Jun 24, 2025

Protocol for Extracting *Amynthas* Species Cocoons

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwbqjzvmk/v1

Maryam Nouri-Aiin[1]

[1]University of Vermont

Maryam Nouri-Aiin
University of Vermont

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwbqjzvmk/v1

Protocol Citation: Maryam Nouri-Aiin 2025. Protocol for Extracting Amynthas Species Cocoons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwbqjzvmk/v1

Protocol status: Working
We use this protocol and it's working

Created: January 30, 2025

Last Modified: June 24, 2025

Protocol Integer ID: 119362

Keywords: Amynthas, cocoon, eathworms, cocoon extraction, soil, protocol for extracting amynthas species cocoon, extracting amynthas species cocoon, extracting cocoon, accuracy in cocoon retrieval, extraction, cocoon retrieval, amynthas species, more bins of water, sieving approach, mesh sieve, water reuse, water conservation, removing large debris, such as wood fragment, minimizing waste, finer sieving, soil, improved efficiency, wood fragment, processing, improving efficiency, reduced water usage, larger debris, benchtop extraction, processing time

Disclaimer

> *This protocol is intended for research and educational purposes only.*

Abstract

This protocol presents an optimized method for extracting cocoons of three *Amynthas* species—*A. tokioensis, A. agrestis,* and *A. hilgendorfi*—building on the techniques described by Ikeda et al. (2015) and Nouri-Aiin & Görres (2019). By incorporating key modifications, this wet-sieving approach enhances **accuracy in cocoon retrieval** while significantly improving **efficiency, ergonomics, and water conservation**.

Key Modifications:

- **Reduced Water Usage:** Instead of using continuous running water in a sink, this method utilizes two or more bins of water (depending on the number of samples), allowing for water reuse and minimizing waste.
- **Improved Efficiency:** A **coarse-mesh sieve** (e.g., Sieve No. 8) is introduced to quickly remove larger debris (such as wood fragments and roots) before finer sieving. This significantly reduces processing time, as removing large debris through Sieve No. 12 alone is much more time-consuming.
- **Ergonomic Advantage:** Unlike previous methods where the person extracting cocoons had to wash soil over a bucket in a sink, this approach allows for a comfortable, seated, benchtop extraction, significantly reducing physical strain.

Guidelines

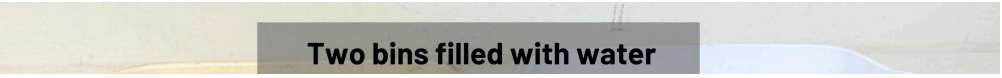
- Use **small soil portions** (~1 cup) per batch for higher accuracy.
- Always stack **Sieve No. 8 on top of No. 12** to prevent cocoon loss.
- **Additional bins** can be used as needed based on the number of samples and the amount of soil being processed.
- Perform a **final rinse in clean water** for better visibility before cocoon extraction.
- A **white background** can improve visibility and make cocoons easier to identify.
- Using **a stereo microscope** can help distinguish cocoons from similar-looking debris.
- The **water in the bins can be reused** for multiple samples; replace it when it becomes heavy with sediment and organic debris, or develops a murky, moldy appearance.

Materials

- **Bins** filled with water X 2
- **Sieve No. 12** (1.7mm)
- **Sieve No. 8** (2.36mm)
- **Forceps**
- **Petri dish**
- **Ruler**

Optional:

- **Sieve No. 14** (1.4mm) – Alternative to No. 12 for finer soil filtration



Two bins filled with water

Safety warnings

- **Do not** submerge the soil completely in water, as this may wash away cocoons.
- **Too much pressure** when handling soil can break cocoons.
- Some **seeds and small soil particles** resemble cocoons—verify by gently squeezing with forceps (cocoons are elastic, while seeds/soil particles break).
- Extracted cocoons should be **kept moist to prevent rapid dehydration**.

Before start

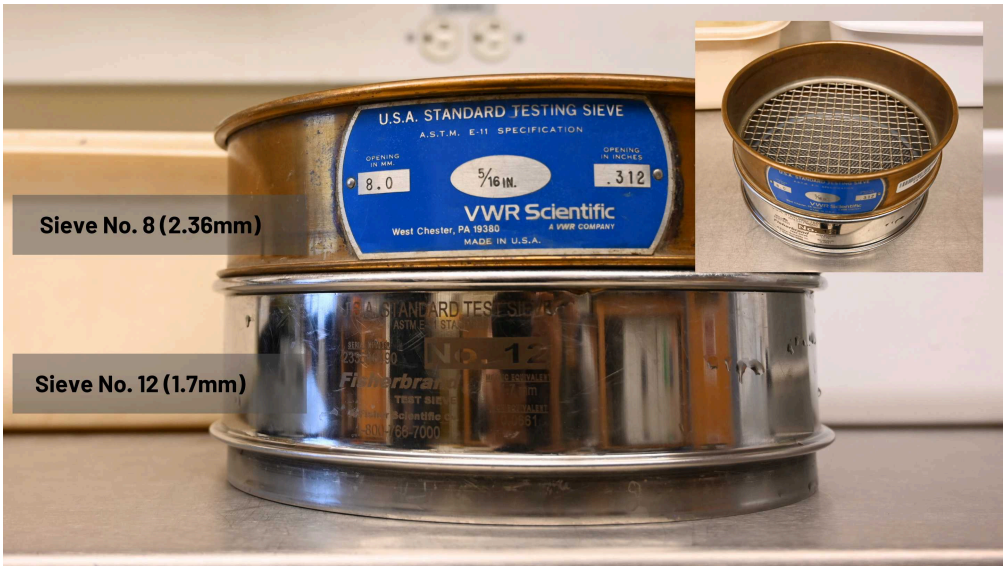
- If the **soil is excessively dry, gradually moisten** it over a period of time appropriate for its dryness. This allows cocoons to rehydrate, preventing shrinkage that could cause them to pass through the sieve mesh.
- **Remove large debris**, such as sticks and leaves, before sieving to ensure a smoother process.
- **Work in a well-lit area** to facilitate accurate cocoon identification.
- **Use a flat, stable surface** to minimize the risk of spills or sample loss.
- Keep **a waste container** nearby for easy disposal of unwanted debris.

Sieve Setup

1 Stack **Sieve No. 8** on top of **Sieve No. 12**, or simply just use Sieve No. 12.

Note

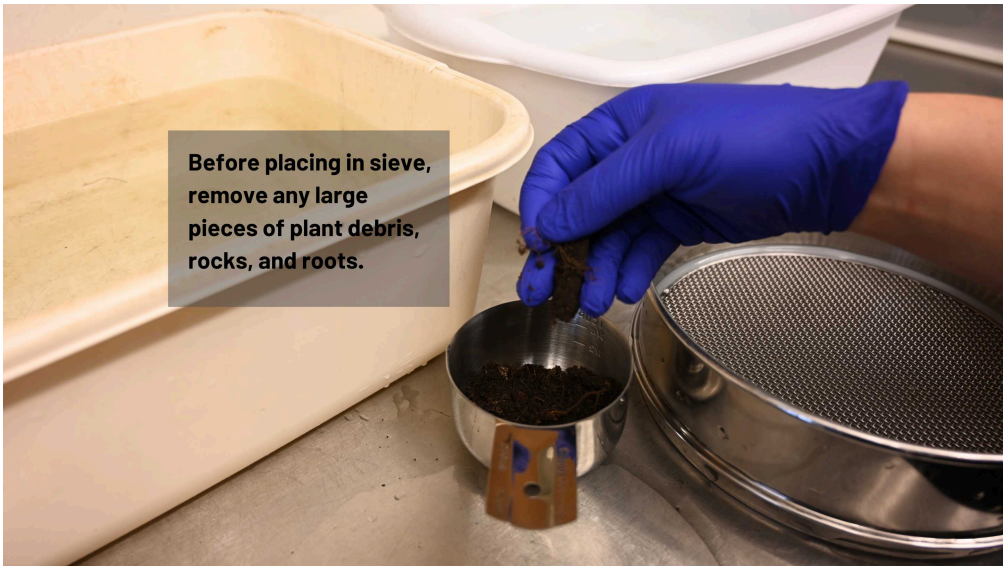
- Sieve No. 12 is the standard size for capturing cocoons of the three *Amynthas* species, ranging from 2.5 ± 0.05mm to 4.5 ± 0.05mm.
- Sieve No. 14 (1.4mm) can be used as an alternative to No. 12; however, its smaller mesh size increases processing time by slowing the removal of soil particles.
- Sieve No. 8 is optional but recommended for removing large debris such as wood fragments and roots.
- If using Sieve No. 8, always ensure it is stacked on Sieve No. 12 to prevent cocoon loss.



Soil Washing

2 Place approximately *one cup* of soil into Sieve No. 8 stacked on Sieve No. 12.

- Using smaller soil portions increases accuracy and ease of cocoon extraction.



3 Submerge the sieves in a bin filled with water.

Note

- Water level should cover the soil but should not fully submerge the sieve.

4 Gently rub the soil to break down larger particles, allowing soil to pass through Sieve No. 8 onto Sieve No. 12.
- This process disperses aggregates, retaining only coarse soil particles and organic debris on the sieve.

5 Remove Sieve No. 8 along with the retained debris.

6 Lift Sieve No. 12 so that water covers the soil but does not submerge it.

This is how the soil captured in Sieve No. 12 should appear after removing Sieve No. 8. Only soil particles should remain in Sieve No. 12.

7 Gently rub the soil against the sieve to separate remaining particles.

Note

- *Avoid applying too much pressure* as it can break cocoons.

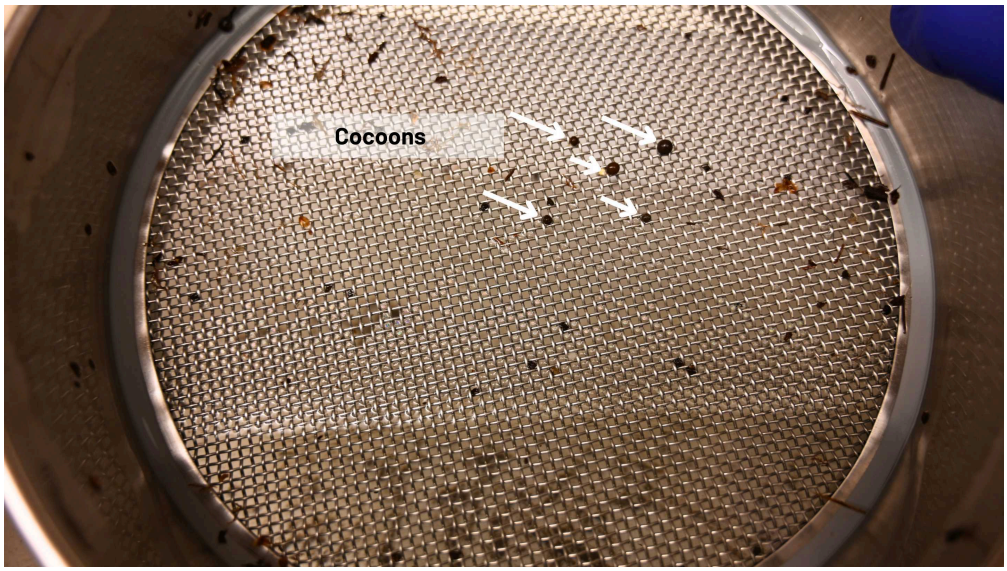
8 After soil removal, perform a final rinse in a second bin filled with clean water.

Cocoon Extraction

9 Extract cocoons using forceps. Small soil particles and seeds may resemble cocoons. To distinguish them:

Note

- Apply *gentle pressure* with forceps.
- *Cocoons are elastic and bouncy*, while seeds and soil particles break apart quickly.
- A white background can improve visibility and make cocoons easier to identify.
- Using a stereo microscope can further aid in distinguishing cocoons from similar-looking debris.



10 Place extracted cocoons in a petri dish filled with water.

Note

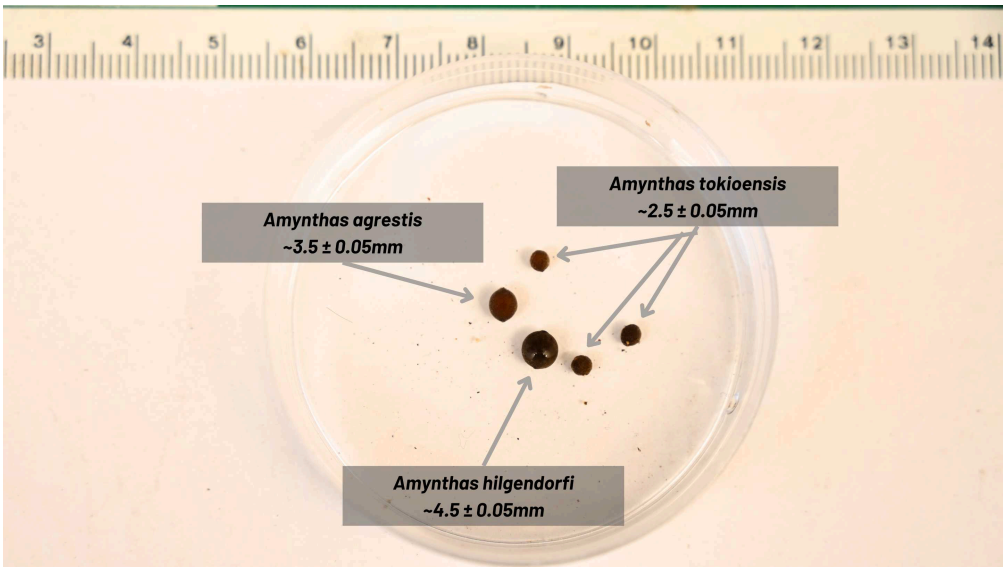
- Cocoons dehydrate quickly if not kept moist.

Cocoon Identification

11 Cocoon size measurements according to **Nouri-Aiin & Görres (2019):**

Note

- *Amynthas tokioensis*: ~2.5 ± 0.05mm
- *Amynthas agrestis*: ~3.5 ± 0.05mm
- *Amynthas hilgendorfi*: ~4.5 ± 0.05mm



12 For specimens that fall between species size ranges, a molecular identification method can be used, as described in **Nouri-Aiin et al. (2021).**

Protocol references

Citation

Ikeda, H., Callaham Jr, M.A., O'Brien, J.J., Hornsby, B.S., Wenk, E.S. (2015)
. Can the invasive earthworm, Amynthas agrestis, be controlled with prescribed fire?. Soil Biology and Biochemistry.

https://doi.org/10.1016/j.soilbio.2014.12.011 LINK

Citation

Nouri-Aiin, M. and Görres, J.H. (2019). Earthworm cocoons: the cryptic side of invasive earthworm populations. Applied Soil Ecology.

https://doi.org/10.1016/j.apsoil.2019.05.004 LINK

Citation

Nouri-Aiin, M., Schall, J.J., Keough, C.A., Wen, Y. and Görres, J.H. (2021)
. Identifying the unidentifiable: a PCR multiplex protocol for the diagnosis of invasive pheretimoid earthworm species, verified by morphological and barcode identification.
Applied Soil Ecology.

https://doi.org/10.1016/j.apsoil.2020.103822 LINK

Citation

Nouri-Aiin, M. (2022). Elements of Biocontrol Strategies for Pheretimoid Earthworms.
The University of Vermont and State Agricultural College..

https://www.proquest.com/docview/2703003513?pq-origsite=gscholar&fromopenview=true&sourcetype=Dissertations%20&%20Theses
LINK

Citations

Ikeda, H., Callaham Jr, M.A., O'Brien, J.J., Hornsby, B.S., Wenk, E.S.. Can the invasive earthworm, Amynthas agrestis, be controlled with prescribed fire?
https://doi.org/10.1016/j.soilbio.2014.12.011

Nouri-Aiin, M. and Görres, J.H.. Earthworm cocoons: the cryptic side of invasive earthworm populations.
https://doi.org/10.1016/j.apsoil.2019.05.004

Nouri-Aiin, M., Schall, J.J., Keough, C.A., Wen, Y. and Görres, J.H.. Identifying the unidentifiable: a PCR multiplex protocol for the diagnosis of invasive pheretimoid earthworm species, verified by morphological and barcode identification.
https://doi.org/10.1016/j.apsoil.2020.103822

Nouri-Aiin, M.. Elements of Biocontrol Strategies for Pheretimoid Earthworms
https://www.proquest.com/docview/2703003513?pq-origsite=gscholar&fromopenview=true&sourcetype=Dissertations%20&%20Theses