National Diagnostic Protocol

For Gypsy Moths (Erebidae: Lymantriinae: Lymantriini: Lymantria),

focussing on *L. dispar asiatica*

NDP 42 V1

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Purpose

National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

- are a verified information resource for plant health diagnosticians
- are consistent with ISPM No. 27 – Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

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

https://www.ippc.int/core-activities/standards-setting/ispms

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.

NDPs are developed and endorsed according to Reference Standards developed and maintained by SPHD. Current Reference Standards are available at https://www.plantbiosecuritydiagnostics.net.au/initiatives/national-diagnostic-protocols/

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

Document status

This version of the National Diagnostic Protocol (NDP) for Gypsy moths: *Lymantria dispar* 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 protocol should be sent to: sphd@agriculture.gov.au

Contents

1 INTRODUCTION

Gypsy moths, members of the insect family Erebidae, are a group of major economic importance as defoliators of a wide variety of trees. A few are crop pests and many are also of medical significance as their larvae have urticating hairs which may cause severe irritation and even anaphylaxis.

Many of the gypsy moths of economic concern are within the genus *Lymantria*, a large, very diverse genus. Schintlmeister (2004) recognised 167 species in 12 subgenera occurring mainly in the Oriental region. All are notoriously difficult to identify. Species separation based on morphological means relies on wing pattern elements and genitalia characteristics made more difficult by strong sexual dimorphism in wing colour and pattern and the occurrence of flightless females with reduced wings.

While this protocol focusses on identification of Asian gypsy moth, *Lymantria dispar asiatica* Vnukowskij, information on other species also exotic to Australia (including *L. dispar japonica, L. mathura, L. monacha, L. umbrosa* and *L. xylina*) is included. All races, subspecies or strains of *L. dispar* are exotic to Australia. Genitalia structures, which are crucial morphological diagnostic characters for this protocol, are indistinguishable between races and subspecies of *L. dispar.*

To avoid confusion, in this protocol, "Gypsy moth" is used as the common name for all forms of *L. dispar* (as per Keena *et al*. 2008) and the scientific name used in preference where possible.

1.1 Host range

Gypsy moths have an extremely wide host range and have been referred to as 'superpolyphagous'. Lists of known hosts for gypsy moth have been provided by Forbush and Fernald (1896), Kurir (1953), Liebhold *et al*. (1995), and Schaefer *et al*. (1986).

Relatively few Australian species (55) have been tested for their susceptibility (Matsuki *et al*. 2001). Oak species (*Quercus* spp.) are considered to be the preferred hosts but heavy defoliation is also observed on *Carpinus*, *Castanea*, *Fagus*, *Malus*, *Populus*, *Prunus*, *Pyrus* and *Salix*.

Known host families include: Aceraceae, Anacardiaceae, Betulaceae, Corylaceae, Ebenaceae, Ericaceae, Fabaceae, Fagaceae, Hamamelidaceae, Juglandaceae, Mimosaceae, Myrtaceae, Nothofagaceae, Oleaceae, Papillionaceae, Pinaceae, Platanaceae, Poaceae, Rosaceae, Salicaceae, Sapindaceae, Taxodiaceae, Tiliaceae, Ulmaceae.

Known host genera include: *Acacia*, *Acer*, *Alnus*, *Betula*, *Callistemon*, *Carpinus*, *Carya*, *Castanea*, *Corylus*, *Corymbia*, *Diospyrus*, *Eucalyptus*, *Eugenia*, *Fagus*, *Fraxinus*, *Glycine*, *Hamamelis*, *Larix*, *Leptospermum*, *Liquidambar*, *Litchi*, *Lithocarpus*, *Malus*, *Nothofagus, Ostrya*, *Picea*, *Pinus*, *Pistacea*, *Platanus*, *Populus*, *Prunus*, *Pseudotsuga*, *Pyrus*, *Quercus*, *Robinia*, *Salix*, *Taxodium*, *Tilia*, *Ulmus*, *Vaccinium*, *Zea*.

More details of major and minor hosts can be found in Appendix 8.2 Major and minor hosts of Gypsy Moth. Basic host plant information on other *Lymantria* spp. attracted to disparlure is included in Appendix 8.1.

2 Taxonomic Information

The classification below follows the recent changes by Zahiri *et al.* (2011, 2012).

Class:	Insecta
Order:	Lepidoptera
Super Family:	Noctuoidea
Family:	Erebidae
Subfamily:	Lymantriinae
Tribe:	Lymantriini
Genus:	*Lymantria*
Species:	*Lymantria dispar*

2.1 Names and synonyms

Common names

Gypsy moth (English), erdei gyapjaslepke (Hungarian), gubar (Romanian), lagarta peluda (Spanish), limantria (Italian), løVstraesnonne (Danish), maimai-ga (Japanese), mniska vel'kohlava (Slovak), Schwammspinner (German), spongieuse (French).

Synonyms

In his complete revision of the genus, Schintlmeister (2004) reviewed the taxonomic history of *Lymantria dispar* complex and was the first to adopt subgeneric taxa as provided in the following list of synonyms:

Lymantria (Porthetria) dispar dispar (Linnaeus, 1758) (*Phalaena*) [Lepidoptera: Lymantriidae], gypsy moth.

= *Ocneria dispar erebus* Mieg., 1886
= *Lymantria dispar asiatica* Vnukowskij, 1926
= *Lymantria dispar praeterea* Kardakoff, 1928
= *Lymantria dispar hokkaidoensis* Goldschmidt, 1940
= *Lymantria dispar koreibia* Bryk, [1948]
= *Lymantria dispar kolthoffi* Bryk, [1948]
= *Lymantria dispar andalusica* Reinig, 1938
= *Lymantria dispar mediterraneae* Goldschmidt, 1940
= *Lymantria dispar bocharae* Goldschmidt, 1940
= *Lymantria dispar chosensis* Goldschmidt, 1940

CABI (2015) provided the following list.

Lymantria dispar (Linnaeus, 1758)
= *Porthetria dispar* Linnaeus
= *Ocneria dispar* Linnaeus
= *Bombyx dispar* Linnaeus

= *Hypogymna dispar* Linnaeus
= *Liparis dispar* Linnaeus
= *Phalaena dispar* Linnaeus
= *Porthesia dispar* Linnaeus

The above list includes just some of the names by which *L. dispar* has been, or is known.

2.2 Taxonomic description of *Lymantria dispar asiatica*

The following taxonomic description is centered on *L. d. asiatica* but illustrations of the major recognised subspecies of *L. dispar* are included for comparison. Note that the subspecies names used in the captions are as supplied with the figures used in Horak *et al*. (2006). Where the subspecies name of *Lymantria dispar dispar* is used in these captions, this should perhaps be *L. d. asiatica*, however this nomenclature cannot be updated here until the matching voucher specimens have similarly been updated. In addition, as DNA sequence data is required to separate subspecies of *L. dispar*, the voucher specimens should also have been sequenced for the gene region of diagnostic interest.

2.2.1 Male

Abdomen ochreous to brownish, never pinkish red (Figures 1-4). Forewings very variable, with ill-defined brownish to blackish markings on ground colour ranging from ochreous to dark grey-brown, with a broad darker band along distal margin unless entire wing dark; subbasal band indicated only near costa; antemedial band diffuse, strongly angled near middle of wing but often developed near costa only, postmedial band only weakly indicated at costa; submarginal spots variably developed V-shaped marks; marginal spots a variably developed series of dark spots; discal spot a well-defined, narrowly crescentic black mark, adjacent to antemedial band. Hindwings very variable, ranging from yellowish, usually with a darker band around margin, to grey-brown, often with an ochreous centre.

Male genitalia (Figures 5, 6): Uncus continuous with tegumen; without lateral tegumen processes. Valva with costa continued as a long, straight, distally tapering, finger-like process, roughly in the same plane as the valva; valva proper widest at ventrodistal angle, with lateral margin inwardly oblique towards costal process; saccus long and very narrow, pointed; juxta a simple transverse band; aedeagus long and slender.

Figure 1 *Lymantria dispar dispar*, male; lab culture from Russian Far East. Vanna Rangsi, CSIRO Ecosystem Sciences.

Figure 2 *Lymantria dispar dispar*, male; lab culture. Vanna Rangsi, CSIRO Ecosystem Sciences.

Figure 3 *Lymantria dispar dispar*, male, from US lab culture, Vanna Rangsi, CSIRO Ecosystem Sciences.

Figure 4 *Lymantria dispar japonica*, male, Japan. David McClenaghan, CSIRO Ecosystem Sciences.

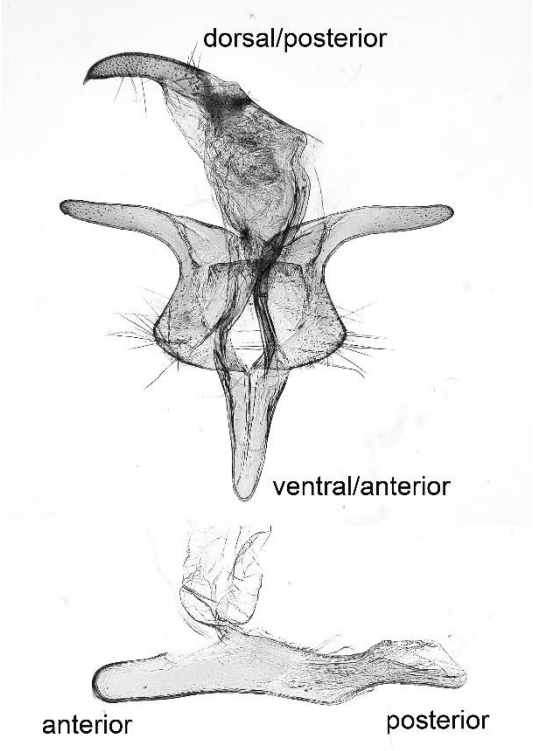

Figure 5 *Lymantria dispar dispar*, male genitalia and aedeagus; culture from Russian Far East. Vanna Rangsi, CSIRO Ecosystem Sciences.

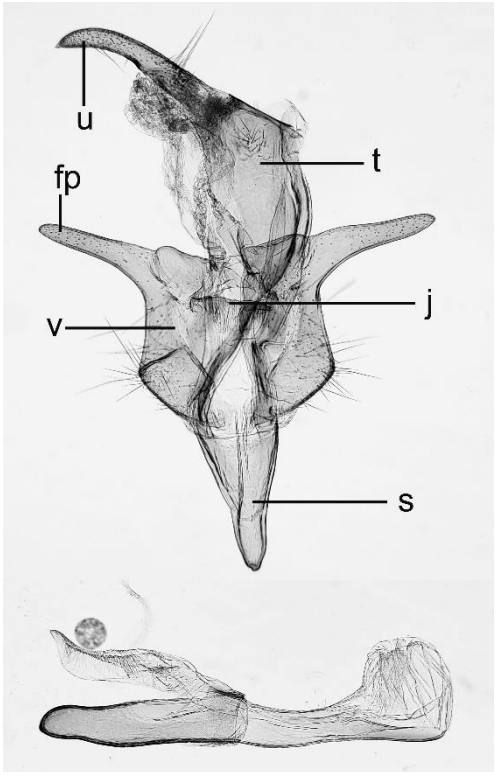

Figure 6 *Lymantria dispar japonica*, male genitalia and aedeagus, Japan (u, uncus; t, tegumen; fp, finger-like valva process; j, juxta, v, valva; s, saccus. Vanna Rangsi, CSIRO Ecosystem Sciences.

2.2.2 Female

Abdomen white (Figures 7, 8). Forewings with few and partly faint brownish markings on whitish to pale ochreous ground; usually a few faint darker spots near base; subbasal band present as a curved brown mark near costa; antemedial band originating from middle of costa and sharply angled, usually weakly developed; postmedial band a faint, thin, zig-zag line, angled and roughly parallel to antemedial band; often faint traces of a V-shaped submarginal mark, a row of small dark marks along outer margin; brown crescentic discal mark very prominent. Hindwing whitish to pale ochreous, with a series of dark small marginal spots, a faint, darker, suffused submarginal band and an indistinct darker mark at end of discal cell.

Female genitalia (Figures 9-11): Ovipositor lobes and additional paired ventral lobes strongly sclerotised. Sterigma a weakly sclerotised broad ring, anteriorly smooth and posteriorly split into two rounded, spinulose lobes, with centre of ring leading to ductus bursae. Posterior most part of ductus bursae with an elongate, barrel-shaped, ventrally split sclerotisation followed by wrinkled thickened membranous portion opening into corpus bursae.

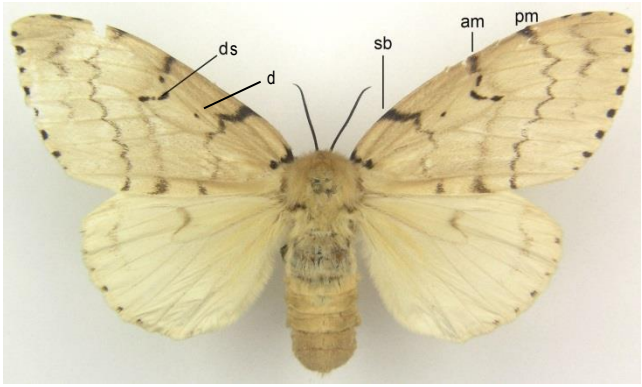

Figure 7 *Lymantria dispar dispar*, female, lab culture (ds, discal spot; d, basal dot or ring; sb, subbasal band; am, antemedial band; pm, postmedial band). Vanna Rangsi, CSIRO Ecosystem Sciences.

Figure 8 *Lymantria dispar japonica*, female, Japan. David McClenaghan, CSIRO Ecosystem Sciences.

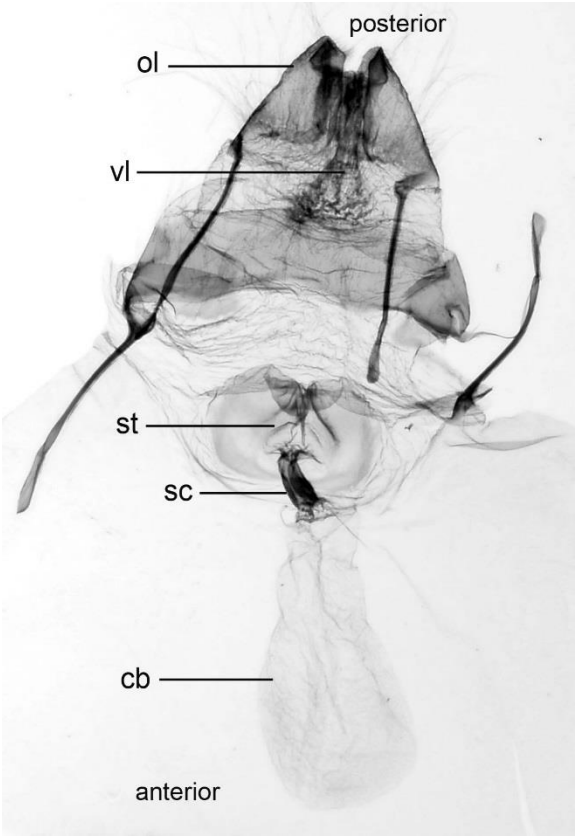

Figure 9 *Lymantria dispar dispar*, female genitalia; culture from Russian Far East (ol, ovipositor lobe; vl, additional ventral lobes; st, sterigma; sc, sclerotised part of ductus bursae, cb, corpus bursae. Vanna Rangsi, CSIRO Ecosystem Sciences.

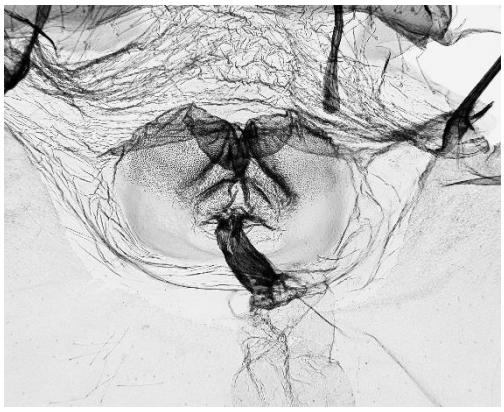

Figure 10 *Lymantria dispar dispar*, female sterigma; culture from Russian Far East. Vanna Rangsi, CSIRO Ecosystem Sciences.

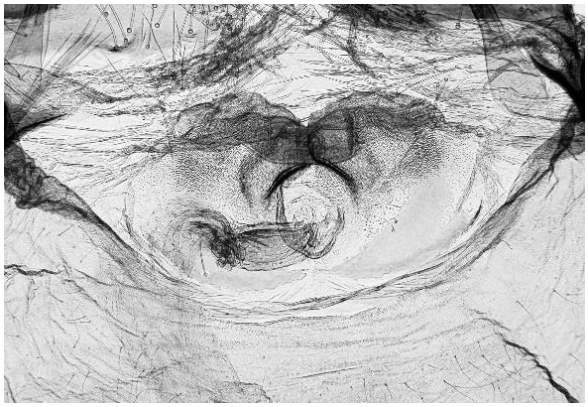

Figure 11 *Lymantria dispar japonica*; female sterigma; Japan. Vanna Rangsi, CSIRO Ecosystem Sciences.

2.2.3 Larvae

The caterpillar is easily recognisable in the latter part of the larval stage: charcoal grey with a double row of five blue and six red dots on its back (Figure 12).

Figure 12 *Lymantria dispar asiatica* larval forms. LHS form most common. USDA APHIS PPQ Archive, USDA APHIS PPQ, Bugwood.org

2.2.4 Eggs

Eggs are laid in batches covered by abdominal scales. Refer Section 4.1.3 for more details

3 DETECTION

3.1 Detection methods

Method of detection will differ depending on life stage suspected of being present. Pheromone traps baited with disparlure will detect adult males, inspection of potential host plants may detect larvae and egg masses. Larvae on foliage are easily distinguishable from other defoliators. Pupae and egg masses on host tree trunks are indicative of gypsy moth infestation. Because adult females can fly up to 40 km before laying eggs and are attracted to lights, eggs masses are often found on forest products, shipping containers, cargo and ship structures.

Details of surveillance trapping methods are not provided in this protocol but note reference to degradation of DNA in Section 3.3.

It is important to note however, that, while males of all strains of *L. dispar* (Asian, European and Japanese gypsy moths) respond to the same lure (Cardé *et al*.1996), other species within the genus also respond to this pheromone to a greater or lesser degree (Appendix 8.1).

3.2 Symptoms

Lymantria dispar larvae (Figures 12, 13) are gregarious defoliators, able to consume whole leaves and sometimes avoid tough veins in older foliage growth. The main symptom of their presence therefore is partial or total defoliation of trees and forests (Figures 14, 15). Suppressed and understorey trees have a greater probability of being defoliated and dying than overstorey trees (Davidson *et al*. 2001). Frass may be evident under host trees if the population density is high (Liebhold and Elkinton 1988a; 1988b).

Figure 13. *Lymantria dispar* larva on narrowleaf ash (*Fraxinus angustifolia*) Photo by Haruta Ovidiu, University of Oradea, Bugwood.org

Figure 14 Damage to English oak, *Quercus robur*, from *Lymantria dispar*, Bihor county North-Western Romania. Haruta Ovidiu, University of Oradea, Bugwood.org.

Figure 15 *Lymantria dispar* infestation of hardwood forest showing complete defoliation of infested trees, USA. Mark Robinson, USDA Forest Service, Bugwood.org

The gypsy moth overwinters in egg masses attached to the bark of trees. The egg masses (Figure 16) are usually about the size of a one dollar coin, are buff to tan in colour and may contain from 100 to 1,000 eggs. The severity of the infestation can be determined by the size of the egg mass. Egg masses tend to be smaller, about the size of a one cent coin, when populations are on the decline. Larger eggs masses are a sign of stable or growing populations.

The eggs hatch when tree buds begin to open. The larval stage (Figure 12), lasting up to seven weeks, is when the insect feeds. Feeding ends by early to mid-summer.

The pupal stage (Figure 17) occurs after feeding and lasts 9-17 days. Pupal cases can be found in the same places as the egg masses.

Adult moths (Figure 18, 19) appear in late summer.

Life cycle and biology information is provided in Appendix 8.3 Distribution and Biology.

Figure 16 *Lymantria dispar* egg masses, Slovakia. Photo by Milan Zubrik, Forest Research Institute - Slovakia, Bugwood.org

Figure 17 *Lymantria dispar* pupae, USA. Photo by Pennsylvania Department of Conservation and Natural Resources - Forestry, Bugwood.org

Figure 18 Female *Lymantria dispar* moths and egg masses, USA. John H. Ghent, USDA Forest Service, Bugwood.org

Figure 19 Male (L) and female (R) *Lymantria dispar* moths, USA. John H. Ghent, USDA Forest Service, Bugwood.org

3.3 Sampling

DNA testing is an effective diagnostic procedure for gypsy moth. Where DNA testing will be undertaken, it is critical that samples are collected regularly from pheromone traps, stored appropriately and submitted quickly to the diagnostic laboratory. Jackson and Molet (2012) recommend no longer than two weeks between visits to traps to reduce degradation of DNA in any specimens caught. High temperatures and high relative humidity also speed degradation of specimens. Specimens should be stored in a cool dry area if immediate dispatch to the diagnostic laboratory is not possible.

3.3.1 Methods

Apart from specific mention of procedures to preserve DNA in the subparagraphs below, standard entomological techniques for collection and preservation of insects apply.

Adults

If possible, pin material with stainless steel insect pins leaving about ¼ of the pin above the moth for handling. Dry as quickly as possible safely away from ants etc. Label specimen with standard collection details and any other relevant information. Preserve 1-2 legs in 95-100% ethanol and securely cross reference to the original specimen for molecular studies. While this step is recommended, Lepidoptera Barcode of Life (2009) showed more than 80% amplification of DNA and sequencing success for specimens up to 10 years old. Success then rapidly decreases with age but special techniques have been developed to deal with older specimens.

Other body parts like antennae or wing veins/membrane are also suitable for that purpose, but legs are usually preferred because they are paired appendages and their removal does not affect the general aspect of the specimen. The posterior end of the abdomen may also be used and the genitalia

can be recovered after DNA extraction. This is only applicable for small individuals since the size of the tissue used for extraction should not exceed 3-4 mm (Lepidoptera Barcode of Life, 2009).

Where pinned material is sent to a diagnostic laboratory, the specimens should be cross-pinned to prevent specimens turning on the pin in transit. The specimen box containing the material should be packed into a larger box and well-padded with shock-absorbing packing beads so that the inner box cannot move.

Preparation of genitalia

The following procedure assumes specimens have been submitted on the sticky base of a pheromone lure trap and is a combination of notes from Horak *et al*. (2006) and Gilligan and Epstein (2009).

Equipment

- Watch glasses or cavity blocks
- Glass tube
- Cotton wool or other suitable stopper
- Beaker
- Heat source
- Safety glasses
- Dissecting equipment including fine forceps and needles
- Labelling equipment (pencil, paper)
- 1% solution of Chlorazol Black
- 30% and 70% ethanol
- 10% potassium hydroxide (KOH) in water
- Citrus oil (any commercial product acceptable)

Procedure

Safety Note: In addition to standard laboratory protective clothing, wear safety glasses when working with KOH. This solution is extremely corrosive!

1. Create temporary labels to track specimen/s through process.
2. Cut piece of sticky trap containing specimen or remove abdomen from specimen.
3. Immerse sticky trap piece of abdomen in citrus oil until specimen is clean of adhesive.
4. Remove abdomen, transfer to a glass tube and cover with 10% KOH in water.
5. Lightly stopper tube and stand in a beaker filled with water to just beyond the KOH level in the tube. Gently bring the water to the boil and keep at boiling point for about 15 mins.
6. Rinse abdomen in several changes of water, removing fat, scales and other debris using needles, brushes and forceps as appropriate. (A very large female abdomen may have to be rinsed and briefly returned to fresh KOH and heated for another 2-3 mins. A drop of soapy water may be needed to break surface tension.)
7. Transfer to 30% ethanol for dissection.

   a. Male: Separate genitalia from the abdominal pelt with fine forceps.
   b. Female: Separate genitalia from the abdominal pelt with fine forceps, leaving the 7th sternite attached to the abdominal tip to make sure the sterigma is not damaged.

8. Staining
   a. Male: Genitalia can be examined without staining.
   b. Female: Stain cleaned abdominal pelt briefly in 70% ethanol to which a few drops of a 1% solution of Chlorazol Black E in 70% ethanol have been added. (Chlorazol Black E is recommended but other stains may be used).
9. To examine the male carefully flatten the genitalia dorsoventrally and open the valva for comparison with the illustrations. Do this in 30% ethanol and fix the flattened position in absolute ethanol. If the valva and uncus cannot be pressed into the correct position during the first few seconds in absolute ethanol with the help of fine brushes or a piece of cover slip, return preparation to 30% ethanol any number of times to soften it and repeat until correct position is achieved. To examine the aedeagus, it can be removed using two pairs of forceps. Withdraw its apex past the juxta while grasping the dorsal juxta margin with a second pair of forceps.
10. Storage: Genitalia preparations can be stored indefinitely in 80-100% ethanol. If a permanent slide is desired, dehydrate the preparation in 2-3 changes of absolute ethanol and then mount in Euparal (or similar mountant) under a cover slip on a genitalia slide.

Useful References: Detailed descriptions of genitalia preparations are provided by Robinson (1976) and Common (1990).

Eggs, larvae, pupae

Lepidoptera Barcode of Life (2009) advises that tissue from eggs, larvae or pupae can be used for DNA studies as long as the specimens are preserved as vouchers (eggs will then require a tissue recovery step). Collect tissues such as the head capsule, a transverse section of a segment or a thoracic leg (or a fragment of it) or any epidermal tissue from a pupa and allow to dry rapidly, or preserve in 95% ethanol. Specimens preserved in high concentration ethanol usually remain suitable for DNA extraction for 5 to 10 years, possibly more if the storage conditions are optimised. Important considerations are ethanol/sample volume ratio, ethanol renewal, protection from UV light and storage of samples either frozen or refrigerated.

Where samples are taken for DNA analysis, avoid gut contents as they may contain contaminants which could impact on test results.

4 IDENTIFICATION

This section includes methods for separation of species based on both morphological and molecular methods. While the focus of this protocol is distinguishing *L. dispar*, sufficient information is provided to support diagnosis of other species of biosecurity concern in the genus *Lymantria*. This is particularly so of the molecular method, DNA Barcoding, which has the potential to diagnose 71 species as at August 2014.

4.1 Morphological methods

Morphological methods will include reference to adults, larvae and eggs. Diagnostic information is concentrated on life stages most likely to be intercepted. For instance, a key is provided to separate males collected in disparlure traps, there is information on *Lymantria* spp. occurring in Australia with which *L. dispar* may be confused and an illustrated section on egg masses. Reference is also made to Palaearctic and African species.

4.1.1 Adults

The forewing pattern of *Lymantria* is based on or derived from a series of more or less parallel, transverse dark bands or lines, usually in zig-zag shape with the segments between the veins inwardly curved and bow-shaped with their ends meeting at the veins in outwardly directed points, (Figures 3, 7, section 2.2). Shape and exact position of these transverse bands vary between species and are strongly sexually dimorphic, and their expression may differ greatly within a species. Generally, three bands are recognisable, a subbasal, antemedial and postmedial one (Figure 7) with the antemedial band in *Lymantria* arising usually at or beyond the middle of the costa. There often is a submarginal row of dark marks or spots between the postmedial band and the margin, and a row of dark spots between the veins along the margin. At the end of the discal cell, at about the middle of an imaginary line from the wing base to the wing tip, there often is a dark, crescentic to V-shaped discal spot, with a dot or ring basal to it, distal to the sub basal band.

The male genitalia of *Lymantria* are very diverse throughout the genus, especially the valva structure, with those of *L. dispar* and related species strongly sclerotised and relatively simple. The pointed uncus is usually continuous with the rest of the tegumen, but in *L. nephrographa* it is separated by a membranous region (Figures 61a, b). The gnathos is absent, but some species including *L. lunata* have small, lateral, paired, finger-shaped tegumen processes. The saccus is tapering, often of diagnostic shape. The aedeagus is simple, straight or lightly sinuate, with the ductus entering subapically.

The female genitalia have an extensile ovipositor which, together with the 8th segment, is as long or longer than ductus and bursa combined. The details of the sterigma are diagnostic, but dissection and slide preparation are difficult. *Lymantria dispar* and the Australian *Lymantria* species lack a signum.

Key to identification of adults

This key is intended for material collected in disparlure traps, a pheromone unique to the genus *Lymantria* and will separate *L. dispar* from known Australian taxa.

The key also separates *Lymantria* females of the same species, even though they will not be caught in a pheromone trap. *L. pelospila* and *L. antennata* key out together.

The first couplet serves to confirm that the material in the trap is a *Lymantria* and not a specimen of another family.

WORDS OF CAUTION

- Disparlure is named after *L. dispar,* the species from which the pheromone was first isolated. However, a total of 14 *Lymantria* taxa, not counting subspecies, are so far known to be attracted to disparlure (Appendix 8.1). This key will not identify those additional taxa. Refer to supplementary information provided for a determination based on morphology although preference should be given to using molecular techniques in such cases.

- Studies on the response of Australian species of *Lymantria* to disparlure between 1996 and 2009 (Ingram 2010) demonstrated that *L. antennata* Walker (in part of its range) was the only one of the four species of *Lymantria,* recognised in Australia, attracted to disparlure.

Glossary of terms

[For more information on male genitalia refer to Sibatani *et al*. (1954)]

- *aedeagus* - penis, intromittent organ of male genitalia
- *antemedial band* - transverse darker band on forewing, in *Lymantria* usually just after middle (Figure 7)
- *bipectinate* - pectinate on two sides
- *corpus bursae* - inflated anterior portion of bursa copulatrix (Figure 9)
- *costa* - anterior margin of wing, but Torre-Bueno (1989) states that in male Lepidoptera the term also refers to the dorsal, marginal part of valva, variously sclerotised, bearing a great variety of structures and processes (Tuxen 1970). The word is used in both senses here.
- *discal cell* - large median cell of Lepidoptera wing
- *discal spot* - mark associated with the crossvein distally closing the discal cell (Figures 7, 31)
- *dorsum* - hindmargin of wing (wing extended at right angle to body)
- *ductus bursae* - posterior narrow portion of bursa copulatrix (Figure 38)
- *frenulum* - single (male) or several (female) bristles from base of hindwing costa linking hindwing to forewing
- *gnathos* - paired processes from tegumen below uncus
- *juxta* - sclerotised plate between valvae, ventral to and hinged with aedeagus (Figure 6)
- *ovipositor lobes* - paired hairy, terminal lobes of female genitalia (Figure 9)
- *postmedial band* - transverse darker band on forewing distal to antemedial band (Figure 7)
- *saccus* – mid-ventral, cephalic evagination of the vinculum, usually somewhat cylindrical (Figure 6)
- *signum* - conspicuous, sclerotised structure in wall of corpus bursae
- *sterigma* - sclerotised structures around entrance to ductus seminalis (Figure 9)
- *subbasal band* - transverse darker band on forewing at about 1/3 costa in *Lymantria* (Figure 7)
- *tegumen* - dorsal portion of male genitalia (Figure 6)
- *uncus* - terminal portion of tegumen (Figure 6)
- *valva* - paired, lateral clasping organs in male genitalia (Figure 6)

Key

1. Coiled tongue visible between palps in ventral view, antenna not bipectinate or if bipectinate, branches each not ending in a long spinule ..... not Erebidae

- No tongue visible between palps, antenna bipectinate and each branch ending in a long spinule (Figure 60) ..... (Erebidae)2

2. Antenna with long pectinations (Figures 1, 42); frenulum a single bristle; males 3

- Antenna with short pectinations (Figure 32); frenulum several bristles; small pair of setose lobes ventrally between ovipositor lobes (Figure 52); females 6

3. Uncus continuous with tegumen (Figure 61a); valva with distal finger-shaped process (Figures 5, 34, 43, 45) 4

- Uncus separated from tegumen by membranous zone (Figure 61b); valva rhomboid, pointed, not with finger-shaped process (Figure 66) ..... *L. nephrographa*

4. Finger-shaped valva process straight and a direct continuation of straight costa (Figures 5, 6); abdomen never with pink scales ..... *L. dispar*

- Costa dorsally projecting in a curve or hump between base and inception of finger-shaped process (Figures 43, 45), or process from middle of outer valva margin (Figures 34, 35); abdomen often with pink scales 5

5. Finger-shaped process from middle of outer valva margin (Figures 34, 35); valva subrectangular with costa and ventral margin straight and parallel; tegumen with lateral, paired, small, finger-shaped processes (Figures 34-36) ..... *L. lunata*

- Finger-shaped process projecting at right angle from inner surface of valva near costa (Figures 43-46); costa and ventral valva margin not straight; tegumen without paired lateral processes (Figures 43-45) ..... *L. pelospila* and *L. antennata*

6. Flightless, with only minute forewing stumps (Figure 59); sterigma (Figure 9) two paired, spinulose, posteriorly rounded lobes (Figures 48, 49) ..... *L. pelospila* and *L. antennata*

   Forewings developed 7

7. Discal spot a whitish crescentic mark with a blackish border, surrounded by other complex pattern elements (Figure 64); hindwing with dark grey scaling in basal half; sterigma two elongate-triangular plates with a row of bristles posteriorly (Figure 65) ..... *L. nephrographa*

- Discal spot a black crescentic mark (Figures 7, 8, 32), sometimes fused to antemedial band (Figures 32, 33), hindwing pale right to base 8

8. Antemedial band (Figure 7) well developed, straight and often wide, as dark as discal spot (Figures 32, 33); sterigma subrectangular spinulose area with pronounced ventral lip with lateral bristles and a central depression leading to funnel-shaped posterior end of ductus bursae (Figure 38) ..... *L. lunata*

- Antemedial band a weak, angled, zig-zag line, paler than conspicuous discal spot (Figures 7, 8); sterigma a broad ring, anteriorly smooth, posteriorly separated into two spinulose lobes (Figures 9-11) with posterior end of ductus bursae barrel-shaped, sclerotised (Figures 9, 10) .... *L. dispar*

4.1.2 Larvae

Newly hatched caterpillars are black and hairy, later developing a mottled yellow to grey pattern with tufts of bristle like hairs and two rows of blue then red spots on their back. Of the different life stages, larvae are least likely to be intercepted as they are not as easily transported as eggs or adults.

Pogue and Schaefer (2007) provide information on preparation and examination of larvae of *Lymantria* by both light and electron microscopy and well-illustrated keys to first instar *L. dispar, L. mathura, L. monacha* and *L. obfuscata* and last instar larvae of these species together with *L. albescens, L. atemeles, L. bantaizana, L. fumida, L. lucescens, L. mimomonis, L. serva, L. umbrosa,* and *L. zylina.*

4.1.3 Eggs

Lymantria dispar females lay their eggs in large batches covered with scales from the abdomen. Several Australian lymantriid and one notodontid species are known to produce egg batches covered with abdominal scales, superficially potentially very similar to those of *L. dispar*. If an egg batch is found on a shipping container after it has arrived in Australia it could have been laid by a female of one of these Australian species.

The structure of the abdominal scales viewed in a scanning electron microscope (SEM) seems to be diagnostic to some extent. Information is provided below to help determine whether the egg mass is from *L. dispar* or an Australian species.

- Figures 20-22 illustrate scales from egg batches of Russian, Japanese and European populations of *L. dispar*,
- Figure 23 those from *L. mathura* Moore and Figure 24 from *L. xylina* Swinhnoe, to give an indication of variation within the genus *Lymantria*.
- Figures 25-31 illustrate scales removed from the abdomen of females of five Australian species with scale covered egg batches; four lymantriid species, the notodontid *Ochrogaster lunifer* and an exotic species not in the genus *Lymantria* as follows
    - Figure 25 illustrates abdominal scales, white cedar moth, *Leptocneria reducta* (Walker)
    - Figure 26 illustrates abdominal scales, mistletoe brown tail moth, *Euproctis edwardsii* (Newman)
    - Figure 27 illustrates abdominal scales, tussock moth identified as *Euproctis* sp.
    - Figure 28 illustrates abdominal scales, omnivorous tussock moth, *Acyphas semiochrea* (Herrich-Schäffer)

- Figure 29 illustrates abdominal scales of white spotted tussock moth, *Orgyia thyellina*, native to Russia Far East, Japan, Korea, Taiwan and China. This species was detected in Auckland, New Zealand, April 1996 and declared eradicated in 1998.

- Figure 30 illustrates abdominal scales, processionary caterpillar or bag shelter moth, *Ochrogaster lunifer* (Herrich-Schäffer)

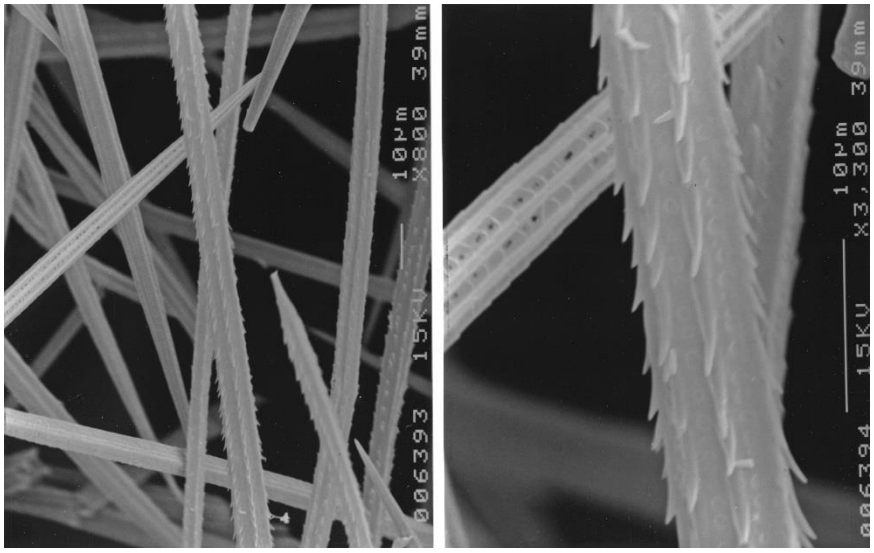

Figure 20 *Lymantria dispar*, Russian population, scales from egg batch. Eric Hines, CSIRO Ecosystem Sciences.

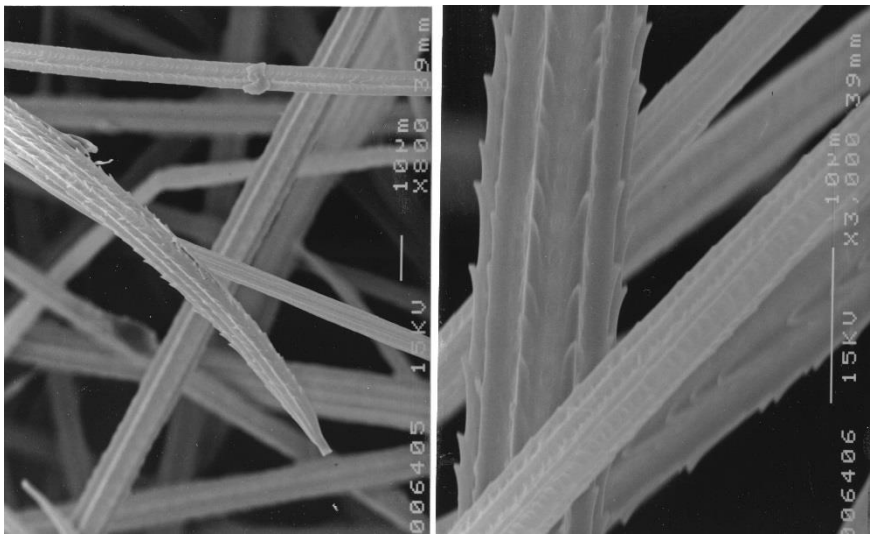

Figure 21 *Lymantria dispar*, Japanese population, scales from egg batch. Eric Hines, CSIRO Ecosystem Sciences

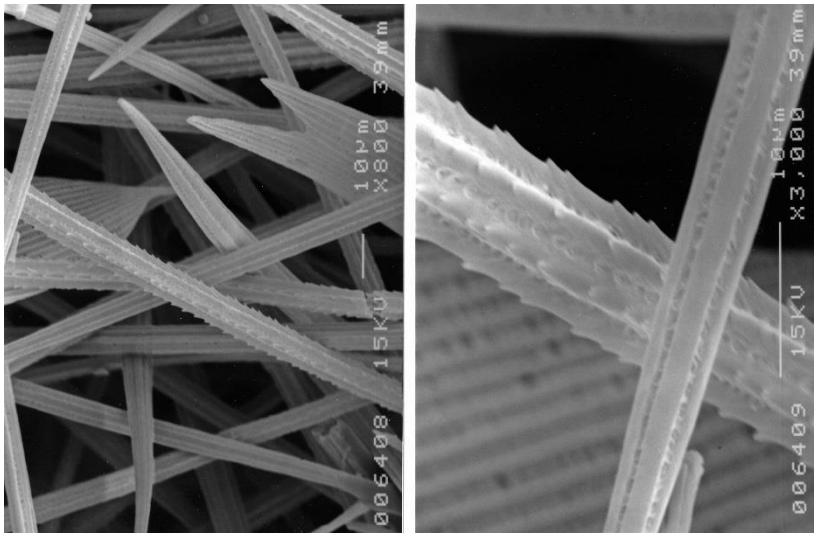

Figure 22 *Lymantria dispar*, population from Sardinia, scales from egg batch. Eric Hines, CSIRO Ecosystem Sciences.

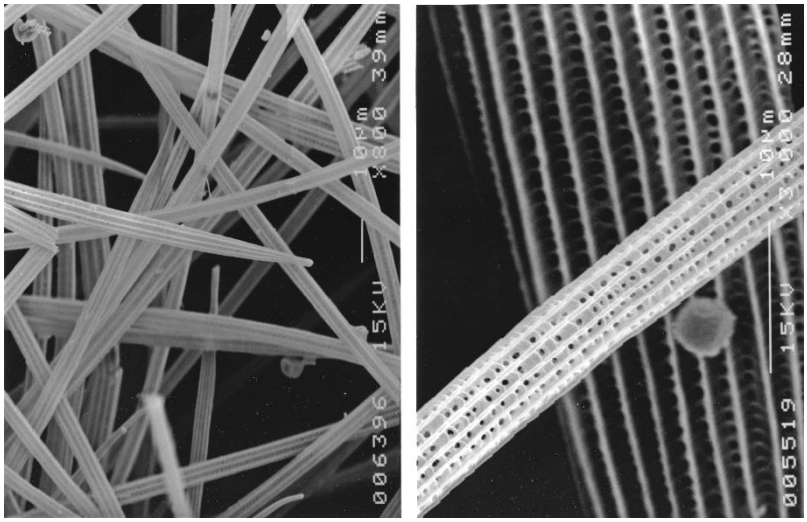

Figure 23 *Lymantria mathura* Moore, scales from egg batch. Eric Hines, CSIRO Ecosystem Sciences.

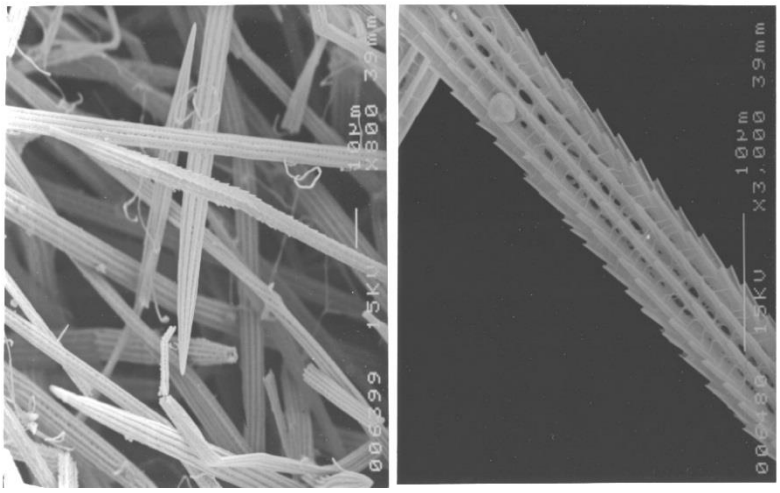

Figure 24 *Lymantria xylina* Swinhoe, scales from egg batch. Eric Hines, CSIRO Ecosystem Sciences.

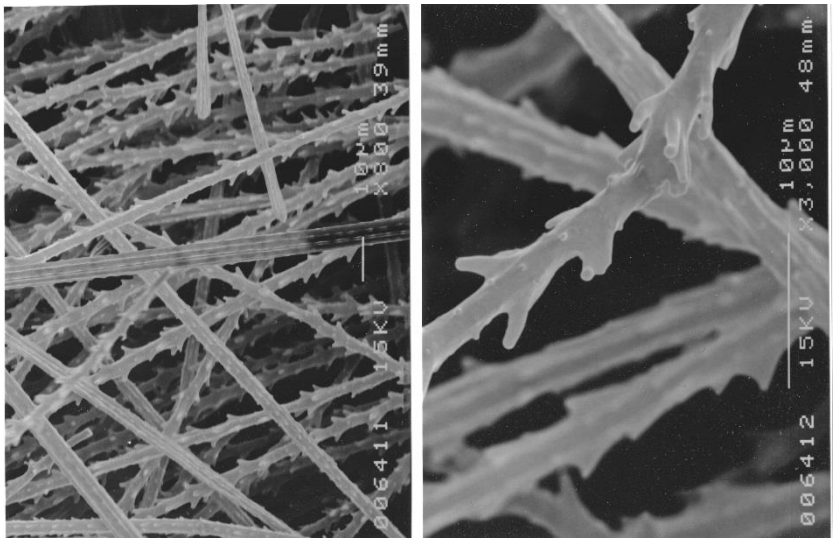

Figure 25. *Leptocneria reducta* (Walker), Australia, scales from abdominal tuft. Eric Hines, CSIRO Ecosystem Sciences.

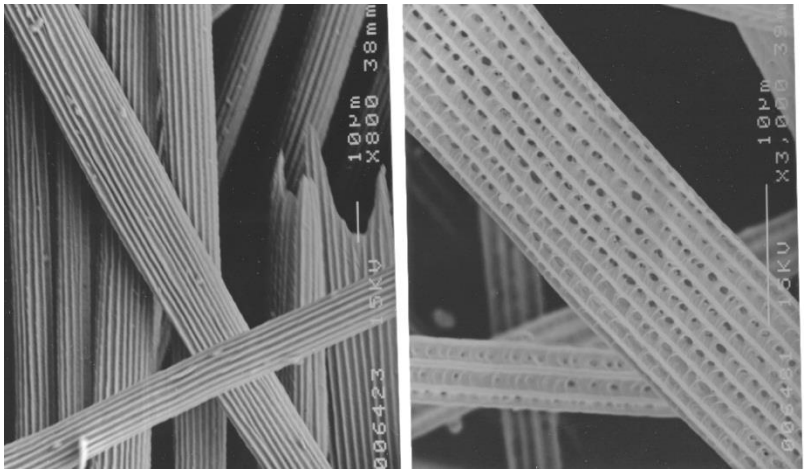

Figure 26 *Euproctis edwardsii* (Newman), Australia, scales from abdominal tuft. Eric Hines, CSIRO Ecosystem Sciences.

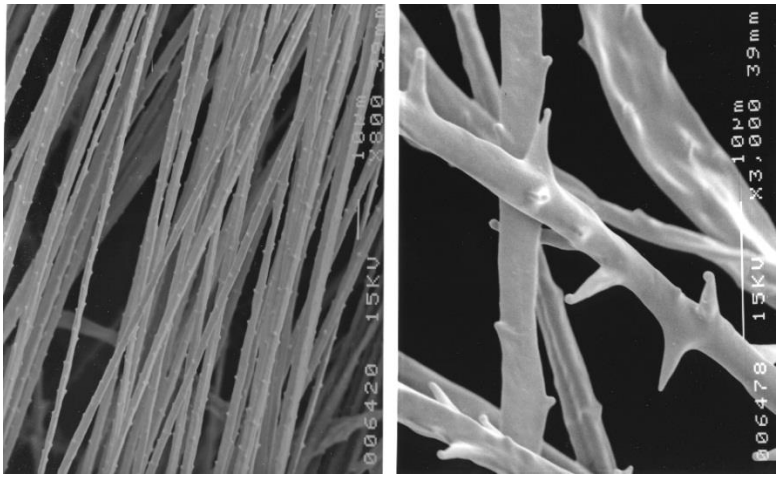

Figure 27 *Euproctis* sp. A, Australia, scales from abdominal tuft. Eric Hines, CSIRO Ecosystem Sciences.

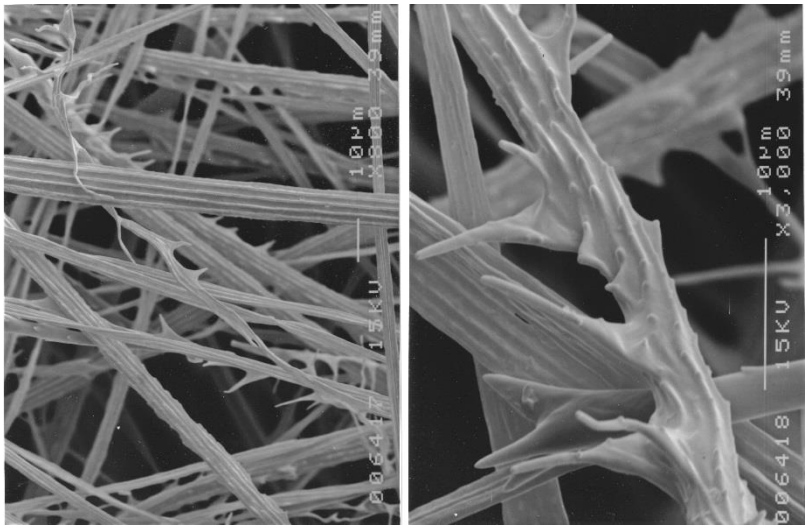

Figure 28 *Acyphas semiochrea* (Herrich-Schäffer), Australia, scales from abdominal tuft. Eric Hines, CSIRO Ecosystem Sciences.

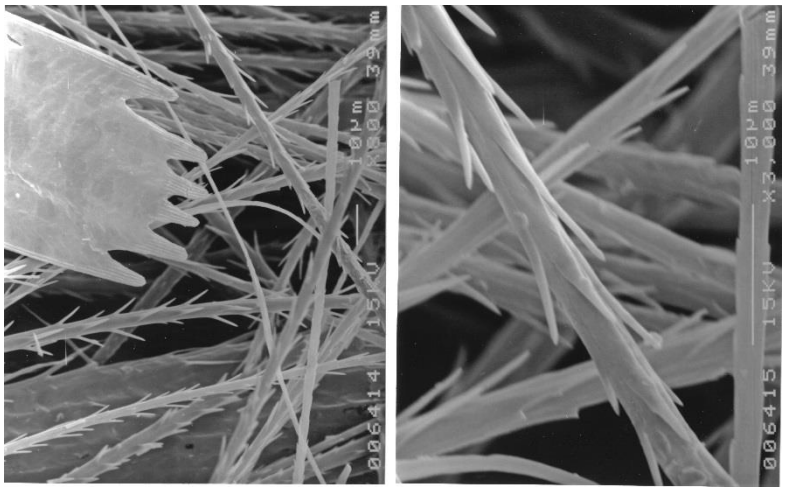

Figure 29 *Orgyia thyellina* Butler, scales from abdominal tuft. Eric Hines, CSIRO Ecosystem Sciences.

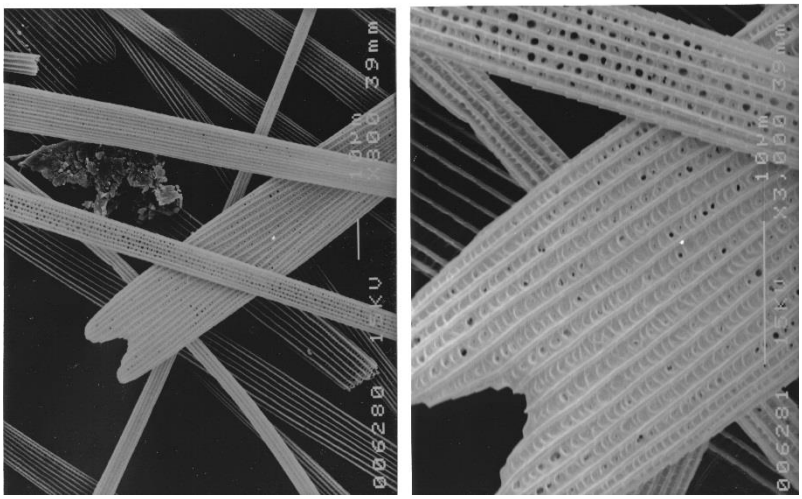

Figure 30 *Ochrogaster lunifer* (Herrich-Schäffer), (Notodontidae), Australia, scales from abdominal tuft. Eric Hines, CSIRO Ecosystem Sciences.

4.1.4 Lepidoptera that occur in Australia with which L. dispar might be confused

Four species of *Lymantria* are currently recognised in Australia, *L. antennata* Walker, *L. pelospila* (Turner), *L. lunata* Stoll and *L. nephrographa* Turner; but see comments below. Some males of the first three species could be mistaken for *Lymantria dispar*. *L. antennata* and *L. pelospila*, both with flightless females with minute wing stumps, are either two distinct species with overlapping distribution and possibly hybridisation in north Queensland, or a single cline in both wing pattern and genitalia morphology ranging from northern New South Wales around the Queensland coast to the Northern Territory and just into Western Australia. Molecular data will be needed to resolve this question. While they are keyed out together, descriptions of the typical form for both taxa are provided below. *Lymantria nephrographa* is quite distinct and much less closely related to *L. dispar*. It is classified as a species *incertae sedis* within *Lymantria* by Schintlmeister (2004) rather than included in one of his 12 subgenera.

Given the wide variability of *Lymantria* wing pattern and the fact that crucial material needing identification will be extracted from pheromone traps which attract males only, the protocol is focused on male genitalia information. However, adults of both sexes and male and female genitalia are figured for gypsy moth and the four Australian *Lymantria* species, and a brief diagnosis is given for these species.

Description of Lymantria lunata (Stoll)

Male (Figure 31): Abdomen at least partly pinkish red. Forewings with ill-defined pale to dark brownish to blackish markings on pale brownish grey ground; a few prominent dark spots near base of wing; three hardly angled transverse bands, each a series of very diffuse spots; a prominent submarginal row of dark diffuse spots preceded by a row of pale V-shaped blotches; a row of small dots along margin; discal mark conspicuous, well-defined, V-shaped, next to the antemedial band, with a small dark spot basal to it. Hindwings yellowish with a darker band around margin, with pink hue next to abdomen.

Female (Figures 32, 33): Abdomen white with pale pink tinge. Forewings with well-defined ochreous to grey-brown markings on white ground; a few faint darker spots near base; subbasal band strongly angled twice, widest at costa; antemedial band straight, from beyond middle of costa to middle of dorsum, broad and gradually widening towards dorsum; postmedial band straight or lightly zig-zag, roughly parallel to but narrower than antemedial one; weak traces of a few submarginal spots, a row of elongate dark marks along outer margin; V-shaped mark prominent, connected to antemedial band. Hindwing whitish with black marginal dots and pink hue next to abdomen.

Male genitalia (Figures 34-36): Uncus continuous with tegumen; with small, paired, finger-shaped processes laterally on tegumen. Valva with dorsal and ventral margins straight, roughly parallel, with a straight, finger-like process from middle of distal margin, roughly in the same plane as the valva; saccus irregular, elongate and rather broad; juxta a simple transverse band; aedeagus long and slender.

Female genitalia (Figures 37-39): Ovipositor lobes and additional paired ventral lobes very weakly sclerotised. Sterigma a weakly sclerotised, subrectangular spinulose area with a pronounced, ventral lip with a few bristles laterally and a central depression leading to ductus bursae. Posteriormost part of ductus bursae a longitudinally wrinkled funnel of thickened membrane.

Note: Schintlmeister (2004) recognised three sub species: *Lymantria lunata ingrami* Schintlmeister, *Lymantria lunata carteri* Schintlmeister, and *Lymantria lunata curvifera* Schintlmeister.

Pogue and Schaeffer (2007) listed all as synonyms of *L. lunata* but did not list any Australian material in the list of specimens examined.

Based on feeding preferences of larvae, Ingram (pers. comm.) believes that *L. lunata* in Australia is sufficiently different to warrant further consideration of this point and of the potential threat posed by non-Australian populations of the species. Ingram says that *L. lunata* in the Philippines attacks 59 tropical fruits, in Indonesia 10 tropical fruits and Australian forms have been recorded eating petals only of mangoes.

Figure 31 *Lymantria lunata*, male; Ingham, Qld (ds, discal spot). David McClenaghan, CSIRO Ecosystem Sciences.

Figure 32 *Lymantria lunata*, female; Cairns, Qld; reared fresh specimen. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 33 *Lymantria lunata*, female; Halifax, Qld; old, rubbed specimen. Vanna Rangsi, CSIRO Ecosystem Sciences.

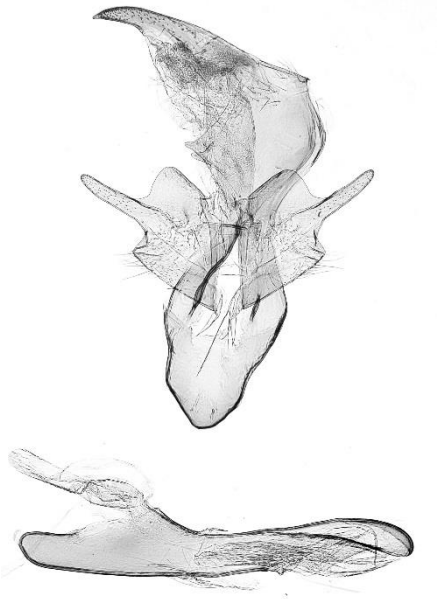

Figure 34 *Lymantria lunata*, male, Papua New Guinea. Vanna Rangsi, CSIRO Ecosystem Sciences.

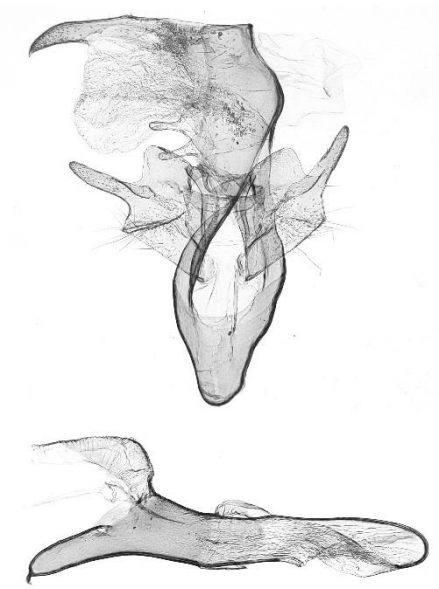

Figure 35 *Lymantria lunata*, male, Cairns. Vanna Rangsi, CSIRO Ecosystem Sciences.

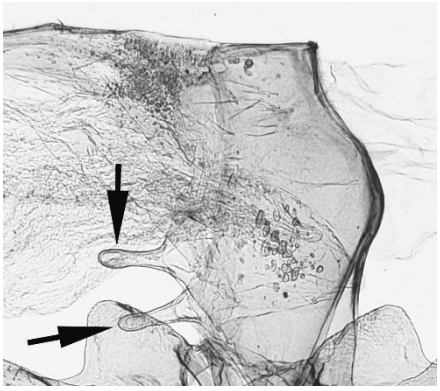

Figure 36 *Lymantria lunata*, male, detail of lateral tegumen processes (arrows). Vanna Rangsi, CSIRO Ecosystem Sciences.

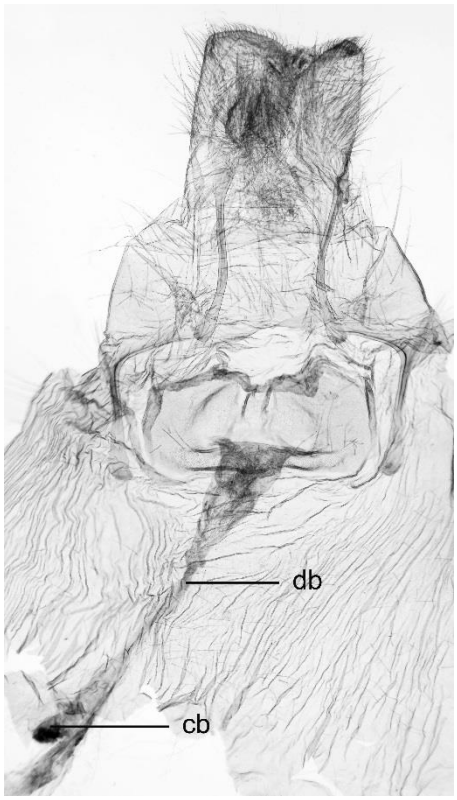

Figure 38 *Lymantria lunata*, female (db, ductus bursae; cb, corpus bursae. Vanna Rangsi, CSIRO Ecosystem Sciences.

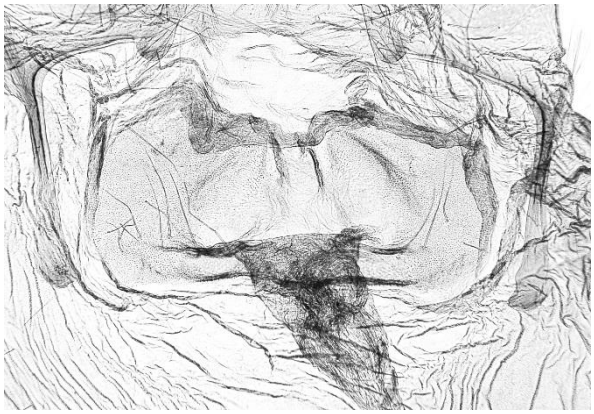

Figure 37 *Lymantria lunata*, female, sterigma. Vanna Rangsi, CSIRO Ecosystem Sciences.

Description of Lymantria pelospila Turner

Male (Figures 39-42): Abdomen whitish or yellowish, often with a pink tinge but rarely pink. Forewings with brownish to blackish markings on white to pale ochreous ground, sometimes markings so pale as to be hardly visible; up to five dark spots in wing base; subbasal band strongest on costa, a zig-zag series of dark marks; antemedial band weakly developed or absent, sinuate and zig-zag from middle of costa; postmedial band prominent, deeply zig-zag and strongly sinuate from 3/5 costa, parallel with antemedial band in dorsal half; a few irregular submarginal spots and an often incomplete row of marginal dots. Hindwings yellowish white, rarely with pinkish hue next to abdomen.

Female: flightless, with only minute wingstumps.

Male genitalia (Figures 43, 44, 46): Uncus continuous with tegumen; without lateral tegumen processes. Valva rounded-rectangular, with a long, straight, finger-like process from close to valva edge protruding at right angle from valva, and with a sharp, toothed ridge from inner surface of valva basal to finger-like process; saccus with truncate apex; juxta a simple transverse band; aedeagus long and slender.

Female genitalia (Figures 49, 51, 52): Ovipositor lobes and additional paired ventral lobes moderately sclerotised. Sterigma weakly sclerotised, entirely spinulose, medially split to form two low, posteriorly evenly rounded lobes with a dimple each at their base, with entrance to ductus bursae in between. Posteriormost part of ductus bursa of strongly wrinkled, thickened membrane gradually opening into corpus bursae.

Description of Lymantria antennata Walker

Male (Figures 53-58): Abdomen nearly always pink. Forewings with brownish to blackish markings on ground colour ranging from whitish to grey; up to five dark spots in wing base; subbasal band strongest on costa, a zig-zag series of dark marks; antemedial band sinuate and zig-zag from middle of costa, often widened and ill-defined, extending towards postmedial band and sometimes fused with subbasal band on dorsum; postmedial band deeply zig-zag and strongly sinuate from 3/5 costa, parallel with antemedial band in dorsal half; very few submarginal spots and a usually complete row of marginal dots. Hindwings ranging from whitish to grey, usually with pinkish hue next to abdomen.

Female (Figure 59): flightless, with only minute wingstumps.

Male genitalia (Figures 44, 45): Uncus continuous with tegumen; without lateral tegumen processes. Valva rounded, with a long, straight, finger-like process from inner surface of valva protruding at right angle from valva, at most with a low hump on inner surface of valva basal to finger-like process; saccus with V-shaped apex; juxta a simple transverse band; aedeagus long and slender.

Female genitalia (Figures 47, 48, 50): Ovipositor lobes and additional paired ventral lobes moderately sclerotised. Sterigma weakly sclerotised, entirely spinulose, medially split to form two high, irregularly rounded lobes, with entrance to ductus bursae in between. Posterior most part of ductus bursa of strongly wrinkled, thickened membrane gradually opening into corpus bursae.

Figure 39 *Lymantria pelospila*, male, Drysdale R., WA. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 40 *Lymantria pelospila*, male, nr Borroloola, NT. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 41 *Lymantria pelospila*, male, Hann River, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 42 *Lymantria pelospila*, male, Kuranda, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

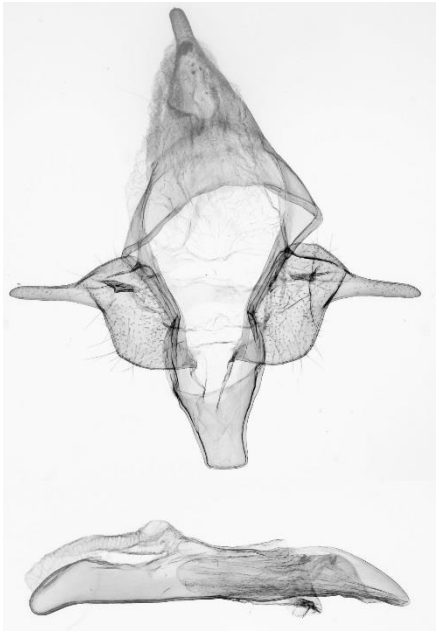

Figure 43 *Lymantria pelospila*, male, Blackmore River Crossing, NT. Vanna Rangsi, CSIRO Ecosystem Sciences.

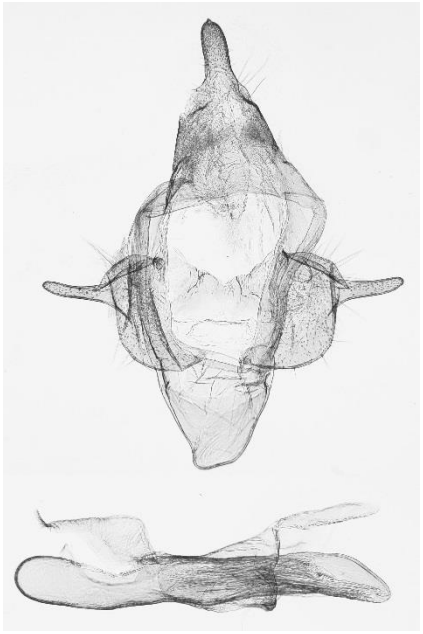

Figure 44 Male, intermediate between typical *L. pelospila* and *L. antennata*. Vanna Rangsi, CSIRO Ecosystem Sciences.

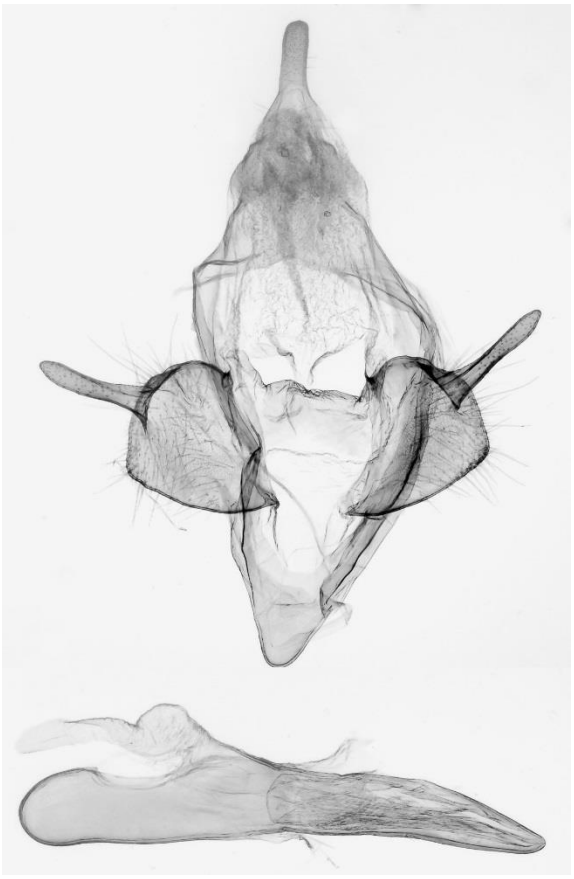

Figure 45 *Lymantria antennata*, male, nr Tallebudgera, Qld. Vanna Rangsi, CSIRO Ecosystem Sciences.

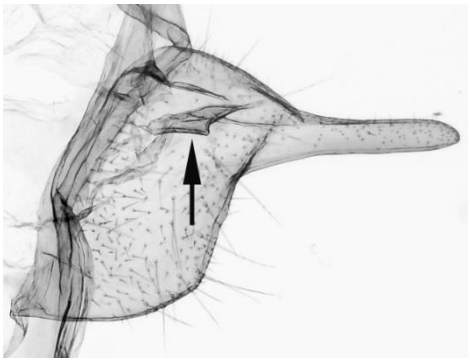

Figure 46 *Lymantria pelospila* valva with sharp ridge from inner surface. Vanna Rangsi, CSIRO Ecosystem Sciences.

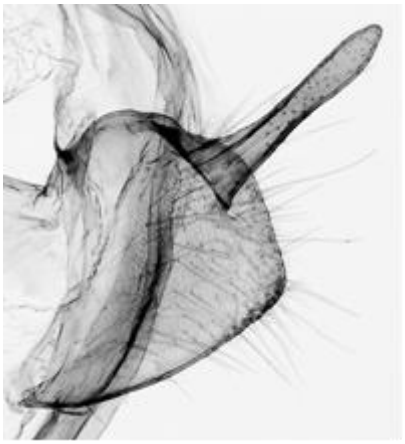

Figure 47 Typical *Lymantria antennata* valva. Vanna Rangsi, CSIRO Ecosystem Sciences.

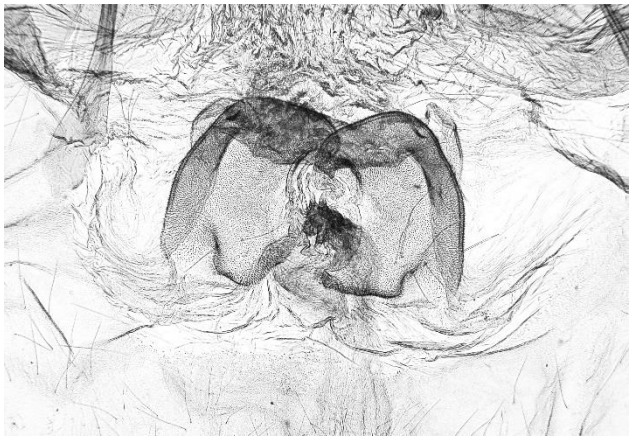

Figure 48 *Lymantria antennata*, sterigma. Vanna Rangsi, CSIRO Ecosystem Sciences.

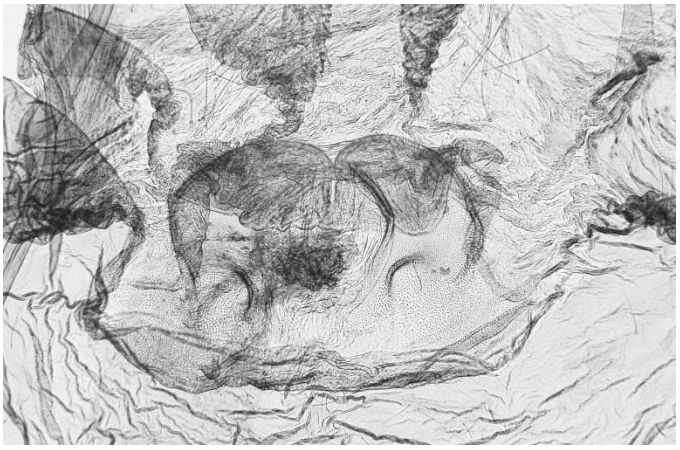

Figure 49 *Lymantria pelospila*, sterigma. Vanna Rangsi, CSIRO Ecosystem Sciences.

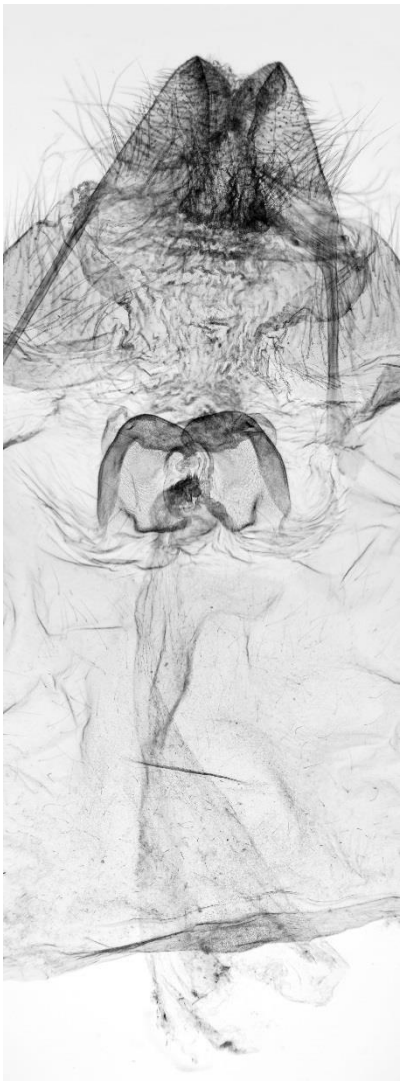

Figure 50 *Lymantria antennata*, female, Townsville. Vanna Rangsi, CSIRO Ecosystem Sciences.

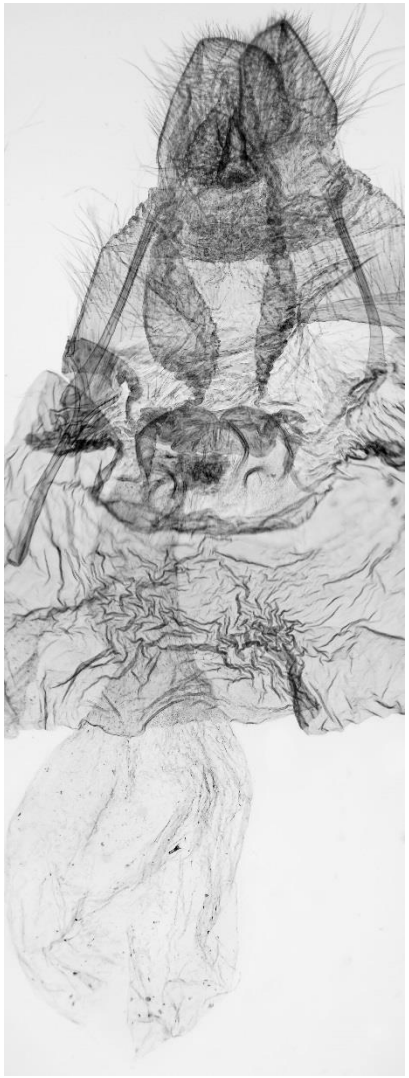

Figure 51 *Lymantria pelospila*, female, Kuranda. Vanna Rangsi, CSIRO Ecosystem Sciences.

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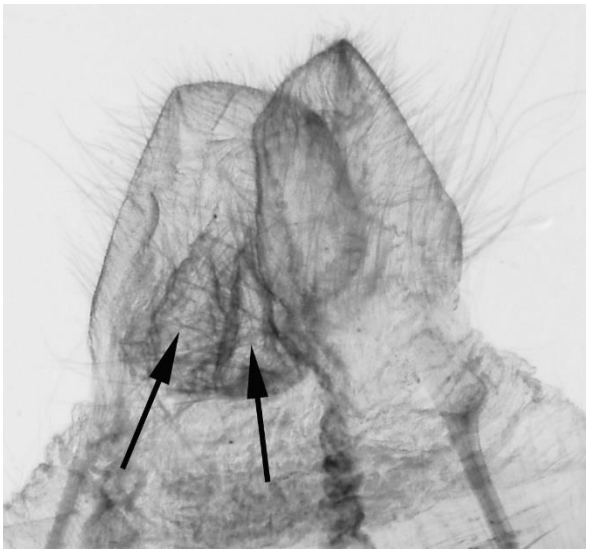

Figure 52 *Lymantria pelospila*, with ventral pair of setose lobes between ovipositor lobes (arrows). Vanna Rangsi, CSIRO Ecosystem Sciences.

Figure 53 *Lymantria antennata*, male, Stannary Hill, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 54 *Lymantria antennata*, male, Brisbane, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 55 *Lymantria antennata*, male, Noosa, Qld. Vanna Rangsi, CSIRO Ecosystem Sciences.

Figure 56 *Lymantria antennata*, male, Grafton, NSW. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 57 *Lymantria antennata*, male, Burleigh Heads, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 58 *Lymantria antennata*, male, Burleigh Heads, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 59 *Lymantria antennata*, female, higher magnification than males. David McClenaghan, CSIRO Ecosystem Sciences.

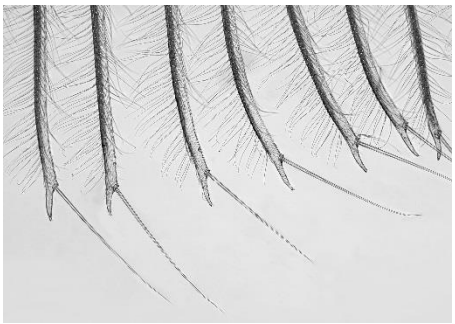

Figure 60 Antenna, tip of branches with long spinules (*Lymantria antennata*, male). Vanna Rangsi, CSIRO Ecosystem Sciences.

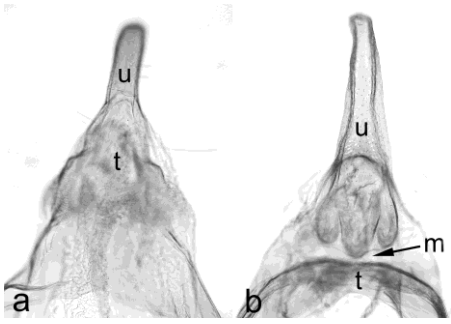

Figure 61 Connection uncus (u) and tegumen (t): a, continuous, *Lymantria antennata*; b, membranous zone in between (m), *Lymantria nephrographa*. Vanna Rangsi, CSIRO Ecosystem Sciences.

Description of Lymantria nephrographa Turner

Male (Figures 62, 63): Abdomen black and white. Forewing with well-defined complex, fine, reddish grey to blackish pattern on whitish ground, with the many scattered U-shaped marks not obviously aligned into transverse bands but the discal spot prominent as an interrupted outline of a crescent with a minute ring basal to it; with a complete row of mostly U-shaped submarginal marks and regular black marginal spots followed by a white fringe. Hindwing with black scales except for a whitish band along costa and around margin with a series of black marginal spots.

Female (Figure 64): Similar to male, but pattern elements larger and more distinctly aligned into transverse lines, with the crescent-shaped discal spot partly fused with elements of the antemedial line. Hindwing blackish in basal half with a crescentic black mark across end of cell, rest whitish with a series of blackish marginal spots.

Male genitalia (Figure 66): Uncus separated from tegumen by membranous area; without lateral tegumen processes. Valva simple, pointed, elongate-rhomboid, without finger-like process; saccus triangular, pointed; juxta an elliptic plate topped with a vertical transverse band; aedeagus short, medially much wider.

Female genitalia (Figures 65, 67): Ovipositor lobes and additional paired ventral lobes moderately sclerotised. Sterigma two well-sclerotised, smooth elongate-triangular plates with a row of large bristles along posterior margins and edged darker along ventral midline, with an irregular, sclerotised domed plate in between at their base leading to corpus bursae.

Figure 62 *Lymantria nephrographa*, male, Upper Allyn River, NSW. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 63 *Lymantria nephrographa*, male, Lamington National Park, Qld. David McClenaghan, CSIRO Ecosystem Sciences.

Figure 64 *Lymantria nephrographa,* female, Acacia Plateau, NSW. David McClenaghan, CSIRO Ecosystem Sciences.

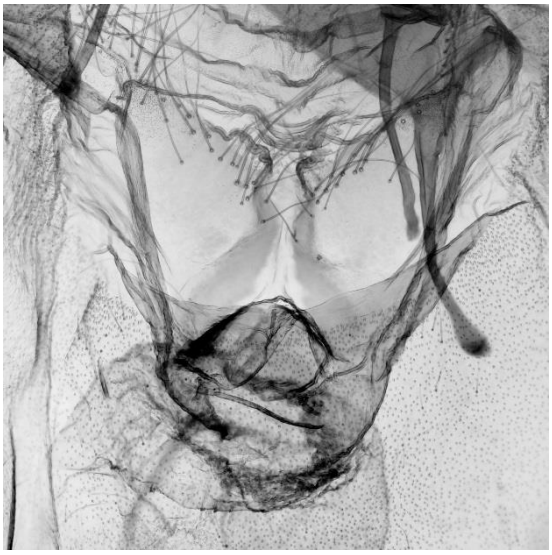

Figure 65 *Lymantria nephrographa*, sterigma. Vanna Rangsi, CSIRO Ecosystem Sciences.

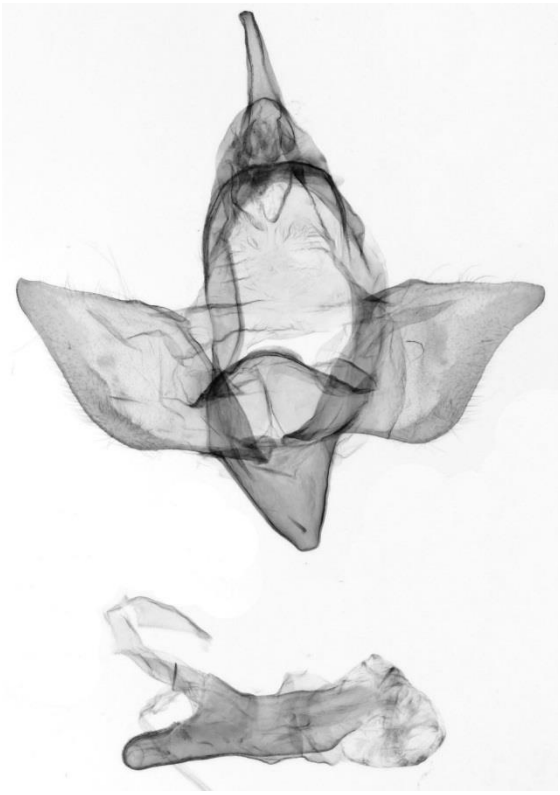

Figure 66 *Lymantria nephrographa*, male genitalia. Vanna Rangsi, CSIRO Ecosystem Sciences.

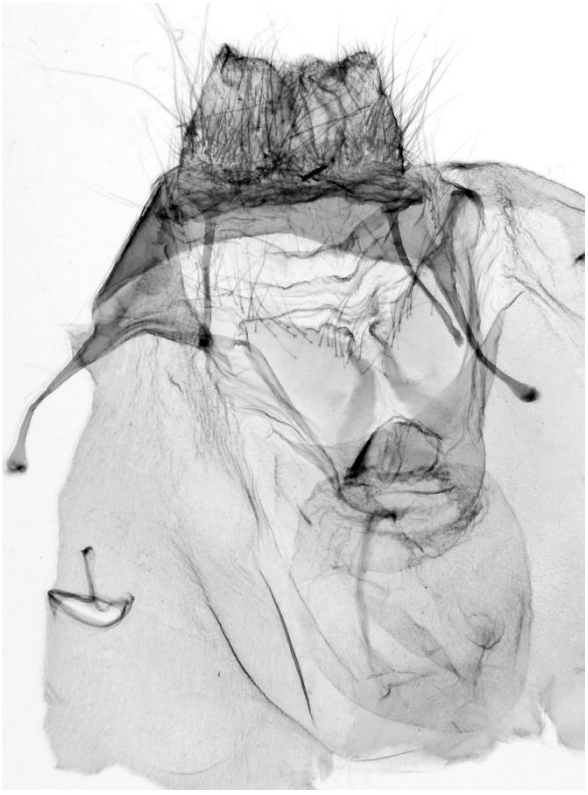

Figure 67 *Lymantria nephrographa*, female genitalia. Vanna Rangsi, CSIRO Ecosystem Sciences.

4.1.5. Palaearctic Lymantria species

A table summarising the diagnostic information presented by Pogue and Schaefer (2007) in their taxonomic review of the economically important *Lymantria* of the Palaearctic region, is included as Appendix 8.4. The review has excellent illustrations of diagnostic characters, including wing maculation patterns and male and female genitalia. A copy of Pogue and Schaefer (2007) should be available as a diagnostic reference tool in any laboratory in Australia responsible for the primary morphological identification of economically important *Lymantria* species.

4.1.6. African Lymantria species

No African species of *Lymantria* have been recorded as pests to date. However the following are important references that may assist should the presence of an African species be suspected.

The African species treated by Schintlmeister (2004) in *Lymantria* subgenera *Griveaudtria* Schintlmeister and *Pyramocera* were treated in more detail by Griveaud (1977) as the genera *Lymantica* Collenette and *Pyramocera* Butler, respectively. Griveaud (1977) illustrated the male and female genitalia of most species of *Lymantica* and also provided black and white photographs of each species. However, Griveaud's key to *Lymantica* species is based almost entirely on descriptions of colour patterns, which can be difficult to interpret.

4.2 Molecular methods

Developments in molecular technology can be traced through the literature for this important insect group as they have been applied to research questions in population studies (Bogdanowicz *et al*.1993; Keena *et al*. 2008) and evolution (Le Roux and Wieczorek, 2009) as well as biosurveillance (Armstrong and Ball, 2005) and species identification (Armstrong *et al*. 2003). DNA fingerprinting techniques such as microsatellite analysis are summarised by Loxdale and Lushai (1998).

Ball and Armstrong (2006) used DNA barcode data to correctly identify 20 species of Lymantriidae, including *L. dispar*, with 100% success and deWaard *et al*. (2010) were able to separate 36 morphologically defined species with a success rate of 97.2%. DNA barcoding is now the method of choice for gypsy moth identification (Armstrong 2010).

The method described below in 4.2.1 has been drawn from Armstrong (2010) and deWaard *et al*. (2010) with reference to the COI primers and amplification methods of Cho *et al*. (2008) and has been successfully verified as part of the protocol review.

However, more recently Mitchell (2015) developed and published primers which work in a wider range of taxa (including, to date, Lepidoptera, Coleoptera, Diptera. Hemiptera, Odonata and Zygentoma) and include primers for amplifying short fragment of COI for old specimens with degraded DNA. Refer to Appendix 8.6. This procedure was not verified as part of the review process.

4.2.1 Lymantria molecular identification using DNA barcoding

Note: Follow appropriate procedures to avoid contamination of diagnostic samples with other DNA templates and amplicons that may be present in the laboratory environment.

DNA extraction

- DNA extraction is most efficiently performed using commercial nucleic acid extraction kits based on proteinase-K digestion of tissue followed by silica-based spin column technology.
- In general terms, up to 20 mg of tissue (a single leg from adult moth, an equivalent amount of tissue for larvae, or even a single ovum) is macerated in buffer and digested with proteinase-K for 1-3 hours (or overnight, which can work better for older specimens) before being applied to a spin column, washed and eluted. Follow the detailed instructions for your chosen DNA extraction kit.
- Suitable DNA extraction kits include the following:
    - Machery Nagel Nucleospin™ Tissue Kit
    - Qiagen DNeasy® Tissue Kit
    - Sigma GenElute™ Mammalian Genomic DNA Extraction Kit
    - Sigma GenElute™ Mammalian Genomic DNA Miniprep Kit
    - Bioline ISOLATE II Genomic DNA Kit

PCR Amplification

The PCR primers used by Ball and Armstrong (2006) were designed and published by Folmer *et al*., (1994):

- LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and
- HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3').

For most of their research deWaard *et al*. (2010) used the primers published by Hebert *et al*. (2004):

- LepF1 (ATTCAACCAATCATAAAGATAT), and
- LepR1 (TAAACTTCTGGATGTCCAAAAA),

However, where molecular work targeting the COI sequence was performed in Australia, the primers used were as described by Cho *et al*. (2008):

- Forward primers LepFm (5'-*GTAAAACGACGGCCAGT*CAATYTATCGCYTAAMTTCAGCC-3') binding to the tRNA-Tyr gene, or
- BC1Fm (*GTAAAACGACGGCCAGT*TCWACWAAY-CAYAARGAYATYGG-3') binding to the COI gene, and
- Reverse primer BC3Rm (5'-*CAGGAAACAGCTATGAC*GWARAATWARAATRTAWACYTCWGG-3').

The latter primers are described by Cho *et al*. (2008) as degenerate versions of K698D (Simon *et al.*, 1994) and LCO1490 and HCO2198 (Folmer *et al.*, 1994 in Ball and Armstrong 2006). (Note the italicized section of each sequence. This is an M13-vector sequence added to facilitate DNA sequencing.)

The subsequent PCR steps outline below were developed from a combination of Brake (2012), Cho *et al*. (2008) and Kumarasinghe pers. comm. (2013). Other standard reagents and quantities may be used if known to work.

PCR solution (Quantity 30 µL)

- 1 µL genomic DNA

Plus: (supplied by Invitrogen, Mount Waverley, Australia (Cho *et al.* 2008))

- 1 X PCR buffer (20 mM Tris-HCl (pH 8.4); 50 mM KCl
- 2 mM $MgCl_2$
- 0.2 mM dNTPs
- 10 pmol of each PCR primer
- 1 unit Platinum® Taq DNA polymerase

PCR Amplification Conditions

- 94˚C for 2 min
- 35 cycles of (94˚C for 30 s, 50˚C for 30 s, 72˚C for 60 s)
- 72˚C for 7 min
- 4˚C hold

PCR Product Visualization

PCR products should be visualized by running a 3µL sample on a 1.5% agarose gel stained with 1 drop of Biotium GelRed (Gene Target Solutions, Dural, Australia) per 50 mL of gel mix. PCR products which yield a visible product of the expected size should be purified and sent for DNA sequencing.

PCR Purification

Purification may not be necessary for sequencing – direct sequencing can be done on dilution of the PCR product. If required, PCR products can be purified using a commercially available kit such as:

- GenElute™ PCR Clean-Up Kit (Sigma-Aldrich, Castle Hill, Australia)
- QiaQuick PCR Purification kit (Qiagen Australia)

Note: *Manufacturer's procedures may need to be modified depending on specific requirements for the sequencing step.*

DNA Sequencing

The purified COI PCR product should be sequenced in both forward and reverse directions. PCR products can be sent to any sequencing facility for sequencing such as Australian Genome Research Facility, Micromon or Macrogen (list is not exhaustive). There is a range of software available that can be used to edit sequences, these may include Geneious, Sequencher (commercial), Bioedit or MEGA (freeware) and other packages. The commercial software packages are preferred as only they are capable of assembling forward and reverse sequence trace files (electropherograms) simultaneously to produce a consensus sequence. Once edited and checked, the consensus sequence is searched against the Barcode of Life Data System (BOLD)(http://www.boldsystems.org).

DNA Barcode Analysis

Go to the 'Identification' heading and choose Animal Identification (COI) and select databases to search, paste in the sequence where indicated and submit. Sequences less than 100 bp long may be searched against the BOLD database, although best results are obtained with sequences meeting the barcode standard, i.e. >485 bp.

The BOLD identification system will deliver a species identification if the query sequence shows a tight match, <1%divergence, to a reference sequence together with access to a species page aggregating all the available information about the species in BOLD. Should the ID engine be unable to deliver a species identification, other results pages are generated as described by Ratnasingham and Herbert (2007). Appendix 8.5 lists the *Lymantria* spp. with data sets represented on the BOLD website to August 2014.

DeWaard *et al.* (2010) demonstrated clearly that COI distinguished *L. dispar* from its most closely related species, *L. umbrosa*. Indeed, their dataset distinguished all of the 34 species of *Lymantria* that they sampled. Their paper lists all the GenBank and BOLD accession numbers of the sequences used in their study in Supplementary Table S1. It is worth noting that the specimens used for DeWaard *et al*.'s study were expertly identified by co-author Michael Pogue, who also authored a taxonomic review of potentially invasive *Lymantria* species (Pogue & Schaeffer, 2007).

Chen *et al*. (2015) sampled additional material from China and Russia (Siberia) and found that the subspecies of *L. dispar* could not be distinguished using COI alone. However, Stewart *et al*. (2016) developed and validated TaqMan PCR assays, based on COI and nuclear gene sequences, which are capable of distinguishing all subspecies of *L. dispar*. In addition, Djoumad *et al*. (2017) demonstrated that use of supplementary mtDNA markers (ND2 and ATP6) can distinguish all subspecies of *L. dispar*, whereas COI alone cannot distinguish the two Asian subspecies from each other.

NOTE: *The BOLD database has grown rapidly in the past few years. Unfortunately, data curation efforts have not kept pace with data deposition and BOLD contains some errors. As a result, it is now more difficult to get an unambiguous identification. Until this is managed, it is advisable to consult with an expert in the area to check the sequence data and analytical methods if there are any ambiguities.*

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Taxonomy and identification of African Lymantriidae

Dr. Ugo Dall'Asta is now retired but is still willing to assist.

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Development of this protocol has had a long history. This version was compiled by Dr Margaret Williams, with molecular input from Andrew Mitchell.

An earlier Diagnostic Protocol prepared for Asian gypsy moth by Horak *et al*. (2006) provided the starting point, including all the taxonomic descriptions of *Lymantria* spp. and the diagnostic key used here to separate known Australian species of the genus *Lymantria* from gypsy moth.

Mitchell (2007), a report of overseas studies during the latter half of 2006, provided the starting points for information on Palaearctic and African species of *Lymantria* and the molecular methods.

Asian Gypsy Moth Pest Risk Review (Plant Health Australia 2006) contributed collated information on host plants for *L. dispar.*

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Dr. Catherine Byrne, Senior Curator of Zoology, Tasmanian Museum and Art Gallery, helped resolve conflicts with terminology.

The protocol was reviewed by Dr Roberta Hitchcock and the molecular section reviewed by Dr Dongmei Li, MPI NZ.

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8 APPENDICES

8.1 *Lymantria* spp. attracted to disparlure.

The following table has been prepared from Pogue and Schaefer (2007), Kamata (2001) and other resources for specific points as identified in the footnotes.

Common Name	Scientific name	Distribution (Principal host)
Okinawa gypsy moth	*Lymantria albescens* Hori and Umeno	Ryukyu Islands of Japan (reared on Eucalyptus in the Laboratory)[1]
Orange winged tussock moth	*Lymantria atemeles* Collenette	Malaysia, Thailand Cambodia, Vietnam (Mango)
Concolorous tussock moth	*Lymantria concolor* Walker	From Pakistan east to south China and Taiwan (reported to include apple, *Pinus kesiya* (benguet pine) *Quercus serrata, Q. incana*)
Asian gypsy moth	*Lymantria dispar asiatica* Vnukovskij	Asia mostly east of the Urals, China Korea (polyphagous)
European gypsy moth	*Lymantria dispar dispar (Linnaeus)*	Europe, western Asia, North Africa, eastern North America (polyphagous)
Japanese gypsy moth	*Lymantria dispar japonica* (Motschulsky)	All main islands of Japan (polyphagous)
Dissolute tussock moth	*Lymantria dissoluta* Swinhoe	China, Taiwan, Vietnam (conifers and deciduous hardwoods)
Red-bellied tussock moth	*Lymantria fumida* Butler	China, Japan, Korea (*Abies, Larix, Keteleeria, Juniperus)*
Dark mango tussock moth	*Lymantria marginata* Walker	Thailand, India, Sumatra, Java (Mango)
	Lymantria narindra Moore[2]	Malaya Java, Sumatra, Borneo (Cinnamon)
Indian gypsy moth	*Lymantria obfuscata* Walker	Northern India, Pakistan, Afghanistan (polyphagous)
Ryukyu gypsy moth	*Lymantria postalba* Inoue	Japan (*Livistonia subglobosa)*
Pulverea tussock moth	*Lymantria pulverea* Pogue and Schaefer	Taiwan (unknown)
	Lymantria singapura Swinhoe[3]	Burma, Malaya, Borneo, Indonesian Archipelago (*Pinus)*
Sinica tussock moth	*Lymantria sinica* Moore	China, Vietnam,Taiwan (not well known)
Hokkaido gypsy moth	*Lymantria umbrosa* (Butler)	Hokkaido (polyphagous)

[1] Nasu Y, Arita Y, Kimura M, and Ogata A (2004) Some Lepidopterous pests of *Eucalyptus* trees from Japan. *Japan Journal Applied Entomology & Zoology* **48**:123–133.
[2] http://www.mothsofborneo.com/part-5/lymantriini/lymantriini_1_1.php
[3] http://www.mothsofborneo.com/part-5/lymantriini/lymantriini_1_6.php

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8.2 Major and minor hosts of gypsy moth

Not an exhaustive list and compiled from many references

Major hosts

Quercus alba (white oak), *Quercus coccinea* (scarlet oak), *Quercus ellipsoidalis* (Northern pin oak), *Quercus garryana* (Garry oak), *Quercus ilex* (holm oak), *Quercus lobata* (California white oak), *Quercus montana* (basket oak), *Quercus muehlenbergii* (Chinquapin oak), *Quercus palustris* (pin oak), *Quercus petraea* (durmast oak), *Quercus robur* (common oak), *Quercus rubra* (northern red oak), *Quercus suber* (cork oak), *Quercus velutina* (black oak)

Minor hosts

Acer (maples), *Acer negundo* (box elder), *Acer platanoides* (Norway maple), *Acer rubrum* (red maple), *Acer saccharinum* (soft maple), *Acer saccharum* (sugar maple), *Alnus* (alders), *Alnus rhombifolia* (white alder), *Betula* (birches), *Betula alleghaniensis* (yellow birch), *Betula lenta* (sweet birch), *Betula papyrifera* (paper birch), *Betula populifolia* (grey birch), *Carpinus* (hornbeams), *Carya* (hickories), *Castanea sativa* (chestnut), *Corylus*, *Eucalyptus camaldulensis* (red gum), *Fagus* (beeches), *Fagus grandifolia* (American beech), *Fagus sylvatica* (common beech), *Fraxinus americana* (white ash), *Fraxinus pennsylvanica* (downy ash), *Glycine max* (soyabean), *Hamamelis virginiana* (Virginian witch-hazel), *Larix* (larches), *Larix kaempferi* (Japanese larch), *Larix occidentalis* (western larch), *Liquidambar styraciflua* (Sweet gum), *Litchi chinensis* (lichi), *Lithocarpus edulis*, *Malus* (ornamental species apple), *Malus domestica* (apple), *Ostrya virginiana* (American hophornbeam), *Picea abies* (common spruce), *Picea jezoensis* (Yeddo spruce), *Pinus* (pines), *Pinus contorta* (lodgepole pine), *Pinus echinata* (shortleaf pine), *Pinus resinosa* (red pine), *Pinus rigida* (pitch pine), *Pinus strobus* (eastern white pine), *Pinus sylvestris* (Scots pine), *Pinus taeda* (loblolly pine), *Pistacia vera* (pistachio), *Platanus acerifolia* (London planetree), *Populus* (poplars), *Populus grandidentata* (Bigtooth aspen), *Populus nigra* (black poplar), *Populus tremuloides* (trembling aspen), *Prunus* (stone fruit), *Prunus armeniaca* (apricot), *Prunus domestica* (plum), *Prunus serotina* (black cherry), *Prunus serrulata* (Japanese flowering cherry), *Pseudotsuga menziesii* (Douglas-fir), *Pyrus* (pears), *Quercus ilicifolia* (bear oak), *Robinia* (locust), *Robinia pseudoacacia* (black locust), *Salix* (willow), *Salix babylonica* (weeping willow), *Taxodium distichum* (bald cypress), *Tilia americana* (basswood), *Tilia cordata* (small-leaf lime), *Vaccinium* (blueberries), *Zea mays* (maize)

8.3 Distribution and biology

The gypsy moth *Lymantria dispar* is native to Europe and north Asia with several strains being recognised including, the European gypsy moth, Asian gypsy moth and perhaps a North American gypsy moth. The European strain was introduced from France to Massachusetts, U.S.A. in 1869 (Montgomery and Wallner 1988; Wallace 2003) but has since shown specialisation. It reached Canada in 1924. It is now permanently established in northeastern U.S.A. and eastern Canada. Keena and Moore (1998) reported there was no mating incompatibility between two European, four Asian and a North American strain. While it has been reported that females of European gypsy moth are flightless, but those of Asian gypsy moth are capable of flight and can disperse up to 40 km (Anon. 1991, Wallner 1996), this is not considered a reliable trait as it is not fixed in most populations (Reinieke and Zeibitz 1998, Keena *et al* 2008). The two strains can be distinguished by genetic markers (Garner and Slavicek 1996). Asian gypsy moth is native to northeastern Asia but now occurs in Europe along with European gypsy moth and has recently been reported from North America (Wallner 1996). Natural hybrids between the two strains occur in Europe; the female hybrid moths can fly. Asian gypsy moth has been the subject of successful eradication campaigns in some areas of North America and more recently New Zealand (USDA-APHIS 2004).

The biology and ecology of both the Asian and European forms of *L. dispar* are similar. The primary differences are: (i) Asian female moths fly (>20 km [>12 mi]) while European female gypsy moths are flightless; and (ii) the Asian strain has slightly different host preferences than the European strain (reviewed in Drooz 1985; Reineke and Zebitz 1998; Charlton *et al*. 1999; reviewed in Wallner 2000).

Gypsy moth egg masses may be found on trees, stones, walls, logs, and a range of outdoor objects. Eggs are laid in autumn. Each egg mass contains from 50 to more than 1000 eggs, and the mass is covered with yellowish fuzz from the abdomen of the female. Hatching begins in the spring depending on the climatic situation. The newly hatched larvae remain one to two days (Asian strain) or up to a week (European strain) with the egg mass before they climb vertically, and produce a strand of silk from their head. The wind catches the silk and the larvae balloon a few hundred metres to several km in the chance of finding a host. First instar larvae are about 3 mm long, and are black with long hairs. Second instar larvae are about 5 mm long, and brown with short hairs. Both first and second instars are capable of ballooning (in the European strain, but in the Asian strain only the first instar larva balloons). During the first three instars feeding occurs during daylight hours. From the fourth instar onwards larvae feed at night descending the tree during daylight to seek resting sites in the litter, or in the bark on the trunks of host trees. During outbreaks, when populations are at high density, feeding continues during the day. Larvae in instars four to six are similar to each other and may be light to dark grey in colour with flecks of yellow and long hairs that may be dark or golden in colour. These late instar larvae all have a very recognisable double row of tubercles along the back, usually five pairs of blue followed by six pairs of red (Figure 15). The larval stage lasts around six to eight weeks. Males usually have five instars and females six. The final-instar larvae are by far the most voracious feeders. On average a single larva consumes about one square metre of green foliage.

Pupation occurs in early summer, lasting two weeks. Larvae find a resting place on the trunk of the host tree, or on rocks or walls and surround themselves with a silken cocoon in which they pupate. Male pupae are about 1.5 cm long and female pupae about 3 cm long. Males emerge one or two days before females and at emergence both sexes are fully mature. Males of all strains are good fliers. There is one generation per year. The Asian gypsy moth female can fly very well, up 40 km, resulting in a four

to five times faster rate of spread than the non-hybrid European form or the North American strain, and is attracted to light. Females of the non-hybrid European strain and North American strain are fully winged but flightless while the hybrid form can fly up to 5 km.

After emergence females crawl to an elevated place, usually a tree trunk, and begin releasing a pheromone to attract males. Mating lasts up to one hour; females usually only mate once. Oviposition of the single egg mass then begins. All adults are short-lived, surviving for about one week. Embryogenesis commences soon after oviposition and fully formed larvae are complete in the eggs after about one month. Eggs undergo obligatory diapause. Any larvae that hatch in summer do not develop. Movement of egg masses attached to pieces of wood, outdoor furniture, vehicles, boats, cargo containers, etc. is a highly effective method of dispersal. Egg mass counting is a common method for monitoring infested areas to estimate population density and predict future outbreaks.

Large eruptive populations occur cyclically in gypsy moth but appear to be more frequent in the Asian form (every eight years which includes two years of devastation and two or three years for population build-up). In the USSR an indication of severe damage to broadleaf forests was given if egg masses contained 1000–1500 eggs and the egg density was over 500 per square metre. The Asian form differs from the European form in diapause requirements (a higher percentage of eggs do not need a diapause), the host range is more extensive, host preferences differ, some pheromone compounds are unique and pupation occurs on foliage instead of litter.

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8.4 Palaearctic Lymantria species

Diagnosis of the economically important *Lymantria* species of the Palaearctic region summarised from Pogue and Schaefer (2007) initially by Mitchell (2007) who had access to the unpublished manuscript. The table was edited as follows by reference to the published paper in preparing this Diagnostic Protocol.

- Each entry was compared with published text in Pogue & Schaefer (2007).
- Common names and authors for each species have been added.
- Nomenclature updated to agree with published names.
- North American *L. dispar dispar* has not been treated separately to the description of European populations of this subspecies.
- Information not considered essential to the purpose of the table has been excluded.
- Where available, information on larval food plants and female flight capability has been included in the Notes.
- Unambiguously diagnostic characters are shown in bold.

(NOTE: This table is a summary only. Pogue & Schaefer (2007) should be consulted for full descriptions of each species and illustrations of the diagnostic characters mentioned.)

Common Name	***Lymantria* Species**	**Distribution**	**Diagnosis**	**Notes (for references in these notes refer Pogue and Schafer, 2007)**
Subgenus: *Porthetria* Hübner, 1819			• Males have the forewing ground colour brown or white, as in most species, with a full complement of fascia consisting of the basal, antemedial, median, postmedial, and subterminal lines. These lines usually are a series of connected chevrons, or curved spots that can vary with intensity and with shading between them. • All species are sexually dimorphic with the females larger and with a white forewing ground colour. • Female forewing shape is more of an elongate triangle than triangulate in the male. The forewing fascia are solid lines and can be reduced in number or nearly absent. • Valve in male genitalia is characteristic having an elongate arm that is either an extension of the costa or can extend from the middle of the valve. • Tymbals on the third abdominal sternite are absent. • Female ovipositor is not telescopic.	Type species: *Phalaena dispar* Linnaeus, 1758:501; designated by Kirby, 1892:475

Common Name	***Lymantria* Species**	**Distribution**	**Diagnosis**	**Notes (for references in these notes refer Pogue and Schafer, 2007)**
European Gypsy Moth, in USA called North American Gypsy Moth	*dispar dispar (Linnaeus)*	Europe, western Asia and N. Africa Eastern North America	• Males generally smaller in *L. d. dispar* than in *L. d. asiatica* and *L. d. japonica*. • Females also smaller, slightly reduced wing size, but not able to fly as in *L. d. asiatica*. • Larvae with area between D verrucae on abdomen of *L. d. dispar* has a prominent white pattern on a less evident solid colour whereas in *L.d. asiatica* solid colour is prominent and white pattern less evident. • The small D_1 verrucae have a black primary seta in *L. d. dispar* and this seta is white in *L. d. asiatica.* • Along the anterior margin of abdominal segments 1-7 are a pair of irregular shaped white spots with a grey centre in *L. d. asiatica* and in *L. d. dispar* these spots are absent. • Infrequent black-backed mutants occur where these colour variations do not hold true.	• Females winged but flightless • No significant difference between *L. d. dispar* and *L. d. asiatica* in either male or female genitalia • Male flight diurnal • Highly polyphagous
Asian Gypsy Moth	*dispar asiatica* Vnukovskij	Asia mostly east of Urals, S & E Siberia, Russia, Korea, north and central China, and Tibet	• Hindwing in some male specimens can be a dark brown and lack the contrasting marginal band that is usually found in *L. dispar.* • Late stage larvae can be found which exhibit the black-backed form but it is very limited in its percentage of the population	• Flight capable females (fly to lights) • Differs from *L. d. dispar* in size, distribution, and the ability of the female to fly.
Japanese Gypsy Moth	*dispar japonica* (Motschulsky)	Main islands of Japan, limited in Hokkaido	• Males are the largest of all [sub]species covered in the review of Pogue and Schaefer (2007) and are the darkest brown of all close relatives. • The males of *L. d. japonica* are very similar to *L d. asiatica,* but *L. d. japonica* has a dark brown marginal band on the hindwing and in *L. d. asiatica* the wing lacks a definite band. • The female wings of *L. d. japonica* have a distinct brown cast and are not as white as *L. d. asiatica*. • In the male genitalia, the saccus of *L. d. japonica* tends to be wider than in *L. d. asiatica* and the apex of the sacculus is curved in *L. d. japonica* and not produced as in most specimens of *L. d. asiatica*.	• Flight capable females

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
			• The male genital capsule of *L. d. japonica* is approximately 1.25–1.5x larger than that of *L. d. dispar.* The dorsal margin of the juxta is concave to slightly convex in *L. d. japonica* and convex in *L. d. dispar.* • Larvae show considerable variation in colour and patterning, being intermediate in colouration and pattern between *L. dispar asiatica* and *L. umbrosa* • Among late stage larvae, the black-backed mutant form is found to a limited extent and is very similar in appearance to that illustrated for *L. d. asiatica*	
Hokkaido Gypsy Moth	*umbrosa* (Butler)	Hokkaido, esp. eastern part	• There is no significant difference (from *L. dispar*) in either male or female genitalia • Forewing ground colour generally cream in *L. umbrosa* as compared to brown in both *dispar*. Markings similar to *L. d. asiatica* being less distinct than in *L. d. dispar*. • Female has more rufous cast to the longer scales of the wings and body. Also fewer forewing markings than either *L. d. dispar* or *L. d. asiatica.* • The hindwing has more white than either *L. d. dispar* or *L. d. asiatica,* and the dark margin contrasts with the lighter ground colour and shares this distinct band with *L. d. dispar*. • The larvae are quite different in *L. umbrosa* than in either *L. d. dispar* or *L. d. asiatica* by possessing the wide dorsal stripe. • In *L. umbrosa* the primary setae on the D_2 verruca are dark brown and white, but are black in *L. d. dispar* and *L. d. asiatica*. • The underside is speckled black and white in *umbrosa* and in *dispar* and *asiatica* it is a solid colour with little or no pattern.	• Flight capable females able to fly to lights at night. • Smaller males. • Crosses between *L. d. dispar* and *L. umbrosa* are male lethal. • Often feeding on Japanese larch, *L. umbrosa*, but its polyphagous nature would allow it to feed on a number of alternate hosts including a variety of oaks.
Indian Gypsy Moth	*obfuscate* Walker	N. India, Pakistan & Afghanistan	• General colouration and pattern is characteristic of *L. d. dispar,* but smaller. • The forewing border is less distinct and resembles the overall colour of the wing in *L. obfuscata,* whereas in *L. d. dispar* there is a definite dark brown border. • The hindwing border is more sharply defined in *L. obfuscata* than in *L. d dispar.*	• Female brachypterous, flightless. • This is the N. India component of *Lymantria* (*Porthetria*). Literature reports of *L. obfuscata* from S. India are based on a misidentification of *L. ampla* (Walker) and those findings should apply to that species (Chacko and Singh 1990). • *L. obfuscata* also is separated from *L. dispar* based on mtDNA data (Ball and

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
			• Male genitalia with saccus a broader V-shape with a more pointed apex in *L. obfuscata.* In *L. d. dispar* the saccus is narrower and the apex tends to be broader. • Sacculus with apex straight and not produced. In *L. d. dispar* sacculus generally has a produced apex. • In the female abdominal hairs that cover the normal egg masses there is a preponderance of hairs that are elbowed, or contain a rather abrupt bend, usually near the proximal end of the seta (Roonwal 1954).	Armstrong 2006) and pheromonal communication evidence (Gries, Schaefer *et al*. unpubl.). • Potential for invasion is minimal because of the inability of females to fly.
Okinawa Gypsy Moth	*albescens* Hori and Umeno	Okinawa, S. Ryukyu Islands.	• Male forewing ground colour is white suffused with grey, especially along the costa and near base. The lines of the forewing vary with intensity from being quite distinct to almost absent. • Forewing outer margin colour is dark grey as compared to brown in *L. apicebrunnea* Gaede. • The marginal band in the hindwing is also variable, being more distinct and well developed in darker individuals and only a diffuse spot near outer apex in lighter specimens (it is less evident in *L. apicebrunnea*). • *L. albescens* and *L. apicebrunnea* share the broad costal margin of the hindwing, but it is dark grey in *L. albescens* and brown in *L. apicebrunnea.* • The distal process in the male genitalia is straight in *L. albescens* and curved dorsally in *L. apicebrunnea*. The distal process is longer and the apex is slightly expanded in *L. albescens* compared to *L. dispar*. • Egg masses are indistinguishable from those of *L. xylina* except for a slight colour tone difference.	• Flight capable females • Has been treated as a subspecies of *L. dispar* but differences in male genitalia sufficient to establish *L. albescens* as a distinct species.
Ryukyu Gypsy Moth	*postalba* Inoue	Japan	• *L. postalba* can be separated from *L. albescens* by its smaller forewing length, 21–27 mm, versus > 30 mm in *L. albescens.* • Forewing ground colour in male is brown in *L. postalba* and white or white suffused with grey in *L. albescens.* • Male with white hindwing	• Flight capable female flying to lights. • *L. postalba* has a more northerly distribution than *L. albescens.* • Invasion potential appears minimal because of the limited native range, the remote island habitat, and minimal levels of commerce.
Non-dispar complex species:				

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
	apicebrunnea Gaede	China (Guangdong, Sichuan and Yunnan Provinces)	• *L. apicebrunea* resembles *L. xylina,* but *L. xylina* lacks the brown forewing margin, has a pink neck, pink on the legs, and a pink underside which *L. apicebrunnea* lacks. • The labial palpus is larger and black in *L. xylina* and smaller and white in *L. apicebrunnea*. • The saccus in the male genitalia is wider and stouter in *L. xylina* than the narrow saccus in *L. apicebrunnea*.	• Larva unknown. • Potential for invasion appears minimal but little is known about the biology and behaviour of this species.
Brown-bordered gypsy moth	*brunneoloma* Pogue and Schaefer	China, Yunnan	• Males very similar to *L. apicebrunnea,* but lacking the small tuft of pink scales just behind the head on the pronotum, found in *L. apicebrunnea.* • Forewing length shorter in *L. brunneoloma* and the outer margin has a much wider brown border than in *L. apicebrunnea.* • The subterminal line is not as deeply scalloped in *L. brunneoloma* as it is in *L. apicebrunnea.* • The postmedial line is discernable only as a faint spot along the posterior margin of the forewing in *L. brunneoloma*, but it is a well-defined scalloped line in *L. apicebrunnea.* • The male genitalia has a straight dorsal process in the valve of *L. brunneoloma* and in *L. apicebrunnea* it is curved toward the apex. • The saccus is short with a truncated apex in *L. brunneoloma* and in *L. apicebrunnea* it is elongate and narrow with a rounded apex.	Female and larva unknown.
Casuarina tussock moth	*xylina* Swinhoe	Japan, Taiwan, and China (Fujian, Guangdong)	• *L. xylina* resembles *L. apicebrunea,* but *L. xylina* lacks the brown forewing margin, has a pink neck, pink on the legs, and a pink underside which *L. apicebrunnea* lacks. • The females of *L. xylina* and *L. apicebrunnea* are more similar than the males. The angulate postmedial line in *L. apicebrunnea* is somewhat more crenulate than the straighter line in *L. xylina.* • The labial palpus is larger and black in *L. xylina* and smaller and white in *L. apicebrunnea*. • The saccus in the male genitalia is wider and stouter in *L. xylina* than the narrow saccus in *L. apicebrunnea*.	• Polyphagous, frequent pest of *Casuarina* windbreaks in Taiwan and China. • Females fly to lights at night and in areas around ports might lay eggs on shipping containers or transoceanic vessels. • Responds to xylinalure

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
Detersa tussock moth	*detersa* Walker	Southern India (Ahmednagar, Bombay, Belgaum, Nagrishpur, Poona, S. Coorg)	• Similar to *L. obfuscata.* • In *L. detersa* the hindwing ground colour is dirty white and in *L. obfuscata* the hindwing ground colour is dark reddish brown. • The male genitalia is similar to *L. obfuscata*, but the juxta is different. Also the distal process is shorter and more robust in *L. detersa* and more elongate and slender in *L. obfuscata*.	• Females brachypterous, flightless. • The brown forewing ground colour and zigzag postmedial line are characters that are shared with some species related to *L. dispar*. • *L. detersa* is a tropical species with similarities to *L. d. dispar* but wings highly atrophied.
Subgenus: *Lymantria* Hübner, [1819]			• Most species have a white forewing with distinct black fascia and a distinct V-shaped spot at the distal end of the discal cell. Some species have a grey or greyish-brown forewing ground colour with less distinct fascia. • The hindwing fringe has a checked pattern. • The valve in the male genitalia has a produced costal process similar to that of the subgenus *Porthetria*, but usually shorter than the valve. • A basal process is present at the base of the valve and is variously shaped and appressed to the inner surface of the valve.	Type species: *Phalaena monacha* Linnaeus, 1758:501; designated by Moore, [1883]:99
Nun moth	*monacha* (Linnaeus)	Eurasia	• Similar to *L. concolor* but *L. concolor* with more strikingly marked forewings and more pink on abdomen. • Tegumen with lateral process present	• In Europe a high percentage of melanistic adults occur while in Japan and Korea only the non-melanistic form is found. • Sex pheromone communication and periodicity of response support the designation of an "Asian form" and a "European form." • This moth appears more of a serious pest in Europe than is *L. d. dispar*.
Pulverea tussock moth	*pulverea* Pogue and Schaefer	Taiwan	• Male genitalia with length of dorsal process of valve half as long in *L. pulverea* than in *L. monacha*. • Basal process of valve more or less fused with the valve and only represented by a small spine-like process in *L. pulverea* but is well developed and thumb-like in *L. monacha*. • Saccus a broad V-shape in *L. pulverea* but narrower and constricted in *L. monacha*.	• Most similar to *L. monacha* and difficult to distinguish without dissection but *L. pulverea* responds to disparlure

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
Concoloro us tussock moth	*concolor* Walker	Pakistan, N. India, Bhutan, Tibet, Nepal, Myanmar, Thailand, south China and Taiwan.	• The forewing markings are fainter in *L. concolor* and heavier and bolder in *L. monacha*. The postmedial and subterminal lines are thin and separated in *L. concolor*, whereas in *L. monacha* these lines are thicker (especially the postmedial line) and closer together. • Female abdomen is pink in the anterior half in *L. concolor* and white becoming pale pink at posterior in *L. monacha*. • Male genitalia are similar, but the dorsal process is shorter in *L. concolor* than in *L. monacha*, and the saccus is wider in *L. concolor* than in *L. monacha*.	• Found up to 7,000 feet elevation
Minomonis tussock moth	*minomonis* Matsumura	Northern India, China, Taiwan, Japan (Honshu, Shikoku, Ryokyu Islands including Okinawa)	• Most similar to *L. concolor* in overall wing pattern and colouration. • Male genitalia most similar to *L. concolor*, but easily separated by size and shape. In *L. minomonis* the dorsal process of the valve is shorter and thicker than in *L. concolor* and the basal projection is rectangular in *L. minomonis* and triangular in *L. concolor*. • The saccus narrow, U shaped.	• *L. minomonis* is more southerly distributed, seemingly replacing *L. monacha*, with which it has been confused in the past.
	similis Moore [While this species was included in the manuscript viewed by Mitchel, it was excluded from the final publication because it is a tropical Asian species and out of scope for the publication (Pogue *pers. comm*. 2013)].	India (Assam, Calcutta and Sikkim), Bhutan, Myanmar, Thailand, China (Yunnan), Indonesia (Sumatra), Papua New Guinea, Philippines	• Male of *L. similis* shares overall forewing pattern of *L. monacha, L. minomonis*, and *L. concolor*, but is easily separated from these species. • *L. similis* is larger than *L. monacha,* the lines in the forewing are heavier in *L. monacha* than in *L. similis,* and the hindwing is white in *L. similis* and grey in *L. monacha.* • Not all of the forewing lines in *L. minomonis* are complete as they are in *L. similis*, the hindwing colour is grey in *L. minomonis* and white in *L. similis*, and the abdomen is pink in *L. minomonis* and white in *L. similis*. • The lines in the forewing are black and heavy in *L. concolor*, but thinner and browner in *L. similis*, the hindwing is grey in *L. concolor* and white in *L. similis*, and the abdomen is pink in *L. concolor* and white in *L. similis*. • Female of *L. similis* is most like that of *L. minomonis*, both having incomplete forewing lines, but the hindwing is grey in *L. minomonis* and white in *L. similis*.	• Potential for invasion appears quite minimal, but females are attracted to outdoor lighting. • The female forewing markings are much reduced as compared to the male. The hindwing submarginal band is barely present in the female. The form *niasica* is a female of *L. similis,* but has a grey hindwing. • Subspecies *L. monachoides* has somewhat bolder and more defined forewing markings and a slightly more smoky grey hindwing than *L. similis* from India. • Subspecies *L. loeffleri* is identical to *L. similis* in forewing maculation and has a white instead of smoky grey hindwing. • The synonym, *L. cara* Butler, is the female of *L. similis.*

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
Umbrifera tussock moth	*umbrifera* Wileman	Taiwan and SW China (Jiangsu, Zhejiang, Hebei, Hunan and Jiangxi Provinces)	• Size of *L. umbrifera* is close to *L. monacha*, but easily distinguished by the grey forewing colour, diffuse dark marginal border in the hindwing, and pink abdomen. *L. monacha* has forewing ground colour white (as do most other species in subgenus *Lymantria).* • The forewing lines are incomplete and are irrorated with dark grey scales giving the overall appearance of "fuzzy" or out of focus fascia. • Male genitalia similar but costal process of valve shorter in *L. umbrifera* than in *L. monacha* and saccus V-shaped in *L. umbrifera* and U-shaped in *L. monacha*.	
Dissolute tussock moth	*dissoluta* Swinhoe	China (Anhui, Jiangsu, Jiangxi, Hubei, Hunan, Guangdong, incl. Hong Kong), Taiwan and Vietnam	• *L. dissoluta* is a small greyish-brown species with faint forewing markings, except for a distinct dark bar along the distal margin of the discal cell. A very slight hint of a marginal band is on the hindwing.	• Since it may cause serious damage to pine species, it represents a potentially dangerous invasive to semi-tropical areas where pines are common.
Sinica tussock moth	*sinica Moore*	China (Shanghai, S. to Kwangtung and inVietnam and Taiwan	• *L. sinica* resembles a pale grey *L. monacha*, but the spot at the end of the discal cell is pronounced in *L. sinica*. In *L. monacha* it is not pronounced but resembles the other forewing markings. • Male genitalia are distinct in *L. sinica*, with the bifurcate ventral process of the valve. No other *Lymantria* have this character.	
Lucescens tussock moth	*lucescens* (Butler)	Japan (Hokkaido, Honshu, Kyushu) and Korea	• In Japan *L. lucescens* is most similar to *L. umbrifera* in overall forewing colouration and maculation. Differences include: forewing length is larger in *L. lucescens*; abdomen in *L. lucescens* is various shades of grey with only a hint of light pink scales laterally, whereas in *L. umbrifera* the abdomen is pink; the discal spot in the hindwing is more distinct in *L. lucescens* than in *L. umbrifera*. • Male genitalia are distinct between these two species. The most obvious difference is the extremely elongate and narrow saccus in *L. lucescens*; in *L. umbrifera* the saccus is a broad triangle.	• Invasion potential is moderate because of the ability of females to fly considerable distances , their tendency to respond to outdoor lighting and production of well-hidden egg masses.

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
	Subgenus: *Beatria* Schintlmeister, 2004		• Shape of the male forewing is characteristic of this subgenus: triangulate, with straight margins and a sharp obtuse angle where the outer and posterior margins meet. • Tymbals are absent in the male abdomen. • The hindwing in the female is white with a wide, black marginal band that can be either solid or with a few white spots. • Male genitalia have a triangular valve with a curved, elongate dorsal projection.	• Type species: *Phalaena Bombyx beatrix* Stöll, 1790:173; original designation.
Dark mango tussock moth	*marginata* Walker	Indonesia, India, Myanmar, N. to Tibet and China	• Female forewing ground colour can be white with black markings or light brown with dark brown markings. • Male hindwing in *L. marginata* is brown with small white patches along margin. • Male genitalia in *L. marginata* have an elongate, curved dorsal process on the valve which lacks a subapical process.	• Considerable sexual dimorphism in *L. marginata* with the small dark male and the large white female.
Orange-winged tussock moth	*atemeles* Collenette	Malaysia, Thailand, Cambodia, and Vietnam	• Male of *L. atemeles* forewing ground colour pale grey to brown with black markings. • Male hindwing in *L. atemeles* is yellow with a broad black marginal band. • Female is more heavily marked and somewhat smaller in *L. atemeles* than in *L. marginata*. • Male genitalia in *L. atemeles*, the dorsal process on the valve has a small subapical process.	• Forewing fascia in males can become browner in older specimens while the yellow hindwings are unique. • This tropical species may have invasive potential among tropical regions providing they share mango cultivation (larva eats mango leaves).
Subgenus *Nyctria* Schintlmeister, 2004			• Male forewing ground colour is variable including white, yellow, greenish-grey, and brown, with a dark brownish-grey pattern. • Female forewing has a white ground colour with a dark brown to brownish-grey pattern. • Hindwing of female is flushed with pink and with a broken submarginal band that can vary from bold to faint. • Male genitalia have a lateral digitate process arising from the ventral margin of the tegumen and the valves are deeply divided.	• Type species: *Lymantria mathura* Moore, 1865:501; original designation

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
Pink gypsy moth	*mathura* Moore	Widespread in E. Asia, N. to Hokkaido, Japan, Nepal, India (Ussuri and Amur), and S. to Vietnam and Sri Lanka	• *L. mathura* and *L. flavida* are very similar but have subtle differences. • Male forewing shape is more pointed in *L. mathura* and more rounded in *L. flavida*; veins are white in *L. mathura* and yellow in *L. flavida*; and the hindwing fringe is white in *L. mathura* and yellow in *L. flavida*. • Female has narrow V-shaped reniform spot at the end of the discal cell in the forewing; this spot is much wider in *L. flavida*. • In *L. mathura* the pink on the dorsal surface of the abdomen extends approximately two thirds the length of the abdomen, in *L. flavida* it extends to about half the abdominal length. • Larvae in *L. mathura* have black setae on XD1 verruca and a yellowish-brown stripe angled from the D verruca on A4 to the L verruca on A5.	• Males of *L. mathura* often have melanic forms. The white of the forewing is pale grey and the wing markings are black. The hindwing is dark brown with black markings. • Polyphagous, hosts include mango. • Attracted to lights.
Flavid tussock moth	*flavida* Pogue and Schaefer	Japan (Okinawa)	• There are very subtle differences in the male genitalia of *L. mathura* and *L. flavida* similar to those in *Lymantria (Porthetria)* species. The lateral process of the tegumen is shorter and the apex is round in *L. flavida*, but in *L. mathura* the lateral process is longer and its apex is slightly narrowed. The ventral process of the valve has parallel sides in *L. flavida*, but in *L. mathura* the sides slightly converge toward the apex resulting in the process being wider at the base than at the apex. • The female ovipositor is shorter in *L. flavida* than in *L. mathura*. The ostium bursae in the female genitalia is a large ovate shape in *L. flavida* and is a smaller ventrally produced flap in *L. mathura*. • Larvae of *L. flavida* have white setae on XD1 verruca and on A5 there are a pair of curved bars on *L. flavida*.	• Females fly strongly and are attracted to outdoor lighting.
Subgenus: *Collentria* Schintlmeister, 2004			• Subgenus *Collentria* has violet-brown forewing ground colour, a brown pattern, and a rounded apex. There is a prominent discal spot and a series of three dashes in the tornal area of the forewing that are part of the subterminal line. These markings are also present on the female. • Male genitalia variable and those of *L. grisea* Moore and *L. fumida* Butler are easily separated.	• Type species: *Lymantria grisea* Moore, 1879:55; original designation
Grisea's tussock moth	*grisea* Moore	Nepal, NE India, Myanmar, N. Thailand, SW (Yunnan) and E.	• *L. grisea* is a much paler species overall than *L. fumida*. The male forewing ground colour is white in *L. grisea* and brown in *L. fumida* and the pattern is virtually identical.	

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
		China, and Taiwan	• The male hindwing in *L. grisea* is white with barely a hint of a marginal band and brown with a marginal band in *L. fumida*. • The male genitalia of *L. grisea* is unique in *Lymantria* with the shape of the valve and the elongate pointed projections on the dorso-medial margin of the tegumen.	
Red-bellied tussock moth	*fumida* Butler	Japan (Honshu, Shikoku, Kyushu), Korea, China (as far SW as Yunnan Province)	• There is some variation in the forewing ground colour in both sexes. The males are generally darker than the females, but light coloured males are similar to dark females. In males the forewing ground colour can be a rich, deep brown to brown; the females can have a similar brown ground colour or it can be white, irrorated with brown scales. • *L. fumida* is darker than *L. grisea* as discussed above. • The valve has multiple projections in the male genitalia of *L. fumida* and only two very small projections in *L. grisea*. The saccus is very narrow in *L. fumida* and very wide in *L. grisea*. The vesica in *L. fumida* is covered with minute cornuti and these are absent in *L. grisea*.	• Stenophagous on Pinaceae and Cupressaceae
Subgenus: *Spinotria* Schintlmeister, 2004			• This subgenus forms a compact group characterized by a ventro-medially fused valve that is divided into dorsal and ventral arms. The ventral arms are longer than the dorsal. • The saccus is evenly curved ventrally.	• Type species: *Bombyx serva* Fabricius, 1793:474; original designation
Ficus tussock moth	*serva* (Fabricius)	Taiwan, Hong Kong, south China (Yunnan Province), Nepal and India	• *L. serva* is similar to *L. hreblayi* in overall forewing colour and pattern. The reniform spot is more distinct in *L. serva* than in *L. hreblayi*. The fine brown line that separates the margin of the hindwing from the fringe in *L. serva* is absent in *L. hreblayi* and the spots on the fringe in *L. hreblayi* are more distinct and darker than in *L. serva*. • Male genitalia different from *L. hreblayi* (see below).	• Nearly stenophagous on *Ficus* spp.
Laszloronkayi's tussock moth	*laszloronkayi* Schintlmeister	northern areas of Vietnam, Laos, Thailand and China (Yunnan and Sichuan Provinces)	• *L. laszloronkayi* has the same general indistinct pattern as *L. serva*, but has a bold black bar subbasally below the M vein, which is absent in *L. serva*. • Male genitalia in *L. laszloronkayi* differ from *L. serva* in the shape of both the dorsal and ventral processes. The dorsal process is short and triangular in shape in *L. laszloronkayi* and in *L. serva* it is longer, with a spine-like apical projection.	• Food plants unknown

Common Name	*Lymantria* Species	Distribution	Diagnosis	Notes (for references in these notes refer Pogue and Schafer, 2007)
Hreblay's tussock moth	*hreblayi* Schintlmeister	northern Vietnam, Yunnan, and Sichuan Provinces in China	• *L. hreblayi* has the same general indistinct pattern as *L. serva*, but is larger. The reniform spot, which outlines the veins at the end of the discal cell is distinct in *L. serva*, in *L. hreblayi* the reinform spot is obscured by the suffusion of black scales that make the other forewing pattern elements indistinct. • This species resembles *L. serva*, but the male genitalia are quite different. In *L. hreblayi* the ventral process is roughly the same width from base to slightly narrower apex as in *L. serva* but this process in wider and the apex has a spine-like apical process, in *L. serva*.	• Food plants unknown
Iris tussock moth	*iris* Strand	China and Taiwan (perhaps Vietnam and NE India – see notes)	• *L. iris* has a more brownish forewing ground colour and is smaller than *L. bantaizana*, which has a greyish ground colour. The black spots on the fringe of the wings are more distinct in *L. bantaizana* being much less obvious in *L. iris*. • *L. obsoleta*, *L. iris*, and *L. serva* have distinct male genitalia. The laterally angled ventral process is unique for *L. iris*.	• Several *Ficus* spp. hosts
Bantai tussock moth	*bantaizana* Matsumura	Japan	• *L. bantaizana* is a grey species with both sexes similar. • Male genitalia differ from *L. iris* by the ventral process of the valve being straight, with a slight medially curved apex. • The male genitalia of *L. bantaizana* and *L. laszloronkayi* are very similar, but the cornuti are larger and more conspicuous in *L. bantaizana* than in *L. laszloronkayi*.	• Stenophagous on Juglandaceae.
Albolunulata tussock moth	*albolunulata* Moore	SW China to the Himalayan regions of N. India, including Bangladesh and Thailand	• *L. albolunulata* is similar to *L. serva*, but the forewing pattern is darker and more contrasting in *L. albolunulata* than the more washed out appearance of *L. serva*. The female abdomens of *L. serva* are pink as compared to brown with some pink in *L. albolunulata*. • The male genitalia of *L. albolunulata* are similar to *L. hreblayi*. The dorsal process is perhaps more bulbous basally and the apex is evenly produced and longer in *L. albolunulata*.	• Larvae reared on *Quercus* spp. (Fagaceae).

8.5 Lymantria spp. listed on the BOLD website as at August 2014.

The list shows gypsy moths of biosecurity concern to Australia, plus the inclusion of *L. similis* identified during this protocol development, species attracted to disparlure and species of the genus recorded in Australia. (Species name hyperlinked to the individual entry in BOLD.)

Lymantria* spp. *in BOLD	**Gypsy Moths of Biosecurity Concern (Australia)**	**Attracted to Disparlure**	**Species recorded in Australia**
Lymantria WS01Th [1]			
Lymantria albescens [13]		Y	
Lymantria alexandrae [1]			
Lymantria ampla [3]			
Lymantria antennata [53]		Y (in NSW, Ingram 2010)	Y
Lymantria atemeles [5]		Y	
Lymantria atlantica [1]			
Lymantria bantaizana [4]			
Lymantria brunneiplaga [1]			
Lymantria caphodes [1]			
Lymantria concolor [6]		Y	
Lymantria dispar [285]	Y	Y	
Lymantria dispar asiatica [10]	Y	Y	
Lymantria dispar dispar [80]	Y	Y	
Lymantria dispar japonica [4]	Y	Y	
Lymantria dissoluta [7]		Y	
Lymantria ekeikei [1]			
Lymantria fergusoni [1]			
Lymantria flavida [6]			
Lymantria fumida [6]		Y	
Lymantria grisea [2]			
Lymantria grisea kosemponis [1]			
Lymantria hollowayi [1]			
Lymantria kinta [2]			
Lymantria libella [2]			
Lymantria liedgensi [1]			
Lymantria lucescens [4]			
Lymantria lunata [8]			Y
Lymantria marginata [3]		Y	
Lymantria mathura [26]	Y		
Lymantria mathura subpallida [1]	Y		
Lymantria meyi [1]			
Lymantria microcyma [6]			

Lymantria* spp. *in BOLD	**Gypsy Moths of Biosecurity Concern (Australia)**	**Attracted to Disparlure**	**Species recorded in Australia**
Lymantria microstrigata [1]			
Lymantria mikkolai [1]			
Lymantria minahassa [1]			
Lymantria minomonis [4]			
Lymantria minomonis sugii [1]			
Lymantria minora [2]			
Lymantria modesta [16]			
Lymantria monacha [87]	Y		
Lymantria naesigi [1]			
Lymantria narindra [1]		Y	
Lymantria nebulosa [1]			
Lymantria nephrographa [7]			Y
Lymantria ninayi [5]			
Lymantria novaguineensis [1]			
Lymantria obfuscata [9]		Y	
Lymantria panthera [2]			
Lymantria pelospila [7]			Y
Lymantria plumbalis [9]			
Lymantria pulverea [2]		Y	
Lymantria rhapdota [1]			
Lymantria roseola [2]			
Lymantria rubroviridis [4]			
Lymantria sarantrija [1]			
Lymantria schaeferi [1]			
Lymantria semperi [1]			
Lymantria serva [2]			
Lymantria similis [1]	Y		
Lymantria singapura [1]			
Lymantria sinica [4]		Y	
Lymantria sp. [6]			
Lymantria strigata [1]			
Lymantria sublunata [4]			
Lymantria subrosea [4]			
Lymantria todara [1]			
Lymantria umbrifera [5]			
Lymantria umbrosa [25]	Y	Y	
Lymantria vacillans [25]			
Lymantria xylina [7]	Y		

8.6 Lymantria DNA barcoding on preserved specimens (Mitchell 2015)

This procedure is published in "Mitchell A (2015) Collecting in collections: a PCR strategy and primer set for DNA barcoding of decades-old dried museum specimens. *Molecular Ecology Resources* 15, 1102–1111." and reproduced with permission. It was not verified as part of the original review process.

Mitchell (2015) found that for insects recently preserved (under 3 years in alcohol) the primer pair AMbc0f1m-AMbc0r1m was successful (Table 1). Older specimens required a two-step amplification strategy:

1. Two PCRs are performed using primer pairs AMbc0f1m - AMbc5r1m and AMbc3f1m-AMbc3r1m which target overlapping 5'- and 3'- amplicons, respectively.
2. The PCR products from step 1 are used as the DAN template for reamplification PCRs using primer pairs M13F-AMbc5r2m and AMbc3f2m-M12R-pUC (-40) respectively.

PCR solution (Quantity 15 µL)

Prepared using Invitrogen Platinum Taq PCR Kit (Life Technologies, Mulgrave Australia)

- 1 X PCR buffer
- 2.8 mM $MgCl_2$
- 200 µM dNTP mix
- 2 pmol of each forward and reverse primers
- 4 µL of genomic DNA extraction (or 1 µL of 1:10 diluted PCR product for reamplification reactions)
- 0.375 U (0.075 µL) Platinum® Taq DNA polymerase

PCR Amplification Conditions

- 94˚C for 2 min
- 40 cycles (35 for reamplification) of :94˚C for 30 s, 50˚C for 40 s, 72˚C for 60 s)
- 72˚C for 7 min
- 10˚C storage

Table 1. Primers developed by Mitchell (2015)

Primer name*	**Primer sequence (5'-M13 tail separated from gene-specific sequence by a hyphen)**	**Notes**
AMbc0f1m	GTAAAACGACGGCCAGT-TCWACWAAYCAYAARRWTATYGG	Based on BC1Fm (Cho *et al.* 2008) but incorporates the additional degeneracy of BC1culicFm (Bellis *et al.* 2013). Binds to the same site as LCO1490 (Folmer *et al.* 1994)
AMbc0r1m	CAGGAAACAGCTATGAC-AAAATRTAWACYTCDGGRTGNCC	Based on Scar-3RDm (Mitchell & Maddox 2010).
AMbc0r2m	CAGGAAACAGCTATGAC-CAAARAAYCARAAYARRTGYTG	Based on JerR2m from Bellis *et al.* (2013) but more degenerate and one base shorter on 5'-end (where the last 3 nt of M13 sequence now matches the COI template, coincidentally). Sequence of JerR2m reported incorrectly in Gopurenko *et al.* (2013).
AMbc5r1m	CAGGAAACAGCTATGAC-GADARWGGNGGRTANACDGTTC	Based on Scar-2RDm reported in Gopurenko *et al.* (2013) but more degenerate. Sequence of Scar-2RDm reported incorrectly in Gopurenko *et al.* (2013).
AMbc5r2m	CAGGAAACAGCTATGAC-GTTCANCCNGTWCCWGCNCC	
AMbc3f1m	GTAAAACGACGGCCAGT-GCHCCHGAYATAGCNTTYCCNCG	Based on Scar-3aFm of Gopurenko *et al.* (2013) but sequence reported incorrectly in Gopurenko *et al.* (2013).
AMbc3f2m	GTAAAACGACGGCCAGT-TTYCCNCGRMTRAAYAAYATNAG	Combines the degeneracy of both miniScarFm (designed for Coleoptera) and miniLepFm (designed for Lepidoptera) so this single primer can be used for both taxa.
AMbc3r1m	CAGGAAACAGCTATGAC-ARYATNGTRATNGCNCCNGC	
M13F	GTAAAACGACGGCCAGT	M13 forward sequencing primer
M13R-pUC(-40)	CAGGAAACAGCTATGAC	M13 reverse sequencing primer

*Naming convention used: "AM" denotes author; "bc" denotes barcode; "0", "5" or "3" denote whether primers target the end of the barcode region (for amplifying the entire region), or the 5'-half or 3'-half only; "f" and "r" denote forward and reverse; "1" or "2" denotes whether the primer is intended for use as a first round (external) primer, or a second round (internal) primer for reamplification reactions; "m" denotes 5'-M13 tail incorporated into sequence.