NEON FSU Field and Lab Protocols for Field Operations 2012 Prototype in Domain 3: Ground Beetle Diversity

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

1.1 Purpose

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

The primary purpose of this document is to provide a change controlled version of Observatory protocols and for external review by subject-matter experts. This document also 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 DOMAIN 3 2012 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 DOMAIN 3 2012 and is TBR.]

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference Documents

RD[01]	NEON.MGMT.NPR.000008.GEN NEON Glossary of Abbreviations
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/QC Plan
	DOORS requirements
	ATBD
AD[01]	FSU Science Requirements
AD[02]	FSU Field Operations Plan
AD[03]	Data Products Level 1-3 Catalog

2.2 Acronyms

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 DOMAIN 3 2012 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 contracted experts for morphological identifications (Taxonomists), Genetic Analytical Laboratories to conduct the bi-directional DNA sequencing during the construction period of the observatory (during operations only uni-directional sequencing should be required for verifying well-circumscribed specimens), and/or to Archives.

For 2012, the expert taxonomist contract is with Michael C. Thomas, Ph.D. Florida State Collection of Arthropods, Florida Department of Agriculture & Consumer Services. The Biodiversity Institute of Ontario is the current bid-winning contract for the Genetic Analytises. The NEON Support Facility at 1635 38th St., Suite 100, Boulder, CO 80301 will be the Archive for the 2012 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”.

Definitions:
Vertebrates = animals with their spine inside their body (e.g. mice, humans)
Invertebrates = animals with their “spine” (exoskeleton) outside of their body (e.g. crabs, spiders, beetles)
Insects = a subset of invertebrates with six legs (e.g. beetles and mosquitoes)

3.1.1 Metadata Collection

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 Data sheet'. 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.

3.2 Science Requirements

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

This protocol fulfills the following Observatory science requirements:

3.3 Data Products

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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

Measurement	Data Product
	Revising catalogue with Sarah Elmendorf and Steve Berukoff; note added 20120517

4 PROTOCOL

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

4.1 Plot Location

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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 DOMAIN 3 2012 and is TBR.]

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

6 DECISION TREE

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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)	YES. Data not comparable to standard collection events	Cannot calculate diversity indices if traps collections are not comparable. If ALL traps move by a day this is less of an issue.
2-7 days	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	YES. 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)	YES. 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)	YES. 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

7 SAFETY

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

8 PERSONNEL REQUIREMENTS

8.1 Field Personnel

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 DOMAIN 3 2012 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).

9 TRAINING REQUIREMENTS

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

See 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 DOMAIN 3 2012 and is TBR.]

For Domain 3 in 2012, IDEALLY the first actual sampling should occur during the week of Wednesday July 11 and continue for 6 weeks through to the week ending Friday, August 17 2012. (Training & Establishment Monday Jun 25 – Tuesday July 10).

Pitfall traps will be checked, emptied, and reset weekly.

Table 2 The approximate sample dates for beetle sampling at all NEON sites during full scale Operations

Domains	Start Week	Stop Week (inclusive)	Total No. Weeks	Approx. Calendar Start Date	Approx. Calendar Stop Date	Total All sampling weeks	No.
1	17	37	21	23-Apr	10-Sep		21
2	15	37	23	9-Apr	10-Sep		23
3,4,7,9,11,14,17,20	14	37	24	2-Apr	10-Sep		192
5,10	20	37	18	14-May	10-Sep		36
6	18	37	19	30-Apr	10-Sep		19
8,15	21	37	17	21-May	10-Sep		34
12	24	37	14	11-Jun	10-Sep		14
13,16	19	37	19	7-May	10-Sep		38
18,19	21	35	15	21-May	27-Aug		30

Beetle sampling is to occur 7 days within 3 hours of a standard time each week.
This means that if a plot is visited on a Thursday morning for the first sampling bout, it must be visited every Thursday morning subsequently so that THE SAMPLES ARE COMPARABLE FROM ONE WEEK TO THE NEXT.
Whichever day is selected to sample each plot is the discretion of the Domain Manager, however, THE SAMPLING MUST BE CONSISTENT FOR THE ENTIRE FIELD SEASON.
Sampling is estimated to require 2 technicians for 12min per trap x 40 traps = 480 min = 8 hours each day of sampling time, plus hiking time and travel (driving) to and from site.

10.2 Contingent decisions

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

See Section 6

10.3 Field Procedure

10.3.1 Equipment and Materials

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Table 3 Materials and supplies required for the Beetle Sampling Procedure at one site for one sampling day.

Item Description	Quantity per sampling event	Hazardous Chemical
Pitfall trap locality labels	3 per trap; 3*40=120	N
Ice packs	2	N
Chests coolers	1	N
Trowel or soil knife	2	N
Sharpies	4	N
Pitfall trap bowls	80 (in ground)	N
Pitfall covers	40 (in ground)	N
Plastic nails for pitfall covers	200	N
Propylene glycol	50 mL per trap	N
Distilled water	50 mL per trap	N
Ethanol		Y
Archival, ethanol-safe pens (Pigma brand, size 01)	4	N
Scissors	2	N
Forceps, fine point	2	N
Whirlpak bags, 13 oz	40	N
300 µm filter mesh organdy pre-cut (9 cm square)	40	N
Modified wide mouth Nalgene bottles (bottom	3	N

portion of bottle cut off)		
Modified wide mouth Nalgene lids as filter tops (top cut out of lid, filter screws onto bottle	3	N
Ethanol squeeze bottle	2	N
Water squeeze bottle	2	N
Carry-all craft tote bag or other field pack	2	N
Specimen cups for PG waste while filtering	3	N
Specimen cups for Ethanol waste while filtering	3	N
Large ziplock bags	12	N
Ethanol waste wide mouth bottle (1000 mL Nalgene)	2	N
PG waste wide mouth bottle (4000 mL jug)	2	N
Latex gloves	1 pair	N
Handwipes	optional	N
Replacement pitfall bowls	10	N
Replacement whirl paks	20	N
Replacement filters	10	N
1000 mL wide mouth Nalgene bottles filled with ethanol	2	N
1000 mL wide mouth Nalgene bottle filled with water	3	N
1000 mL wide mouth Nalgene bottle filled with PG	6	N
Duct tape	1 roll	N
Flagging	1 roll	N
Bug spray	optional	Y
Mosquito protective clothing	optional	N
Sun block	Optional	N
First-aid kit	1	N
Camera	1	N
GPS/Trimble	1	N
PDA	1	N
Datasheets	10	N

10.3.2 Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

10.3.2.1 Plot Establishment

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 plastic 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. This bowl has large holes drilled into it so that any excess moisture has an exit rather than building up and causing the top bowl to float. The second bowl has a line at the 100 mL mark and has the preserving fluid. This top bowl is picked up and changed during sampling. The nestled bowls are then protected from weather by a cover while the trap is in operation. Covers are best made using a drill press (in advance of the field season) to get appropriate sized holes in each lid so that the plastic nails can be readily driven through the covers in the field. IF THE HOLES ARE NOT THE RIGHT SIZE THE COVERS WILL FALL DOWN AND CLOSE THE TRAP AT VARIOUS POINTS THROUGHOUT THE SAMPLING WEEK. TRAP SAMPLES ARE THEN NOT EQUIVALENT.

For 2012, 1/8" (3.17 mm) plexi-glass lids were cut to 8" (203 mm) square from a plastics company, Colorado Plastic Products. ¼" (6.35 mm) plastic garden spikes were used and so 17/64" (6.75 mm) holes were requisition 5/8" (15.88 mm) in from each edge.

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. All sampling fluids must be brought in and out from the lab each visit.

Once in field:

1. Select the first trap location by moving 9 m north of the center plot marker.

2. Dig a small hole with a soil knife to accommodate the pitfall bowl.

   [Figure 1]

3. Set the bottom bowl (with holes) into the hole. Set the top bowl into bottom bowl. Ensure that the lip of the top bowl is flush with the ground (this ensures that insects fall into the trap rather than walk around trap). Also ensure that there is no gap between the bowls so that specimens do not accumulate in the bottom bowl. Again, THE TOP BOWL MUST BE FLUSH WITH GROUND LEVEL SO THAT SPECIMENS ACCUMULATE IN THE TOP BOWL ONLY. THE TOP BOWL IS THE ONLY PART OF THE TRAP WHERE SPECIMENS SHOULD BE COLLECTED FOR THE SAMPLE.

4. Fill top bowl with 100 mL of PG:water solution. The bowl should have been prepared in the lab with a line around the outside circumference of the bowl for guidance in filling to the right volume. If it is not possible to fill to this volume THEN DO NOT TRAP. SHUT LID AND RESET WHEN THERE IS SUFFICIENT MATERIAL. THE VOLUME MUST BE THE SAME FOR EACH TRAP OR THEY ARE NOT COMPARABLE.

5. Position cover with four plastic nails so that it is straight and 1.5 cm above the surface of the bowls.

   [Figure 2]
   [Figure 3]

6. The second trap is 9 m east, the third is 9 m south, and the fourth is 9 m west of the center plot marker. Repeat until all four traps are set within a plot. THE POSITIONING OF THE TRAPS MUST BE COMPARABLE ACROSS THE OBSERVATORY. Pitfall traps should be in the same location from year to year. (Backfilled holes should be excavated anew each year).

10.3.2.2 Generating Sample Labels

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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)
- “collector” (e.g. Domain manager name, RNelson)
- a collection code which is comprised of the following parts: NEON.FSU.Domain.Plot.Trap, e.g. NEON.FSU.D10.BD01.4 for biodiversity trap in the 4th position at biodiversity plot 01, Domain 10.

To prepare labels:

a. Do NOT change to template set of labels; instead, rename the file name with the appropriate month and year THEN in this new file, select ‘Edit’ > 'find' and 'replace' to replace the month in this new set of labels.

b.

c. Once labels have been printed, cut labels into long columns using a snap-off blade knife and ruler. There should be no obstructions on the cutting mat or the ruler while doing this. Using a snap-off blade is essential to making clean cuts that DO NOT ADD ANY additional whitespace to the edges of labels. Unfortunately, this outcome is inevitable with scissors when making a cut of this length.

d. Cut labels with as little white space on all sides of the text as possible (see picture).

e. These cuts result in many labels in a vertical strip.

[Figure 18]

f. Store labels this way, and then when needed, cut individual labels across the row with scissors; again, leaving as little white space around as possible.

[Figure 19]
[Figure 20]

g. 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.

10.3.2.3 Preparing Field Equipment

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

10.3.3 Sample Collection in the Field

10.3.3.1 Trap Collection

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 to protect your hands from the materials used. Gloves can be reused.

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. One specimen cup should be used per trap to ensure that there is no sample loss or confusion of samples among traps.

   [Figure 9]
   [Figure 10]

4. Pour used PG solution into a 1 L storage bottle for disposal in the lab.

5. Rinse animals on filter with water into waste container.

   [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 >/= 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 Datasheets' the general number of invertebrates using the scale 0/few/many (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). Recording the general number helps with issue tracking.

11. Place all 4 whirl pak bags from a single plot are into a ziplock bag and label the outside of the bag with date and plot # before leaving the plot.

12. Ziplock bags are then placed into hard bottom carrying device ensuring that the whirl pak bag openings are upward. (For example, an airtight plastic container)

If sampling is continuing for another week:

1. Refill the PG mixture in the bowl to 100 mL. The bowl should have been prepared in the lab with a line around the circumference of the bowl for guidance in filling to the correct volume. If it is not possible to fill to this volume NOTE THIS IN THE DATASHEET AND TRANSCRIBE IT TO THE DIGITAL DATASHEET. THE VOLUME MUST BE THE SAME FOR EACH TRAP OR THEY ARE NOT COMPARABLE.

2. Replace the cover so that it sits 1.5 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.
2. Backfill holes with sand or appropriate local substrate. These same holes will be excavated in following years for pitfall trapping.

10.3.4 Sample Preservation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 lining the bottom) in the field vehicle.

10.3.5 Data Handling

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Field data collected should be carefully entered into the 'NEON Ground Beetle Diversity Datasheets' on the P drive within as short a time as possible from the field (preferable each day, required by the end of the week). Make sure to note deviations or any other points of interest in the digital datasheet.

10.3.6 Equipment Maintenance, Cleaning, and Storage

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

11 LAB STANDARD OPERATING PROCEDURE

11.1 Timing

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

11.2 Lab Procedure-Sorting

11.2.1 Equipment and Materials

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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

Item Description	Quantity per sampling event	Hazardous Chemical
Color Separation Guide and Gray Scale	1	N
Ethanol		Y
Plastic petri dishes for sorting specimens under microscope	2	N
Sharpies	5	N
Archival, ethanol-safe pens (Pigma brand, size 01)	5	N
No. 3 pins archival quality	Variable	N
50 mL nonsterile centrifuge tubes	Variable	N
Specimen cups - bottoms	5	N
Specimen cups - lids	5	N
Schmitt boxes	Variable	N
Point punch	1	N
Fumigant (moth crystal) boxes	1 per drawer or Schmitt box	N
Archival, ethanol-safe pinned specimen label paper point paper (Strathmore archival quality bristol board)	1 sheet	N
Pinning block (anodized; height is better than wood)	1	N
Jewelers forceps	3	N
96 well microwell plates	Variable	N
96 well microwell caps	To fit plates	N
Hardware/software (i.e. Clemex Captiva)	1	N
Microscope	2	N
Canon Powershot camera	1	N
Fumigant (paradichlorobenzene - moth crystals)	Variable, ~6.5 g per box	Y
Packing tape	variable	N
Shipping supplies (boxes, padded envelopes)	Variable	N
Cardboard specimen mailers	Variable	N
Self healing cut mat (30 cm x 46 cm)	1	N

Scissors	2	N
Exacto knife	2	N
Metal ruler	1	N
Unit trays for inside Cornell drawers (48 per cabinet)	48 per cabinet	N
Cornell drawer (pine - hardboard bottom)	6 per cabinet	N
Cornell cabinet (6 drawers, unassembled)	2	N
Balsa wood for point preparation surface	4	N
Secondary containment bins for pouring liquids	1	N
Drill press (for pitfall covers)	1	N
Latex gloves	Variable	N
DNA barcode unique numbers	Variable	N
Adhesive shipping labels	Variable	N
Ethanol waste drum	1	N
PG waste drum	1	N
Tupperware for temporary storage of each bout of whirl paks	10	N
Tupperware for photo box of large beetles	1	N
Styrofoam for photo box	1	N
Plain white paper (for pointing specimens under microscope)	1	N
Tissue paper for photo box background	1	N
LED lights (microscope, photos)	3	N
Vellum as light diffusers (photos)	1	N
Elmer's white glue	2	N
Ethanol lamp (sterilizing)	1	N
Lighter	1	N

11.2.2 Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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. Preferably beside point extractors to diminish inhalation of ethanol fumes.

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.

11.2.3 Sample Processing in the Lab

11.2.3.1 Ethanol Processing (first change from field)

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

The first change of ethanol must occur within 24 h of sample collection from the field if unable to sort and transfer into fresh ethanol within 24 h of sample collection.

[Figure 23]

1. Put on gloves.

2. Pour initial ethanol (poured in whirl pak in field) with the specimens through an unused filtration device into individual 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 as little airspace as possible.

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 an airtight plastic 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).

11.2.3.2 Sorting Ground Beetles from Bycatch

This activity can be done in conjunction with the first change of ethanol time permitting. Do not begin sorting unless CERTAIN that the change of ethanol can be completed for ALL samples.

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

1. Sort each sample one at a time for ground beetle specimens.
2. Suspend specimens in ethanol in the whirl pak bag so that they are uniformly floating throughout.

3. Pour them into a large Petri dish marked with a grid.

4. It is important that each sample remains clearly labeled and is not left unattended for any length of time.
5. Sorting is best done under the microscope.

[Figure 27]

6. Beetle samples are sorted to select all adephagan (a suborder of Coleoptera) beetles. Adephaga contains aquatic groups (which are unlikely to fall into pitfall traps) and all of the beetles of interest to NEON.
7. 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]
8. Ground beetles are sorted into temporary holding cups with a single locality label from the three initially put in the pitfall bowl.
9. The other 2 locality labels remain in the sorting dish with the rest of the specimens at all times.
10. Additional locality labels may be required for large samples with many specimens that do not all fit into a single 50 mL centrifuge tube.
11. 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 for a particular sampling bout, a maximum of 20 individuals of that species 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). ENSURE THAT THE LID ON THE 50 mL TUBE IS TIGHTLY SHUT. These data are to be recorded in the 'NEON Ground Beetle Diversity Datasheet.' For example, from 4 pitfall traps at a single Biodiversity plot for one sampling bout: if there were 10 specimens of species X in pitfall trap 1 – they are all pinned; 25 specimens of species X in pitfall trap 2 – 20 are pinned, 5 are placed separately in ethanol; 9 specimens of species X in pitfall trap 3 – they are all pinned; 36 specimens of species X in pitfall trap 4 – 20 are pinned and 16 are placed separately in ethanol.
12. Morphospecies designations are made with unique letters 'MorphA','MorphB','MorphC'….'MorphZZ' if a direct match to a species name in the teaching collection cannot be made. Add a leading label with each unique 'Morph' designation to specimens in the teaching collection. This will help other parataxonomists to follow the logic for each designation made. Additionally, a morphospecies notice sheet, provided by FSU, will include the initials of the person who designated it as which particular morphospecies along with the features that make it unique.

11.2.3.3 Processing Bycatch Samples in Ethanol

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

2. Vertebrate bycatch that does not fit in 50 mL centrifuge tubes should be kept in 16oz wide mouth straight sided plastic or glass jars available from headquarters FSU staff scientists.

   [Figure 31]
   [Figure 32]
3. Each bycatch 50 mL centrifuge tube should have three individually cut locality labels (date filled in).
4. Label the lid of tube with sharpie with the collection code (NEON.FSU.Domain.Plot.Trap) and date.
5. Processed samples in ethanol are optimally stored at -20°C.
6. If there is no access to -20°C, they may be stored at 4°C
7. If there is no access to 4°C they may be stored temporarily (less than one year) at room temperature in the flammables cabinets.

11.3 Lab Procedure - Processing

11.3.1 Equipment and Materials

Item Description	Quantity per sampling event	Hazardous Chemical
Color Separation Guide and Gray Scale	1	N
Ethanol		Y
Plastic petri dishes for sorting specimens under microscope	2	N
Sharpies	5	N
Archival, ethanol-safe pens (Pigma brand, size 01)	5	N
No. 3 pins archival quality	Variable	N
50 mL nonsterile centrifuge tubes	Variable	N
Specimen cups - bottoms	5	N
Specimen cups - lids	5	N
Schmitt boxes	Variable	N
Point punch	1	N
Fumigant (moth crystal) boxes	1 per drawer or Schmitt box	N
Archival, ethanol-safe pinned specimen label paper point paper (Strathmore archival quality	1 sheet	N

bristol board)		
Pinning block (anodized; height is better than wood)	1	N
Jewelers forceps	3	N
96 well microwell plates	Variable	N
96 well microwell caps	To fit plates	N
Hardware/software (i.e. Clemex Captiva)	1	N
Microscope	2	N
Canon Powershot camera	1	N
Fumigant (paradichlorobenzene - moth crystals)	Variable, ~6.5 g per fumigant box	Y
Packing tape	variable	N
Shipping supplies (boxes, padded envelopes)	Variable	N
Cardboard specimen mailers	Variable	N
Self healing cut mat (30 cm x 46 cm)	1	N
Scissors	2	N
Exacto knife	2	N
Metal ruler	1	N
Unit trays for inside Cornell drawers (48 per cabinet)	48 per cabinet	N
Cornell drawer (pine - hardboard bottom)	6 per cabinet	N
Cornell cabinet (6 drawers, unassembled)	2	N
Balsa wood for point preparation surface	4	N
Secondary containment bins for pouring liquids	1	N
Drill press (for pitfall covers)	1	N
Latex gloves	Variable	N
DNA barcode unique numbers	Variable	N
Adhesive shipping labels	Variable	N
Ethanol waste drum	1	N
PG waste drum	1	N
Tupperware for temporary storage of each bout of whirl paks	10	N
Tupperware for photo box of large beetles	1	N
Styrofoam for photo box	1	N
Plain white paper (for pointing specimens under microscope)	1	N
Tissue paper for photo box background	1	N
LED lights (microscope, photos)	3	N
Vellum as light diffusers (photos)	1	N
Elmer's white glue	2	N
Ethanol lamp (sterilizing)	1	N
Lighter	1	N

11.3.2 Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

11.3.2.1 Beetle Archival Dry Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

1. Whole ground beetle specimens stored dry are to be either pinned or labeled.
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 appears that it will be destroyed by directly pinning it. 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]

11.3.2.2 Pointing Small Beetles (<5mm)

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 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. Ensure that the point is at a 90 degree angle from the pin and not angled up nor down. It is easiest to prepare these in batches of 40 or so.

   [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 2nd and 3rd 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 down on the point at the correct angle.
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]

8. Attach the locality label. Ensure that the proper details are filled in appropriately.

Think of the locality label as protection for 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).

9. Attach a unique number to each specimen.

When all of the specimens are mounted and labeled:

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).

11.3.2.3 Direct Pinning Large Beetles (>5mm)

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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. Ensure that the proper details are filled in appropriately on the locality label.
6. Attach a unique number to each specimen.

   [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 Data sheet'. 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.

11.3.2.4 Removing a Sub-Sample for DNA Barcoding

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

After all of the ground beetles have been prepared, mounted and properly labeled for the field season:

1. Prepare the 'NEON Ground Beetle Diversity Datasheets' and 96 well microplates so they are within easy reach.

2. Wipe down the work area with 70% ethanol.

3. Assess the total richness represented, including the 'Morph' designations in the provided spreadsheet. The number will likely be on the order of 25 different types of specimens per site. At 3 specimens to be sequenced for each of 25 different morphospecies this would be 75 beetle species per site. This gives 225 specimens per Domain for a season. The maximum number of beetle specimens to be sent per site is 330 (based on budget) in general. Likely fewer than this will require sequencing in general. For the 2012 Field season the budget is for 285 ground beetle specimens. The following is the rationale for selecting specimens:
    - Beetle specimens are selected first by checking whether there are 5 or more public sequences represented for the particular species of interest in the BOLD database.
        - Point your browser to: http://www.boldsystems.org/views/login.php?&
        - Select 'Taxonomy browser' at the top left of the page
        - Select 'Arthropoda'> 'Insecta' > 'Coleoptera' > 'Carabidae'
        - Look for the genus of interest in the 'Genera' category and select it
        - The number in square brackets beside each species name is the number of sequences
        - Click on the species name and then click on the species progress link
        - Fill in the number of public sequences > 500 bp in the appropriate spreadsheet tab within the 'NEON Ground Beetle Diversity Datasheets'
        - If there are fewer than 10 public sequences > 500 bp for a species add at least 3 more specimens (if possible) from the season's field sampling
        - If there are more than 10 public sequences only one specimen is required from the season's field sampling

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. Flame sterilize the forceps in the ethanol lamp. (If there is difficulty with the wick cut a small piece off. If there is too much air movement in the room for the flame to remain stable, create an aluminum foil covered barrier to prevent the draft from affecting the flame).
2. Excise a leg sample from the first specimen.
3. Place the beetle specimen's leg tissue sample in the well.

[Figure 45]

[Figure 46]

4. Place the forceps into a specimen cup filled with ethanol.
5. Record the unique number in the 'NEON Ground Beetle Diversity Data sheet'.
6. Periodically sterilize gloves by spraying them with 70% ethanol – AWAY FROM THE ETHANOL LAMP. WAIT until gloves are air dry (not by waiving them, simply by waiting) before proceeding.

DO NOT place any foreign objects (e.g. labels, hair) into sampling wells. Before proceeding to the next sample, ensure that no residual tissue is present on the forceps by flame sterilizing them with the ethanol lamp.
If something accidentally falls into a well (e.g. eyelash), note it, do not place a specimen sub-sample into that well and move to the next well. TRY TO AVOID THIS.

Well caps can be applied to wells that are not in use. Note strip caps are numbered and correspond to numbering on plates.

Well plates can be stored at room temperature.

11.3.2.5 Digital Photography

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Follow all directions that arrive with camera and microscope for proper set up. Specifically for these protocols, the scale bars should be matched between camera and microscope.

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 Data sheet'.

Small specimens (<10mm) are measured with the camera. Larger specimens (>10mm) are measured with a ruler. Specimens should be measured from the tip of the elytra to the end of the head (clypeus). This does not include the mandibles which can be in variable positions during death.

11.3.2.6 Photographing Smaller Beetles (<10mm)

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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\DOMAIN 3 2012\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 Data sheet'.

11.3.2.7 Photographing Larger Beetles (>/=10mm)

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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. 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 (so there are no bright spots on the beetle).

[Figure 52]

7. 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.
8. 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.
9. Adjust the manual focus of the camera until the specimen is in focus.
10. If necessary, adjust the specimen height until the entire beetle can be captured without a person touching the camera.
11. 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.

11.3.2.8 Processing Images

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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\DOMAIN 3 2012\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. From Start menu select 'Run'
2. Open command prompt: Open Run & type in **cmd**.
3. 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.
4. Navigate to desired folder.
5. 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\DOMAIN 3 2012\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.
6. 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\DOMAIN 3 2012\Beetle Pictures\edited>**dir /b *.jpg>list.txt**). Note: spaces are required after dir and b in the command.
7. 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. Attempt to 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\DOMAIN 3 2012\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\DOMAIN 3 2012\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\DOMAIN 3 2012\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.

11.3.3 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]

11.3.4 Sample Shipping

11.3.4.1 Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

11.3.4.2 Taxonomists

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

1. Using the appropriate spreadsheet in the 'NEON Ground Beetle Diversity Datasheets' designate no more than 400 specimens per domain to send to Taxonomists. Ideally at least one specimen from each species or morphospecies is sent to Taxonomists. Further, it is ideal to send THE SAME or as similar as possible specimens to the Taxonomist as have been sent for DNA barcoding.
2. Prepared dry (pinned/pointed) beetle specimens are shipped overnight to Taxonomists with a hard and digital copy of the 'NEON Ground Beetle Diversity Datasheets'.
3. Any large beetles (typically 1.5 cm or larger, but as required for smaller than this suggested size but heavy bodied specimens) should be brace-pinned on either side of the label to prevent them from moving during transit.
4. Once the mailers are prepared for shipping take a photo of each one (with specimens visible) for tracking purposes. Upload these photos to the same location as the 'NEON Ground Beetle Diversity Datasheets'.
5. Cut a cardboard insert to size to place on top of specimens. Make a packing tape tab to easily pull on the cardboard insert (otherwise it can become wedged in the box and difficult to remove).

6. Do NOT include moth crystals in shipments. Moth crystals are a hazardous material that must NOT be shipped.

[Figure 53]

[Figure 54]

7. Ensure that all of the specimens are firmly glued to their points by tapping the box to test.
8. A specimen dissociated from its locality label is worthless.
9. 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]

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

11.3.4.3 Genetic Analytical Laboratories

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 Data sheet'.

[Figure 57]

11.3.4.4 Archives

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 Data sheet'.
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 Data sheet'.
5. Only 3 specimens of each species should remain in the teaching collection at a Domain facility. All other specimens should be shipped to the Archive.

11.3.5 Data Handling

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

At the end of each field and lab day, all information from field and lab data sheets must be entered into the digital data sheets daily. All data from the image processing must also be backed up and uploaded to the NEON server daily.
ENSURE THAT ALL INFORMATION FROM THE HARD COPY SHEETS IS ENTERED INTO THE DIGITAL SYSTEM. THIS INCLUDES ALL NOTES ABOUT ANY DEVIATIONS FROM THE PROTOCOL. WHAT WAS DONE AND WHY. (e.g. not enough PG to fill trap so no sampling was conducted).

11.3.6 Refreshing the Laboratory Supplies

11.3.7 Laboratory Maintenance, Cleaning, Storage

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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. Cornell drawers should be filled with empty unit trays so that trays that do contain specimens are unable to slide around.
6. The teaching collection should NEVER be left out unattended and should be kept inside an airtight Cornell cabinet with a fumigant box of moth crystals anchored in the corner. Moth crystals should be checked and topped up as needed.

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

12 DEFINITIONS

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

13 REFERENCES

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX A Field & Lab Data Sheets

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 Datasheets' which includes several tabs which includes several sheets where 'P' indicates a version for print and 'D' indicates a digital version.

Sheet	Purpose
FieldSheet_P	Printable sheet for data entry in field. The 0/few/many estimates help to ensure that samples are sorted appropriately in the lab
FieldSheet_D	Digital sheet for entry of the field data
PitfallTrapSorting_D	Digital sheet for entry of specimen numbers required for analyses
MorphospeciesLab_P&D	Print and digital sheet for ascribing particular specimens or groups of specimens to a group not currently recognizable from the teaching collection or other materials
BiodiversityDataEntry_P	Printable sheet for entry of the biodiversity information required for upload to BOLD and analyses
BiodiversityDataEntry_D	Digital sheet for entry of the biodiversity information required for upload to BOLD and analyses
SpecimenSelection	Digital sheet for the selection of specimens to DNA barcode and to send to Taxonomist
ListInfo	Locked sheet with drop down list choices for ease of filling out sheets; includes coordinates and elevation for the plots and autofills them in other sheets
TaxInfo-Reference	Locked sheet with taxonomic information as an aid for spelling species names and for filling in higher level taxonomic information in the Biodiversity Sheet

The columns in each tab are TBR but currently look as follows:

FieldSheet_P – See sheet, similar fields as digital sheet different layout

FieldSheet_D

Week

Date

Field sheet filled out by:

Site

Plot ID #

Landcover

GPS lat

GPS long

Arrival at plot

Departure from plot

Specimens (0/Few/Many)

Notes

Additional notes

Data entered by

Date of data entry

Time elapsed before sorting (ex.10 h)

PitfallTrapSorting_D

Plot

Trap

Week

Date of Collection (Month/Day)

Sorter's Initials

morpho-species

Adephagans pinned/pointed

bycatch tubes

If > 20 (ethanol) species: _____

If > 20 (ethanol) species: _____

If > 20 (ethanol) species: _____

Notes

MorphospeciesLab_P&D

Morphospecies name

Unique features that differentiate this group vs. other

in teaching collection?

staff name who designated it

date designated

BiodiversityDataEntry_P – See sheet, similar fields as digital sheet different layout

BiodiversityDataEntry_D

Unique #

Collector

Collection Date

Collection Method

Plot #

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

Date ready to ship to taxonomist

Date received back from taxonomist

Date ready to ship to barcoding institution

SpecimenSelection

Species List

# of seq >500 bp in BOLD

at Jones

from Jones to sequence

from Jones to Taxonomist

at Disney

from Disney to sequence

from Disney to Taxonomist

at Ordway

from Ordway to sequence

from Ordway to Taxonomist

TOTAL to sequence. Must not be over 285 for all sites in 2012.

TOTAL to send to Taxonomist. Must not be over 400 for all sites in 2012.

ListInfo

Site

Region

Landcover

Plot ID

Latitude

Longitude

Elevation (m)

specimens

Field Techs Names

Field Techs Initials

Basis of ID

TaxInfo-Reference

Order

Genus

Species

Species author and year

Subfamily

Tribe

Notes

Reference

Date

APPENDIX B Considerations for implementation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX C Procedure Checklist

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX D Tables

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX E Figures

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.
Central Plains EXPER RNG.
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NEON.FSU.D10.

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

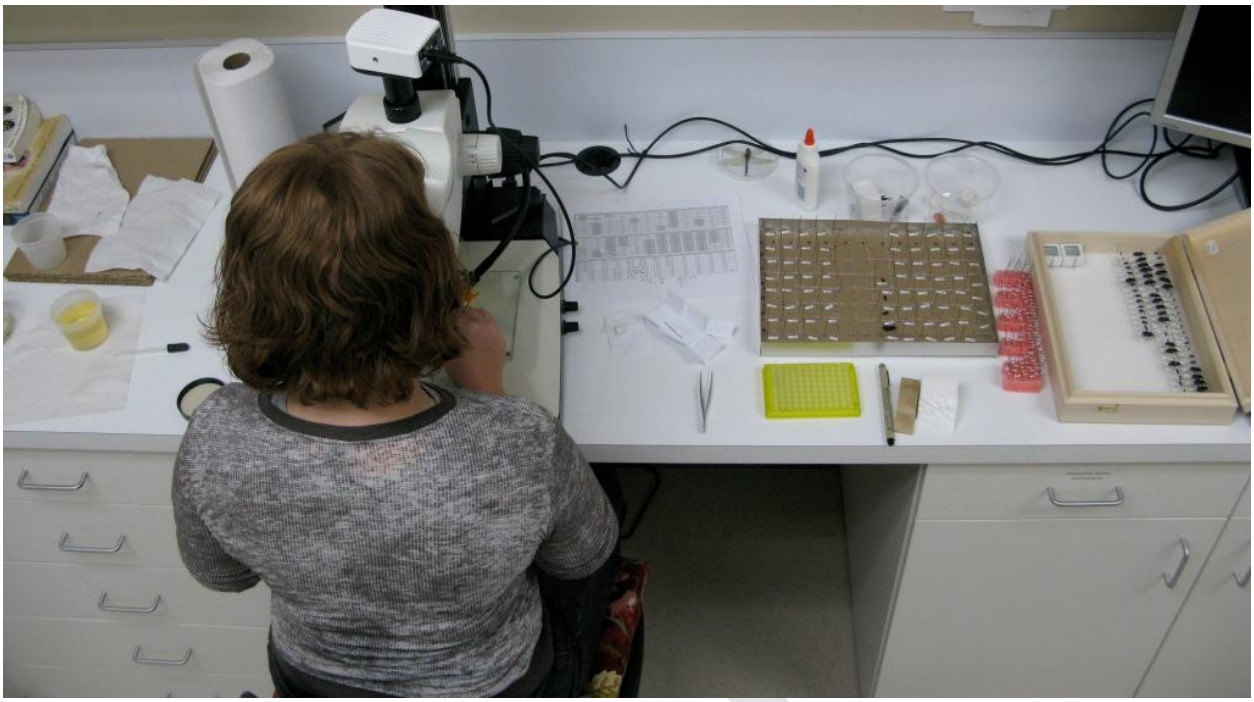


Figure 21

Figure 22

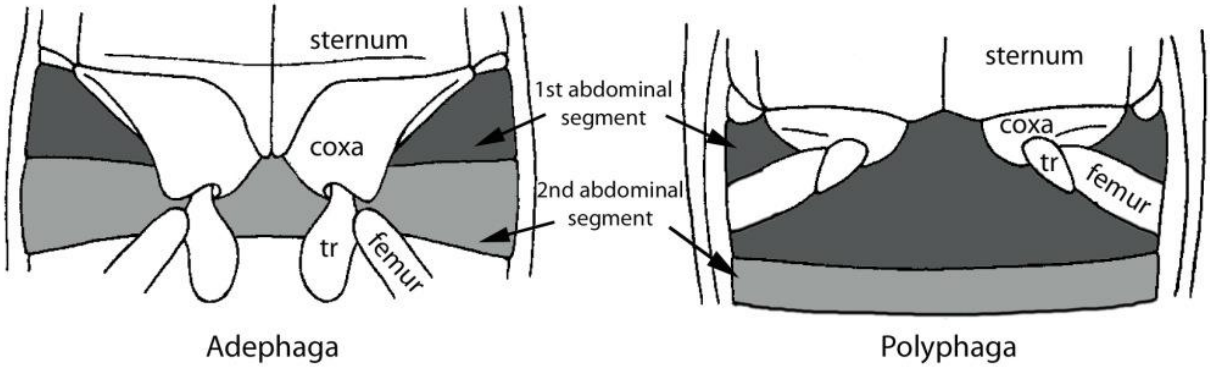
Figure 23

Figure 24

Figure 25

Figure 26

Figure 27



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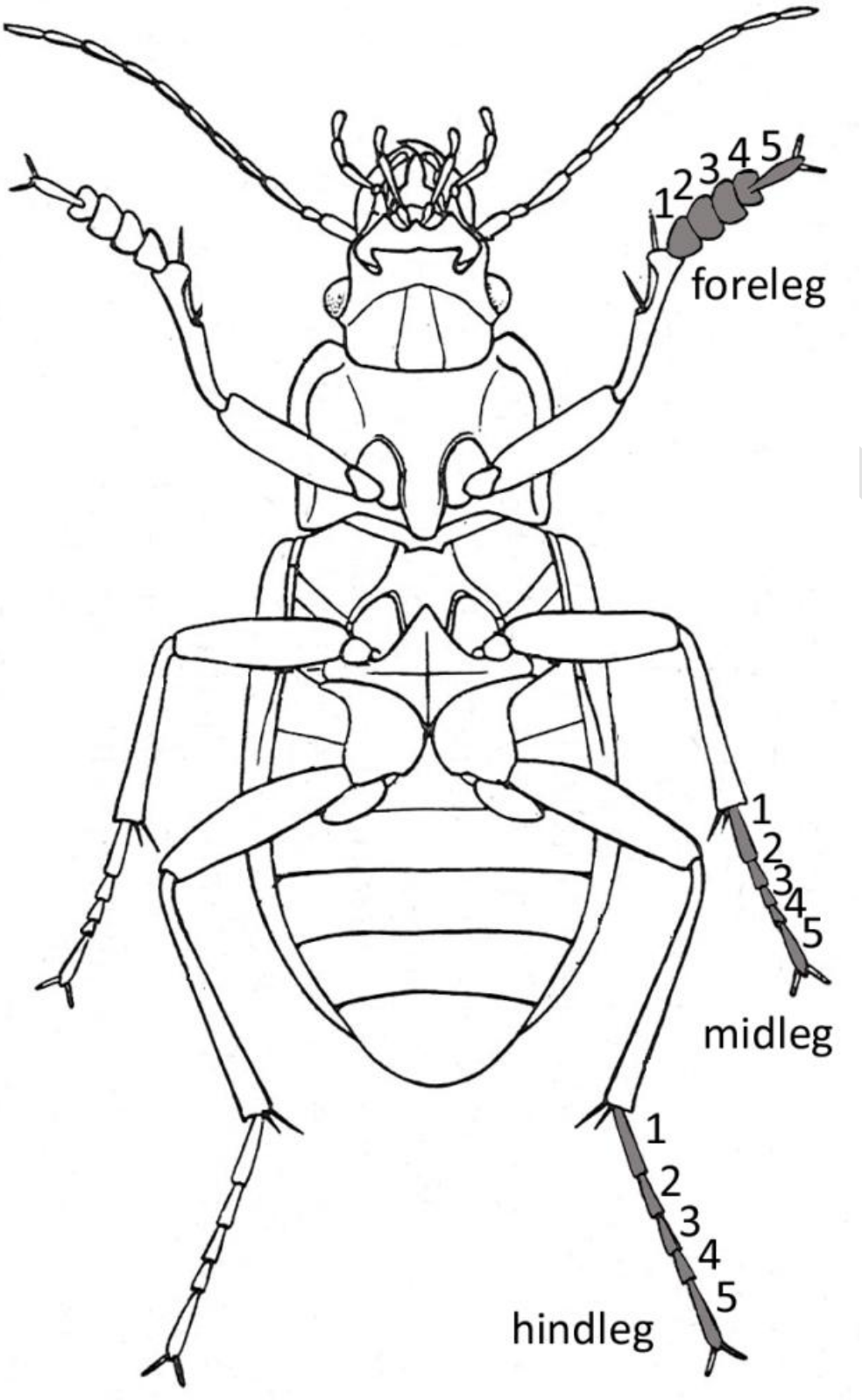

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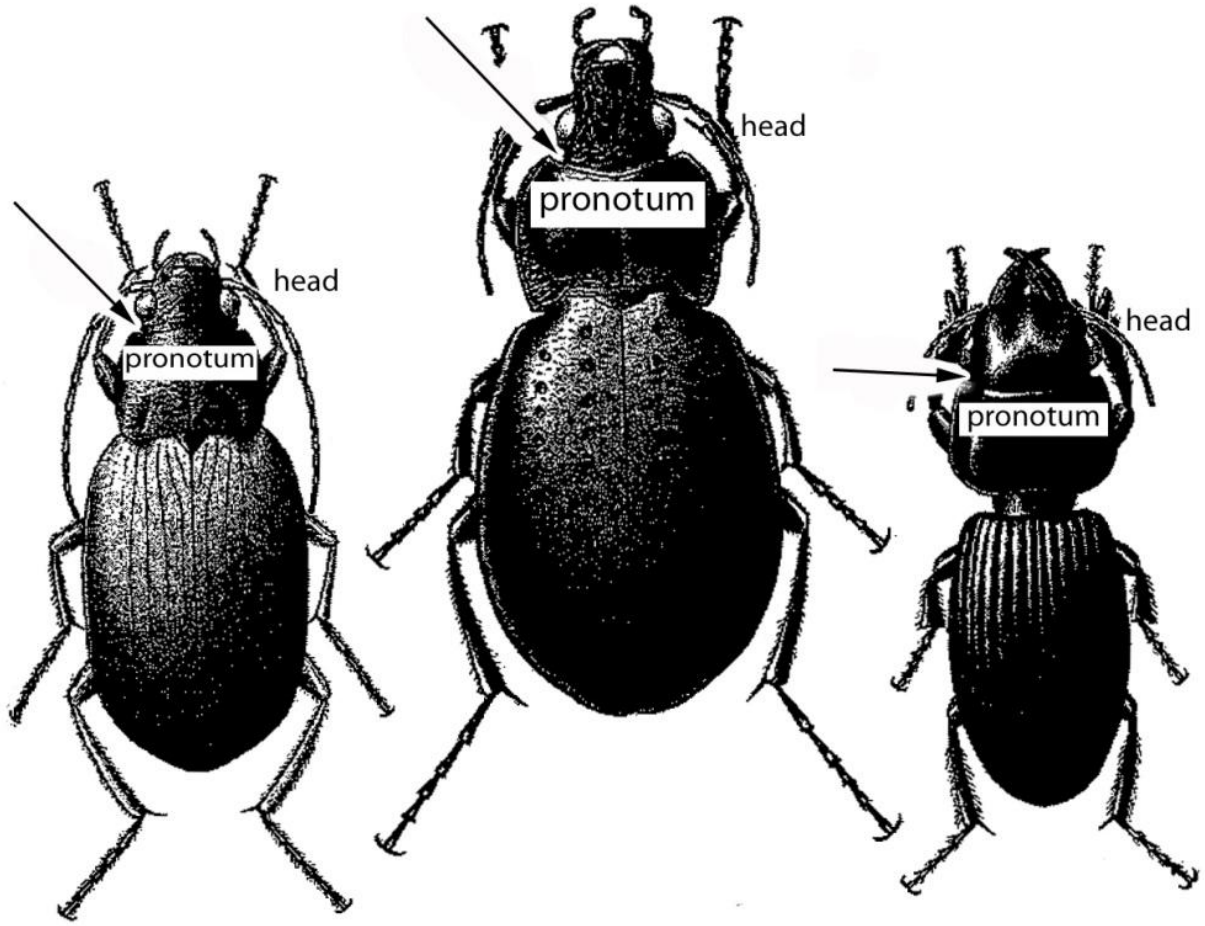
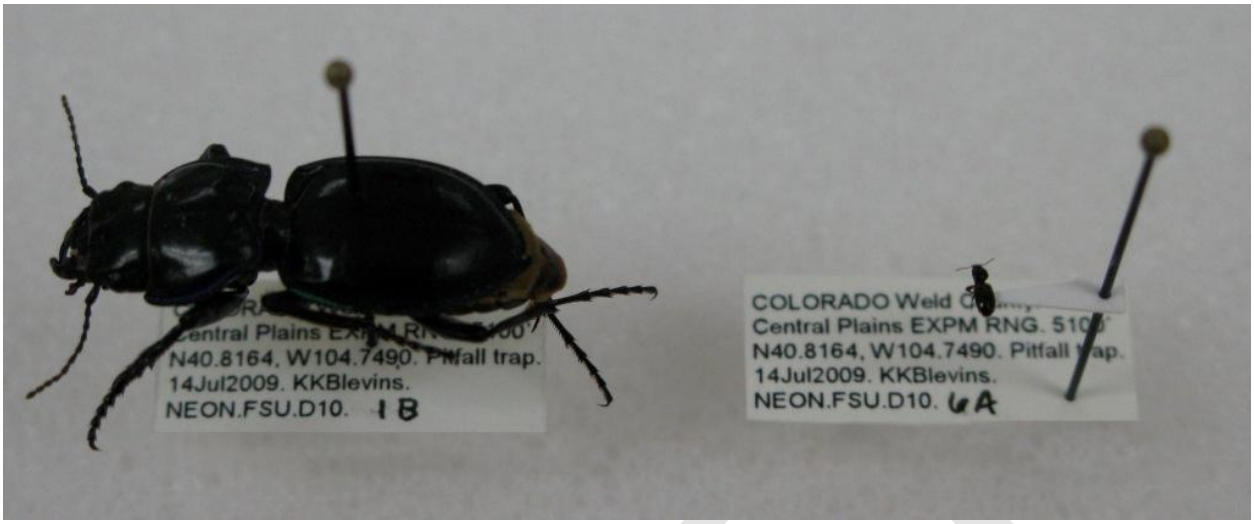


Figure 30

Figure 31

Figure 32



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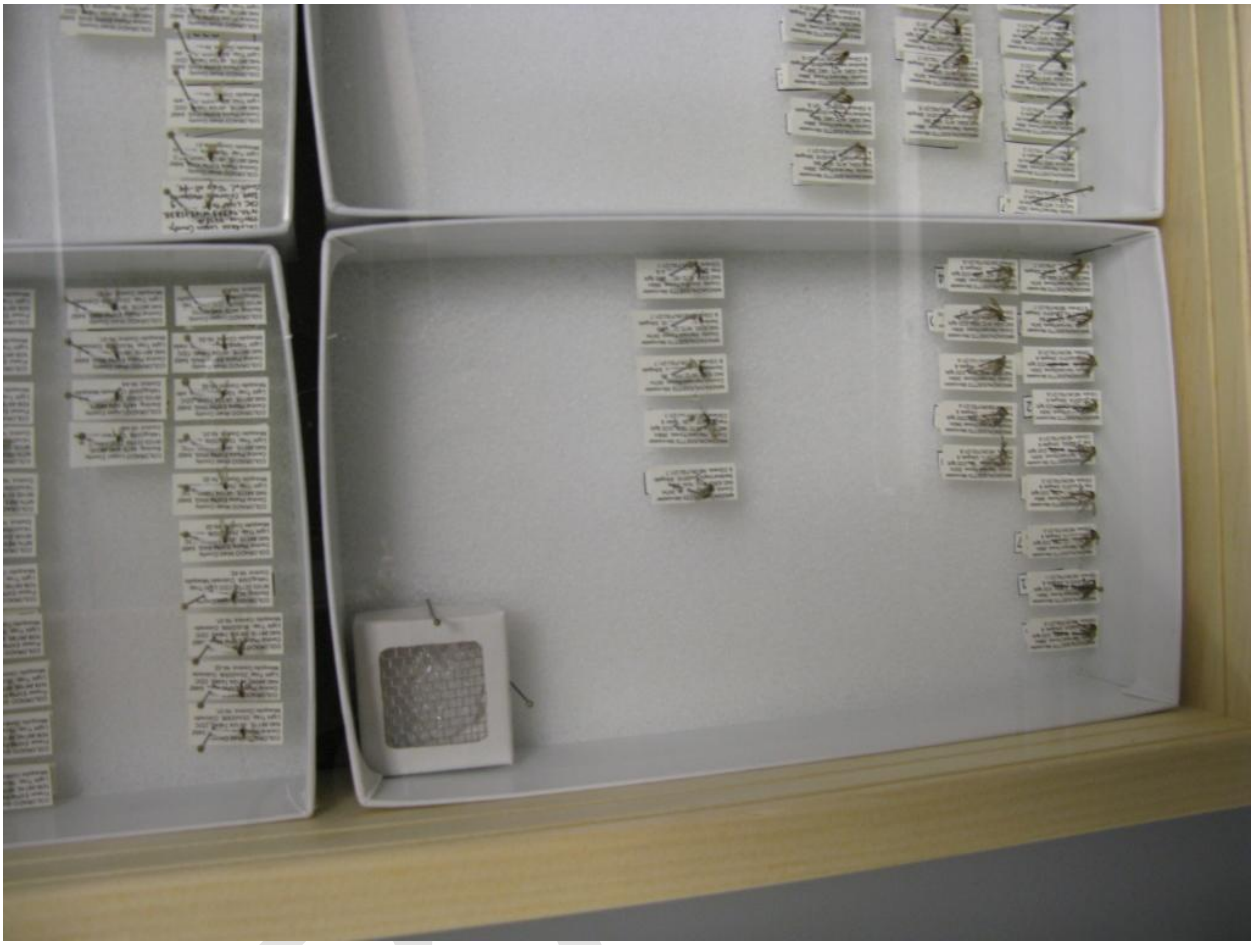

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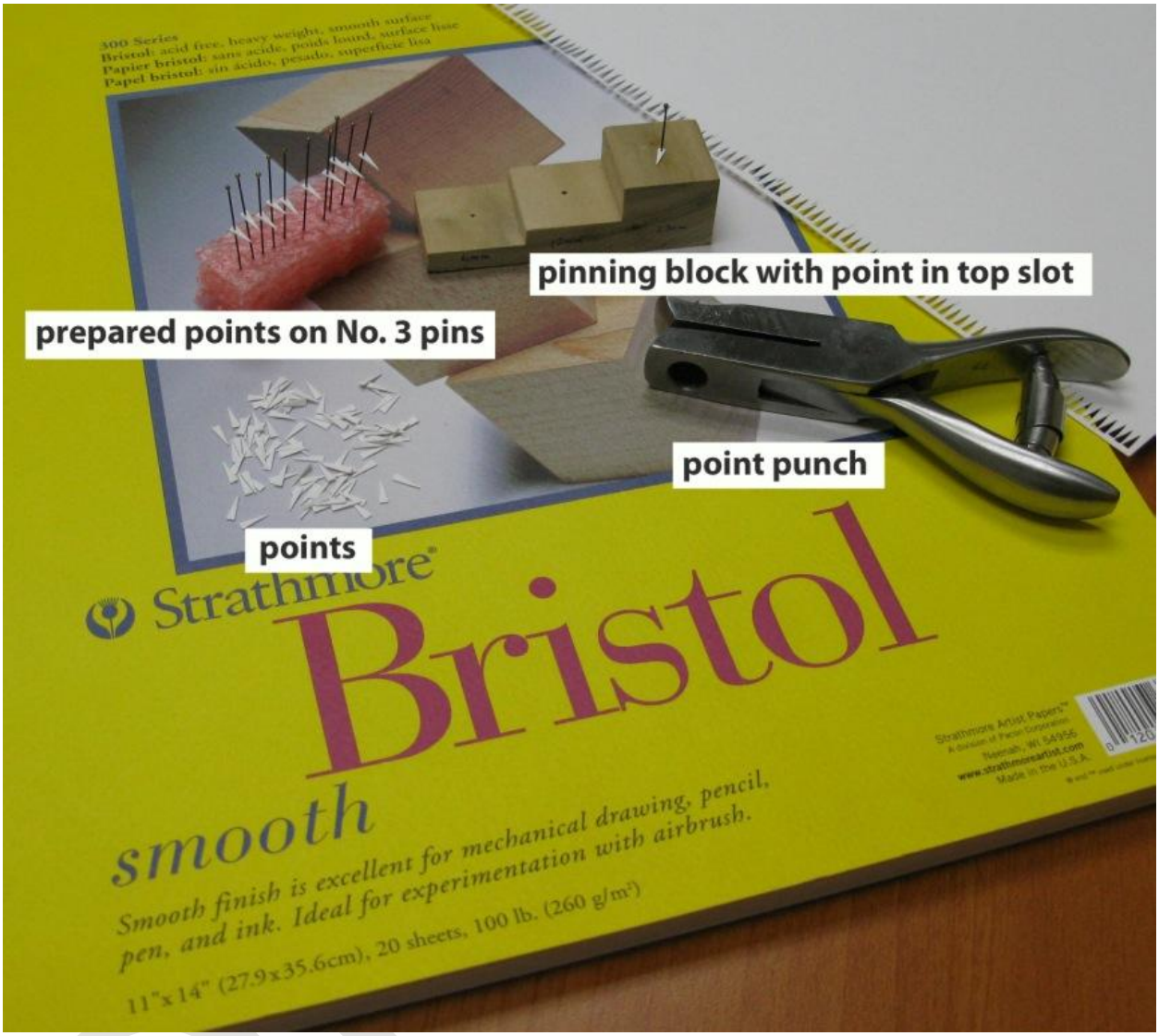
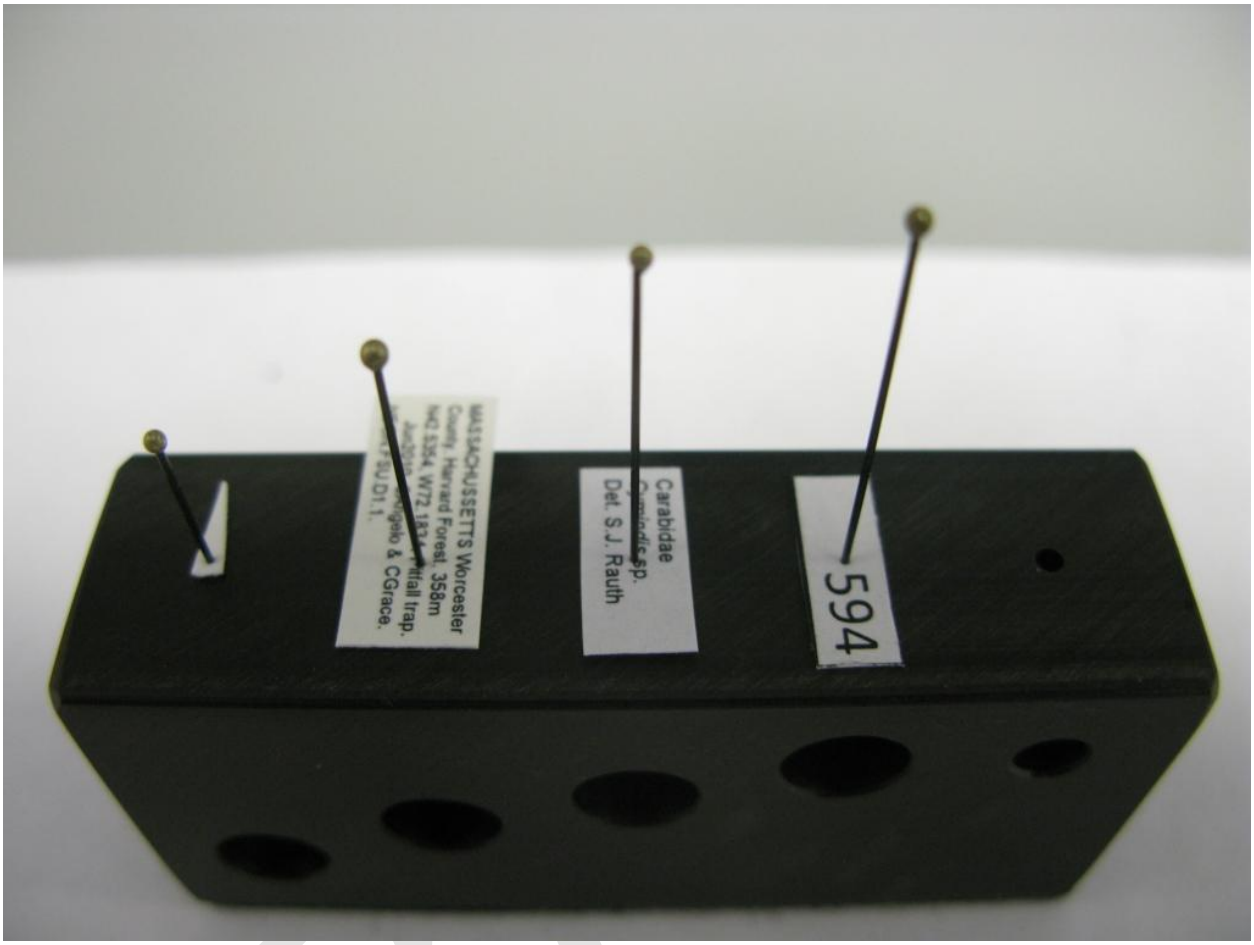

pinning block with point in top slot
prepared points on No. 3 pins
point punch
points
Strathmore
Bristol
smooth
Smooth finish is excellent for mechanical drawing, pencil, pen, and ink. Ideal for experimentation with airbrush.
11"x14" (27.9x35.6cm), 20 sheets, 100 lb. (260 g/m²)

Figure 35

Figure 36



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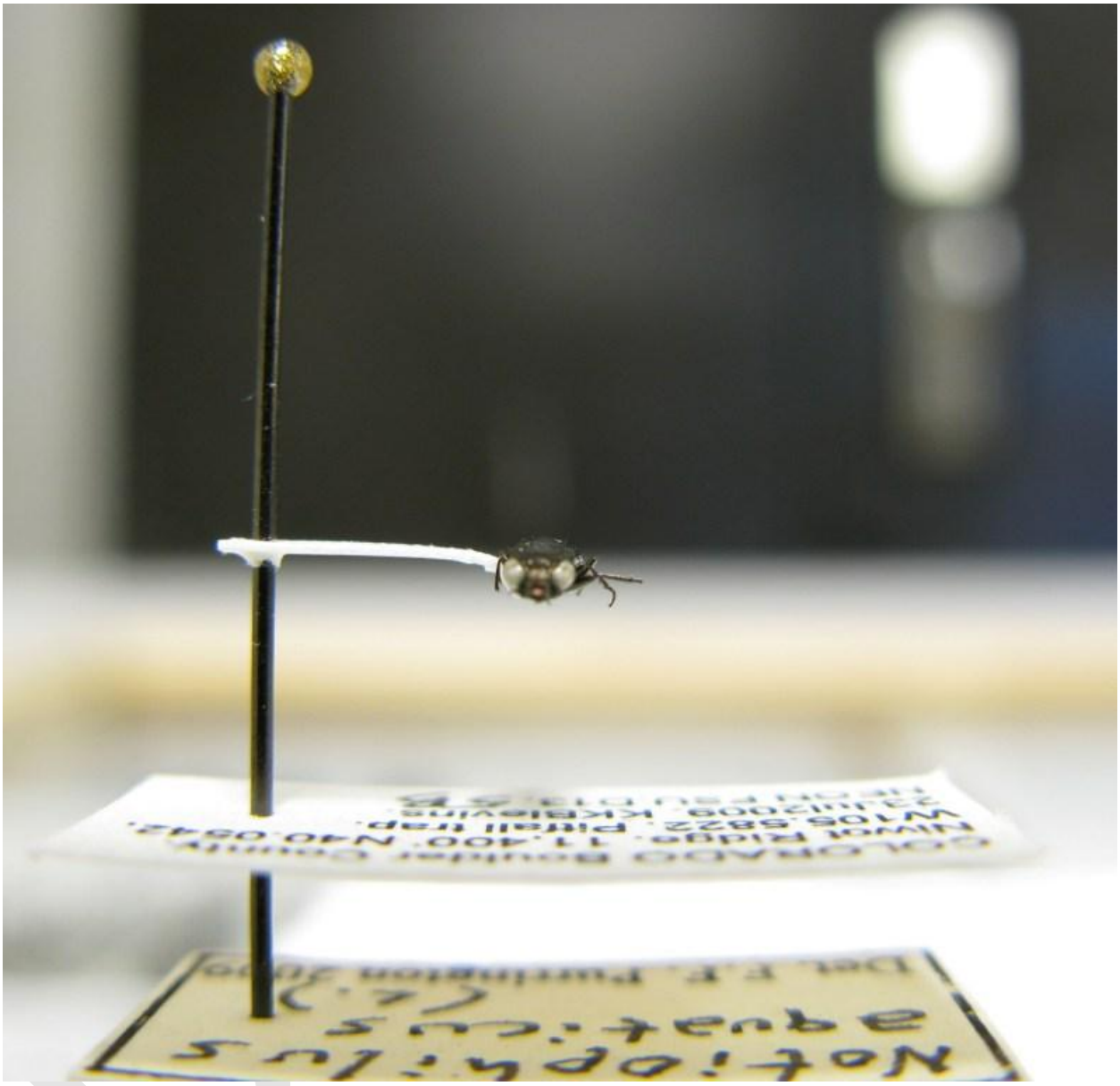

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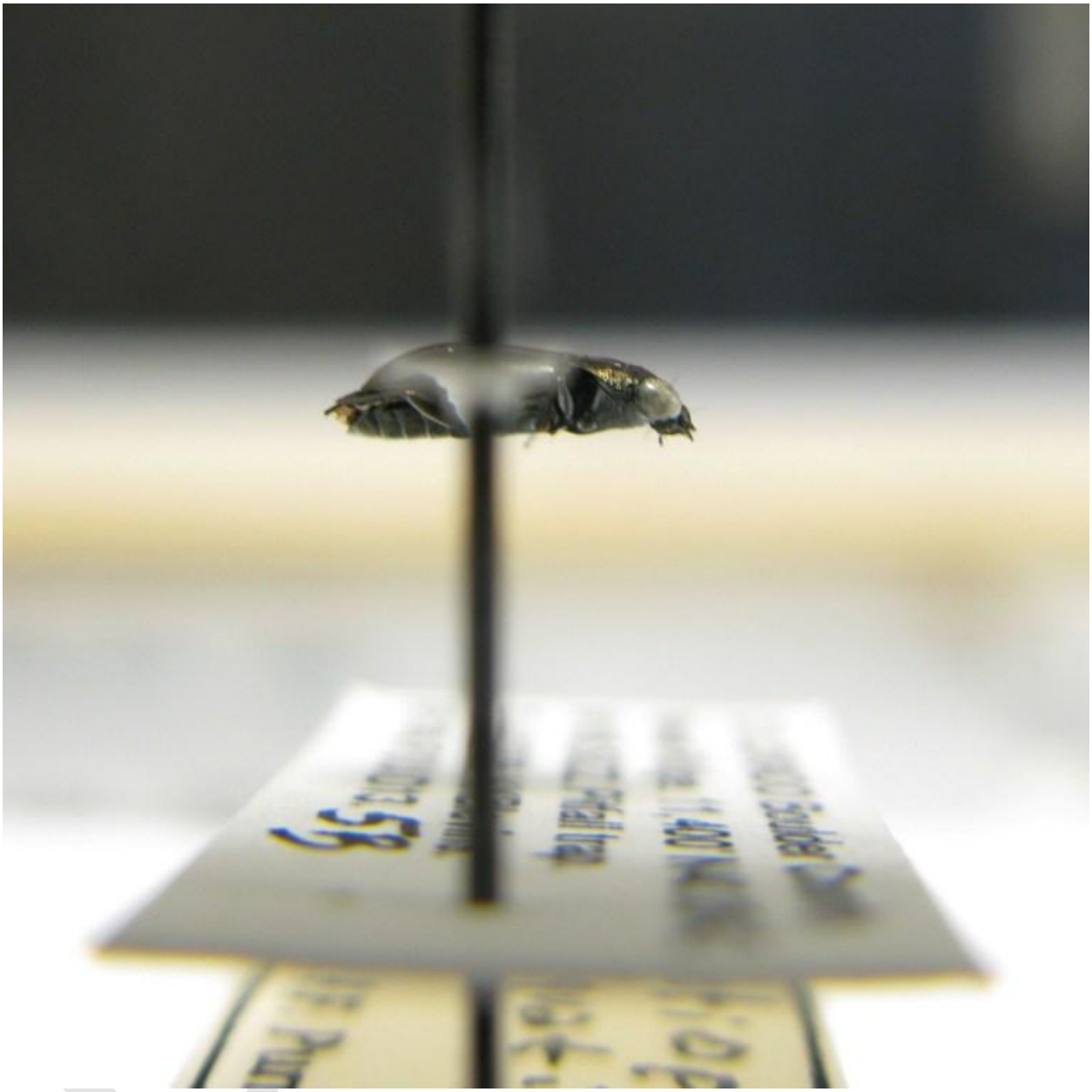

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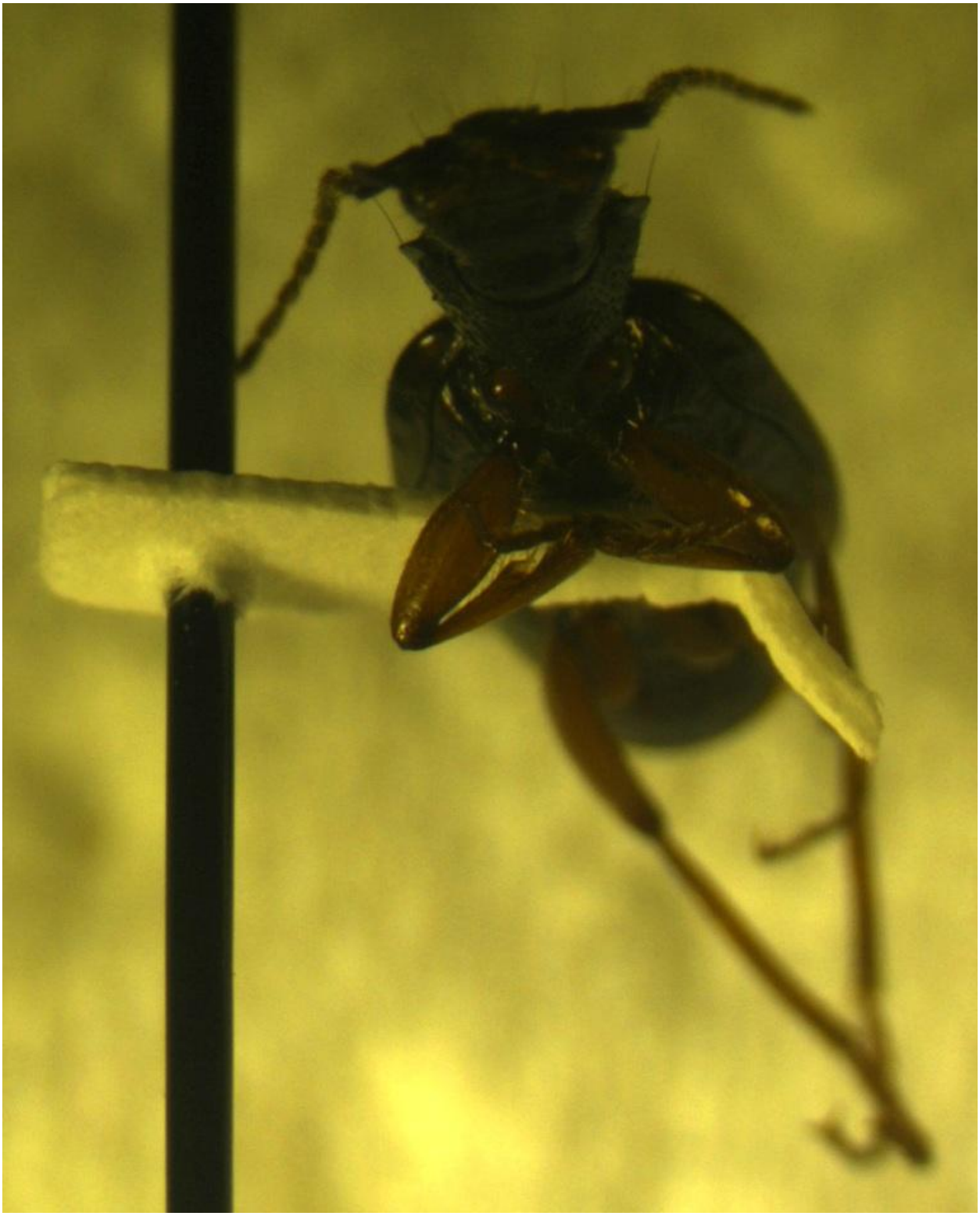

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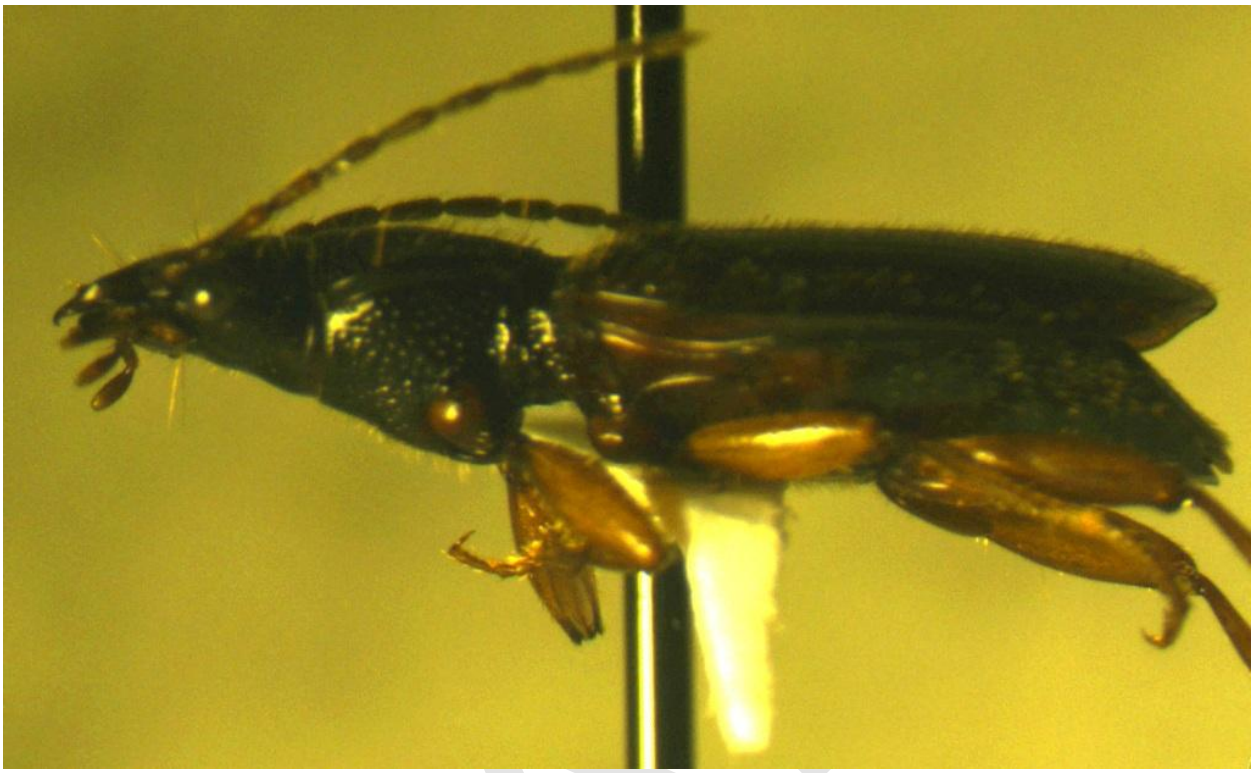

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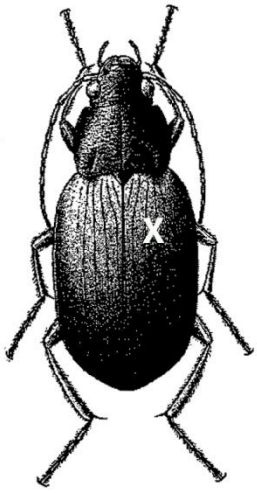


Figure 42

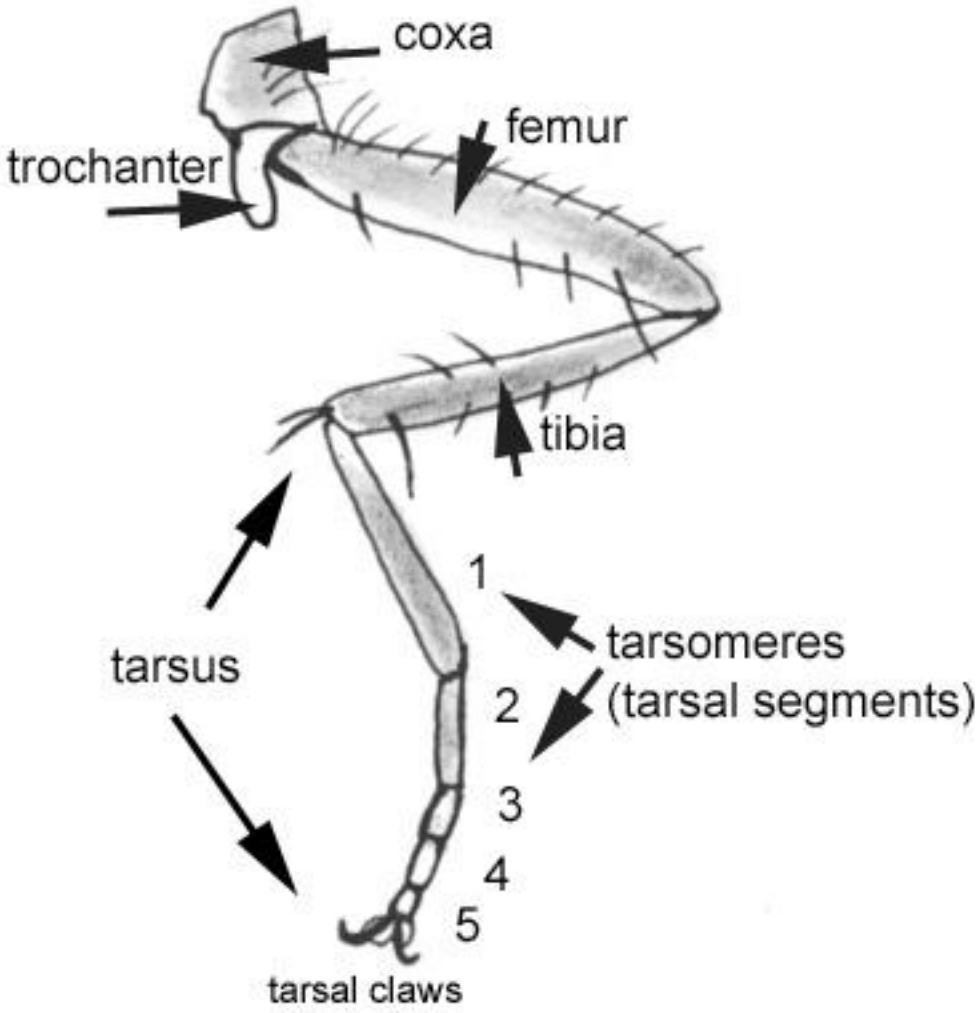
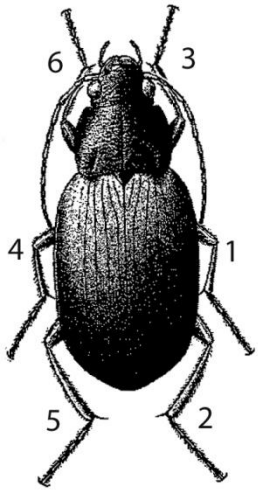

coxa
femur
trochanter
tibia
1
tarsomeres
2
(tarsal segments)
tarsus
3
4
5
tarsal claws

Figure 43



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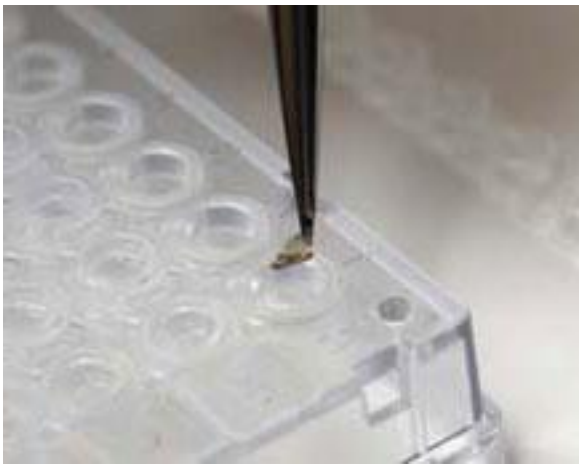

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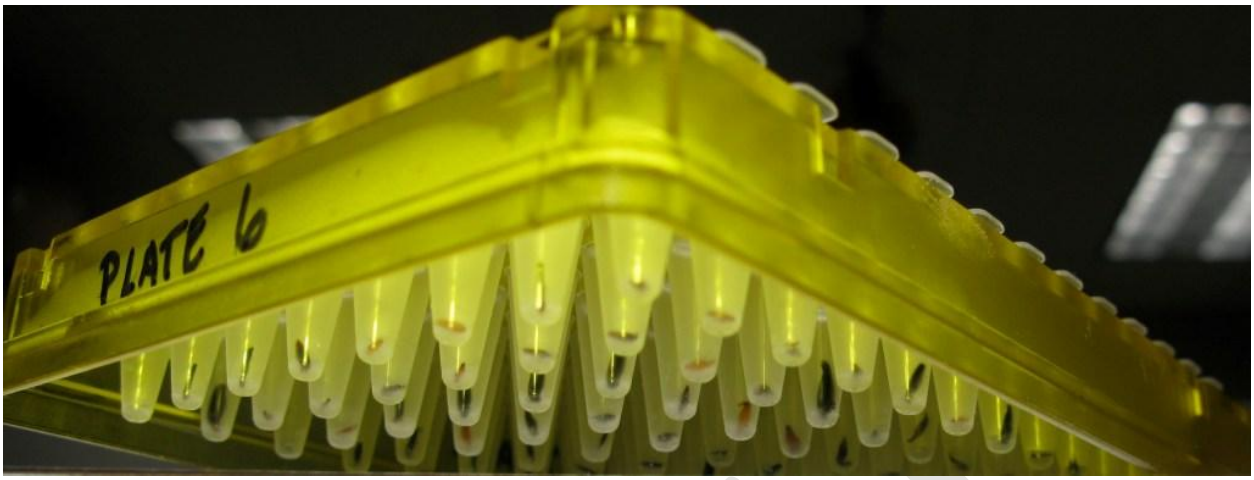

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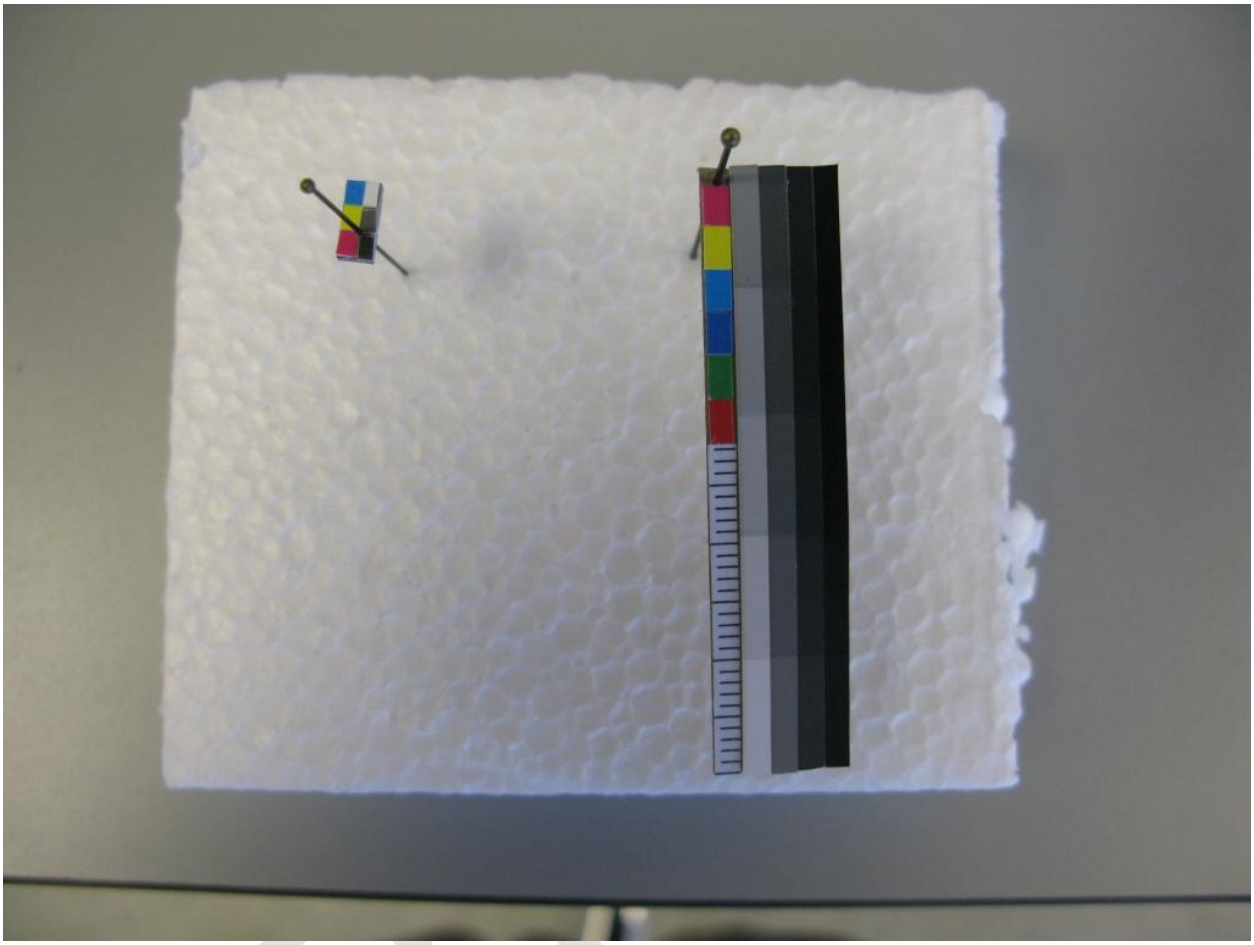
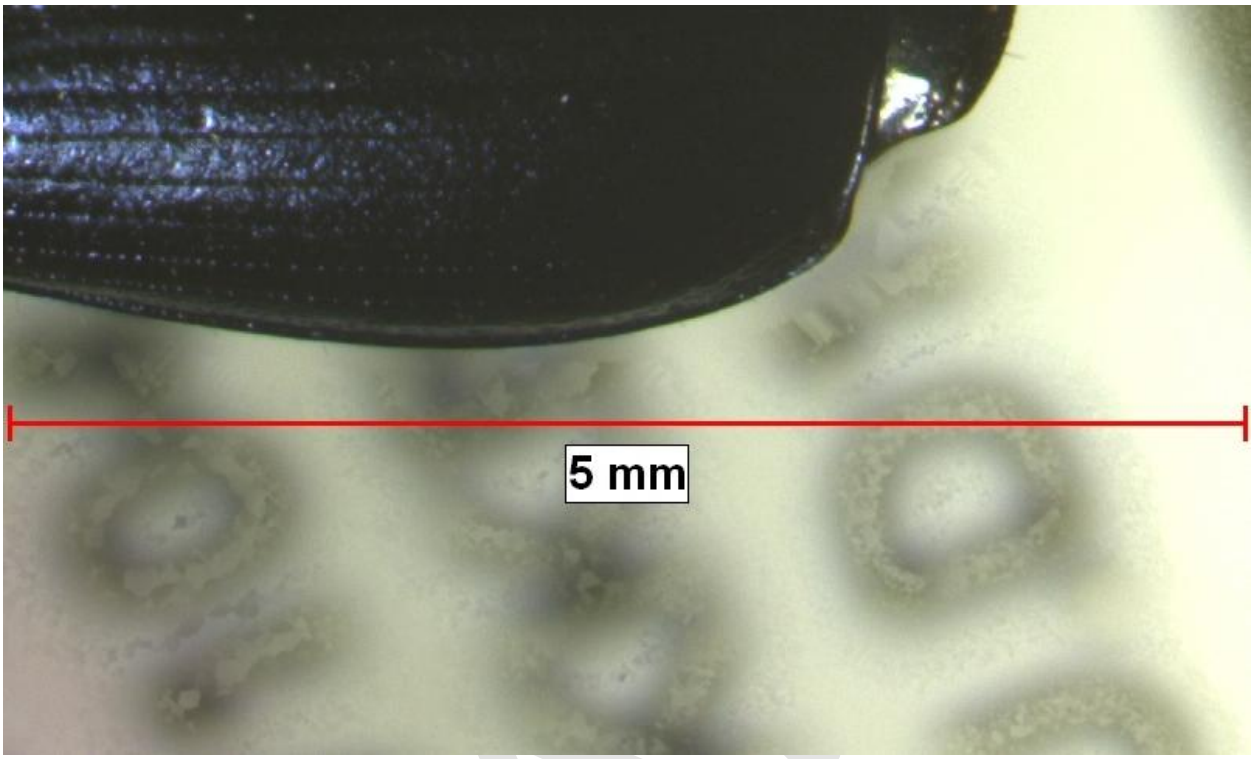


Figure 47

Figure 48



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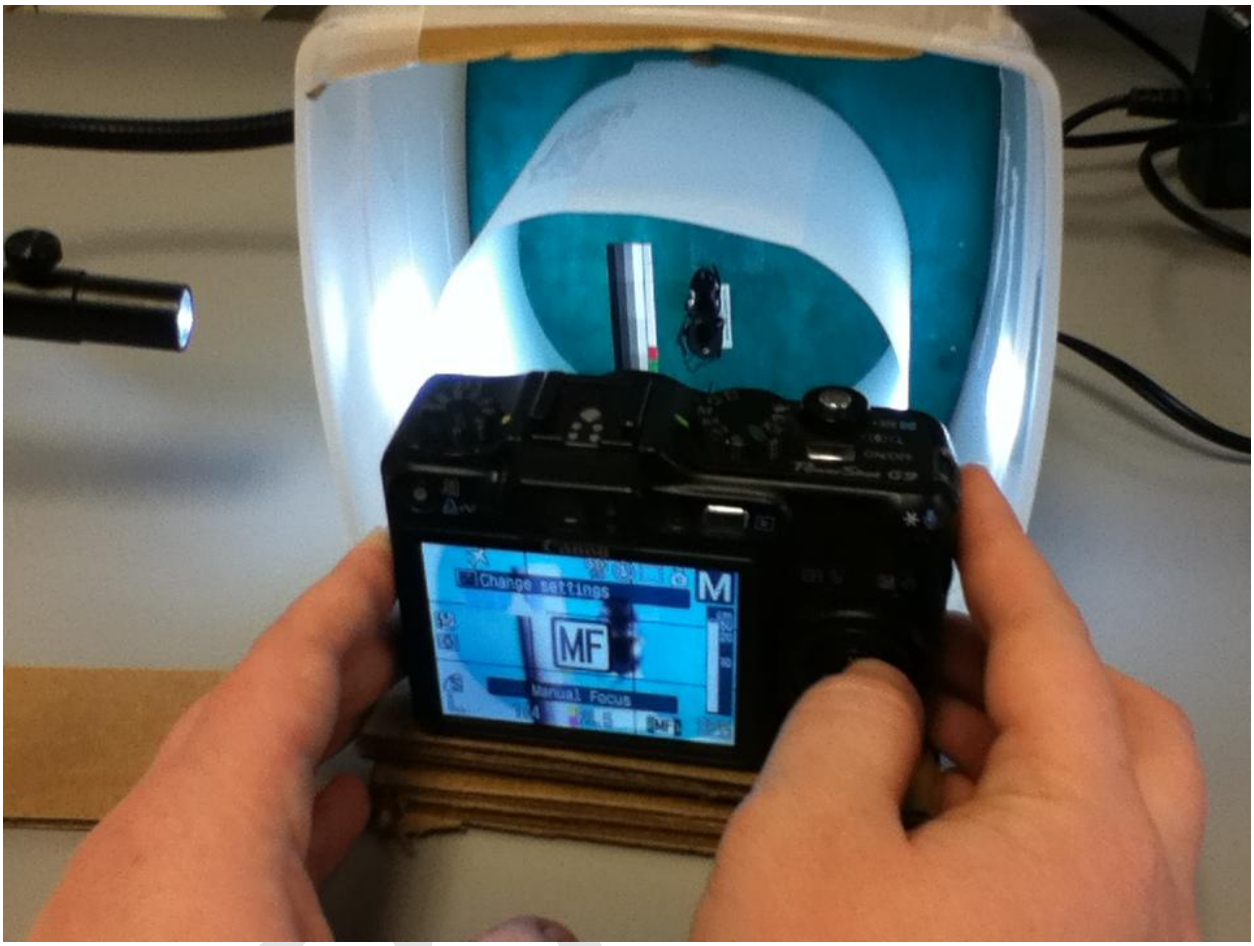

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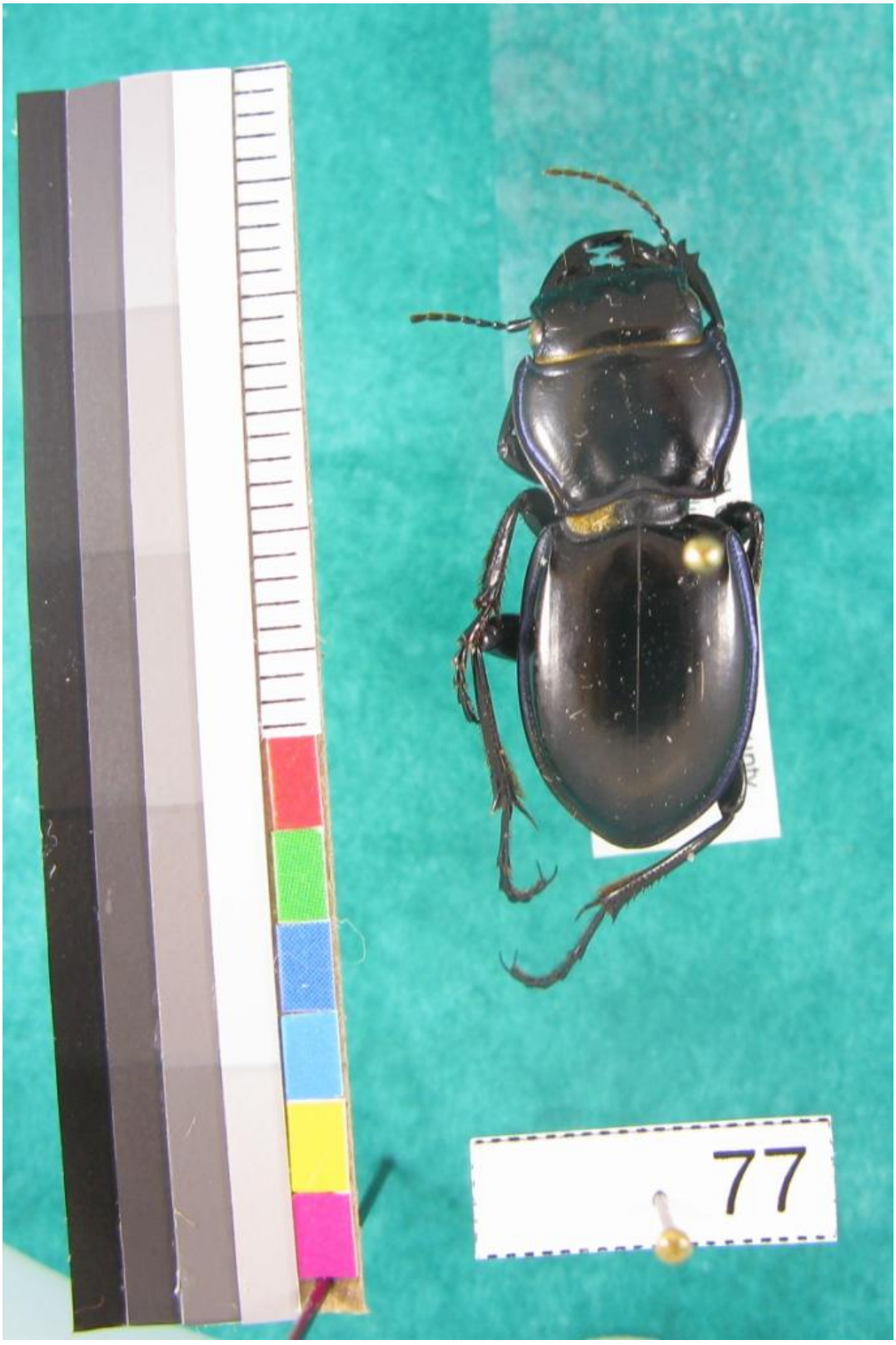

Figure 51

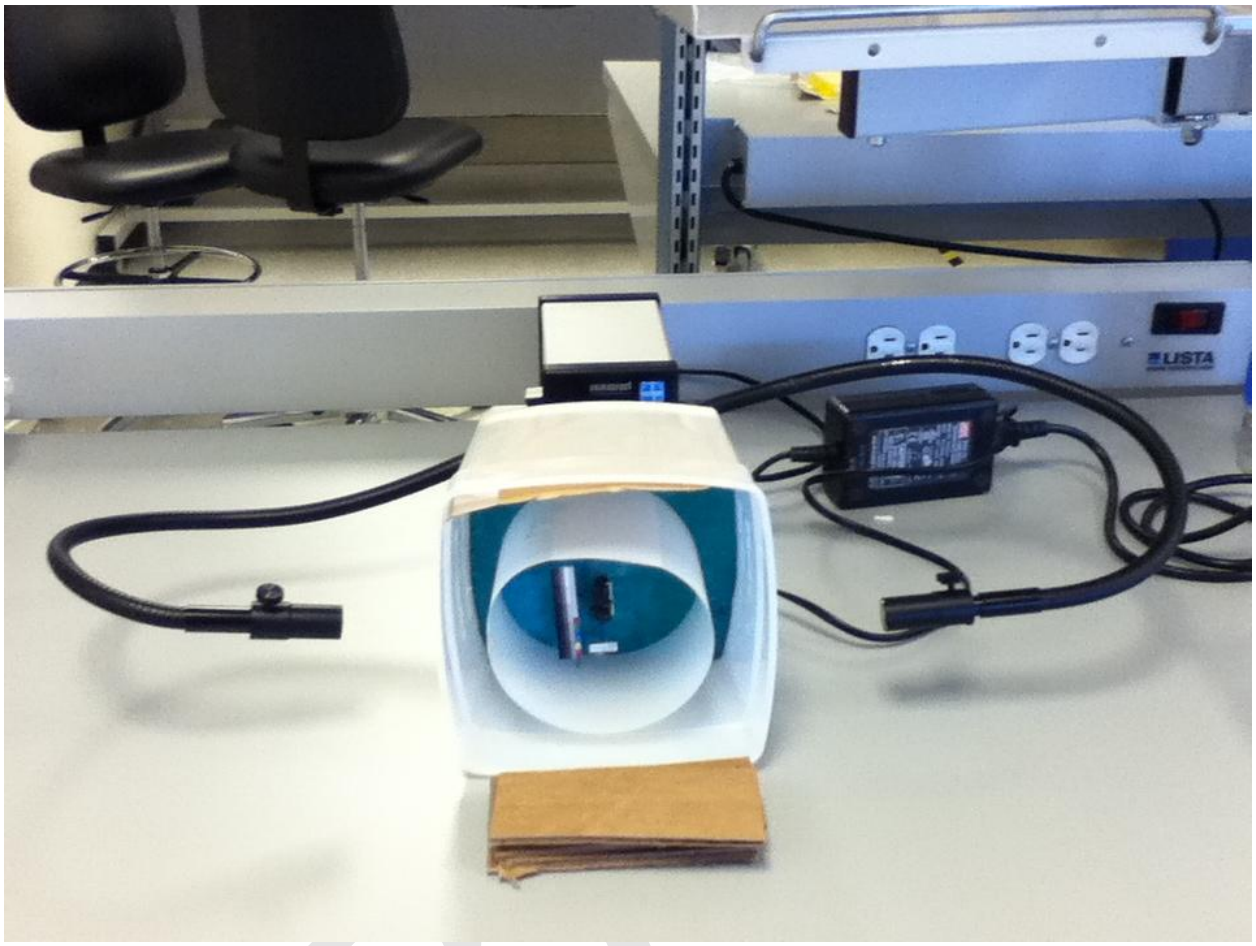

Figure 52

Figure 53

Figure 54

Figure 55

Figure 56

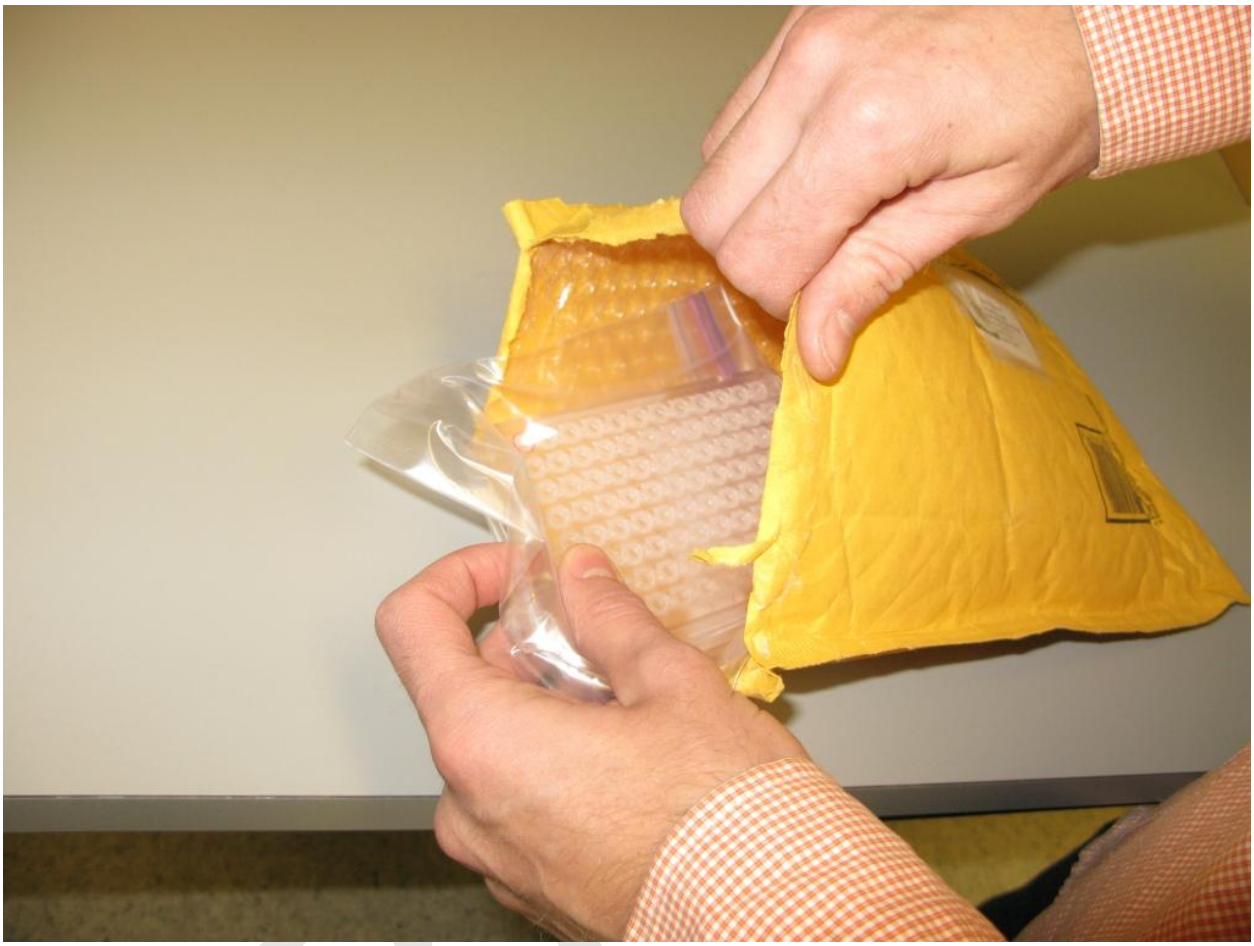

Figure 57