

# Methods For Collecting Eumycetozoan Substrates in the Field



# Some Definitions

- Site: a collecting stop.
- Substrate Collection (for protostelids and myxomycetes): an individual bag of substrate. Ideally, it will consist of about two good handfuls of substrate that is air dried and stored in a small *paper* bag (e.g., lunch bag). **Never** store collections in plastic bags.
- Paired Substrate Collection: Two collections, 1 from aerial dead plant material (e.g., grasses, forbs, twigs, dead leaves, tree bark) and 1 of the same type of material that has fallen to the ground below. If it is not possible to get only the material of interest from the ground, get what you can. Designate the each collection in the pair with the same collecting number followed by "a" for the aerial substrate and "g" for the ground substrate.
- Collection Set: 5 to 10 paired collections from a site.

# More Definitions

Specimen Collection: A specimen of a myxomycete fruiting body collected in the field and mounted in a small box. Fruiting bodies of many myxomycetes are large enough to be easily seen in the field and can be brought back to the lab for identification and preparation for storage in an herbarium.



# At the Site

- Take and record a GPS reading. (If you move more than 500 meters from the initial reading for one collection set, take and record another if possible.)
- Record the type of habitat and include some general physical and geographic information.
- For Protostelid and Myxomycete Substrates: Begin collecting paired collections and record the type of substrate with the collection number. (It is easy to use a collecting number that starts with an abbreviation for the country or state/province, the last two digits of the year, the number of the collection in order, and the designation "a" or "g" for aerial or ground, respectively.) For example, SA03-1a and SA03-1g could represent the first paired samples from South Africa in 2003. Record the collection number on the collecting bag and in the collecting notebook. It is not necessary to always collect paired samples. Samples of bark, pieces of downed wood, or dung may be collected singly. Paired samples are most appropriate when collecting the same type of substrate in both the aerial and ground microhabitats (e.g., dead grasses that are still standing and dead grasses that have fallen over and are decomposing on the ground).

## Taking a GPS Reading



## Recording Habitat Data



Bagging Substrates  
(Be sure all bags are labeled with a collection number.)

# Suitable Substrates for Protostelids and Myxomycetes

Bark of living trees



Plant litter on the ground



Aerial plant litter



Standing dead wood or stumps



Dead but still attached herbaceous plant parts such as old inflorescences



Downed and decayed wood or bark



# Bark Samples

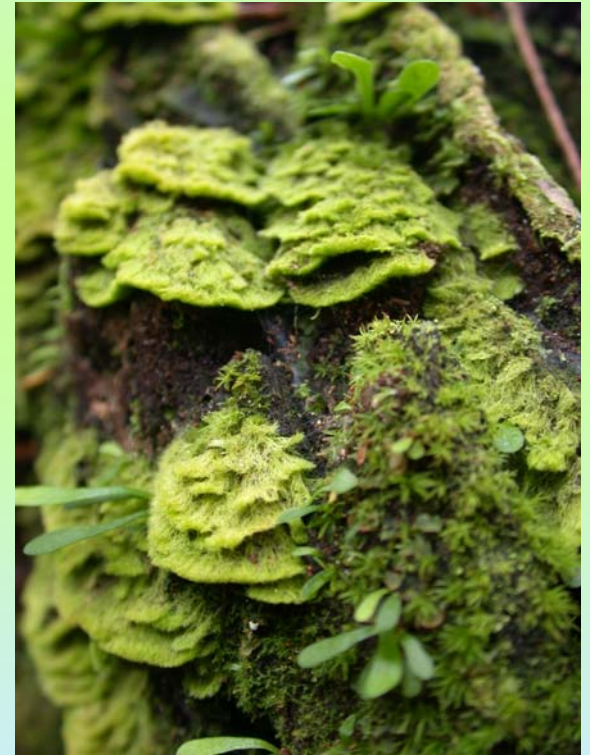
- Bark samples should be collected from the dead outer bark of living trees.
- The individual pieces of bark collected should be no larger than about 2 to 4 cm across.
- If possible, collect pieces of bark from more than a single tree of each species.
- The sample for a given species should consist of enough pieces to cover a total area equal to the size of your hand (palm and fingers).

# Other Aerial Microhabitats

Large inflorescences of tropical herbaceous plants



Epiphytic moss and algal communities on tree bark



# Care of Substrate Collections

- Collections of substrate material should be as dry as possible when collected. If they are moist or wet, they should be air dried in the bags with the tops of the bags open before the bags are rolled closed and packed away.



# Collection of Substrates for Dictyostelid Isolation

From each site, collect 5 to 10 collections of humus-rich soil from the litter/soil interface. Each collection should contain about ten soup spoons (20-50 grams) of material and should be kept in a sterile plastic bag such as a Whirl-Pak. The collections should be stored in a cooler at approximately 15C if possible.

One also should look for specialized microhabitats in which soil-like material can be found above the ground, such as (1) on epiphytic plants with structures that tend to retain decaying plant matter and various types of organic debris or (2) beneath mats of vascular epiphytes and/or bryophytes.

# Typical leaf litter habitat



# Remove Intact leaves





**Scraping  
soil/litter into a  
sterile bag**

# Other not so typical places to find soil-like material

Around the roots of vascular epiphytes



Associated with other portions of various types of epiphytes



10-50 g  
soil/litter in a  
sample bag



# What happens to the samples you send in?

- We carry out various methods for isolating each of the three different groups in the laboratory, but each method has an element in common: a high humidity chamber or “moist chamber” is set up to allow the organisms to form fruiting bodies in culture.

# Methods for Isolating Protostelids



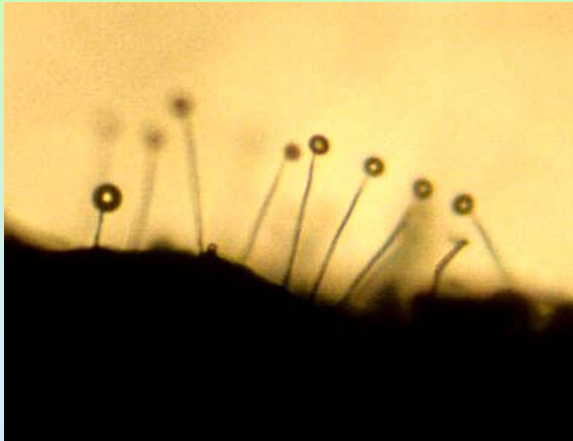
Substrates are broken into small pieces and soaked in sterile water.



Then they are arranged on agar containing very low nutrient levels.



Next the plates are scanned to find and identify protostelids.



This is the reward a researcher gets upon finding a protostelid. This species is *Schizoplasmodiopsis micropunctata*.

# Methods for Isolating Myxomycetes



Moist chambers are prepared by placing filter paper in a Petri dish.



Substrates are arranged in the chamber and water added.



Plates are scanned with a stereoscope.

Finally, one is rewarded with a fruiting like this one of *Arcyria denudata*.

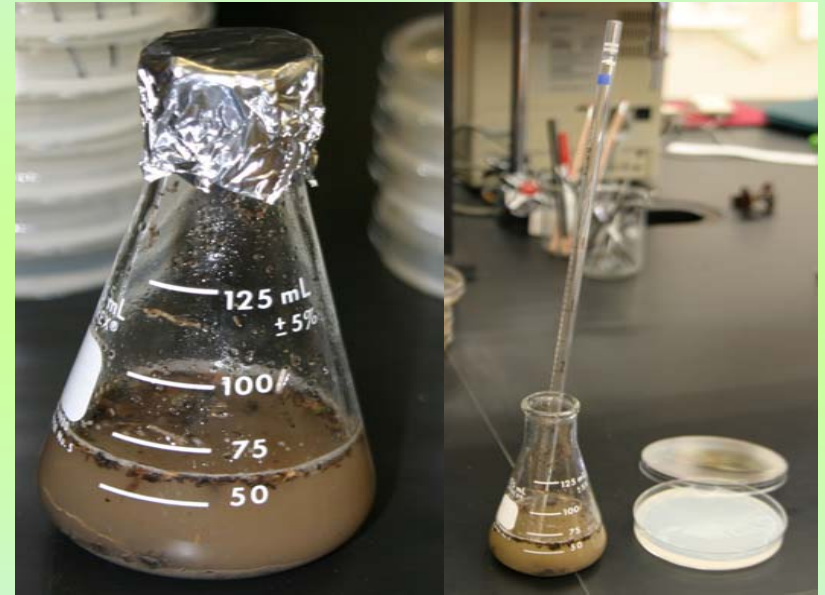


# Methods for Isolating Dictyostelids

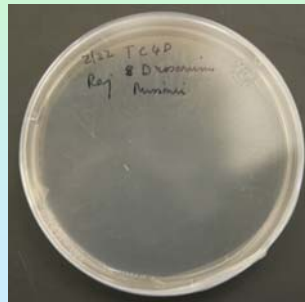
Part of the sample is weighed.



Then the weighed sample is placed in sterile water and diluted and a portion is placed on a plate of hay infusion agar.



Plates are scanned with a stereoscope and species are noted.



Some dictyostelids are subcultured for closer inspection and identification.

If interested in making collections  
for our project please email:

Steven L. Stephenson: [slsteph@uark.edu](mailto:slsteph@uark.edu)

or

Frederick W. Spiegel: [fspiegel@uark.edu](mailto:fspiegel@uark.edu)

or

John D. Shadwick: [jshadwi@uark.edu](mailto:jshadwi@uark.edu)

For more information, please visit our website:

<http://cavern.uark.edu/ua/mycetozo/>