



<i>Title: NEON FSU Field and Laboratory Protocol for Operations Central Plains Experimental Range 2011: Ground Beetle Abundance and Diversity</i>	<i>Author: C.Gibson</i>	<i>Date: 09/23/2011</i>
NEON Doc. #: NEON.DOC.014050		<i>Revision: A_DRAFT</i>

NEON FSU Field and Laboratory Protocol for Operations Central Plains Experimental Range  
2011:  
Ground Beetle Abundance and Diversity

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DRAFT

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## **1 INTRODUCTION**

### **1.1 Purpose**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The primary purpose of this document is to provide change controlled version of Observatory protocols and is the version used for external review by subject-matter experts. This document provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change controlled document, not through field manuals or training materials.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

### **1.2 Scope**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices (i.e. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (i.e. fill the car with gas)

It does identify procedure-specific safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

### **1.3 Acknowledgements**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*If a protocol is based closely on the work of another program or author, note that here.*

## 2 RELATED DOCUMENTS AND ACRONYMS

### 2.1 Reference Documents

*(If you want to reference other procedural documents (e.g. associated Protocol document), drawings, etc. then include filenames in the following sections.)*

RD[01]	NEON.DOC.000008 NEON Acronym List
RD[02]	EHS Safety Policy and Program Manual
RD[03...]	<primary science design docs explaining/justifying this protocol/these procedures>
RD[04]	NEON Sampling Design Document
RD[05]	Training Plan
	QA/PA Plan
	DOORS requirements
	ATBD
AD[01]	FSU Science Requirements
AD[02]	FSU Field Operations Plan
AD[03]	Data Products Level 1-3 Catalog
	NEON.DOC.000243 NEON Glossary of Terms

### 2.2 Acronyms

*Insert table for definitions of acronyms used in this document.*

NEON	National Ecological Observatory Network
FSU	The NEON Fundamental Science Unit at Headquarters
P&P	Procedure and Protocol

### **3 BACKGROUND AND OBJECTIVES**

#### **3.1 Background**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The purpose of this section is to inform the activities involved with planning and scheduling ground beetle diversity field sampling for the Field Operations Prototype. This section of the document outlines the field and laboratory procedures to collect, process, and maintain the integrity of the ground beetle diversity samples.

The purpose of the ground beetle diversity sampling design is to capture inter- and intra-annual variation of ground beetles (Coleoptera: Carabidae) in the NEON purview. Ground beetles were chosen as a focal terrestrial invertebrate taxon for several reasons. They are relatively easy to sample and well known taxonomically. They are relatively common in many habitats and form well-defined gradients in species richness across North America. They have been used as indicators of environmental change as they are sensitive to habitat disturbance. They are generally predacious and can influence trophic structure. They also represent other feeding types (scavengers, frugivores, etc.), and prey for other animals, therefore, changes in the proportion of each guild can indicate significant changes in the local ecological community.

The following sub-sections contain draft protocols that provide detailed guidance for setting pitfall traps in a subset of the plant Biodiversity Plots, collecting the ground beetles and invertebrate (and at times, vertebrate) bycatch, laboratory processing of the collected animals and storage and shipment of samples to Taxonomists, Genetic Analytical Laboratories, and/or to Archives.

Taxonomists' contracts are TBD. Pisces Molecular and The Biodiversity Institute of Ontario are the bid-winning contracts for the Genetic Analytical Laboratories. The NEON Support Facility at 1635 38<sup>th</sup> St., Suite 100, Boulder, CO 80301 will be the Archive for the 2011 sampling year.

Pitfall traps serve to capture ground dwelling invertebrates (insects and their allies, e.g., spiders, scorpions) that fall into them. The animals (invertebrates and vertebrates) become preserved by a liquid mixture of DNA safe preservative in the bottom of the trap. Animals collected in these traps other than ground beetles are termed "bycatch".

This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.

Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length <1 page.

#### ***Metadata Collection***

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Data about the samples will be compiled into fields outlined in a spreadsheet to be supplied by FSU called 'NEON Ground Beetle Diversity Datasheet'. These fields follow the Barcode of Life Datasystems

(BOLD) standards and include some additional fields in order to be compliant with other global biodiversity projects.

*This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.*

*Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length <1 page.*

### **3.2 Science Requirements**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

This protocol fulfills the following Observatory science requirements:

*List science requirements from DOORS that are met by this protocol.*

### **3.3 Data Products**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*List Level 0 data products measured by protocol.*

**Table 1** A summary of field and related lab measurements and the associated NEON Data Products

<b>Measurement</b>	<b>Data Product</b>

## **4 PROTOCOL**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Summarize the science rationale (e.g. experiment design), include key citations. Briefly summarize the procedure included in this document and variations in how NEON is implementing this protocol in different locations throughout Observatory (e.g. above ground tree biomass for temperate vs tropical zones). If the protocol is based on existing published procedures, reference those here (e.g. “These methods are based on the amazing work of John Updike (1964). No one has come up with anything better since then.”).*

*A protocol is a formal summary description of a procedure and its related rational. A protocol includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result; can also be called a method.*

Read

more:

[http://wiki.answers.com/Q/What\\_is\\_the\\_difference\\_between\\_a\\_method\\_and\\_a\\_procedure#ixzz1Flnc5Na](http://wiki.answers.com/Q/What_is_the_difference_between_a_method_and_a_procedure#ixzz1Flnc5Na)

Identify assumptions or known-unknowns of the chosen protocol.

Identify and summarize quantitative aspects of the procedure (timing, # plots, # samples, location of sensors).

Provide a simple timeline diagram or table if pre-field activities occur the day prior to the field day, or if the field procedure is a multi-day task. Plot Location

FSU is responsible for determining plot locations. Pitfall traps will be placed adjacent to Biodiversity Plots (within the destructive sampling buffer zone). Ten Biodiversity Plots will each have four pitfall traps located around them at the north, south, east, and west points of the circular Biodiversity Plot (40 traps total per site).

## 5 QUALITY ASSURANCE AND QUALITY CONTROL

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

Summarize QA/QC plan and reference QA/QC document.  
See QA/QC plan and reference QA/QC document.

## 6 DECISION TREE

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

Delay	Action	Adverse Outcome?	Outcome for Data Products
hours	Collect traps asap; note duration & cause	None	None
day	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	Data not comparable to standard collection events	Likely none
2-7 days	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	Data not comparable to standard collection events; integrity of specimen DNA becoming compromised	May not be readily able to obtain DNA barcodes; affects Abundance&Diversity measurements
8-13 days	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	Data not comparable to standard collection events; integrity of specimen DNA suspect	May not be readily able to obtain DNA barcodes; affects Abundance&Diversity measurements
2 or more weeks	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	Data not comparable to standard collection events; integrity of	May not be readily able to obtain DNA barcodes; affects Abundance&Diversity

		specimen DNA suspect	measurements
--	--	----------------------	--------------

See Decision Tree document

*Summarize for the field technician or manager any implementation decisions regarding the protocol.*

## **7 SAFETY**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Materials Safety Datasheets are provided at training and are available in the lab for the following chemicals used in this work: propylene glycol, ethanol and paradichlorobenzene (moth crystals).

Personnel working at a NEON site should be familiar with and practice safe field work as outlined in the EHS Safety Policy and Program Manual. Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

*Any safety issues specific to the procedure should be detailed here, along with references to any pertinent safety standards.*

## **8 PERSONNEL REQUIREMENTS**

*Include special skills or subject matter expertise required (e.g. able to identify regionally specific plants by visual inspection, through use of dissecting microscope and through use of dichotomous key)*

### **8.1 Field Personnel**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

A minimum of two field technicians is required for Ground Beetle Diversity sampling in the field.

### **8.2 Laboratory Personnel**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

A minimum of two laboratory technicians are required to process Ground Beetle Diversity samples ideally within six months of collection from the field (at most one year)

- Fine manual coordination (in order to properly handle pinned specimens).
- Small, neat handwriting.
- Knowledge and ability to work with a dissecting microscope

## 9 TRAINING REQUIREMENTS

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

See NEON Training Plan document relevant to this method.  
Reference NEON Training Plan document relevant to this method.

## 10 FIELD STANDARD OPERATING PROCEDURE

### 10.1 Sampling Frequency and Timing

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

This first actual sampling occurs on Monday May 16 and continues for 16 weeks through to the week ending Friday September 2, 2011. (Training during Monday May 2 – Friday May 13). Pitfall traps will be checked, emptied, and reset weekly.

**Table 2** The approximate sample dates for beetle sampling at all NEON sites

Domain	Date	Frequency
10	May16-Sept2	Weekly

Beetle sampling occurs every 7 days within 3 hours of standard time each week. We estimate sampling requires 2 technicians for 12min per trap x 40 traps = 480 min = 8 hours each sampling day plus hiking time and travel to and from site.

*Based on sampling frequency information, list estimated dates that correspond with timing rules or estimated dates when timing rules are fulfilled for each NEON site where this procedure is implemented. Include range of scientifically acceptable sampling timing. If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.*

### 10.2 Contingent decisions

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

See Section 6

*Summarize what-if decisions (how to accommodate site-specific or changing conditions).*

*Example: If it starts raining halfway through the microbe sampling, work must stop for the day. 24 hours after the rain stops, work can continue from the previous stopping point (i.e. work does not need to be repeated).*

*Example: If you are unable to begin checking small mammal traps prior to 9am, field technicians should open all traps to release animals and avoid heat-induced mortality. Work should start over that night by resetting the traps.*

**10.3 Field Procedure**

Write the procedure as if a PDA were not available. This way, when the asteroid hits the earth and disrupts all electrical equipment, the field personnel can reference the “old school” procedure and collect data. These non-PDA procedures also help CI to define PDA and data ingest requirements, and provide sufficient information for an external reviewer to assess the procedure without the need of a PDA.

**Equipment and Materials**

Include all standard and unique equipment and capabilities required to execute the procedures in this document, including:

- A detailed list of materials (e.g. equipment, sampling gear, sample containers, chemical preservatives) used in the field.
- Do not include materials used for separate but related activity; that will be included in the Procedure for that activity (i.e. lab vs. field materials).
- Describe the chemicals being used or as a preservative when samples are immediately returned to the field – exact chemical constituents and strength, and bottle size. Leave a place-marker in the draft if unknown.
- Illustrations of materials (e.g. sampling gear) – all in jpeg format, 3 inches wide
- Can be in bullet point or table format, but be consistent throughout the document.

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

**Table 3** Materials and supplies required for the Beetle Sampling Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Pitfall trap locality labels		
Ice packs		
Chests coolers		
Trowel or soil knife		
Sharpies		
Pitfall trap bowls		
Pitfall covers		
Galvanized nails for pitfall covers		
Propylene glycol		
Distilled water		
Ethanol		Y
Archival, ethanol-safe pens (Pigma brand, size 01)		
Scissors		
Forceps		
Whirlpak bags		
300 µm filter mesh organdy pre-cut (9 cm square)		

Modified wide mouth Nalgene bottles (bottom portion of bottle cut off)		
Modified wide mouth Nalgene lids as filter tops (top cut out of lid, filter screws onto bottle)		
Ethanol squeeze bottle		
Water squeeze bottle		
Bin to keep some materials at plot		
Carry-all craft tote bag or other field pack		
Specimen cups for PG waste while filtering		
Specimen cups for Ethanol waste while filtering		
Large ziplock bags		
Ethanol waste wide mouth bottle (1000 mL Nalgene)		
PG waste wide mouth bottle (4000 mL jug)		
Latex gloves		
Optional knife or Leatherman		
Handwipes		
Replacement pitfall bowls		
Replacement whirl paks		
Replacement filters		
1000 mL wide mouth Nalgene bottles filled with ethanol		
1000 mL wide mouth Nalgene bottle filled with water		
1000 mL wide mouth Nalgene bottle filled with PG		
Duct tape		
Flagging		
Bug spray		Y
Mosquito protective clothing		
Sun block		
First-aid kit		
Camera		
GPS/Trimble		
PDA		
Datasheets		

### **Preparation**

- Describe all activities that must occur prior to arrival in the field, for example equipment calibration or preparation.
- A detailed list of tasks, using the numbering format shown below. Be consistent.
- Break each step down into actionable steps
- Described and list the tasks in chronological order
- If pre-field tasks occur over multiple days, break Section 7.1 down further (e.g.:
  - One week prior to sample collection, do this
  - Two days prior to sample collection, do this

- Be explicit and use language geared toward 3<sup>rd</sup> yr undergraduate student. Assume the user has no previous knowledge of the activity.
- Include a description of what sampling gear, equipment, etc. they need to verify are in working order and properly packaged for the field day.
- Define safe practices (e.g. use gloves to mix chemicals in the fume hood), where necessary at each step. Do not assume this information is written elsewhere – include all relevant safety procedures in every document. Each document should be written as a stand-alone document.
- Illustrations or photographs of tasks that are complex or would benefit from an illustration – jpeg format, 3” wide.

### 10.3.1.1 Plot Establishment

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The field crew is responsible for establishing traps at each pre-defined location. Pitfall traps are deployed at ground level with the lip of the bowl flush with the ground.

The pitfall trap design currently consists of two wide, shallow bowls (7 cm deep with an 11 cm diameter, 540 mL total volume) nestled within one another. One bowl ensures that the hole stays in the ground as the second bowl with the preserving fluid is picked up and changed during sampling. The nestled bowls are then protected from weather by a plywood cover while the trap is in operation. Covers are best made using a drill press (in advance of the field season) to get equivalent holes in each lid. A 6.4 mm drill bit should be used. This facilitates driving the nails through the covers in the field.

The killing fluid and preservative used in the pitfall traps is a distilled water and propylene glycol (abbreviated PG) mixture (1:1). Propylene glycol is a non-toxic antifreeze, MSDS: mild irritant, non-toxic. Some replacement items may be left in a storage bin at the plot (e.g. whirl pak bags). All sampling fluids must be brought in and out from the lab each visit.

Once in field:

1. Dig a small pit (about the size of two fists) with a trowel or soil knife to accommodate the pitfall bowl.

[Figure 1]

2. Set two bowls into the trap ensuring that the top of the bowl is flush with the ground (this ensures that insects fall into the trap rather than walk around trap)..
3. Fill top bowl with 100 mL of PG:water solution.
4. Position cover with four 15 cm galvanized nails and 3-5 cm above the surface of the bowls.

[Figure 2]

[Figure 3]

5. Repeat until all traps are set within a plot.

### 10.3.1.2 Generating Sample Labels

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Six different kinds of sample labels are required. Each is dealt with in turn below.

1. FIELD LOCALITY LABELS: Locality labels for samples should be created before the field campaign begins in order to expedite processing and reduce opportunities for error.

Laboratory technicians are responsible for generating sample labels on archival, ethanol-safe paper. A template file will be provided from FSU for locality labels to be adjusted following the procedure below.

Locality labels include particular information in order to be consistent with collection records from other global campaigns.

- State
- County
- Town
- Elevation (m)
- GPS coordinates N and W with four degrees reported in WGS84
- trap type (e.g. pitfall or CO2)
- space left for the day, the month and the year are printed (e.g. \_\_\_Aug2008)
- collectors (e.g. KKBlevins)
- a collection code which is comprised of the following parts: NEON.FSU.Domain.Plot.Trap

To prepare labels:

- a. Cut labels into long columns using an exacto knife and ruler.
- b. Do this with as little white space on either side of the text as possible.
- c. These cuts result in many labels in a vertical strip.

[Figure 18]

- d. Store labels this way, and then when needed, cut individual labels across the row with scissors.

[Figure 19]

[Figure 20]

- e. Add date numbers with archival, ethanol-safe ink as needed. This is a key step, as this particular information and kind of ink is required. Prompts are found throughout the protocols for each time this is required.

2. LAB LOCALITY LABELS: Storage containers for long term sample archive in voucher collections (e.g., 50 mL centrifuge tubes) will require locality labels inserted into them in triplicate (three individually cut labels).
3. DNA BARCODE UNIQUE NUMBER LABELS: A file will be provided from FSU with the reserved unique numbers for specimens that are to be DNA barcoded. These numbers are to be applied to pins below locality labels on each specimen to be DNA barcoded.
4. TEMPORARY LABELS: Consumables that only require temporary labels (e.g., waste ethanol cups, cardboard mailers) will be marked with laboratory tape and sharpie makers.
5. MICROPLATE LABELS: Ninety-six well microplates will have 'NEON#' written in sharpie on multiple sides of the plate. These numbers are in series and are to be designated by FSU.
6. SHIPPING LABELS: Shipping labels with the relevant details for addressor (Domain contact) and addressee (the external facility) should be pre-made to expedite shipping throughout the field season.

### **10.3.1.3 Preparing Field Equipment**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

All necessary field equipment will be assembled at least one day prior to field sampling to ensure spreadsheets are uploaded or printed and that all consumables are available. PDA must be loaded with correct map and database information. Laboratory space and appropriate consumables must be available for use within 24h of sample collection.

The Ground Beetle Diversity Field Crew is responsible for transferring the appropriate files to the handheld PDA before field sampling and / or printing out appropriate datasheets. These files will include mapping tools to find the Biodiversity Plots and the designated pitfall trap locations, and fields for entering GPS waypoints, metadata, and comments during sample collection.

Ahead of going into the field:

1. Prepare one liter bottles of propylene glycol:water solution(500 mL PG : 500 mL distilled water) in chemical hood above secondary containment bin.
2. Prepare 95% ethanol for the field by pouring it into 1 L wide mouth Nalgene bottles in chemical hood above secondary containment bin.
3. Prepare space for pitfall traps and sampling liquids in field vehicle and the lab.
4. Prepare space in flammables storage cabinet for ethanol.
5. Prepare storage space for propylene glycol.
6. Ensure access to sink (in any lab) for preparing propylene glycol mixture and rinsing waste change out (of ethanol) containers (minor & acceptable amounts of each going down sink – on order of mL).
7. Clear space in freezer, fridge or flammable materials cabinet for samples .
8. Clear space in chemical hood (with secondary containment bins) for propylene glycol and ethanol waste.
9. Clear lab bench space (2.4 m or equivalent) in clean lab or dirty lab if no conflicting activities (e.g. soil sieving or other dust) for ethanol change.
10. Ensure access to propylene glycol and ethanol waste storage.

11. Ensure access to sink for washing materials with residual residue.
12. Clear storage space in freezer, fridge or flammable materials cabinet for samples.
13. Clear storage space for pinned insects in Cornell cabinets and / or Schmitt boxes.

### ***Sample Collection in the Field***

#### **10.3.1.4 Trap Collection**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Upon arrival at the trap:

1. Place three copies of locality labels into the bowl (see section on generating labels). The labels can be treated in the same way as the trap catch in the subsequent steps without harm. This procedure is vital to reducing error.

[Figure 4]

2. Put on gloves

Collecting insects from trap,

[Figure 5]

1. Insects collected from the pitfall traps are filtered from the PG using a 300 µm mesh filter and a modified Nalgene bottle with a screw cap lid.

[Figure 6]

[Figure 7]

2. Pick up the top bowl containing sample and, holding the filter above an empty specimen cup, pour the contents of the bowl through/onto the filter

[Figure 8]

3. PG solution is collected into the specimen cup.

[Figure 9]

[Figure 10]

4. Pour used PG solution into a 4 L storage bottle for disposal in the lab.

5. Rinse animals on filter with water over open ground.

[Figure 11]

6. Rinse animals on filter with 95% ethanol over an ethanol waste container.

[Figure 12]

7. Place animals on filter (include filter) in  $\geq$  100 ml of 95% ethanol in a whirl pak bag.

[Figure 13]

[Figure 14]

[Figure 15]

8. Roll the whirl pak bag shut and apply the bag's twist ties so that there is minimal opportunity for the ethanol to leak.

[Figure 16]

[Figure 17]

9. Double check that the appropriate locality labels are in the whirl pak bag.
10. Note in the 'NEON Ground Beetle Diversity Datasheet' the general number of invertebrates using the scale 0/1/2/3 (numbers increasing with catch size) taken from the trap and any other issues (e.g. trap was damaged by bears, wind blew cover off, trap flooded with rainwater).
11. All 4 whirl pak bags from a single plot are put together into a ziplock bag before leaving the plot.
12. Ziplock bags are then placed into hard bottom carrying device ensuring that the whirl pak bag openings are upward.

If sampling is continuing for another week:

1. Refill the PG mixture in the bowl to 100 mL.
2. Replace the plywood cover so that it sits 3-5 cm above the surface of the bowls.
3. Repeat for other traps.

If sampling is complete (at the end of the field season):

1. Bring in all trap components to the lab.

### **Sample Preservation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

**Collected animals must not be exposed to direct sunlight or extremely high temperatures during the remainder of the field work. Use a chest cooler (with ice packs) in the field vehicle.**

*Detail the task in order from start to finish*

*Include how long (time/days/yrs/indefinitely) sample can be preserved in this mode.*

*Only include activities that relate to the Field Task.*

*If a sample is processed at the Domain Lab, that work is written in the next Section or as a separate document if complex.*

### **10.3.5.2 Sample Transport and Storage Prior to Processing**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

- All 4 whirl pak bags from a single plot are put together into a ziplock bag before leaving the plot.
- Ziplock bags are then placed into hard bottom carrying device ensuring that the whirl pak bag openings are upward.
- Collected animals must not be exposed to direct sunlight or extremely high temperatures during the remainder of the field work. Use a chest cooler (with ice packs) in the field vehicle.

### **Sample Shipping (may not be applicable for all Field SOPs)**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

- Provide detail on shipping specifics (e.g. wrap the sample containers in a plastic bag, seal the top. Place containers upright in cooler labeled “Elephant Samples”, include four reusable ice packs. Seal the container.
- In addition to the shipping label, the following hazmat labels are required:
- Check with EHS for label requirements

### **Data Handling (may not applicable for all Field SOPs)**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Briefly explain data upload steps (e.g. enter data into excel file name “XXXXX”, plug in PDA, etc., etc.).*

Field data collected should be carefully entered into the ‘NEON Ground Beetle Diversity Datasheet’ on the P drive within as short a time as possible from the field (preferable each day, required by the end of the week).

### **Refreshing the Field Sampling Kit**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Provide detail on how to restock the sampling kit with non-perishable items. Best practice is to restock the sampling kit after the sample event, with a check at the start of each sample event that the kit is appropriately stocked*

*Reference the materials list, above*

*Be explicit and ensure information does not overlap or refute early info (ex. If preservatives used in the field have to remain cold, then the ‘refreshment’ of the preservative is detailed in Section 7.2 and not here. )*

### **Equipment Maintenance, Cleaning, and Storage**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Include maintenance of sampling gear as they relate to this Procedure, such as battery recharge. Do not include vehicle maintenance or maintenance of gear commonly used in the field such as a mosquito net or boots. Maintenance of common field items will be a different document. Include relevant safety issues and practices  
Be explicit  
Illustrations or photographs of tasks that would benefit from an illustration, jpeg 3" wide.*

**11 LAB STANDARD OPERATING PROCEDURE**

*Write the lab procedures as if a PDA were not available. Use the same sections as the field protocol, above.*

*Separate the lab procedures into multiple sections and add on to the above title. For example:  
11 Lab Standard Operating Procedure - Plant identification and drying  
12 Lab Standard Operating Procedure - Plant mounting*

**11.1 Timing**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Pitfall traps will be checked, emptied, and reset weekly. The first change of the ethanol must occur within 24 h of collection from the field. The specimens must be completely prepared within one year of collection from the field.

*Provide details on preferred timing of sample processing at the domain labs AND the maximum time between field collection and lab processing If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.*

**11.2 Lab Procedure-Sorting**

***Equipment and Materials***

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

**Table 4** Materials and supplies required for the Beetle Lab Procedure.

<b>Item Description</b>	<b>Quantity per sampling event</b>	<b>Hazardous Chemical</b>
Color Separation Guide and Gray Scale		
Ethanol		
Plastic petri dishes for sorting specimens under microscope		
Sharpies		
Archival, ethanol-safe pens (Pigma brand, size 01)		

No. 3 pins archival quality		
50 mL nonsterile centrifuge tubes		
Specimen cups - bottoms		
Specimen cups - lids		
Schmitt boxes		
Point punch		
Fumigant (moth crystal) boxes		
Archival, ethanol-safe pinned specimen label paper point paper (Strathmore archival quality bristol board)		
Pinning block (anodized; height is better than wood)		
Jewelers forceps		
96 well microwell plates		
96 well microwell caps		
Hardware/software (i.e. Clemex Captiva)		
Microscope		
Canon Powershot camera		
Fumigant (paradichlorobenzene - moth crystals)		
Packing tape		
Shipping supplies (boxes, padded envelopes)		
Cardboard specimen mailers		
Self healing cut mat (30 cm x 46 cm)		
Scissors		
Exacto knife		
Metal ruler		
Unit trays for inside Cornell drawers (48 per cabinet)		
Cornell drawer (pine - hardboard bottom)		
Cornell cabinet (6 drawers, unassembled)		
Balsa wood for point preparation surface		
Secondary containment bins for pouring liquids		
Drill press (for pitfall covers)		
Latex gloves		
DNA barcode unique numbers		
Adhesive shipping labels		
Ethanol waste drum		
PG waste drum		
Tupperware for temporary storage of each bout of whirl paks		
Tupperware for photo box of large beetles		
Styrofoam for photo box		
Plain white paper (for pointing specimens under microscope)		
Tissue paper for photo box background		
LED lights (microscope, photos)		

Acrylic plastic as light diffusers (photos)		
Elmer's white glue		
Ethanol lamp (sterilizing)		
Lighter		

### **Preparation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Lab bench space (2.4 m or equivalent) in clean area or dirty area if no conflicting activities (e.g. soil sieving or other dust) for ethanol change.
2. Access to propylene glycol and ethanol waste storage.
3. Access to sink for washing materials
4. Storage space in freezer, fridge or flammable materials cabinet for samples.
5. Clear storage space for pinned insects in Cornell cabinets or Schmitt boxes.

### **Sample Processing in the Lab**

#### **11.2.1.1 Ethanol Processing (first change from field)**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The first change of ethanol must occur within 24 h of sample collection from the field.

[Figure 23]

1. Put on gloves.
2. Pour initial ethanol (poured in whirl pak in field) through an unused filtration device into temporary holding (do this for each trap, one at a time. This ensures that specimens from different traps are not poured together).

[Figure 24]

3. Pour waste ethanol in clearly labeled temporary ethanol waste containers.
4. Place animals on filter (include filter) in the same whirl pak bag from the field.

[Figure 25]

5. Pour fresh 95% ethanol into each whirl pak bag ensuring that all of the insects are submersed and close bag with little airspace.
6. Double check that the appropriate labels are still in the whirl pak bag .

7. Put each sampling bout (all the plots from a single date) into a Tupperware container and seal tightly until able to be processed following the steps outlined below.

[Figure 26]

8. Store these samples in the freezer (-20°C optimal) or refrigerator (4°C).

#### **11.2.1.2 Sorting Ground Beetles from Bycatch**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Sort each sample one at a time for ground beetle specimens.
2. It is important that each sample remains clearly labeled and is not left unattended for any length of time.
3. Sorting is best done under the microscope on a Petri dish that has been marked with rows on the bottom (marking the surface that touches the microscope stage so that it is not worn off by ethanol).

[Figure 27]

4. Beetle samples are sorted to pull all adepagan (a suborder of Coleoptera) beetles out. Adephaga contains aquatic groups (which are unlikely to fall into pitfall traps) and all of the beetles of interest to NEON.
5. This group is easily identified by the manner in which last pair of legs insert into the beetle's underside and the tarsal formula (5-5-5). The former feature is denoted by the last pair of legs completely separating the first abdominal segment.

[Figure 28]

The latter feature is denoted by each of the beetle's tarsi (or feet) being comprised of 5 segments. Foretarsus = 5 segments; Midtarsus = 5 segments; Hindtarsus = 5 segments.

[Figure 29]

[Figure 30]

6. Ground beetles are sorted into temporary holding cups with a single locality label from the three initially put in the pitfall bowl.
7. The other 2 locality labels remain in the sorting dish with the rest of the specimens at all times.
8. Additional locality labels may be required for large samples with many specimens that do not all fit into a single 50 mL centrifuge tube.
9. All specimens are pinned/pointed (described elsewhere) from each trap, however in the event that there are a long series of what appear to be the same species collected from a particular trap, a maximum of 20 individuals of should be prepared (pinned/pointed) from that trap. In this event, the remaining individuals should be counted, recorded, and placed in 95% ethanol in a 50 mL plastic centrifuge tube along with three locality labels (date filled in). These data are to be recorded in the 'NEON Ground Beetle Diversity Datasheet.' Morphospecies designations are made with unique letters 'MorphA', 'MorphB', 'MorphC' .... 'MorphZZ' if a direct match to species name in the teaching collection cannot be made. A running tally of morphospecies is to be kept with one individual from each as an exemplar in a designated Cornell drawer and the initials of

the person who designated it as the morphospecies. (This morphospecies notice sheet will be provided by FSU)

### 11.2.1.3 Processing Bycatch Samples in Ethanol

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Bycatch samples are stored in as few 50 mL centrifuge tubes as possible but ensuring that all specimens are fully covered with 95% ethanol. This will be a variable amount of ethanol depending on the volume of animals collected.

[Figure 31]

[Figure 32]

2. Each bycatch 50 mL centrifuge tube should have three individually cut locality labels (date filled in).
3. Label the lid of tube with sharpie with the collection code (NEON.FSU.Domain.Plot.Trap) and date.
4. Processed samples in ethanol are optimally stored at -20°C.
5. Failing this, they may be stored at 4°C
6. Failing this they may be stored temporarily (less than one year) at room temperature in the flammables cabinets.

### 11.3 Lab Procedure-Processing

#### *Equipment and Materials*

Item Description	Quantity per sampling event	Hazardous Chemical
Color Separation Guide and Gray Scale		
Ethanol		
Plastic petri dishes for sorting specimens under microscope		
Sharpies		
Archival, ethanol-safe pens (Pigma brand, size 01)		
No. 3 pins archival quality		
50 mL nonsterile centrifuge tubes		
Specimen cups - bottoms		
Specimen cups - lids		
Schmitt boxes		
Point punch		
Fumigant (moth crystal) boxes		
Archival, ethanol-safe pinned specimen label paper point paper (Strathmore archival quality bristol board)		
Pinning block (anodized; height is better than wood)		
Jewelers forceps		

96 well microwell plates		
96 well microwell caps		
Hardware/software (i.e. Clemex Captiva)		
Microscope		
Canon Powershot camera		
Fumigant (paradichlorobenzene - moth crystals)		
Packing tape		
Shipping supplies (boxes, padded envelopes)		
Cardboard specimen mailers		
Self healing cut mat (30 cm x 46 cm)		
Scissors		
Exacto knife		
Metal ruler		
Unit trays for inside Cornell drawers (48 per cabinet)		
Cornell drawer (pine - hardboard bottom)		
Cornell cabinet (6 drawers, unassembled)		
Balsa wood for point preparation surface		
Secondary containment bins for pouring liquids		
Drill press (for pitfall covers)		
Latex gloves		
DNA barcode unique numbers		
Adhesive shipping labels		
Ethanol waste drum		
PG waste drum		
Tupperware for temporary storage of each bout of whirl paks		
Tupperware for photo box of large beetles		
Styrofoam for photo box		
Plain white paper (for pointing specimens under microscope)		
Tissue paper for photo box background		
LED lights (microscope, photos)		
Acrylic plastic as light diffusers (photos)		
Elmer's white glue		
Ethanol lamp (sterilizing)		
Lighter		

**Preparation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Full lab bench space (4.6 m or equivalent) in clean lab with 2 microscopes (1 dissecting and 1 compound) connected to computer and room for one additional dissection microscope.
2. Lab bench space (2.4 m or equivalent) in clean lab for preparation of dry archival samples.

[Figure 21]

[Figure 22]

3. Clean lab bench space (no dust, wild air movement) for putting tissue samples into wells for genetic analysis (to be sent to external facility).
4. Access to sink for rinsing equipment.
5. Storage space in clean lab for pinning materials.
6. Storage space for pinned ground beetles.
7. Cold storage space for ethanol preserved specimens.
8. Storage space for shipping materials.

#### **11.3.1.1 Beetle Archival Dry Preparation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Whole ground beetle specimens stored dry are to be either pinned or pointed.
2. Generally specimens 5 mm and greater are pinned, and specimens smaller than 5 mm are pointed. However, pointing is a good default option for any specimen if it seems it will be destroyed by pinning. A good example video of pinning and pointing specimens resides at: <http://www.nhm.ac.uk/nature-online/collections-at-the-museum/making-part-collection/making-pinning/index.html>

[Figure 33]

#### **11.3.1.2 Pointing Small Beetles (<5mm)**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Pointing is probably the most widely used method of mounting small dried insects. This method consists of attaching the specimen with adhesive (Elmer's white glue) to the tip of a small triangle (archival quality cardstock) (known as a 'point'). Points are transfixed by a No. 3 entomological archival pins (number refers to thickness of pin). Points are cut with a special, standardized triangle-shaped punch.

Prior to pointing:

1. Prepare a batch of triangles using the point punch over a balsa wood (or other malleable) surface.

[Figure 35]

2. Press No. 3 archival quality pins into the points equidistant from the three sides at the back of the triangle base.

[Figure 36]

3. Push the point up the pin until it is 15 mm from the top of the pin.

[Figure 37]

4. Check the height using the highest hole on a pinning block (for ease of using the collection at a later date).

Pointing is best done under the microscope. To mount a specimen on a point:

1. Using forceps, put the beetle or beetles (it may be easier to do them in batches) on their left side on a slip of plain white paper so that their heads are facing the right and their legs are toward the edge of the bench.
2. Slide the slip of paper with the beetles to the center of the microscope stage.
3. Take the prepared point and touch the tip of the point in a bit of glue. (Use the minimum amount of glue that will attach the specimen firmly to the point) .
4. Narrow in on the target spot for the point on the thorax between the bases of the 2<sup>nd</sup> and 3<sup>rd</sup> pair of the beetle's right legs and tack the point down.
5. Lift and angle so that the weight of the specimen is leaning directly into the point.
6. Place the pin in a block of Styrofoam in a manner that ensures that gravity is holding the specimen to the point.
7. Adjust the specimen with forceps or a pin. Adjusting the specimen should cause it to bind firmly to the point. A heavy specimen that rotates on the point should be straightened as the glue thickens but before it completely hardens.

Although the point and glue will obscure one side of the thorax, the other side will be free for examination. Neither the point nor the glue should extend onto the top or the bottom of the beetle, nor touch the head, abdomen, or wings. The body of the beetle must be at a right angle to the length of the point when it is mounted. Note: The sides of the thorax can slope inward to differing degrees, therefore, the tip of the point can be bent down with forceps to accommodate the size and shape of each particular specimen. For example, a specimen that has a flat-bottomed thorax can be mounted on a flat (unbent) point. A specimen that has the sides of the thorax vertical must have the tip of the point bent downward at a right angle.

[Figure 38]

[Figure 39]

[Figure 40]

[Figure 41]

Finally, the locality label can protect the specimen. Pointed specimens bisect the type. Therefore the pin will pierce the label in a different location in order to accommodate the beetle being in the center of the label (as different beetles are differing widths).

When all of the specimens are mounted:

1. Examine the specimens under the microscope to see that they are all attached securely to the points.
2. To test, place the specimens in a Schmitt box or unit tray.
3. Tap the box or tray.

The specimens should remain fixed. A specimen dissociated from its locality label is worthless. Re-attach any specimens (as long as it is clear which is its/their locality labels).

### **11.3.1.3 Direct Pinning Large Beetles (>5mm)**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

No. 3 archival quality pins are also used for direct pinning. Finer pins are too flexible and vibrate in the specimen, destroying them from within. Thicker pins damage specimens as they are inserted. It is important to insert the pin in the correct location in the beetle's body. This results in the pin being

positioned halfway between the midline of the beetle's body and the edge of the beetle where the elytra (wing shells) begin to round.

1. Insert the pin vertically through the top layer of the body.

[Figure 42]

2. Pause and look at the specimen from the side BEFORE shoving the pin all of the way through.
3. Ensure that the pin is at a right angle to the beetle's body.
4. Push the specimen up the pin until its top surface is 15 mm from the top of the pin. This leaves enough space above the insect for the pin to be a useful handle and below for data and other labels. Specimens at a uniform height are easier to examine and compare with one another.
5. Finally, the locality label can protect the specimen. Pinned specimens line up with the long axis of the body parallel with the type.

[Figure 33]

Once all field collections have finished, all of the pinned and pointed specimens are sorted to morphospecies as a whole. All species designations are to be recorded in the 'NEON Ground Beetle Diversity Datasheet'. Morphospecies designations are made with a lead label at the left of the series. This lead label designation applies to all specimens to the right and in subsequent rows until a new lead label is reached. Specimens are prepared for DNA barcoding and /or shipped to the Taxonomist.

#### **11.3.1.4 Removing a Sub-Sample for DNA Barcoding**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Ideally this procedure happens after examination by a Taxonomist. However, it may be more convenient in instances for the leg samples to be removed first.

After all of the ground beetles have been mounted and properly prepared:

1. Prepare the 'NEON Ground Beetle Diversity Datasheet' and 96 well microplates so they are within easy reach.
2. Apply a unique DNA barcode number to each specimen.

The leg priority for removal for DNA barcoding is as follows: insect's right side midleg, first priority (1), if missing, then midleg left side, second priority (2), if missing, then right hindleg (3), left hindleg (4), right foreleg (5), left foreleg (6).

[Figure 43]

[Figure 44]

If the beetle is small (2-5 mm) then the whole leg is excised and placed into a 96 well microplate well. If the beetle is greater than 5 mm, than a subsample of the leg (tibia and tarsus only or femur only, 2-4 mm length) is made with forceps and placed into the sampling well.

1. Excise a leg sample from the first specimen.
2. Place the beetle specimen's leg tissue sample in the well.

[Figure 45]

[Figure 46]

3. Record the unique number in the 'NEON Ground Beetle Diversity Datasheet'.

DO NOT place any foreign objects (e.g. labels) into sampling wells. Before proceeding to the next sample, ensure that no residual tissue is present on the forceps by rinsing them in 95% ethanol and then flame sterilizing them with an ethanol lamp.

#### **11.3.1.5 Digital Photography**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

One photograph of each specimen to be sent for DNA barcoding shall be archived to maintain visual records. This also creates a database of images to use for training. These photos will be taken with a standard Color Separation Guide and Gray Scale, measurement scale and standard camera settings. These specimens should also be measured and the length (mm) in the 'NEON Ground Beetle Diversity Datasheet'.

#### **11.3.1.6 Photographing Smaller Beetles (<10mm)**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

If a specimen is small enough to be captured completely in one shot, the picture shall be taken with the microscope camera (Leica DM1000 microscope, Clemex Captiva 5.0 camera software).

1. Pin the beetle to a small (approximately 3.5 x 5 cm) Styrofoam block covered with green tissue, dorsal side of beetle parallel to the block, head facing to the left.
2. Pin a second copy of the specimen's unique number label next to the specimen at a height that it will be easily viewed in the photograph.
3. Set the magnification as high as possible while still being able to view the whole specimen, its unique number, and the 'Color Separation Guide and Gray Scale'.

[Figure 47]

4. Adjust focus to the highest point at which part of the specimen is still in focus.
5. Using the camera software, capture a multi-layer image.
6. Choose the 'Capture Multi-Layer' option in the 'Advanced' menu.
7. Manually focus through the entire specimen using the fine focus knob on the microscope.
8. Change focus as little as possible while still capturing the full depth range of the entire specimen. A progress bar appears in the lower left-hand corner of the image window when the 'Capture Multi-Layer' is initiated.
9. Manually click 'Done' once finished focusing through the specimen. This is recommended as the software shuts down if left idle for too long.

After the picture is taken but before it is saved:

1. Place a digital image of a scale bar on the bottom right of the image, parallel to the side of the beetle with the end of the scale bar in line with the end of the specimen.

[Figure 48]

[Figure 49]

2. Activate the 'Scale Line' icon in the task bar of the Image window.
3. Change the units used on the scale bar in the 'Options' menu under 'Preferences'.
4. Use millimeters.
5. Change the number of units that appear on the scale bar by adjusting the number in the 'Scale Line Object Attributes' window that appears when the scale bar is clicked on.
6. Change the number of units that appear on the scale bar to either 1, if the specimen is less than 5 mm, or 5, if the specimen is equal to or greater than 5 mm.

Saving the Image:

1. Open a viewer window in the Clemex window.
2. Click and drag the image into this viewer window. This is the only way to save the image with the scale bar.
3. Save the image in viewer window.
4. Click on the save icon and save the image.
5. Save images to the P drive at P:\FSU\Invertebrates\CPER 2011\Beetle Pictures\unedited. The raw file is named 'carabid' and the unique number of the specimen and then the letter 'r' to indicate it is the raw file (e.g. For specimen number 777, the file name is carabid777r.jpg). Raw files can be accumulated and periodically processed. This and all subsequent file paths should be entered into the 'NEON Ground Beetle Diversity Datasheet'.

### 11.3.1.7 Photographing Larger Beetles ( $\geq 10\text{mm}$ )

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

If a specimen is too large to capture in one shot on the microscope, the picture shall be taken with a Canon Powershot G9 PC1250 digital camera. The best shots of pinned specimens are taken when the specimen is placed inside a light diffusing box. This can be achieved through the use of a translucent Tupperware container.

[Figure 50]

1. Cut styrofoam to the appropriate size to fit in the container and cover with green tissue paper.
2. Place the styrofoam on the bottom of the container until this false bottom is high enough that the entire beetle can be captured by a camera positioned at the container lip (without using the digital zoom). Pin the beetle to the false bottom with the dorsal (top shell or rounded back of the beetle) side parallel to the block.
3. Orient the beetle inside the container so that the dorsal side of the beetle is facing the camera and the beetle's head is to the left.
4. Pin a second copy of the specimen's unique number label next to the specimen at a height that it will be easily viewed in the photograph.

[Figure 51]

5. Pin a 'Color Separation Guide and Gray Scale' next to the beetle so the scale portion of the card is below and adjacent to the specimen. Adjust the card to the same height as the specimen.
6. One more piece of Styrofoam with a circular hole cut in the center is used as a lid, the center hole being the proper size to allow the camera lens to fit inside the box completely.

7. Angle two flexible LED lights so that they are shining on the two sides of the box, but neither is angled so light shines directly on the beetle.

[Figure 52]

8. Diffuse the light further if necessary (for example if beetle is particularly shiny), with sheets of clear acrylic plastic that are cut into strips and taped into cylinders to encircle the specimen. More than one of these acrylic plastic cylinders may be required.
9. Place the camera lens through the center hole in the Styrofoam lid and set the camera on a base so that it can capture the picture without being held.
10. Adjust the manual focus of the camera until the specimen is in focus.
11. If necessary, adjust the specimen height until the entire beetle can be captured without a person touching the camera.
12. Set the camera with the macro setting on, the flash off, and the two second timer on.

#### Saving Images:

Once satisfied with the photograph, save only one picture per specimen.

1. Upload it to the computer
2. Save it using the file naming scheme and in the location described earlier (i.e. carabid 777r) in 'Photographing smaller beetles' section.

#### 11.3.1.8 Processing Images

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

After the raw image has been saved it must be processed. When editing groups of pictures, copy the original raw files of the group of pictures to be edited and paste them to the edited pictures folder at P:\FSU\Invertebrates\CPER 2011\Beetle Pictures\edited.

Make sure to complete all of the steps below or delete any files that have not been edited from the folder before leaving or changing to a new task.

To Batch rename files, generate a list of current file names in 'edited' folder:

1. Open command prompt: Open Run & type in **cmd**.
2. Navigate to the P drive within the command prompt: Prompt will show which drive is in use (e.g. if in H drive will say H:\>). To change to a different drive, type the name of that drive (e.g. **P:**) and hit enter.
3. Navigate to desired folder.
4. Type in the command **cd** followed by a space and then the complete file path to the folder containing the pictures to be compressed. (e.g. P:\>**cd FSU\Invertebrates\CPER 2011\Beetle Pictures\edited**). Note: to copy and paste into command prompt, short cuts will not work (e.g. Ctrl+V); right click must be used.
5. Use the command prompt to generate a list of the file names in the compressed pictures folder: type in the command **dir /b \*.jpg>list.txt** - this will create a text file with a list of the file names in the folder. (e.g. P:\>FSU\Invertebrates\CPER 2011\Beetle Pictures\edited>**dir /b \*.jpg>list.txt**). Note: spaces are required after dir and b in the command.

6. The newly formed text file (list.txt) will be located in the edited pictures folder.

Create an excel spreadsheet to generate commands for new file names:

1. Create an excel spreadsheet and save it to the 'edited' folder under the name 'rename'. Label Column A "Original," Column B "New," and Column C "Command."
2. Copy the list of file names from the list.txt file into Column A.
3. In Column B fill in the new file names, which are made different from the old file names by replacing the r with an e (e.g. carabid2100r.jpg becomes carabid2100e.jpg). The easiest way to do this is to copy the original file names into the second column, highlight the second column, choose replace and replace r.jpg with e.jpg.
4. In Column C, paste this formula into C2: **=CONCATENATE("ren ",A2," ",B2)**. Fill this formula in for the remaining rows, it should automatically fill the correct values, but check to make sure the formula is filling correctly (A3,B3, A4,B4, etc.).
5. Select and copy the cells containing the commands that are desired (i.e. C2:C...).
6. Right click in the command prompt, make certain that the correct folder is being used, and paste the commands in and then hit enter. The files should all be renamed.
7. Open the pictures in Microsoft Office Picture Manager.
8. Go to edit pictures and crop.
9. Fix aspect ratio to '4x6'.
10. Crop out empty space so the images include just the beetle, the scale bar, color scale and number.
11. Edit all of the pictures and then choose File>Save All when they are finished.

#### *Compressing Pictures:*

When compressing a group of edited pictures, copy the edited files of the group of pictures to compress and paste them to the compressed pictures folder at P:\FSU\Invertebrates\CPER 2011\Beetle Pictures\compressed. Make sure to complete all of the steps below or delete any files that have not been compressed from the folder before leaving or changing to a new task.

To batch rename files, generate a list of current file names in 'compressed' folder:

1. Open command prompt: Open Run & type in **cmd**.
2. Navigate to the P drive within the command prompt: Prompt will show which drive currently in use (e.g. if in H drive will say H:\>). To change to a different drive, type the name of that drive (e.g. **P:**) and hit enter.
3. Navigate to desired folder: Type in the command **cd** followed by a space and then the complete file path to the folder containing the pictures to be compressed. (e.g. **P:\>cd FSU\Invertebrates\CPER 2011\Beetle Pictures\compressed**). Note: to copy and paste into command prompt, short cuts will not work (e.g. Ctrl+V); right click must be used.
4. Use the command prompt to generate a list of the file names in the compressed pictures folder: type in the command **dir /b \*.jpg>list.txt** - this will create a text file with a list of the file names in the folder. (e.g. **P:\>FSU\Invertebrates\CPER 2011\Beetle Pictures\compressed>dir /b \*.jpg>list.txt**). Note: spaces are required after dir and b in the command.

5. The newly formed text file (list.txt) will be located in the compressed pictures folder.

Create an excel spreadsheet to generate commands for new file names:

1. Create an excel spreadsheet and save it to the 'compressed' folder under the name 'rename'. Label Column A "Original," Column B "New," and Column C "Command."
2. Copy the list of file names from the list.txt file into Column A.
3. In Column B, fill in the new file names, which are made different from the old file names by replacing the e at the end of the file name to c to the end (e.g. carabid2100e.jpg becomes carabid2100c.jpg). Copy the original file names into the second column, highlight the second column, choose replace and replace e.jpg with c.jpg.
4. In Column C, paste this formula into C2: =CONCATENATE("ren ",A2," ",B2). Fill this formula in for the remaining rows, it should automatically fill the correct values.
5. Check to make sure the formula is filling correctly (A3,B3, A4,B4, etc.).
6. Select and copy the cells containing the commands that are desired (i.e. C2:C...).
7. Right click in the command prompt, making sure you are still operating in the correct folder, and paste the commands in and then hit enter. The files should all be renamed.
8. Open the newly renamed pictures in Microsoft Office Picture Manager.
9. Select all the pictures by going to the multiple picture view, clicking on the first one, then holding down shift and clicking on the last one. They should all be highlighted when selected.
10. Go to edit pictures and then Compress Pictures.
11. Under Compress Pictures, choose Web Pages, then click OK. Once compressed, the thumbnails will have an asterisk in front of the file name.
12. Go to File & Click Save All. All the pictures should now be compressed. To check, look at the file sizes, they should all be less than 100 KB.

### **Sample Preservation**

1. Prepared, pinned or pointed, ground beetle specimens are to be stored in airtight Schmitt boxes or in unit trays in Cornell cabinet drawers with a small amount of moth crystals (~6.5 g) in a cardboard fumigant box.
2. The fumigant box must be firmly secured in the corner of the Schmitt box or unit tray with pins.

[Figure 34]

### **Sample Shipping**

#### **11.3.1.9 Preparation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Storage space for shipping materials (cardboard mailers, cardboard boxes, packing tape, etc.).
2. Space for preparing packing for shipping.
3. Hazardous Materials certification for shipping ethanol.

#### **11.3.1.10 Taxonomists**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Prepared dry (pinned/pointed) beetle specimens are shipped overnight to Taxonomists with a hard and digital copy of the 'NEON Ground Beetle Diversity Datasheet'.
2. Any large beetles should be brace-pinned on either side of the label to prevent them from moving during transit.

[Figure 54]

3. Ensure that all of the specimens are firmly glued to their points by tapping the box to test.
4. A specimen dissociated from its locality label is worthless.
5. Prepared dry material is shipped in cardboard mailers in outer cardboard boxes with ample packing material to ensure that the specimens are not jostled.

[Figure 55]

[Figure 56]

6. These outer boxes should be clearly labeled with 'Fragile' and 'Dead Insects for Scientific Study'.

#### **11.3.1.11 Genetic Analytical Laboratories**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Ship 96 well microplates dry overnight at room temperature (20°C) to external facilities along with a hard and digital copy of the 'NEON Ground Beetle Diversity Datasheet'.

[Figure 57]

#### **11.3.1.12 Archives**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Archives are voucher collections of a variety of materials that represent many different kinds of storage (e.g. dry in cabinets, -80°C, specimens in ethanol). Each of these differing kinds of facilities should receive a hard and digital copy of the 'NEON Ground Beetle Diversity Datasheet'.
2. Ship prepared dry material in cardboard mailers packed in outer boxes with ample packing material to ensure that the specimens are not jostled.
3. These outer boxes should be clearly labeled with 'Fragile' and 'Dead Insects for Scientific Study'.
4. Ship Genomic DNA extractions overnight dry to cryogenic facilities to be stored at -80°C. These facilities should aliquot and send sample location and storage parameter data to NEON. This information should be entered into the 'NEON Ground Beetle Diversity Datasheet'.

#### **Data Handling**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Preferably at the end of each field and lab day, all information from field and lab data sheets must be typed up and saved to the NEON server as directed by the Field Operations Manager. All data from the image processing must also be backed up and uploaded to the NEON server as soon as possible.

## ***Refreshing the Laboratory Supplies***

### ***Laboratory Maintenance, Cleaning, Storage***

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Empty and wash out waste receptacles (e.g. ethanol, PG) and leave on lab bench to dry on paper towels.
2. All materials should be put away in clearly marked receptacles or cabinets after each bout of laboratory work.
3. All flammables must be returned to the appropriate cabinets.
4. Ethanol preserved specimens should be clearly labeled in 50 mL tubes (collection code and date) and in cool storage.
5. Pinned material should be placed in the appropriate Schmitt box or Cornell cabinet.

[Figure 53]

6. Temporary waste holding containers must be emptied into proper (clearly labeled; no abbreviations) receptacles.
7. Pack equipment and consumables for subsequent field work and store neatly.

## **12 DEFINITIONS**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.

*Define all protocol specific technical terms in alphabetical format.*

## **13 REFERENCES**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Use Ecology style

## APPENDIX A Field Data Sheets

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

See 'NEON Ground Beetle Diversity Datasheet' which includes several tabs (Field Data Collection, Field Data Entry Logistics). The columns in each tab are TBR but currently look as follows:

*Include copies of all data sheets – jpg format*

*(data sheets are useful for CI to define PDA and data ingest requirements)*

Activity  
Names  
Month  
Day of the Month  
Day of the Week  
Year  
Start Location  
Start Time (in car, departing start location)  
Arrival at site (in car, arrival on site)  
Plot  
GPS Location  
Arrival at plot  
Collection start (from first trap)/CO2 set  
# specimens in each trap (0/few/many)  
Collection end (from last trap)/CO2 pickup  
Notes  
Freezer time in  
Freezer time out  
Total mosquitoes (0/few/many)  
Bloodfed mosquitoes (0/few/many)  
Bycatch (0/few/many)  
Time of ethanol change for each trap  
Departure from site (in car, leaving)  
End Time (arrival at end location)  
End Location  
Start Location  
Start Time  
Arrival at site  
Plot # for 1st - 10th plot (order of visitation)  
Arrival time at each plot  
Collection time start each plot  
Collection time end each plot  
Departure time from site  
End Location  
End Time  
Drive time to site

Drive time from site  
Total time at site  
Average Collection time at each plot  
Average Travel time between plots  
Plot  
Trap  
Institution Storing  
Collectors  
Collection Date  
Collection Method  
Plot #  
Trap #  
Continent  
Country  
State  
Region  
Exact Site  
Latitude  
Longitude  
Elevation (m)  
Other Info  
Phylum  
Class  
Order  
Family  
Subfamily  
Tribe  
Morphospecies  
Genus  
species  
Species Authority  
Basis of ID  
Date of ID  
Taxonomist Identifier  
Technician Identifier  
Length (mm)  
Sex  
Reproduction  
Life Stage  
Notes

## APPENDIX B Lab Data Sheets

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

See 'NEON Ground Beetle Diversity Datasheet' which includes several tabs (Beetle Trap Sorting Data Entry, Biodiversity, 96 well plate, DNA barcode). The columns in each tab are TBR but currently look as follows:

*Include copies of all data sheets – jpg format*

*(data sheets are useful for CI to define PDA and data ingest requirements)*

Week  
Date (Month/Day)  
Sorter's Initials  
Time Trap Collected (ex. 8:15 AM)  
Time Ethanol Changed (ex. 6:30 PM)  
Time between Collection & Ethanol Change (ex: 10:15)  
# morpho-species  
# Adephagans pinned/pointed  
# bycatch tubes  
If > 20 (ethanol) species: \_\_\_\_\_  
If > 20 (ethanol) species: \_\_\_\_\_  
If > 20 (ethanol) species: \_\_\_\_\_  
Unique #  
Collectors  
Collection Date  
Collection Method  
Plot #  
Trap #  
Continent  
Country  
State  
Region  
Exact Site  
Latitude  
Longitude  
Elevation (m)  
Other Info  
Phylum  
Class  
Order  
Family  
Subfamily  
Tribe  
Morphospecies  
Genus  
species  
Species Authority  
Basis of ID

Date of ID  
Taxonomist Identifier  
Technician Identifier  
Length (mm)  
Sex  
Reproduction  
Life Stage  
Notes  
Well positions  
NEON Plate #  
CCDB Plate #  
Well  
NEON Specimen #  
Sample ID  
Institution Storing  
Collectors  
Collection Date  
Collection Method  
Plot #  
Trap #  
Continent  
Country  
State  
Region  
Exact Site  
Latitude  
Longitude  
Elevation (m)  
Other Info  
Phylum  
Class  
Order  
Family  
Subfamily  
Tribe  
Morphospecies  
Genus  
species  
Species Authority  
Basis of ID  
Date of ID  
Taxonomist Identifier  
Technician Identifier  
Length (mm)  
Sex  
Reproduction  
Life Stage  
Notes

## **APPENDIX C Considerations for implementation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Indicate activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure.*

*Describe any component of the process that may interfere with the accuracy of the final product.*

*Discuss how to avoid common errors in sampling or common ways samples can be contaminated.*

*Clearly flag things that might impact their work or the scientific data that aren't covered in the procedural pieces (stupid examples: "We're measuring nitrates, if you are exposed to or using nitrates at home on your lawn, trace amounts might contaminate our data"; "If it's raining, sky water getting into the samples before you seal them could alter results" )... i.e. call out weird issues and folklore explicitly. See: [http://en.wikipedia.org/wiki/Phantom\\_of\\_Heilbronn](http://en.wikipedia.org/wiki/Phantom_of_Heilbronn)*

## **APPENDIX D Procedure Checklist**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

## **APPENDIX E Tables**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

## **APPENDIX F Figures**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*



Figure 1



Figure 2



Figure 3



Figure 4



Figure 5



Figure 6



Figure 7



Figure 8



Figure 9



Figure 10



Figure 11



Figure 12



Figure 13



Figure 14



Figure 15



Figure 16



Figure 17

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

COLORADO Weld County.  
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COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

Figure 18

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

Figure 19

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. KKBlevins.  
NEON.FSU.D10.

Figure 20



Figure 21



Figure 22



Figure 23



Figure 24



Figure 25



Figure 26



Figure 27

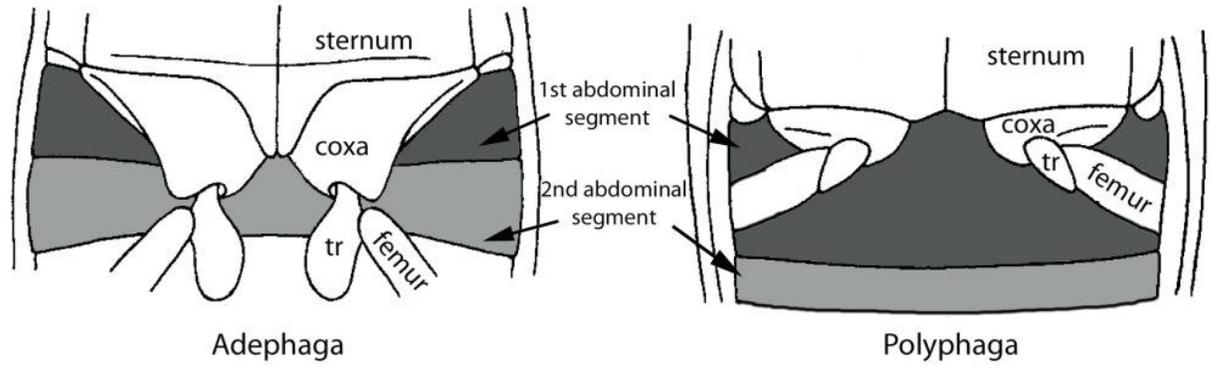


Figure 28

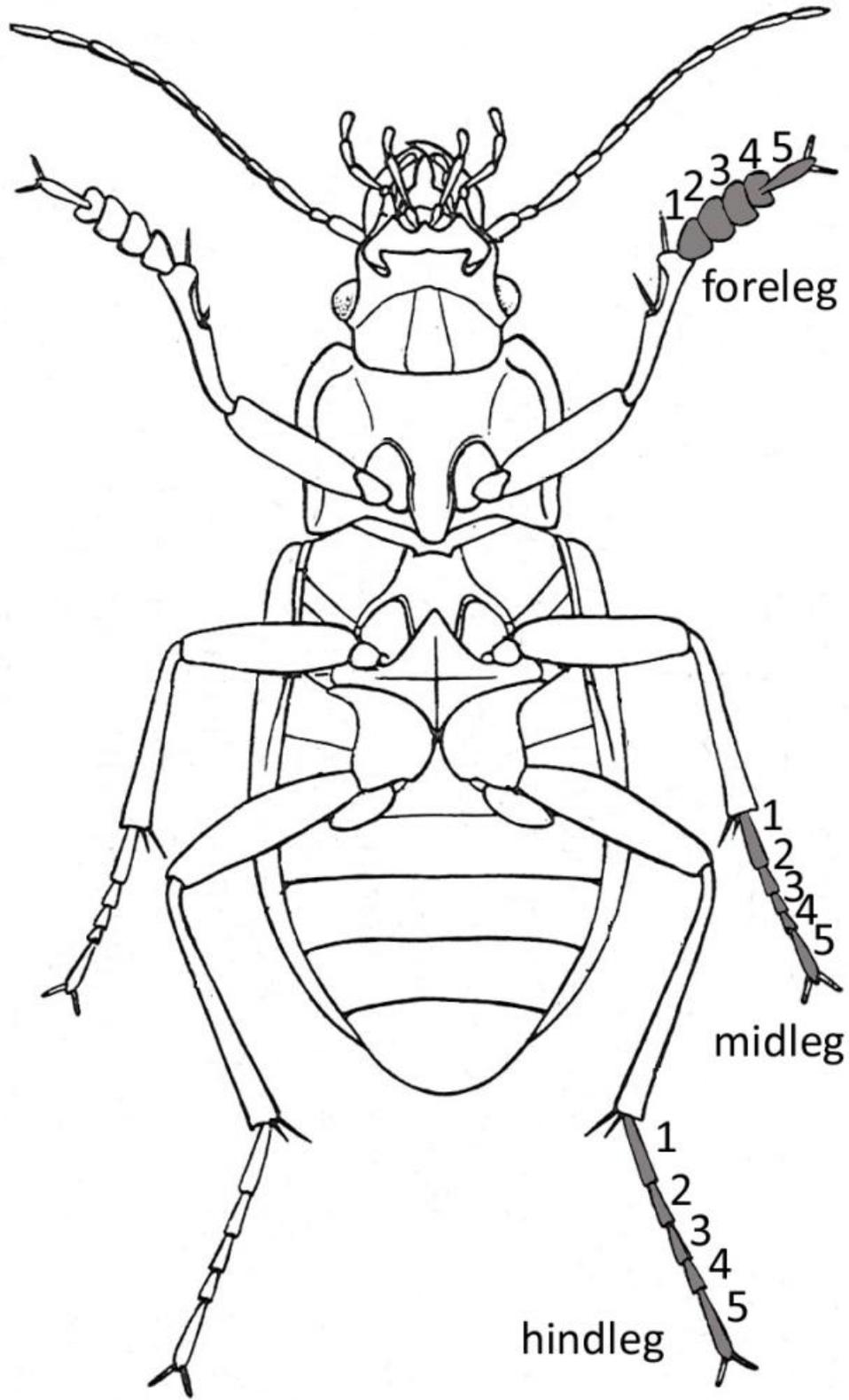


Figure 29

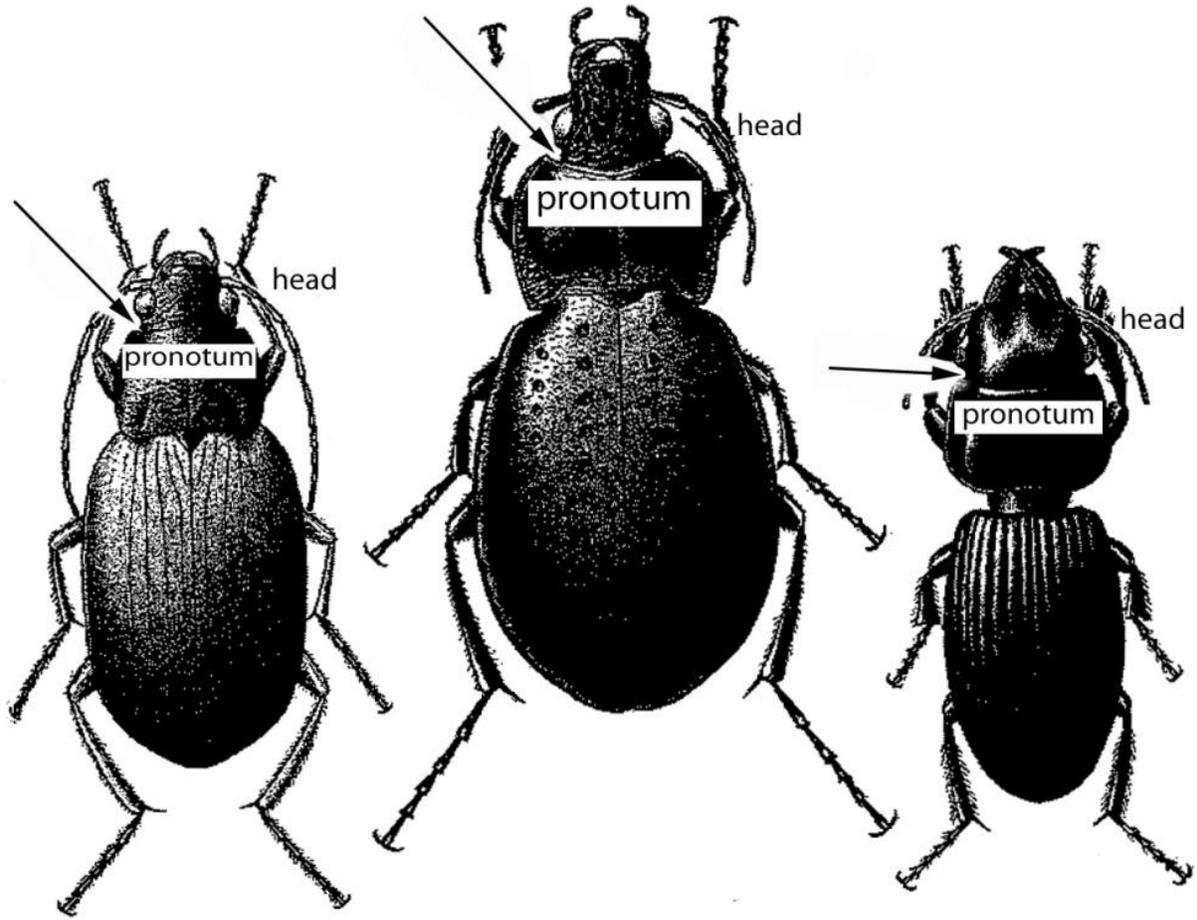


Figure 30



Figure 31



Figure 32



Figure 33

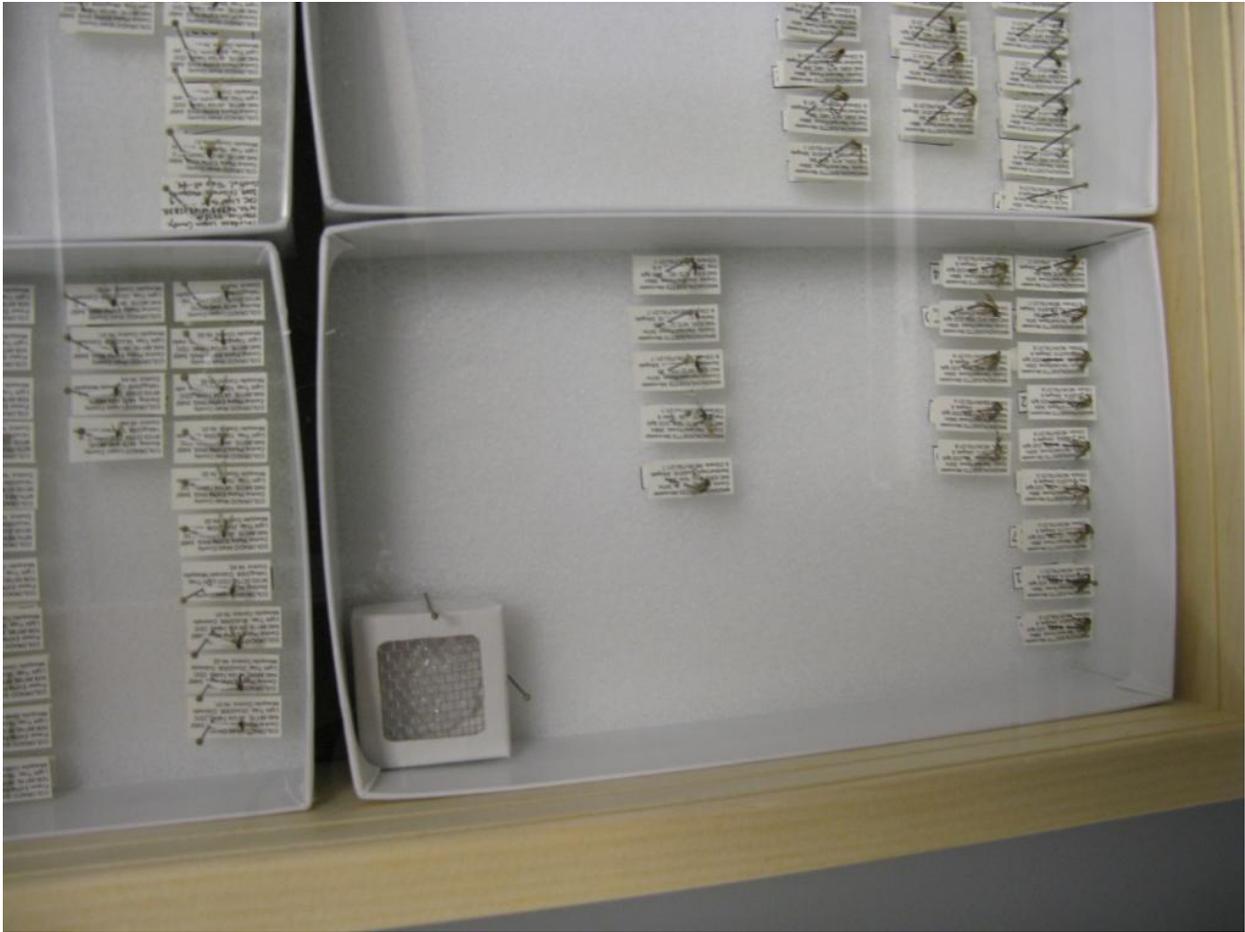


Figure 34

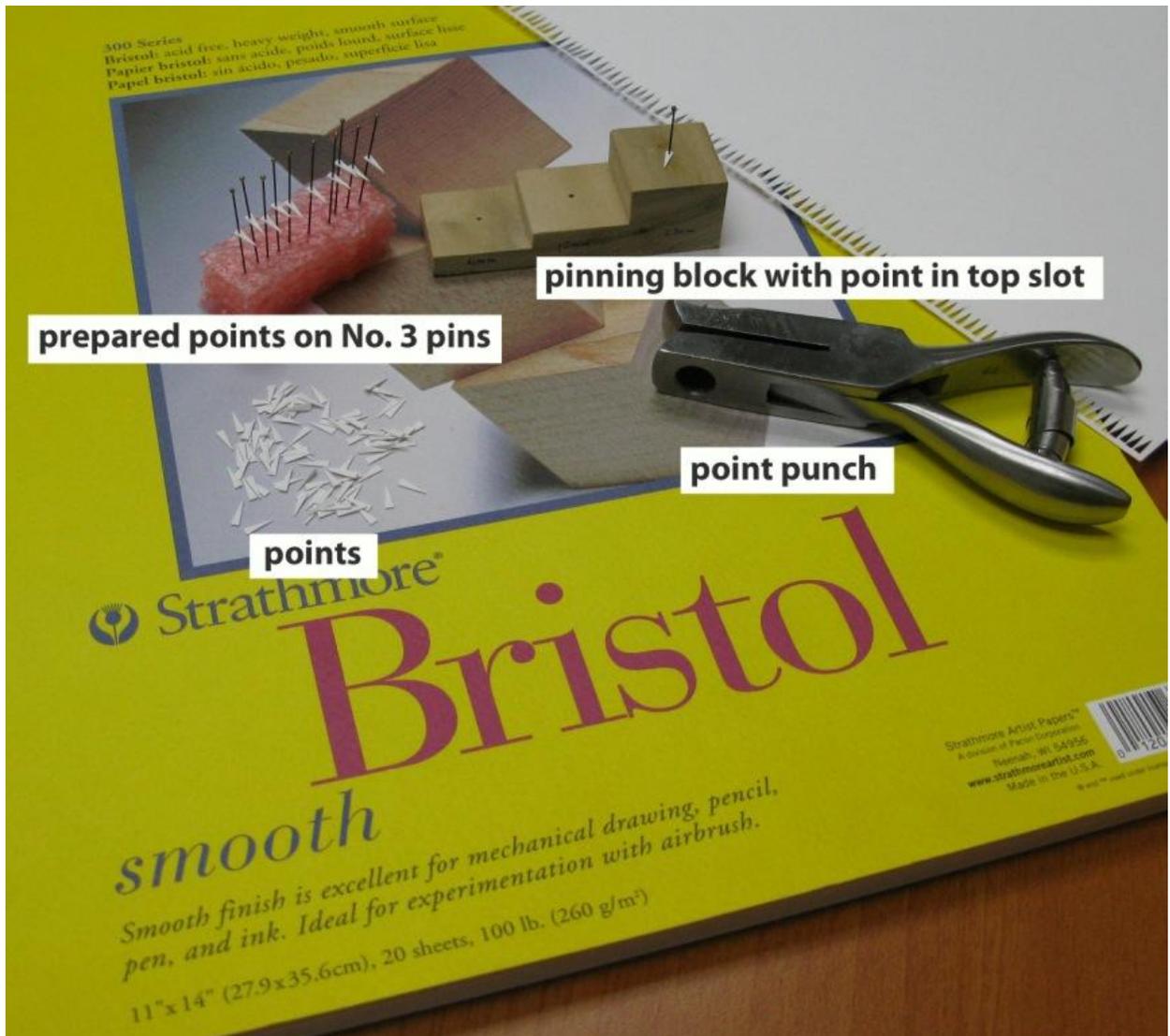


Figure 35

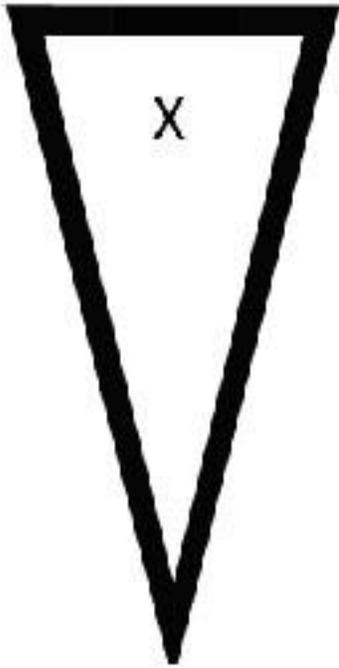


Figure 36

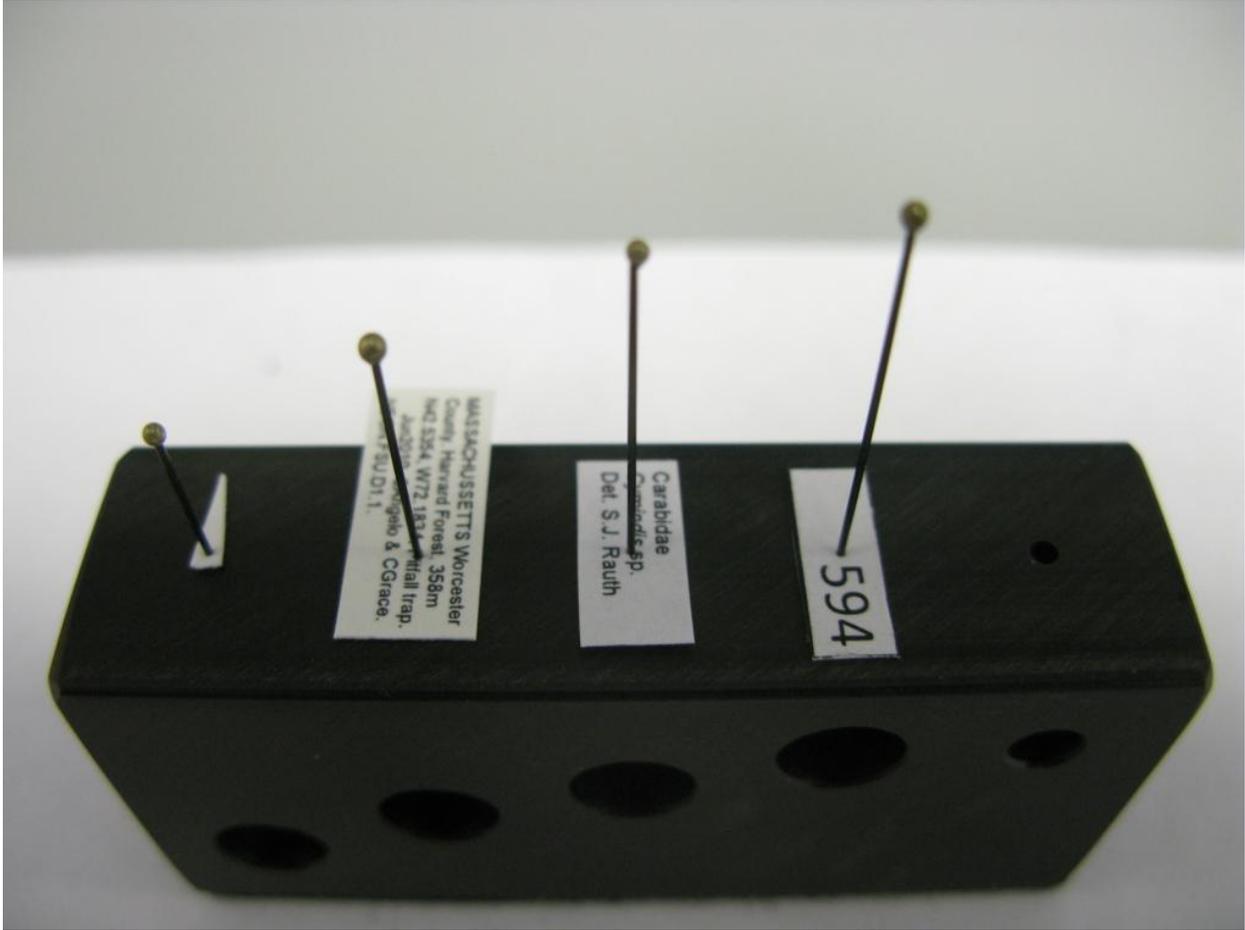


Figure 37

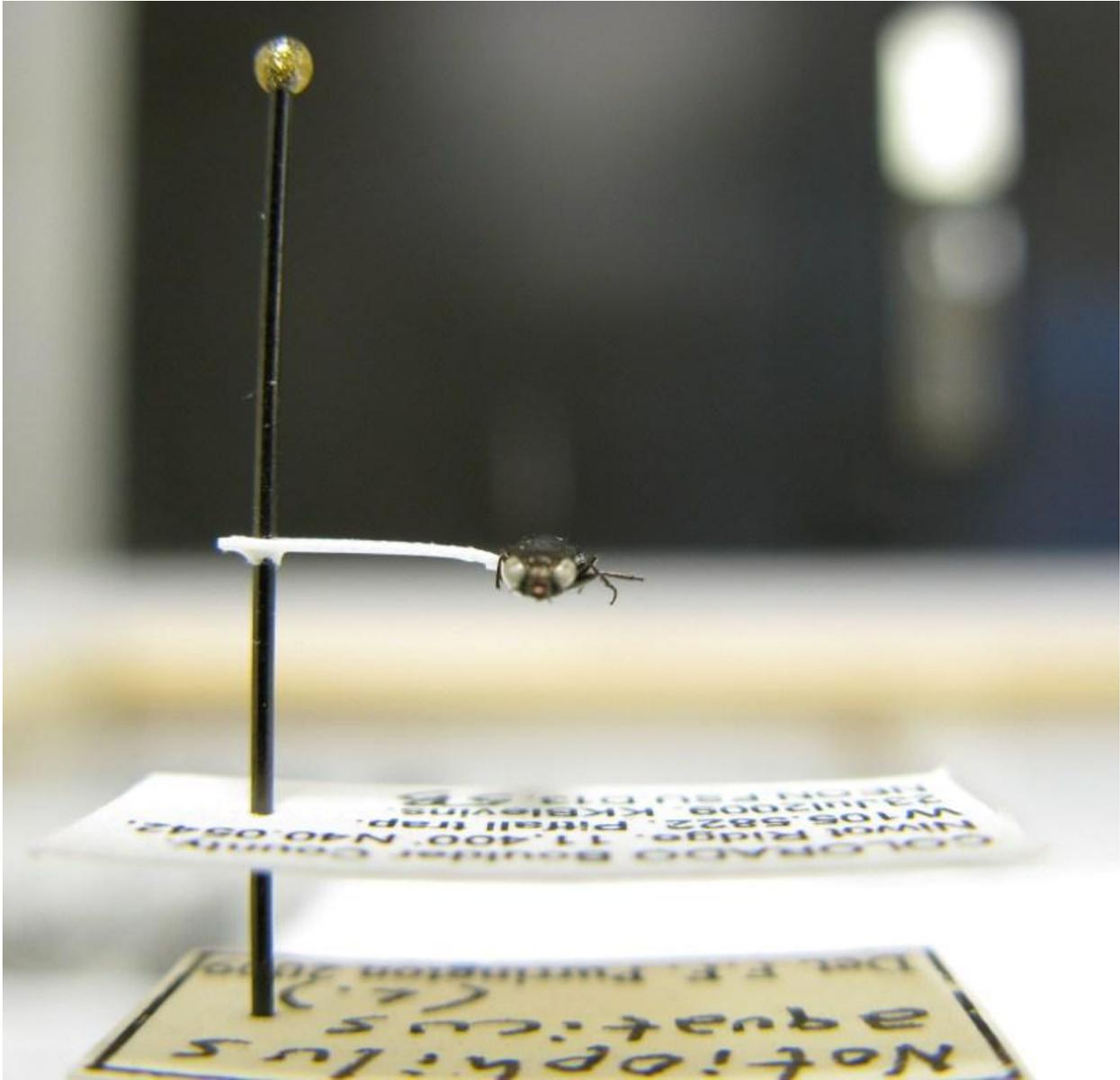


Figure 38

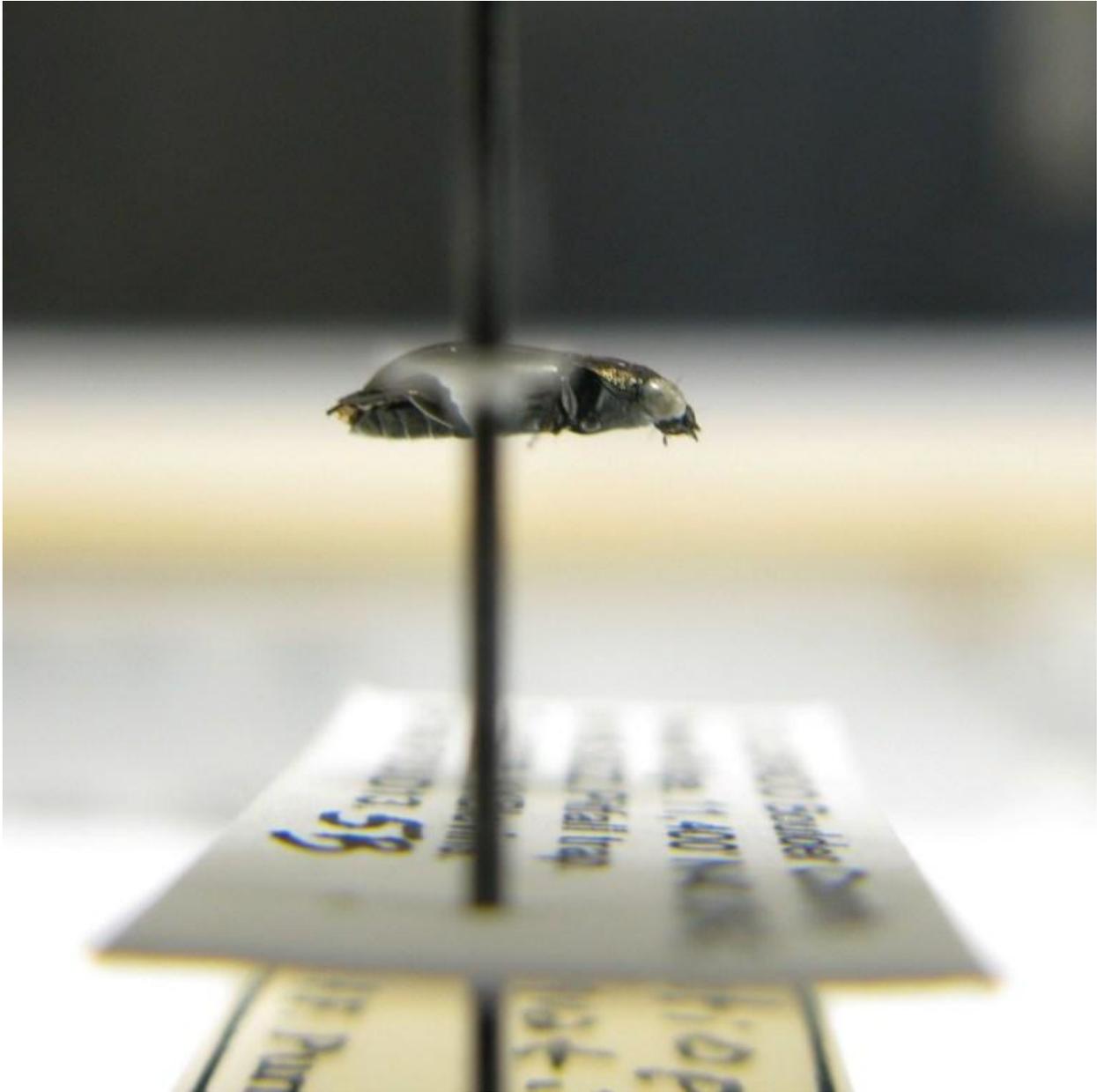


Figure 39

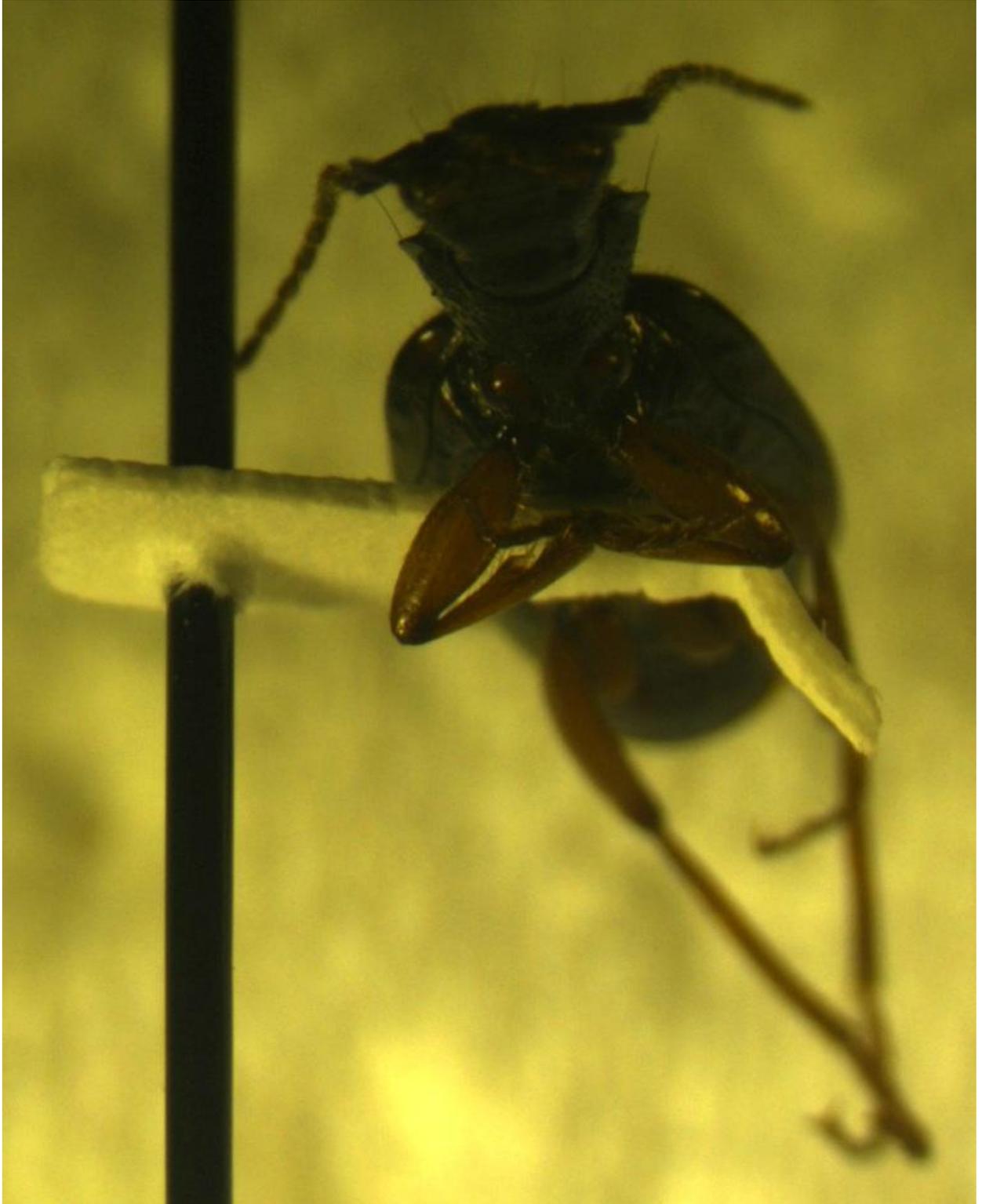


Figure 40



Figure 41

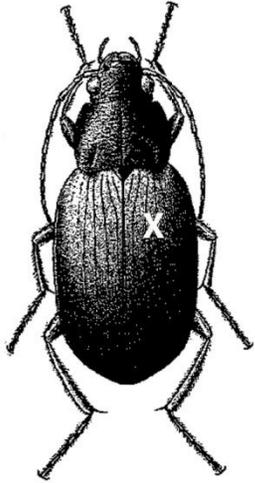


Figure 42

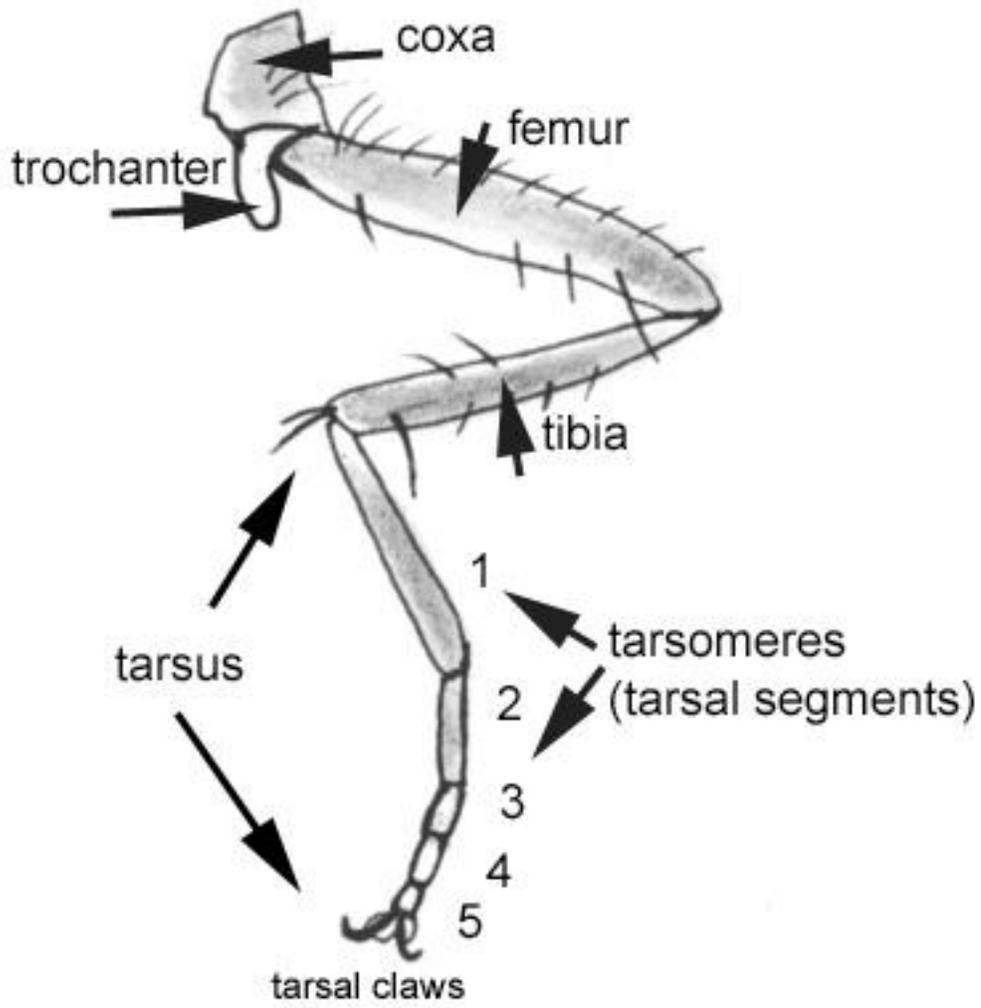


Figure 43

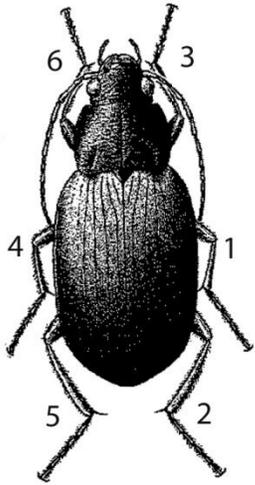


Figure 44

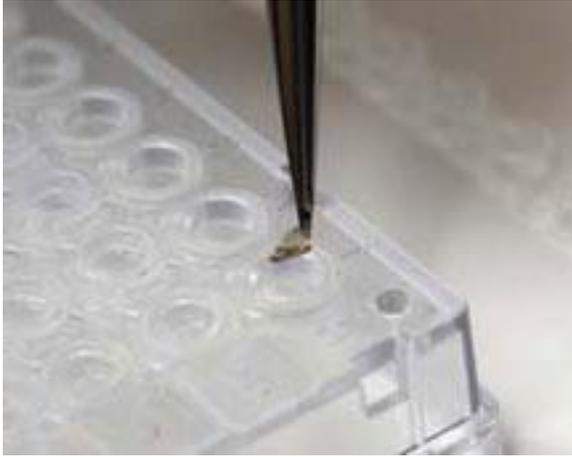


Figure 45

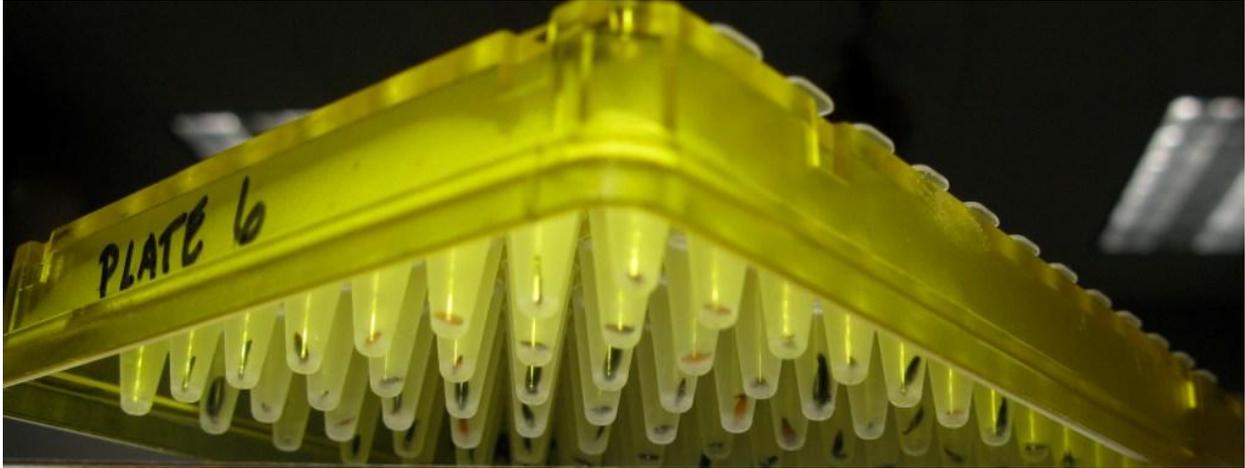


Figure 46

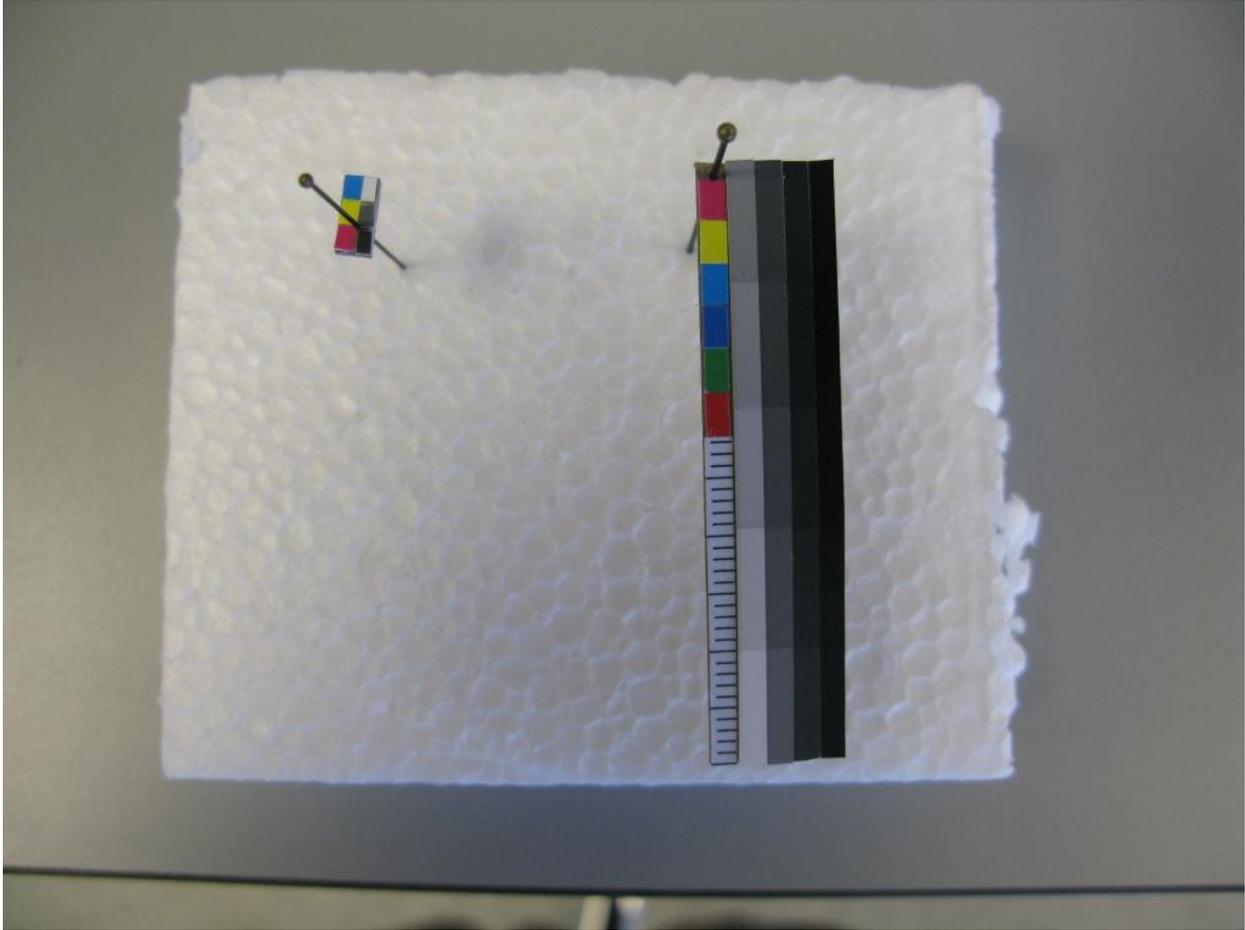


Figure 47



Figure 48

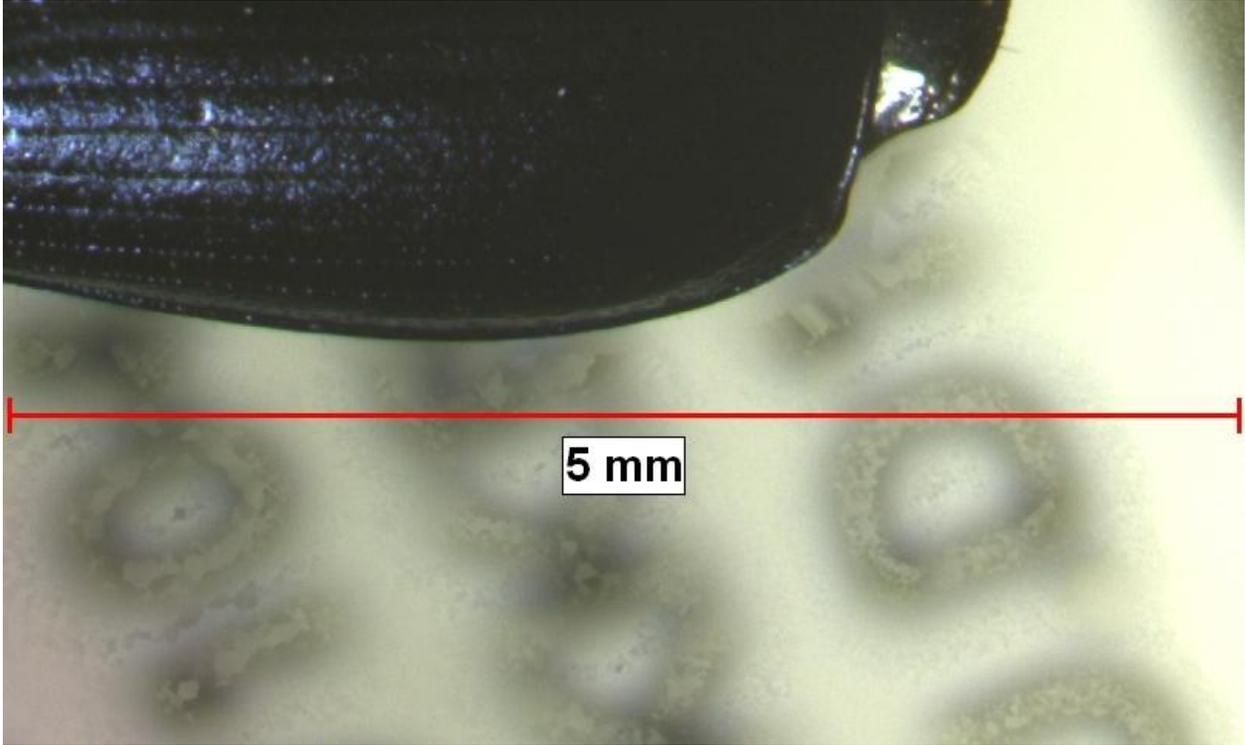


Figure 49



Figure 50

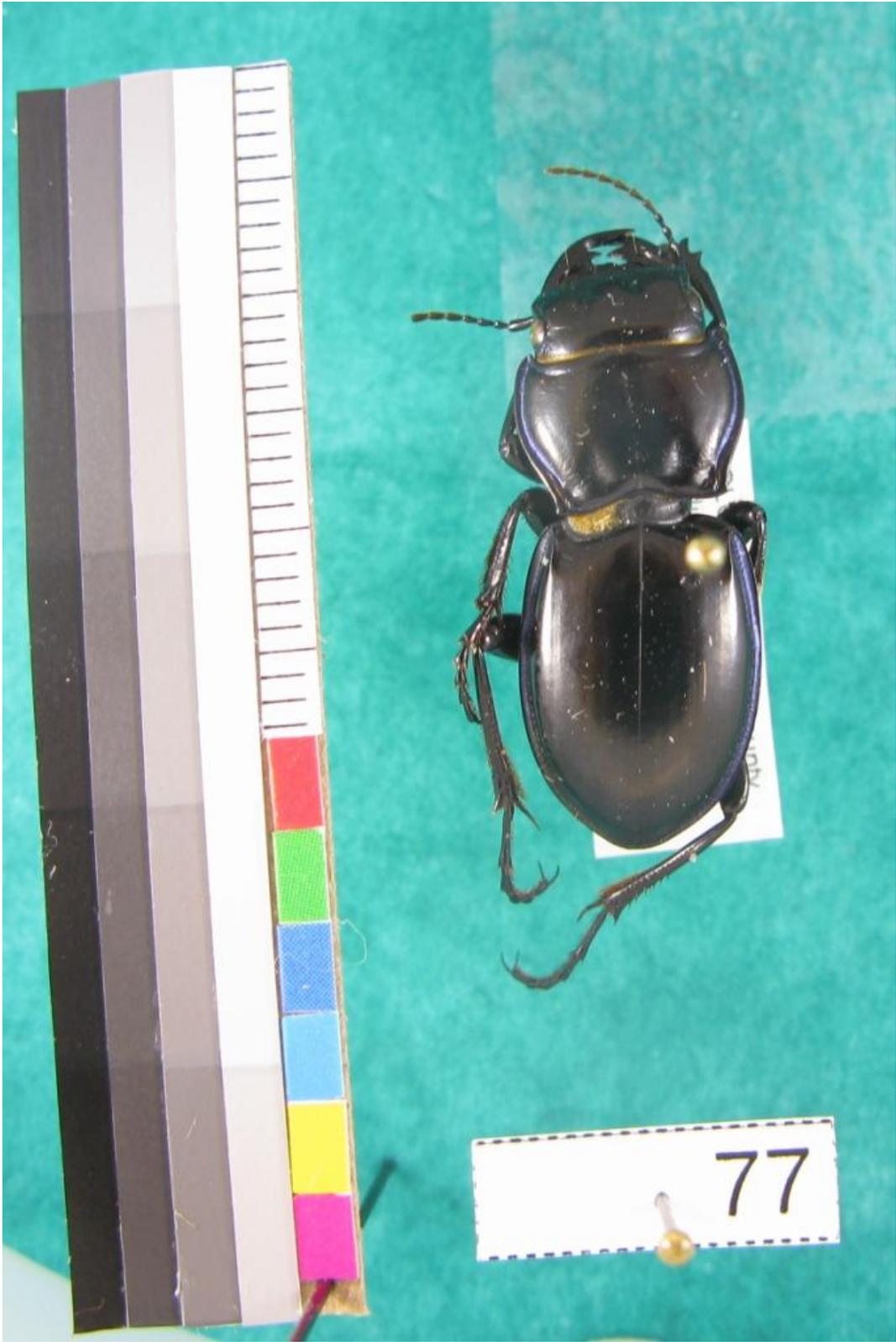


Figure 51

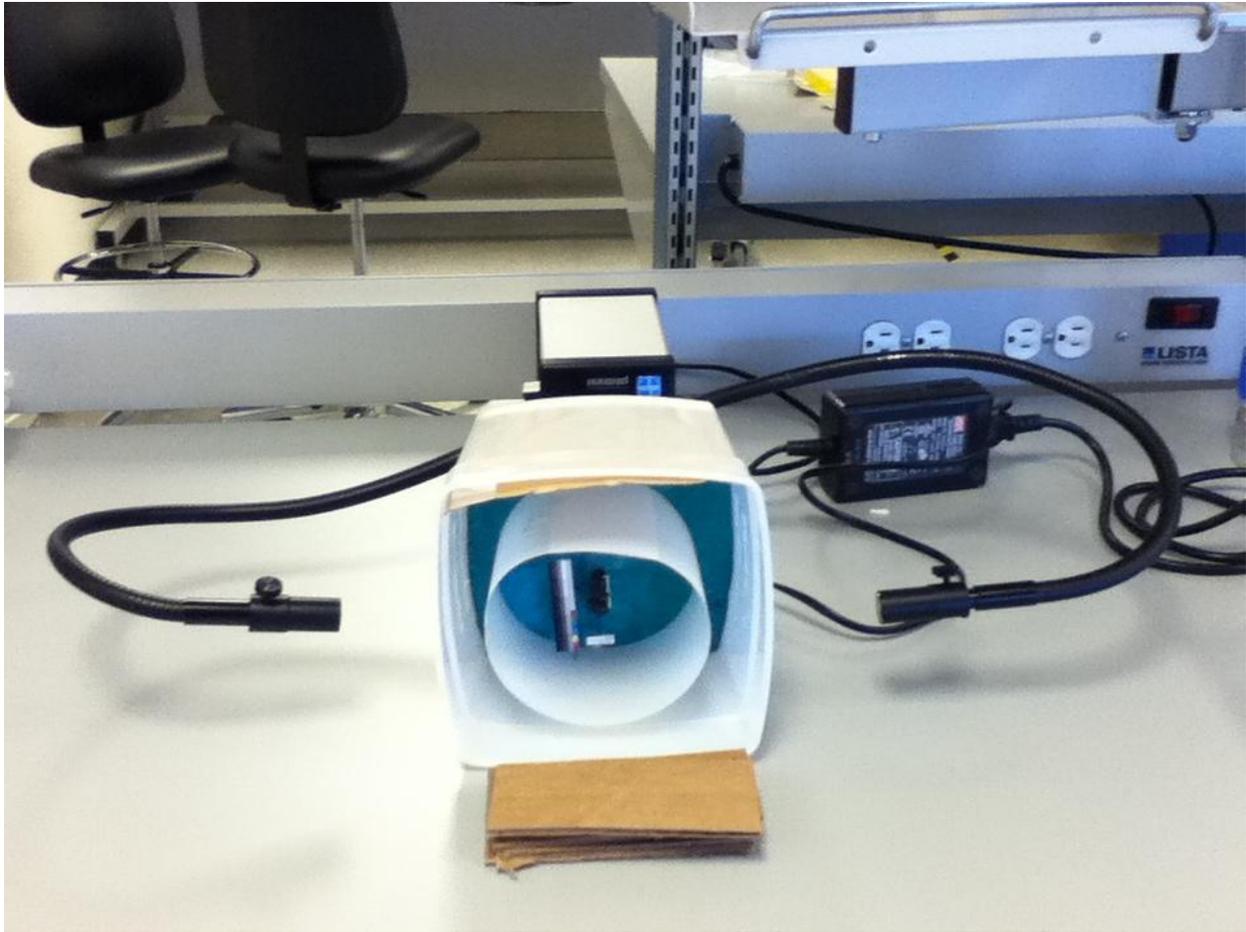


Figure 52



Figure 53



Figure 54



Figure 55



Figure 56

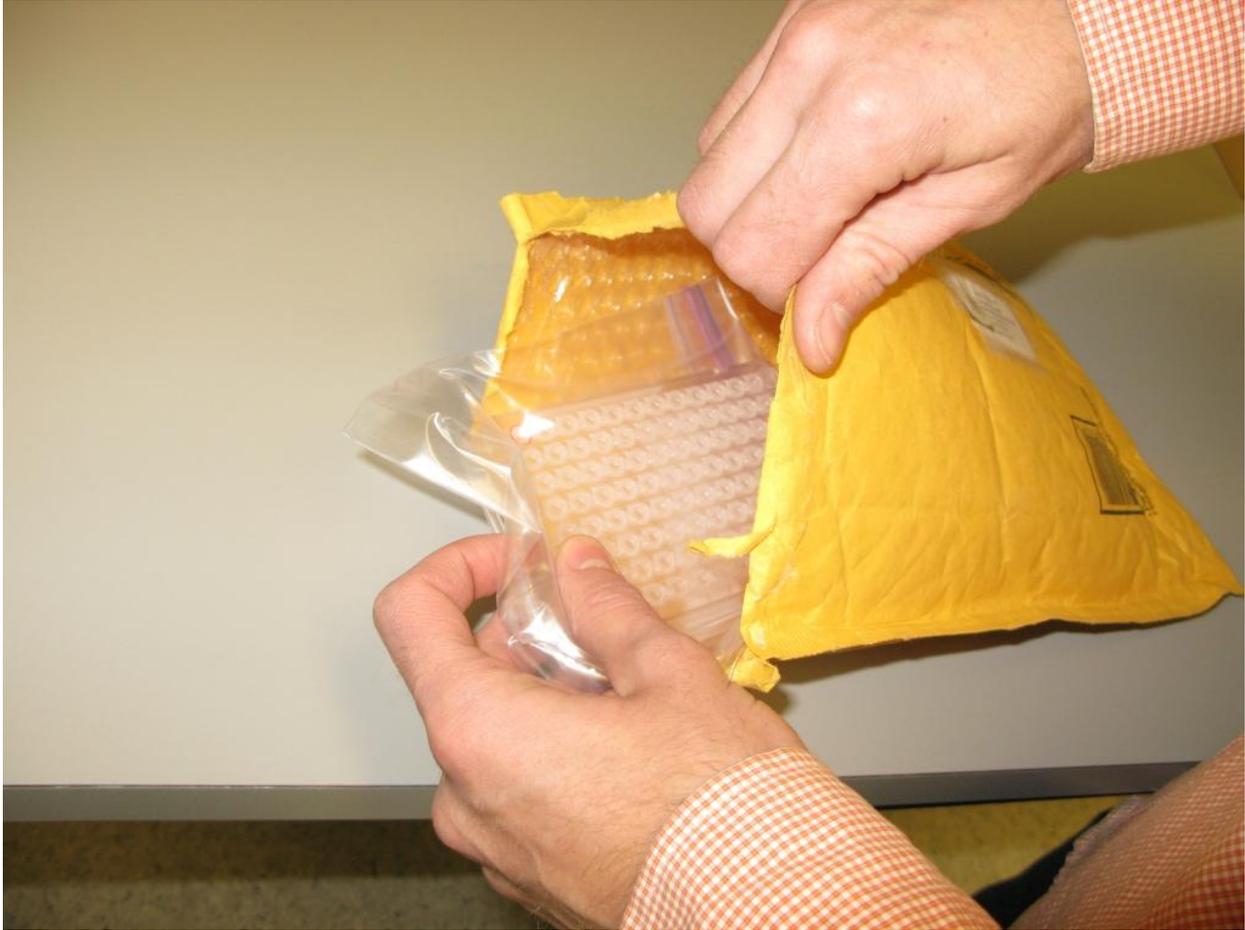


Figure 57