# Essig Museum of Entomology

University of California, Berkeley

Version 6

Standard Operating Procedures and Protocols for Ground Beetle Morphological Identification and Museum Services

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## **Table of Contents**

1.0 Traceability of Analysis	1
1.1 Keys and References	1
1.2 Relevant Experience	1
1.3 Training Policy	1-2
2.0 Procedures	2
2.1 Receipt, tracking and storage	2
2.2 DNA sampling	22
3.0 QAQC Protocols	3

# 1.0 Traceability of Analysis

## 1.1 taxonomic keys/references used for identification

The Kipling W. Will (KWW) lab library and/or in the holdings of the University of California, we have access to all of the references listed in the most current NEON identification references list. The three primary references to be used are by Lindroth (1969. The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, parts 1-6. Opuscula E. Entomoligiska Sallskapet.), Ball and Bousquet (in Arnett Jr., R. H., and M. C. Thomas. 2001. American Beetles Archostemata, Myxophaga, Adephaga, Polyphaga: Staphyliniformia. 1st Vol. (C. Press, Ed.). 1st Ed. Boca Raton, FL.), and Bousquet (2010. Illustrated Identification Guide to Adults and Larvae of Northeastern North American Ground Beetles).

We also have the Essig Museum's carabid specimen collection for comparison and full access to the California Academy of Sciences collections and library.

# 1.2 experts working on analysis and summary of years of experience on relevant work

All identifications will be finalized by KWW, Associate Professor in the Department of Environmental Science, Policy, and Management, who has >25 years of experience in carabid beetle research, worldwide.

Initial sorting may be done by Roberta L. Brett (RLB), who has been working with insect collections and frequently with carabid beetles, since 1987.

#### 1.3 training policy if non-experts (technicians/graduate students) are working on analysis

Students may be used for unpacking and repacking specimens. They will be taught safe specimen handling procedures and basic museum methods either during coursework prior to hiring or under direct supervision of the Essig Museum collection manager, KWW or RLB.

Particularly skilled students might provide preliminary identifications, but all final identifications will be checked by KWW. Students are typically taught to assist in determining the sex of identified specimens.

#### 2.0 Procedure

# 2.1 Receiving, sample tracking, storage procedure

Details for sample processing procedures are detailed in this section and illustrated in Figure 1.

- 1. On arrival, shipments are photographed and any apparent damage noted. The NEON team will be notified of any damage that may have resulted from improper shipping methods and what measures can be taken to reduce damage in the future.
- 2. Counts and manifest checks of specimens received are done at this point and receipt documentation is uploaded to the NEON Portal. Any discrepancies will be noted on the receipt documentation and the NEON team will be notified via the shipping receipt notification email chain.
- 3. All specimens will be placed in a -80 C freezer for a minimum of three days.
- 4. After freezer treatments, specimens will be moved from specimen boxes into foam-bottomed unit trays and then into standard insect drawers in the collection.
- 5. Specimens are assembled by eye into tribes, and where possible to genus or species and placed in unit trays and into Essig Museum of Entomology Collection EMEC drawers for processing.
- 6. A tracking tag is added to each unit tray to record progress through the specimen identification workflow.
- 7. Specimens are identified by KWW using a Leica MZ12S or similar microscope.
- 8. Selected specimens are dissected as needed for identification. Genitalia are placed in a capsule on the pin under the specimen.
- 9. Sex is determined by inspection using a Leica MZ6 or similar microscope.
- 10. Identification labels are formatted, printed, and applied to all specimens.
- 11. All final identification, reference, and sex data are entered into the standard NEON CSV files and uploaded to the NEON Portal
- 12. All data and specimen counts/numbers are double-checked when specimens are returned to the NEON Biorepository and/or domains as appropriate. The NEON team will be notified of any specimens retained by the Essig Museum for reference. Occurrence data for retained specimens will be requested from the NEON biorepository. All NEON CSV files of specimens retained for reference will be uploaded to the Essig Museum online database.
- 13. Shipping documentation will be uploaded to the NEON Portal listing specimens to be archived to the NEON biorepository.
- 14. Specimens to be archived at the NEON biorepository will be repacked and returned in NEON shipping materials and packed using standard Museum protocols to ensure safe shipment.

## 2.2 Sample submission for DNA barcoding procedures

- 1. Specimens selected by NEON for DNA barcoding are transferred to museum drawers with foam bottoms marked with a grid that corresponds to row and well number on 96-well microplate.
- 2. Right mesothoracic leg (or best alternative as necessary) of each specimen is extracted and placed in the appropriate well which has been pre-filled with 30 microliters of 95-100% ethanol using the protocols set forth by the Canadian Centre for DNA Barcoding (CCDB).
- 3. Green paper printed with "Tissue sampled for DNA barcoding" and the current project year is attached to each specimen selected for barcoding..
- 4. Well location coordinates, eg. A01, of samples in microplates are entered into MS Excel file CCDB-00000\_Record.xls sample data input sheet template received from CCDB along with the corresponding NEON sample ID following CCDB protocol.
- 5. Microplates and accompanying documentation are packaged and mailed via FedEx next day to the Canadian Centre for DNA Barcoding following CCDB protocol.

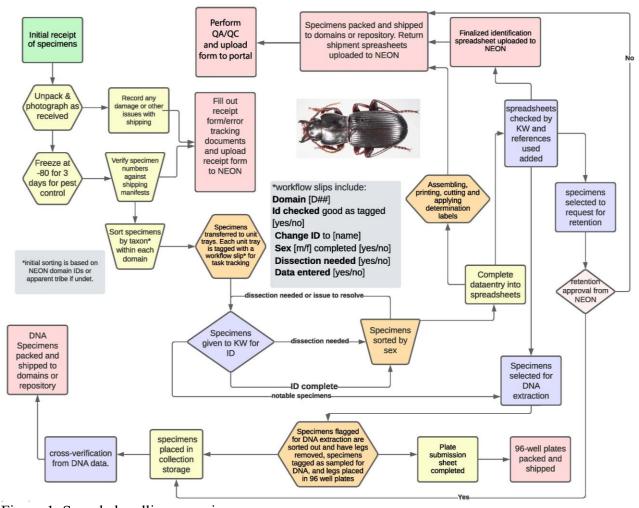


Figure 1. Sample handling overview.

# 3.0 QA/QC Protocols

QAQC is performed utilizing a randomization script generated in Microsoft Excel to select 5% of all sample identifications across all domains and using the formula below as outlined in Exhibit A SOW 2023.

The cause of identification uncertainty will be noted and rectified on a case-by-case basis. After determination by KWW, and during the post-ID processing by RLB and students, specimens are returned to KWW if they appear in conflict with information on the tracking tag, look incongruous with other specimens in the series, or if there is a specimen number counting error. KWW will then reidentify or confirm the specimen's status.

Equation 1, Percent Taxonomic Disagreement (PTD):

$$PTD = \left(1 - \frac{agreements}{N}\right) \times 100$$