Texas A&M University's

Terrestial Parasite Tracker Procedure: Ectoparasite Vial Processing

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Background:

Ectoparasites are parasites that are collected from the external surface of a vertebrate (usually) host. These parasites can be collected through various methods. For example, the collector or specimen preparator may individually pick ectoparasites off selected hosts using forceps or other tools. Alternatively, collectors or specimen preparators may brush all or part of the hosts' body to collect the ectoparasites. Individual ectoparasites or brushings are often stored in vials or tubes, either dry, in ethanol, or some other preservative.

Oftentimes, ectoparasite specimens are stored without any identification or sorting. Recommendations moving forward are to sort through the ectoparasite samples, separating by type of ectoparasite and clearly marking the collection tube with collector name, type of ectoparasite, host information, and storage medium. A label containing the same information can also be placed inside the tube in the event that labels on the outside are damaged or removed. The collector or specimen preparator should always provide an electronic file with all relevant collection data (date of collection, locality, host taxonomy, host voucher information, etc.).

Below, we recommend a process for sorting and organizing ectoparasite collections for installation in natural history collections. Before beginning a similar process, please discuss with the intended repository in advance in order to follow preferred procedures.

Supplies Needed:

Tools and reagents recommended before processing ectoparasite collection tubes:

- Computer
- Barcodes (if used by natural history collection)
- Barcode Scanner (if used by natural history collection)
- Microscope/ Microscope Light
- Microscope slides or weigh boats
    - Note: Paper placed underneath the weigh boat makes it easier to move the boat while under the microscope
- Kimwipes (to wipe slides)

- Tube racks or plastic boxes (storage for collection tubes)
- Collection tubes (usually 2ml; NUNC or similar brand)
    Note: Try to reuse original ectoparasite collection tubes or vials when possible

- Ethanol (95% or higher) or some other preservative
    Note: Determine preservative with natural history collection
- Squirt bottle or plastic or disposable pipettor for ethanol or preservative
- Paper Towels/Napkins
- Sharpies (to label the outside of the plastic boxes)
- Collection Labeling Pen - Ultra fine point permanent marker (to label the outside of the collection tube)
- Plastic or Disposable Pipettors
- Paintbrush
- Forceps

Procedure:

1. Take the ectoparasite collection tube or vial and empty the contents into a weigh boat or onto a microscope slide (depending on preference)
    a. If the tube sample is dry, empty all or some of the contents directly into a weigh boat
    b. If the tube sample is wet, use ethanol (or storage medium) and a pipette to rinse the tube into the weigh boat or use a paint brush to pick up and place the ectoparasite into the weigh boat or onto a slide
    c. Before proceeding, make sure the tube or vial is empty
        i. Method 1: Use a paintbrush to wipe the inside of the tube to remove all specimens
        ii. Method 2: Place tube under microscope and zoom out in order to see the whole tube, then rotate the tube in order to see the sides, corner, and lid more closely

2. Examine contents under a microscope and sort
    a. If no ectoparasites are present, move to the next collection tube
        i. Throw out brushings and possibly the collection tube if it cannot be reused
    b. If ectoparasites are present:
        i. Use a paintbrush or other tool to separate ectoparasite contents into the different orders

Note: Each type of ectoparasite found (e.g., lice, mites, ticks, fleas, flies, other insects) should go into a separate tube and have a unique catalog or barcode number (if natural history collection uses barcodes)

ii. Place each ectoparasite order into different tubes (see Step 4)
iii. Label tubes (see Step 4)
iv. Assign a barcode

3. Prepare the Barcode Scanner by plugging the scanner into a computer or laptop USB port (specific step for work with TAMUIC; may apply to other collections)
    a. Wait for the 3 beeps before beginning use
    b. To use:
        i. Select cell in Excel sheet
        ii. Have your barcode out that you would like to scan
        iii. Press and hold the yellow button (a red + should appear)
        iv. Position the scanner to where the red + goes over the barcode
            Note: The scanner may have to be moved in different directions in order for it to pick up the barcode
        v. Once the barcode has been scanned there will be one beep, the red + will go away, and the barcode number will appear in the selected cell (see Step 5)

4. Once contents are ready to be placed into a new tube:
    a. Label the outside of the tube with a unique identifier (the collector's initials, preparation/catalog/museum voucher number, etc.) and the barcode number
        i. Example: JEL 122
            X1660717
    b. Fill the tube halfway (~1mL) with preferred preservative
    c. Place the barcode into the tube so the number is read from top to bottom

5. Once the tube is capped, place the tube in the box and enter the box name, cell location of the tube, and collector number in the spreadsheet
    a. Example:

Barcode	Box Name	Cell Location	Collector Number
X1660717	Light Ectos 3	I7	JEL 122

Parasite Tracker Procedure - Data Transfer to Spreadsheet

Background:

It is not necessary to have all host voucher information prior to processing, but it could be helpful. It is most useful to have host voucher information in an Excel data sheet. Host voucher information includes:

- Museum voucher information (museum code and catalog number), if available
- Voucher locality data (country, state, county, specific locality, latitude, longitude, elevation)
- Voucher collection data (day, month, year)
- Collector information (initials and catalog number)

Note: The collector or specimen preparator may have already indicated the number and type of parasites in a collection tube. If these specimens were used for research, the numbers and types of parasites may need to be updated in the spreadsheet to reflect any changes

Note: Fields and format may differ depending on natural history collection. Please discuss first with the natural history collection where ectoparasites will be deposited.

Procedure:

1. Verify Collector Number from other institution's databases/spreadsheets to the Collector Number entered at the time the barcode was assigned
    a. Example:

Barcode	Box Name	Cell Location	Collector Number
X1660717	Light Ectos 3	I7	JEL 122

2. Enter collecting date as a standard date, then day month year format, and then divided in separate columns, or follow the natural history collection preferred format
    a. Example:

Verbatim Collecting Date	Collecting Date	Start Date Month	Start Date Day	Start Date Year
5/1/2002	1 May 2002	May	1	2002

3. Enter Method of Identification (Method ID), Method Name, Collector, and Determiner (who identified the ectoparasites)
    Note 1: Enter last name first
    Note 2: Determiners name may be the same as the collector or different
    a. Example:

Method ID	Method Name	Collector	Determiner
Brushed from host	Dissecting Microscope	Light, J.E.	Fischer, S.F.

4. Identify the ectoparasites to the lowest taxonomic level possible
    a. If needed, add taxonomic authority (this may be best left to a taxonomic expert)
        i. Example: Johnson 1900
    b. Example:

ID	Name	Taxonomic Authority	*Genus* ID	*Species* ID
Fahrenholzia ehrlichi	*Fahrenholzia ehrlichi*	Johnson 1900	*Fahrenholzia*	*ehrlichi*

5. Enter Order of the sample under the Higher Classification ID column
    a. Example:
        Psocodea -> Lice
        Acari -> Ticks and Mites
        Siphonaptera -> Fleas
        Diptera -> Flies
        Insecta -> Insects

b. Example:

Higher Classification ID
Psocodea

6. For Biological Attributes, count/roughly estimate how many ectoparasites are placed in the tube
    a. Enter number, plus sex (if possible)
    b. Add additional taxonomic identification, if available. For example, louse suborders (now nanorders) such as Anoplura, Ischnocera, Amplycera, etc.

7. For Stage Name, identify, if possible, the life stage of the ectoparasites (adult, nymph, both)
    a. Example:

Biological Attributes	Stage Name
1F, 1M, 2N	Adult, Nymph
1N Ischnocera	Nymph

8. In comments section, add notes about the specimen
    a. For example: the sample is mangled (not identifiable), tangled, in a clump, body parts are separate or missing, unknown sex, etc.
    b. Example:

Comments
1 Mangled

9. For locality, break down information by Country, State, County, Locality, and Verbatim Locality (putting all information together, separated by colons)
    a. Some countries, like Mexico, do not have counties, so just leave column blank
    b. Example:

Country	State	County	Locality	VebatimLocality
USA	Louisiana	Iberville Parish	Corner of Pecan Dr. and Bayou Paul Dr., St. Gabriel	USA: Louisiana: Iberville Parish: Corner Pecan Dr. and Bayou Paul Dr. St. Gabriel
Mexico	Puebla		3 km NE Tilapa (off Mexico 160)	Mexico: Puebla: 3 km NE Tilapa (off Mexico 160)

10. Add latitude and longitude coordinates, if available. Please note if these localities have already been georeferenced with information about certainty, etc.
    a. Convert to decimal degrees if lat/long is in degrees, minutes, seconds format
        i. Website 1: https://www.pgc.umn.edu/apps/convert/
        ii. Website 2: https://www.rapidtables.com/convert/number/degrees-minutes-seconds-to-degrees.html
    b. If no verbatim coordinates, enter the decimal degrees or leave blank
    c. Example:

Verbatim Lat	Verbatim Lon	Decimal Coordinates Lat	Decimal Coordinates Lon
34° 7'33.5"N	106° 52'21.5"W	34.125972	-106.872639
		26.32125	-97.82275

11. For Elevation, enter as meters
    Note: If elevation is in feet, convert to meters
    a. Example:

Verbatim Elevation	Elevation Units
1438	meters

12. Break down the host information by Order, Family, Genus, and Species
    Note: Host's Genus and Species name need to be entered in italics
    a. Example:

Host Order	Host Family	*Host Genus*	*Host Species*

Rodentia	Heteromyidae	*Liomys*	*irroratus*

13. Add Host Museum Voucher and Host Catalog Number if present
    a. Example:

Host Voucher Museum	Host Catalog Number
LSUMZ	36243

14. In the Notes section enter any extra general information that pertains to the sample

	Notes
	Mites present; No host voucher