneric species, (including 5 undetermined), publicly available.

***Xyphon fulgidum* Nottingham, 1932**

There are **no DNA barcode** sequences of *X. fulgidum* recorded on the BOLD systems or NCBI GenBank databases and no other gene sequences available for this species.

However, there are COI gene sequences of 3 congeneric species, (*Xyphon flaviceps* (Riley, 1880), *X. reticulatum* (Signoret, 1854), *X. triguttatum* (Nottingham, 1932)), publicly available on BOLD.

Australian endemic genera

Cofana

On the BOLD systems database, *Cofana* has 8 species with COI gene sequences and 7 species with barcode sequences available. This includes all three species (*C. perkinsi* (Kirkaldy, 1906), *C. spectra* (Distant, 1853) and *C. unimaculata* (Signoret, 1854)) present in Australia and other exotic species (*C. klossi* (Distant, 1914), *C. polaris* Young, 1979, *C. subvirescens* (Stål, 1870), *C. yasumatsui* Young, 1979 and one undetermined species). However, many of these sequences are from unpublished sources.

Conoguinula

There is only one COI gene sequence of *Conoguinula coeruleopennis* (Fabricius, 1803) publicly available on the BOLD systems database and through NCBI GenBank (Accession No. MN345088). The reliability of the identification of the specimen sequenced is not known and the fragment length is only 403bp (not the full barcode sequence). Additionally, the specimen sequenced is from Papua New Guinea (PNG) and there are no sequences available of Australian *C. coeruleopennis* specimens. It is also noted in Young (1986) that there is some variability of the male genitalia displayed between specimens from and Australia and PNG. A careful taxonomic study of this species is needed to confirm that the Australian and PNG specimens are the same species.

Ishidaella

Of the nine species known in the *Ishidaella*, only one species *I. albomarginata* has a COI gene sequence available on the NCBI GenBank (Accession No. **MK321084**) and there are no publicly available COI sequences available on the BOLD systems database. This is based on data from a phylogenetic study Feng *et al*. 2023.

8.4 Reference specimens

Table 8. List of reference specimens of exotic leafhopper *Xylella* vector species represented in Australian Collections

Genus	Species	Australian Reference Collection with reference specimens (for morphological examination)
Acrogonia	*Terminalis*	
Cicadella	*Viridis*	ASCU, DPIRD, VAIC
Dilobopterus	*Costalimai*	
Draeculacephala	*Minerva*	ASCU, VAIC
Graphocephala	*Atropunctata*	ASCU
Homalodisca	*Vitripennis*	ASCU, VAIC
Oncometopia	*Facialis*	
Xyphon	*Fulgidum*	ASCU

Institutions Listed:

ASCU – Agricultural Scientific Collections Unit, Biosecurity Collections, NSW Department of Primary Industries and Regional Development, Orange, NSW

DPIRD – Department of Primary Industries and Regional Development, South Perth, Western Australia

VAIC – Victorian Agricultural Insect Collections, Agriculture Victoria, Bundoora, Victoria

Note: This is not a comprehensive list, further specimens may be available in Australian collections.

9 DIAGNOSTIC PROCEDURES TO SUPPORT SURVEILLANCE

9.1 Introduction

The diagnostic surveillance procedures outlined below are adapted from two other significant *Xylella fastidiosa* vectors including, SPHD (2013) Glassy–winged sharpshooter, *Homalodisca vitripennis* (Germar) NDP 23 (currently being updated), and SPHD (2025) Meadow Spittlebug NDP 54 and additionally other exotic plant pest species such as the SPHD (unpublished) Maize Leafhopper, and SPHD (unpublished) *Hyalesthes obsoletus* NDP. These NDPs can be requested from the NDP coordinator.

The following section provides a guideline for the surveillance and high–throughput testing of some highly invasive exotic *Xylella* vector species (see Table 9 below for a description of tests, testing times and outputs). Detailed laboratory procedures can be found in Section 4 of this NDP.

In summary, the current tests available to identify the exotic *Xylella* vectors include:

1) Morphological testing (section 4.1): Used to identify adult males and females to the subfamily, tribe and genus level. A key, description and images are available in section 4.1 to identify specimens as one of the seven exotic species presented in this protocol or determine whether or not, specimens belong to one of the 3 endemic genera of Cicadellinae known to occur in Australia. However, morphological identifications to species level can only be verified by dissection and examining the male genitalia structures under a stereo–microscope. Care must be taken to also screen against congeners of the exotic species presented in this protocol. Specimens that do not match any of the genera/ species covered in this protocol should be further tested with keys in relevant literature (such as Young 1968, 1977 and 1986) or using molecular methods (DNA barcoding). Females need to be tested using molecular methods for confirming species identification.

2) Molecular testing (section 4.2): Used to identify all life stages to genus/ species level but especially necessary for eggs, nymphs, and females. DNA barcoding is the only molecular method available for identification of these species. Currently 6 of the 7 exotic species covered in this protocol have barcode sequence data (5' region) available online in the BOLD systems or GenBank databases (for *Acrogonia terminalis*, *Cicadella viridis*, *Dilobopterus costalimai*, *Draeculacephala minerva*, *Graphocephala atropunctata* and *Oncometopia facialis*) but there is no data for *Xyphon fulgidum*. Morphological examination of the male genitalia is the only method to confirm the identification of the latter species. DNA sequence data is available for the Australian species of *Cofana* and *Conoguinula* but only one of 9 species of *Ishidaella* (*I. albomarginata* (Signoret, 1893) is barcoded. Therefore, generic and species identification of *Ishidaella* by molecular testing is limited.

Note: a large number of Cicadellini and all Proconiini are exotic to Australia. Any exotic species detected (even if not one of the target species in this protocol), needs to be reported through the appropriate official channels (see Section 4.3).

There are currently no known molecular in–field tests for identifying sharpshooter leafhoppers and definitive identification in the field is not possible. However, visual aids and general diagnostic features may help with sample triage (see section 9.3).

Refer to Tables 9 and 10 for a description of identification tests and outputs.

Different types of samples, require different treatment and processing times prior to commencing the tests. For example, searching for eggs or nymphs on a plant sample, removing specimens from sticky traps or card mounting specimens (following instructions in section 4.1.1) adds to the total processing and testing times provided.

Table 9. Morphological tests for laboratory–based identification, recommended for Cicadellinae sharpshooters.

Test	**Identification Level**	**Life stage**	**Identification confidence**	**Required Time**	**Level of expertise**	**Throughput [No. of specimens]**
Morphological Test (A) External characters **(sections 4.1.4 –4.1.5)**	TO SUBFAMILY/ TRIBE Cicadellinae: Proconiini or Cicadellini	Adults (males & females)	High (99%+)	1 DAY	Experienced Diagnostician (able to recognise superfamilies of Auchenorrhyncha and family Cicadellidae)	MEDIUM (≤ 200)
Morphological Test (B) External characters **(section 4.1.6)**	TO GENUS/ SPECIES identified as one of the 7 exotic genera or species presented in this protocol or one of the three genera in Australia	Adults (males and females) To further confirm species id.: - For males go to Morphological Test (C) - For females go to Molecular test (D)	Medium (90–95%)	1 DAY	Experienced Diagnostician	LOW (≤40)
Morphological Test (C) Examination of male genitalia	SPECIES ID CONFIRMED Identified as one of the 7 exotic species presented in this protocol or to a	Adult males only	Medium to High (95–99%+)	1 DAY	Experienced Diagnostician/ Auchenorrhyncha specialist	LOW (≤40)

(sections 4.1.1 and 4.6)	species of one of the three Australian genera.					

Table 10. **Molecular tests for laboratory–based** identification, recommended for Cicadellinae sharpshooters.

Test	Identification Level	Life stage	Identification confidence	Required Time	Level of expertise	Throughput [No. of specimens]
Molecular Test (D) DNA (COI) barcoding **(section 4.2)**	TO SPECIES ID	All life stages (Eggs, nymphs, adults)	Medium to High (95–99%+) (only for those species where a barcode is available)	3–5 DAYS	Experienced molecular diagnostician/ technician	MEDIUM (≤ 200)

! Note: These are estimated times only and may vary depending on a number of factors such as condition and number of specimens, expertise of diagnosticians and laboratory facilities. It is recommended to consult with the diagnostic laboratories prior to sending specimens to discuss processing capacity. Time is based on a standard working day.

9.2 Sampling methods

Adult specimens are the most readily detected life stage, compared with eggs and nymphs, because the adults are relatively large and active. Adults (specifically male specimens) are required for morphological identification to species and are most important to collect, if present. Male and female specimens can sometimes be distinguished by their size, where males are generally slightly smaller than females. They can also be recognised by examining the ventral aspect of the terminal segments of the abdomen, with females having a distinct ovipositor and males with a pair of plates. However, these features are difficult to see in the field, even with a 10x hand lens. Therefore, it is recommended that **all** suspect specimens should be collected, regardless of their sex or life stage and can be collected following the procedures recommended below and in Table 11. Adult females, nymphs and eggs require molecular testing to confirm species identifications.

To collect specimens, it is important to know **when** the different life stages are found, on **what** host plants and **where** on the plant they are likely to be present. The biology and behaviour of the insect may also help in its detection (see Table 12 for life cycle and seasonal information).

Targeting the preferred host plants is important, although many sharpshooters are polyphagous and the different life stages may feed on different plant species. Furthermore, they may switch host plants depending on the time of year and availability of the food source. Of the seven species covered in this

NDP, at least three (*D. costalimai*, *G. atropunctata* and *O. facialis*) are polyphagous, on ornamental plants, fruit trees and perennial plants. *Acrogonia terminalis* is found in the canopy of *Citrus* trees but also on woody plants and perennials. *Xyphon fulgidum* and *D. minerva* mostly feed on grasses although they are also polyphagous on other plants. *Cicadella viridis* is known mostly from rushes (Juncaceae) in humid habitats.

The plants targeted for surveillance will depend on the sharpshooter species of concern or on the affected crop. Refer to the list of main host plants for each exotic species covered in this NDP (see Table 6, Appendix 8.2 and also Table 12 below). When conducting surveys, it is recommended to search not only the crop plants of concern, but also the adjacent vegetation.

Like other leafhoppers, sharpshooter adults and nymphs can hop when disturbed (this is a trait of most Auchenorrhyncha). This characteristic movement can help to detect specimens. If the survey also requires sampling plant material to be collected for pathogen testing, sweeping for the insect vector should be conducted first to prevent disturbing specimens on the plants which will readily jump and/ or fly (adults only) away.

Look for symptoms on plants which can be an indicator of the presence of sharpshooters e.g. the presence of the sugary excrement covering fruit or plant foliage, which appears as a white powder when dry (Figure 1A).

Collecting Methods

Information about general collecting methods for Auchenorrhyncha can be found in Wilson and Turner (2021), Löcker *et al*. (2018), CDFA (2020) and IPPC (2018). The SPHD (unpublished) *H. obsoletus* NDP (in section 3.2.2) describes collecting methods in detail, such as sweeping, beating and sticky trap methods. Also, for more details about collecting techniques and preservation of specimens see section 3.2.2 and 3.2.3 in this NDP.

Sweeping or beating and collecting with an aspirator are the best methods to collect *Xylella* vectors and are recommended to complement visual inspections (Varela *et al*. 2001, IPPC 2018). Sweeping is useful when insects are becoming a little active but can be quickly scooped up in the net. Shrubs and bushes should be swept on all sides if accessible but noting that once the plant is disturbed the leafhoppers may jump away from the plant. Care must be taken to avoid sweeping plants with spines (such as some *Citrus* varieties, blackberries or native plants), as this will quickly damage the net. In this case, beating is an alternate collecting method. Beating involves hitting the branches with a solid pole or stick and collecting insects which drop from the plant, into a tray or onto a cloth below the branches or into a sweep net. Beating is most useful in the cooler times of the day (around 15 °C) when insects are less active and can be swiftly knocked from the plants. Beating is also a good option for collecting nymphs and cast skins. Otherwise, a vacuum trap can be used for bushy or spiny plants.

Sticky traps can be used for longer term surveillance. However, of the methods proposed, sticky traps are the least preferred by diagnosticians because it is difficult and time–consuming to remove specimens from the traps and specimens may get damaged in the process. Furthermore, sticky traps are not considered as effective as other collecting methods, for collecting xylem feeders (Purcell *et al.* 2014). According to the CDFA (2020), sticky traps are less effective for attracting sharpshooters when temperatures are below 18 °C, as this effects their flight behaviour. Some sharpshooter species, such *D. minerva* and *X. fulgidum*, are not attracted to yellow sticky traps (Varela *et al*. 2001). Additionally, traps are less likely to capture the nymphal stages. However, sticky traps can be a good method to use in combination with other surveillance collecting techniques listed above. See also sections 3.2 for further comments regarding use of sticky traps.

Light trapping at night can also be useful for collecting sharpshooters. This involves hanging a light (either a Mercury Vapour light or a UV led light) in front of a white sheet draped vertically. Some species of sharpshooters are known to be collected at light (Cabrera–La Rosa *et al*. 2014).

Malaise trapping can also collect a large number of specimens and can also be useful for long term surveillance but requires longer processing times for diagnosticians to sort through samples and like sticky traps and light trapping, host plants are not recorded.

Some additional tips for sampling sharpshooters can be found in the CDFA (2020) and IPPC (2018).

While dry preserved specimens are preferred for morphological examination (see section 3.2.3), it may not be the most practical method for preserving specimens for large–scale surveillance. Specimens can otherwise be placed into a container with 70–80% ethanol (for morphological examination but can also be used for DNA barcoding if stored short–term) or 95–100% ethanol (preferred for molecular testing and specimens can also be used for morphological examination, although may get brittle over time).

Any of these preservation options can be used (for the short term), regardless of testing methods to be used (Marquina *et al*. 2021, Carew *et al*. 2018), but it's best to speak with the receiving diagnostic laboratory for further advice. Preservation information should be clearly stated with submission of the sample, along with host, locality and date of collection.

Adults and nymphs

Surveys should focus on collecting adults as this life stage is best for morphological identification. Both adults and nymphs can be detected by the visual examination of plant stems, particularly young, fresh shoots, which is where they typically feed. Sharpshooters may also be detected resting on the leaves. Nymphs are found on the underside of leaves and leaf petioles should also be checked.

Little is known about the life history of some species (see Table 12). Sharpshooter adults generally tend to be more active over the warmer months in spring and summer or in tropical regions, during the wet season. This is the time that surveillance for adults is most effective, particularly if using flight interception traps. Some species (*D. minerva* and *G. atropunctata*), overwinter as adults (are present in autumn and winter), therefore they can be collected year round, although perhaps with more difficulty and in lower numbers in cooler months. *Graphocephala atropunctata* is known to overwinter in riparian habitats often adjacent to vineyards (EFSA 2015). Cicadellinae species in Europe overwinter in the egg stage and adults are not usually found until spring/ summer.

In Brazil, in citrus nurseries where population studies have been conducted, sharpshooter numbers (of *A. terminalis*, *D. costalimai*, and *O. facialis*) start to increase around December and persist to July (summer, autumn, early winter = wet season) and drop off or disappear over July to October (winter = dry season). Water availability seems to be an important factor effecting population numbers (Garcia *et al*. (1997)).

Nymphs are normally present in spring and summer but for some species such as (*D. minerva*) which have multiple generations (up to 6) per year in their native range (California, USA), the nymphs may also be present in winter.

Eggs

The egg stage is difficult to detect, and it is not advisable to focus on this life stage for surveillance. Eggs cannot be readily identified morphologically to species or to genus. However, if found, eggs can be tested using molecular DNA barcoding. Leaves with suspect eggs can be removed from the plant and placed in a plastic bag or container with a piece of slightly moistened paper and kept cool.

Sharpshooter eggs, which are long, narrow and elliptical, are inserted into plant tissues. In some species, such as in *X. fulgidum*, the eggs are laid deeply within the tissue, appearing as a blister on the leaf (EPPO 2025a). *Dilobopterus costalimai* and *D. minerva* eggs are also endophytic (laid beneath the leaf or stem tissue). *Graphocephala atropunctata* lays a single egg in the distal parts of the plant. The eggs are not readily found for this species unless nymphs are bred from the leaf (EPPO 2025c). The eggs are very difficult to detect by eye in the field and any leaves with suspicious blisters should be removed from the plant, for dissection and examination under a microscope, while in other species, such as *A. terminalis*, the eggs are exophytic (protruding from the leaf) (EPPO 2025b). Some sharpshooter species, such as *Homalodisca* and *Oncometopia*, lay their eggs in masses on the underside of the leaves. These eggs are more obvious. The underside of recently matured leaves should be checked for eggs. See Figure 1B in this NDP and CABI (2022) for more images of *Homalodisca vitripennis* egg masses as an example.

Cicadella viridis eggs are laid under bark of trees and shrubs where they overwinter and in summer, the adults lay their eggs in grasses.

Table 11. Sampling summary for adults, nymphs and eggs.

Specification	**Description**
Sample type	Adult, nymphs and eggs
What to sample	1) Adults and nymphs – Examine by eye, leaves, stems (particularly new growth) and fruit of host plants (see also Table 6). Collect specimens directly into a specimen tube or aspirator. 2) Adults and nymphs – Sweep or beat (for spiny plants) host plants. If surveying around orchards, sweep adjacent weeds and vegetation. Collect specimens with an aspirator or collect as bulk samples into a jar. 3) Adults – Alternatively, use sticky traps, vacuum suction trap or light trapping at night. 4) Eggs – Collect leaves with suspect blisters/ eggs and place into a plastic bag with damp paper towel (see instructions in section 3.2.3).

Equipment required	Sweep net; beating net or tray; aspirator; ethyl acetate; killing jar; specimen jars and tubes; 70–80% ethanol; 95–100% ethanol; vacuum suction sampler; white sheet; MV or UV lamp; malaise trap.
Sample size	Collect **all** suspect specimens of all life stages.
Sample preservation	See section 3.2.3 for further details. 1) Aspirated adult or nymph specimens can be kept **live** in specimen tubes; **OR** 2) Aspirated individual specimens, bulk swept samples or vacuum samples killed with ethyl acetate or frozen, should be preferably transferred to a **dry container** within layers of dry paper towel (preferred for morphological examination) OR placed into a container with **70–80% ethanol** (for morphological examination) or **95–100% ethanol** (preferred for molecular testing). **OR** 3) Malaise trap jars with specimens in preservative (ethanol) can be sent to the laboratory for sorting. **OR** 4) Yellow sticky traps can be covered in a plastic sheet protector and placed in a zip locked bag. Keep flat. Retain sticky traps in a cool environment (such as a refrigerator or esky). Any of these methods can suffice for short–term preservation
Sample transport	Specimens should be kept cool in a styrofoam esky until delivered to a diagnostic laboratory. If samples cannot be delivered on the same day as collection, all samples should be placed in a refrigerator or freezer until delivery can be made.

Table 12. Temporal and host data for all life stages.

This table lists the biology where data is known but does not cover data for all the different localities of the species distribution range. Life cycle seasonality may differ in Australian conditions if the sharpshooter were to be introduced. *No or little life history data is known for some species in their natural environment.

Species	Number of generations	What type of host to sample:	When best to sample (based on studies in native range)	References

Acrogonia terminalis	Not known	*Citrus* (plus other crop plants)	* life history in natural environment is not known for this species. **Adults**: In nursery conditions – numbers peak between late summer (wet season in Brazil) to autumn, population drops in winter (dry season in Brazil).	Garcia *et al.* (1997)
Cicadella viridis	1–3 (Europe, China)	Sedges and rushes (but generally polyphagous also found on woody plants). In humid habitats.	**Adults**: Late spring to Autumn; **Nymphs**: mid spring to summer; **Eggs**: Late autumn to Early spring (overwinters as egg, under bark of woody trees and shrubs) and summer (in grasses).	Chu & Teng (1949); Shah *et al.* 2019
Dilobopterus costalimai	Not known	*Citrus* x *aurantium* var. *sinensis*; *Coffea* sp.	* life history in natural environment is not known for this species. **Adults**: In nursery conditions generally numbers can peak between late summer (wet season in Brazil) to autumn, population drops in winter (dry season in Brazil).	Garcia *et al.* (1997)
Draeculacephala minerva	3–6 (California, USA)	Grasses, sedges and weedy vegetation (but is a generalist feeder)	**Adults**: all year round (overwinters as adult); **Nymphs**: spring and summer, autumn, winter (temperature dependant); **Eggs**: Late winter, Late spring and mid summer.	Daane *et al.* (2011); EPPO (2025d)
Graphocephala atropunctata	1 (California, USA)	*Citrus* x *aurantium* var. *sinensis*; (polyphagous	**Adults**: all year round (overwinters as adult);	EPPO (2025c)

		on other plants)	**Nymphs**: spring and summer only; **Eggs**: spring.	
Oncometopia facialis	Not known	*Citrus* x *aurantium* var. *sinensis*; *Coffea* sp., *Lantana camara* (but polyphagous with wide host range)	* life history in natural environment is not known for this species. **Adults**: In nursery conditions numbers can peak between late summer (wet season in Brazil) to autumn, population drops in winter (dry season in Brazil).	Garcia *et al*. (1997); Redak *et al*. (2004)
Xyphon fulgidum	Not known	Grasses and weedy vegetation	*life history not known for this species. Data of closely related species suggest that the species possibly overwinters as adults or eggs.	EPPO (2025a)

9.3 In–Field Tests

There are currently no in–field molecular tests or morphological field guides for the identification of sharpshooters and a definitive morphological diagnosis in the field is not possible as this requires a high level magnification (e.g. a stereo microscope with at least 40x magnification) and examination of the male genitalia or otherwise a DNA barcode test.

However, field surveillance officers can be trained to recognise by eye, some general physical characteristics (such as general shape, size and coloration), and the behaviours of sharpshooters to identify these from other insects and hemipterans and to assist with the triage of specimens.

Visual aid

The general form of cicadellids, is shown in Figure 20A, next to other (non–Auchenorrhyncha) Hemiptera taxa (Figures 20B–D). Also check Section 4.1.3 for features and a key used to recognise Cicadellidae from other Auchenorrhyncha families.

Visual recognition of sharpshooters in the field may require a 10x hand–lens. Features to look for include: 1) size – adults can range from 5 to 15mm in length; 2) may be colourful, wings may have white patches (dried brochosomes) on either side at certain times of the year (Hix 2001); 3) eyes bulge laterally (in some species), head usually wider than pronotum and 4) head with a distinctly swollen face. See also identification features of Cicadellinae in section 4.1.4 of this protocol. Colour and size can be useful in the field to help narrow down suspect specimens but can not be relied on as a definitive feature for identification. The colour and pattern may vary slightly within the same species of sharpshooters.

For example in *C. viridis* males may have blackish, bluish/ green or green wings, while females normally have green wings; in *G. atropunctata* the head may bear a pair of black spots or otherwise black lines. *O. facialis* can be variable in colour where the head and thorax can appear reddish/ brown or purplish and the tegmen can appear brown with pale veins or brown with bright yellow veins and a mottled yellow pattern between the veins (see Figs 14A–C). Colour variation has not been documented for the other exotic *Xylella* vector species covered in this protocol.

Nymphs which are similar in shape to adult leafhoppers (being broader at the head and tapering at the apex of the abdomen) and also bear rows of spines on the hind tibiae, can be recognised from the adults by the presence of wing buds (wings are not yet fully developed).

Sharpshooter eggs are generally long and elliptical, see section 3.1 (Figure 1B), but see comments about egg detection in sections 9.2.

There are also some excellent online resources which show sharpshooter images such as PaDIL – Walker (2005) https://www.padil.gov.au/pests-and-diseases/pest/143194, Wilson *et al*. (2020), Wilson & Turner (2021) and Fletcher (2009 and updates) which could be useful to help recognise the target species.

As repeated throughout this protocol, confirmation of sharpshooter identification requires dissection and examination of the male genitalia under a stereo microscope, otherwise molecular testing to confirm for females, nymphs and eggs. Therefore, it is recommended that any suspect Cicadellinae leafhoppers detected in the field be sent to a diagnostic laboratory for further identification.

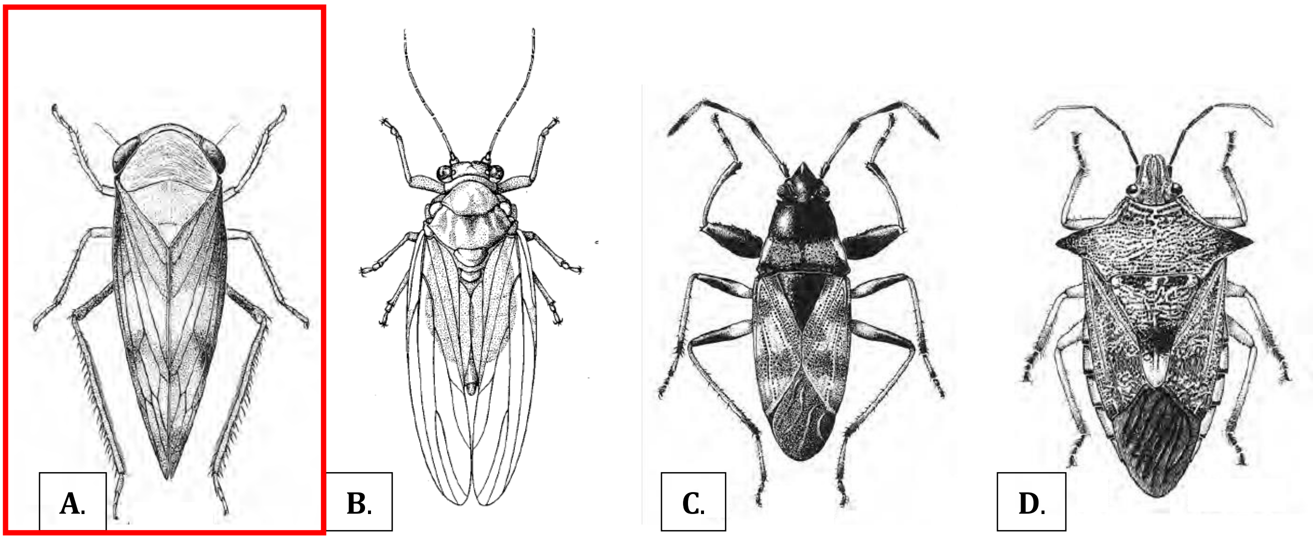

Figure 20. Identifying Cicadellidae from other Hemiptera. A) Cicadellidae, (target group highlighted by red box); B) Psyllidae; C) Lygaeidae; D) Pentatomidae, (source of images: Carver *et al*. 1991).

9.4 Laboratory Tests

For morphological identification of exotic Cicadellinae sharpshooters, refer to section 4.1 (particularly 4.1.6 which includes a key and description of the 7 target exotic species). Any suspect female specimens, eggs or nymphs need to be tested using a DNA barcoding test as stipulated in section 4.2.

A guidance to laboratory tests and throughput times are listed in Table 9 (section 9.1, morphological tests A–C) and Table 10 (molecular test D).

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