



HANDBOOK

PROTOCOLS FOR SAMPLING, GENERAL SOIL
CHARACTERIZATION AND SOIL BIODIVERSITY ANALYSIS

EDITED BY DAVID FERNÁNDEZ CALVIÑO, DIEGO
SOTO GÓMEZ, KRISTIAN KOEFOED BRANDT AND LIEVEN
WAEYENBERGE

JUNE 2020

SOIL SAMPLING
PHYSICAL ANALYSIS

CHEMICAL ANALYSIS
BIOLOGICAL ANALYSIS

WHEAT QUALITY
ANALYSIS

This handbook is a deliverable of WP3: Soil biodiversity assesment in European cropping systems of the SoildiverAgro project financed by the European Union's Horizon2020 research and innovation programme under grant agreement N°. 817819.

Edited by Universidade de Vigo (UVigo).

Design by Fundacion Empresa Universidad Gallega (FEUGA).

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JUNE 2020
1st Edition



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817819

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4.1. EARTHWORM DIVERSITY

BY MARIA J. I. BRIONES (1), VISA NUUTINEN (2) AND STEFAN SCHRADER (3)

(1) DEPARTAMENTO DE ECOLOGÍA Y BIOLOGÍA ANIMAL, UNIVERSIDAD DE VIGO, 36310 VIGO, SPAIN

(2) SOIL ECOSYSTEMS, NATURAL RESOURCES INSTITUTE FINLAND (LUKE), TIETOTIE 4, 31600 JOKIOINEN, FINLAND

(3) THÜNEN-INSTITUTE OF BIODIVERSITY, BUNDESALLEE 65, 38116 BRAUNSCHWEIG, GERMANY

PRINCIPLE AND APPLICATION

Earthworm populations are usually characterized according to different criteria: i) developmental stage to quantify the proportion of adults and juveniles; ii) ecological groups to describe the feeding and burrowing behaviour; iii) species identification to determine species richness.

This protocol describes how:

- to identify earthworm species
- to assign each specimen to one of the established ecological groups (Bouché, 1977): epigeic, anecic and endogeic,
- to distinguish developmental stages: adult, subadult and juvenile
- to separate incomplete specimens and fragments from all others
- to determine total abundance (individuals m^{-2}) and biomass ($g\ m^{-2}$)

PROCEDURE

Wear disposable protective gloves and use plastic forceps when handling earthworms. The specimens from each replicate sample should be placed into a plastic container filled with cold tap water. Before the species identification under the stereo microscope and weighing, each specimen should be cautiously dried on tissue paper.

Developmental stage

Earthworms are first separated according to their developmental stage by looking at their sexual maturity:

- Adults have a fully developed clitellum (glandular thickened section of the body wall in the middle or towards the anterior end of the body)
- Subadults do not have a developed clitellum but tubercula pubertatis (glandular thickenings on the ventral surface) are present
- Juvenile worms do not show any visible signs of clitellum nor tubercula pubertatis.

Figure 4.1.1. shows some of the morphological characters for identifying earthworms to species level, and includes the position of the clitellum and the tubercula pubertatis, two distinct characteristics of fully adult specimens.

Ecological groups

Most earthworm individuals (adults and sub-adults but often also juveniles) can be assigned to their ecological groups according to their dorsal pigmentation:

- Epigeic species are heavily pigmented small earthworms.
- Anecic species are large earthworms and moderate to heavily pigmented on their front end (until some segments behind the clitellum);
- Endogeic species are unpigmented or nearly unpigmented; in some species, the epidermis is translucence showing through inner organs



EQUIPMENT AND MATERIAL

- Disposable protective gloves
- Tissue paper or paper towels
- Plastic forceps
- Flat plastic containers (vol.: ca. 1 L) with cold fresh tap water
- Plastic cups with cold fresh tap water
- Stereo microscope (magnitude: ca. 10x to 50x)
- Analytical balance (precision: 0.01 g)
- Identification keys
- Data sheets



REMARKS

All fragments of earthworms in the sample must be included for determining total earthworm biomass. However, only those fragments that include the head and as best also the clitellum can be identified to the species level and if this is the case, they can be used for total abundance and diversity estimates. Any other fragments are listed as “unidentified fragments”.

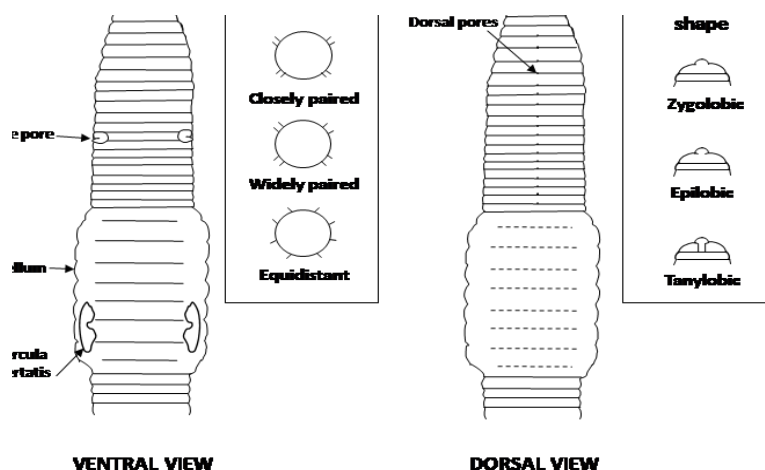


Figure 4.1.1. Some of the main morphological characters that are used in identifying developmental stage and species of earthworms

Figure 4.1.2. shows one example species, its typical pigmentation and type of burrow system for each ecological group.

Taxonomical identification

Adult earthworms can be identified to species level by using a stereo microscope (magnitude: 10x to 50x) and a taxonomic identification key. For example, the identification guide by Sims and Gerard (1999) and key to the earthworms of the UK and Ireland by Sherlock (2018) are useful for identifying the majority of the common European species. Because they do not cover completely European earthworm fauna, national or regional keys should also be used when necessary.

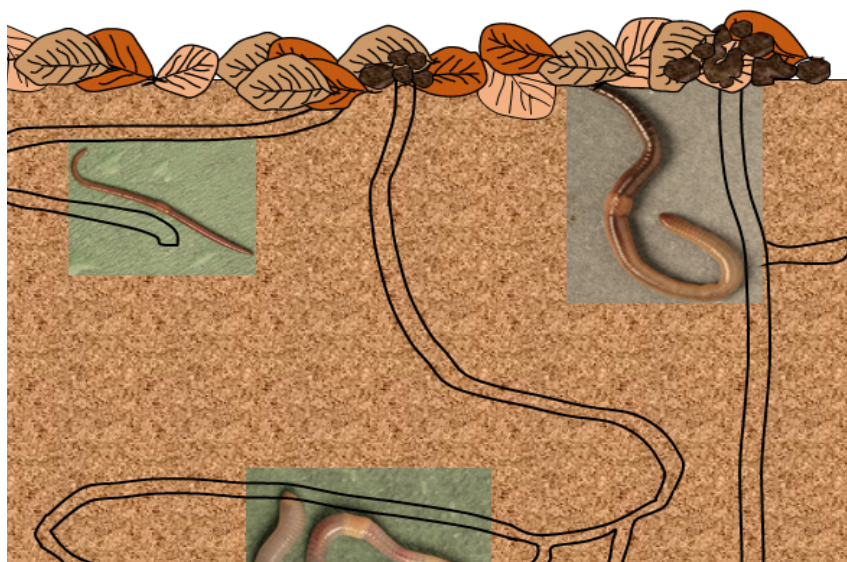


Figure 4.1.2. Earthworm ecological groups (Bouché, 1977): epigeic (*Lumbricus castaneus*; top left), anecic (*Lumbricus terrestris*; right), and endogeic (*Octolasion cyaneum*; bottom) (the size of the species not to scale). Photo credit: Guide to British earthworms (<https://www.opalexplornature.org/earthwormguide>).

The most important diagnostic characters are the position and shape of the clitellum and location of the tubercula pubertatis (see Figure 4.1.1.). Other important diagnostic characters are the shape of prostomium (in front of the first segment) and the clitellum (annular, saddle), setae pattern (distance between setae on each segment) (Fig. 4.1.1.). Subadult earthworms can be identified to species in many cases, however, juvenile earthworms can only be assigned to ecological groups (see above). If the specimen cannot be accurately identified, then annotate all available information and classify it as 'unknown'.



CALCULATIONS

Use a balance with precision of 0.01g for determination of the preserved earthworm biomass (g). Determine the biomass per species, per ecological group and in total for each sample. Express all abundance (of individual species and ecological groups) and biomass data as individuals per square meter (ind. m^{-2}) and grams per square meter (g m^{-2}), respectively. In case of a 50 cm x 50 cm sampling quadrat, multiply the density and mass in the sample by 4. In case of a 25 cm x 25 cm sampling quadrat, multiply by 16.

REFERENCES

- Bouché, M. B. (1977). “Strategies lombriciennes,” in Soil Organisms as Components of Ecosystems, eds U. Lohm and T. Persson (Stockholm: NFR, Swedish Natural Science Research Council), 122–132.
- Sherlock E. (2018). Key to the Earthworms of the UK & Ireland (2nd edition). Field Studies Council,
- Sims RW, Gerard BM (1999) Earthworms: Keys and Notes for the Identification and Study of the Species. Rev. ed., Field Studies Council, Shrewsbury.