

## Isolation of the Corrophilous Fungus, *Pilobolus*, from Wayne County, Indiana

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### Introduction

*Pilobolus* is a microscopic zygomycete that grows on the dung of herbivores. And, while it appears to be widely distributed, it has not been widely recorded. Records of *Pilobolus* from North America are particularly uncommon.

*Pilobolus* has been recorded from Ohio (4), Michigan (1), New York and Pennsylvania (5), but no records of *Pilobolus* from Indiana exist. In this study samples of dung were collected in Wayne County, Indiana and examined for isolates of *Pilobolus*.

### Methods and Materials

Beginning mid-winter, collections of *Pilobolus* were made from samples of dung of herbivores collected at 35 locations in Wayne County, Indiana. These samples were collected from sheep, beef and dairy cattle, horses, ponies, llamas and deer. Only fresh samples of dung were collected. These collections were made aseptically in plastic baggies and were transferred within hours to sterile preparation dishes lined with water saturated filter paper. All cultures were maintained at room temperature under cool white fluorescent lights with an intensity of 320 foot candles with a 12 hour photoperiod until growth with visible sporangiophores appeared.

Upon maturity *Pilobolus* discharge their sporangia. These sporangia adhered to the tops and sides of the preparation dishes because of the gelatinous layer around the sporangium. Isolates were obtained by removing single sporangia from the sides or tops of the preparation dishes with a sterile inoculating needle. Each sporangium was transferred to a petri dish containing dung agar (6). After 1 to 1 1/2 weeks growth would fill the petri dish depleting the media. At such times hyphal tips were transferred to fresh media. Active hyphal growth could be seen within 24 hours after transfer.

Development of *Pilobolus* sporangia is influenced by light, so sporangia were collected between 9 am and 1 pm daily (the photoperiod was set between 8 am and 8 pm). Sporangia were collected from the lids of petri dishes or from sporangiophores with sterile inoculating needles or microforceps, and mounted in lactophenol. Spores were also observed in the lactophenol mounting preparation. Pressure on the coverslip broke the sporangial wall releasing spores for observation and measurement. Columella were observed by removing sporangia with microforceps.

The following characteristics were observed and measured:

1. Spore size, shape, color, and wall thickness
2. Sporangium size, shape, and ornamentation
3. Sporangiphore length
4. Trophocyst size and shape
5. Subsporangial swelling size, shape, and color
6. Columella shape

Measurements of taxonomic structures were made both from the original isolates on dung and later from the growth on dung agar.

### Results

The genus *Pilobolus* has rarely been seen to reproduce sexually. Its normal method

of asexual reproduction is by the production of sporangiospores within a sporangium. The structure of this asexual reproductive complex, and the component structures are the primary taxonomic characteristics for the genus.

In nature, *Pilobolus* grows submerged in dung with its sporangial apparatus rising above the surface. The sporangial apparatus of *Pilobolus* is unique. It consists of the sporangium, containing sporangiospores, the subsporangial swelling, the sporangiophore, and the trophocyst. The sporangium is covered with a thick cutinized wall that is darkly pigmented and rests at the apex of the sporangiophore. This sporangium contains thousands of sporangiospores. Unlike many zygomycetes, the spores within the *Pilobolus* sporangium remain together and act as a sporangial unit. Upon maturity the sporangium with all of its spores is forceably discharged from the sporangiophore and travels as far as 8 feet. This characteristic gives *Pilobolus* its name "hat thrower" (2).

The subsporangial swelling is the portion of the sporangiophore located just below the sporangium. It is a widened area of the sporangiophore which is light sensitive and acts in 'aiming' or directing of the sporangium prior to discharge. Below the subsporangial swelling is the long, slender sporangiophore. The sporangiophore measures from 1 mm to several centimeters in length in different species and holds the sporangium above the surface of the substratum.

The trophocyst, a structure unique to *Pilobolus*, is located at the lower end of the sporangiophore. It is embedded in the substratum and anchors the sporangial apparatus. The trophocyst may be elongated, somewhat oval, or turnip shaped.

These structures: the sporangium, sporangiospores, subsporangial swelling, sporangiophore, and trophocyst are the primary characteristics used in the taxonomy of *Pilobolus*.

From twenty-eight isolates of *Pilobolus* from Wayne County, Indiana, four different species were recovered. These species were: *Pilobolus crystallinus*, *Pilobolus kleinii*, *Pilobolus longipes*, and *Pilobolus roridus*.

#### *Pilobolus crystallinus* Tode (7)

*Pilobolus crystallinus* sporangiophores develop in 3 to 4 days, are 1 to 5 mm long, and are clear to pale yellow in color. Trophocysts develop submerged in the substratum and are usually 500  $\mu\text{m}$  long by 350  $\mu\text{m}$  wide. Sporangiospores are pale yellow ellipses which measure  $9.83 \pm 0.90 \mu\text{m}$  in length by  $6.05 \pm 0.75 \mu\text{m}$  in width producing a length to width ratio of 1.62.

Sporangia are covered with a dark, cutinized wall and range from 100-750  $\mu\text{m}$  in diameter, with a mean of  $205.7 \pm 49.7 \mu\text{m}$ . About 1/3 of the isolates recovered have polygonal reticulations as described by van Tieghem (8).

*Pilobolus crystallinus* was isolated in 15 locations in Wayne County during March through August from the dung of sheep, cattle, donkey, goat, llama, and pony.

#### *Pilobolus kleinii* van Tieghem (8)

*Pilobolus kleinii* sporangiophores measure 2-3 cm in length and arise from dark yellow turnip shaped trophocysts measuring 300-500  $\mu\text{m}$  in diameter. The trophocysts are often partially submerged within the substratum. Sporangia are dark, smooth, and cutinized. They measure 100-300  $\mu\text{m}$  across with a mean of  $146.25 \pm 98.8 \mu\text{m}$ , and are about 2/3 as high as wide. The columella are conical and extend deeply into the sporangia. Sporangiospores are yellow and elliptical, measuring  $12.14 \pm 1.16 \mu\text{m}$  in length by  $7.57 \pm 0.59 \mu\text{m}$  in width with a length to width ratio of 1.60.

*Pilobolus kleinii* was isolated in 5 locations in Wayne County between March and August from the dung of cows, sheep, and goats.

#### *Pilobolus longipes* van Tieghem (8)

*Pilobolus longipes* sporangiophores range from 5 mm to 3 cm (sometimes longer) and

develop from large trophocytes often 1 mm or more in length. Sporangioophores growing from freshly collected dung are much longer than those growing from isolates transferred to samples of sterilized dung or to dung agar. Sporangia are nearly globose, smooth, dark, cutinized and vary greatly in size from 100 to more than 400  $\mu\text{m}$  in diameter. However, the mean diameter for sporangia is  $226.3 \pm 53.7 \mu\text{m}$ . Sporangiospores are subglobose to globose, dark yellow to orange in color and measure  $12.23 \pm 1.59 \mu\text{m}$  by  $11.23 \pm 1.52 \mu\text{m}$  with a length to width ration of 1.09.

*Philobolus longipes* was isolated at 7 locations in Wayne County during May through July. All isolates were taken from horse dung.

*Pilobolus roridus* (Bolt.) Pers. (3)

*Pilobolus roridus* sporangioophores are 1 to 2 mm long. The sporangia are smooth and hemispherical, and average  $260.0 \pm 22.4 \mu\text{m}$  in diameter. The trophocysts are 250-300  $\mu\text{m}$  in diameter, nearly spherical, and bright orange in color. The sporangiospores are pale yellow to colorless, oval in shape and measure  $5.79 \pm 0.68 \mu\text{m}$  in length and  $3.07 \pm 0.35 \mu\text{m}$  in width. The length to width ratio is 1.89.

*Pilobolus roridus* was isolated in Wayne County during August from deer dung.

### Discussion

Even though *Pilobolus* has been isolated in many places, this is the first record of isolates from Indiana. The source of the substratum from which the organism was isolated has been recorded. Even though there seems to be some relationship between the species of *Pilobolus* isolated, and the type of dung on which it was found, there has not been a direct correlation shown. It is, however, interesting to note that *P. longipes* was isolated seven times in this study and in all instances it was isolated from horse dung. The time of year each collection of dung was made was recorded. During the winter it was difficult to obtain isolates of *Pilobolus*. Dung of herbivores not obtaining at least part of their food by grazing on pasture included no isolates of *Pilobolus*. It is easy to speculate that *Pilobolus* can be isolated only from the dung of animals grazing on open pasture. Certainly, this was the case in this study.

Many of the taxonomic characters traditionally used with *Pilobolus* are of questionable value in separating the various species. This is because of the wide variation that occurs within a single isolate. Sporangium size, subsporangial swelling size and shape, and to some degree length of the sporangioophore have little value. The fluctuation in these characters makes them almost useless.

The sporangiospores seem to be the most constant and thus exhibit the most valuable taxonomic characteristics. The size, shape, and coloration of the sporangiospore are reliable taxonomic characteristics. Regardless of the size of the sporangium, the sporangiospores contained within remain remarkably constant.

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