Chapter 5. Collection, preservation, slide-mounting, labelling and vouchering of scale insects.

Chapter · July 2022

DOI: 10.1079/9781800620643.0005

2 authors:

Takumasa Kondo
Agrosavia
311 PUBLICATIONS **2,137** CITATIONS
SEE PROFILE

Gillian W Watson
Natural History Museum, London
153 PUBLICATIONS **2,533** CITATIONS
SEE PROFILE

Note. This is a Preprint. Please cite this Book Chapter as follows:

Kondo, T. & Watson, G.W. (2022) Chapter 5. Collection, Preservation, Slide-mounting, Labelling and Vouchering of Scale Insects. *In*: Kondo, T. & Watson, G.W. (Eds.). Encyclopedia of Scale Insect Pests. CABI, Wallingford, Oxfordshire, pp. 548–558.

Collection, preservation, slide-mounting, labelling and vouchering of scale insects

Takumasa Kondo[1] and Gillian W. Watson[2]

[1]Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Centro de investigación Palmira, Diagonal a la intersección Cra. 36ª con Cll. 23, Palmira, Valle del Cauca, Colombia.
[2]Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.

The first step towards any pest management strategy is the correct identification of the insect. Only a handful of scale insects may be identified with any degree of confidence by studying only their appearance in life (e.g., colour, shape, patterns of wax on the cuticle), host plant identity or the position of the infestation. Traditional identification of a scale insect is through microscopy of mostly cuticular features of slide-mounted specimens. However, some species may be identified only with their DNA sequences (i.e. DNA barcoding). It is important to recognise that identification always is provisional against future updates in taxonomy, and appropriate vouchering (placement of published specimens in appropriate repositories) is essential.

The taxonomy of scale insects is based mainly on the morphology of the adult females, thus most taxonomic keys are prepared for identification of this sex and stage. Reasons why the adult female stage is preferred include: (i) not all species have males because many are parthenogenetic, and a few are hermaphroditic with males being very rare; (ii) adult females are the most commonly collected stage, due to their longer lives, larger size and sedentary habit; (iii) adult females cause most damage to the host plant; and (iv) because amongst the growth stages, adult females have more identifiable features (character states) for separating species than immature stages. However, for a handful of species there are also taxonomic keys for the identification of immature stages and males. The present chapter describes some common collection, preservation and slide-mounting methods, and recommendations on the labelling and vouchering of slide mounts.

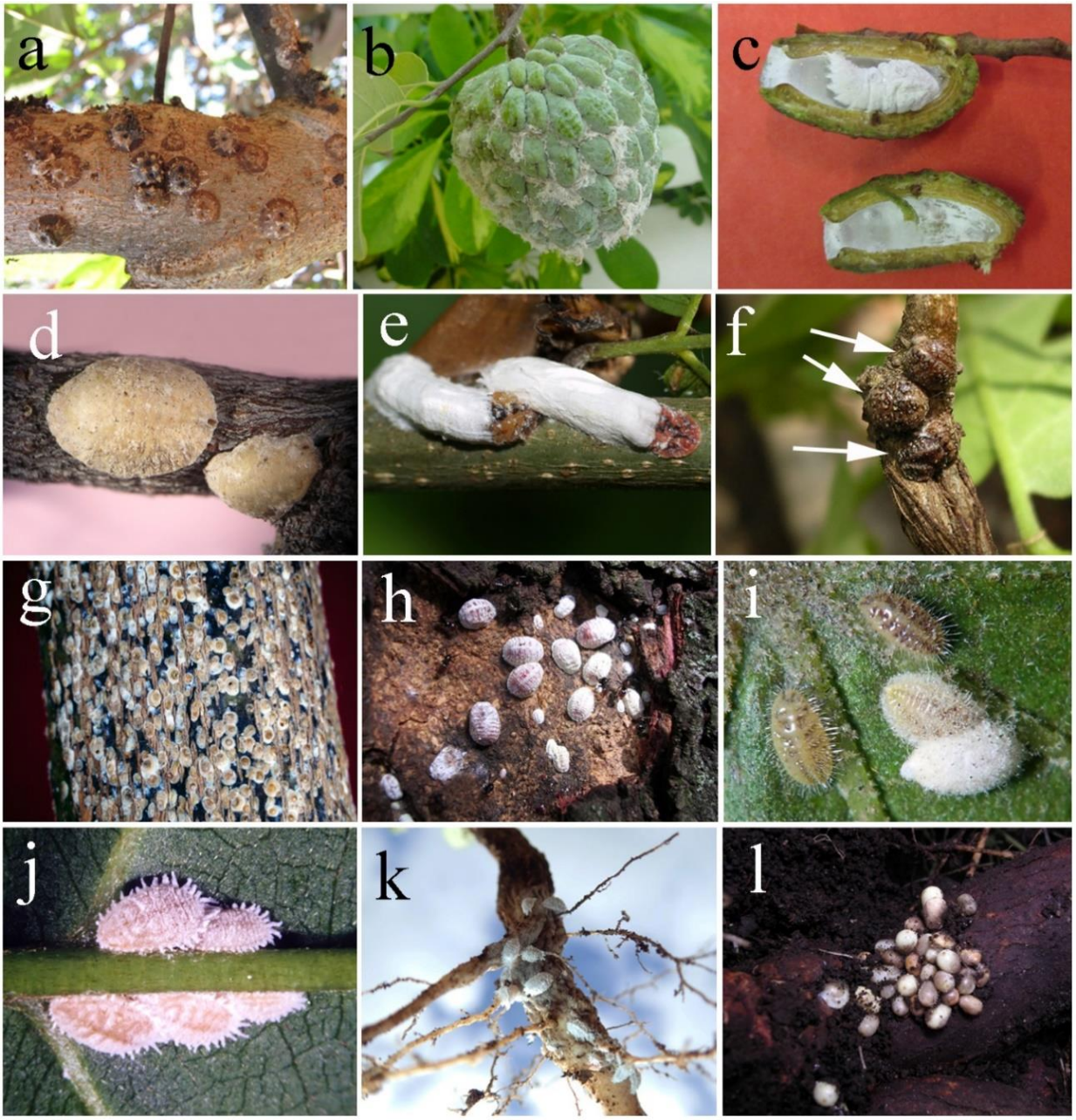

Figure 1. a) *Hemilecanium guanabana* Kondo & Hodgson (Coccidae) on soursop branch; **b)** *Ferrisia dasylirii* (Cockerell) (Pseudococcidae) on cherimoya fruit; **c)** *Apiomorpha* sp. (Eriococcidae) inside gall on *Eucalyptus*; **d)** *Lecanodiaspis rufescens* (Cockerell) (Lecanodiaspididae) on twig of host; **e)** *Pulvinaria* sp. (Coccidae) with long ovisacs on twig of host; **f)** cryptic specimens of *Nidularia* sp. (Kermesidae) on twig of oak host (see arrows); **g)** *Aonidiella* sp. (Diaspididae) on twig of host; **h)** *Crypticerya abrahami* (Newstead) (Monophlebidae) on trunk after removal of bark; **i)** eriococcids, *Eriococcus* sp. (Eriococcidae) on host leaf; **j)** citrus mealybug, *Planococcus citri* (Risso) (Pseudococcidae) on leaf underside; **k)** *Puto barberi* (Cockerell) (Putoidae) on roots of a weed; **(l)** *Eurhizococcus colombianus* Jakubski (Margarodidae) on root of blackberry, *Rubus glaucus* (Rosaceae). Photographs: T. Kondo.

Collection

Scale insects may occur on any part of their host plants, on branches (Fig. 1a), fruit (Fig. 1b), in galls (Fig. 1c), on twigs (Fig. 1d, e), trunk (Fig. 1g, h), leaves (Fig. 1i, j), under bark, and roots (Figure 1k, l). Grass-infesting species may be found on leaf surfaces, under / inside the leaf sheaths or on the root system. In sexual species, males are often found on different parts of the host plant from females. Some species migrate between different parts of the plant at different stages of development. Scale insects come in all colours and sizes, ranging from about 1 mm to up to 25 mm, but some species may grow up to 40 mm long; in some the body is bare, whereas others are covered by different types of tests, ranging from thin and waxy and attached to the insect body (Fig. 1a), thin and waxy and detached from the insect (Fig. 1g), thick wax (e.g. *Ceroplastes*), felt-like (Fig. 1i), glassy, or mealy (Fig. 1j, k); and adult females of some species produce ovisacs (Fig. 1e), while others do not. Some are conspicuously coloured while others are very cryptic (Fig. 1f); some species are found inside hollow branches tunnelled by other insects; others live inside galls (Fig. 1c) that they induce, and some look like leaf buds (Fig. 1f); others burrow under the bark (Fig. 1h) or live underground (Figure 1k, l). Scales are commonly found in protected and shaded parts of the leaves, trunk, twigs, bark crevices, in twig axils, often on sheltered parts of the plants. It is fair to assume that most plant species are likely to be host to one or more scale insect species. Perennial plants tend to have more scale insect species than annuals. Due to their small size and cryptic habits, scale insects often go unnoticed. Here we do not discuss the collection or preparation of fossil scale insects, which are often found in amber deposits.

Collection of scale insects from aerial parts of plants

There is no single collecting method for all scale insect groups. Grasses, indoor plants, weeds and small plants can be destructively inspected, including pulling up the whole plant to inspect the root system. On small trees and shrubs, the most common collecting method involves pruning the lower twigs, especially those in the shade. Collecting random branches and examining them under a dissecting scope is a very effective method of finding cryptic species, especially armoured scales. Dark beating sheets are especially effective for collecting mealybugs. In tropical forests, tree canopies more than 30 meters above the ground harbour a wide variety of scale insects that may only be reached using canopy towers or special climbing equipment; otherwise small branches may be brought down by shooting them with a rifle. Telescopic tree pruners have been used for scale insects high up on the branches, twigs and leaves of trees. Commonly, commercially available telescopic pruners or ‘cherry pickers” enable access to tree crowns 2.4 to 5.0 meters above ground, but pruners on longer poles may be available.

Phloem-feeding scale insects eliminate sugary honeydew that often attracts attendant ants, bees, wasps and other insects; intensive ant or bee activity is often associated with scale insect infestations. Ants attending honeydew-producing scales often build carton shelters over them. Sooty moulds grow wherever honeydew accumulates on plant surfaces and even on the ground; thus, the presence of ants, bees and sooty mould is often an indication of the presence of scale insects.

The basic scale-insect collection kit should contain the following items. A pair of garden scissors (Fig. 2a) is used for cutting infested leaves and smaller twigs, and a foldable pruning saw (Fig. 2b) or the small saw of a multipurpose pocketknife is for cutting infested branches. The pocketknife (Fig. 2m) helps remove infested plant parts like bark, and for cutting open galls and twigs. A

magnifying glass (Fig. 2f) allows inspection of plants in the field. Samples of infested leaves and branches may be folded into Kraft wrapping paper or newspaper (Fig. 2c) or put in paper bags (Fig. 2d) and/or plastic bags, including ziplock bags (Fig. 2e). It is essential to write all collection data on the wrapping paper or bag with a permanent pen (Fig. 2l). The scale insect-infested plant material should be unwrapped and air dried in the lab as bagged samples develop mould quickly and become unusable, so they should be processed rapidly either by slide-mounting, preservation in ethanol or by drying in an incubator. Soft-bodied scale insects, especially species that walk once disturbed, may be preserved in the field in labelled vials (Fig. 2g) containing 70% (Fig. 2h) or 98% ethanol (Fig. 2i), respectively. Specimens preserved in 70% ethanol are useful for slide-mounting, and specimens in 98% ethanol may be used for DNA extraction. A small paper label (Fig. 2j) with the collection data written in pencil (Fig. 2k) or permanent ink (Fig. 2l) should be placed inside the vial with the sample. The collection data should include latitude, longitude and altitude, preferably taken using a Global Positioning System (GPS) (Fig. 2n). Small and medium-sized plastic containers or an insulated food box are useful for transporting samples from the field to the laboratory without them getting crushed.

Figure 2. Scale insect collection materials: **a)** garden scissors; **b)** foldable pruning saw; **c)** newspaper; **d)** paper bags; **e)** zip-lock plastic bags; **f)** magnifying glass; **g)** sample vials; **h)** 70% ethanol; **i)** 98% ethanol; **j)** acid-free paper; **k)** pencil; **l)** permanent-ink pen; **m)** pocket knife; **n)** GPS.

Generally, in specimens preserved in 98% ethanol the tissues become fixed, so they are not good for slide-mounting. However, DNA extraction protocols provide good vouchers for slides from specimens collected into 98% ethanol, post-extraction of the DNA. It is recommended to use different types of vials (either by size or cap colour) for 70% and 98% ethanol. Vials of different sizes are useful for collecting scale insects of different sizes. When possible, collect the scale insects on the plant material to which they are attached, as attempts to detach them in the field

often damages the mouthparts and delicate derm. Experienced collectors take samples of plant material to study closely under a stereomicroscope in the laboratory; this increases the chances of detecting cryptic species that otherwise would go unnoticed in the field. It is recommended to collect plenty of specimens of an assortment of sizes, to help understand any intraspecific variation. Immature stages and males should be collected because of their increasing importance in research on scale insect taxonomy. Close-up photographs of the scale insects, the infestation, damage symptoms caused, and collection site provide important morphological, biological and ecological information.

Collection of hypogaeic scale insects

Members of the family Rhizoecidae are hypogaeic, and the Margarodidae are completely adapted for their underground life, only appearing above ground for short periods for reproduction. Several other scale-insect families include hypogaeic species, e.g. Coccidae, Diaspididae, Eriococcidae, Ortheziidae, Pseudococcidae, Putoidae and Xenococcidae. The roots of small plants, weeds and grasses may be pulled out of the soil manually or using a hand shovel. The roots of shrubs and trees can be dug out with a shovel. The roots, root crown and rootlets should be inspected closely in good light; often there are traces of wax in the soil around an infestation. Honeydew-producing scales are often attended by ants; those of genus *Acropyga* associate with underground scale insects, especially those of the family Xenococcidae. Heavy infestations of scale insects on the underground parts often result in the weakening of the plant, leaf chlorosis and premature leaf drop, and plants with these symptoms should be inspected. Many species of Rhizoecidae and Ortheziidae have been collected from leaf litter and soil samples as bycatches from Berlese funnel trap and Winkler apparatus samples targeted at other soil arthropods, but they have the disadvantage that the host-plant's identity cannot be determined.

Collection of male scale insects

Many scale insect species are parthenogenetic and thus have no males. Male scales closely associate with females, allowing identification through the female morphology. Synthetic sex pheromones may attract the males of some pest species. Males of certain species fly in the early hours of the day or at dusk and may be collected in suction traps, sticky traps or at light sources. However, when males are collected separately from females it is almost impossible to correlate them with the conspecific females except through molecular analysis, or, rarely, if a published key is available to males. Malaise traps used for trapping flying insects, particularly Hymenoptera and Diptera, sometimes catch flying male scale insects.

Collection of immature scale insects

As for the adult males, immature stages can be difficult to associate with the appropriate adult female. Several species of scale insect may co-occur so associating immature stages with the relevant adult female should be made with caution. Intermediate immature females often resemble the adult to some extent. First-instar nymphs are often found beneath the parent female; there are some taxonomic keys to this developmental stage, for identification to family level and in some cases, to genus and species.

Collection permits

Collectors should be aware that it is important to collect insects legally, although laws vary between countries. Collecting on private land requires the permission of the owner, and collecting

in State Parks, National Forests, National Parks, Nature Reserves, National Wildlife Refuges, National Monuments, Recreation Areas, Land Trusts and Private Reserves may require official permits. When describing species new to science, many scientific journals require that the authors state that the specimens were obtained legally. Major museums also require a written statement accompanying specimen donations. Collecting without a permit may result in penalties such as confiscation of specimens, fines and jail time.

Preservation of scale insects

Scale insects may be preserved in three main ways: in various liquids (wet preservation) (Fig. 3a), as dried specimens (dry preservation) (Fig. 3d, e) or as slide mounts (Fig. 3c, f). These methods are described below.

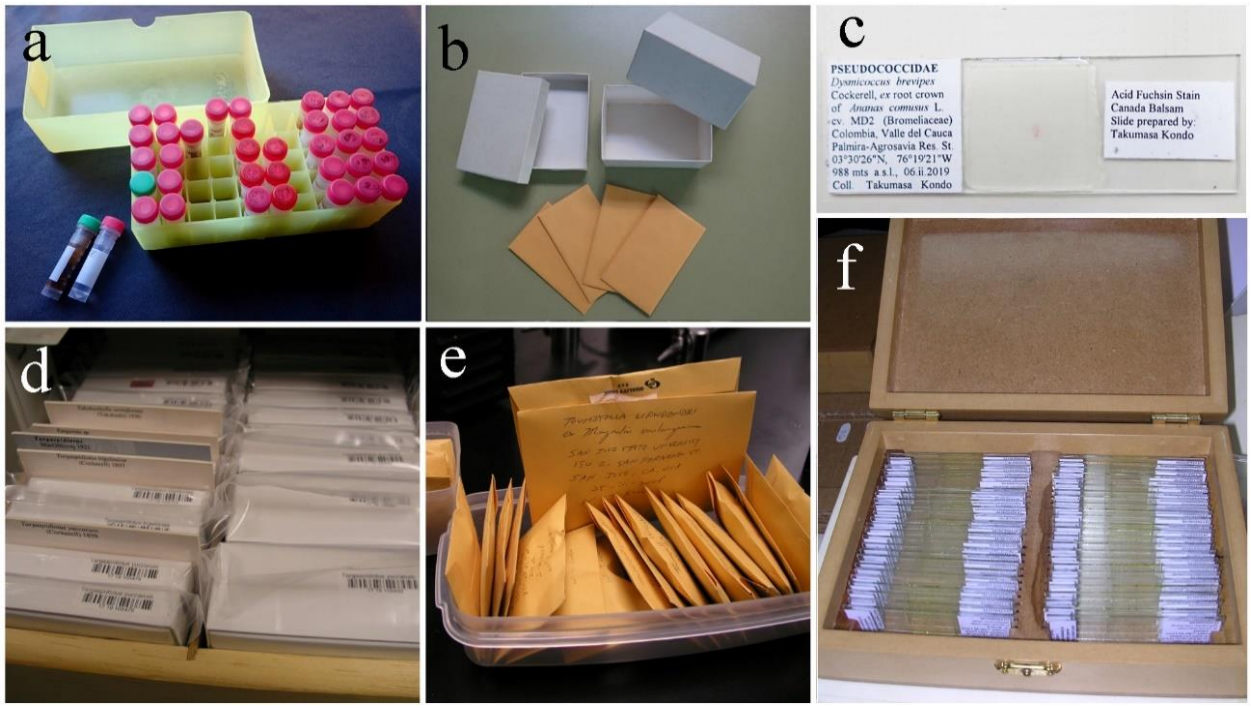

Figure 3. Scale insect preservation methods: **a)** alcohol vials (green cap: 70% for slide-mounting; red cap: 98% for DNA extraction); **b)** boxes and envelopes for preserving dry material; **c)** a permanent slide; **d)** a museum file cabinet with sealed boxes of dry material; **e)** envelopes with dry material in a plastic box; **f)** a wooden box for storage and transportation of slide mounts. Photographs: T. Kondo.

Wet preservation

Scale insects may be preserved in various liquids for long-term storage. The most common preservatives include 70% - 80% ethanol (C_2H_5OH) (EtOH), and acid ethanols (acetic acid ethanols or lactic acid ethanols) (Table 1). Isopropanol (C_3H_7OH) has advocates for its lower volatility / flammability: methanol (CH_3OH) should not be used alone or in combination with EtOH ('denatured') for its DNA degrading properties.

Table 1. Common liquids used for preservation of scale insects

Preservative name	Contents
70% ethanol	7 parts EtOH + 3 parts distilled water
80% ethanol	8 parts EtOH + 2 parts distilled water
Acetic acid ethanol	4 parts 98% EtOH + 1 part glacial acetic acid
Lactic ethanol	2 parts 95% EtOH + 1 part 75% lactic acid

Preservation in ethanol (Fig. 3a) is a popular method for storing scale insects, but it comes with the risk of evaporation and the sample drying out over time. Vials should be checked regularly, and the storage liquid should be topped up or changed periodically to prevent the contents drying out or deteriorating. Each vial should contain a label with detailed collection data written in pencil or permanent ink on acid-free paper. Vials should be filled to the top with liquid to avoid specimen damage by bubbles during transport. Transport of bulk volumes of flammable material is prohibited in many mailing and courier services so the liquid may have to be removed before samples are shipped and replaced immediately after arrival at the destination. However, an agreement between the International Air Transport Association (IATA) and Museums allows

transport of small volumes under Special Provision A180 applies (IATA Dangerous Goods Regulations) and this declaration accompanied by 'Dead (non-infectious) preserved insects, containing small quantities of UN1170' is the current (2020) regulation for shipment regulations for small volumes of ethanol (IATA). Ethanol or isopropanol-preserved material is good for molecular analysis but specimens stored in liquid more than 6 months can make poor slide mounts.

Dry preservation

Although less popular than wet preservation, dry preservation has its advantages. Dry specimens stored in cool, dry conditions can be made into excellent slide mounts, often better than those preserved in liquid, even if they were collected more than one hundred years ago. Care should be taken to dry the specimens thoroughly, preferably in a drying oven. Once dry, storage in a sealed container containing some silica gel helps maintain low humidity to avoid deterioration due to fungal growth. Care should be taken to avoid attack by dermestid beetles, book lice and other dry storage pests. Dried material in museums is often wrapped in tissue paper and stored in cardboard boxes (Fig. 3b, d), glass or plastic containers, paper envelopes (Fig. 3b, e), or sealed in plastic bags (Fig. 3d). Naphthalene may be used to deter storage insect pests, but the fumes are toxic. Caution should be taken when handling dried insects since they are very brittle and can easily lose setae and limbs. Dry material stores well and does not need to be checked frequently, in contrast with wet-preserved specimens. Boxes of dried material should have an external label visible without the need of opening the box, to avoid damage; a second label should go inside with the dry material. Dry samples should be kept preferably in well-built insect-proof cabinets. When shipping dry material, if there is plenty then it is recommended to send only part of it, to avoid a complete loss in case it does not reach the destination due to accidents during the mailing process. The samples should be packed very carefully to avoid crushing, and using plenty of cushioning material like tissue paper to ensure that they do not move about during shipment.

Host plant identification

The identification of the host plant is an important part of the collecting process of scale insects. A portable plant press may be used for plant samples, including flowers, fruits, leaves, and given

identical collecting number or code so the host plant can be identified later. It is useful to carry plant field guides for identification of the host plant *in situ.*

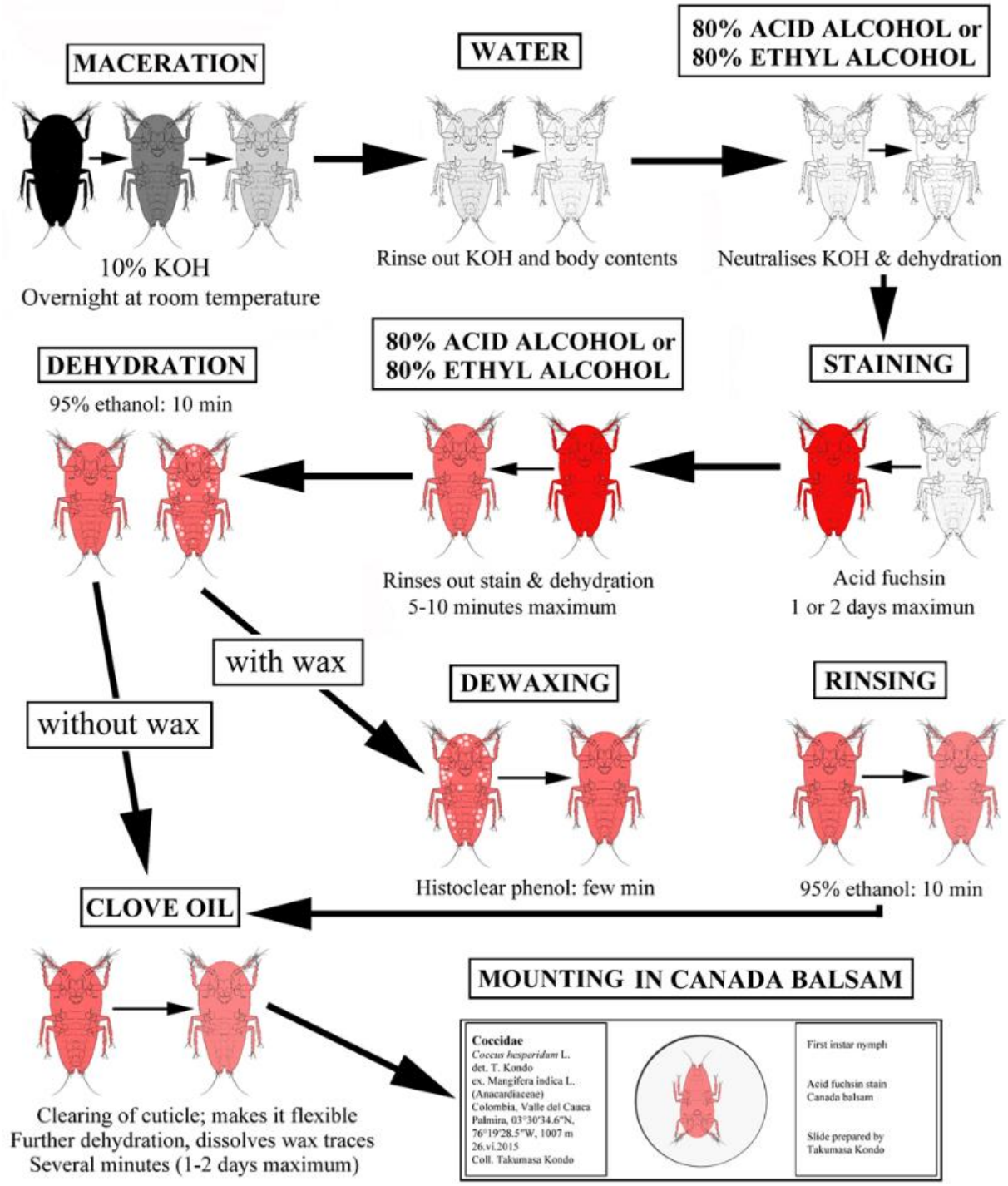

Figure 4. A flow diagram of a method for preparing permanent scale insect slide mounts. The black specimen represents the opaque live insect, which subsequently becomes clearer during maceration in 10% KOH. The red colour of the insects represents different degrees of uptake of stain. The white dots in the body represent body fat and wax.

Slide-mounting scale insects

The identification of scale insects is based on the cuticular morphology of slide-mounted adult females. The best slide mounts are from teneral adult female specimens (i.e. that have recently had their last moult); at this stage they are unsclerotised, the body has not yet expanded and there are no eggs inside. They also tend to be free of endoparasitoids and take up stain better than sclerotised specimens. However, inclusion of some older and larger specimens is important to determine the size range and to show the sclerotisation patterns that develop in more mature specimens. There are numerous slide-mounting methods available in the scientific literature, including both temporary mounts and permanent mounts. Here we describe and illustrate a preferred method of making permanent mounts.

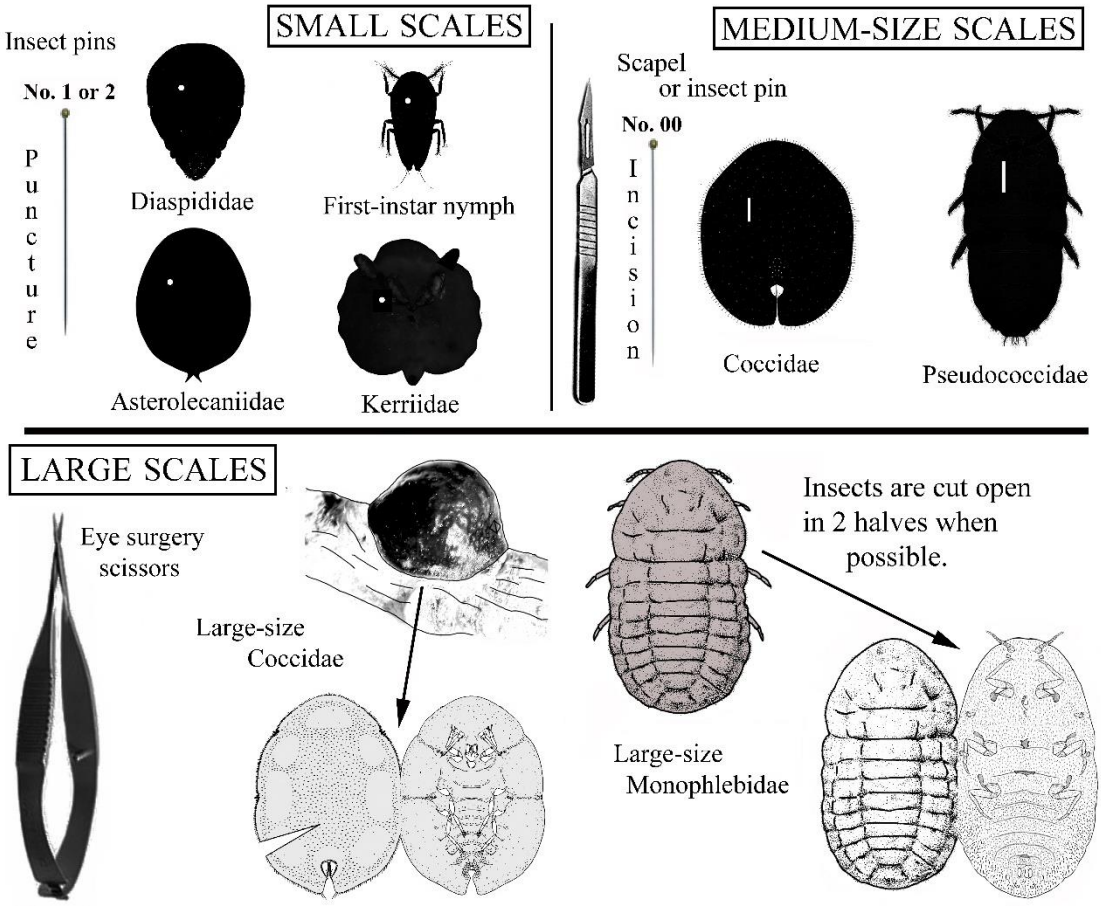

Figure 5. Puncture points and incision lines in smaller scale insects, and cutting open of larger scale insects with a pair of eye surgery scissors.

Steps for making permanent slide-mounts

Freshly collected material makes the best slide mounts; however, dried and wet-preserved scale insects can be prepared as slide mounts. The slide-preparation process is summarised in Figure 4. Specimens must not be allowed to dry out or air gets trapped in the limbs.

1. Rehydration
Dried and wet-preserved material should be rehydrated in distilled water with a drop of detergent or liquid soap at room temperature for a day or two. Freshly collected material does not need to go through this step.

2. Maceration
To help the body contents to clear, it is best to make a small puncture with a No. 1 or No. 2 insect pin to one side of the mid-line in small scales (e.g. Diaspididae, first-instar nymphs, etc.), or a small longitudinal incision using a No. 00 insect pin or a scalpel for medium-sized scale insects (Fig. 4). It is recommended that highly convex specimens (e.g. large Coccidae) and large scale insects (e.g. large Monophlebidae) be cut around the margin and opened into two halves, as shown in Fig. 5.

Place specimens in 10% potassium hydroxide (KOH) and leave at room temperature overnight (overheating can damage the insect cuticle) or heat at 60 °C until the body contents become translucent and droplets of liquified fats become visible through the cuticle; the body contents should be expelled carefully using a microspatula and/or a blunt mounted needle or a small paint brush.

3. Rinsing and acidification
10% KOH solution is a potent base (i.e. strong alkali) and it is necessary to wash it all away with distilled water. Add a drop of detergent or liquid soap to the distilled water to prevent the insect from floating. If the KOH solution is not rinsed away properly, specimens may not stain well or become decolourized after some time. At this stage, any unsightly tracheae and eggs remaining in the body may be carefully extracted through the hole or slit using insect pins, microspatula, small paint brushes etc., ensuring that the body is left in the appropriate shape and dorso-ventrally flattened. After soaking in water for 5-10 minutes, the specimens are then transferred to acidified 80% alcohol (isopropanol or ethyl alcohol) for at least 10 minutes to acidify the cuticle, neutralize any remaining KOH, and start the dehydration process.

Table 2. Common reagents used for staining scale insect cuticle

Reagent	Contents
Essig's aphid fluid	20 parts lactic acid (reagent grade 85%) + 2 parts phenol (liquefied) + 4 parts glacial (100%) acetic acid + 1 part distilled water. Heat at 56-60°C for 30-60 minutes. Label and store solution in a dark bottle.
Acid alcohol	20 parts glacial acetic acid + 80 parts 50% ethanol
Acid Fuchsin	Acid Fuchsin powder (0.5 g) + 10% HCI (25 ml) + H_20 (300 ml)
Acid Fuchsin stain	3 parts acid alcohol + 1 part acid Fuchsin.

4. Staining
The clean specimens are stained in a mixture of acid Fuchsin stain and Essig's aphid fluid for 12-24 hours; this mixture has the advantage that it will not dry up, so it can be heated at 60 °C for half an hour to several hours to speed up the staining process. The staining mixture can be reused many times. Alternatively, specimens may be stained more quickly in acidified 80% alcohol (but this

evaporates rapidly and there is a risk of damaging the specimens if they dry out). If specimens do not stain well (due to residual traces of KOH), a few drops of glacial acetic acid added to the dish usually darkens the stain. See Table 2 for the preparation of liquids used in the staining process.

5. Removing excessive stain, dehydration and stain fixation
Newly-stained specimens are full of deep red liquid and are usually overstained. They should be briefly rinsed in acidified 80% isopropanol or ethanol until the membranous cuticle becomes pink while thick cuticle remains red. Then they should be quickly transferred to 95% isopropanol or ethanol to fix the stain, and soaked for about 10 minutes to dehydrate the cuticle.

6. Dewaxing
Specimens without any wax or oil droplets within the body can go straight to step 7. However, those that contain wax lumps or oil droplets should be transferred to Histoclear for at least 5 minutes to dissolve the lipids, then rinsed in 95% isopropanol or ethanol to remove dissolved waxes and to keep the cuticle dehydrated.

7. Clearing in clove oil
The dewaxed, dehydrated specimens are transferred to high quality, anhydrous clove oil to soak for at least 10 minutes, to clear the cuticle. Clove oil also removes any remaining water and lipids. Provided it is kept clean and anhydrous, clove oil can be re-used many times.

8. Temporary labelling
It is essential that each sample remains associated with the collection data at all times; specimens without data are of no scientific value. Each slide should be marked with some form of unique sample identifier (e.g. sample number) using an indelible pen or a diamond-point marker before the specimen is mounted on it. Full data labels can be printed and affixed after the mountant has dried.

9. Mounting in Canada balsam
The Canada balsam should be the consistency of liquid honey; if it is too thick, mix in clean 100% xylene until it is adequately soft. Place a small spot of clove oil where the insect will go. Transfer an insect to the clove oil spot and position it with the ventral side up and head towards the front of the microscope stage (away from the top line of the label) and arrange its limbs (when present). [This positioning of the insect is so that when the slide is placed on a compound microscope stage, the data on the label is readable and, when observed through the eyepieces, the insect will appear with the head away from you.] Soak up any excess clove oil with a tissue; add a drop of Canada balsam and slowly lower a glass coverslip on to it to seal the insect in. For thick insects, slide-mount some specimens with the dorsal side up so to be able to study the dorsal features. Dry slides at 39 °C on a hot plate or in an oven for 3 months. Glass cover slips may be circular or rectangular, the most common sizes being 15-22 mm wide. Special, larger-size coverslips may be required for slide-mounting very large scale insects such as monophlebids.

10. Permanent labelling
Slides should be labelled with data printed on acid-free paper or archival-quality thin card. Labels should be glued to the slide (Fig. 6) using polyvinyl acetate (PVA) glue, which is not water soluble

once dry. Some researchers prefer liquid white glue because it is water soluble, however there is a risk of detachment of the labels. Each slide should carry the full collection data. The basic

collection data includes: (i) the place (country, department or state, county or municipality, or other local political unit, distance and direction to the nearest city, town or other place that can be easily found on a map, latitude, longitude and altitude may be taken with a GPS, (ii) the collection date, and (iii) the collector´s name. Other additional useful information include the name of the host plant, on which part of the plant the insect was collected (e.g. leaf, twig, under bark, inside gall, on roots, etc), association with other insects (e.g. if the insect was tended by ants or if it was found inside ant carton, etc.), and some details of the collecting site (e.g. near ditch of a pine forest, etc).

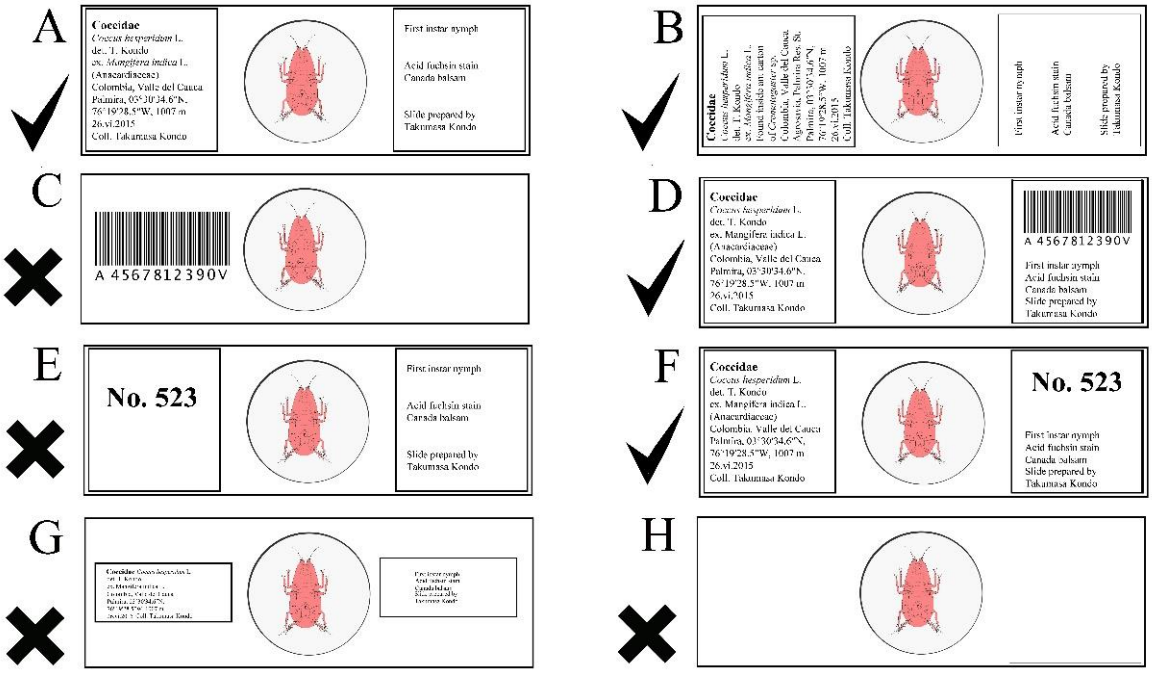

Figure 6. Labelling examples. **A.** A label aligned vertically (left label) with a right label with information on the insect stage, staining liquid used, mounting medium and name of who prepared the slide. **B.** A label aligned horizontally. **C.** Slide with a single barcode. **D.** A good label with bar code. **E.** A slide with a number. **F.** A good label with a museum / voucher number. **G.** Bad labels with tiny unreadable letters. **H.** Slide with no labels. **Note.** Good examples are marked with a check mark (tick) and bad examples are marked with a cross.

The best labels are those that use the full breadth of the slide, and include full collection data written in a letter font size (5-6 pt) that can be read without the aid of a magnifying glass (Fig. 6A, B, D, F). The direction of the label may be either vertical (Fig. 6A), or horizontal (Fig. 6B) if the label is too long and would not fit vertically. Labels that do not give the full information should be avoided. For example, slides labelled with a bar code are not useful (Fig. 6C) unless a label also provides full collection data (Fig. 6D). Slides with a number (Fig. 6E) are also useless unless the collection data are provided (Fig. 6F). Too-small labels in tiny print (Fig. 6G) are almost as bad as labels written in illegible handwriting. Unlabelled slides (Fig. 6H) are useless scientifically. Some museums carve a museum entry number on the glass slide with a diamond-point marker at the time of the slide preparation to ensure that the correct label is put on the correct slide.

Temporary slide mounts

Common temporary slide-mounting media include Hoyer's medium and polyvinyl alcohol (PVA). One of the ingredients in Hoyer´s medium is chloral hydrate, a controlled substance. Temporary media are water based, so stained specimens will soon lose their colour. Once the specimens have been dehydrated in strong alcohol they can be mounted in temporary media; do not use clove oil before mounting, as it will not mix with the aqueous mountant.

11. Vouchering specimens

The process of collection, slide preparation, subsequent identification and (often) publication of details is incomplete unless the material is preserved for posterity by retention in a suitable repository. This is especially true of specimens from which DNA has been extracted, and for nomenclaturally important material such as new species, but is equally so for pests that subsequently may require re-examination after the discovery of cryptic species and new taxa of quarantine significance. The repository should be of national or international status with a legal mandate to maintain access to the collections. The laboratory bench and slide boxes of a solitary investigator in a university or research laboratory do not have such mandated status. All too often, historically valuable specimens have been discarded after cessation of the research due to changed institutional priorities or retirement or death of the researcher. It is critical that important private collections are relocated prior to demise, or legal instructions are given for post-mortem transfer to an appropriate repository mandated to curate and care for the material for posterity.

Main information sources

Ben-Dov, Y., & Hodgson, C. J. (1997). 1.4 Techniques. 1.4.1 Collecting and mounting. In Y. Ben-Dov, & C.J. Hodgson (Eds.), *Soft scale insects: Their biology, natural enemies and control, vol. 7A* (pp. 389-395). Amsterdam & New York, Elsevier.

Green, E. E. (1896). *The Coccidae of Ceylon. Part I*. Dalau & Co., London, UK.

Gullan, P. J., & Cranston, P. S. (2014) *The Insects: An Outline of Entomology. 5th edition*. Wiley-Blackwell.

McKenzie, H. L. (1957). The armored scale insects of California. *Bulletin of the California Insect Survey, 5*, 1-209.

Newstead, R. (1903). *Monograph of the Coccidae of the British Isles. Vol. 2*. Ray Society, London, UK.

Sirisena, U. G. A. I., Watson, G. W., Hemachandra, K. S., & Wijayagunasekara, H. N. P. (2013). A modified technique for the preparation of specimens of Stenorrhyncha for taxonomic studies. *Tropical Agricultural Research Journal, 24*(2), 139-149.

Watson, G. W. (2002). *Arthropods of economic importance: Diaspididae of the world. An illustrated identification guide and information source.* CD-ROM. Amsterdam, Netherlands: Expert Center for Taxonomic Identification (ETI), University of Amsterdam. https://diaspididae.linnaeus.naturalis.nl/linnaeus_ng/app/views/species/taxon.php?id=113132&epi=155

Watson, G. W., & Chandler, L. R. (2000). *Identification of mealybugs important in the Caribbean region. Second edition*. Egham, Surrey, UK: CABI Bioscience.

Wilkey, R. F. (1990). 1.5.1. Collection, preservation and microslide mounting. In D. Rosen (Ed.), *Armored scales Insects, their biology, natural enemies and control. Vol. 4A.* (pp. 345-350). Elsevier, Amsterdam, The Netherlands.

Williams, D. J., & Granara de Willink, M. C. (1992). Mealybugs of Central and South America. CAB International.

Williams, M. L., & Kosztarab, M. (1972). Morphology and systematics of the Coccidae of Virginia with notes on their biology (Homoptera: Coccoidea). *Research Division Bulletin, Virginia Polytechnic Institute and State University, 74*, 17-21.