SOP 03: Specimen Preparation Guide

Frost Entomological Museum Curator & Interest Group

4 April 2022

Preamble

Proper collecting, specimen preparation, and curation methods are fundamental skills for any entomologist. This SOP document provides guidance on current best practices and the standards used at the Frost Entomological Museum. You may also wish to consult the U.S. Department of Agriculture manual, updated by Schauff *et al.* [1], and other resources for more details regarding the approaches described or alluded to below.

1 Ethical considerations

Museum staff must adhere to the principles outlined in the Insect Collector's Code [2].

2 Supplies and equipment

The majority of insect preparation supplies can be purchased through an entomological supply store, VWR or similar science materials supplier, or Fine Science Tools, Inc.

- Vials, 4 dram with polyseal caps
- 70–80% ethanol, for preserving insects
- Polyvinyl alcohol adhesive, for point-mounted insects; we prefer MONO Aqua Liquid Glue (SKU 52180)
- Point punches and Bristol board for point-mounting
- Stainless steel, size 2 pins (*e.g.*, Entochrysis)
- label paper (acid free, lignin free, archival card stock)
- archival 3×5 cards and cellophane envelopes for Odonata
- identifier labels, which one can think of as unique serial numbers for each specimen or lot; see section on labels below
- Soft-tipped forceps
- Waterproof pen for labels, *e.g.*, Sakura size 005 Pigma Micron pen (black)

- Spreading board
- Acetone for Odonata

3 Preparing Hexapoda

A rough guide to preservation preferences—which taxa are pinned *vs.* slide-mounted, *etc.*—is provided in Appendix 1. One should also refer to manuals published by the USDA [1, 3]. Some general rules of thumb: If it has wings it'll likely need to be pinned. If it's soft-bodied and relatively large you will put it in ethanol. If it's small and relatively soft-bodied if should be slide-mounted (but putting in ethanol is a good first step).

3.1 Specimen handling and curation meta-practices

As you set out to prepare new specimens or handle those in an existing collection, consider these issues:

- The health and safety of the specimen is paramount. Handle specimens with extreme care, so as not to damage them or otherwise affect data quality
- Specimens should be prepared in a way that maximizes their observation potential while minimizing their footprint in the storage environment

3.2 Pinning

Pinning is the best way to preserve hard-bodied, medium to large pterygotes (*i.e.*, winged insects but not usually Odonata; see below and Appendix 1). One should use specially made insect pins, rather than common pins used in sewing and other crafts. Insect pins range in size from 000 (VERY thin and mostly unmanageable) to 7 (very thick and longer than most pins). We recommend sizes 1, 2 (especially), and 3 for general use. The best way to pin an insect is to:

1. Hold the dead insect between your index finger and thumb
2. Pass the pin vertically through the mesonotum (usually), slightly to the right of center (except in Lepidoptera, which gets the pin through the center of the mesonotum), such that it emerges near the right mid coxa. Pin placement often varies slightly by taxon (see Figure 1)
3. Slide the pin far enough through the body such that approximately 1 cm of pin is left between the insect and the pin head (Figure 2); this forms the "handle" for manipulating specimens

Lepidopterans, especially the larger species, need to have their wings spread by using a spreading board (see Figure 3). Note that Odonata are never pinned, as they take up too much room when spread out. See Appendix 1. Also, bees that were collected in ethanol or otherwise look matted can be restored to fluffiness using a process developed by Droege [4]; see Appendix 2.

See Appendix 1 for some taxon-specific recommendations. In general, though, the following guidance should be helpful when preparing dry-mounted insect specimens:

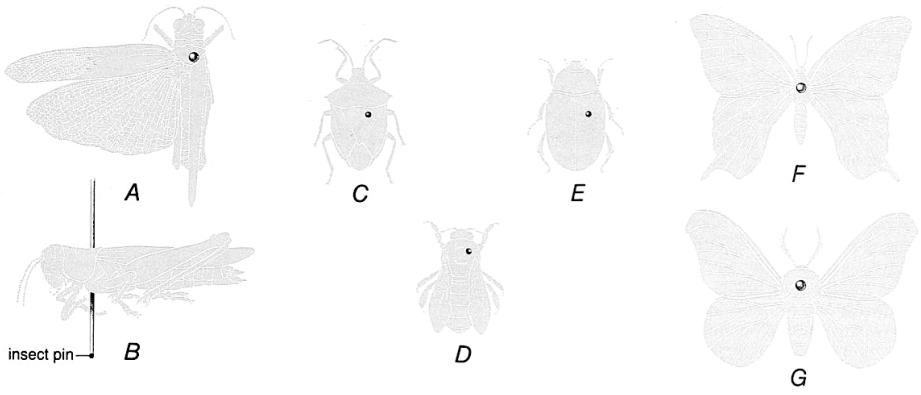


Figure 1: Ideal pin placement on different kinds of insects [modified from 3, Fig. 17]

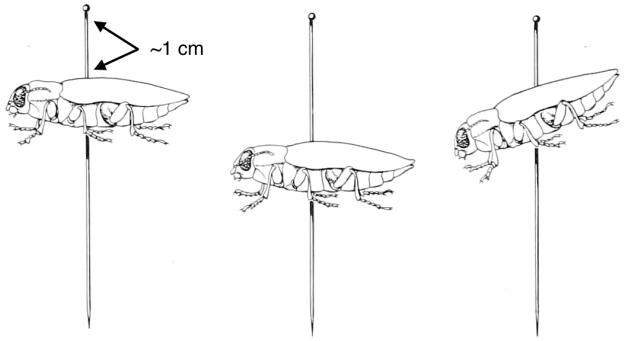


Figure 2: Ideal specimen placement on pin (left) and poor placements (middle specimen is too low, right specimen is catawampus) [modified from 3, Fig. 16]

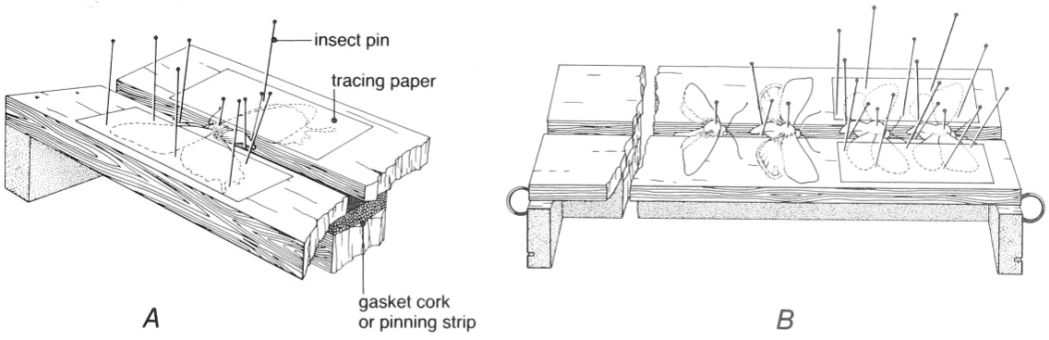


Figure 3: How to prepare lepidopteran specimens using a spreading board [3, Fig. 20]

– Most taxa are best mounted with the fore legs oriented anteriorly (*i.e.*, pointing forwards) and the middle and hind legs oriented posteriorly (*i.e.*, pointing backwards)

– Many insects (e.g., crickets, katydids, longhorn beetles, some wasps) have extremely long antennae; try to gently align them with the insect's body during the mounting process and allow them to dry fully before handling. You may need to use pins to brace the antennae

while they dry

- Mount specimens against thick styrofoam or another soft material, or use extra pins for propping appendages in place while specimens dry; this strategy will prevent legs, antennae, and other structures from hanging down and interfering with subsequent label placement
- Once you've placed a pin through an insect, don't try to remount it! A crooked specimen is far better than a specimen with multiple holes through it
- Allow specimens to fully dry before putting them into collection boxes; specimens that still have moisture can grow mold which can spread to other specimens in the same area
- Never mount a specimen using a damaged pin or damaged cardstock point
- Heavy or large specimens should be "braced" by placing pins on multiple sides of the body to prevent them from spinning and damaging adjacent specimens
- When organizing specimens in a storage box, leave sufficient room between individual specimens and between specimens and the edges of unit trays to avoid potential collisions
- Never cram specimens into small spaces; see Figure 4 (far right) for a good distribution of specimens within a single unit tray
- If a specimen breaks *do not* try to repair it with adhesive. Consult with a curator on strategies to keep the broken parts associated with the remainder of the specimen and its labels. Gelatin capsules can be used for this purpose. See section on breakage below

3.3 Pointing

Smaller insects are usually glued to a triangle (a "point") cut or punched from acid-free cardstock or Bristol board (preferred). Place a drop of archival adhesive (*e.g.*, MONO Aqua Liquid Glue), on the tip of a point that has already been pinned. Touch the point tip to the mesosternum of the insect, usually between the fore and mid coxae. The pointed insect should be oriented in a similar position to that of a pinned insect (Figure 5).

3.4 Double mounts

Microlepidopterans and scaly flies (*e.g.*, Culicidae) need to be double-mounted using minuten pins. Essentially this is the same as pinning, except one uses a very small pin (the minuten) to mount the specimen. That mount is then affixed to a normal-sized insect pin via a small piece of foam or silicone (Figure 6). A more detailed description is provided by [5].

3.5 Alcohol

Soft-bodied hexapods, including spiders and all immature and aquatic insects (except perhaps Coleoptera), must be preserved in alcohol, rather than being pinned or pointed. Hexapods preserved in >95% alcohol are best for DNA extraction, especially if they are kept cold. Unfortunately,

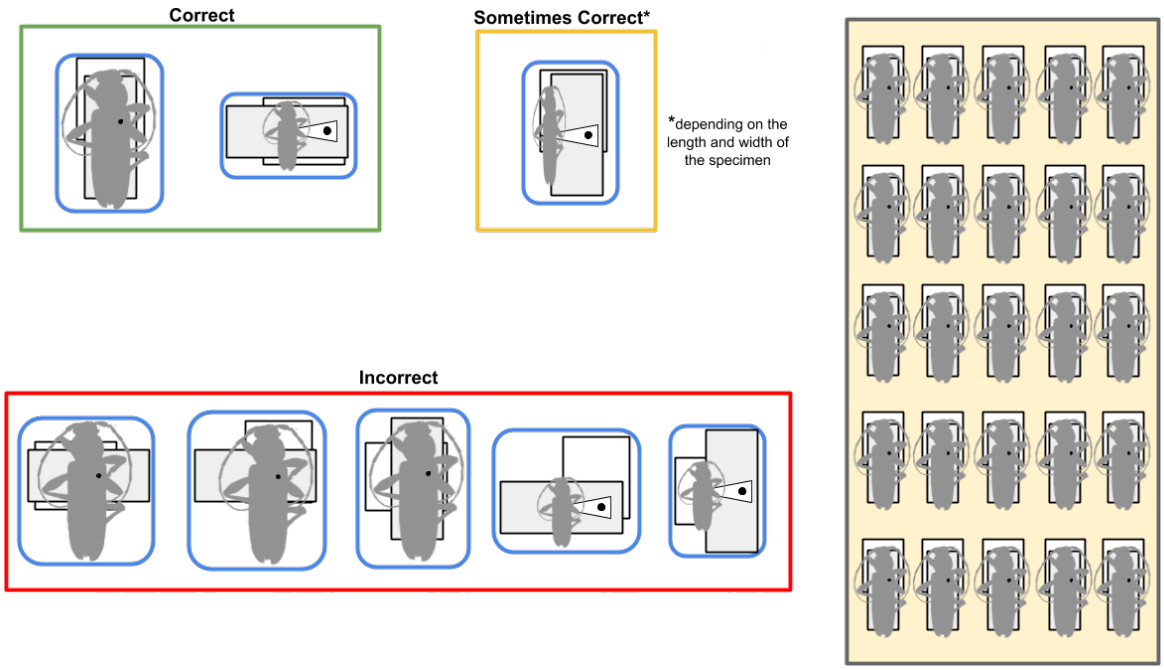
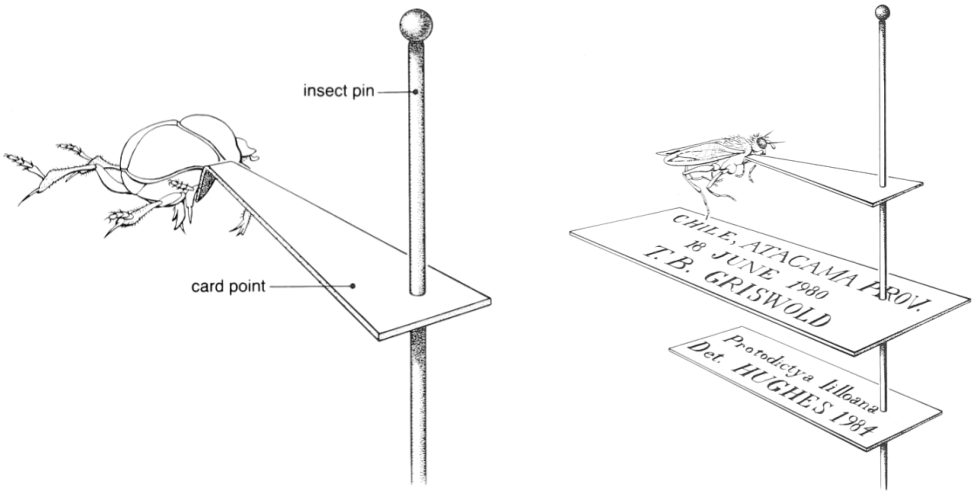


Figure 4: Label orientation and specimen spacing. Figures by Hailey Majewski (Cleveland Museum of Natural History; figures used by permission)



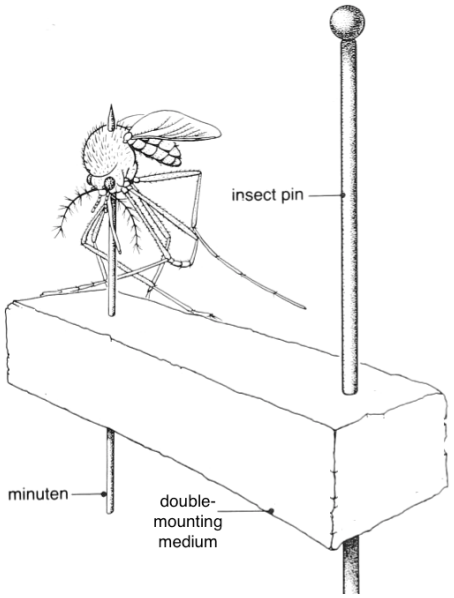
(a) Coleopteran on point [3, Fig. 18C]

(b) Dipteran on point [3, Fig. 24]

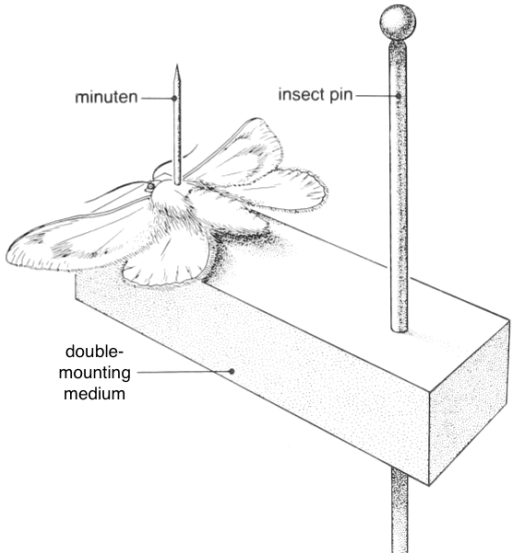
Figure 5: Point-mounted insects

high concentrations of alcohol also tend to make specimens a bit fragile, by dehydrating them. Concentrations below 70% are generally not recommended, as specimens have a better chance of rotting.

We use 70–80% ethanol in the lab, which works well for nearly any kind of hexapod. Preparation procedure:



(a) Dipteran on double mount [modified from 3, Fig. 18A]



(b) Lepidopteran on double mount [modified from 3, Fig. 18D]; this specimen is arguably too close to the medium but is oriented correctly

Figure 6

1. Soft-bodied arthropods are best killed in boiling water, which will usually result in a body that is "spread out" and partially fixed. Once killed, place them inside a shell vial or screw cap vial. There should be one species per vial
2. Insert locality label and identifier label. See label standards below
3. Fill with 80% ethanol
4. If using a shell vial insert cotton wad or polyethylene stopper. Otherwise cover with screw cap. Be sure all air bubbles are removed
5. Place shell vial on cotton pad inside jar. Ideally a jar would contain a single species or several species in a genus. If using a screw cap vial make sure cap is on tight and place into storage box. Make sure jar/box is numbered and labeled with taxon and fill date

Adult Lepidoptera should not (ideally) be preserved in ethanol, as the scales will detach.

3.6 Slide Mounting

Slide-mounted preparations are critical for proper diagnosis of certain taxa, especially small insects. The methods are typically intensive and are covered by another SOP document [6].

4 Specimen labeling

4.1 Label order on pins

Label order should *always be preserved*, as it provides a historical record for that specimen. We typically deal with two kinds of scenarios in the collection:

- Historical specimens that need identifier labels and/or new determination (det.) labels. In these cases always put the identifier below the last historical label and any new determination labels below the identifier.
- Recently prepared/labeled specimens that previously had no labels. In these cases put the identifier label on first, then the collecting event, then the determination label.

When is it okay to discard a label? Assume *never*. Some labels, however, do not offer any obviously important data—a ripped, non-archival label that simply offers the family name, for example—and may be discarded at the discretion of the Director or Collection Manager.

4.2 Identifier label

Every specimen or lot should have a unique code associated with it, and ideally the string of numbers and letters would be (somewhat) meaningful and globally unique. For the ENT 532 class one should create a code that combines the class name + student initials + year + unique sequential number. A student named Ke Chung Kim, for example, could use labels that look like this:

ENT532_2022
KCK_1234

ENT532_2022
KCK_1235

For specimens to be accessioned into the Frost Museum's research collection, sheets of pre-made identifiers are available. All are prefixed with "PSUC_FEM" and include a data matrix code for scanning. Ideally the data matrix portion of the label should be visible from above. These labels are safe to use in ethanol and other liquid preservatives.

4.3 Collecting event label

Collecting event (CE) labels can take many forms, but you generally want to adhere to the following formats:

1. Labels always begin with the country (sometimes in **bold** or ALL CAPS) and continue with finer scale details of the locality. One should always include the latitude and longitude—using decimal based degrees is preferred over minutes and/or seconds, as they are easier to database.
2. The date should be formatted such that the month is in lowercase Roman numerals (*e.g.*, October would be "x"): 12.x.2022 or 11–12.x.2022 or 11.x–12.xi.2022
3. The collector's name(s) should also be included, as should the collecting method. Common abbreviations include: MT = Malaise trap, YPT = yellow pan trap, FIT = flight intercept trap, SS = screen sweep. Collectors' names are sometimes followed by "leg.", which is short for the Latin "lego", to gather or collect.

4. A sans-serif font (like Arial Narrow) makes the label more readable when the size gets small. Most people use 4 pt for the font size. The finished label should be informative, with a minimal amount of abbreviations, but also reasonably small in size. The information should also be presented in a symmetrical label that minimizes white space:

   USA: PA: Centre County:
   Pine Grove Mills, 40.730,
   -77.884, ± 500m 15.iv.2021
   A.R. Deans, sifted litter

5. Labels that seem to be excessively large can be cut into two labels. Specimens are prone to multiple labeling from future studies (voucher label, determination label, accession numbers, barcodes, *etc.*), so it's desirable to keep the label number to a minimum.

6. Use cotton rag, acid free cardstock for printing labels.

4.4 Determination Labels

Determination ("det") labels can also vary in their appearance, but it's important that they include the taxon name(s), including the author if it's a species-level determination, the name of the determiner, and the year (or date) the determination was made. Det labels for fluid preserved specimens should be in a slightly larger font and more elongate (see Section 4.5).

Amphibolips confluenta
(Harris, 1841)
Hymenoptera: Cynipidae
det. A.R. Deans 2022

4.5 Labels for fluid-preserved Specimens

Same suggestions apply to labels for fluid-preserved specimens, but one should make the labels slightly larger (maybe 6 pt) and more elongate:

USA: PA: Centre County: Pine Grove Mills
Slab Cabin Run, 40.730, -77.884, ± 250m
hand collected off rocks, 15.iv.2021 A.R. Deans

Small labels act almost like blades and can damage wet-preserved specimens as the vial gets moved around.

5 Breakage

Insects are relatively fragile, especially dried specimens, and breakage is not a rare phenomenon. Broken parts should be treated in a similar fashion to dissected parts, *i.e*, by reassociating them with the primary specimen. For example, if one or more appendages breaks off from a body it/they can be glued to a point or small cut of archival card stock and pinned with the primary specimen. Alternatively one can place them inside a gelatin capsule or genitalia vial that is then pinned with the primary specimen. For Odonata in envelopes, the broken pieces can be placed inside a mini-envelope, cut from a larger envelope, and inserted inside the primary envelope that holds the specimen.

6 How to cite

To cite this document one can use the following format:

Frost Entomological Museum Curator & Interest Group (2022) SOP 03: Specimen Preparation Guide. Available at https://doi.org/10.26207/a1kq-xy95 Accessed <date>

References

[1] M. E. Schauff, ed. *Collecting and Preserving Insects and Mites, Techniques & Tools*. Systematic Entomology Laboratory, USDA, Washington, DC, 2005. http://www.ars.usda.gov/Main/docs.htm?docid=10141&page=1.

[2] Carolyn Trietsch and Andrew R. Deans. The insect collector's oath, version 1. *figshare*, 2016. Retrieved: 00 33, Aug 04, 2016 (GMT).

[3] George C Steyskal, Jr Murphy, William L., and Edna M Hoover. *Insects and mites: techniques for collection and preservation*. U.S. Department of Agriculture, Washington, DC, 1986. https://archive.org/details/insectsmitestech1443stey.

[4] Sam Droege. Rewashing old bee specimens so they are clean and fluffy and good for picture taking. https://listserv.unl.edu/cgi-bin/wa?A2=ECN-L;618813d.1708B, 2017. Accessed: 17 August 2017.

[5] Chris Grinter. Spreading microlepidoptera. http://www.theskepticalmoth.com/techniques/spreading-microlepidoptera/, 2016. Accessed: 20 July 2016.

[6] István Mikó and Andrew R. Deans. Frost Entomological Museum standard operating procedure: Slide-mounting Arthropoda. http://bit.ly/SlideMountingInsects, 2013. Accessed: 20 July 2016.

Appendix 1. Insect collection and preservation guide

The table below should not be considered as the definitive guide to insect preservation but rather as a quick reference for how these insects are typically preserved—*i.e.*, a "What can I do now that I caught this hexapod?" guide.

Entognatha (proturans, diplurans, springtails). *Habitat:* leaf litter, under rocks/logs. *Collecting method:* Winkler extractor, Berlese funnel. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Traditionally slide-mounted, but can also be stored in vials with ethanol (>70%) as the preservative.
Photo: Andy Murray (CC BY-SA 2.0) `https://flic.kr/p/bJCR9e`

Archaeognatha (bristletails). *Habitat:* leaf litter, on logs at night. *Collecting method:* Aspirator, forceps. *How to euthanize:* Submerge in ethanol or boiling water or put in kill jar. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: Henry Lydecker (CC BY-NC 2.0) `https://flic.kr/p/dgRtaW`

Zygentoma (silverfish, firebrats). *Habitat:* leaf litter, caves, inside buildings. *Collecting method:* Aspirator, net. *How to euthanize:* Submerge in ethanol or boiling water or put in kill jar. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: Jean-Raphaël Guillaumin (CC BY-SA 2.0) `https://flic.kr/p/czibHd`

Ephemeroptera (mayflies). *Habitat:* Immatures are aquatic; adults fly. *Collecting method:* Use D-net for immatures; aspirator, net, and/or sheet at light for adults. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Preserve all stages in vials with ethanol (>70%); note that these specimens will be fragile!
Photo: Magnus Hagdorn (CC BY-SA 2.0) `https://flic.kr/p/ffsx8c`

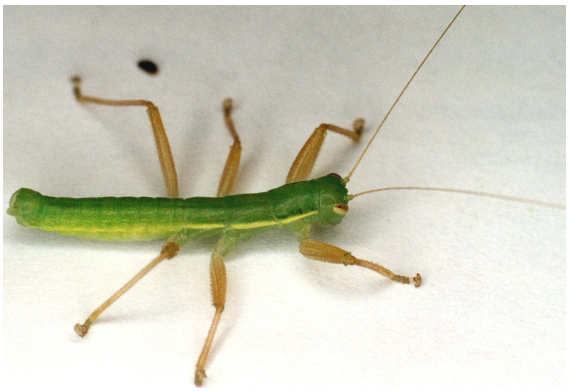
Odonata (dragonflies and damselflies). *Habitat:* Immatures are aquatic; adults fly. *Collecting method:* Use D-net for immatures, aerial net for adults. *How to euthanize:* Adults should be kept alive in glassine envelope or paper triangle until they can be euthanized by submersion in acetone. Larvae are euthanized in ethanol or boiling water, like other insects. *Prepare specimens:* Soak adults in acetone overnight, then let air dry. Dried specimens are preserved with a 3×5 card (with locality label), inside a cellophane envelope. Larvae are always preserved in vials with ethanol (>70%).
Photo: Andy Deans (CC BY 2.0) `https://flic.kr/p/oa95N7`

Phasmatodea (walking stick, stick insects, leaf insects). *Habitat:* Trees, fence posts. *Collecting method:* By hand, net. *How to euthanize:* Freeze or use kill jar with ethyl acetate. *Prepare specimens:* Nymphs are preserved in vials with ethanol (>70%). Adults are pinned through mesothorax, with legs, wings (if present) and antennae pinned near body to minimize overall specimen size.
Photo: Norman Walsh (CC BY-NC 2.0) `https://flic.kr/p/3gcGh5`

Orthoptera (grasshoppers, crickets, katydids). *Habitat:* Grasses, trees, shrubs. *Collecting method:* By hand, net, with baits. *How to euthanize:* Freeze or use kill jar with ethyl acetate. *Prepare specimens:* Nymphs and soft-bodied taxa (*e.g.*, Gryllidae) are preserved in vials with ethanol (>70%). Adults are pinned through mesothorax, with legs and antennae pinned near body to minimize overall specimen size. Note that the guts of large specimens should be removed prior to pinning (insert sharp forceps under the posterior edge of pronotum, grab gut and pull out). Left wings could be spread.
Photo: Andreas Kay (CC BY-NC-SA 2.0) `https://flic.kr/p/mR5QLa`

Grylloblattodea. *Habitat:* Snow pack, glaciers of western North America. *Collecting method:* By hand. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: Alex Wild (CC0) `https://goo.gl/DAU1HJ` (Wikimedia Commons)

Mantophasmatodea. *Habitat:* Grasses, shrubs, and rocks of southern Africa. *Collecting method:* By hand. *How to euthanize:* Freeze or submerge in ethanol. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: P. E. Bragg (CC BY-SA 3.0) `https://goo.gl/kpnn99` (Wikimedia Commons)



Dermaptera (earwigs). *Habitat:* Under rocks and logs. *Collecting method:* By hand, on sheet at light. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Pin through mesothorax.
Photo: Mick E. Talbot (CC BY-NC-SA 2.0) `https://flic.kr/p/baT4zp`

Non-isopteran Dictyoptera (cockroaches, mantids). *Habitat:* Under rocks and logs; also inside buildings. *Collecting method:* By hand, on sheet at light. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Pin through mesothorax and keep appendages close to body; male genitalia are useful for species-level diagnosis, so maximize their visibility.
Photo: Martin Grimm (CC BY-NC-SA 2.0) `https://flic.kr/p/aVJPH8`

Dictyoptera: Isoptera (termites). *Habitat:* Under rocks and logs; also inside buildings. *Collecting method:* By hand, with aspirator, with net, on sheet at light. *How to euthanize:* Submerge in ethanol or boiling water. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: Stevenw12339 (CC BY-NC 2.0) `https://flic.kr/p/fAdvLc`

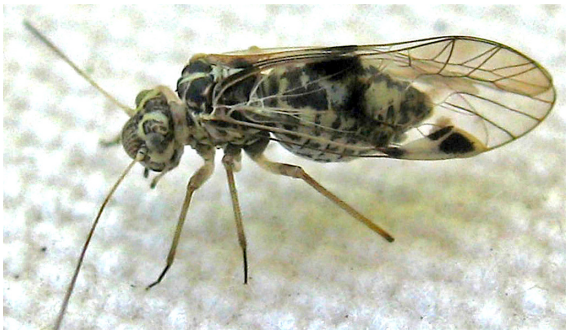
Embioptera (webspinners). *Habitat:* Under rocks and logs, on tree trunks. *Collecting method:* Look for silken galleries and collect by hand; may also come to lights. *How to euthanize:* Submerge in ethanol or boiling water. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: Bill & Mark Bell (CC BY-NC-SA 2.0) `https://flic.kr/p/cCJB7S`

Zoraptera. *Habitat:* Under logs, especially in or near sawdust. *Collecting method:* By hand or with Winkler extractor or Berlese funnel. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Preserve in vials with ethanol (>70%).
Photo: David Maddison (CC BY 3.0) `http://goo.gl/hSP3EW` (tolweb.org)

Plecoptera (stoneflies). *Habitat:* Immatures are aquatic, especially in cold, highly oxygenated streams; adults fly. *Collecting method:* Use D-net for immatures; aspirator, net, and/or sheet at light work for adults. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Preserve all stages in vials with ethanol (>70%).
Photo: Bernard DuPont (CC BY-SA 2.0) `https://flic.kr/p/dKDmYT`

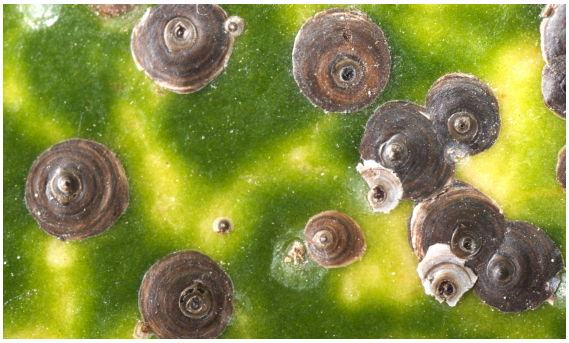
Thysanoptera (thrips). *Habitat:* On leaves, flowers, under fallen logs. *Collecting method:* Aspirator, net, Winkler extractor, Berlese funnel. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Normally slide-mounted but can be preserved in vials with ethanol (>70%).
Photo: Katja Schulz (CC BY 2.0) `https://flic.kr/p/q6LxBS`

Psocodea (bark lice, book lice, parasitic lice). *Habitat:* On leaves and trunks, around books; parasitic species on mammals and birds. *Collecting method:* Aspirator, forceps. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Normally slide-mounted but can be preserved in vials with ethanol (>70%).
Photo: Ken Schneider (CC BY-NC 2.0) `https://flic.kr/p/mX6h9D`



Hemiptera: Heteroptera, Auchenorrhyncha (true bugs, hoppers, cicadas). *Habitat:* On plants, under logs, in/on water, on host mammals and birds; diverse and nearly ubiquitous. *Collecting method:* Almost any method, depending on habitat targeted. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Nymphs preserved in vials with ethanol (>70%). Adults of most species are pinned through thorax, dorsal to mesothoracic legs. Note that wing venation, mouthparts, and antennae are important for family-level diagnosis.
Photo: NY State IPM Program (CC BY 2.0) `https://flic.kr/p/guTgFA`

Hemiptera: Sternorrhyncha (scale insects, aphids). *Habitat:* On plants, especially leaves and stems *Collecting method:* Aspirator, by hand. *How to euthanize:* Freeze, submerge in ethanol. *Prepare specimens:* Usually slide-mounted but can be preserved in vials with ethanol (>70%).
Photo: Jon Sullivan (CC BY-NC 2.0) `https://flic.kr/p/oSAp1Y`



Mecoptera (scorpionflies). *Habitat:* On plants, on snow near trees. *Collecting method:* Sweep net/aspirator, by hand, Malaise trap. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Larvae and all Boreidae are preserved in vials with ethanol (>70%). Adults are pinned through mesothorax.
Photo: Orest Shvadchak (CC BY-SA 2.0) `https://flic.kr/p/945m9F`

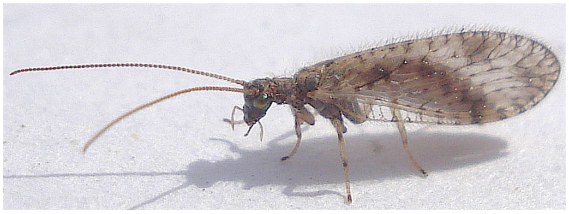
Siphonaptera (fleas). *Habitat:* Larvae live in nests of their hosts. Adults usually found on host mammals and birds. *Collecting method:* Aspirator, forceps. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Normally slide-mounted but can be preserved in vials with ethanol (>70%).
Photo: AFPMB (CC0) `https://flic.kr/p/9bKUYn`

Diptera (flies, gnats, mosquitoes). *Habitat:* Diverse and nearly ubiquitous in most habitats. *Collecting method:* Almost any method, depending on habitat targeted. Malaise traps and yellow bowls are especially effective for adults. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Larvae and soft-bodied adults (*e.g.*, Cecidomyiidae) preserved in vials with ethanol (>70%). Adults of most species are pinned through thorax, dorsal to mesothoracic legs, or pointed dextrally. Adults should be pinned/pointed in a way that one can view all sclerites (*i.e.*, legs away from body). Wing veins and bristle patterns are important for diagnosis. Scaly flies, like mosquitoes, should be double-mounted, with the minuten entering the thorax dextrally.
Photo: Troup Dresser (CC BY-NC 2.0) `https://flic.kr/p/cYaAPo`

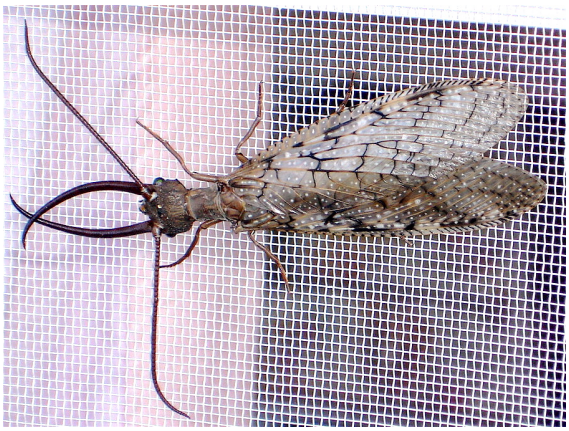
Lepidoptera (moths, butterflies). *Habitat:* Diverse and nearly ubiquitous in most habitats. *Collecting method:* Almost any method, depending on habitat targeted. Lights and baits are especially effective at luring adults adults. *How to euthanize:* Freeze or use kill jar with ethyl acetate. *Prepare specimens:* Larvae are preserved in vials with ethanol (>70%). Adults of most species are pinned medially through thorax, dorsal to mesothoracic legs. Small specimens should be double-mounted, with minuten entering thorax similar to a normal pin. Wings of all specimens must be spread.
Photo: Andy Reago & Chrissy McClarren (CC BY 2.0) `https://flic.kr/p/ofEZW6`

Trichoptera (caddisflies). *Habitat:* Immatures are aquatic; adults fly. *Collecting method:* Use D-net for immatures; aspirator, net, and/or sheet at light for adults. *How to euthanize:* Submerge in ethanol. *Prepare specimens:* Preserve all stages in vials with ethanol (>70%)
Photo: Macroscopic Solutions (CC BY-NC 2.0) `https://flic.kr/p/o4e7U7`

Neuroptera (lacewings, antlions, mantisflies). *Habitat:* Immatures are terrestrial or aquatic; adults fly. *Collecting method:* Aspirator, net, and/or sheet at light for adults. *How to euthanize:* Submerge in ethanol, freeze, or use kill jar with ethyl acetate. *Prepare specimens:* Larvae go in vials with ethanol (>70%). Adults are pinned through the mesothorax or pointed and positioned in a way that minimizes their size (legs and antennae close to body).
Photo: Mick E. Talbot (CC BY 2.0) `https://flic.kr/p/6mWUDw`



Megaloptera (dobsonflies, fishflies, alderflies). *Habitat:* Immatures are aquatic; adults fly. *Collecting method:* D-net for larvae; forceps, net, and/or sheet at light for adults. *How to euthanize:* Submerge in ethanol (especially larvae), freeze, or use kill jar with ethyl acetate. *Prepare specimens:* Larvae go in vials with ethanol (>70%). Adults are pinned through the mesothorax and positioned in a way that minimizes their size (legs and antennae close to body).
Photo: Ronald Orosz (CC BY-NC 2.0) `https://flic.kr/p/4TEtzq`



Raphidioptera (snakeflies). *Habitat:* Larvae and adults are frequently associated with trees; in the USA they're found exclusively in the west. *Collecting method:* Forceps, net. *How to euthanize:* Submerge in ethanol (especially larvae), freeze, or use kill jar with ethyl acetate. *Prepare specimens:* Larvae go in vials with ethanol (>70%). Adults are pinned through the mesothorax and positioned in a way that minimizes their size (legs and antennae close to body).
Photo: Tab Tannery (CC BY-NC-SA 2.0) `https://flic.kr/p/92UMz6`

Strepsiptera (twisted-wing parasites).*Habitat:* Larvae, pupae, and adult females are usually found with their hosts (*e.g.*, aculeate Hymenoptera, many hemipterans). *Collecting method:* Malaise traps sometimes get males; other methods haphazardly collect these insects by collecting their hosts. *How to euthanize:* Submerge in ethanol or use kill jar with ethyl acetate. *Prepare specimens:* All stages go in vials with ethanol (>70%).
Image: J. Sowerby (CC0), via Biodiversity Heritage Library `https://flic.kr/p/a9W5Qa`

Coleoptera (beetles). *Habitat:* Diverse and nearly ubiquitous in most habitats. *Collecting method:* Almost any method, depending on habitat targeted. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Larvae preserved in vials with ethanol (>70%). Adults are pinned through right elytron, dorsal to mesothoracic legs, or pointed dextrally. Adults should be pinned/pointed in a way that one can view antennae, all legs, and the ventral sclerites.
Photo: Gilles San Martin (CC BY-SA 2.0) `https://flic.kr/p/hCtNmf`

Hymenoptera (sawflies, wasps, ants, bees). *Habitat:* Diverse and nearly ubiquitous in most habitats. *Collecting method:* Almost any method, depending on habitat targeted, but yellow bowls and Malaise traps are especially effective. *How to euthanize:* Freeze, submerge in ethanol, or use kill jar with ethyl acetate. *Prepare specimens:* Adults are pinned through mesothorax, dorsal to mesothoracic legs, or pointed dextrally. Head characters, wing venation, and ventral sclerite morphology can be important for family-level diagnosis. Small, dainty hymenopterans with soft cuticle (e.g., most Chalcidoidea) and larvae should be preserved in vials with ethanol (>70%).
Photo: Patrick_K59 (CC BY 2.0) `https://flic.kr/p/peXyQ8`

Appendix 2. Rewashing old bee specimens so they are clean and fluffy and good for picture taking (as described by Sam Droege [4])

Old, dried ugly specimens, even those decades old can be revived using the following procedure:

1. Pull tags off specimen
2. Drop specimen into centrifuge tube or vial with HOT water with high concentration of dish soap
3. Shake for about a minute ... more if super goopy and matted
4. Dump into hand
5. Wash under hot running water
6. Put on paper towel to pull off water
7. Drop in tube of acetone
8. Shake for around 30 seconds
9. Drop on paper towel and blot off excess acetone
10. IMMEDIATELY take to a station that allows you to blow compressed air on specimen (Droege uses a latex hose that connects the lab's compressed air nozzle on a lab bench and then connects to the extracted top of a lab wash bottle ... the type with a long tube that goes through the cap into the bottle ... attach the latex tube using a binder clamp or it will slip off)
11. Turn on air a SMALL amount
12. Use your the wash bottle nozzle to blow air on specimen. *NB*: If you blow air directly from compressed air fitting the wings will shred
13. Blow until hairs are dry and separated
14. For best results do under a microscope

See `https://flic.kr/p/XBLmfg` for a good before/after example.