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Title of resource
GN_06: Slide mounting
Authored by
When using this protocol, the following should be referenced: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3.
DOI
10.13140/RG.2.2.14879.41129
Description
Procedure for slide mounting arthropods for permanent storage as morphological voucher specimens. Protocol from the Gnatwork Bangladesh workshop, September 3-6th 2018.
Intended use
Scientific research use and training purposes.
Restrictions on use
Content is not to be redistributed in the public domain (e.g. presentation, lecture, online or in publications).
Resource history
Updated version of: Harrup, L.E. (2014). The Pirbright Institute *Culicoides* DNA Barcoding Protocols, Version 2, available at www.ibvnet.com

GN_06: Slide mounting

Citation: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3

Equipment Required

- Stereomicroscope (x10 – x40 magnification)
- Timer
- Watchmaker No. 3 fine-point stainless steel forceps
- Pipette (20-200 µl)
- 2x microscalpel (e.g. Interfocus Micro Knife Angled 22.5˚ (part number 10316-14)
- Angular or straight needle (optional)
- 6 Glass and/or plastic 250-500 ml bottles (50:50 clove oil : ethanol mix must be kept in a glass bottle, ethanol dilutions can be kept in plastic or glass bottles)
- 1-2 mm outside diameter glass rod between 5-10 cm long, toothpick or other suitable implement

Consumables Required (Figure 1)

- 76 mm x 26 mm x 1 mm ground edge high-optical clarity microscope slides
- 5-10 mm wide round or square No (0.13-0.16 mm thick) or No. 0 (0.08-0.10 mm thick) glass coverslips
- Slide labels
- Solvent resistant fine-point pen
- Paper towel
- Reaction tubes, PCR plate micro centrifuge tube or other suitable container
- 1ml glass bijous or other tube/container compatible with clove oil
- No.1 fine paintbrush (optional)

Reagents Required

- Euparal mountant
- Analytical grade 100% ethanol
- Autoclaved distilled water
- Clove oil
- 10% (w/v) or 2 M acetic acid Solution (optional only if additional specimen clearing required)
- 10% (w/v) potassium hydroxide solution (optional only if additional specimen clearing required)

Source. Equipment, consumables and reagents required (*stereo microscope, 10% (w/v) or 2 M acetic acid solution and 10% (w/v) potassium hydroxide solution not shown)

Preparation

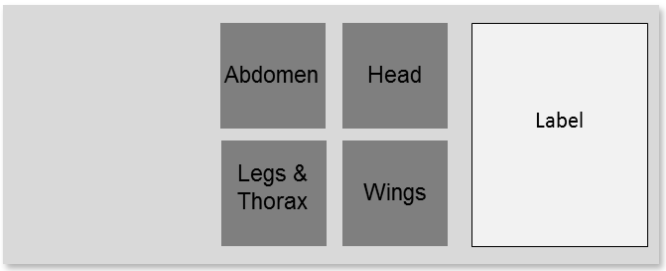
1. Prepare stock of 50%, 70%, 80% and 90% dilutions of ethanol (dilutions made with sterile distilled water).

2. Prepare specimens for mounting by incubating specimens individually in 200 µl tissue digest solution as per steps C1 through C14 of protocol GN_08 (non-destructive DNA extraction of Dipteran specimens).

3. Under a stereomicroscope at between 10-40x magnification, place the specimen to be mounted in a drop of ethanol on a petri dish or microscope slide. If further clearing of the specimen is required follow method A, if no further clearing is required follow method B (typically specimens cleared by incubating in tissue digest solution as per protocol GN_08 do not require additional clearing).

A. IF CLEARING IS NEEDED

1. Label a clean microscope slide with the following information:
   a) Species (if known)
   b) Sex (Male; Female)
   c) If female, the status (Non-Pigmented; Pigmented; Blood-fed; Gravid)
   d) Geographical coordinates of collection site (Latitude; Longitude)
   e) Type of trap used to make collection
   f) Name and affiliation of identifier
   g) Type of mounting media used
   h) Sample ID.



Source. Standard layout for slide mounted specimens

2. Under a stereomicroscope approximately 10-15x magnification, using the toothpick/glass rod or another suitable implement place one drop of Euparal on the microscope slide at the bottom right corner of a square approximately 1.5 cm wide on the central area of the microscope slide.

3. Prior to further clearing the specimens wings must be removed and mounted as they are too delicate to survive intact additional cleaning). Hence, in a drop of 50% clove oil 50% ethanol solution to the right hand side of the drop of Euparal position the specimen dorsal side up and starting with the left wing using the microscalpel separate the wing from the thorax and place it dorsal side up in the centre of the top half of the bottom right-hand square of Euparal i.e. with the basal potion of the wing to the right. Repeat for the left wing placing it dorsal side up below the left wing in the bottom half of the bottom right-hand square of Euparal i.e. with the basal portion of the wing to the left.

4. Using your toothpick/glass rod or another suitable implement, spread the Euparal drop until it is very thin and covering an area about the size of the cover slip. Using forceps select a cover slip and ease the cover slip down from right to left over the coverslip. Without crushing the specimen, gently press the coverslip to flatten. Minor adjustments to the position of the specimen under the coverslip can be made by gently tapping in the appropriate area; the vibrations in the Euparal will help the specimen flow away from the source of the tapping.

5. To complete clearing, place the remaining part of the specimen (now minus its wings) in a 500 µl microcentrifuge or other suitable container with 200 µl 10% KOH overnight (approximately 16 hours). Label the tube/container with the appropriate Sample ID using a solvent resistant pen.

6. Neutralise the KOH by moving the specimen to a new 500 µl microcentrifuge tube or other suitable container containing 200 µl of 10% acetic acid for 30 minutes. Label the tube/container with the appropriate sample ID using a solvent resistant pen.

7. Proceed with protocol B step 8.

B. AFTER CLEARING OR IF NO CLEARING IS NEEDED

1. Label a clean microscope slide with the following information (as above):
   a) Species (if known)
   b) Sex (Male; Female)
   c) If female, the status (Non-Pigmented; Pigmented; Blood-fed; Gravid)
   d) Geographical coordinates of collection site (Latitude; Longitude)
   e) Type of trap used to make collection
   f) Name and affiliation of identifier
   g) Type of mounting media used
   h) Sample ID.

2. Dehydrate the specimens to be mounted individually in a series of ethanol washes at 50%, 70%, 80%, 90% and 100% ethanol for 60 minutes per concentration if mounting directly after removing from a tissue digest solution (Protocol GN_08). If specimens have been stored in 70% ethanol following treatment in tissue digest solution (Protocol GN_08), only ethanol washes at 80%, 90% and 100% for 60 minutes per concentration are required. Use 200 µl of each ethanol concentration in a reaction tube, microcentrifuge tube, microplate or any other suitable container. Label tube/container appropriately with sample ID using solvent resistant pen.

3. Transfer specimens individually to a glass tube/container containing 200 µl of a 50:50 solution of clove oil : 100% ethanol for 60 minutes. (clove oil will 'melt' the majority of plasticwares e.g. Elisa microplates, in general PCR plates have been found to be compatible with clove oil however, before use check the plasticware is compatible with clove oil) Label tubes/containers with appropriate sample ID's using a solvent resistant pen.

4. Transfer specimens individually to a tube/container containing 200 µl clove oil for at least three days. Label tubes/containers with appropriate sample IDs using solvent resistant pen.

5. Under a stereomicroscope at between 10-15x magnification, using the glass rod place four drops of Euparal on the microscope slide at the corners of a square approximately 1.5 cm wide on the central area of the microscope slide.

6. Transfer the individual specimen to be mounted from the clove oil, briefly touching the individual to a clean paper towel to remove excess clove oil, and place in the lower left drop of Euparal on the appropriate microscope slide.

7. In the lower left drop of Euparal position the specimen dorsal side up and starting with the left wing using the microscalpel separate the wing from the thorax and place it dorsal side up in the centre of the top half of the bottom right-hand square of Euparal i.e. with the basal portion of the wing to the right. Repeat for the right wing placing it dorsal side up below the left wing in the bottom half of the bottom right-hand square of Euparal i.e. with the basal portion of the wing to the left.

8. With a micro-scalpel remove the head and place it in the centre of the upper left-hand square of Euparal, with the antennae to the left. Specimens cleared using tissue digest solution (Protocol GN_08) cannot support the weight of a cover slip so place 3 or 4 small pieces of broken cover slip around the head to support the cover slip.

9. Reposition the remainder of the specimen and either:

    a. Using the microscapel place the abdomen ventral side up at the centre of the top left square of Euparal, reposition if required and mount the remainder of the thorax and legs laterally in the bottom left-hand square of Euparal.

    Or

    b. With extreme care (and only if the mesonoteum is required for taxonomic purposes) position the remainder of the specimen ventral side up and using the microscalpel slice off the mesonotum together with the scutellum to separate the abdomen and place the abdomen ventral side up at the centre of the top left square of Euparal. Leave the mesonotum, dorsal side up, with the rest of the thorax and legs in the bottom left-hand square of Euparal.

10. Check the Euparal has not set too much in any of the four areas, add more using the toothpick/glass rod or other suitable implement before placing coverslips if required. Coverslips may also be applied immediately after each section is dissected and positioned, this is recommended if drying of the Euparal is experienced due to time take to dissect/mount other portions of the specimen.

11. Using forceps select a cover slip and starting with the upper right-hand drop of Euparal ease the cover slip down from right to left, so that the antennae flow out and away from the head to ease identification. Without crushing the specimen, gently press the coverslip to flatten. Minor adjustments to the position of the specimen under the coverslip can be made by gently tapping in the appropriate area; the vibrations in the Euparal will help the specimen flow away from the source of the tapping. Repeat for the remaining two drops of Euparal.

12. Reduce the magnification so that all four coverslips can be easily seen and check there is sufficient Euparal to cover to the edges of the coverslip if not use a glass rod with a very small amount of Euparal on the end to carefully touch the edge of the coverslip, capillary action will pull the Euparal under the coverslip.

13. Clean any excess Euparal from the slide using a fine paintbrush and 100% Ethanol.

14. Position the slide horizontally and allow slides to dry/set completely before handling. Check the slides about one week after mounting to ensure the Euparal has not receded from the edges of the coverslips. If shrinkage of the Euparal has occurred, add a small volume of fresh Euparal to the edge of the affected cover slip using a toothpick/glass rob (or other suitable implement) and capillary action will draw the Euparal into the gap, recheck the affected slides again in a about one week, repeat if required.

15. For storage place the slide in the appropriate cardboard slide tray and place the slide tray back in its box. Ensure the slides are stored in the dark (i.e. the slide tray is kept in its box) and flat, as the Euparal may take several weeks to fully harden). Once slide are completely dry and hardened slides may be transferred to slide storage boxes where they are stored vertically.

16. Check slides about 1 week after mounting to ensure Euparal has not receded from the edges. If this occurs, add some fresh Euparal to the edge of the cover slip and capillary action will draw the Euparal into the gap.

Tips & Troubleshooting

- Regularly wipe forceps, microscalpels etc to prevent build-up of Euparal while mounting specimens.
- If labels must be hand-written two labels, one either end of the slide, may be used to allow all the required information to be included. Round coverslips can be easier to achieve an edge to edge coverage of Euparal than square coverslips.
- Labels with permanent adhesive should be used, if non-laminated paper labels are used they can be sealed by brushing a fine layer of PVA adhesive over the label(s) and at the edges of the label(s).
- If pre-cut 5 to 10 mm square coverslips are not available, larger coverslips may be cut using a diamond knife to the required size. Round coverslips can be easier to achieve an edge-to-edge coverage of Euparal than square coverslips.
- If the glass rods for dispensing mounting media are not available, toothpicks or the plunger of a 1 mm syringe (with the rubber stopper removed) can be used.
- Unlike in other mounting media types small air bubbles trapped in slide preparations are absorbed by the Euparal during drying, although this may take several days.
- Significant shrinkage of Euparal may occur during drying. In moderately thick preparations, this results in shrinkage away from the edges of the cover slip. This is counteracted by using a wider area of mountant and a slightly larger 10 mm cover slips when Euparal is used in comparison to when specimens are slide mounted with Canada Balsam (where 7 mm diameter coverslips are typically usually used), but an excess of any medium around the edge of the cover slip is undesirable. It may also be necessary to top up the Euparal mountant where required as described in step 17.
- Remounting of specimens should be avoided wherever possible, however, if required, Euparal mounted specimens may be removed by soaking in absolute Ethanol, Euparal essence or xylene.
- The Euparal mounting medium is relatively fast-drying. Allowing the slide to remain overnight in a drying oven set at about 35° C, over a slide hot plate or in the open at room temperature for a few days will yield usable and permanent preparations. Slides should however always be stored horizontally at least for the first few months to prevent slippage of any media which has not fully set. The drying progress can be checked by very lightly pressing on the centre of the coverslip and watching to see if there is any movement of the specimen in the medium.
- Purchase pre-washed/pre-cleaned microscope slides to limit the dirt, dust etc on slides, a microfiber cleaning cloth or propan-2-ol (isopropanol) applied using a lint-free foam/cotton swabs may be used to further clean slides as required.