Appendix I: Morphological Terms of Orientation and Position

In order to describe morphological structures accurately and unambiguously, one needs to know the meanings of the following terms. Terms of direction and position do not necessarily indicate that the structure to which they are applied lies near or nearer the part suggested by the term. They mean that the structure lies in the direction taken by a particle moving from the center of the body toward and continuing beyond the part in question. Thus "cephalic" related to the cephalon or head but he second segment of the antenna which projects anteriorly beyond the head is cephalic to (or cephalad of) the first segment, although it is farther away from the head.

Orientation/Position:

Anterior — refers to the forward or head end of the animal. In some insects the anterior end of the body is formed by the mouthparts, in others by the vertex, the mouthparts being directed ventrally or even posteriorly.

Caudal — pertaining to the posterior or "tail" end.

Cephalic — pertaining to the head; anterior.

Distal — refers to the free end of a part or appendage, or the end furthest away from the point of attachment.

Proximal — refers to the base of a part or appendage; the region nearest the base.

Dorsal — pertaining to the dorsum or back.

Ectal — pertaining to the outer surface of the body.

Ental — pertaining to the inside of the body

Lateral — pertaining to the sides.

Meson — the sagittal plane dividing the insect into right and left halves; the midline.

Mesal— pertaining to the meson.

Posterior — refers to the rear end of the animal.

Ventral — pertaining to the venter.

Movement/Direction (suffix -ad):

Caudad — posteriorly; in the direction of the posterior or "tail" end. [caudad of = posterior to]

Cephalad — anteriorly; in the direction of the head.

Distad — toward the distal end.

Dorsad — dorsally; toward the dorsum of the back.

Ectad — toward the outer surface. Used to indicate the relative position of internal structures.

Entad — toward the inside; i.e. the long axis.

Laterad — toward the sides (right or left).

Mesad — toward the meson.

Ventrad — towards the venter.

Terminology used in reference to sections through the body of an animal:

sagittal section — passing through the plane which bisects the body along the midline into right and left halves (meson).

parasagital section — a section parallel to the sagital section but offset slightly from the midline to the right or left.

cross section — passing through the body at right angles to the long axis of the animal.

frontal section — a section at right angles to a cross section and a sagital section.

Terminology used to refer to muscle position and connection:
origin — the proximal or unmoved site of attachment of a muscle fiber.
insertion — the distal or moved site of attachment of a muscle fiber.

Taken in part from Duporte, 1977. Manual of Insect Morphology, pp. 6-7; See also Nichols & Schuh, 1989. The Torre-Bueno Glossary of Entomology.

Appendix II: Dissection Tools and Techniques

For high quality observation of your dissections we recommend the following pieces of equipment. First, a good quality dissecting scope with a range of magnification from 10x to 60x. For drawing, scopes should have an ocular grid or an attached camera lucida (see Appendix III for sketching techniques, including use of the camera lucida). At least one pair (and preferably two pairs) of high quality dissecting forceps (Dumont #5 is standard) will be essential for manipulating specimens. We recommend Fine Scientific Tools (Fine Scientific Tools, Inc., 373-G Vintage Park Dr., Foster City, CA, 94404 [800] 521-2109) as a source for dissecting instruments. For dissections we also recommend some sort of dissecting tray or pan with a wax bottom into which pins can be pushed. Simple and cheap dissecting pans can be made with plastic petri dishes (20x100 mm) lined with a thin layer (2-4 mm) of black dissecting pan wax (available from Carolina Biological Supply Co., 2700 York Rd., Burlington, NC, 27215-3398 [800] 334-5551). Minutin pins (available from most entomological supply houses, including BioQuip Products, 17803 LaSalle Ave., Gardena, CA, 90248-3602 [310] 324-7931) are essential for positioning insects during dissections and for holding certain organs and appendages out of the way during dissections.

Minutin pins can also be mounted on the ends of small dowels (3-4 mm in diameter) for micro-dissecting tools. To do this, soak the tip of a dowel in water for 10 minutes to soften it and then, holding the minutin pin with some pliers, jam the dull end of the minutin pin into the end of the dowel. Allow the dowel to dry and the minutin pin will be permanently fixed in place, with the sharp end pointing outward. These sharply pointed micromanipulators can be modified for many different types of dissecting instruments.

Small dissecting scissors (available from Fine Scientific Tools) are nice to have but are not essential. A scalpel and sharp blades or single edge razor blades will be needed for cutting specimens in sagittal, parasagittal or cross-section.

Insect dissections are best performed under some fluid medium. For preserved material and freshly killed specimens we recommend 70% ethanol. Make sure the specimen is totally immersed so that there is no optical distortion from parts of the specimen projecting out of the ethanol. For internal anatomy we recommend, it is available, using live material. Internal organs are far more clearly visible in live rather than dead specimens (and they move as well!). In preserved specimens the fat body becomes an amorphous mass of connective tissue which obscures the relationships among the other organs. For dissection of live specimens we recommend using an insect Ringers solution (see Appendix VI) which will keep the cells and organs of anesthetized insects alive for up to an hour during dissections. Insects can be anesthetized by chilling in the freezer for 5 or 10 minutes or by knocking them out with CO_2 or chloroform. (Chloroform should be handled with rubber gloves in a fume hood.)

Appendix VI: Stains, Fixatives, Slide Mounting Media, etc.

Fixation & Storage

1. Dietrich's (Kahle's) Fluid:

For gross morphology: Place living insect into cold fluid, inject or slit if possible (especially necessary with hard-bodied insets). Preserve in Dietrich's Fluid. Dissect under water or in 50% ethanol, store after dissection in 70% ethanol or permanent preservative fluid.

For histology: Place living insect in fluid, heat fluid on hotplate under hood under it begins to give off vapor, but is not bubbling. Slit or inject insect. Replace insect in fluid and remove from heat, cover and allow to stand for up to 24 hours under hood. Wash in 50% ethanol, then in 70% ethanol. Store in 70-80% ethanol or permanent preservative fluid.

Formula	**Dietrich's**
formalin	6 ml
95% ethyl alcohol	15 ml
glac. acetic acid	1 ml
dist. water	80 ml

Formula	**Permanent Preservative Fluid**	
	70%	80%
95% ethyl alcohol	70 ml	80 ml
distilled water	25 ml	15 ml
glycerin	5 ml	5 ml

2. F.A.A. Solution (Smith's Modification): Place living insects into cold fluid, inject or slit if possible. Rinse and store in 80% 2-propanol (isopropyl alcohol) when convenient, after 24 hours.

Formula	
glacial acetic acid	10 ml
formalin	10 ml
80% 2-propanol	80 ml

3. **Duboscq-Brasil (=alcoholic Bouin's) Fluid**: Place living insect in cold fluid, heat fluid on hotplate under hood until it begins to give off vapor, but is not bubbling. Slit or inject insect. Replace insect in fluid and remove from heat, cover and allow to stand for 3-24 hours under hood. Wash in 70% ethanol until washing fluid no longer stains yellow. Store in 70-80% ethanol or permanent preservative fluid. Stiff insects may be relaxed for a few minutes in warm water before dissecting.

	Formula
Picric acid	1 gr
40% formalin	60 ml
glacial acetic acid	15 ml
80% ethyl alcohol	150 ml

4. **Aqueous Bouin's Fluid**: Fix 4-24 hours in cold fluid. Wash in 50% ethyl alcohol, then into several washes 70% ethyl alcohol, changing daily until yellow color ceases. Store in 70% permanent preservative fluid. ***Better method***: Heat Bouin's Fluid to 70 C, remove from heat, and add insect.

	Formula
picric acid saturated aqueous solution (about 1 gr. picric acid will saturate 75 ml water)	75 ml
40% formalin	25 ml
glacial acetic acid	5 ml

5. **Carnoy's Second Formula Fluid**. Fix 10 min. to 3 hours, use cold. Transfer to 95% ethyl alcohol, several changes. Store in 95% ethyl alcohol.

	Formula
absolute ethyl alcohol	60 ml
chloroform	30 ml
glacial acetic acid	10 ml

6. **Weaver & Thomas' Solution**: Drown insect in fixative and slit or inject with solution. Specimen should be stored in fixative; do not use alcohol before or after fixation. Insects may be dissected under water after fixation to avoid fumes.

	Formula
40% formalin	5 ml
glacial acetic acid	2.5 ml
chloral hydrate	20 gr
add distilled water	100 ml

DISSECTING LIVING MATERIAL:

Insect Ringer's solution. Narcotize insect with chloroform, ether, or CO2. Dissect in Ringers.

Formula (for cockroaches)

sodium chloride	14 gr
calcium chloride	0.4 gr
potassium chloride	0.2 gr
sodium bicarbonate	0.2 gr
distilled water	1 liter

BLEACHING

1. **Sodium hypochlorite solution**. To remove dark pigments from the cuticle of specimens to be cleared. Place the alcohol-preserved specimen in a closed container of the bleaching agent for a few hours to several weeks, until pigment is removed. Replace agent every 2 days. Wash well in 70% ethanol after bleaching is completed.

Formula

70% ethanol	10 ml
"Clorox"	3 or 4 drops

2. **Mayer's Chlorine Method**: Used as above, may injure delicate speciments. ***Preparation*:** Put few crystals potassium chlorate into test tube, add 2-3 drops concentrated hydrochloric acid. When greenish chlorine gas is emitted, add 5-10 ml of 70% ethanol. Transfer specimen from 70% ethanol to solution, leave until bleached (few minutes to day or more). Rinse in several changes of 70% ethanol. Water may be used in place of alcohol.

CLEARING

1. **Potassium (or Sodium) Hydroxide**. For use with skeletal characters only. Place specimen in test tube of 10% potassium hydroxide, allow to sit 1 hr 1 day until sufficiently clear. Wash several times in distilled water. If specimen is to be mounted in resin permanent mount, wash in acidified 35% ethyl alcohol, then into more concentrated ethyl alcohol (70% usually). ***Fast method***: Place tube of 10% KOH with specimen into beaker of boiling water, watch carefully until clear. 10% NaOH is more delicate than KOH and sometimes preferred.

Formula: Acidified Alcohol

35% ethyl alcohol	50 ml
hydrochloric acid	3 drops

2. **Acetic Corrosive**: Used for skeletal structures only, often used for mites. Place specimens in cold acetic corrosive (tightly covered) overnight or until clear. Mount in Hoyers.

Formula

glac. acetic acid	162 ml
chloral hydrate	160 gr
distilled water	180 ml

3. **Lacto-phenol**: Used for skeletal structures and mites, as with acetic corrosive. Use as above, or for faster action gently warm solution and specimen on hotplate (do not allow to boil). 30% solution (below formula at half strength) may be used for more delicate specimens. To avoid crystals when mounting specimens in Hoyers, place specimen briefly in drop of Hoyers before transferring to fresh Hoyers for mounting.

Formula

85% lactic acid	40 ml
molten phenol	20 ml
distilled water	20 ml

4. **Xylene**. Use from 100% ethyl alcohol. Leave in xylene until clear, from less than an hour to 6 hours. Mount in balsam, or into 100% ethyl alcohol to mount in diaphane.

5. **Clove Oil**. Use from 95% ethyl alcohol. Leave in oil until clear, transfer to balsam for permanent mount. ***Kugler Technique***: Place specimen in equal parts clove oil and 95% alcohol overnight; alcohol evaporates, leaving clove oil. Disadvantage: specimen becomes brittle.

6. **Cedar Oil**. Use same as #4; slower acting; can be stored in cedar oil for some time.

7. **Terpineol**. Use same as #5; also slow acting.

TEMPORARY MOUNTS

Place cleared specimen in small glass spot-plate or depression slide in drop of glycerol, adequate to cover specimen. May be transferred from water or 70% alcohol. For delicate specimens, run through

graded water- or alcohol-glycerol series. Preserve in glycerol in small corked "genitalia vial" attached to pin of specimen.

PERMANENT MOUNTING MEDIA

1. **Canada Balsam**. Mount from xylene or cedar oil, clove oil, or terpineol. Place cleared specimen into drop of balsam on clean microscope slide, cover with cover slip, leave dry at room temperature for several days or over low heat. If specimen is thick, cover slip must be supported by celluloid or glass strips or cells.

2. **Permount**. Use as #1.

3. **Diaphane**. Mount from 100% ethyl alcohol or, less satisfactorily, from 95% ethyl alcohol. Otherwise use as #1. Tends to fade stains.

4. **Euparal**. Use as #3.

5. **Hoyer's Mounting Medium**. Widely used in mite work. Mount from water or clearing agents #2 or #3; dry in 50 C oven. After slide is dry, ring twice with varnish, Zuts, or Glyptal. *Disadvantage*: Hoyer's may crystallize with age; specimen must be remounted.

6. **Glycerine Jelly**. Use from glycerol (see Temporary Mounts), after glycerol has replaced water or ethanol in specimen. Jelly is heated to 60 C and spread on warmed slide to form a spot the diameter of the coverslip. The specimen is transferred to the jelly and positioned as the jelly cools and solidifies. The coverslip is coated with a thin layer of hot jelly and inverted over the specimen. *Advantage*: Specimen may be accurately positioned. *Disadvantage*: Not used with stains (low refractive index), not too permanent (ring well).

STAINS

1. **Acid Fuchsin**. Use from water. Widely used as cuticle stain. Leave specimen in stain for 5 min. or until stained; wash in water. Good with Weaver's fixative: replace fixative by water, then add stain. After about 5 min., wash specimen 3-4 times with tap water, replace fixative. Does not destain in fixative.

Formula

acid fuchsin	0.5 gr
10% hydrochloric acid	25 ml
distilled water	300 ml

2. **Grenacher's Alcoholic Borax-carmine**. Use from 50-70% ethyl alcohol. Stain until object is thoroughly penetrated (about 10 min.), then into 70% acidified alcohol until bright transparent, then into 95% ethyl alcohol.

Formula (quick method)

carmine	3 gr
borax	4 gr
distilled water	100 ml
boil for 30 min., cool	
70% ethyl alcohol	100 ml

Filter after 2-3 days

Acidified Alcohol

70% ethyl alcohol	100 ml
conc. hydrochloric acid	4 drops

3. **Chlorazol Black E**. Use from 70% ethyl alcohol, into stain until stained (about 20 min.), wash 95% ethyl alcohol, into 95% ethyl alcohol to stand. Good for chitin. Can also be used in aqueous solution.

Formula (alcoholic)
chlorazol black E saturated solution
in 70% ethyl alcohol

4. **Methylene Blue**. Methylene blue is often used as a vital stain (to stain living insects), especially useful for delineating neurons. Dilute stain (0.001-0.01%) in Ringers should be used if specimen should be kept alive. More concentrated stains (0.2% aqueous) can be used otherwise; immerse dissected, fresh specimen in stain, watch until desired structures take up dye, then rinse in water. Stain is temporary.

5. **Toluidin Blue O**. Use as 0.2% aqueous solution; same technique as Methylene Blue.

Many other stains are available. Especially to be noted are Delafield's and Heidenhain's Hematoxylins, which are somewhat more complicated to use. Consult the references to microscopial technique.