

Guideline for Field Collecting and Preserving Sphaeriidae Clams, for DNA and Taxonomic Research.

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(Photo on cover page are 3 *Pisidium adamsi* taken by A. Frankiewicz.)

Index:

- Goals..... 03
- Sphaeriidae General Description..... 03
- Sphaeriidae Habitat 04
- Equipment List 04
- Methods for Collecting and Preserving Specimens..... 04
- Description and Photos of each Genus..... 06
- Corbiculidae 09
- References..... 10
- Field Sheet..... 11

Goals:

The objective of this sampling protocol is to provide a guideline for collecting and preserving Sphaeriidae clams for future taxonomic and DNA analysis. This protocol will give a brief description of the ideal habitats for clams, equipment and methods for qualitative sampling, how to identify specimens in the field, and the different ways to preserve and transport specimens for DNA analysis.

Sphaeriidae General Description:

Sphaeriidae, commonly called fingernail, pea, or pill clams, are the smallest and most poorly understood freshwater bivalves. Their shell length ranges from < 3 mm in the smallest species (*Pisidium conuentus*) to approximately 25 mm for the largest (*Sphaerium simile*). Because of their small size and cryptic nature, they are usually overlooked during field collection or mistaken as pebbles or plant seeds. But with practice and patience, the field crew can separate clams from other debris by looking for: 1) an object that is not transparent (similar to a grain of sand) 2) Is hard like a pebble (though some species have delicate shells), and 3) has smooth sides with either very distinct or fine striation. The specimen should also have a distinct line that separates the two shells. For mature specimens, the shell will have a hump or umbo on the dorsal side of the shell (Fig.1).

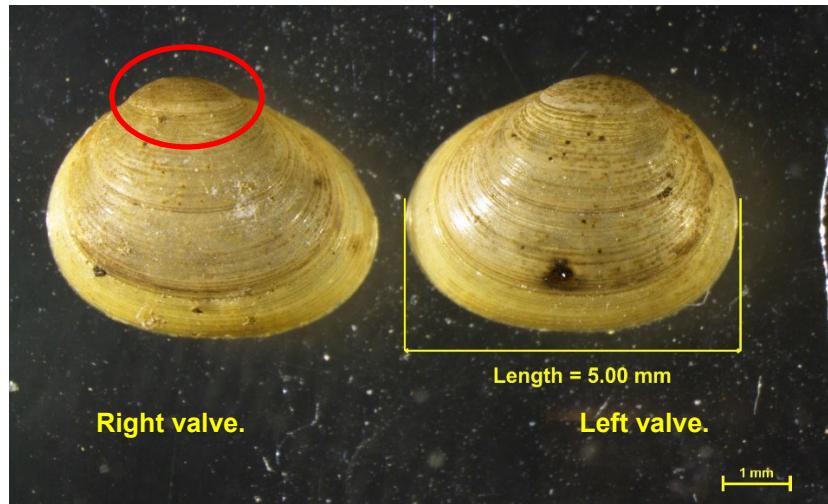


Fig. 1, Is a photograph of *Pisidium casertanum* right an left valves, the Red circle shows the location of umbo, photo was taken by A. Frankiewicz.

The Sphaeriidae shells are usually rounded or oval, but some species have more of a trigonal or tetragonal shape. Shell coloration ranges from pale yellow or white to chestnut or a dark brown tint. Soft anatomy will take on the coloration of the water from where the specimen was collected. For example, I have found clams from vernal pools that appear red or orange despite their shell being white. Many of the paler color clams have a distinctive orange tint at the

umbone, this can make them easier to separate from other material such as plant seed or sand grain.

Sphaeriidae Habitat:

Sphaeriidae can be found in almost any type of aquatic habitat, from large lakes and rivers to springs, bogs, and vernal pools. Generally, the greatest diversity can be found in lakes, ponds, and small rivers. Other habitats, such as vernal pools or small creeks, are usually colonized by a small number of species, but may contain unique species such as *Sphaerium occidentale*. Like mussels, Sphaeriidae are burrowers, and thus only a few species are found in areas with coarse sediment like rocks, gravel, and hard sand (such as the invasive *Pisidium amnicum*). Most Sphaeriidae species prefer living in detritus or in finer sediment such as silt, clay, mud, muddy sand. For example in vernal pools I found more clam on top of the leaf litter then in the sediment, but in some lake I found the opposite where there was more clam in the sand sediment than in the vegetation.

Equipment list:

- Dip-nets
- Round pointed shovel (optional but useful)
- Drag dredge (If available)
- Sorting sieve with a 500 µm mesh
- 10 to 12 quart pail for secondary containment
- Flat tubs for picking
- Disposable plastic pipette with opening of >5 mm
- Forceps with soft or curved tip
- Vials ranging from 5 ml to 30 ml or larger, depending on the size and number of specimen
- Cotton, wool or other soft absorbent material to place in vial
- Cooler (for storage of specimens)
- Foam vial holder (needs to fit in cooler)
- Ethanol (95 % proof)

Methods for Collecting and Preserving Specimens:

This protocol focuses on qualitative sampling techniques using dip-nets and shovels. If available, a drag dredge can be used for collecting in depths > 5 feet. Sphaeriidae, like all bivalves, are found primarily near the surface of the benthic zone in aquatic habitats. However, some can also be found in other zones clamped onto debris or submerged plants. When collecting sediment in 2ft or less, a round pointed shovel or dip-net can be used to skim the surface of the soil. When collecting from depths deeper than 2ft, or when the sediment at the site is very soft, the dip-net is

used. Collecting with the dip-net follows similar protocol as collecting for other benthic invertebrates except field collectors may have to skim deeper into the substrate than typical. When using a drag dredge to collect samples from depths deeper than 5ft (usually being dragged behind a boat) samples are emptied into a tub.

Once sediment samples are collected, the contents should be carefully cleaned with a 500 µm mesh sieve in a 10 to 12-quart pail as secondary containment. Sieving the material will remove most of the fine debris and make picking easier. If no container is available then the sieve can be placed in relatively clear water, where the field crew should slowly and carefully sieve the material. Once samples are cleaned, specimens can either be picked from the sieve or the contents can be place in a picking tub. Fine sediment from the secondary container should be sieved again to ensure no specimens were accidentally slipped out when sieving.

When picking, specimens must be handled with care! The shell is very important for taxonomic identification and any damage to the shell will make it difficult to key a species. Large specimens such as *Sphaerium* or *Musculium* can be collected by hand, but for most clams use forceps with a soft or curved tip. Most dissection forceps are also acceptable, but remember to be careful to only put enough pressure to hold the specimen. To collect smaller clams safely and effectively, use a disposable plastic pipette with an opening of 5mm or larger. If the specimen are to be kept cool then pipetting excess water or other material is not only acceptable but could also help the specimen stay alive longer. But if putting the specimen in ethanol try to avoid pipetting extra water which could risk diluting the preservative.

Sphaeriidae should be preserved and transported one of two ways to allow future DNA work: placing specimen in 95% ethanol or freezing the specimen. When using 95 % ethanol, remove any excess material or vegetation from the specimen of the shell. Excess organic material and water will dilute the ethanol and compromise preservation. Replace the ethanol at the end of the collecting day (or when needed). When freezing, clams should be placed in vials with a small amount of water and soft material—such as wet cotton wool, grass, or moss—to help prevent fragile shells from being damaged when transported. Label vials and place into a foam vial holder, or appropriate container that can protect and hold the vials for transport. Place the container into a cooler with ice at a temperature roughly around 5°C (41°F) or lower. Most species of *Sphaeriidae* can survive for several weeks or even months in cold temperatures; although it is worth noting that others may not have the tolerance for such conditions and will perish. Regardless of their survivability, the cold temperature will aid in maintaining the integrity of the specimen's DNA.

Although ethanol is an easy way to preserve specimens, problems do arise because of improper preservation and tendency for clam muscle to stiffen. This stiffness makes it challenging to open the shell for identification. Because of these issues, the field crew is encouraged to use the freezing method. Only use ethanol if the clam's shell becomes damaged while collecting or if options are limited.

It is acceptable to put multiple specimens in one vial, but be considerate of the size of the vial, number of individual clams that will be put into the vial, and the size of the clams. There is no formal guideline for this, but generally, the smaller the clam and the larger the vial the more that can be added; the larger the clam and the smaller the vial, less should be added. Vial sizes may fluctuate, but generally keep to no more than 50 small specimens (1 – 6mm) per vial, < 20 medium specimens (7 – 15mm) per vial, and < 10 larger specimens per vial.

Descriptions and Photos of Each Genus

There are an estimated 39 species of Sphaeriidae in North America, and 34 of those species are found around the Great Lakes region. The family is split into 4 genera: *Sphaerium*, *Musculium*, *Pisidium*, and the fourth, *Euperinae*, is only found in the Southern United States. Genera can be separated in the field using the following descriptions.

Sphaerium: Adults are easy to moderately easy to spot in samples because of their large size, with adults ranging from 7 – 25mm in length. Depending on the species, shell thickness can range from thick to thin, which either have fine or coarse striations. Coloration range from yellowest chestnut to dark brown. (Fig. 1 and 2).



Fig. 2. *Sphaerium Corneum*, photo taken by J. Szokalová (2010).



Fig. 3. *Sphaerium simile*, photo taken by A. Frankiewicz.

Musculium: These are easy to moderately difficult to spot due to their small to medium sizes, adults ranging from 7 – 15mm in length and their very thin shell which makes them appear semi-transparent. **Care should be taken when handling!** Their most distinctive feature is the umbone, which is separated from the rest of the shell by a distinct sulcus or ridge which forms a cap on the top of the shells (Figs. 3 and 4).

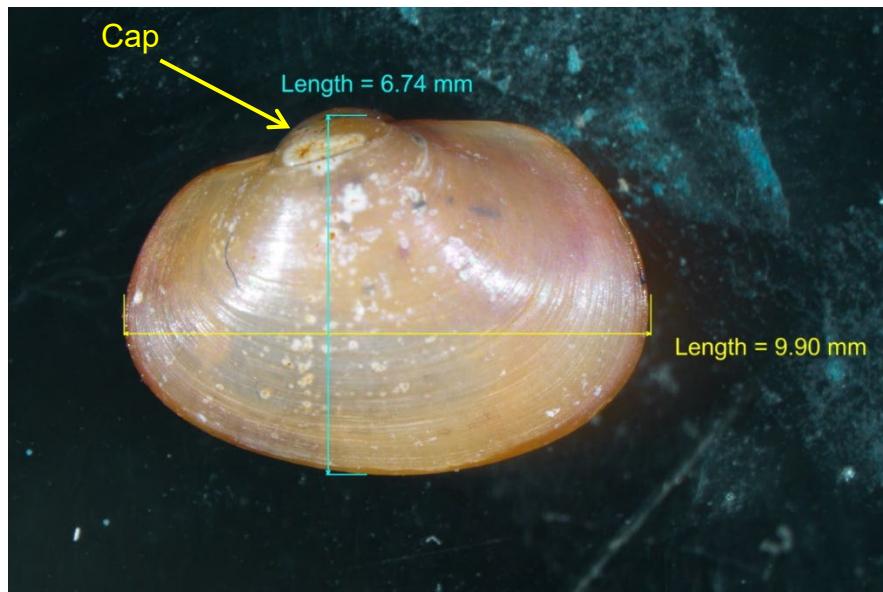


Fig. 4. *Musculium transversum*, right valve. Arrow indicating the shell cap, Photo taken by A. Frankiewicz.



Fig. 5. *Musculium partiumeum* on a cattail leaf, photo by M. Leppin (2009).

Pisidium: Although some species of *Pisidium* can be easily spotted (such as *P. amnicum*), most are very difficult to find in collected samples due to their small size, adult ranging from 2 – 7mm and sometimes pale shell coloration, this makes them often mistaken as pebbles or plant seeds. Thus, **field crews must take caution and care when sorting through small material**. In relation to their size, most species have thick shell with distinctive striation while others have thin shell with fine striae, coloration range from pearl white or transparent to dark brown (Fig. 5 and 6).



Fig. 6. *Pisidium ventricosum* resting on a man's index finger, photo taken by L. Clarfeld (2014).



Fig.7. Photographs of 2 different shells of *Pisidium amnicum*, photo by A. Frankiewicz.

Corbiculidae:

Although Corbiculidae (Asian clam) are not targeted for this research, they do belong to the same superfamily as Sphaeriidae (Corbiculacea) and thus specimens may be useful for comparisons and can be collected.

Corbiculidae, genus *Corbicula*: are very easy to spot from samples and separate from Sphaeriidae due to their size, adult ranging from 13 – 30mm in length and triangular shaped shells, adult's height usually = length. Shell are thick and with very distinctive Striations and have a light to dark brown coloration (Fig.7.)

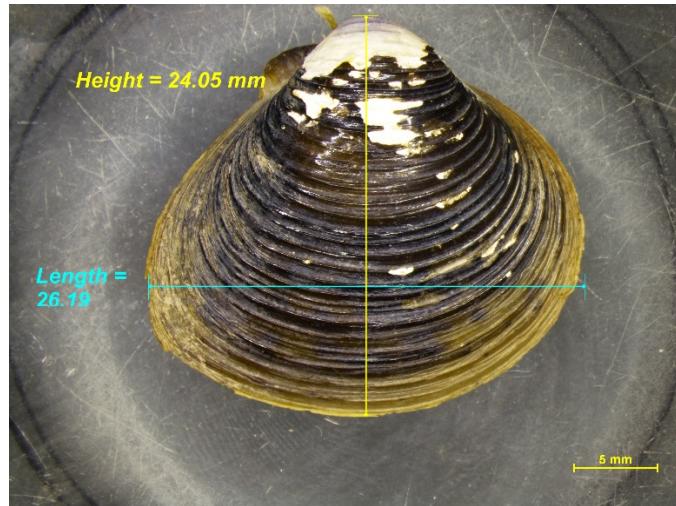


Fig. 8. *Corbicula fluminea*, photo by A. Frankiewicz.

References

- **Photographs:**

Clams on ruler or leaf: <https://hiveminer.com/Tags/sphaeriidae/Recent>

Photo of clam on finger: <https://www.inaturalist.org/observations/645491>

Sphaerium on hand: <https://www.biolib.cz/en/image/id177785/>

- **Articles and Books:**

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Collecting Sphaeriidae Field Sheet

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1. Collecting samples: sample can be collect with either using a shovel or dip-net to skim the surface of the sediment, digging in roughly 1 to 4 inches into the soil.
 - a. Shovels are used in < 2 feet of water unless the sediment is soft then use a dip-net (although a dip-net can for other sediments as well).
 - b. When depths are > 2 feet, use a dip-net.
 - c. If available, a drag dredge should be used when the water depth is deeper than 5 feet.
 - d. Be aware when collecting for other inverts that some clams can also be found in plant vegetation, so keep an eye out when sampling these areas.
2. Cleaning samples: collected samples need to be cleaned with a 500 µm mesh sieve this will remove materials such as muck, silt, organic debris, and other fine particles and make it easier for field crew to pick through samples.
 - a. It is ideal to sieve samples in a pail or secondary container that can catch any specimens that may slip out of the sieve. If no container is available, sieve the sample in relatively clear water.
 - b. Samples must be carefully sieved moving the content back and forth slowly. If there is material that will not sieve (leafs, rock, or filamentous algae), it can be removed from the sieve, examine carefully for clams or other inverts, and then placed into a picking container or back into the environment where it was collected.
 - c. After cleaning sample, contents of the sieve can either be placed in a picking container or the sieve itself can be picked.
 - d. If a container was used to sieve in then the “waste” content should be sieved again to insure no specimen were missed from the first sieve.

- 3. Pick specimens:** **Please handle specimens with care!** Some species of clam are very delicate and may crack if too much pressure is applied.

 - a. Use your finger to collect larger specimens. You can use thin nitrile gloves, but you want to feel the clam in your finger so you know how much pressure you are applying.
 - b. Other size clam can be pick with soft or curved tip forceps (dissecting forceps work too just remember to put just enough pressure to hold the specimen).
 - c. A disposable plastic pipette with a large opening (> 5 mm) works very well for collecting smaller specimen. Remember if the specimen is placed in ethanol, avoid excess much water into the vial which would dilute the concentration of the ethanol.
- 4. Preserving and transporting:** Specimen will either be preserved in 190 proof ethanol (95 % Alc.) or specimen will be chilled in vials and transported in freezers. We want to freeze the specimens instead of using ethanol, so use ethanol method if the specimen is damaged or if options are limited.

 - a. Ideally, individual specimen should be placed in separate vials, but some cases this may not be reasonable so it is acceptable to put multiple specimens in one vial. Field crew should use their best judgement with regard to how many specimens should go in one vial.
 - b. When using ethanol, the vial should be half full of ethanol (depending on the vial), more if needed. The exception to this is if you have a few small clams and a big vial, then less can be used. Specimens should be cleaned as best as possible before putting into ethanol. Ethanol in vial should be replace with clean ethanol after collecting day or if need, you can tell if it needs clean ethanol if it's not clear or the alcohol lost its odor.
 - c. When freezing, clam will be placed in vial that have small amount of water (1/10 the volume of the vial) and something that is soft that can protect the clam shell when transporting such as wet cotton wool, wet grass, or moss (moss is supposedly the best option). Vials are then placed into a foam vial holder or some other container that can protect the vial while transporting. Container are then placed in coolers with temperature roughly around 5°C (41°F) or colder.