Preservation and mounting techniques for mosquitoes

1. WHOLE LARVAE
    a. Kill larvae in hot water (not boiling), remove promptly.
    b. Store in small vial containing 75-80% ethanol (ethyl alcohol)
    c. Transfer specimens from alcohol to cellosolve for 15 minutes or more (dark specimens can be stored in cellosolve for 8 hours or overnight).
    d. Lift the specimen from cellosolve[1] and place on the center of a glass microscope slide with the dorsal side up.
    e. Drop a small amount of Canada Balsam[2] on the specimen. Mount the specimen dorsal side up with the head pointing down, arrange the head, thorax and abdomen in a natural position, then cut the abdomen between segment VI and VII (using surgical blade No. 25). Place the terminal segments with the siphon to the left in culicine larvae or segment X to the right in anopheline larvae (See Plate I).
    f. Add more Canada Balsam on the specimen and check the arrangement of setae and larval position, then carefully cover the specimen with a 22 mm rectangular cover glass.
    g. Dry in a drying oven at 45^0 – 55^0 C for 2 weeks or more.
2. WHOLE PUPAE
    a. Follow steps 1-5 described for mounting whole larvae.
    b. Separate the cephalothorax from the metanotum and abdomen. Mount the specimen pointing down, place the metanotum and abdomen dorsal side up then turn the cephalothorax left side up and place it below the metanotum. (See Plate I).
    c. Add more Canada Balsam on the specimen, check the specimen for the correct position. Cover the specimen with a 15 mm round cover glass.
    d. Dry in a drying oven at 45^0 – 55^0 C for 2 weeks or more.
3. LARVAL AND PUPAL EXUVIAE

    The 4^{th} larval and pupal exuviae from an individually reared adult should be mounted on the same slide.

    a) Store in 75 – 80 % alcohol
    b) Transfer the specimens into cellosolve for 15 minutes.
    c) Lift the specimen from cellosolve, placing it on a glass microscope slide, the larval exuviae on the left and the upal exuvia on the right (pointing head down and dorsal side up).
    d) Drop a small amount of Canada Balsam on the specimens. Arrange and spread the body and setae of laval exuvia into a better position, then separate the pupal cephalothorax just chephalad of wing sheath, leave metanotum attached to the abdomen. Open the cephalothorax

[1] Dilute cellosolve may turn white when adding Canada balsam on specimen. To avoid this problem, use facial tissue to blot off the excess cellosolve from the specimen before adding Canada Balsam on the specimen (Tough specimen very carefully and softly with a small piece of facial tissue.
[2] Euparal can be used instead of Canada Balsam. Thick Canada Balsam can be thinned down with xylene and Euparal with Euparal essence.

and mount the ventral side up, place it below the metanotum (See Plate I).

e) Add more Canada Balsam, check the position of the larval and pupal exuviae, then cover the specimens with a 15 mm round cover glass.
f) Dry in a drying oven at $45^0 – 55^0$ C for 2 weeks or more.

4. ADULTS
    a. After emergence, adults should be held for at least 24 hours before killing. Dried specimens should be relaxed in a relaxing jar for at least 2 hours so that the appendages will not break when handled.
    b. Kill in chloroform[3] killing bottle. (Ethyl acetate keeps specimens relaxed longer)
    c. Using a small amount of 'Ambroid' cement[4] on tip of paper point, glue the specimen on the right side of the thorax with the legs toward the pin, wing and body should not be attached with paper point and glue, and pint should not project beyond the scutum. (See label, Plate I).
    d. Pinned specimens should be kept in Schmitt boxes. Paradichlorobenzene (See 3) or naphthalene(See 3) is needed for each box to protect specimens from being eaten by beetles, cockroaches, mites or other insects. The former has both repellent and insecticidal properties, but does not last as long as the latter which basically acts as a repellent.
5. GENITALIA
    a. Place the adult in a relaxing jar for 3-5 hours [5] (Can use a jar with boiling-steaming water for 20-30 minutes)
    b. Clip the abdomen between the 6^{th} and 7^{th} segments using a fine dissecting scissors.
    c. Place the genitalia in a small screw cap vial containing 10% KOH[6] and a small drop of 40% detergent to sink the genitals in the KOH.
    d. Put the vial in a drying oven for $45^0 – 55^0$ C for 1 hour.
    e. Transfer the genitalia to cellosolve for 10-15 minutes.
    f. Place the specimen on the center of a glass microscope slide. Dissection of the genitalia should be made in cellosolve. Dissect the genitalia from the 7^{th} and 8^{th} abdominal segments and place below the segments, tergal side up with the gonostylus pointing down (See Plate I)
    g. Position each piece of the genitalia in a minute drop of Canada Balsam. Dry slide in oven overnight.
    h. Add more Canada Balsam over the specimen and place 2-3 tiny pieces of broken covr glass surrounding the specimen and cover with a 12 mm round cover glass.
    i. Dry in a drying oven at $45^0 – 55^0$ C for 2 weeks or more.

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[3] CAUTION! Chloroform and Ethyl Acetate are toxic and dangerous to breathe. These chemicals are stored in liver tissues and may cause health problems if used frequently. Always use in well-ventilated areas.

[4] 'Ambroid' cement should be thinned down with amyl acetate.

[5] Specimens are normally mounted on points or pinned before this procedure.

[6] Potassium hydroxide

6. STAINING GENITALIA
    a. Follow genitalia mounting techniques steps 1-4.
    b. Remove and place genitalia in 5% acetic acid and 1-2 drops of acid fuchsin[7] solution overnight or leave in the oven for another hour.
    c. Remove and place in cellosolve (can be stored in this).
    d. Follow genitalia mounting techniques.
7. LABELING (PLATE I)
    a. Label the slide with 2 labels
        i. Left label contains
            1. County, collection number
            2. Province, date collected
            3. Location
            4. Collector's names
            5. Habitat
            6. Slide number
        ii. Right label contains:
            1. Genus
            2. Subgenus
            3. Species
            4. Person making determination
    b. Label the adult with 2 labels. Each label should be ¼" by ½" in size or smaller
        i. Upper label contains:
            1. Country, province
            2. Location
            3. Collection number
            4. Date/Year
        ii. Lower label contains:
            1. Genus, subgenus and species
            2. Sex
            3. Person making determination

[7] Acid fuchsin solution
acid fuchsin - .05 gm.
10% hydrochloric acid – 25 cc.
distilled water – 300 cc

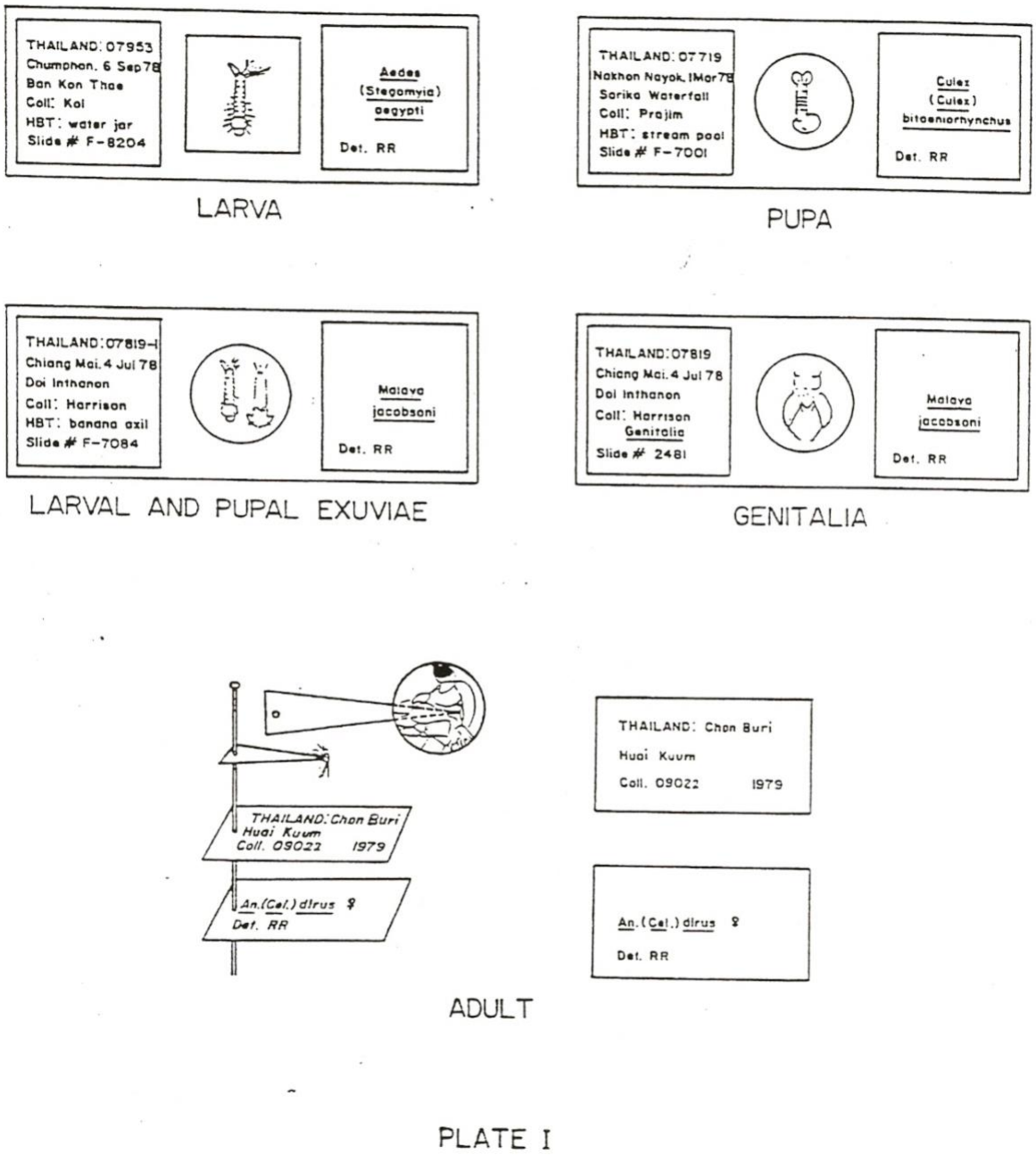

Figure 1: Mosquito Systematics, Vol.14(3)1982