Biological Monitoring at Intervale Lowlands:

Earthworms

Sampling Protocol, Revised October, 2013

Ecological Research as Education Network

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FACTORS AFFECTING THE DISTRIBUTION OF NORTH AMERICAN EARTHWORMS

SAMPLING PROTOCOL, AS REVISED OCTOBER 2013

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Background

Assemblages of earthworms in North America often consist of a combination of native species and species that have arrived from elsewhere at various times in the past and from various places (Gates, 1982; Hendrix and Bohlen, 2002). Nearctic species in previously glaciated landscapes re-colonized these areas after they were excluded by ice in the Pleistocene. Species exotic to North America spread across the landscape from the particular places where they were introduced. So, biogeographic barriers and corridors may play a role in determining the presence of certain species and composition of earthworm assemblages at particular locations. This is likely to vary regionally because of difference in the pattern of human colonization and differences in the structure of the landscape (e.g., topography, hydrology). By examining earthworm assemblages in a variety of areas across North America, we can better assess the extent to which biogeographic factors, rather than habitat quality, affect worm assemblages in different places. Determining the role of biogeographic barriers in affecting worm distributions is potentially important to land managers, who may want to prevent the introduction of certain worms to areas currently not inhabited.

Objective

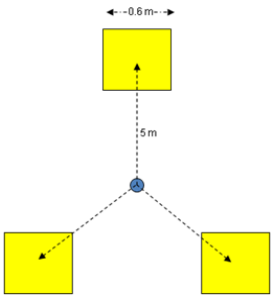
We aim to better understand the factors affecting worm distributions using a geographically broad sampling program powered by the EREN network of collaborators. Work is amenable to class-lab projects and independent-study work involving undergraduate students.

Site and Quadrat Placement

Because of the strong influence of people in distribution of certain earthworm species, it is desirable to have sites that are variously removed from intensive human land use. Investigators might consider adopting a sampling design that includes some sites that have a long history of land use (e.g., urban woodlots) along with sites that are more remote. Forested and non-forested sites may be sampled. Lawns make interesting (and often productive) sample locations. Participants in the EREN Permanent Forest Plot Project (http://erenweb.org/project/carbon-storage-project/) are encouraged to use their established forest plots as a context for worm sampling. Indeed, this might be particularly desirable for two reasons: first, the abundance and diversity of worms may help to explain observations of forest attributes (e.g., Asshoff et al., 2010), and second, desired variability among permanent forest plots (e.g., urban – rural contrast) are also potentially interesting sources of variability for worms.

Once a habitat has been selected for sampling, the specific location of sampling should be chosen randomly (or at least arbitrarily) to avoid bias due to micro-habitat. Consider tossing a tennis ball or something else into the site to determine the exact location for the central point of the sampling arrangement. In the case of permanent forest plots, an object might be softly tossed more-or-less straight up from the center of the plot. Mark the determined central point using a stake flag or something similar.

Three quadrats should be sampled if possible to integrate some of the micro-habitat variation anticipated at most sites. We have developed a sampling arrangement that facilitates the sampling of three 0.36-m^2 quadrats per site. Participants should try to follow this arrangement whenever possible to simplify analyses. However, if only one or two quadrats are sampled per site, the resulting data may be used as long as the number of quadrats is clearly recorded. Also, investigators wishing to use the plot size indicated in the protocol designed by the Great Lakes Worm Watch (www.nrri.umn.edu/worms; 0.1225-m^2 quadrats) can do so without biasing the data. Abundance data will be adjusted to indicate density (e.g., number of earthworms per square meter). However, we recommend the use of the larger quadrats, when possible, to maximize chances of detecting uncommon species.



Place quadrats 5 meters from the central point toward the bearings 0°, 120°, and 240° as illustrated in the diagram to the right. At these quadrat locations, place the sampling frame. You may use a square frame that is 0.6 m × 0.6 m or a round frame with a radius of 34 cm. (If you are using 0.1225- m^2 quadrats, the square version would have a side of length 35 cm and the round version would have a radius of 20 cm.) The frame can be made of any material; ours are made of 1-×-4-inch nominal lumber as shown in the picture below. The three quadrat samples need not be taken simultaneously; however, the date and time of each sample should be recorded to facilitate possible consideration of the effect of weather on sampling.

Earthworm Collection

Worms should be collected using mustard vermifuge. Additionally, time-constrained searching can be used to supplement samples and ensure detection of litter-dwelling species. Although not strictly required for this protocol, field sampling may be made slightly more effective by taking students into the field to practice each of these techniques prior to sampling.

Most earthworms of North America are perennial species, and adults may be sampled at any time of the year. However, fall sampling is best for finding mature stages of species with an annual life cycle, such as members of the genus *Amynthas*, in temperate areas. Early spring is a

particularly good time of the year to find sexually-mature (and easily identifiable) individuals of most perennial species. Also, when climatic conditions are very cold or dry, sampling may be ineffective. Repeated sampling of the same location at different times of the year will likely demonstrate different patterns in abundance. Because the emphasis of this project is on presence and composition of worm assemblages, our conclusions should be relatively robust to intra-year variability.

Mustard vermifuge

Preparation of Solution.—Add 60 g ground hot mustard seed powder (See Materials and Cost List) to 6 liters of water and mix well. (This is the same concentration indicated by the Great Lakes Worm Watch.) Two doses of 6 liters are required for each 0.36-m^2 sample quadrat. If you are using 0.1225- m^2 quadrats, two doses of 2 liters of this solution should be prepared for each quadrat.)

As you may have gathered, the hauling of large volumes of water is a major concern for sampling remote sites. Consider that ambient water (e.g., from a stream) may be used. In the absence of ambient water, we have hauled water into remote sites using 20-liter plastic carboys. Working with strong students is a plus in these cases. It is best to prepare mustard solution within 24 hours of use, and it can be used immediately after preparation. The solution becomes foul with prolonged storage. We mix the solution in buckets, which can be easily rinsed out (rather than in the carboys).

Preparing the quadrats.—Clip all vegetation and manually remove loose leaf litter. Clippers are useful in removing vegetation from old-field and lawn quadrats. It is not necessary to clip all grass from quadrats in mowed lawns; however, the grass should be short enough to allow you to see the ground surface.

Vermifuge—At each sample quadrat, apply the first dose of the solution to the area within the frame. Pour the solution slowly, allowing it to infiltrate the soil. Wait and watch for 5 minutes, collecting any worms that emerge. Pour a second dose of the solution and wait another 5 minutes.

Quadrats on steep slopes present challenges to the protocol because of the tendency for the solution to run off rather than infiltrate. Solution must be applied very slowly in these cases. The sample frame can be solidly pressed against the ground (e.g., by standing on it) to prevent runoff.

Earthworms should be collected with fingers or flat forceps. Wait for worms to completely emerge to avoid damage to the animals or scaring them underground and missing them. This is especially important for *Lumbricus terrestris*, the common night crawler.

Time-constrained Searches

Litter-dwelling worms are often more conspicuous under forest structure (e.g., logs) than in the soil and might be missed during soil vermifuge. Either after or before the mustard vermifuge, worms can be searched for under rocks, logs, and other forest debris for a total of 0.5 person-hour at each site. If four students are

doing the searching, then each should search for 15 minutes and so on. The area covered in these searches is not prescribed, only the time spent searching. Sampling should be conducted within the same habitat as the vermifuge samples. That is, personnel should not cross any conspicuous ecotones as they move around during this sampling. A good rule of thumb for these samples is that they should not extend beyond about 50 m from the center point of the vermifuge sampling arrangement. These worms should be collected and stored in a different container than those collected using the mustard solution.

Stabilization and storage of worms

Worms should be rinsed with water in the field, wrapped with moist paper towels and placed in labeled containers. Containers should be stored in a cooler on ice until return to the lab.

In the lab, worms should be blotted dry and weighed. Animals should be described as fully as possible at this point. Special note should be made about color, which will change after preservation, and behaviors. Photographs should be taken, if possible.

To make or confirm specific determinations, worms will have to be sacrificed and preserved. The three-step procedure below is partially based on the technique developed by and described by Fender (1985).

Euthanasia.—Worms should be killed by immersing them in dilute isopropanol (about 10%) until immobile. (This should just take 1-2 minutes.)

Fixation.—Worms should be transferred to 10% buffered formalin (=37% formaldehyde). Environmental health precautions must be taken with formalin. Animals should remain in the fixative for at least 2 days.

Preservation.—Animals should be rinsed with distilled water and placed in 70% isopropanol. Because the bodies of earthworms are large and contain a lot of fluid, the preservative should be replaced after 2 days to avoid dilution. Thereafter, it need not be replaced again. Animals can be retained for long-term storage in this solution.

Identification of Worms

Tentative identification of specimens can be completed when worms arrive at the lab. However, many characters are difficult to see until the worms are fixed and preserved.

Reynolds (1977) and Fender (1985) are recommended reading for investigators involved in the project. These papers not only include useful keys, but they also include a lot of great information and diagrams regarding identification.

In my experience the most useful keys to use in identification are Reynolds (1977), Schwert (1990), and Fender (1985). For those working outside the Northeast, Reynolds et al. (1974), James (1990), and Fender and McKey-Fender (1990) might also be useful.

The most user-friendly key to earthworms is the little field guide by Hale (2013). It also includes a key, but it is likely not comprehensive in any one particular area. A series of papers have been completed by John Reynolds, cataloging the species in particular states.

Maryland – Reynolds (1974)
New Jersey – Reynolds (2007a)
New York – Reynolds (2008a)
Ohio – Reynolds (2007b)
Pennsylvania – Reynolds (2008b)
Tennessee – Reynolds et al. (2004)
West Virginia – Reynolds (2007c)

In the classroom, it is likely best to just use Hale (2013) and the key included there. This will allow the identification of most material in most locations. Instructors and advanced students can run worms through more sophisticated keys to confirm identifications or revise. See the attached appendix for a list of taxonomic codes for species found in New York State. Participants should use these codes when possible. When referring to a species not on this list, participants can develop a unique code, which I can add to a running list. Specimens should be tallied separately for samples yielded by vermifuge and time-constrained-searching.

Identification Support and Archival

Samples – either in their entirety or representative specimens – may be sent to the Museum of the Chenango Valley at Colgate University[1] for confirmation of determinations and permanent storage. Once they are properly preserved, as described above, worms can be wrapped in cheese cloth saturated with 70% alcohol and sealed in plastic bags ≥ 2 mil thick. If heavy-duty "zip lock" bags are used, they should be sealed across the "zip" with glue. A better option is to use a bag heat-sealer, which can be purchased for about $100 (See Materials and Cost List). All worms from a site may be sent together as a batch or may be individually wrapped and sealed with their collection data or individually tagged by tying the tag around the animal toward its back end. Determinations will be sent back to participants and animals accessioned into the collections of the museum, unless it is requested that specimens be returned.

Measuring Organic Biomass of Worms

The emphasis of this initial EREN Network project is on species composition. However, it is my hope that others will extend the data set in the direction of ecological function. It might be helpful, therefore, to estimate biomass. Please feel free to participate in the project without measuring biomass.

[1] Tim McCay, Museum of the Chenango Valley, Colgate University, 13 Oak Drive, Hamilton, NY 13346

Because worms consume mineral soil and contain variable amounts of water, their crude mass does not always accurately reflect their organic, dry biomass. There are several ways to get around this, but the two most common approaches are (1) to measure the ash-free dry mass (AFDM) of earthworms using the protocol below,[2] and (2) to estimate biomass from length. Calculating AFDM obviously results in the sacrifice of the specimen. So, it should only be used in cases where further identification is not desired.

Protocol for the Measurement of AFDM of Earthworms

1. Dry specimen at 60°C for 24-48 hours or until mass is constant
2. Measure dry mass
3. Place animal in a muffle furnace within an appropriate container at 500°C for at least 4 hours
4. Weight the ash, which is the mineral component of your specimen, and subtract that amount from the dry mass to get the AFDM

Fortunately, there exist allometric relationships between length and biomass of earthworms that have been described for a variety of earthworm taxa (Hale et al. 2004, Greiner et al. 2010). Take a look at these original papers to get a better sense for the advantages and limitations of this approach and obtain the actual equations relating length to biomass. Length should be measured on preserved specimens after they have been straightened but not stretched.

Soil Sampling and Analysis

Soil quality – especially pH and organic content – can strongly affect earthworm presence and abundance. Conversely, the presence and abundance of worms can have a strong effect on litter depth. Investigators participating in this project should measure soil pH and organic content, if possible. If participants are unable to complete these analyses on their campuses, these techniques may be outsourced to other participants. Please contact Tim McCay with questions regarding this. Measurement of litter depth is optional but can be very interesting, especially when comparing worm-colonized to un-colonized sites.

Soil and Litter Sampling

A soil sample should be taken from a location adjacent to each quadrat, for a total of three samples per site. The samples can be taken with a professional soil corer or a simple garden bulb planter. In any case, the soil should be sampled to a depth of 10 cm. Samples should be combined, homogenized (mixed), "screened" (rubbed through a ~ 2 mm mesh filter), and air-dried.

Protocol for the Measurement of Soil pH[3]

1. Place 10 g of air-dried soil into a 30 ml beaker and add 20 ml of deinonized/distilled water;

[2] Protocol is adapted from the Great Lakes Worm Watch (www.nrri.umn.edu/worms/research/methods_ash.html)
[3] Protocol adapted from Hendershot et al. (1993)

2. Stir vigorously about once every 5 minutes for 30 minutes;
3. Let stand about 1 hour; and
4. Immerse electrode of a calibrated pH meter into the supernatant and record.

The organic content of soils is calculated most easily by burning off the organic fraction and determining the difference. Measure the organic content of your sample using the following steps:

Protocol for the Measurement of Soil Organic Content[4]

1. Oven dry a small amount of your sample at 105°C for 24 hours to produce about 2 g of oven-dried soil;
2. Determine the mass of an appropriate container (e.g., porcelain crucible);
3. Place about 2 g of oven-dried soil into the container and mass again to determine the exact mass of your sample;
4. Ash the sample for 6 hours at 600°C;
5. Cool in a dessicator;
6. Mass again and determine to mass lost during combustion; and
7. Calculate the organic content fraction as (mass lost during combustion) / (mass of original sample)

Protocol for Measurement of Litter Depth

Litter depth may be recorded directly with a ruler. To do this, gently move the end of the ruler through the forest litter until resistance changes, indicating the underlying mineral soil (A Horizon). View the ruler graduations from a perspective close to the litter surface. Do this at three places, one adjacent to each of your vermifuge samples, as there is much variability in litter depth.

Location and Landscape Analyses

Location should be determined as specifically as possible so that landscape attributes can be measured in the lab using ArcGIS, Google Earth or other geographic information system. A GPS unit is best, but location also can be determined from a 1:24,000 topographic map.

Participants can conduct analyses related to landscape attributes if it fits within curricular aims and institutional capacity. If participants are unable or do not wish to complete these analyses, they can be completed centrally based on provided locations.

[4] Protocol adapted from Karam (1993)

The following attributes will be measured for each site – specifically, the center point of the site (see data form for units, etc.):

Habitat type
Elevation
Slope
Aspect
Distance to nearest road (and type of road)
Distance to the nearest stream (and type of stream)

Data Compilation, Analysis, and Publication

A copy of data sheets can be sent by email (a scanned copy) or US Post to Tim McCay, Biology Department, Colgate University, Hamilton, NY 13346. Ultimately, data will be recorded using an online database. Until then, once a sufficiently large dataset has been built, updates will be posted at a secure website, which can be accessed by participants in accordance with the EREN Data Sharing policy (http://erenweb.org/project/data-sharing-policy/).

This project is designed to illuminate the factors that affect worm presence, abundance, and species composition. To address this aim, a variety of multiple-variable techniques (e.g., multiple logistic regression) will be used to relate earthworm presence/absence, abundance, diversity, and other community metrics to habitat attributes. In a project in central New York involving 50 sites, both attributes related to habitat quality (particularly soil pH) and landscape attributes (including proximity of roads) were important. It will be interesting to see whether this holds true across a broad geographic range and whether these relationships differ between glaciated and non-glaciated landscapes.

Authorship and other details regarding the preparation of papers from this work will follow the EREN Authorship Guidelines (http://erenweb.org/project/authorship-guidelines/). I invite investigators to use the anticipated data set in other projects and to suggest modifications to increase the usefulness of this protocol in other work. For example, an amendment to this protocol might be proposed that would allow genetic analysis of collected material.

Current and Future Support

I have received modest internal funding to support a student to aid in the processing of materials, jars and vials for storage, and so forth. There exist no funds that might be used to subvent sampling or shipping costs of collaborators.

Curricular Adaptations

The adaptation of this protocol to particular curricular aims is not developed in this document. See additional documents posted at the EREN website for more information.

Materials and Cost List

Materials are detailed here for site establishment and earthworm collection, preservation, and shipping. Materials for worm biomass and soil analysis are not provided here. Please feel free to contact me for details if you are going to conduct these analyses.

Required for Site Establishment and Earthworm Sampling

Item	**Use**	**Cost and Source**
Stake flag	Mark the location of the center of the quadrat arrangement	About $7 per 50; Ben Meadows (benmeadows.com)
Sampling quadrat – this may be variously constructed. Materials necessary to make 1 of our lumber quadrats: 1-by-4 lumber (8 foot); decking screws (ca 20); 2" corner braces (4)	Define the boundaries of the earthworm sample	About $5, one 8-foot 1-by-4 (nominal dimensions) is sufficient for each quadrat; about $1, 20 decking screws per quadrat; about $4, four corner braces. Any local hardware store.
Compass	Determine the direction of sampling quadrats	About $15; Ben Meadows
Fiberglass Tape Measure	Determine the distance to sampling quadrats from the center point	About $20 for a 50-m tape; Ben Meadows
Ground hot mustard seed	Make the vermifuge solution (120 g needed for each quadrat; 360 g needed for each site). One pound of mustard powder can make approximately enough to sample 1.25 quadrats.	$5.60 per 1-pound bag; Penzey's Spices (penzeys.com, product 45410)
5-gallon buckets	Needed for mixing of solution (at least 1)	About $4; most local hardware stores
2.5-gallon collapsible plastic carboys	Carrying water into sites (need 1 per each site)	About $11; Grainger (grainger.com; see item number 6CTF5)
Flat forceps (optional, but useful) – recommend spade-tipped butterfly forceps	Picking up earthworms during sampling	About $4; Bioquip (bioquip.com; see item 4747)
Plastic cups with lids (need at least 2 per site sampled)	Temporarily holding earthworms	About $1 a piece. We use Fisherbrand specimen storage containers 8 and 16 oz (http://www.fishersci.com; see items 14-955-115A and 14-955-117A)

Required for Earthworm Preservation, Identification, and Shipping

10% isopropanol	Worm euthanasia	Variable. Isopropal alcohol often is most economically purchased as 95% and cut to the desired concentration. Most institutions order isopropanol in bulk. For example, 95% isopropal alcohol is available at my institution for about $8 per 4 liters.
70% isopropanol	Worm long-term storage	See above.
Distilled water	Dilution of various solutions	About $0. Typically, distilled water is freely available at universities. If not, it can be purchased at grocery and "big box" stores.
Formalin (10% buffered)	Worm fixation: Alternative to Lavdowsky's Fluid (optional)	Varies with quantity and vendor, but about $40 per 4 liters (Sigma-Aldrich # HT501128)
Dissecting microscope	Worm identification	Expensive. Minimally, one should have a stereomicroscope capable of 40× magnification with good lighting.
Heat sealer	Shipping of worms (optional)	Varies with vendor, but about $100 (grainger.com, see item number 4LT36)
Clear poly bags for the heat sealer, use at least 2 mil thickness	Shipping of worms (optional)	Varies with vendor, but about $32 per 1000 (grainger.com, see item number 5ZW10)
Extra-thick "ziplock style" bags. For shipping, I recommend at least 2 mil (and better 4 mil) reclosable bags.	Alternative to heat sealer for shipping of worms (optional)	A few cents each if purchased in bulk. I use www.consolidatedplastics.com, see item number 90760, 4 mil 4 × 6 inch bag, $67/1000.
Superglue or other that adheres to plastic	Alternative to heat sealer for shipping of worms, safe closure of plastic bag (optional)	A few dollars at any grocery, hardware, or "big box" store.

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EREN Worm Network

Sample and Environmental Data Form

Unique Sample Code: _____ - _____

Date and time of Sample: _____ / _____ / _____ _____ HRS

Latitude: _____ Longitude: _____

Other Locality Data:

_____

_____

Habitat Type: _____

Elevation: _____ m ASL

Dominant Vegetation:

_____

_____

Soil description:

_____

_____

Slope: _____

Aspect: _____

Distance to the nearest road: _____ is this road paved? _____

Distance to the nearest water body: _____ type of water body _____

Soil pH: _____

Soil organic matter %: _____

Did you follow the EREN Worm Network Sampling Protocol? □ Yes □ No

If not, please describe the technique you used in sampling:

_____

_____

EREN Worm Network

Sample and Environmental Data Form (instructions)

Unique Sample Code: This code should begin with the last name of the investigator and end with a three-digit unique number to identify the sample. For example, a sample might be labeled "McCay – 028."

Date and time of Sample: Date and time that the sample was taken.

Latitude/Longitude: When possible, a GPS should be used to determine the specific location. Use latitude and longitude and decimal degrees when possible.

Other Locality Data: This should include but not be restricted to the county and state/province from which the sample was taken. Township, village, nearest cross-street, and so forth are also strongly desired.

Habitat Type: forest (vegetation community dominated by trees), shrubland (vegetation community dominated by shrubs), old-field (a sort of transitory habitat that has resulted from the abandonment of agricultural use, typically dominated by non-woody plants but including various invading woody species), agricultural - grazed (habitat dominated by non-woody plants and currently used for grazing livestock), agricultural – cultivated (habitat dominated by non-woody plants and currently used for cultivation), or lawn (habitat dominated by grasses and regularly mowed).

Elevation: Number of meters above mean sea level.

Dominant Vegetation: The most common species in the over-story vegetation should be given here along with the most common ground-floor or shrub-layer plants.

Soil description: The soil type and series should be given here along with any other available information regarding the history of the soil (e.g., agricultural history).

Slope: Slope in degrees. This can be calculated onsite using a clinometer or later using geographic information systems

Aspect: Direction of slope, expressed as a directional bearing. This can be calculated onsite using a compass or later using geographic information systems

Distance to the nearest road: Distance in meters. This can be calculated onsite using a meter tape or later using geographic information systems.

Is this road paved? Answer yes if the road has a paved surface

Distance to the nearest water body: Distance in meters. This can be calculated onsite using a meter tape or later using geographic information systems. If there is no water body within 1000m, then indicate > 1000m.

Type of water body: ephemeral stream, perennial stream, river, lake, marine coast, or other (provide description)

Soil pH: The pH of the soil measured using the standard EREN protocol.

Soil organic matter: The percent organic matter of the soil measured using the standard EREN protocol.

Did you follow the EREN Worm Network Sampling Protocol? Indicate "Yes" only if the protocol was followed exactly.

If not, please describe the technique you used in sampling: If the EREN protocol was not followed exactly, indicate the exact protocol used.

EREN Worm Network

Identification Data Form

Unique Sample Code: _____ - _____

Sample Type: _____

	Taxon	Con-fidence	Length (mm)	Live mass (g)	AFDM (g)	Biomass technique	Notes
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EREN Worm Network

Identification Data Form (instructions)

It is absolutely essential that a unique sample code is indicated, which links these data to a specific Sample and Environmental Data Form.

Sample Type – vermifuge or time-constrained

Each animal sampled should be given a separate row on the data form. If more than 30 animals are collected, multiple forms may be used.

Taxon – species or other taxonomic determination; use species codes where possible (see appendix to EREN protocol)

Confidence – level of confidence in identification: high, medium, low

Length – length to the nearest mm of the animal after fixation and preservation

Live mass – mass of the live animal after rinsing and blotting dry

AFDM – ash-free (organic) dry mass of the animal (see protocol for details)

Biomass technique – if AFDM was estimated from length using the model provided in the protocol based on Hale (2004), indicate Model; if AFDM was measured directly, indicate Direct.

Notes – notes regarding unusual morphologies, etc.

Appendix, Earthworm Species Codes (earthworms recorded for New York State)

Lumbricidae	
Allolobophora chlorotica	ALCH
Aporrectodea icterica	APIC
Ap. longa	APLO
Ap. rosea (=*Eisenia rosea*, =*Allolobophora rosea*)	APRO
Ap. trapezoides	APTR
Ap. tuberculata (=*A. caliginosa*)	APTU
Ap. turgida	APTR
Bimastos parvus	BIPA
B. tumidus	BITU
Dendrobaena octaedra	DEOC
Dendrodrilus rubidus	DERU
Eisenia foetida (=*E. fetida*)	EIFO
Eisenia hortensis	EIHO
Eiseniella tetraedra	EITE
Eisenoides lönnbergi	EILO
Lumbricus castaneus	LUCA
L. rubellus	LURU
L. terrestris	LUTE
Octolasion cyaneum	OCCY
O. tyrtaeum	OCTY
Magascolecidae	
Amynthas agrestis	AMAG
Am. diffringens	AMDI
Am. hilgendorfi	AMHI
Am. hupiensis	AMHU
Am. morrisi	AMMO
Metaphire californica	MECA
M. levis	MELE
Sparganophilidae	
Sparganophilus eiseni	SPEI