# Earthworms and Vermicomposting

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**Abstract**

Vermicomposting, the conversion of organic waste into vermicompost, is mediated by the combined action of earthworms and microorganisms. This interesting and attractive alternative to regular composting turns organic waste into a substrate that can be used as a soil amendment and as a growing medium for use in horticulture. Soil is not required in vermicomposting as the organic matter acts as both the substrate and food, and therefore only epigeic earthworms can be used in the process. Several earthworm species have been evaluated for their potential use in vermicomposting, including *Eisenia fetida* (Savigny), *Eisenia andrei* (Bouché), *Dendrobaena veneta* (Rosa), *Dendrobaena hortensis* (Michaelsen) *Eudrilus eugeniae* (Kinberg), and *Perionyx excavatus* (Perrier). The species most commonly used in vermicomposting and vermiculture facilities worldwide are *Eisenia andrei* and *Eisenia fetida*. This chapter reviews and updates the controversy surrounding the taxonomic differentiation between *E. andrei* and *E. fetida*, and between *D. veneta* and *D. hortensis*, showing that these are all different species and emphasizing the importance of maintaining pure cultures in vermicomposting systems. In the final section, methods of cultivating epigeic earthworms to ensure high rates of growth and reproduction are described.

**Keywords:** earthworms, *Eisenia andrei*, *Eisenia fetida*, *Dendrobaena veneta*, *Dendrobaena hortensis*, vermicomposting, vermiculture, epigeic, red worms, tiger worms, earthworm culture

## 1. Introduction

Earthworms (Crassiclitellata) are terrestrial oligochaetes that usually live in the soil. These invertebrates constitute the largest animal biomass in most temperate ecosystems, where they strongly influence the physical, chemical, and biological properties of soil. They play a key role in modifying soil structure and accelerating the decomposition of organic matter and

nutrient cycling, ultimately shaping the structure and composition of the aboveground plant community.

Earthworms have a burrowing lifestyle and simple body structure, leading to the commonly held belief that there is only one type of this not very pretty soil creature. However, earthworms constitute a highly diverse group of burrowing annelids, including more than 6000 extant species. For the vast majority of these, only the name and morphology are known, and nothing is known about their biology and ecology. Different species of earthworms have different life strategies and occupy different ecological niches. Earthworms have thus been classified on the basis of their feeding habits and the part of the soil profile that they inhabit into three main ecological categories: epigeic, anecic, and endogeic. These categories can be difficult to establish and some species cannot be accurately assigned to any of them. In agricultural soils, earthworms usually burrow deeper than they do in grasslands and forest soils. Epigeic earthworms live in the organic horizon, on or near the soil surface, and they mainly feed on decaying organic matter such as vegetable and animal debris. They are usually small, pigmented, and have high metabolic and reproductive rates that allow them to adapt to the changing environmental conditions of the soil surface. They also display high rates of consumption, digestion, and assimilation of organic matter and play a key role as litter transformers, producing holorganic casts. Epigeic lumbricids include the species *Dendrobaena veneta*, *Dendrobaena hortensis*, *Dendrobaena octaedra*, *Eisenia fetida*, *Eisenia andrei*, *Dendrodrilus rubidus*, *Eiseniella tetraedra,* and *Allolobophoridella eiseni*. By contrast, endogeic earthworms live deeper in the soil profile and feed mainly on soil and the associated organic matter. These worms have little pigmentation and construct highly branched horizontal galleries, which become filled with excrement as the worms move along the organic-mineral horizon of the soil. Endogeic earthworms have lower reproduction rates and longer life cycles than epigeic earthworms and are more resistant to unfavorable conditions such as drought and lack of food. Most earthworms, including *Aporrectodea caliginosa*, *Aporrectodea rosea,* and *Octolasion lacteum*, belong to this category. Anecic earthworms live more or less permanently in vertical galleries, which can extend for several meters throughout the soil profile. These species surface at night to feed on litter, feces and decomposing organic matter, which they transport to their galleries. They deposit their excreta on the surface, at the opening of their galleries, in the form of conspicuous earthworm casts. These earthworms are usually large and dark brown in color. They have relatively low reproductive rates and long life cycles. The night crawler *Lumbricus terrestris* is a typical anecic earthworm.

Vermicomposting, the transformation of organic waste into vermicompost, is a biooxidative mesophilic process in which detritivorous earthworm species interact with microorganisms, strongly affecting decomposition processes, accelerating the stabilization of organic matter, and greatly modifying its physical, chemical, and biological properties [1–4]. Vermicomposting and vermiculture are well established worldwide and are important for economic and environmental reasons [5]. As organic matter acts as both the substrate and food in vermicomposting, and soil is not involved, only epigeic earthworms can be used in the process. Among the epigeic earthworms, *Eisenia andrei* and *Eisenia fetida* are the species most widely used in vermicomposting and vermiculture facilities worldwide.

In nature, epigeic species occupy unpredictable and unstable habitats, characterized by highly variable environmental conditions, food availability, and predation pressures. When conditions are unfavorable, epigeic earthworms suffer high mortality, the population density oscillates widely (**Figure 1**), and the reproduction rate increases greatly [6]. Under these
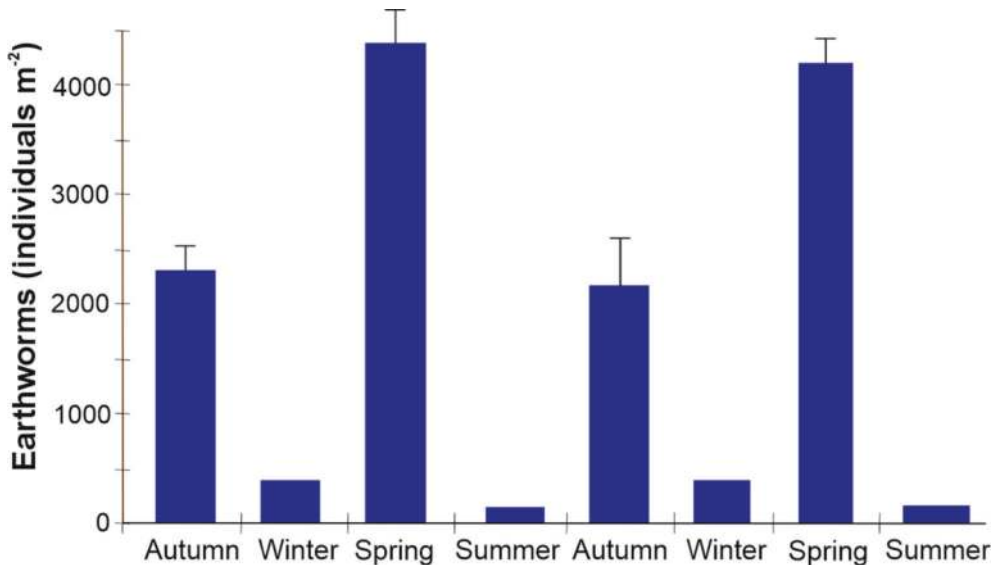
**Figure 1.** Seasonal fluctuations in earthworm density in a field population of the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae) in Vigo (Pontevedra, Spain).

circumstances, the ability to grow and reproduce exponentially is critical. From the point of view of their life history, epigeic earthworms are typical "r-strategists" or fast developers in the slow-fast continuum. Fast or r-selected organisms have typically short life cycles, are small, attain sexual maturity rapidly, and have high metabolic rates. Under unfavorable environmental conditions, high reproduction rates will ensure population survival, and the formation of cocoons may enable the worms to resist until conditions become more favorable, thus explaining the fluctuations in population density.

The favorable, stable conditions, and high reproduction rates enable earthworm populations to reach extremely high densities in vermicomposting facilities (more than 20,000 individuals $m^{-2}$, [7]).

## 2. *Eisenia fetida* and *Eisenia andrei* are different species

The importance of taxonomy is well recognized by most scientists and, indeed, without reliable taxonomy, most ecological studies are irrelevant [8]. In many species of earthworms, taxonomic identification based on morphological characteristics is difficult due to the structural simplicity of the earthworm body plan, which lacks anatomical complex structures or highly specialized copulatory appendages [9, 10]. *Eisenia fetida* and *Eisenia andrei* (**Figure 2**) are closely related species of earthworms that are widely used in vermicomposting systems to recycle organic waste, as well as in ecotoxicological, physiological, and genetic studies. These species are widely used because they are ubiquitous, have short life cycles, high reproductive rates, are tolerant to a wide range of temperature and humidity, and are relatively easy to handle Domínguez [1] and Domínguez and Edwards [11].

**Figure 2.** Photographs of *Eisenia andrei* (top panel) and *Eisenia fetida* (bottom panel) collected in Vigo (Pontevedra, Spain).

Both species were originally described as different morphotypes of *E. fetida* according to differences in body pigmentation [12]. Bouché (1972) later gave these earthworms subspecific status, naming them *E. foetida foetida* and *E. foetida unicolor* [13]. Although many authors now accept that *E. fetida* and *E. andrei* are different species, the oldest literature and also much current literature refer to these species collectively as *E. fetida* or *E. foetida*, an incorrect version of the original *E. fetida* [14, 15]. *Eisenia fetida* is the striped morph and the area between the segments has no pigmentation or is yellow or pale yellow, hence its common name of striped worm or tiger worm. By contrast, *E. andrei*, the common red worm, is uniformly red in color. Apart from the differences in pigmentation (**Figure 2**), the species are morphologically similar (**Figures 3** and **4**) with no differences in biological parameters, especially in relation to reproductive potential and life cycles, although the rates of growth and cocoon production are somewhat higher in *E. andrei* than in *E. fetida* [16]. The life cycles of *E. fetida* and *E. andrei* are well known and their population biology and ecology have been investigated by several authors and summarized by Domínguez [1] and Domínguez and Edwards [11].

A long-standing research project conducted in the soil ecology laboratory at the University of Vigo has resolved the problem of the taxonomic status of these two species; however, in much of the current literature, both species are still indiscriminately referred to as *E. fetida*, and it
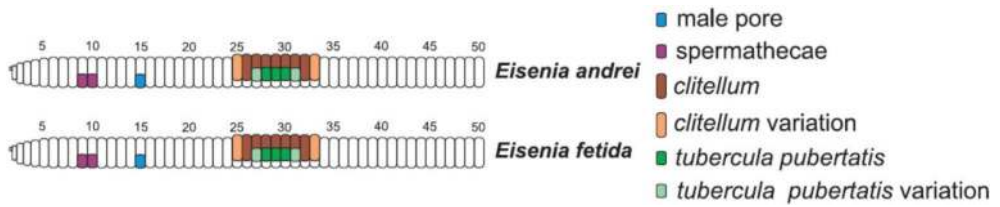
**Figure 3.** Diagram of the external morphology of *Eisenia andrei* and *Eisenia fetida*, showing that the two species are morphologically similar.
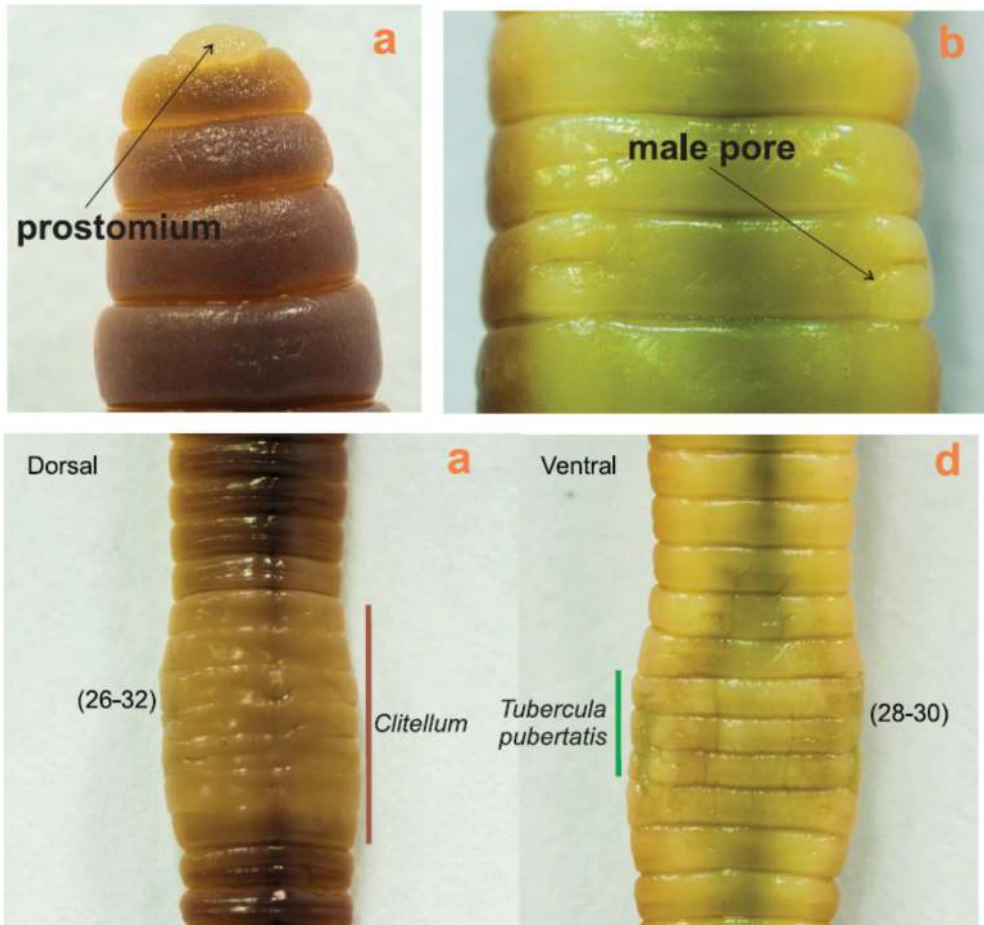


**Figure 4.** External morphology of the red worm *Eisenia andrei*. (a) Dorsal view of prostomium, peristomium, and first segments. (b) Male pores in the ventral side of segment 15. (c) Dorsal view of the clitellum in segments 26–32. (d) Ventral view of the tubercula pubertatis in segments 28–30. These external morphological characters are commonly used to distinguish between earthworm species.

is often not clear which of the two species is actually being considered. The objective of our research was to determine whether *E. andrei* and *E. fetida* are biologically and phylogenetically different species. We conducted laboratory experiments to determine the existence of any prezygotic or postzygotic reproductive barriers by comparing cocoon and hatchling production in interspecific and intraspecific crosses of the two species. We then used molecular phylogenetic methods data based on mitochondrial and nuclear DNA sequences to identify any differences between populations of *E. fetida* and *E. andrei*.

Four different populations of worms were used to study reproductive isolation: one population of *E. fetida* from Vigo (northwestern Spain) and three populations of *E. andrei* from Vigo, Madrid, and Brazil. Juveniles (<150 mg f.w.) were maintained in individual Petri dishes until maturity, to ensure that worms did not store sperm from previous copulations. The worms were supplied with food in the dishes, which were held in incubated chambers at 20°C and relative humidity 90%. When the worms reached sexual maturity, individuals from different populations were crossed. Individuals were randomly assigned for crossing, although the weight of the partners at each crossing was similar. Each pair of worms was held in a Petri dish for 7 days. Each worm was then placed in its original Petri dish, and cocoon production, incubation time, viability rate, and the number of hatchlings per cocoon were recorded weekly for 15 weeks. For the phylogenetic delimitation, 20 individuals of *E. andrei* from 4 populations (Brazil, Ireland and Spain [Vigo and Madrid]) and 11 individuals of *E. fetida* from 3 populations (Ireland and Spain [Vigo and Santiago de Compostela]) were used. Six individual specimens of *E. eiseni* (Levinsen, 1884) from Spain (Vigo and Santiago de Compostela) were used as outgroup.

The biological definition of a species is a group of individuals that can reproduce with one another in nature and produce fertile offspring. The crossbreeding experiment demonstrated that *E. fetida* and *E. andrei* are reproductively isolated as their crosses do not produce viable offspring (**Figure 5**). Although there were no significant differences in the rate of cocoon production in the intra and interspecific crosses of *E. fetida* and *E. andrei*, there were significant differences in cocoon viability. Thus, only the intraspecific crosses of both *E. fetida* and *E. andrei* produced viable cocoons (i.e., cocoons that produced hatchlings) [8].

In another crossbreeding experiment (*E. andrei x E. fetida*, *n* = 15; food: cow manure) carried out in the laboratory in 2016, the interspecific crosses did not produce cocoons. The study findings reject the possible existence of a single polymorphic species of *E. fetida* (including *E. andrei*), and we suggest that, as both phenotypes can be easily distinguished, the "good species" status can be applied to the studied taxa. Furthermore, our findings reveal that the reproductive isolation between *E. andrei* and *E. fetida* occurs post copulation and is probably postzygotic, with no efficient mechanism preventing interspecific copulations. In fact, both the interspecific and intraspecific crosses of the species produced similar numbers of cocoons, revealing that there are no mechanisms preventing copulation or cocoon production.

Although they are very similar, *E. andrei* and *E. fetida* are biologically different species and, as a consequence, the coexistence of both species in mixed cultures inevitably leads to poorer functioning of the vermicomposting system. The abundance and frequency of citations in the specialized and nonspecialized literature that indiscriminately refer to *E. andrei* and *E. fetida* as different names for the same species suggest that mixed cultures of both species are also quite common. In mixed cultures, the reproduction rate and biological efficiency will be much lower than in pure cultures because earthworms will waste energy in carrying out unsuccessful copulations.
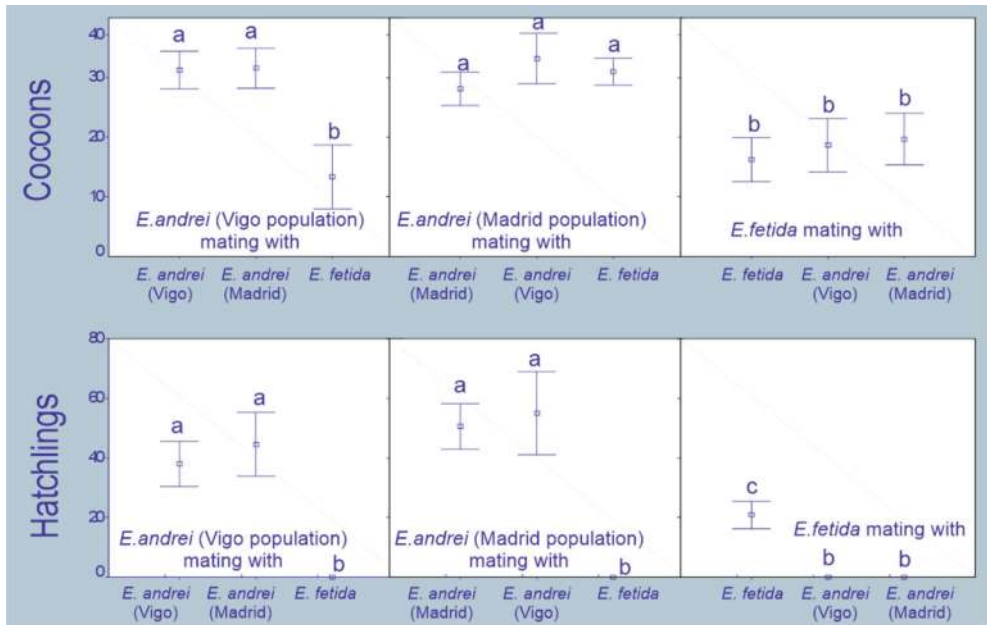
**Figure 5.** Results of crossbreeding experiments with the red worm *Eisenia andrei* and the tiger worm *Eisenia fetida*. Upper panel: Cocoon production (number of cocoons per earthworm) over a period of 15 weeks in the intra- and interspecific crosses. Lower panel: Hatchling production (number of hatchlings per earthworm) over a period of 15 weeks in the intra- and interspecific crosses.

This argument also applies to another two earthworm epigeic species often used in vermiculture and vermicomposting: *Dendrobaena veneta* and *Dendrobaena hortensis*. Although the names are often considered synonyms, *Dendrobaena hortensis* and *Dendrobaena veneta* are actually phylogenetically different species (see **Figure 8**; [17]). *Dendrobaena veneta* is two times larger (50–150 mm) than *D. hortensis* and the body color is also different. The dorsal side of *D. hortensis* has red-violet stripes and the ventral side is pale red, whereas *D. veneta* is uniformly red and is not striped. However, apart from the differences in pigmentation and size, both species are morphologically similar (**Figure 6**) and their biological parameters are not well known, mainly due to this taxonomic confusion.

These species can also be confused with *E. andrei* and *E. fetida* on examination by the naked eye. When more than one species coexist in vermicomposting systems, the reproduction rates
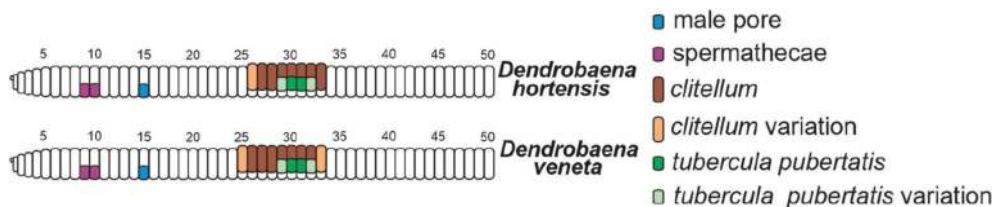


**Figure 6.** Diagram of the external morphology of *Dendrobaena veneta* and *Dendrobaena hortensis*, showing the morphological similarities between the two species.
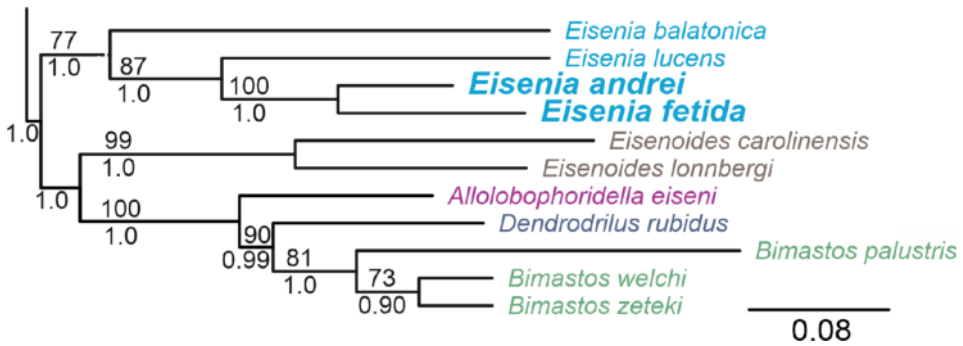
**Figure 7.** Clade including the species *Eisenia andrei* and *Eisenia fetida* extracted from the maximum likelihood molecular tree of the family Lumbricidae. The genus *Eisenia* is monophyletic and *E. andrei* and *E. fetida* are phylogenetically different species. Modified from [17].
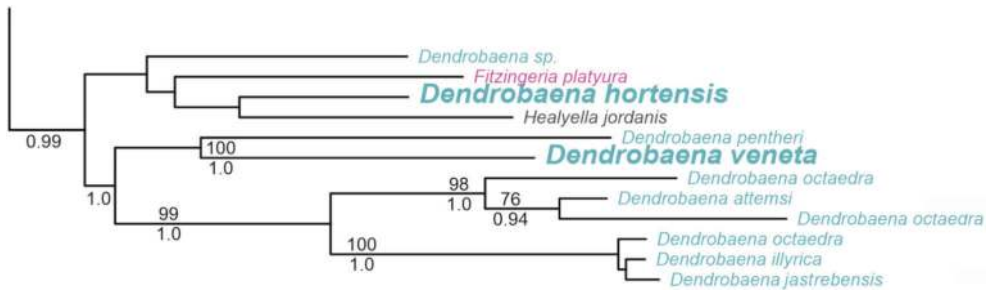


**Figure 8.** Clade including the species *Dendrobaena hortensis* and *Dendrobaena veneta* extracted from the maximum likelihood molecular tree of the family Lumbricidae. The genus Dendrobaena is not monophyletic and *Dendrobaena hortensis* and *Dendrobaena veneta* are phylogenetically different species. Modified from [17].

and ultimately the functioning of the process will be much less efficient. In summary, it is very important to determine which species are present in the cultures and to prevent the existence of mixed earthworm cultures.

The phylogenetic study demonstrated that *E. fetida* and *E. andrei* are phylogenetically different species. Phylogenetic analysis of maximum parsimony, maximum likelihood, and Bayesian (BMCMC) of the sequences of genes 28S and cytochrome c oxidase I (COI) and of the combined sequences (28S-COI) showed *E. fetida* and *E. andrei* to be monophyletic [18]. These results have been confirmed by other authors and by our group in a genus-level phylogeny of the family Lumbricidae (see **Figures 7** and **8**, [17]) and through a DNA barcoding study [19].

# 3. Laboratory culture of epigeic earthworms

Laboratory culture of epigeic earthworms should be rapid and easy to carry out, thus enabling (1) study of earthworm growth and reproduction; (2) identification of the demographic parameters of populations of different species and in different types of organic matter and organic

**Figure 9.** Laboratory culture of the epigeic earthworm *Eisenia andrei* (the red worm).

waste; (3) determination of the rate of consumption of organic matter; and (4) collection of casts to study the changes that take place in the organic matter during transit through the earthworm intestine (**Figure 9**).

Culture and maintenance of epigeic earthworms is quite simple and can be carried out in different ways and at different scales. However, it is important to establish some standard conditions to ensure success in culturing different species of epigeic earthworms.

### 3.1. Moisture and temperature

Epigeic earthworms require a substrate with a relatively high moisture content. High growth rates will be ensured by a moisture content of between 80 and 85%, which can be determined manually: the substrate should be damp, but when a handful is squeezed by hand, scarcely any water should escape. The temperature of the substrate should be between 20 and 25°C for optimal development of the vast majority of epigeic earthworms. The worms will also breed successfully under these conditions. However, they will not tolerate large variations in temperature, and the use of controlled temperature chambers is recommended. If this is not possible, the cultures should be maintained at a relatively constant temperature, and variations in temperature should be recorded with a minimum-maximum thermometer.

### 3.2. Culture dishes, recipes, and boxes

Different types and sizes of containers can be used for culturing earthworms, depending on the purpose of the culture.

### 3.2.1. Stock boxes

Relatively large populations of the different epigeic species can be maintained in stock boxes for later use (for different purposes) (**Figure 10**). The size of the boxes is not limited, except for the height, which should not exceed 50 cm. The bottom of the boxes should be perforated or formed by a grid of mesh size 0.5–1 cm. The boxes should not be in direct contact with the ground, and a container of vegetable waste can be placed underneath the box to collect the leachate. To start the culture, the box should be filled with a bed of vermicompost into which the initial population of worms is inoculated. This bed should be at least 10 cm high. The food material, for example, animal manure, is then added to the box. As the worms eat, they ascend through the food/substrate. More food is added in successive layers not exceeding 5 cm in height. When the boxes are almost full, plastic netting (mesh size 1 cm) is then placed on top of the box and covered with a new layer of manure. After some time, most of the earthworms will rise above the net. The net (plus worms) is then removed and can be used to start a new culture in another box. The surface of the substrate should be covered by a perforated plastic cover to prevent light entering and to preserve the moisture.

### 3.2.2. Petri dishes

Petri dishes are suitable for holding individual specimens or small groups of earthworms (**Figure 11**). Plastic petri dishes allow gas exchange while also maintaining good moisture conditions in the substrate. Some vermicompost containing earthworm(s) is placed on the bottom of the plates, which are then filled with food. The food is renewed as it is consumed. Cocoon production by mature individuals can also be monitored in Petri dishes. Dishes of different diameters can be used depending on the size of the species and the number of individuals to be cultured per dish.



**Figure 10.** Stock culture of earthworms (*Eisenia andrei*) fed with grape marc in the greenhouse facilities of the Animal Ecology Group at the University of Vigo (Spain).

**Figure 11.** Left: Petri dishes containing different densities of earthworms used to study growth and reproduction. The dishes are held in a laboratory environmental chamber under controlled conditions of temperature and humidity. Right: Detail of a petri dish with an individual specimen of *Eisenia fetida*.
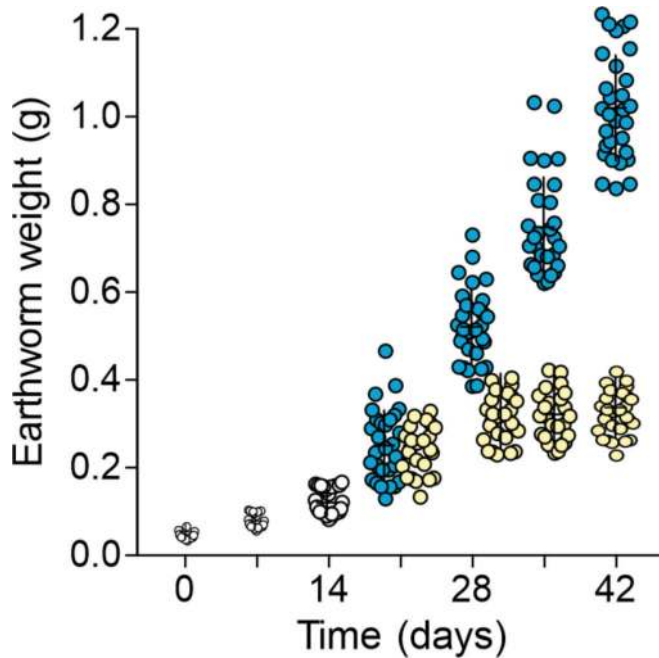


**Figure 12.** Growth curves of *Eisenia andrei* reared at (1) low population density (blue circles) and (2) high population density (yellow circles). High population density is usually reached when the vermicomposting system is performing at peak levels. Figure based on data from different experiments with different types of food for earthworms.

When environmental conditions are suitable and sufficient food is available, the growth of epigeic earthworm fits logistic curves, with a long phase of exponential growth (**Figure 12**, blue points). Earthworm growth is density-dependent, and individual growth and earthworm weight are lower in crowded conditions (as in vermicomposting systems) than in optimal conditions, although total earthworm biomass is greater. Earthworms reared in crowded conditions reach sexual maturity at smaller sizes than earthworm reared under conditions of low population density (**Figure 12**, yellow circles).

### 3.2.3. 96-well plates

Use of 96-well plastic plates to rear earthworms is recommended for studying reproduction and reproductive parameters related to cocoons, such as viability, time to hatching, and the number of juveniles hatched per cocoon (**Figure 13**). The cocoons should be washed with water and handled carefully with flat, blunt tweezers, to prevent damage. One cocoon is placed on top of moistened cotton wool in each well of the plate, each identified by a code number (e.g., A5 or F3). The plates are covered with plastic film (such as Parafilm M). The film over each well is pierced with a pin to make a small hole to allow gas exchange. In addition to reducing evaporation, the plastic film also prevents mixing among the hatchlings emerging from different cocoons.
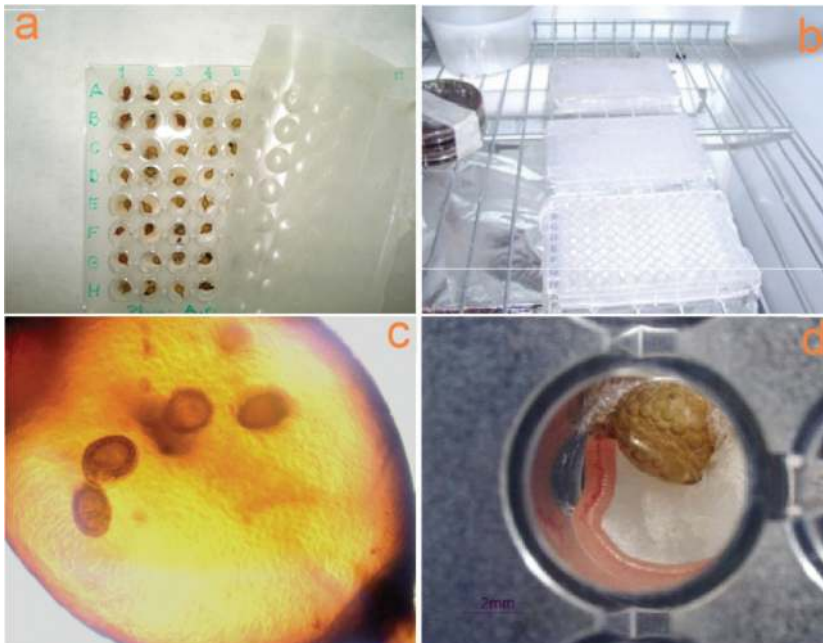


**Figure 13.** Methods used to study reproductive parameters related to earthworm cocoons. (1) 96-well plates with one cocoon in each well. (2) Plates covered with covered with plastic film (Parafilm M), (3) earthworm (red worm) embryos inside the cocoon, and (4) new earthworm hatchling (red worm) emerging from the earthworm cocoon inside the well.

The plates are checked daily to monitor cocoon development. Plates with cocoons should be placed in an incubated chamber at a temperature between 18 and 22°C in darkness until they hatch, which in the case of the red worm takes place between 18 and 26 days after cocoon production, with 2–3 new hatchlings typically emerging per cocoon [11]. A cocoon is considered viable when it produces at least one earthworm. The newly emerged hatchlings are then placed in Petri dishes, with food provided ad libitum, to study the first stages of growth (**Figure 13**).

## 4. Conclusions

The ideal earthworm species for rapidly transforming organic waste into vermicompost, from the point of view of the rapid return of nitrogen to the ecosystem and adjustment of the C/N ratio of the waste, should combine a short life cycle with a high metabolic rate. *Eisenia fetida* and *Eisenia andrei* are suitable for use in vermicomposting as both species are small, r-strategists, and have a short life cycle and high reproductive rates. Indeed, these are the most widely used earthworm species in vermicomposting and vermiculture facilities throughout the world because they are ubiquitous, naturally colonize diverse types of organic waste, tolerate wide temperature and humidity ranges, and they are strong, resistant, and easy to handle.

*Eisenia fetida* (tiger worm) and *Eisenia andrei* (red worm) are phylogenetically and biologically different species and do not interbreed. *Dendrobaena veneta* and *Dendrobaena hortensis*—other species used in vermicomposting—are also separate species. As these differences are not generally known, the existence of mixed cultures is quite common in commercial and domestic earthworm culture facilities. The presence of more than one species in mixed cultures leads to lower reproduction rates and a less successful vermicomposting system.

In summary, for optimal functioning of the vermicomposting process, the earthworm population should comprise a single species, optimal environmental conditions should be maintained, and food should be provided ad libitum.

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