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Standard Operating Procedures for Oribatid Mites: Processing, Taxonomy and Curation

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Table of Contents

Summary	
Timeline for Laboratory Training	6
Week 1: Training	6
Week 2: Training	7
Week 3: Training	8
Subsequent Weeks: Sorting to Morphospecies	8
Data Management	9
Transferring Data from the Sample Tracking Log to the Sorting Database	9
Data Entry During Sorting	9
Checking and Storing Data During Sorting	10
Data Entry During Advanced Identification	
Transferring Data from the Taxonomic Expert to the Information Center	
Specimen Management	
Specimen Transfer from the Field to the Processing Center	.11
Specimen Transfer from the Processing Center to the Taxonomic Expert	
Specimen Transfer from the Taxonomic Expert to the Processing Center	
Long Term Specimen Curation at RAM	
Sample Processing	.12
Supervision and Quality Control	
Specimen Extraction	
Extractor Assembly and Sample Logging	
Extraction Setup	
Extraction Takedown	
Sorting to Morphospecies	
Sieving	
Sorting	
Slide Mounting	
Slide drilling	
Specimen Preparation	
Mounting	
Advanced Identification	
Selecting the Expert	
Identifying Mites	
Post Advanced Identification	
Storing Residuals	
Vial Check	
Consolidating Vials	
Vouchering	
Troubleshooting	
Multiple Species	
Mites Sent for SEM or DNA Barcoding	
Data Discrepancies	
New Data Entries	
Missing Specimens	
Appendix 1: Sample Tracking Log	
Appendix 1: Sample Tracking Log Appendix 2: ABMI Mites Data Portal - Field Status Entry	
Appendix 2: ABMI Writes Data Fortal - Fred Status Entry	
Appendix 5: Advir writes Data 1 oftar - Specifien Information and Identification	
Appendix 4: Organic Son Processing Labers	
Appendix 5: Via Check List	
	·10

Summary

This document outlines the training, procedures, and resource materials used to process and identify mites for the ABMI. During spring data collection, ABMI mite technicians receive and track mineral and organic soil samples as they are sent from the field to the ABMI Processing Centre, and use modified Tullgren funnels to extract soil arthropods from the organic soil samples. The extractions are sieved, and the adult oribatid mites collected in a 300 µm sieve that are over 300 µm in body length are sorted into morphospecies groups. The ABMI mite technicians use the Oribatid Morphotaxa sorting manual to perform species identifications for oribatids that can be identified to species with a dissecting microscope. They identify the remainder to family or genus, grouping these by morphospecies, and slide-mounting 1-2 individuals of each morphotype. Advanced identifications are completed by the ABMI mite taxonomist, with the Almanac of Alberta Oribatida and available published literature as reference. The identifications undergo a verification process, and then all specimens are vouchered for accessioning into the Royal Alberta Museum invertebrate zoology collection (PMAE).

Timeline for Laboratory Training

ABMI mite technicians will be hired for mite processing. Ideally, new technicians will commence at the beginning of May, at least 2-3 weeks prior to scheduled arrival of ABMI samples to allow sufficient time for training. The laboratory component of ABMI field work is designed for maximum efficiency with sufficient redundancy to minimize loss of data. After becoming familiar with oribatid mites and the sorting process each technician should be able to process ten ABMI sites per week (average two sites/day) with an accuracy of \geq 95%.

If returning second or third year technicians are confident in their morphospecies identification and sorting after reviewing the material, they can start to sort ABMI sites if available, or assist with other duties, such as preparing extractors for the field season. New technicians must complete the full training schedule before they start sorting ABMI samples.

Week 1: Training

Goals and Expectations

- ABMI mite technicians will understand the general biology of oribatid mites.
- Technicians will begin to learn the structures and terminology needed to sort oribatid mites to morphospecies.
- Technicians will learn how to handle incoming samples, set the samples up for extraction, process the samples through sieves, and sort specimens from field samples.

Training Schedule

Introduction to Oribatid Mites (Mites 101)

- 1. The mite taxonomist or an experienced mite technician will give an introductory lecture on the ABMI, the Processing Centre's role for ABMI, and the relevance of oribatid mites to ABMI objectives.
- 2. Read standard operating procedures: a) Terrestrial Field Data Collection; b) Oribatid Mites: Processing, Taxonomy and Curation.
- 3. Scan the Oribatid Morphotaxa coarse sorting guide, including glossary and morphological structures sections.

Sorting Oribatid Mites

- 1. *Collecting and Extracting Mites:* Weather and time permitting, each new technician will collect several organic soil practice samples and will set them up on the modified Tullgren funnel extractors according to the ABMI protocols. If there are many already-extracted practice samples waiting to be sorted, the sorting supervisor may choose to review the process of collecting and extracting mites with new technicians without collecting any more samples.
- 2. Sample Sieving: The new technician will observe and practice sample sieving procedures to separate the extracted sample into 50-300 μ m and >300 μ m components. Sieve training will highlight the labelling procedures for scintillation vials, and the significance of accurate and consistent labelling.
- 3. *Searching for Oribatids*: The sorting supervisor will discuss main morphological features of mites, how to tell when a mite is an oribatid, and how to discern an adult from a nymph. For the first practice sample, the new technician will begin by grouping all adult oribatids together, and setting those aside in a separate petri dish. The sorting supervisor will check both petri dishes to confirm that the new petri dish only contains oribatid adults (no oribatid juveniles, no other mite taxa), and that the remaining sample contains no adult oribatids. This step clarifies what an adult oribatid looks like, and provides the new technician with practice manipulating mites under the dissecting microscope with probe and pipette. It also reinforces the importance of a detailed, methodical search method while sorting. Once checked, the new technician will temporarily move the oribatids to a vial, so that they can begin sieving another practice sample.
- 4. *Oribatid Identification*: When the new technician has satisfactorily sorted several samples for adult oribatid mites (at the discretion of the sorting supervisor), they will use the glossary and reference diagrams in the Oribatid Morphotaxa sorting manual to begin identifying oribatid mites. The sorting supervisor will be present to clarify terminology, provide hints, and point out key diagnostic features.

Quality Control

- A qualified sorting supervisor or the taxonomic expert will verify all morphospecies identifications during training to ensure specimens are sorted with ≥95% accuracy.
- The sorting supervisor will confirm that proper procedures are being followed for sieving and sorting samples. Multiple samples will be checked to ensure that new technicians have developed a thorough and accurate approach to sorting adult oribatids from sample residuals.

Week 2: Training

Goals and Expectations

- Technicians will learn the structures and terminology needed to sort oribatid mites to morphospecies.
- Technicians will learn how to make and label microscope slides.
- As ABMI samples begin to arrive for extraction, technicians will prioritize sample logging and extraction due to time-sensitivity of field samples.
- By the end of the week technicians will be expected to show an understanding of the ABMI protocols and to demonstrate proficiency in sorting oribatid mites to morphospecies.

Training Schedule

- 1. Lab technicians will sort practice samples into morphospecies and then ask the sorting supervisor to check their identifications.
- 2. The sorting supervisor will demonstrate the procedures to determine which mites should be slidemounted, and how to clear, slide-mount, and label specimens.
- 3. Each technician is encouraged to develop their own personal identification tools as needed to complement the lab protocols and oribatid sorting key (i.e. written notes and/or sketches of key traits for the morphospecies learned).
- 4. If ABMI samples arrive for extraction, technicians will log and set up these samples for extraction under the supervision of the sorting supervisor.

Quality Control

- For technicians that are training, a qualified sorting supervisor or the taxonomic expert will verify all morphospecies identifications during training to ensure specimens are sorted with $\ge 95\%$ accuracy.
- On the last day of Week 2, the sorting supervisor or taxonomic expert will question each new technician about protocols to ensure they understand what to do and why they are doing it. Then the sorting supervisor will provide test samples for the technician to sort and will give each technician an evaluation of their performance.
- Depending on how quickly new technicians learn the morphospecies, they may be required to spend extra time training and/or acquire assistance from the sorting supervisor before sorting ABMI sites.
- For technicians that have begun to sort ABMI sites, a qualified sorting supervisor or the taxonomic expert will verify all morphospecies identifications for the first two ABMI sites to ensure specimens are sorted with ≥95% accuracy.

Week 3: Training

Goals and Expectations

- Technicians will be able to process their practice samples with minimal assistance.
- Technicians will be able to competently receive and extract ABMI field samples, while keeping the Sample Tracking Log updated.
- Technicians may begin sorting ABMI samples as they become available.
- Returning technicians can proceed to Subsequent Weeks: Sorting to Morphospecies Groups (below).
- ABMI samples that arrive will be prioritized for sample logging and extraction due to time-sensitivity of field samples.

Training Schedule

- New technicians that are experiencing difficulty achieving 95% accuracy will work more closely with experienced lab staff and/or the sorting supervisor until the target is consistently attained.
- Sorting supervisor will provide feedback on technician's slide-mounting procedure, help to troubleshoot issues, and suggest alternative techniques.

Quality Control

- The sorting supervisor or taxonomic expert will ensure that new technicians are following extraction protocols and will verify all morphospecies identifications to ensure ≥95% sorting accuracy.
- Samples that do not meet the 95% accuracy target will be reworked until they do.
- Spreadsheets will be checked by the sorting supervisor to ensure that data has been properly recorded, including any samples with no mites.
- Microscope slides will be checked by the sorting supervisor to make sure they have been properly made and labelled.

Subsequent Weeks: Sorting to Morphospecies Groups

Goals and Expectations

- Based on the training and instructional protocols learned in previous weeks, new lab technicians should be comfortable sorting ABMI sites with minimal supervision at an average rate of two sites per day.
- ABMI samples that arrive will be prioritized for sample logging and extraction due to time-sensitivity of field samples.

Quality Control

- The sorting supervisor or taxonomic expert will continue to check the morphospecies identifications of technicians as vial racks are sent for advanced identification to ensure that the ≥ 95% accuracy target is being met.
- The sorting supervisor or taxonomic expert will work with new technicians to clear up any consistent problem areas in the morphospecies identifications.
- For new technicians that have begun to sort ABMI sites, a qualified sorting supervisor or the taxonomic expert will verify all morphospecies identifications of all staff for the first two ABMI sites to ensure specimens are sorted with ≥ 95% accuracy.

Data Management

Transferring Data from the Sample Tracking Log to the Data Portal

All organic soil samples received by the Processing Center (RAM) are tracked using the Sample Tracking Log (Appendix 1). The ABMI system analyst will upload the Sample Tracking Log data into the Field Status Entry (Appendix 2), which populates the year, site, and quadrant fields within the online Mites Data Portal (Appendix 3). As the oribatid mite sort occurs simultaneously with sample logging and soil extraction, updated versions of the Sample Tracking Log will need to be sent to the system analyst, including the final version upon completion of the field season.

Data Entry During Sorting

Lab technicians are responsible for entering sorting data into the online Mites Data Portal (https://newportal.abmi.ca/login) while sorting specimens. Access to the data portal via personal password and username will be set up by the taxonomic expert. Each time they sign in, users should select the "Remember me on this computer" option directly below the input fields for Username and Password; this prevents the portal from logging users out after 15 minutes of portal inactivity. From the Mites home page of the ABMI Workbench, technicians will navigate through "Data Entry" to the "Mite Data Entry" page, and select to expand the appropriate Year and Site/Subsite. Each subsite collected each year has its own data window. Clicking on the empty bottom line (highlighted in orange) on the right-hand side of a data window generates a new data entry row directly above. One row of data should be created for each unique taxonomic grouping identified by the lab technician.

Data that should be included for each record: ID Date, ID Person, Year, Site, Quadrant, Scientific Name, Species Count, Comments (if applicable), Original Vial Location, Number Remaining in Vial, Total Slide Number (recorded as #individuals/#slides - eg. "2/1") and TSN. Each time a user logs into the portal, their personalized subsite data entry window will have re-set, and some unnecessary data entry columns (fields) may be showing in the subsite data entry window, while other necessary columns may be hidden. To adjust the visible data entry columns, the user must right-click anywhere on the column headings. A drop-down list of all possible columns will appear, from which appropriate columns can be selected or de-selected. It is important to check and adjust which data entry fields are showing after each portal sign-in, because data could be omitted if some of the necessary fields are not displayed. Some of the fields should always auto-populate: ID Date, ID Person, Year, Site, Quadrant, and TSN. Generally, users will not need to alter the auto-populated information at all; however, if for example a technician sorted a subsite but was not able to enter the sorting data into the portal until the following day, then the autopopulated ID Date would be incorrect, and the technician would need to change it to the previous day's date on each data line.

The Scientific Name field is a drop-down list of most of the potential taxonomic classifications, in alphabetical order. As the technician begins to type the Genus, the drop-down list will display the section of the list that is applicable, and the technician can select the appropriate name. Many genera are unidentifiable to species in ethanol, but it may be possible to notice significant differences in size or morphology between mites of the same genus in a sample. When this happens, the mites should be separated into "morphotypes" or "morphospecies", and each group should have a unique line of data created. There is no option in the taxonomy drop-down list for morphotypes, so technicians will need to manually type, for example, "Nothrus sp. – Morphotype 1" for the first distinct group of *Nothrus* individuals, and "Nothrus sp. – Morphotype 2" for the second distinct group of *Nothrus* in a sample. Likewise, an identification to Superfamily may be required (if it is not possible to confidently identify a tricky mite to Family), and Superfamily names are also not in the taxonomy drop-down list. The portal will highlight any manual Scientific Name entries in red to indicate an error, but it will still allow them to be saved.

Occasionally the online data portal is unavailable due to maintenance or technical issues. In the meantime, technicians should enter their sorting data into an Excel spreadsheet; this data must then be entered into the data

portal as soon as possible. It is not possible to import or copy/paste the data; it will need to be entered field by field into the portal.

Checking and Storing Data During Sorting

Technicians must ensure that all information is recorded accurately in the database and that all applicable data fields are filled in. Generally, simply clicking or tabbing to a different field in a row will auto-save the previous field. The text will **bold** to indicate it is in the process of saving, and return to regular formatting once the save is complete. Occasionally the data portal will stall while saving and the text will remain bolded. If this happens, technicians must refresh their data entry page to make sure previous fields loaded and were saved into the database correctly. If after refreshing, the most recent data fields are either empty or displaying incorrectly, the technician will need to re-enter the affected data. The data portal is the user interface for the ABMI database, which is stored remotely on University of Alberta servers and backed up regularly.

Data Entry During Advanced Identification

In the online Mites Data Portal, the lower half of the data entry window is for Species Identification (i.e. Advanced Identification). Each data row added in the upper half automatically generates a data row in the lower half for advanced species identification. The taxonomic expert enters advanced identification data for each morphospecies identified by the mite technicians. ABMI requires that each row of data represents the information for a single species. If more than one species is identified for a sorted morphospecies, the expert inserts a new row within the upper half window, ensures that all required fields are entered, modifies the Species Count, Number Remaining in Vial, and Total Slide Number columns within both the original and new columns to accurately represent the number of specimens per species in both rows, and adds information in the Comment fields to ensure that readers understand that there are two species in one vial and/or slide. If a single species has been split into more than one morphotype, the taxonomic expert will transfer all specimens in ethanol into a single vial, merge all data manually into a single row in the data portal, add information in the comments section to describe the modifications, and delete the redundant rows. When entering the Scientific Name, it is important to ensure that the name is chosen from the drop-down list. If the name is missing from the list, the taxonomic expert must log into the "ABMI Taxonomic Workbench" database to ensure that the taxa is included in this database, and to enter the appropriate information if it is a new record or missing. Upon entry into the ABMI Taxonomic Workbench, the taxa name will automatically populate and appear in the Scientific Name drop-down list in the ABMI Mite Data Portal.

Transferring Data from the Taxonomic Expert to the Information Center

Once the advanced identifications and vial check (described below) have been completed, the taxonomic expert exports the data into an excel spreadsheet to check for and correct omissions or errors. The taxonomic expert will inform the Information Centre that the data is available, and the data will undergo additional quality control by the Information Centre before subsequent analyses. The taxonomic expert will work with the Information Centre to answer questions and ensure that any errors are corrected. Vouchering (described below) typically occurs after the initial data has been released to the Information Centre. The vouchering process is also completed in the online data portal and offers an additional opportunity to verify the data. The taxonomic expert will inform the Information Centre when vouchering is complete, as it represents an updated version of the data.

Specimen Management

To ensure that samples are not lost, all ABMI samples received by the Processing Center (RAM) are tracked using the Sample Tracking Log. This data is used to populate the year, site, and quadrant data within the online Mite Data Portal, thereby allowing the mite technicians and taxonomic expert to ensure that all samples are processed during sorting and advanced identification.

Specimen Transfer from the Field to the Processing Center

- Every three days, sample bags containing organic and mineral soil samples are packaged into coolers by field crews, the sample information is recorded onto a flash drive, and the coolers and flash drives are shipped via courier. (see Terrestrial Field Protocols for information on how samples are collected and processed in the field).
- In order to accommodate the space requirements of the mite extractor units, the extractors are stationed at a subsidiary warehouse of the RAM, called Acheson Warehouse ("Acheson"), in Acheson AB. Field samples are couriered to Acheson, where temporarily-relocated mite staff receive and extract them.
- Samples are logged in when they arrive in Acheson. Data from the flash drives are copied into separate Mineral Soil and Organic Soil worksheets within Sample Tracking Log. This data includes the year, soil type, ABMI sites and subsites where the samples were collected, field collection dates, collectors, collection status, and any field comments made by the field technicians. The mite lab technicians add information to the Sample Tracking Log for each sample, including date received, extractor number, extractor start and end dates, and comments.
- Lab technicians ensure that the data sent by the field technicians matches the data and samples received at the Royal Alberta Museum. Missing samples or incongruous data issues are sent to the ABMI lab coordinator at the RAM, who contacts field crew supervisors for clarification and resolution. If missing samples are confirmed to have been sent, then the ABMI lab coordinator contacts the appropriate courier company to expedite tracking and delivery of the wayward cooler(s).

Specimen Transfer from the Processing Center to the Taxonomic Expert

- Lab technicians transfer identified and sorted mites to the taxonomic expert for advanced identification to the lowest taxonomic level possible.
- If samples must be shipped to the taxonomic expert, vial racks are packed within an inner carton, absorbent material, plastic liner, foam packing, and an external cardboard box. Boxes are labelled and shipped by courier in accordance with current regulations for shipping dangerous goods (i.e. ethanol).

Specimen Transfer from the Taxonomic Expert to the Processing Center

- If specimens were shipped to the taxonomic expert all specimens and materials must be returned to the Processing Center after the advanced ID has been completed. Samples are packed and shipped in the same manner as listed above.
- Lab technicians check to ensure that all samples have been returned and are properly labelled. Samples are then organized for storage at the Processing Center.

Long Term Specimen Curation at the RAM

- All specimens and residual materials collected by the ABMI are gifted to, and where appropriate curated by, the Royal Alberta Museum (RAM).
- RAM retains all ABMI materials for at least two years. This includes specimens sorted to species, specimens sorted to broader taxonomic groups, and residual material including non-sorted specimens and debris.
- After two years, voucher specimens from each species (or broader taxonomic group if the specimens were not identified to species) and training specimens are retained by the RAM for use by the ABMI. All other ABMI specimens can be loaned, traded, distributed, or disposed of as the RAM sees fit.

Sample Processing

This protocol is designed to extract arthropods from the organic soil samples, and determine the presence and abundance of oribatid mite species.

Laboratory Equipment:

ABMI Laboratory Protocols and Oribatid Morphotaxa sorting key Almanac of Alberta Oribatida Part 1 – updated digital version Modified Tullgren funnels with 20 watt light bulbs (4 per site) held in moveable extraction racks Lab desktop or laptop computer Absolute ethanol 500mL squeeze bottles #50 cheesecloth 7.1 - 115x dissecting microscopes with cold (fiber optic) light sources and ocular micrometers Drying oven with thermometer 300µm and 53µm sieves Glass and plastic petri dishes Excavated blocks Pasteur pipettes with blue bulbs Fine tipped probes and forceps 20 mL scintillation storage vials and caps with cone-shaped seal 2.0 mL micro vials (uncoloured) with rubber o-ring sealed lids (uncoloured and red) Micro vial racks and rack lids Short barrel funnels Specimen cups with lids 2.5x1.9cm removable labels Small circular custom-printed sticker labels numbered 1-96 Ultra fine-tipped Sharpie markers Scotch tape Microscope slides and round #1.5 coverslips (0.17mm thickness) PVLG (polyvinyl alcohol, lactic acid, glycerol) (i.e. phenol-free PVA) mounting medium in glass dropper bottles Lactic acid (85% w/w) in glass dropper bottles

Supervision and Quality Control

- Lab technicians will be trained in soil arthropod sorting and identification by a qualified sorting supervisor.
 - Qualified sorting supervisors are one of the following:
 - 1. They are the ABMI taxonomic expert on mites, or
 - 2. They meet the following criteria:
 - Greater than two months experience in the identification and sorting of soil arthropods
 - Two or more days of training in soil arthropod identification and sorting under the supervision of the identified expert acarologist/entomologist, and
 - Successful completion of an exam (demonstrating >95 % proficiency) consisting of identification and sorting of mites of Alberta.

Specimen Extraction

Extractor Assembly and Sample Logging

- A large room is required with enough electrical circuits to run the modified Tullgren funnels (each funnel has a 20 watt light bulb). The setup consists of movable shelving units each holding 54 funnels and run with three transformers (Figure 1). The number of shelving units required is dependent on the number of field sites to be visited during the field season. For example, four shelving units containing a total of 216 funnels has been historically sufficient for extracting soil from 100 field sites. Funnels are numbered sequentially.
- Assemble and check that the equipment is working before the arrival of field samples. Replace spent equipment.

PROCESSING MITES VERSION 2021

- When samples arrive, confirm in the Sample Tracking Log that the samples received match the collection data sent by the field technicians. Sample bags are only sent for collected subsites. Subsites where no organic soil was collected are listed in the Sample Tracking Log with red text. Any sample or data problems are recorded in the 'lab comments' column and the corresponding rows are highlighted in blue. The log is backed up on the network every night.
- Lay out the soil samples in numerical order by site and alphabetically by subsite within each site (i.e. NE, NW, SE, SW). This is the order in which the samples will be placed onto the modified Tullgren funnel extractors.
- All organic soil samples are transferred onto the modified Tullgren funnel extractors within seven days of being collected. Record the extraction start date in the Sample Tracking Log.

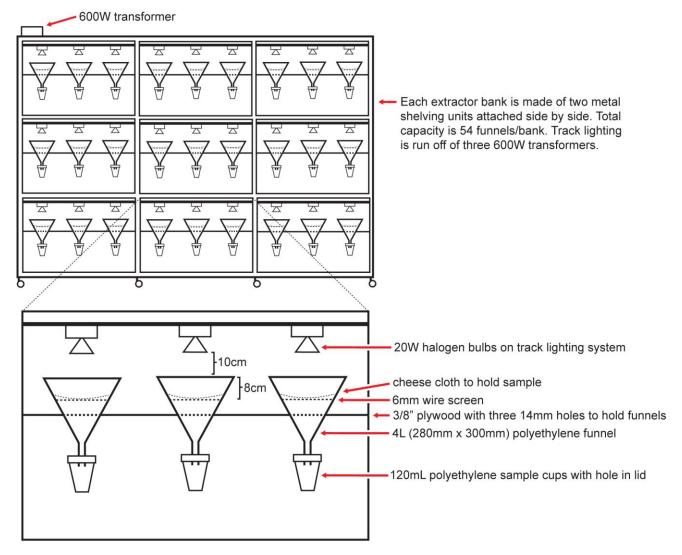


Fig 1. Bank of modified Tullgren funnels for extracting soil arthropods, with enlarged view of one three-funnel segment.

Extraction Setup

• Print out two labels that include the RAM lot number, ABMI site and subsite, collection date and collector initials, funnel number, extraction start date, and the designations 'Organic Soil' and '7 Day Extraction':

Organic Soil Lot: ABMI.2012.491NW.OS ABMI Site #: 491 NW Coll: 24-May-2012, AHI Funnel: 28, 28-May-2012 7 day extraction

Labels are created by filling out the required sample information in the template provided (Appendix 4) and are printed on standard white paper using a laser printer (Verdana 8pt). One label is placed on top of the soil sample and the other is placed into the sample cup. Labels for $50-300\mu m$ and $>300\mu m$ residuals may be printed from the same template at the same time to save time later in the sorting process.

- Place a piece of moistened cheese cloth onto a clean laboratory table. Remove the organic soil sample from its bag and place the sample on the cheese cloth being careful not to lose any soil.
- Wrap the corners of the cheesecloth around the sample then place it onto the wire mesh of the modified Tullgren funnel. Samples are always placed on the funnels prior to adding the sample cups to minimize the amount of fine particulate matter that passes through into the sample cups.
- Wipe the laboratory table clean with a damp cloth before repeating the procedure to prevent carry over.
- Add 50mL of absolute ethanol to the sample cup, insert the label and then screw the cup into the lid attached to the funnel stem.
- Place the original labeled soil sample bag below the funnel.
- Turn on the light bulb above the funnel and run the extractor for seven days. Repeat the setup process for all other soil samples. Check the extractors twice a day (first thing every morning and last thing every evening) during extraction to ensure that the ethanol levels are sufficient and that the funnels continue to function properly (i.e. power has not failed, light bulbs burnt out, etc.). Add more ethanol if levels are low. Do not allow the ethanol in the sample cups to dry up completely.
- To minimize the amount of particulate matter falling into the specimen cups it is important not to bump or otherwise disturb the extractors during the extraction process. The cleaner the samples are, the easier it is to sort the specimens.

Extraction Takedown

- On the seventh day, turn off the light bulbs, remove the specimen cups, and discard the soil sample and cheese cloth into the garbage. Add lids to the specimen cups and store them by site until they can be sorted. Write the site and subsite on the lid of each specimen cup using a Sharpie marker. Record the date each sample was removed from the extractor in the Sample Tracking Log.
- If the soil samples are to be sent to other researchers for use, carefully place each soil sample (still in its cheesecloth) with its funnel label and cloth sample bag into a large Ziploc bag.
- Use a handheld vacuum to carefully remove dirt and debris from the extractor shelves (jostling and bumping of the extractor unit will cause excess debris to fall into the extractor cups still on another part of that same extractor unit, and samples with more debris take longer to sort). Take down and rinse the funnels under running water to prevent any carry-over. Reassemble the extraction rack and prepare it for the next set of samples.
- Extracted samples are typically processed within two to four months of collection.

Sorting to Morphospecies

Using the Laboratory Protocols and Oribatid Morphotaxa sorting key, lab technicians will learn how to handle, sort, and identify adult oribatid mites (Acari: Oribatida) 300µm or more in length to the taxonomic level suggested by the taxonomic expert. Lab technicians will be able to process on average two sites per day by the fourth week of sorting. Lab technicians will sort the mites for a single subsite at a time to prevent carry-over.

Sieving

- Assemble a stacked sieve array by placing a 300µm sieve on top of a 53µm sieve. Use a pair of forceps to remove the label from a sample cup. In a container, pour the contents of a sample cup through the sieve array. Rinse the specimen cup and label over the sieve array with absolute ethanol to ensure all sample material passes through the sieves.
- Rinse the sample material trapped by the 300µm sieve well with ethanol to ensure that any soil, organic material, and soil arthropods less than 300µm in length have been washed through the sieve. This will improve the ease of sorting. Rinse the material trapped by the 300µm sieve to one side of the sieve and then back rinse it into a petri dish. Briefly check the sieve following the transfer to ensure that no organisms remain trapped in the sieve.
- Transfer the material trapped by the 53µm sieve to a 20mL scintillation vial with a short barrel funnel. Place a label into the vial that includes the RAM lot number, ABMI site and subsite, collection date and collector initials, funnel number, extraction start date, and the designations 'Organic Soil' and 'Residue (50-300µm)':

Organic Soil Lot: ABMI.2012.491NW.OS ABMI Site #: 491 NW Coll: 24-May-2012, AHI Funnel: 28, 28-May-2012 Residue (50-300µm)

Labels are printed using the template provided (Appendix 4, Verdana 6pt) on standard white paper using a laser printer (Verdana 6pt) and are placed inside the vials. Write the site and subsite on the lid along with the designation '50-300 μ m' using a Sharpie marker, then tape over the label with scotch tape. The 50-300 μ m residual vials are collected in scintillation vial trays as they accumulate during the sorting process. These residuals are stored at the Royal Alberta Museum (see Specimen Management above and Storing Residuals below).

- The waste ethanol and anything that passes through the 53 μ m sieve are discarded. Waste ethanol is discarded by running it down the drain with large amounts of water.
- The sieves, funnel, and empty sample cup should all be rinsed in warm water so that they are clean and ready for the next sample. The sieves are more effective if they are allowed to dry between samples; it may be necessary to place them in the slide oven to speed up drying.

Sorting

- Using a 7.1 115x dissecting microscope with a cold (fiber optic) light source, sort through the contents of the petri dish containing the material from the 300µm sieve. Separate the target oribatid mites from the other material by using a probe (forceps are not used as they crush the mites).
- Target oribatids are: adult, ≥300µm, not 'pre-dead'. Only oribatid mites that meet all of these criteria should be included in the ABMI data. The size requirement of ≥300µm refers to ventral length; use the microscope's ocular micrometer to check the length of mites if in doubt. Adult oribatid mites are fully sclerotized (there are a few uncommon exceptions within the lower oribatids) the sorting supervisor will point out nymphs (juveniles) during training. It is also important to distinguish between individuals that are live-extracted, and dead individuals that also fell into the sample cup during extraction (ie. 'pre-dead' mites), because 'pre-dead' mites do not represent current mite populations. 'Pre-dead' mites lack any internal contents or structures, and appear clear (see-through). The sorting supervisor will also clarify pre-dead mites during training. Transfer all <300µm adult oribatids to the 50-300µm residual vial; return any other non-target organisms to the >300µm residual vial.

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 Once all the target oribatid mites have been removed, transfer the remaining residual material from the petri dish to a 20mL scintillation vial. Place a label into the vial that includes the RAM lot number, ABMI site and subsite, collection date and collector initials, funnel number, extraction start date, and the designations 'Organic Soil' and 'Sieved Sample (>300µm)':

Organic Soil

Lot: ABMI.2012.491NW.OS ABMI Site #: 491 NW Coll: 24-May-2012, AHI Funnel: 28, 28-May-2012 Sieved Sample (>300µm)

Labels are printed using the template provided (Appendix 4) on standard white paper using a laser printer and Verdana 6pt font and are placed inside the vials. Write the site and subsite on the lid along with the designation '>300 μ m' using a Sharpie marker, then tape over the label with scotch tape. The >300 μ m residual vials are collected in scintillation vial trays as they accumulate during the sorting process. These residuals are stored at the Royal Alberta Museum (see Specimen Management above and Storing Residuals below).

- Technicians must group to the finest possible taxonomic level and then count the number of specimens in each group. Record the number of individuals of each morphospecies in the Mite Data Entry section of the ABMI online data portal (<u>http://new.portal.abmi.ca/login</u>), for the appropriate Year/Site/Subsite. Document how many individuals will be slide-mounted and/or placed in a micro vial, and record the rack and vial number.
- Use a Pasteur pipette to transfer each individual morphospecies of oribatid mites into a separate micro vial containing 1mL of absolute ethanol. If specimens are too large to fit into the pipette then gently grasp them by one leg with fine forceps to affect the transfer. Avoid picking up a mite by its notogaster or prodorsum they are much more likely to be crushed.
- *** It is very common for mites to remain trapped in the pipette, meaning that they can be accidentally transferred to a subsequent vial. Check the micro vial, and the pipette, to ensure that specimens are not lost during transfer and that each vial contains only one morphospecies in clean ethanol. ***
- Label the vial cap with the vial's number using a white, numbered sticker dot. In each vial rack, the vials are numbered from one to ninety-six from the top left corner to the bottom right corner column by column (Figure 3). Vial numbers should face towards the front of the vial rack.
- Once one subsite has been sorted, repeat the process for the next subsite. Only process one subsite at a time to prevent contamination and data errors. All lab equipment must be rinsed with water between subsites to prevent specimen carry over. Dry all petri dishes and excavated blocks before reusing them to prevent the mixing of ethanol and water. This will improve the ease of sorting.
- Once an entire rack of 96 vials has been completed, print out three sets of sorting labels that include the RAM lot number, ABMI site and subsite, collection date and collector initials, sorting date and sorter initials, morphospecies ID, number of specimens, vial ID, and the designation 'Oribatida':

Oribatida

ABMI.2012.491.M Site: 491 NW Coll: 24-May-2012 AHI Sort: 5-Jun-2012 SPL Achipteria sp. 1 x 6 Vial: M-SPL-01-03

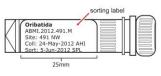


Fig 2. Labeled micro vial

Vial IDs are created as follows: M(for mites)-sorter initials-rack number-vial number (e.g. M-DEW-04-15 would be the fifteenth vial in the fourth rack of mites sorted by DEW). Sorter initials are typically the first initial of first name, and the first two initials of last name, eg. Ashley Thorsen = ATH. Sorting labels are generated via the Online Mites Data Portal. To generate labels, data must first be filtered to a single ABMI sorting year on the Mite Portal. To do this, select the filter icon on the top left portion of the screen, then under the section titled 'Year' put in the desired year and select filter to generate the desired years' data. To

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generate the sorting labels, select the down arrow beside the 'More' tab, then select 'Create Sorting Labels'. The sorting labels will then be exported to a PDF document. The labels generated on the PDF document should then be copied to and printed using the template provided (Appendix 4) and Verdana 4pt font. One label is printed on standard white paper and is inserted into the vial with the print facing out so it can be seen through the plastic. The second label is printed on a 2.5x1.9cm sticky label and is attached to the outside of the vial. Both labels are placed vertically with the left edge of the label facing the bottom of the vial (Figure 2). Vials are placed in vial racks with the outside label facing the front of the rack.

• Label the micro vial racks as follows: M(for mites)-sorter initials-rack number (e.g. M-DEW-04 would be the fourth rack of mites sorted by DEW). When a vial rack is full, tape a label on the left side of the rack (use Comic Sans 11pt font) that indicates the rack number (e.g. M-DEW-04), and a label on the front of the rack (use Times New Roman 10pt font) that indicates the rack number and ABMI sites by vial. The front label should note when a site continues into the next rack, for example:

M-DEW-04 Sites

344(01-28), 758(29-60), 1402(61-84), 1109(85-96 continued M-DEW-05 vials 01-09)

Rack labels are printed on standard white paper using a laser printer and are taped onto the rack using a strip of scotch tape that covers the entire label. The scotch tape should be trimmed so there is minimal overhang. Racks are numbered sequentially for each sorter (Figure 3).

- Print an extra set of sticky labels for the vials in each rack. These will be used for slide mounted specimens (see Slide Mounting below). At the end of the slide mounting process all remaining extra labels should be placed into 19.2x13.9cm envelopes by rack. The envelopes are labeled with the same labels that are taped to the vial rack. These labels are taped to the center of the envelope using a strip of scotch tape that covers the entire label. The scotch tape should be trimmed so there is minimal overhang. The envelope flap should be on the right when the envelope is labeled and should be folded into the envelope.
- Once vial racks have been filled and labeled they are sent to the taxonomic expert for advanced identification along with any slide mounted specimens (see Slide Mounting below) and the envelopes of extra vial labels (see Sample Management above).

						1	micro via	al					
	Тор	first	vial			/	/ lid ı	number l	abel				
				4 25 27 28 29 31 31 32									last
	Front				rack nu	umber a	nd sites	label					
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	Left Side			ra	ack num	nber lab	el						
ç	I		M-D										
			O	- ** - *									

Fig 3. Labeled micro vial rack showing positioning of labels on top, front, and left side.

Slide Mounting

Many species of oribatid mites require high magnification to determine species identity; and therefore, they must be cleared in lactic acid and mounted on microscope slides in PVLG medium rather than left in ethanol. The taxonomic expert provides a list (see below) of which specimens need to be slide-mounted, what type of slide to use (normal, shallow-well, medium-well), how many of each should be mounted (1-2 specimens if available), what position they should be mounted (D = dorsal view, V = ventral, L = lateral). Lab technicians will slide-mount these specimens before sending them to the taxonomic expert for advanced identification.

In general, spherical mites need to be visible in all three orientations (D, L, V) and they should be mounted in a shallow well-slide or, depending on size, a medium well-slide. If unsure and only one specimen is available, then mount it with its dorsum up (D). Small mites or those that are dorso-ventrally flattened can be mounted on a normal microscope slide in both D and V positions. As well as following the list below, mount representative specimens of any mite of which you are unsure or you think new. Use normal slides for the list below except where noted.

Oribatid Genera (or Families) to Slide-mount for Advanced ID:

Ametroproctus (D + V) shallow slide Anachipteria (D) Banksinoma (D) only if unsure of species Bipassalozetes (D) Ceratozetes except gracilis (D) *Cultroribula* (D) Damaeidae except *Epidamaeus* sp. 2 and sp. 3 (D); NB legs must be visible; shallow slide Diapterobates except humeralis (D) shallow slide *Dometorina* (D) Dorycranosus (D) only if unsure of species; medium slide Eniochthonius (D or L) Eporibatula (D) *Eremaeus* (D + V)Eueremaeus (D + V) NB more than one species may co-occur Eupelops (D) shallow slide *Eupterotegaeus* (D) Galumna (D) shallow slide Gymnobates (D) if unsure of species *Hemileius* (D) Heterozetes (D) shallow slide Hoplophthiracarus (L) only if unsure; shallow slide Hydrozetes (D) NB tarsal claws & femur IV must be visible; shallow slide Jacotella (D) Joshuella (D) Lepidozetes (D) shallow slide Limnozetes (D) shallow slide Malaconothrus (D + V)*Mucronothrus* (D + V/L) only if unsure of genus Mycobates (D) except incurvatus; shallow slide Neoribates (D + V) only if unsure of species; shallow slide Nothrus (D) NB tarsal claws must be visible **Oppioidea** except *Aeroppia* and *Banksinoma* (D) Oribatella except jacoti (D) shallow slide, normal slide for small species Parachipteria (D) shallow slide Peloribates except pilosus (D) shallow or normal slide *Pleodamaeus* (D) Propelops (D) only if unsure of species; shallow slide *Protoribotritia* (L) Roynortonella (D) only if unsure of species Scapheremaeus (D) Scheloribates (D) only if unsure of species Scutozetes (D) if unsure (Lepidozetes similar); shallow slide Sphaerozetes (D) shallow slide Suctobelbidae (D) Svalbardia (D) only if unsure *Tectoribates* (D) *Trhypochthonius* (D + V) **NB more than one species may co-occur** Trichoribates (D) shallow slide Trimalaconothrus (D + V)Zetomimus (D) shallow slide Zygoribatula (D) NB more than one species may co-occur

AND any other oribatids that cannot be confidently identified to genus level

Slide Drilling

- Well slides are used to mount most specimens to prevent them from being crushed during the mounting process and to reduce the filling of air bubbles. Normal slides may be used to mount specimens that are dorsoventrally flattened or small species and thus unlikely to be crushed. Well slides are drilled by hand using a dremel mounted in a dremel press and fitted with a #7103 Dremel Diamond Point drill bit. Two depths of well slides are needed: shallow and medium. Fifteen to twenty boxes of each should be drilled prior to the start of the field season.
- The dremel press is fitted with a block of wood and thumb tacks to hold the slide in place during drilling. With the dremel turned off, place a slide in the dremel press then adjust the press so that the drill bit just touches the surface of the slide when the handle of the press is fully lowered.
- Ensure that personal protective equipment is on, including safety glasses, before turning on the dremel.
- Place a slide in the dremel press. Add one drop of water to the center of the slide to collect the glass dust.
- Turn on the dremel. Lower the handle of the press until the drill bit touches the surface of the slide. Release the handle once a well has been drilled in the center of the slide to the required depth. Depth is determined by the circumference of the well. To drill shallow slides briefly touch the drill bit to the surface of the slide then release. To drill medium slides apply more pressure to the handle and hold the drill bit in contact with the surface of the slide for one to two seconds.
- Remove the drilled slide from the dremel press and wipe off the water using a kimwipe. Repeat this process until an entire box of slides has been drilled to a specific depth then turn off the dremel.
- Fill a small beaker with a mixture of soap and warm water. Using a toothbrush scrub the well of each slide to remove any additional glass dust. Rinse the slides in clean water then dry them with paper towel to prevent them from sticking together and return them to their box. Label the top of the lid and the front side of the lid and box with the depth of the slide well (shallow or medium).

Specimen Preparation

- Mites should be slide mounted during the sorting process for best efficiency. For species in the above list one to two mites from each sample should be mounted on a single slide in the orientations specified unless otherwise indicated by the taxonomic expert. If only one mite is present in the sample it should be slide mounted in the dorsal orientation unless otherwise indicated for that species in the list above . If multiple mites are present choose one to two that are clean, intact, lighter in colour, that sit relatively flat, and have few gut boluses for best mounting success.
- Add enough lactic acid to a small excavated block to cover the bottom of the well. Lactic acid is used to clear mites so that light can pass through them. While sorting a sample, identify the mites present that must be slide mounted, then use a pipette to transfer these specimens from the petri dish to the excavated block with as little ethanol carry over as possible. Using a probe make sure that the specimens are fully immersed in the lactic acid and are stuck on the sides of the well.
- Using a Sharpie marker label the glass cover of the excavated block with the ABMI site and subsite, the morphospecies present and number of mites from each. Mites from multiple samples may be combined in a single excavated block as long as each individual morphospecies is found in only one sample to prevent any data mix ups (e.g. do not place *Anachipteria* from two subsites in one excavated block because it will be impossible to tell which *Anachipteria* came from which subsite). Place the cover over the excavated block and let sit at least overnight.
- Place a red lid on each vial that specimens are taken from to show that mites have been removed for slide mounting. In the sorting database record the number of mites still in the original vial and the number of mites per slide (e.g. 2/1 = two mites on one slide, 4/2 = four mites on two slides, etc.).

Mounting

• Choose a slide based on the mites that are being mounted. Shallow slides are typically used for mounting single small to medium spherical mites or two small mites. Medium slides are typically used for mounting single large mites or two medium mites. Normal slides are typically used for mounting small to medium dorsoventrally flattened mites. Place one drop of PVLG mounting medium in the center of the slide, over the slide well if one is present.

- Under a 7.1-115x dissecting microscope use a flattened minutin probe to transfer the mites from the excavated block to the PVLG on the slide with minimal lactic acid carry over. Use the probe to maneuver the mites so they are in the right mounting orientations (i.e. dorsal, ventral, lateral) and are touching the bottom of the slide well or surface of the slide. This will help prevent them from rolling when the cover slip is placed.
- Using a pair of fine tipped forceps place a round cover slip over the mites by touching one edge of the cover slip to the mounting medium and slowly lowering the cover slip. Applying gentle pressure to the top of the cover slip with the forceps as it settles will help prevent the mites from rolling, but be careful not to crush the mites. If the mites roll, remove the cover slip, add more PVLG, reposition the mites, and try again using a new cover slip to prevent the formation of air bubbles. Be sure to only use one single coverslip (the coverslips have a tendency to stick together).
- Use a fine-tipped Sharpie to label the slide with the site and subsite information, species identification, slidemounting date, and your initials.
- Place the labeled slides on a metal slide tray and place the tray in a drying oven set at 55°C. The slides must remain in the drying oven for seven days to allow the mounting medium to harden. Each morning remove the slides from the oven and check them. If the mounting medium pulls away from the edge of the cover slip and an air bubble forms under the cover slip place a drop of PVLG at the edge of the cover slip and allow it to fill the air bubble. Fine forceps or probes may be used to coax the mounting medium under the cover slip. The sooner after mounting air bubbles are caught, the easier they are to fix.
- Once seven days have elapsed remove the slides from the drying oven and label the slide with a sticky label that includes the RAM lot number, ABMI site and subsite, collection date and collector initials, sorting date and sorter initials, morphospecies ID, number of specimens, vial ID, and the designation 'Oribatida'. This label should be the same as that used on the original vial. Extra vial labels are printed during the sorting process (see Sorting above). The label is placed vertically against the left edge of the slide with the left edge of the label facing the bottom of the slide (Figure 4). Send them to the taxonomic expert for advanced identification along with the vial racks and envelopes of extra vial labels. These labels will be used by the taxonomic expert to label any new slides they create while doing the advanced identifications.

Oribatida

ABMI.2012.491.M Site: 491 NW Coll: 24-May-2012 AHI Sort: 5-Jun-2012 SPL Achipteria sp. 1 x 6 Vial: M-SPL-01-03

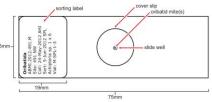


Fig 4. Labeled microscope slide

Advanced Identification

- Lab technicians will transfer all sorted samples to an appropriate expert in oribatid mite taxonomy for identification to the lowest taxonomic level possible. Samples can be sent via registered mail or courier service if required. The taxonomic expert will access the sorting data using the ABMI mite data portal. The taxonomic expert should enter identifications and comments into the lower half of the data entry page, in the Species Identification section.
- If specimens were shipped to the taxonomic expert, all specimens and materials must be returned to the Processing Center after the advanced ID has been completed.

Selecting the Expert

- The ABMI will select a taxonomic expert who is a known specialist in oribatid mite taxonomy. To ensure the highest standards of accuracy and maintain the credibility of the ABMI, the ABMI will only select experts who can meet at least one of the following criteria:
 - 1. The expert is endorsed by the Royal Alberta Museum or an associated museum (i.e. Canadian Museum of Nature, etc.) as being capable of expert identification of mites with ≥95% accuracy.
 - 2. The expert is endorsed by two members of the scientific community recognized in the field of entomology as being capable of expert identification of mites with ≥95% accuracy.
 - 3. The expert completes an ABMI certification exam consisting of 200 known mite specimens from more than 50 species and at least ten broadly separated areas throughout Alberta. The expert must identify the specimens with an accuracy of ≥95% (i.e. a maximum of ten wrong) to pass the exam.

Identifying Mites

- The ABMI defines target taxa as adult oribatid mites (Acari: Oribatida) ≥300µm in length. All target taxa must be identified to the lowest taxonomic level possible. Species names are determined based on the most recent version of the *Almanac of Alberta Oribatida* and the papers referenced within. They should be supported by a species author or authority entered by the taxonomic expert into the ABMI Taxonomic Workbench.
- Whenever possible specimens are to be identified to the species level. Species must be identified with ≥95% accuracy. The taxonomic expert enters all required information into the online ABMI Mites Data Portal while working on the advanced identification. In the case of incomplete identifications:
 - 1. When a specimen is recognizable as a distinct species but cannot be assigned to a named species either because it is undescribed or the identification is currently problematic (e.g. poor original description, no recent revisions, unavailability of types, etc.) the identification is listed in the format: Genus sp. + alphanumerical identifier + identifier initials. For example:
 - Pilogalumna sp. 1 DEW = species 1 of Pilogalumna as identified by David Evans Walter Hydrozetes sp. E RAN = species E of Hydrozetes as identified by Roy A. Norton
 - 2. If the specimen cannot be identified to the species level because it is damaged or for any other reason, the identification is listed as sp. at the lowest level of identification (i.e. *Propelops* sp. = undetermined species of genus *Propelops*).
- The taxonomic expert can rearrange the slide mounted material as required to assist with identifications. Using slide trays to rearrange the slides by taxa, and then by site/subsite within each taxa has been found to be the most efficient for using the identification keys, assessing taxa across sites and against the reference collection, and for entering the data into the online portal. It is most efficient to write the species name in pencil on the slide label and then to enter the data into the portal in small batches (e.g. by taxa or single daily entry) while the slides maintain the order of taxa-site-subsite. The exception is if comments are required or rows in the portal needed to be added or deleted (i.e. multiple species on a slide or single morphotype on multiple slides); in these situations, enter the changes and comments into the portal immediately to reduce accidental errors or omissions.
- The taxonomic expert can slide mount additional specimens from the alcohol collection if needed for identification.
- Taxa that are in alcohol but not yet identified to species (e.g. *Neoribates, Pilogalumna, Cepheus*) will need to be examined under the dissecting microscope. It is easiest to export the data from the online portal into an

excel spreadsheet to assist in determining where these taxa are located in the racks. Sort by species and highlight all rows that are still at genus level with no slides, then sort by vial number to obtain the order of the vials in the racks. Pull these specimens from the racks and place them in a new rack (keeping in order by rack-technician), identify to species, write the species name in pencil on the vial label, and enter the data into the portal as the identifications are completed. Once this step is complete, use a highlighter to colour the tops of all of the microvials and then place them in order back into the original racks. For any vials that can not be identified to species using the dissecting microscope, the lab technicians or taxonomic expert will clear and slide mount specimens and the taxonomic expert will complete the identifications using a compound microscope. For these vials, replace clear lids with red lids to indicate that there is a slide associated with the vial.

- If the taxonomic expert is to do the Vial Check (it is also possible for a qualified technician to do so, as per Vial Check section below), it is best to incorporate this step here, as per the following. Export data from the portal and create a spreadsheet containing all rows of species listed in the 'Vial Check' list given below. Highlight 5 random rows for each species per technician. Sort the spreadsheet by vial number and then go through the racks consecutively and place the vials that are required to be checked into a new rack (keep in order by rack-technician). Check the identification of these specimens and enter the QA cells within the Species Identification section (lower half) of the portal (e.g. 'verified', 'incorrect'). Once complete, colour the tops of the vials with a highlighter and return to original racks. After it is confirmed that 5 vials per technician per species are identified correctly, filter for each of these species within the portal and enter 'overlooked' in the QA area for all rows that were not verified. Reorder the spreadsheet that was created for the vial check by the original vial number. Using this spreadsheet as reference, go through each rack and colour the tops of the lids of the remaining vials for these species, as an identifier that they are completed and no additional check is required. Export data again and check that the QA section was filled out for each species, and that there are 5 verified and the remaining overlooked for each species (per technician). This step helps to ensure that the data was entered correctly.
- After the vial check, go through all remaining vials that do not have highlighted lids and confirm species identifications. Once a rack is complete and identifications complete, highlight the tops of the remaining vial lids for the rack to indicate that they have been checked. For any vials requiring additional work, remove them from the racks and place in the same order in a new rack until species identifications are completed, so that they are not overlooked
- A note that the steps of highlighting the tops of vials and removing vials from the original racks have been added to the procedures as it is not possible to flag rows to be revisited within the portal (previously, it was possible to highlight and flag rows to revisit within the Excel spreadsheet that was used for species identifications) or to easily view the Species Identification section (only one row can be opened at a time within the portal to view the species identification section and this section also does not export to Excel completely). If other more efficient methods are developed to ensure that all IDs and quality checks are completed then it is encouraged to use them.
- Identified mites that remain in the original vial retain their original label, vial ID, and position in the vial rack. In the case of identification or labeling errors by lab technicians, or when vials are being merged (more than one vial from one subsite contains a single morphotype), the taxonomic expert will correct the outside vial label in pencil and the information in the online mite data portal. All vials will receive a corrected label as part of the vouchering process so the original label does not need to be replaced at this point.
- All mites removed from the original vial to be slide mounted must receive the same label as the original vial.
- On a regular basis an effort is made to further verify the advanced identifications. Protocols for the verification process will be produced as needed. In the past these have included DNA barcoding.

Post Advanced Identification

Following the advanced ID, slides and vials are chosen as vouchers for each identified species at each ABMI site sampled during the field season. These vouchers are retained by the RAM for use by the ABMI. All other slides and vials are designated as duplicates and after being retained by the RAM for two years can be loaned, traded, distributed, or disposed of as the RAM sees fit. Before the vouchering process can begin the residuals must be prepared for storage, a vial check must be conducted, and the vials must be consolidated.

Storing Residuals

- During the sorting process 50-300µm and >300µm residuals are placed in 20mL scintillation vials and are collected in scintillation vial trays as they accumulate (see Sorting above). Before the residuals can be boxed and placed into storage they must be organized by site number and residual type. Print out a list of all ABMI sites and subsites for which organic soil was collected during the field season. This list will be used as a reference and to double check that all residuals are accounted for.
- Starting with the 50-300µm residuals transfer all scintillation vials from their original trays to new trays so they are arranged numerically by increasing site number, and alphabetically by subsite within each site. ABMI sites are ordered first followed by any ALPAC sites and then any OG sites. Scintillation vials are placed in trays from the top left corner to the bottom right corner column by column. Place vials in the trays so the lid labels are facing the front of the tray. Each tray holds one hundred vials.
- Once all of the trays have been filled, label the front of each tray with the field season (e.g. ABMI 2012), the designation '50-300µm residuals', the sites that are present in the tray (e.g. sites 1597-A602-2), and the tray number (e.g. 5/6). Trays are numbered sequentially based on the sites they contain, starting with the tray containing the lowest site numbers and ending with the tray containing the highest site numbers. Labeling is done using a Sharpie marker.
- Once the 50-300 μ m residuals have been ordered in labeled trays, repeat the same process for the >300 μ m residuals using the designation '>300 μ m residuals' on the tray labels. Trays are numbered separately for 50-300 μ m and >300 μ m residuals (i.e. if there are six trays of 50-300 μ m residuals numbered 1 to 6, there will also be six trays of >300 μ m residuals numbered 1-6).
- Once all trays have been filled and labeled they are ordered numerically by tray number and placed in plastic boxes (Sterlite #1933, 32 Quart Gasket Box, 47 x 37.8 x 28.3 cm; drill several small holes through the handle region to allow air flow). Each box can hold four trays of scintillation vials. 50-300 μ m and >300 μ m residuals should be placed in separate boxes when possible, but to save boxes and storage space the final box may contain both 50-300 μ m and >300 μ m residuals (e.g. if there are ten trays each of 50-300 μ m and >300 μ m residuals, two boxes with four trays each of >300 μ m residuals, two boxes with four trays each of >300 μ m and >300 μ m residuals.
- Print labels (Avery shipping labels, #5168, 3 $\frac{1}{2}$ " x 5") with the following information: field season (e.g. ABMI 2012), the designation 'Organic Soil Residuals', the type of residuals (i.e. '50-300µm' or '>300µm'), the sites that are present in the box (e.g. sites 3-1580), and the box number (e.g. 4/5). If the final box contains both 50-300µm and >300µm residuals that must be indicated in the label. Boxes are numbered sequentially based on the trays they contain, starting with the 50-300µm boxes, then the >300µm boxes, then the final combined box if there is one. Adhere the labels to the small side (37.8 cm) of each box.
- Use the template in Appendix 6 to print out two ethanol warning labels per box. Adhere one label to the box, on the same end as the collection label so that both labels are facing out and can be read when the box is in storage. Adhere the second label to the box lid.
- Once all of the boxes have been labeled they can be moved to their permanent storage location in the RAM ethanol storage room. Place the boxes on the shelves with labels visible.
- On a regular basis the ethanol levels in the residual vials from previous years will be checked. Any vials that are low on ethanol will be topped up and any vials that have dried up completely will be refilled or replaced as appropriate, and a note will be made on the vial. Any vial that is refilled will have a red colour coding dot placed on the lid to indicate that the vial may have a hairline crack and should be monitored and replaced as needed. Protocols for this process will be produced as needed.

Vial Check

Some mite species are easier to identify to the species level in ethanol than are others. Following training, lab technicians are responsible for correctly identifying all of the species in the list below. The vials identified as containing these species will be subject to a random test by the taxonomic expert early in the advanced identification process and the expert will provide feedback to the mite sorters. Once the advanced ID has been completed a random check of these vials must be performed to determine the accuracy of the technicians' identifications. If the random check turns <95% correct identifications, then all of the vials sorted by a particular person should be checked. This check can be done by an experienced technician or by the taxonomic expert. If completed by the taxonomic expert, this check is easier if incorporated into the advanced identifications as described above (Advanced Identifications: Identifying Mites). If completed by an experienced technician after the advanced identifications are completed, follow the instructions below.

Species Identifications to Check:

Achipteria coleoptrata Achipteria sp. 1 DEW Aeroppia sp. 1 DEW Astegistes sp. 1 DEW Atropacarus striculus Banksinoma lanceolata canadensis Banksinoma spinifera Camisia biurus *Camisia biverrucata* Camisia sp. 1 DEW Carabodes granulatus Carabodes labyrinthicus *Carabodes* polyporetes *Ceratoppia bipilis* Ceratoppia quadridentata arctica *Ceratozetes* gracilis Chamobates cuspidatus Dentizetes ledensis Dentizetes rudentiger Diapterobates humeralis Dorvcranosus acutidens Epidamaeus sp. 2 DEW Euphthiracarus flavus *Exochocepheus eremitus* Fuscozetes fuscipes Gustavia sp. 1 DEW *Gymnodamaeus ornatus* Hafenferrefia sp. 1 DEW *Hafenferrefia* sp. 2 DEW Heminothrus longisetosus Hermanniella robusta *Hypochthonius luteus Hypochthonius rufulus* Lucoppia burrowsii Mainothrus badius Mucronothrus nasalis *Mycobates incurvatus* Nanhermannia sp. 1 DEW *Neogymnobates luteus* Neonothrus humicola *Neoribates* sp. 1 DEW

Neoribates sp. 2 DEW Oribatella jacoti Oribatodes mirabilis *Oribatula* sp. 1 DEW *Platynothrus peltifer* Pelopsis bifurcatus Peloptulus sp. 1 DEW Peloribates pilosus *Pergalumna* sp. 1 DEW Phthiracarus borealis Phthiracarus boresetosus Phthiracarus validus *Platynothrus peltifer* Platynothrus yamasakii Podoribates longipes Propelops alaskensis Propelops canadensis Propelops sp. 3 DEW Protoribates haughlandae Protoribates robustior Punctoribates palustris Rhysotritia ardua Roynortonella gildersleeveae Roynortonella sp. 1 DEW Scheloribates laevigatus Scheloribates pallidulus Scutozetes lanceolatus *Tectocepheus sarekensis* Tectocepheus velatus Tegoribates americanus Unduloribates dianae

- Obtain the list of species identifications to check from the taxonomic expert. Export data from the portal and use the data sort option to sort the rows first alphabetically by genus and species, then numerically by increasing site number.
- For the first species on the list count the total number of vials identified as containing that species in the datasheet, then use a random number generator (<u>www.random.org</u>) to select five of the vials at random. For species with five or fewer vials, check all the vials.
- Pull the specified vials from the vial racks and one at a time empty the vials into a small petri dish containing ethanol and check their contents under a 7.1-115x dissecting microscope.
- If all five vials contain the indicated species, and no additional species, replace the vials and move to the next species on the list. If one or more of the vials do not contain the indicated species, use the random number generator to select five more vials and check those as well. Repeat this process until no new identification errors have been found in a set of five vials, or until all the vials have been checked. Replace all vials when finished and move to the next species on the list.
- While checking the vials, maintain a list of which vials were checked for each species and what the outcome was for each vial (Appendix 5). In case of any identification errors, record the correct species identification and change the information in the sorting datasheet and on the vial label. Once the vial check has been completed send an electronic copy of the list to the taxonomic expert. The taxonomic expert may also ask for a more detailed error rate report.

Consolidating Vials

- Before the oribatid mites from a field season can be vouchered, all the vials still containing mites must be consolidated and ordered in racks numerically by site number. In the portal, select the Export option on the top left navigation bar, and filter to the appropriate year to download the most recent copy of the sorting data for that year. This should download as an Excel spreadsheet: sort the rows first numerically by increasing site number, then alphabetically by subsite, and then by Original Vial Location to maintain the original order of the vials within each site.
- Transfer all the vials that still contain mites from their original vial racks to new vial racks so they are arranged numerically by site number, then alphabetically by subsite within each site, and then by original vial location to keep the vials in the same order as they were sorted. ABMI sites are ordered first followed by any ALPAC sites and then any OG sites. Vials are placed in the vial racks from the top left corner to the bottom right corner column by column. Racks are numbered sequentially as follows: year-rack number (e.g. 2012-04 is the fourth rack of mites from the 2012 field season).
- While transferring the vials, visually check any vials that are indicated as empty in the database, or that have a black x drawn on the lid by the taxonomic expert, to make sure they are actually empty, then set them aside. All empty vials must be kept until vouchering has been completed in case any problems occur (see **Troubleshooting** below).
- As the vials are placed into their new racks keep the labels on the outside of the vials but remove the numbers from the vial lids. Vials should be placed in the new vial racks with the original sorting label on the outside of the vial facing the front of the vial rack.
- Once all the vials have been placed in new racks, re-number the vial lids using the numbered circular stickers. The vials in each rack are numbered from one to ninety-six from the top left corner to the bottom right corner column by column. Each vial is also assigned a new consolidated vial ID as follows: year-rack number-vial number (i.e. 2012-04-15 would be the fifteenth vial in the fourth rack of mites from the 2012 field season). These new Vial ID numbers are then incorporated into the final vouchering label as the last line of data.
- For each vial rack, tape a label on the left side of the rack (Comic Sans 11pt) that indicates the rack number and year (e.g 2012-04), and a label on the front of the rack (Times New Roman 10pt) that indicates the rack number and ABMI sites by vial. The front label should note when a site continues into the next rack, for example:

2012-04 Sites 136 (01-03), 154 (04-12), 163 (13-35), 164 (36-54), 165 (55-74), 182 (75-94), 192 (95-96 continued 2012-05 vials 01-06)

Rack labels are printed on standard white paper using a laser printer and are taped onto the rack using a strip of scotch tape that covers the entire label. The scotch tape should be trimmed so there is minimal overhang (Figure 3). Racks are numbered sequentially by increasing site number.

Vouchering

After the vials have been consolidated, vials and slides are designated as vouchers or duplicates. One slide or vial is chosen as a voucher for each species for each ABMI site that is sampled each year. All other slides and vials are designated as duplicates. When slides have been made they are chosen preferentially over vials as vouchers. When multiple slides have been made for one species at one site, the first slide in the database (alphabetically by subsite) is chosen as the voucher unless that slide is damaged or poorly mounted. As part of the vouchering process all slides and vials are assigned a TMS number for entry into the RAM database. Vouchering is typically done by an experienced lab technician.

• In the data portal, click on the appropriate year in the left navigation bar to select all of the sorting data for the year. Select the Voucher option along the top of the data entry window; additional vouchering data entry columns should appear (eg. 'Voucher Date', 'Voucher TMS Number', 'Voucher Location'). Click on Advance Sort along the top of the data entry window to sort the rows first alphabetically by genus and species, then numerically by site and alphabetically by subsite.

- Starting alphabetically with the first species in the database, select the voucher for the first site. For each site the first entry for a species is typically chosen as a voucher unless the first entry is a vial when a slide is also present, or when the specimens are in poor condition or cannot be found.
- Once the voucher has been selected, fill out the appropriate data columns. In the 'Voucher Person' column put the initials of the person who selected the voucher. In the 'Voucher Date' column put the date that the voucher was selected. The 'Number Voucher Specimens' is the number of individual mites on the slide or in the vial that was selected as the voucher. The 'Voucher Location' is indicated as 's' for slides and 'v + vial ID' for vials (e.g. v 2012-14-66). For vials use the consolidated vial ID not the original vial ID. The 'Voucher TMS number' is an eight digit number starting with an M (e.g. M00010834). When beginning the vouchering process start with the first available TMS number that has not been previously assigned to a specimen, and assign TMS numbers sequentially to both vouchers and duplicates.
- TMS numbers are automatically generated through the online Mite Portal. To update the desired starting TMS number in the sequence, first select the 'More' dropdown, then 'Update TMS Sequence'. Beside the corresponding year put in the starting number of the desired TMS sequence. Then select 'Update'. The TMS numbers should auto generate as voucher and duplicate information is input into the data portal. Care should be taken to ensure that numbers are not skipped by refreshing the page to ensure all data is input correctly after each entry.
- After the voucher has been selected for one species at one site, designate all other slides and vials of that species at that site as duplicates and fill out the 'Dup' columns (located in the bottom right window of the Mite Data Portal; entitled 'Duplicated Voucher Specimen') in the database similar to above. The 'Voucher Person' and 'Voucher Date' columns are filled out for duplicates as well as vouchers. There are situations where there can be multiple duplicates present in a line of data (e.g. multiple slides or a combination of slides and vials from one subsite). A new line should be made for each duplicate slide and/or vial in the 'Duplicated Voucher Section'. The 'Duplicate Number' column should be used to note the number of duplicates present (i.e., the first duplicate slide or vial should have a 1 placed in the column, the next duplicate vial or slide should have a 2 placed in the column, and so on for each duplicate present).
- If an entry row contains only a voucher or only a duplicate, fill out either the voucher or duplicate columns for that row as applicable. <u>Note that if only a duplicate is present for the row of data, the 'Voucher Person' and 'Voucher Date' columns must be manually filled in for that data on the main window</u>. If an entry row contains both a voucher and a duplicate, fill out both the voucher columns in that row and duplicate columns in the bottom right window (titled 'Duplicated Voucher Specimens'). An example of a situation with multiple duplicates would look something like this:

For site 1440 there are two entry lines for Anachipteria howardi:

Row 1 (NE): one slide (1 mite) = voucher

Row 2 (NW): one slide (2 mites) + one vial (3 mites) = two duplicates The information for the two duplicates is entered into the data portal window called 'Duplicated Voucher Specimens' as follows:

'Duplicate TMS Number': M00010953
'Number Duplicate Specimens': 2
'Duplicate Location': s
'Duplicate Number': 1
'Duplicate TMS Number ': M00010954
'Number Duplicate Specimens': 3
'Duplicate Location ': v 2012-16-86
'Duplicate Number': 2

- While filling out the voucher/duplicate information in the data portal, find the actual slides and vials in the collection that correspond to the entries in the database and set them aside for re-labeling. It is best to create the new voucher and duplicate labels while filling out the database to avoid confusion. The most efficient method is as follows:
 - 1. Using the database as a reference choose a species then find and pull all vials and slides containing that species from the collection. Line up the vials and slides in order by site and subsite. Find the vials

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by looking through the vial racks by site and subsite and checking the vial labels. Find the slides by looking in the slide box for the selected species and checking the slide labels.

- 2. Start going through the entries in the database for the species, checking the information in the database against the vials and slides to make sure they are all accounted for.
- 3. Set out three vial racks (80 vials/rack). Fill these racks with vials and slides as they are checked against the database and designated as vouchers and duplicates. Place the vials in the racks in order, leave spaces for slides, and pile the slides in order to one side of the rack.
- 4. As each vial or slide is designated as a voucher or duplicate and placed in the rack, fill in the data portal columns.
- 5. Once all three racks have been filled, print the labels for all the vials and slides in the racks, re-label them, and return them to the collection. Once all vials and slides have been returned to the collection, repeat the process with the next species on the list.
- Vouchers and duplicates must be re-labeled once designated. Each slide or vial receives a new label that includes the TMS number, modified RAM lot number, latitude/longitude, collection date and collector initials, determiner, species name, and the designation 'Oribatid Voucher' or 'Oribatid Duplicate':

Oribatid Voucher

TMS: ABMI.M00010863 CAN.AB.ABMI.491 NW 57.17693/-117.56903 Coll: 24-May-2012 AHI Det: Dave Walter Achipteria sp. 1 DEW

To print labels, select the drop-down option 'More' along the top of the data entry window, and click on "Create Voucher Labels". A new PDF document will open with all the voucher labels entered thus far for that year. Select the labels needed, or copy/paste the required set of labels into a Microsoft Word document if any re-formatting or editing is required. The finished voucher labels are then printed, in the style of the template provided, onto 2.5x1.9cm sticky labels (Appendix 4, Verdana 4pt). For vials the new label is attached to the outside of the vial over top of the original sorting label and just below the consolidated vial ID number. It is placed vertically with the left edge of the label facing the bottom of the vial. For slides the new label is placed vertically against the right edge of the slide with the right edge of the label facing the bottom of the slide (Figure 5).



Fig 5. Vouchered vial and slide showing positioning of voucher labels.

- Continue to work through the data portal row by row designating vouchers and duplicates and adding new labels to the vials and slides until the end of the database is reached. This process typically takes one to two months to complete (see Troubleshooting below for common problems). Once the end of the database is reached a sweep of all vials must be done to look for any that were missed (see Missing Specimens below).
- Upon completion of the vouchering process ensure that all vials and slides are returned to their racks and boxes, that lids are added to all vial racks, and that all vial racks and slide boxes are moved to their permanent storage location in the ethanol collections room at the RAM.
- Verbally explain any persistent issues with the voucher data (see **Troubleshooting** below). Once the taxonomic expert has checked the database and any remaining issues have been resolved, inform the Information Center that vouchering is complete so that they are aware that the mite data has changed.

Troubleshooting

There are several common problems that may occur during the vouchering process, including multiple species, mites sent for SEM or DNA barcoding, data discrepancies, new data entries, and missing specimens. The protocols for resolving these problems are outlined below.

Multiple Species

- Sometimes a single slide or vial will contain mites belonging to two different species, and there will be two entry rows in the database for the slide or vial, one for each of the species. It is easiest to fill out both of these rows at the same time. In the 'Voucher Comments' column in both lines make the note: 'two species on one slide' or 'two species in one vial'.
- When there are two species on one slide or in one vial, the slide or vial receives one label with the information for both species. This means that the label will have two TMS numbers and the names of both species but will otherwise be the same as a normal label. For example:

Oribatid Voucher TMS: ABMI.M00011034/ABMI.M00011035 CAN.AB.ABMI.OG-1558 NE 50.061662/-111.540351 Coll: 11-Jun-2012 KWA Det: Dave Walter *Camisia biverrucata/Camisia horrida*

- For vials print a single copy of the label, attach it vertically to the outside of the vial over top of the original sorting label as typical, and return the vial to the collection.
- For slides print two copies of the label. The first label is placed along the right edge of the slide as typical and the slide is filed in the collection under the first species name on the label. Using a microscope slide as a template cut a slide shaped piece from a file folder. The second label is placed along the right edge of the file folder piece as it would be on a slide, and the location of the actual slide is written on the center of the file folder piece (Figure 6). This file folder piece is then filed in the collection under the second species name on the label as if it were an actual slide so that someone looking for the slide will be able to find it.



Fig 6. Labeled file folder piece showing positioning of labels.

• Sometimes a single slide or vial will contain mites from more than two species. The same steps as above are followed in the case of more than two species.

Mites Sent for SEM or DNA Barcoding

- Sometimes all the mites in a vial will have been sent for SEM or DNA barcoding, so while there is an entry row in the data base there are no actual specimens left to designate as vouchers or duplicates. Leave the voucher database columns empty, including the 'Voucher Person' and 'Voucher Date' columns. In the 'Voucher Comments' column make the note: 'no voucher (or duplicate), all to SEM (or DNA barcoding)'.
- Occasionally vouchered and/or duplicate specimens are sent out for DNA barcoding. Specimens that are sent back to the RAM from barcoding should be slide mounted for ID verification and put into the slide collection. The label for these specimens is similar to the labels made for vouchered specimens except for an added line with the DNA barcoding information. This line should be formatted in red font on the label. The finished label is then printed, in the style of the template provided, onto 2.5x1.9cm sticky labels (Appendix 4, Verdana 4pt) and attached to the slide. For example:

Oribatid Duplicate TMS: ABMI.M00022507 CAN.AB.ABMI.628 SW 56.837563/-115.345043 DNA: CCDB-16567 F01 Coll: 29-May-2016 LHA Det: Lisa Lumley Pergalumna sp. 1 DEW

Data Discrepancies

- Sometimes not all of the information on the slide or vial label will correspond with the information in the sorting database. The most common issue is a discrepancy in the subsite (e.g. the database says 459 NW but the vial label says 459 NE). Discrepancies in the number of mites or the species name may also occur.
- The information in the database is taken as correct unless there is a specific reason why the database is thought to be wrong (e.g. the database says *Achipteria coleoptrata* while the vial says *Achipteria* sp. 1 and a check of the vial contents confirms the mites are *Achipteria* sp. 1 so the database is wrong). Make the correction to the new vouchering label or the database information as required. In the 'Voucher Comments' column make a note of what the discrepancy was and what change was made to fix it (e.g. vial originally labeled NE, changed to NW).

New Data Entries

- Sometimes a slide or vial may be found in the collection that does not have a corresponding entry in the database. This most often occurs for vials that are found when sweeping for missed vials at the end of the vouchering process (see Missing Specimens below).
- For these vials or slides check the contents to verify the original sorting labels then add the information from the labels to the database as new entry rows and fill out the voucher columns. In the 'Voucher Comments' column make the note: 'vial (or slide) not originally in spreadsheet'. Create voucher or duplicate labels for these slides or vials, re-label them, then return them to the collection.

Missing Specimens

- Sometimes the slide or vial that corresponds to an entry in the database cannot be found. The first step is to double check all the vials for the site and all the slides for the species because slides may be mislabeled as vials or vice versa, or slides and vials may be mislabeled as different subsites or species. If, after double checking, the slide or vial still cannot be found, in the 'Voucher Comments' column make the note: 'slide (or vial) not found'. It is best to deal with all missing specimens once vouchering has been completed since they may show up during the vouchering process.
- Once vouchering has been completed and all slides and vials have been returned to the collection, return to any entry lines with comments that indicate as missing and use the following steps to fix as many problems as possible.
- Systematically check all vials looking for vials that have not been given a new voucher or duplicate label and pull these from the collection. Check the labels and contents of these vials. In many cases there will be labeling errors and these will be some of the missing vials. For these vials fill in the voucher columns in the database and create new and corrected voucher or duplicate labels. After relabeling the vials return them to the collection, then remove the comments from the row in the database. Sometimes vials are found that do not correspond to any line in the database (see **New Data Entries** above for what to do).
- If there are still vials missing go through the empty vials that were removed from the vial racks during the consolidation process and look for any vials that still contain mites. Check the contents and labels of these vials. For vials that correspond to the missing entries in the database, fill in the voucher columns in the database and create new voucher or duplicate labels. These vials will be placed at the end of the last vial rack and must be labeled accordingly (add new lid and consolidated vial ID numbers). The label on the vial rack must also be changed to reflect the addition of these vials. Once the vials have been labeled and placed in the collection, remove the comments from the corresponding rows in the database.

- For any missing slides double check the slide boxes for that species as well and any other species in the genus as slides may be mislabeled or misfiled. Also check to make sure that no slides have been left on any slide trays or in the drying ovens. If missing slides are found, fill in the voucher columns in the database and create new voucher or duplicate labels. Once the slide has been labeled and placed back in the collection, remove the comments from the corresponding rows in the database.
- After every effort has been made to locate all missing slides and vials there may still be some vials and slides that cannot be found. It is not uncommon for up to five vials or slides per year to still be missing. These specimens may have been taken for SEM or DNA barcoding without record, been misplaced in the collection, been lost during transfer to the slide or vial, or may simply be data entry errors. For the slides and vials that cannot be found, ensure that there is comment in the 'Voucher Comments' section that indicates that 'no slide or vial found during vouchering'. These rows may be removed from the database at the discretion of the taxonomic expert.

Appendix 1: Sample Tracking Log

In the Sample Tracking Log a separate worksheet is used for logging in each type of field sample. The example below shows only the worksheet that is used for the receiving and processing of organic soil. The columns have been filled in to demonstrate the formatting.

Page	1	of 2
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Project	Year	Sample Code	Date Received	Sample Type	Field Comments	Site	Field Date	Crew	Quadrant
ABMI	2012	OS	29-May-2012	Organic Soil		4	25-May-2012	ESC	NE
ABMI	2012	OS	29-May-2012	Organic Soil		4	25-May-2012	ESC	NW
ABMI	2012	OS	29-May-2012	Organic Soil		4	25-May-2012	ESC	SE
ABMI	2012	OS	29-May-2012	Organic Soil		4	25-May-2012	ESC	SW
ABMI	2012	OS	29-May-2012	Organic Soil	No data collected. quadrant in open water	5	27-May-2012	JTH	NE
ABMI	2012	OS	29-May-2012	Organic Soil	dry area on an outcropping of granite shield rock	5	27-May-2012	JTH	NW
ABMI	2012	OS	29-May-2012	Organic Soil	Quadrant in open water. Tree stage is a glitch, cant delete it.	5	27-May-2012	JTH	SE
ABMI	2012	OS	29-May-2012	Organic Soil	quadrant in open water. tree stage is a glitch	5	27-May-2012	JTH	SW
ABMI	2012	OS	29-May-2012	Organic Soil		17	27-May-2012	ESC	NE
ABMI	2012	OS	29-May-2012	Organic Soil		17	27-May-2012	ESC	NW
ABMI	2012	OS	29-May-2012	Organic Soil		17	27-May-2012	ESC	SE
ABMI	2012	OS	29-May-2012	Organic Soil		17	27-May-2012	ESC	SW
ABMI	2012	OS	29-May-2012	Organic Soil		35	26-May-2012	TWI	NE
ABMI	2012	OS	29-May-2012	Organic Soil		35	26-May-2012	TWI	NW
ABMI	2012	OS	29-May-2012	Organic Soil		35	26-May-2012	TWI	SE
ABMI	2012	OS	29-May-2012	Organic Soil		35	26-May-2012	TWI	SW

Page 2 of 2

Collection Status	Extractor Number	Extractor Start Date	Extractor End Date	Sample Removed	Lab Comments
С	94	29-May-2012	5-Jun-2012	5-Jun-2012	
С	95	29-May-2012	5-Jun-2012	5-Jun-2012	
С	96	29-May-2012	5-Jun-2012	5-Jun-2012	
С	97	29-May-2012	5-Jun-2012	5-Jun-2012	
VNA					
С	98	29-May-2012	5-Jun-2012	5-Jun-2012	
VNA					
VNA					
	99	29-May-2012	5-Jun-2012	5-Jun-2012	No electronic file sent for site 17
	100	29-May-2012	5-Jun-2012	5-Jun-2012	No electronic file sent for site 17
	101	29-May-2012	5-Jun-2012	5-Jun-2012	No electronic file sent for site 17
	102	29-May-2012	5-Jun-2012	5-Jun-2012	No electronic file sent for site 17
None					
С	103	29-May-2012	5-Jun-2012	5-Jun-2012	
None					
С	104	29-May-2012	5-Jun-2012	5-Jun-2012	

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Appendix 2: ABMI Mites Data Portal - Field Status Entry The Field Status Entry is the online version of the Sample Tracking Log that populates the Mites Data Portal (Appendix 3) with field information.

	Field	Status								
	< 1 leid	olulus								
				Excel Download		atus For Year 2	240			
	2		—	Excel Download		alus For Year 2	118			
Year	Year	Site	Quadra	n Date	Crew	Collection Sta	Field Comment	Lab Comment	≜ Tablet Date	Tablet Crew
2008				17.1						J. M. J.
2009	2010	1244	INVV	01-MdA-5010	JUAZ	L				
2010	2018	1244	SW	31-May-2018	JCA2	С				
2011	2018	1244	NE	31-May-2018	JCA2	С	Very rocky, cereal crop just germinated			
2012	2018	1244	SE	31-May-2018	JCA2	С				
2013	2018	1245	NE	27-May-2018	JBO	с	Tame pasture			
2014	2018	1245 1245	SE	27-May-2018	JBO JBO	c c	Tame pasture, tame			
2015	2018	1245	SW	27-May-2018	JBO	c	tame pasture			
2016	2018	1245	NW	27-May-2018 26-May-2018	SBO	c	Juniper dominates about 50 percent of canopy over soil survey area			
2017	2018	1268	SW	26-May-2018	SBO	c	Survey was on streambank [different than creek] downslope [toe] from recently harvested			
2018	2018	1268	SE	26-May-2018	SBO	c	Dense patches of tall Sw and dense patches of short Sw, canopy is variable and trees that			
2019	2018	1294	NE	27-May-2018	SMC	c	bene patenes of can sw and dense patenes of shore sw, canopy is variable and nees that			
	2018	1294	NW	27-May-2018	SMC	c				
	2018	1294	SE	27-May-2018	SMC	c	Sw and PI mix, 5 cores required to get enough LFH [less than 500ml after first 4]			
	2018	1295	NE	28-May-2018	DEN	с	close to cutblock to east, extra light. deep lfh			
	2018	1295	SE	28-May-2018	DEN	с	reforested cutblock, replanted trees around 5m height.			
	2018	1295	sw	28-May-2018	DEN	с	lone spruce tree above sample location also			
	2018	1295	NW	28-May-2018	DEN	с				
	2018	1296	SE	29-May-2018	AWI2/JMA3	с	Clearcut block, planted with pine about 5 years ago. Area mostly covered with labrador tea			
	2018	1296	SW	29-May-2018	AWI2/JMA3	с	No mineral collected because organic depth greater than 50cm. Bottom of core #3 partially			
	2018	1296	NW	29-May-2018	AWI2/JMA3	с				
	2018	1296	NE	29-May-2018	AWI2/JMA3	с	Cut block,planted with pine about 5 years ago			
	2018	1320	NE	19-Jul-2018	NVE	с				
	2018	1320	NW	19-Jul-2018	NVE	с	Vegetated base of scree slope. Very rocky. Could only get a tiny bit of LFH with each samp			
	2018	1320	SE	19-Jul-2018	NVE	С				
	2018	1320	SW	19-Jul-2018	NVE	С				
	2018	1323	sw	30-May-2018	DEN	с	Mix of fir, Sb, Pl and Sw.			
	2018	1323	SE	30-May-2018	DEN	с	Located at hole in canopy. Some Sb but mostly young and old firs. No dec.			
	2018	1323	NE	30-May-2018	DEN	С				
	2018	1323	NW	30-May-2018	JMA3	С				
	2018	1324	NE	31-May-2018	DEN	С	mostly mix of sw and pl, some aw			
	2018	1324	NW	31-May-2018	DEN	с				
						-	· · · · · · · · · · · · · · · · · · ·			

Appendix 3: ABMI Mites Data Portal - Specimen Information and Identification The Mites Data Portal allows for online entry of the oribatid mite data.

C 🔒 new-p	ortal.abmi.ca/da	taentry,	/soilmite																			☆ 🧠	*
<	Specimen Infor	mation N	lame:Lisa Lumley on 2018 1229	•																			
	2 Protect Gr	d 🦉	🀬 💮 😌 🕤 Speci	es ID Vouch	er Location	More 👻	Lisa Lumley or	n 2018 1229															
Export	Year Site	Quad	Advanced ID	qualifier		Advanced ID	Advanced ID Person	Advanced Date	ID Lab Commen	TSN	Original Vi Location			Total Slide	Voucher Person	Voucher Date	Voucher Tms	Number Voucher	Voucher Location	new Vial Location	Voucher Comments	Date Entered	Resi Loca
'ears						Comments	Person	Date	Commen	5	Location			Num		Date	Number	Specimens	Location	Location	Comments	In Tms	LUCa
D							~		~						~								
	2018 1229	NE	Epidamaeus sp. 5 DEW		1		Lisa Lumlev	2019-02-28	1	99000017	N/A		1/	/1 \	/ictoria Giacobbo	2020-08-1	M00036460	1	s				
5	2018 1229		Trhypochthonius tectorum	d	1		Lisa Lumley	2018-12-06		736713	N/A				victoria Giacobbo				s				
5	2018 1229		Ceratoppia quadridentata arctic	-	3		Victoria Giacobbo			99000064	M-VGI-08-8	1 3			/ictoria Giacobbo				v2018-11-26	2018-11-26			
3	2018 1229		Propelops canadensis	-	2		Victoria Giacobbo			741504	M-VGI-08-8				victoria Giacobbo				v2018-11-27				
028	2018 1229		Eueremaeus foveolatus		7		Lisa Lumley	2018-12-13		738580	M-VGI-08-8				victoria Giacobbo				s	2018-11-28			
062	2018 1229		Parachipteria sp. 1 DEW		10		Lisa Lumley	2019-04-09		99000096	M-VGI-08-8				/ictoria Giacobbo				s	2018-11-29			
166	2018 1229		Oribatella yukonensis		3		Lisa Lumley	2019-04-12		99000038	M-VGI-08-8		-		/ictoria Giacobbo				s	2018-11-30			
167	2018 1229		Cepheus sp. 1 DEW		1		Lisa Lumley	2019-06-12		99000007	M-VGI-08-8				victoria Giacobbo				v2018-11-31				
181	2018 1229		Eupterotegaeus rostratus	cf	2		Lisa Lumley	2019-04-10		99000086	M-VGI-08-8		1/		/ictoria Giacobbo				s	2018-11-32			
182	2018 1229		Pleodamaeus plokosus		2		Lisa Lumley	2019-04-10			M-VGI-08-8		-1		/ictoria Giacobbo				s	2018-11-33			
183 198	2018 1229		Epidamaeus sp. 5 DEW		- 3 A	Amalgamate		2019-02-28			M-VGI-08-8				victoria Giacobbo			-		2018-11-34			
198	2018 1229		Eueremaeus tetrosus		1		Lisa Lumley	2018-12-13		738597	N/A				victoria Giacobbo			1	s				
200	2018 1229		Eueremaeus foveolatus		12		Lisa Lumley	2018-12-13		738580	M-VGI-08-9	0 10			/ictoria Giacobbo			-		2018-11-35			
213	2018 1229		Eupterotegaeus rostratus	cf	3		Lisa Lumley	2019-04-10		99000086	M-VGI-08-9				/ictoria Giacobbo					2018-11-36			
214	2018 1229		Diapterobates humeralis		1		Victoria Giacobbo			742211	M-VGI-08-9				/ictoria Giacobbo			1	v2018-11-37				
215	23 rows loaded				-					,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,						2020 00 .		-		2010 11 0/			
228		cation:Fi	ueremaeus foveolatus														Duplicated Vo	ucher Specim	en				
229 230	-		I													`) 🕥 nu	r			
243																							
244	Advanced ID		Final Scientific Nar	ne Final Is	n Qualifier	ID Count		Advanced ID Person	Advanced ID Date	Reference	TSN Q/	A Code	QA Person	1 QA	Date QA Com	nents	Id Par Id	ent Duplicat Location	i Dup		plicate TMS mber	Duplicate Number	
266	Eueremaeus Mo	photype	2			12	١	/ictoria Giacol	2018-10-11		-1 Ver	ified Li	isa Lumley	2018	8-12-13		38287 1991	0 s	2	M00	036469	1	
267	Eueremaeus fov	eolatus				12	L	lisa Lumley	2018-12-13		738580						38288 1991	0 v2018-11	-35 10	M00	036470	2	
268							1	Lisa Lumley	2021-03-04								1991	10					
294																							
295																							
296																							
320 323																							
1323																							

Appendix 4: Organic Soil Processing Labels

Organic soil sample data is entered into the 'Sample Information' worksheet and the sample cup and residual labels are generated automatically. Micro vial labels are created separately using a sticky label template. Examples are shown below. The labels have been enlarged for easier viewing.

	Enter Sample Information			Enter Funnel Numbers
Organic Soil				
Lot:	ABMI.2012.	7 Day Extraction		
ABMI Site#:	1515	Residue (50 - 300 µm)		
Coll:	22-Jul-2012	Seived Sample (>300 µm)	NE	19
Funnel:	25-Jul-2012	Residue (>300 µm)	NW	20
Sorted:			SE	21
Det:			SW	22
Organic Soil				
Lot:	ABMI.2012.			
ABMI Site#:	1532			
Coll:	22-Jul-2012		NE	23
Funnel:	25-Jul-2012		NW	24
Sorted:			SE	25
Det:			SW	26
Organic Soil				

Sample Information Worksheet

Sample Cups Labels (Verdana 8pt)

Organic Soil	Organic Soil	Organic Soil	Organic Soil				
Lot: ABMI.2012.1515NE.OS	Lot: ABMI.2012.1515NW.OS	Lot: ABMI.2012.1515SE.OS	Lot: ABMI.2012.1515SW.OS				
ABMI Site#: 1515 NE	ABMI Site#: 1515 NW	ABMI Site#: 1515 SE	ABMI Site#: 1515 SW				
Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012				
Funnel: 19, 25-Jul-2012	Funnel: 20, 25-Jul-2012	Funnel: 21, 25-Jul-2012	Funnel: 22, 25-Jul-2012				
7 Day Extraction	7 Day Extraction	7 Day Extraction	7 Day Extraction				
Organic Soil	Organic Soil	Organic Soil	Organic Soil				
Lot: ABMI.2012.1532NE.OS	Lot: ABMI.2012.1532NW.OS	Lot: ABMI.2012.1532SE.OS	Lot: ABMI.2012.1532SW.OS				
ABMI Site#: 1532 NE	ABMI Site#: 1532 NW	ABMI Site#: 1532 SE	ABMI Site#: 1532 SW				
Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012				
Funnel: 23, 25-Jul-2012	Funnel: 24, 25-Jul-2012	Funnel: 25, 25-Jul-2012	Funnel: 26, 25-Jul-2012				
7 Day Extraction	7 Day Extraction	7 Day Extraction	7 Day Extraction				

50-300µm Residual Labels (Verdana 6pt)

Organic Soil	Organic Soil	Organic Soil	Organic Soil
Lot: ABMI.2012.1515NE.OS	Lot: ABMI.2012.1515NW.OS	Lot: ABMI.2012.1515SE.OS	Lot: ABMI.2012.1515SW.OS
ABMI Site#: 1515 NE	ABMI Site#: 1515 NW	ABMI Site#: 1515 SE	ABMI Site#: 1515 SW
Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012
Funnel: 19, 25-Jul-2012	Funnel: 20, 25-Jul-2012	Funnel: 21, 25-Jul-2012	Funnel: 22, 25-Jul-2012
Residue (50 - 300 µm)	Residue (50 - 300 µm)	Residue (50 - 300 μm)	Residue (50 - 300 μm)
Organic Soil	Organic Soil	Organic Soil	Organic Soil
Lot: ABMI.2012.1532NE.OS	Lot: ABMI.2012.1532NW.OS	Lot: ABMI.2012.1532SE.OS	Lot: ABMI.2012.1532SW.OS
ABMI Site#: 1532 NE	ABMI Site#: 1532 NW	ABMI Site#: 1532 SE	ABMI Site#: 1532 SW
Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012
Funnel: 23, 25-Jul-2012	Funnel: 24, 25-Jul-2012	Funnel: 25, 25-Jul-2012	Funnel: 26, 25-Jul-2012
Residue (50 - 300 μm)	Residue (50 - 300 µm)	Residue (50 - 300 μm)	Residue (50 - 300 µm)

>300µm Residual Labels (Verdana 6pt)

Organic Soil	Organic Soil	Organic Soil	Organic Soil
Lot: ABMI.2012.1515NE.OS	Lot: ABMI.2012.1515NW.OS	Lot: ABMI.2012.1515SE.OS	Lot: ABMI.2012.1515SW.OS
ABMI Site#: 1515 NE	ABMI Site#: 1515 NW	ABMI Site#: 1515 SE	ABMI Site#: 1515 SW
Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012
Funnel: 19, 25-Jul-2012	Funnel: 20, 25-Jul-2012	Funnel: 21, 25-Jul-2012	Funnel: 22, 25-Jul-2012
Sieved Sample (>300 μm)	Sieved Sample (>300 µm)	Sieved Sample (>300 µm)	Sieved Sample (>300 µm)
Organic Soil	Organic Soil	Organic Soil	Organic Soil
Lot: ABMI.2012.1532NE.OS	Lot: ABMI.2012.1532NW.OS	Lot: ABMI.2012.1532SE.OS	Lot: ABMI.2012.1532SW.OS
ABMI Site#: 1532 NE	ABMI Site#: 1532 NW	ABMI Site#: 1532 SE	ABMI Site#: 1532 SW
Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012	Coll: 22-Jul-2012
Funnel: 23, 25-Jul-2012	Funnel: 24, 25-Jul-2012	Funnel: 25, 25-Jul-2012	Funnel: 26, 25-Jul-2012
Sieved Sample (>300 μm)	Sieved Sample (>300 µm)	Sieved Sample (>300 µm)	Sieved Sample (>300 µm)

Sorting and Vouchering Micro Vial Labels (Verdana 4pt)

Oribatida	Oribatida	Oribatida	Oribatida	Oribatida
ABMI.2012.491.M	ABMI.2012.491.M	ABMI.2012.491.M	ABMI.2012.491.M	ABMI.2012.491.M
Site: 491 NE	Site: 491 NW	Site: 491 NW	Site: 491 NW	Site: 491 NW
Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI
Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL
Achipteria sp. 1 x 1	Euphthiracarus x 1	Achipteria sp. 1 x 6	Pilogalumna x 1	Ceratozetes gracilis x 1
Vial: M-SPL-01-01	Vial: M-SPL-01-02	Vial: M-SPL-01-03	Vial: M-SPL-01-04	Vial: M-SPL-01-05
Oribatida	Oribatida	Oribatida	Oribatida	Oribatida
ABMI.2012.491.M	ABMI.2012.491.M	ABMI.2012.491.M	ABMI.2012.491.M	ABMI.2012.491.M
Site: 491 SE	Site: 491 SE	Site: 491 SE	Site: 491 SE	Site: 491 SW
Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI	Coll: 24-May-2012 AHI
Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL	Sort: 5-Jun-2012 SPL
Euphthiracarus x 10	Achipteria sp. 1 x 12	Ceratozetes gracilis x 1	Damaeidae x 1	Euphthiracarus x 5
Vial: M-SPL-01-06	Vial: M-SPL-01-07	Vial: M-SPL-01-08	Vial: M-SPL-01-09	Vial: M-SPL-01-10
Oribatid Voucher	Oribatid Voucher	Oribatid Voucher	Oribatid Voucher	Oribatid Voucher
TMS: ABMI.M00010832	TMS: ABMI.M00010833	TMS: ABMI.M00010834	TMS: ABMI.M00010835	TMS: ABMI.M00010836
CAN.AB.ABMI.84 SE	CAN.AB.ABMI.445 NE	CAN.AB.ABMI.477 NW	CAN.AB.ABMI.493 SE	CAN.AB.ABMI.829 SE
59.45861/-114.16654	57.10264/-112.58428	56.86831/-111.96440	57.08706/-116.83402	54.67247/-110.54654
Coll: 11-Jun-2012 KTE	Coll: 30-May-2012 ANI	Coll: 25-May-2012 MMA2	Coll: 29-May-2012 GGI	Coll: 24-May-2012 JSA
Det: Dave Walter	Det: Dave Walter	Det: Dave Walter	Det: Dave Walter	Det: Dave Walter
Achipteria coleoptrata	Achipteria coleoptrata	Achipteria coleoptrata	Achipteria coleoptrata	Achipteria coleoptrata
Oribatid Voucher	Oribatid Duplicate	Oribatid Duplicate	Oribatid Voucher	Oribatid Voucher
TMS: ABMI.M00010837	TMS: ABMI.M00010838	TMS: ABMI.M00010839	TMS: ABMI.M00010840	TMS: ABMI.M00010841
CAN.AB.ABMI.182 NE	CAN.AB.ABMI.182 NW	CAN.AB.ABMI.182 SE	CAN.AB.ABMI.272 SE	CAN.AB.ABMI.398 NW
58.56110/-110.64372	58.56110/-110.64372	58.56110/-110.64372	57.99360/-110.46628	57.73985/-118.07789
Coll: 9-Jun-2012 TWI	Coll: 9-Jun-2012 TWI	Coll: 9-Jun-2012 TWI	Coll: 13-Jun-2012 KBE	Coll: 29-May-2012 A2
Det: Dave Walter	Det: Dave Walter	Det: Dave Walter	Det: Dave Walter	Det: Dave Walter
Achipteria sp. 1 DEW	Achipteria sp. 1 DEW	Achipteria sp. 1 DEW	<i>Achipteria</i> sp. 1 DEW	Achipteria sp. 1 DEW

Appendix 5: Vial Check List

This is an example of the list that must be made during the vial check. The vials checked for each species are listed and any corrections are shown in red.

Achipteria coleoptrata

M-SPL-11-63 M-SPL-2-63 M-SAW-10-69 M-SAW-2-6 M-SPL-14-41

Achipteria sp. 1

M-SAW-1-56 M-SAW-1-14 M-SAW-7-92 M-SPL-16-13 M-SAW-6-83

Atropacarus striculus

M-SPL-2-75 M-SPL-13-87 M-SPL-1-49 M-SAW-5-96 M-SAW-6-15

Carabodes labyrinthicus

M-SPL-7-14 M-SPL-10-92 M-SPL-9-12 M-SPL-1-80 M-SAW-1-38

Ceratoppia quadridentata arctica

M-SPL-7-50 Ceratoppia bipilis M-SAW-3-67 M-SPL-7-57 M-SAW-2-19 M-SPL-11-78 M-SPL-10-87 M-SPL-10-60 M-SPL-5-72 M-SPL-9-75 M-SPL-7-12

Ceratozetes gracilis

M-SPL-14-85 M-SAW-1-64 M-SPL-4-92 M-SAW-6-27 M-SAW-7-30

Appendix 6: Ethanol Warning Label

This is an example of the label that must be adhered to boxes used to store the ethanol collection, including the oribatid mite collection and residuals.

