

**A Beginner's Guide to Identifying
The Common Protostelids**

by
Frederick W. Spiegel
John D. Shadwick
and
Lora Lindley-Settlemyre

University of Arkansas, Fayetteville
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Introduction

Protostelids are members of a monophyletic group of organisms known as Eumycetozoa, a group of slime molds that also contains the myxomycetes, or plasmodial slime molds, and dictyostelid cellular slime molds. The protostelids are the least well known and most easily overlooked members of the eumycetozoans. This is because almost every species has delicate, microscopic fruiting bodies and because relatively few researchers have studied them since their recognition in the early 1960s.

The amoeboid trophic stages of protostelids occur as predators of bacteria and fungal cells on decaying vegetation that may be standing (aerial) or in the litter. The decaying plant tissue may be primary (leaves, stems, inflorescences, etc) or secondary (wood and bark). Occasionally protostelids may occur on herbivore dung or the humus layer of soil. There is some recent evidence that the trophic cells may occur on submerged decaying vegetation in aquatic habitats.

Protostelids are recognized when the amoebae develop into sporocarps, or fruiting bodies. Sporocarps are found by the microscopic examination of bits of substrate that have been kept moist for several days on an agar plate. A typical protostelid sporocarp consists of a delicate stalk that supports a single spore, although there are a few species that regularly have 2 or 4 or even 8 spores. Almost all species of protostelids may be identified to species on the basis of sporocarp morphology.

There are 36 species of eumycetozoans that have been described as protostelids and perhaps twice as many more that have been observed but not described. The most commonly encountered species number about 15-20 and are described. This guide is designed to help the user recognize the most common species and to identify them to species.

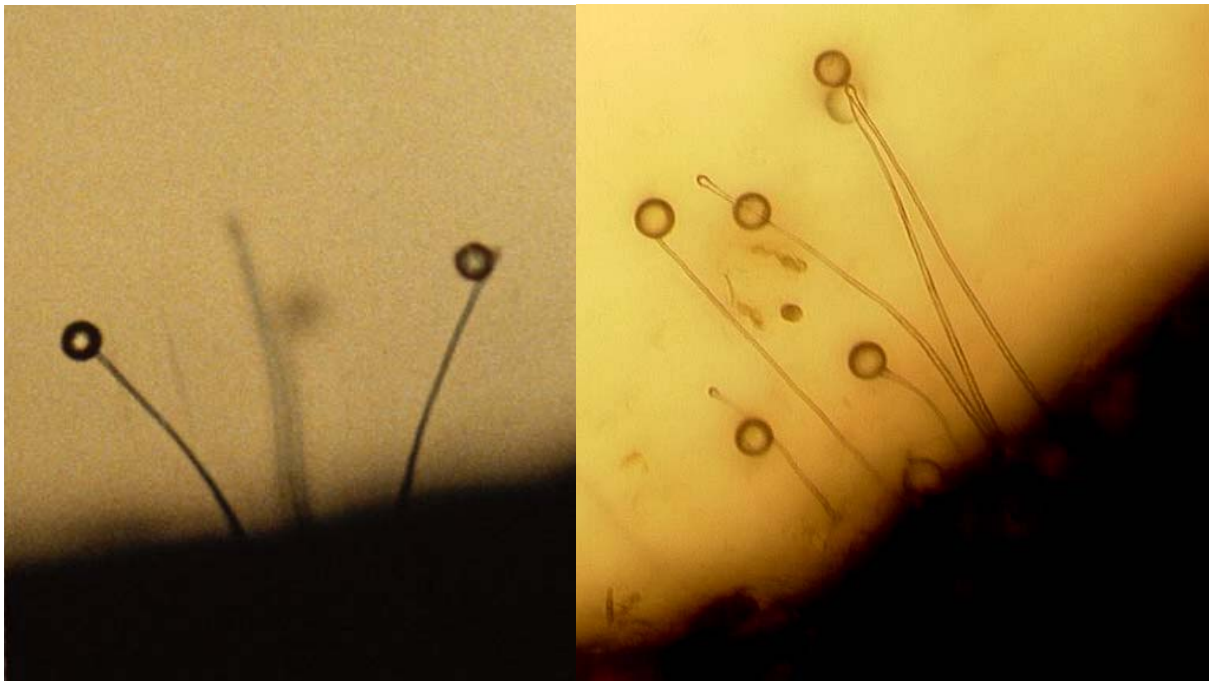
All species covered in this guide are shown fruiting on primary isolation plates. These are agar plates on which pieces of natural substrates have been incubated. When one is looking for protostelids, this is the quickest way to find them and record their presence. While culturing of protostelids is not too difficult, it is time consuming. Therefore, the ability to identify them on primary isolation plates allows most workers to survey them much more effectively.



A typical habitat in a temperate deciduous forest with all the microhabitats in which protostelids occur: standing dead plants, forest floor litter, bark of living and dead trees, and rotting logs.

This guide is prefaced with a section on how to collect and prepare substrates for observation of protostelids and an illustrated guide to the terminology used for sporocarp morphology. It is followed by a visual guide to common protostelids arranged on the basis of sporocarp features. As this is an identification guide, there is no discussion of taxonomy or classification, and closely related species may not be placed together.

The images of sporocarps taken with bright field microscopy as they grow on primary isolation plates with either a 10x or 20x objective. In order to show whole sporocarps or groups of sporocarps in focus, these images are montages compiled with the Auto-Montage system produced by Syncroscopy. The user, to see all the features in these images, would be expected to focus up and down on a specimen. Each taxon is illustrated with an image or images indicating its diagnostic features, and the images are accompanied by a brief description of how common the taxon is and the habitats and microhabitats in which it is most likely to be encountered. Because trophic cells are often difficult to observe in primary isolation plates, there will be few images pointing out features of amoebae.



Single (left) and montaged (right) images of clusters of sporocarps of *Nematostelium gracile*. Note the much greater amount of detail that is in focus in the montaged image.

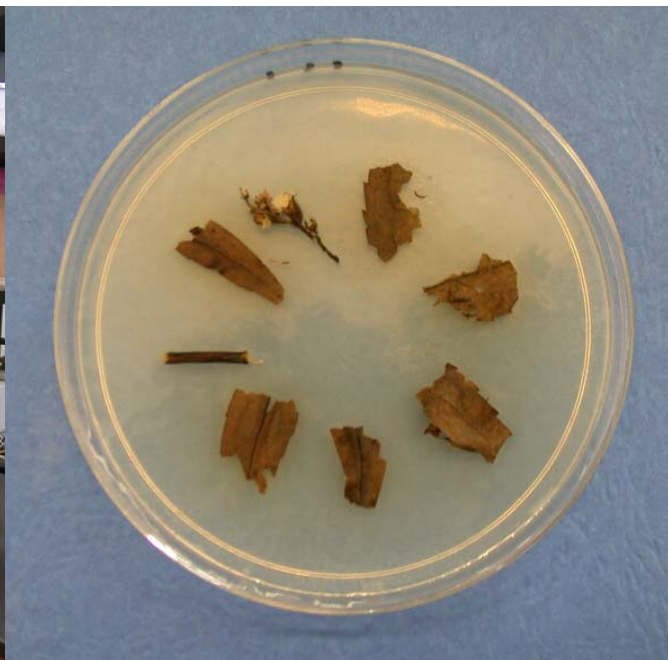
Collecting and Plating Protostelid Samples

1. Go to a terrestrial habitat that is likely to contain protostelid substrates, and collect bits of substrate and place them in a *paper* bag or envelope. Label the collection with a unique collection number, and record locality data (preferably with GPS), habitat type, microhabitat, substrate, and collector. If the collection is to be plated and observed at a later date, air dry it before storage. If the collection is damp, it will rot and become overrun with filamentous fungi.



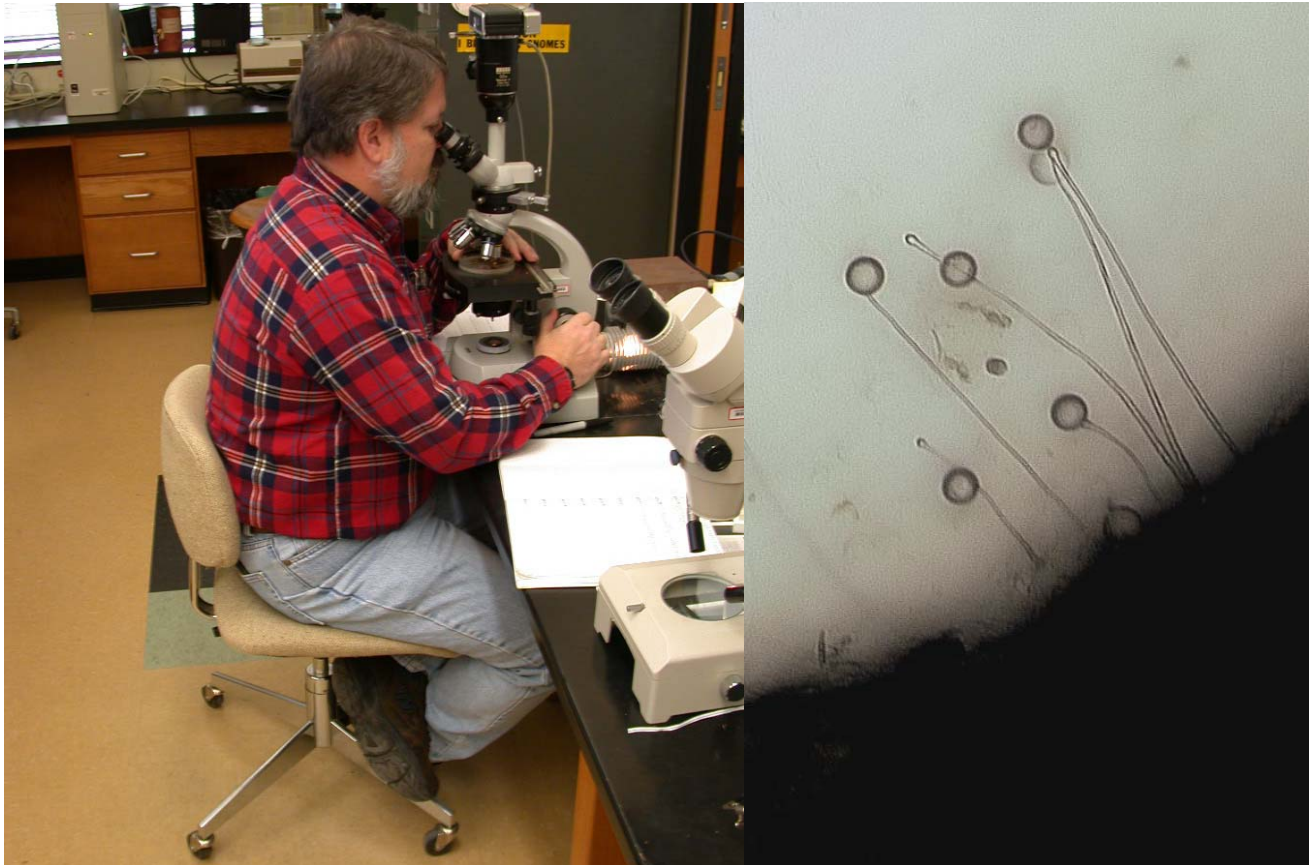
Natasha Jones collecting protostelid substrates along a roadside in New Zealand. In the background, Steve Stephenson is taking a GPS reading.

2. Upon return to the lab, cut the substrate into pieces about 1-1.5cm on a side using sterilized scissors, soak them completely in sterile, distilled water, and plate ca. 8 pieces in a circle about half way between the center and edge of a 15cm petri plate of wMY agar (0.02g malt extract, 0.02g yeast extract, 0.75g dibasic potassium phosphate, 15g agar/L distilled water)



John Shadwick plating out substrates in the lab (left). A primary isolation plate with eight pieces of substrate (right).

3. Incubate the primary isolation plates at room temperature for at least 3 days, then examine them with a compound microscope using a 10x-20x objective. Scan the edge of each piece of substrate and the agar immediately surrounding it for sporocarps of protostelids. Record the species that occur on and around each piece. The greatest abundance of protostelids typically occurs between 5 and 14 days after the substrates are plated. It is usually a good idea to examine each plate once or twice during this period.



Fred Spiegel examining a primary isolation plate for protostelids with the compound microscope (left). A cluster of sporocarps of *Nematostelium gracile* fruiting from the side of a piece of substrate (right).

Morphological Features to Look For on a Protostelid Sporocarp



Spore (Sp): Shape, Number, Relative Size, Deciduous or Nondeciduous, if Deciduous then Actively or Passively Shed, Hilum (H) Present or Absent, Sheath (Sh) Features

Stalk (St): Long (> 2 spore diameters in length) or Short (≤ 1.5 spore diameter in length), Taper, Relatively Thick or Thin, Straight or Sinuous, Stiff or Flexuous, Apophysis Present or Absent, Basal Disc (B) appearance, Stalk Present or Absent after Spore Shed

Other Features: Shape of Prespore Cell, if seen, Gregarious or Scattered

Note: A single species can be very variable in size, but sporocarp proportions tend to be fairly constant over the size range. For instance, the spores of *N. ovatum* can range from less than 5 μ m to over 15 μ m.

Identification Guide

The following section includes images of sporocarps of all the most commonly encountered protostelids along with some less common species. The plates are arranged based upon the number of spores, whether the spore is deciduous, and the relative length of the stalk.

Each species is illustrated in habit in a Primary Isolation Plate (PIP), always along with some details in supplemental images in most cases. Image A always presented at the same size in all the plates with the scale bar equal to 100um. For most of the details, the scale bar equals 50um and is so indicated.

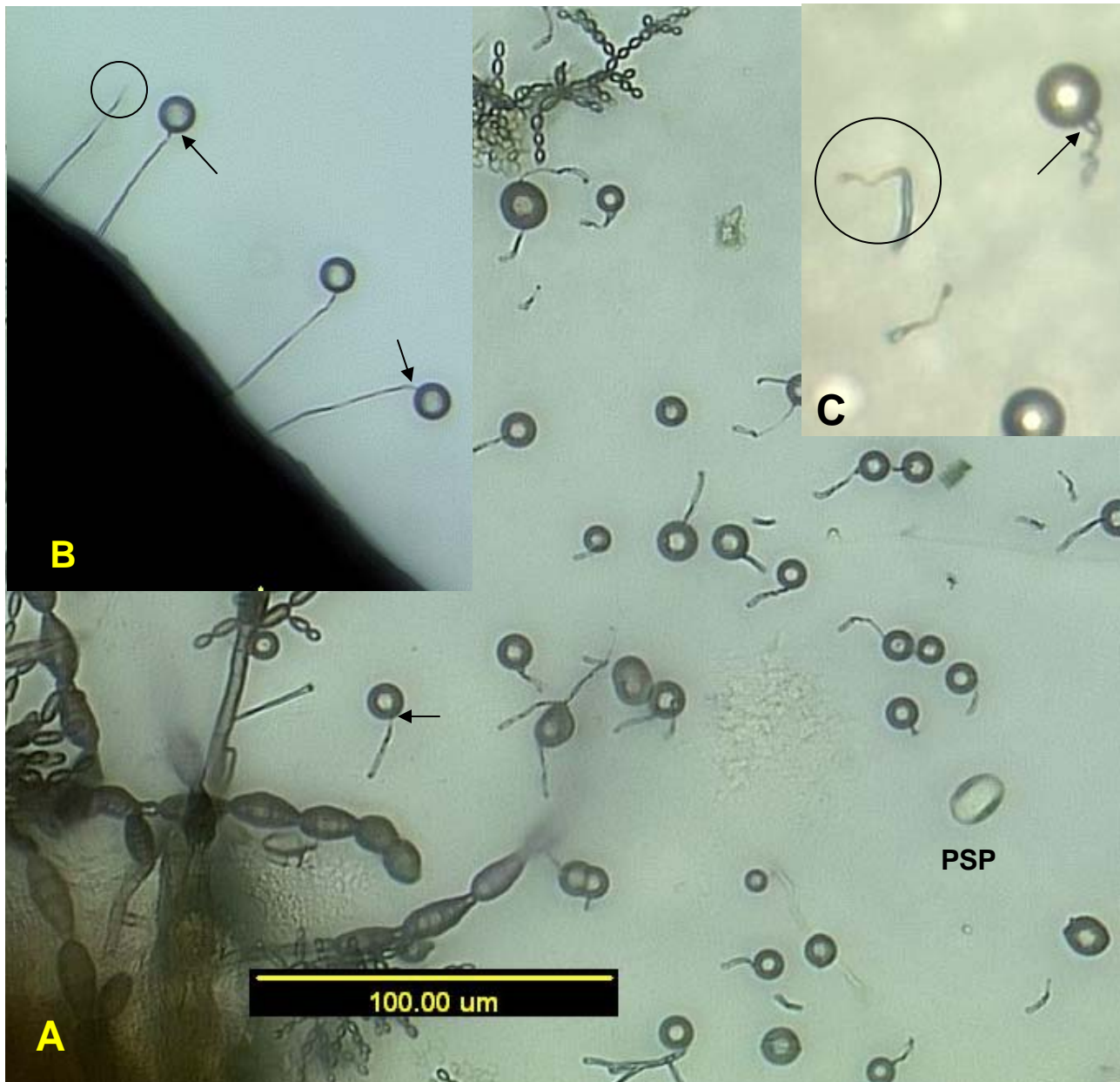
Each plate of images is followed by a description along with hints on how to recognize the species.

The section of species plates is followed by a quick reference guide showing the species together in side by side comparison.

The users of this guide should keep the following general hints in mind when trying to observe protostelids on PIP:

- 1. Use the 10x objective on a compound microscope to make observations.** This allows one to scan the area around a piece of substrate with enough resolution to identify protostelids to species. High power dissecting scopes lack the necessary resolution for a beginner and objectives higher than 10x can easily bump into the pieces of substrate.
- 2. Develop a search image.** Protostelids are easy to see once one learns what to look for. Protostelid sporocarps look superficially similar to the conidiophores of some fungi. The best way to distinguish protostelids from fungi is to remember that protostelid spores have a refractive index similar to a water drop. Fungal spores are more opaque. Also, protostelid stalks tend to be more delicate than conidiophores. If the stalks are on the agar, focus down into the agar, a conidiophore will be continuous with a hypha, a protostelid stalk will rest on a basal disk. Remember, sporocarps float. If in doubt, flood an area with sterile distilled water, and the sporocarps will float on the surface on their hydrophobic basal disks.
- 3. Learn to keep moving.** On PIP, make observations while focusing up and down while moving around the piece of substrate. Protostelids tend to be easier to see this way. Focusing helps to give one a picture of the whole sporocarp, and it tends to “jump out” when one focuses through it. Remember, the images here are montages of several planes of focus.
- 4. Remember, sporocarps are spore dispersal structures.** They are evanescent. Many species drop spores or actively discharge them. Other microorganisms in PIP may disrupt them. It is good to go back and look at a patch of protostelids to see if some sporocarps are developing whether some have shed spores, etc. It is amazing how much the “landscape” can change over a day or so. Myxomycete plasmodia and nematodes, common members of the PIP “community” can have a huge effect on the ease with which protostelid sporocarps can be observed. One really has to look hard to see protostelids on a plate that is “myxo stomped.”
- 5. Don't look for trophic cells.** They are often mixed in with lots of other protists, buried in colonies of bacteria or yeasts, or just plain hard to see. If trophic cells are to be observed accurately, the protostelid of interest must be cultured.
- 6. Protostelids mix.** Don't be surprised to see 2 to 5 different species fruiting intermixed with each other. If species are being recorded this means all sporocarps have to be looked at carefully.

Spores single, deciduous; stalks long



Protostelium mycophaga Olive & Stoianovitch

Sporocarp size variable but constant in proportions, degree of spore deciduousness variable.

Stalk: tapered at maturity, flexuous, often waving in air currents, often with a kink and small apophysis (arrows), with upper portion remaining straight or shriveling with slightly swollen tip when spore shed (circles in B & C). Presence of *P. mycophaga* may be detected by stalks alone.

Spore: spherical smooth, may appear faintly orange (C).

Prespore Cell (PSP): Elliptical when viewed from above, often present in primary isolation plates (PIP).

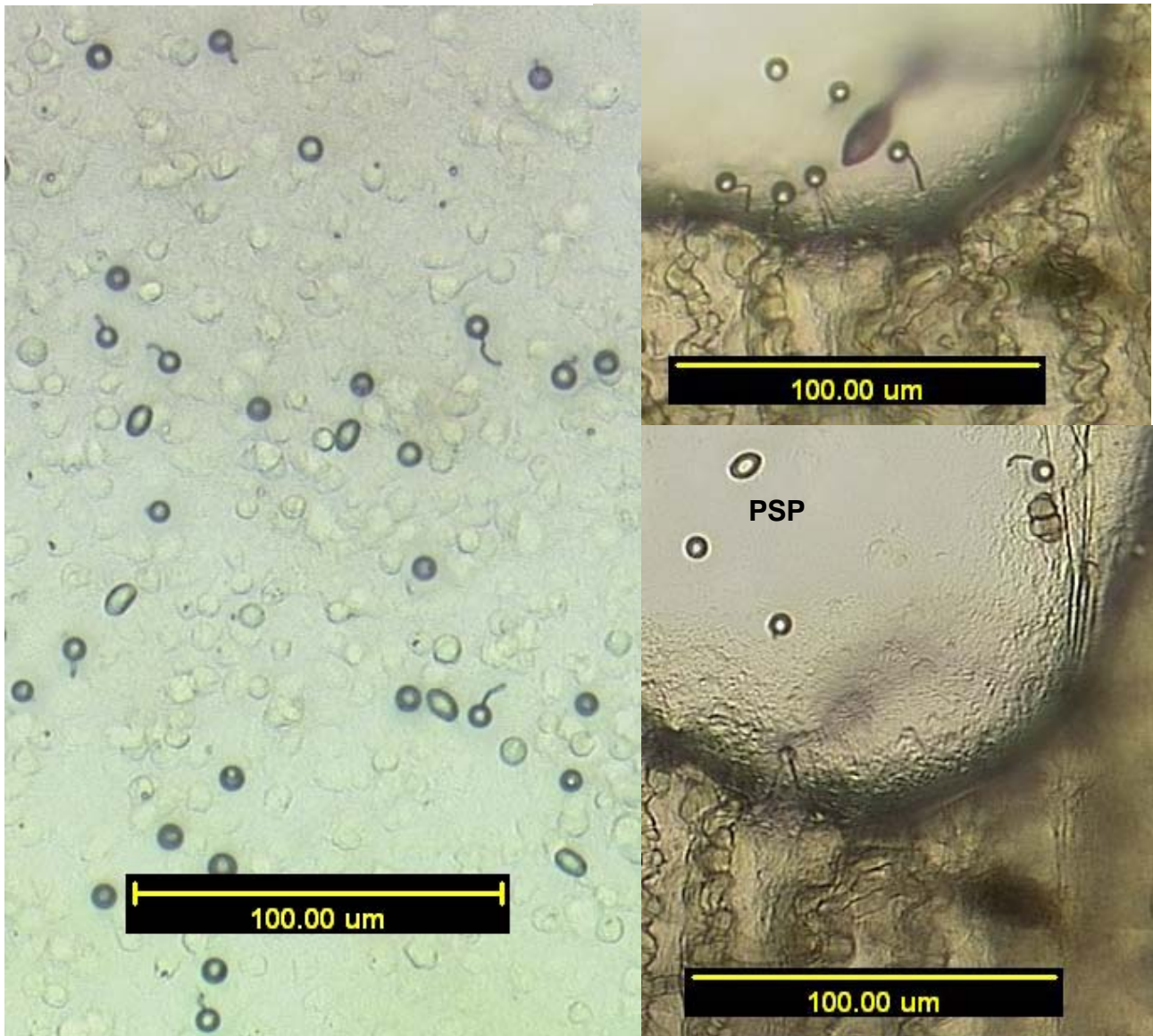
Scale bar: 100μm, A, B; 50μm, C.

Comments: The most common species of protostelid worldwide. Occurs on all types of substrates on which protostelids are found but is most common on dead primary tissues of standing plants.

May be found singly or in dense fruitings on PIP. May be a species complex.

Similar species: The rare *Planoprotostelium aurantium* O. & S., which is nondeciduous.

Spores single, deciduous; stalks long



Protostelium nocturnum Spiegel

This species is ballistosporous, and the entire stalk disappears when the spore is discharged. All one sees is developing and mature sporocarps, but never any empty stalks.

Stalk: similar in shape and proportion to *P. mycophaga*, but smaller. Appears turgid at first, then becomes more contorted and twisted. Disappears suddenly at spore dispersal.

Spore: spherical and smooth, often found on lower surface of petri dish lid above a heavy fruiting. Usually discharged within an hour of maturing. Fig. B = T₀. Fig. C = T_{90 min}.

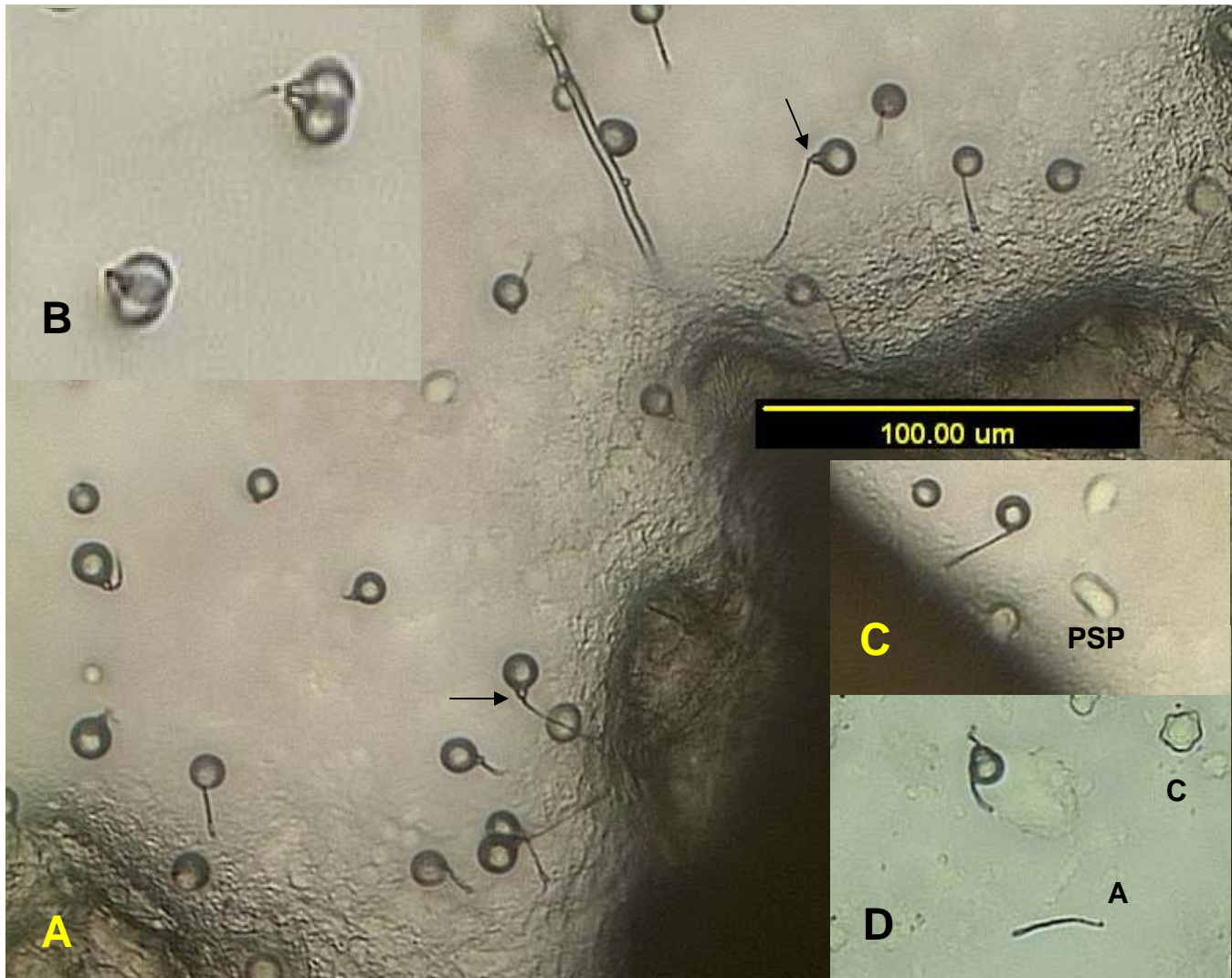
Prespore Cell (PSP): similar to *P. mycophaga*, but smaller.

Scale bar: 100µm in all.

Comments: Relatively common; among the smallest of protostelids; often seen most abundantly in the hours before and after dawn. Should be checked about an hour after the first observation to see that whole fruiting body has disappeared. Found on same types of substrates and microhabitats as *P. mycophaga*.

Similar species: *P. mycophaga* at the small end of its size range, but *P. mycophaga* stalks are persistent after spores fall.

Spores single, deciduous; stalks long



Protostelium pyriformis Olive & Stoianovitch

Size may vary along same range as *P. mycophaga*. Swinging motion of spores at tip of stalk (B, multiple exposure of spores) very eye catching.

Stalk: narrow, gently tapered, straight to gently curved, not flexuous, with small knob-like apophysis (A in Fig. D) inserted into socket in base of spore. Develops in invagination in rising sporogen.

Spore: obpyriform, campanulate, with socket in narrow basal part of spore. Usually swings freely back and forth in air currents prior to falling from stalk.

Prespore Cell (PSP): elliptical, superficially similar to that of *P. mycophaga* Fig. C.

Microcyst: irregular to stellate, often hard to see on PIP. C in Fig. D

Scale bar: 100μm, A, C, and D; approximately 40μm, B.

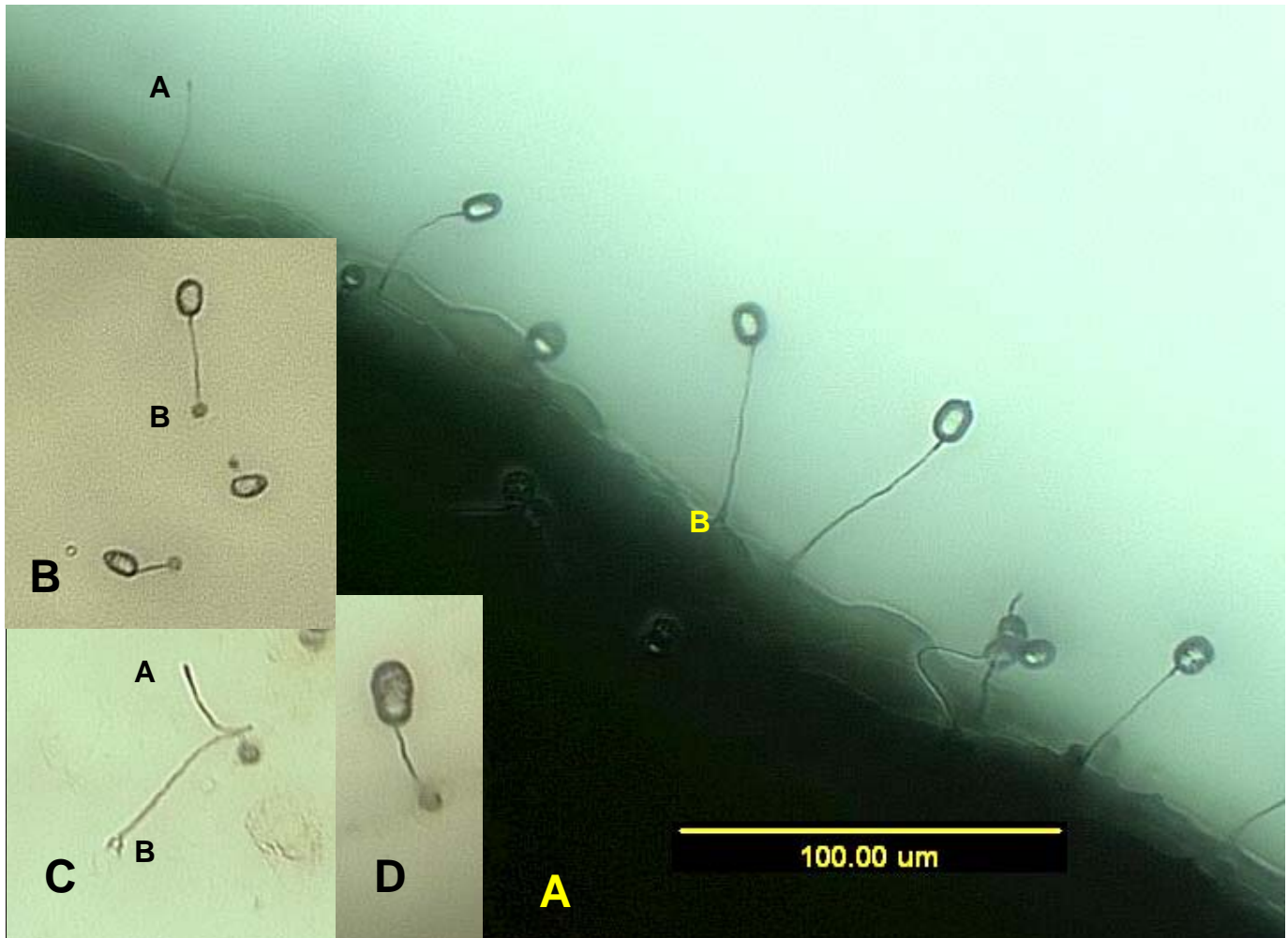
Comments: Not uncommon, it is more abundant in the tropics than in temperate regions.

Found most often on decaying primary tissues on both standing plants and in litter.

Occasionally on bark or dead twigs.

Similar species: About the same size as *P. mycophaga*, but spore shape different, stalk stiffer. Spore shape and movement similar to *E. zonatum* (which see) but smaller and stalk smooth, not regularly beaded.

Spores single, deciduous; stalks long



Protostelium arachisporum Olive

A very distinctive species that varies considerably in size (compare Figs. A, B and D).

Stalk: narrow with little taper, widens noticeably just above basal disk (B in Figs. B and C), with small knob-like apophysis that extends into small socket in spore. Length may vary from 1.5 to 5 times the length of the spore.

Spore: ovate to elongate and constricted such that it looks like a peanut, occasionally almost spherical, often reflexed. May swing at top of stalk like *P. pyriformis* or *E. zonatum*. Small socket at base of spore fits over apophysis (only seen at high power if spore mounted on slide).

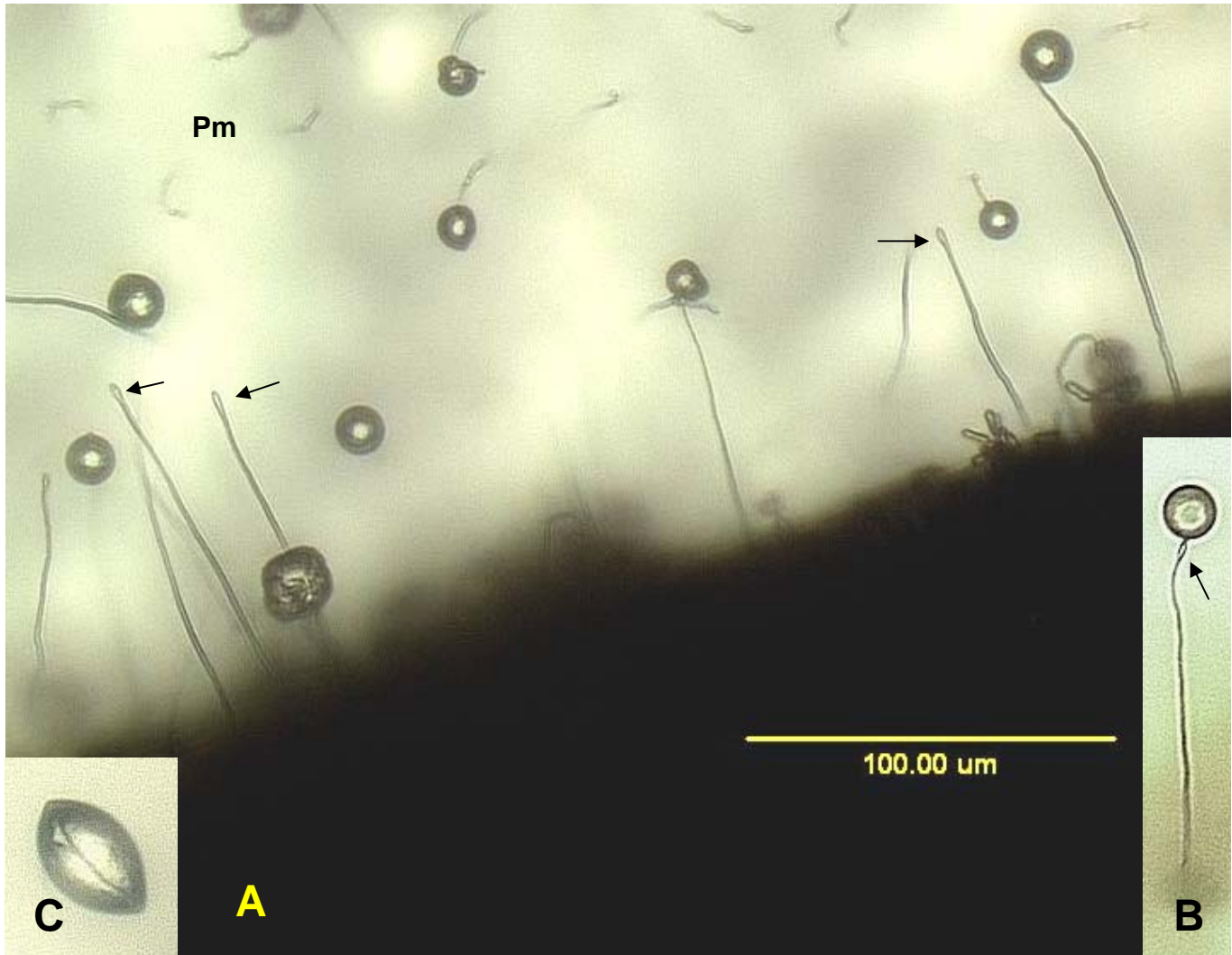
Prespore Cell (PSP): not shown, round in outline.

Scale bar: 100um in all.

Comments: This fairly common species appears to be more abundant in the tropics than in temperate regions. It occurs on all kinds of substrates from both standing dead plants and litter. Though it varies a lot in size and spore shape, it usually fruits abundantly with many sporocarps having the peanut-shaped spores.

Similar species: When spores are nearly spherical, this species may, on cursory examination, be mistaken for *P. mycophaga*. When its spores are more ovate, the sporocarps superficially resemble a small *N. ovatum* (which see), but that species has a more robust stalk with a pronounced bulbous apophysis. With familiarity, this species is easy to recognize despite its degree of variability.

Spores single, deciduous; stalks long



Soliformovum irregularis (Olive & Stoian.) Spiegel

This is one of the largest, most common species in temperate regions. It often fruits intermixed with other species, especially *P. mycophaga* whose stalk tips are visible at the top of Fig. A (Pm).

Stalk: very long with gentle taper and a hastate apophysis (arrows, Fig. A, B) at the tip, straight to gently curved, not flexuous. Easily recognized even after spores dropped.

Spore: spherical, readily dropped from stalk. In open plates often drying into “football” shape at tip of stalk (Fig. C), readily recovers shape when plate closes and relative humidity increases.

Prespore Cell (PSP): not shown but identical to that of *S. expulsum* (which see); a readily seen key character for the genus, the PSP rounds up and develops a raised, refractile mound such that the early PSP looks like a “sunny side-up” fried egg.

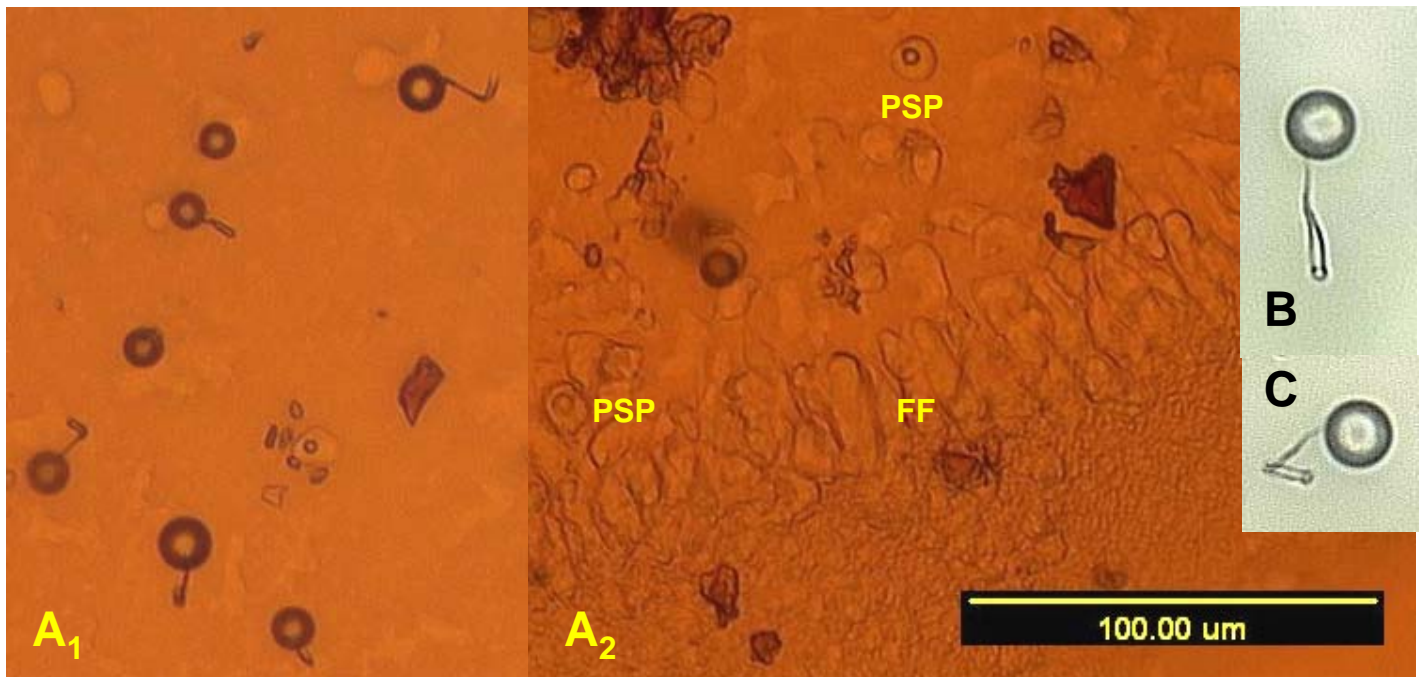
Trophic Cells: not shown, but if in a rich colony of food bacteria, will form a “palisade” feeding front as in *S. expulsum*.

Scale bar: 100um, A, B; 50um, C

Comments: This species is one of the three or four most common species encountered in any survey worldwide. It greatly prefers attached dead primary tissue on standing plants. Originally described as *Protostelium irregularis* O. & S.

Similar species: None if examined carefully. It is usually larger than *P. mycophaga* and more delicate than *Nematostelium gracile*. Once the apophysis is seen, there is no doubt.

Spores single, deciduous; stalks long



Soliformovum expulsum (Olive & Stoian.) Spiegel

Like several other species, this one is ballistosporous. Its stalk disappears when the spore is dispersed.

Stalk: bipartite with a thick basal portion one third to one half the length of the stalk and a thin upper section that is usually sharply reflexed with respect to the basal section. Disappears suddenly when spore is discharged.

Spore: spherical, proportionally large.

Prespore Cell (PSP): goes through “fried egg” stage (Fig. A₂).

Trophic Cells: often found in “palisade” feeding front (FF, Fig. A₂) in colonies of prey bacteria.

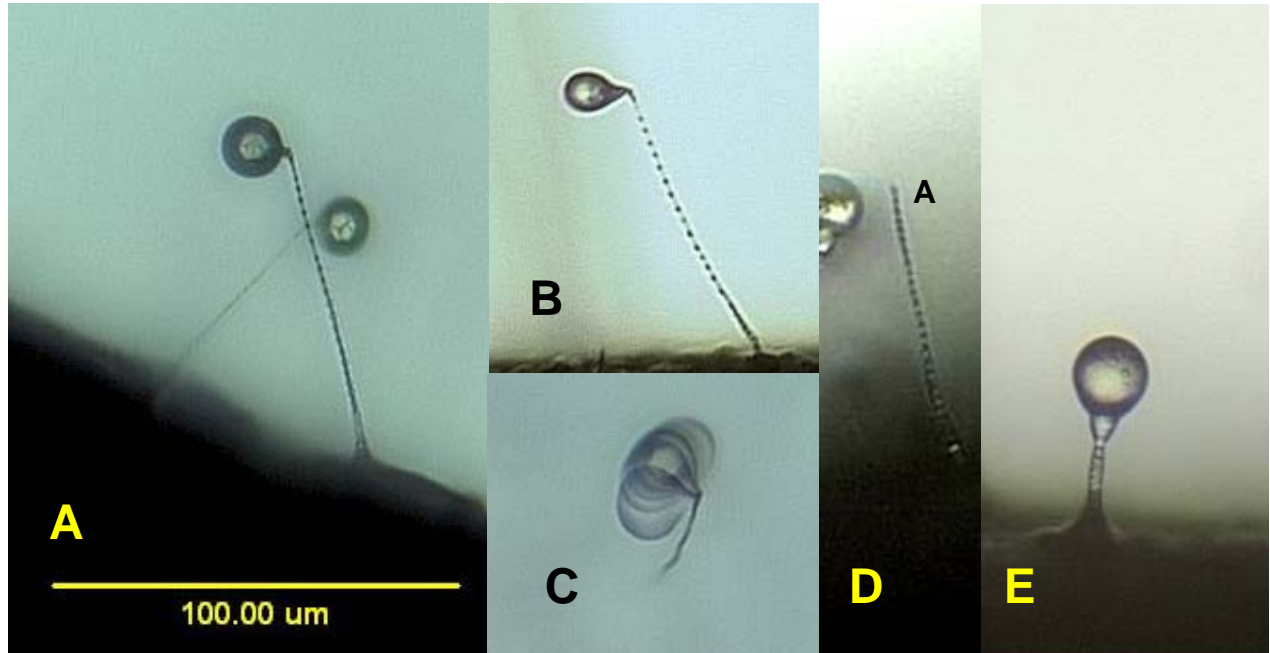
Scale bar: 100μm, A; 50μm, B and C

Comments: Not uncommon, this species is found on dead primary tissues on both standing plants and in litter. Somewhat more abundant in the tropics than in temperate habitats.

Originally described as *Protostelium expulsum* O. & S.

Similar species: None, if carefully examined. No other species has such a reflexed, bipartite stalk. If viewed from the wrong angle, a sporocarp might be mistaken for one of *P. mycophaga*, but there will be no empty stalks around, and there will almost always be some “fried egg” PSP nearby.

Spores single, deciduous; stalks long



Endostelium zonatum (Olive & Stoian.) Olive, Bennett & Deasey

This reasonably common species is the easiest of all protostelids to identify by its beaded stalk.

Stalk: long, quickly tapering from the base then tapering more gradually to the apex, beaded with alternating thick “vesicles” and thin “internodes”; with a slightly swollen apophysis (A in Fig. D). Developing in an invagination in the tapered base of the sporogen (Fig. E). Readily recognizable even after spore is shed (Fig. D)

Spore: obpyriform to nearly spherical with extension with a socket that fits over apex of stalk, campanulate, freely swinging in air currents at apex of stalk (multiple exposure of swinging spore in Fig. C).

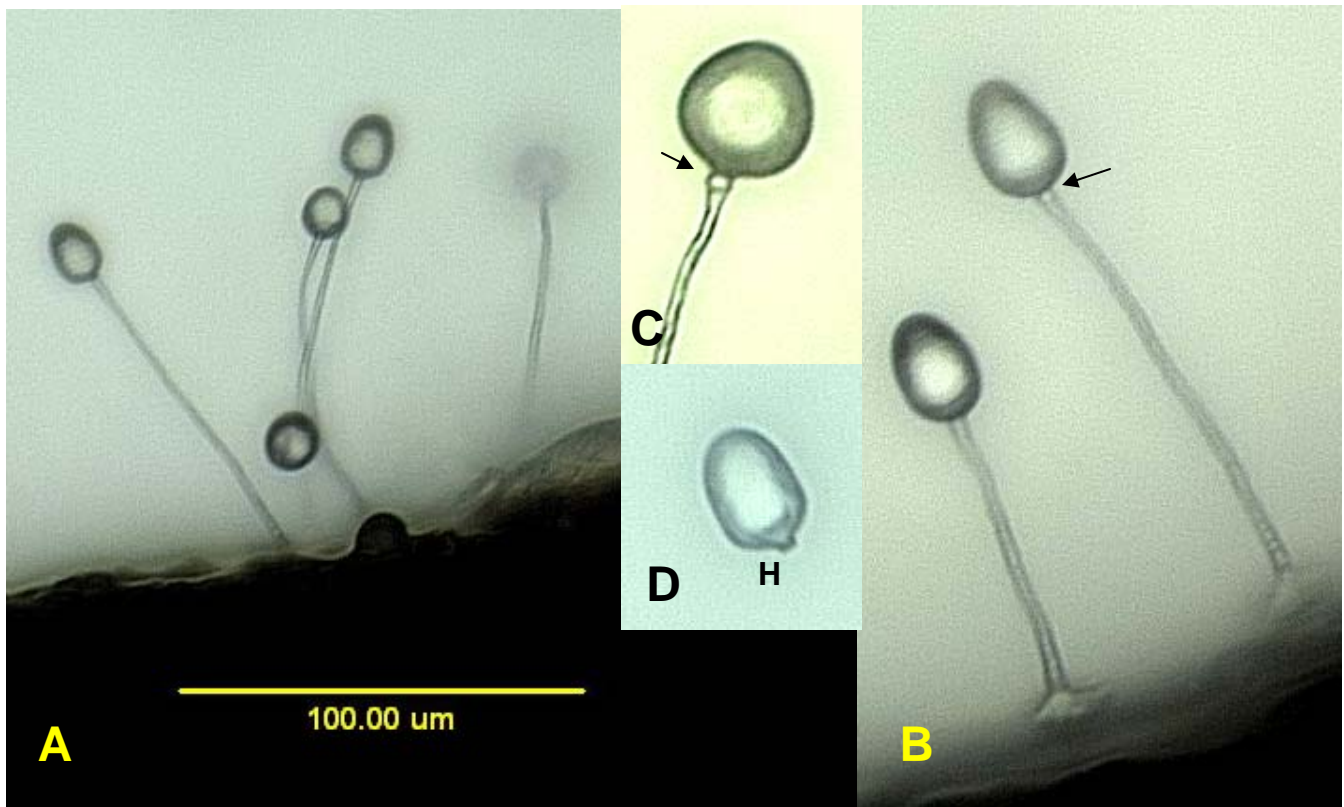
Prespore Cell (PSP): not shown. Circular in outline.

Scale bar: 100μm in all.

Comments: This species is often found with lots of widely spaced fruiting bodies. It is most often found on substrates of primary tissues on standing plants. Frequently, when it is found, the substrate was collected from a relatively dry habitat that is exposed to direct sunlight. Originally described as *Protostelium zonatum*.

Similar species: None. If the beads on the stalk are not really pronounced, it may be mistaken for the less common *E. amerosporum* (not in this guide), but that species has a much coarser, lumpy sporogen. *E. zonatum* is always larger than *P. pyriformis*.

Spores single, deciduous; stalks long



Nematostelium ovatum (Olive & Stoian.) Olive & Stoianovitch

Along with *E. zonatum*, this is one of the easiest species of protostelids to identify.

Stalk: long (5-10 times the length of the spore), robust, appears refractive along entire length, even with 10x objective (most long stalked protostelids have a stalk that appears as a black line at this magnification), with a distinct, knob-shaped apophysis (arrows in Figs. B, C).

Spore: Ovate to nearly ellipsoid, with distinct, ring-shaped hilum with a raised edge that articulates with apophysis (H in Fig. D)

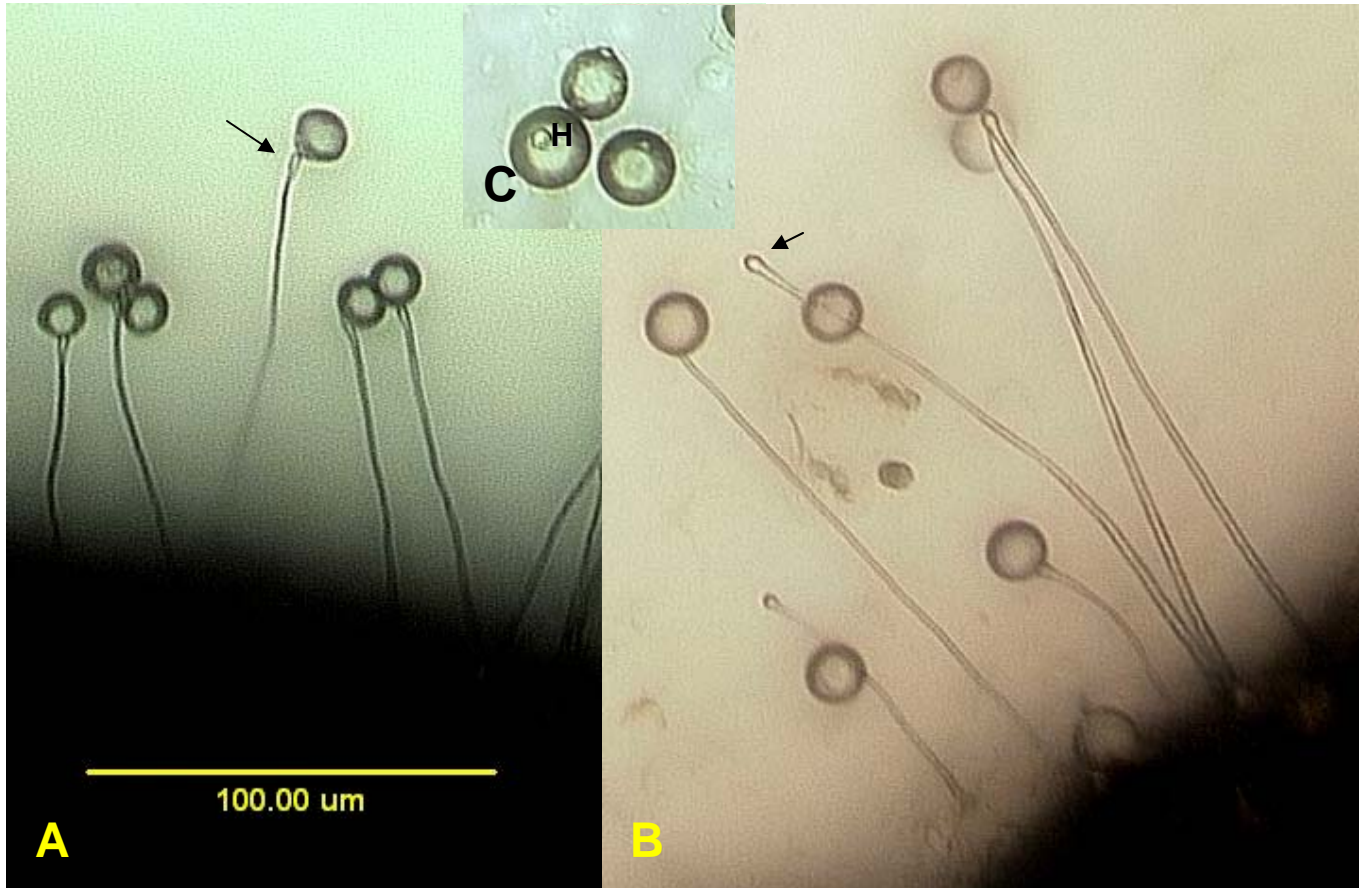
Prespore Cell (PSP): not shown, round in outline, often in clusters arising from fragmentation of plasmodial trophic cell.

Scale bar: 100um, A; 50um, B-D

Comments: This is a relatively common species in litter in temperate regions. It is less common on standing dead plant parts. It occurs a bit more frequently in temperate latitudes than in the tropics. Usually it is encountered in clusters with the stalks angling away from each other as in Fig. A. These clusters arise following the fragmentation of the plasmodium into several closely spaced prespore cells. Originally described as *Schizoplasmodium ovatum* O. & S.

Similar species: None. At the smaller end of its size range it may first appear to be a large *P. arachisporum*, but its stalk is much more robust. If empty stalks are encountered, fallen spores must be found to distinguish it from *N. gracile* (which see).

Spores single, deciduous; stalks long



Nematostelium gracile (Olive & Stoian.) Olive & Stoianovitch

This is one of the most robust and easily seen of the long-stalked protostelids.

Stalk: essentially identical to that of *N. ovatum*, though not always as obviously refractive along its entire length when viewed with a 10x objective (Fig. A), with similar knob-shaped apophysis (arrows, Fig. A, B).

Spore: spherical to slightly turbinate with distinct ring-shaped hilum with a raised edge that articulates with apophysis (H in Fig. C).

Prespore Cell (PSP): not shown, identical to those of *N. ovatum*.

Scale bar: 100um, A, C; 50um, B.

Comments: This species is often one of the three or four most common species in samples from the lowland tropics where it is found on decaying primary tissues on both standing plants and in the litter. It is fairly common in the litter in temperate regions and uncommon on standing plants. It is almost absent at high latitudes and above 2500m. Clusters of fruiting bodies are the rule as with *N. ovatum*. Originally described as *Schizoplasmodium gracile* O. & S.

Similar species: This species is identical to the rarer *Ceratiomyxella tahitiensis* O. & S. These species usually must be distinguished in culture, but culturing attempts show that over 95% of the cultures are *N. gracile*. Can be distinguished from *S. irregularis* by more robust stalks, shape of the apophysis, and hilum on spore. When empty stalks are encountered, fallen spores must be found to distinguish this species from *N. ovatum*. When fallen spores are found, stalks must be found to distinguish it from *Schizoplasmodium cavostelioides* (which see).

Spores single, deciduous; stalks short



Schizoplasmodium cavostelioides Olive & Stoianovitch

This common species is ballistosporous, and its large spore is rarely seen on the stalk. This is the only short stalked species with deciduous spores.

Stalk: I very short and thick, with a distinct cup-shaped apophysis (arrows, Figs. A and B) that appears as a distinct ring when viewed from above (ST, arrows, Fig. A). Usually seen after spore discharge, often grouped in clusters.

Spore: Spherical, smooth, with distinct, ring-shaped hilum with raised edge (H in Fig. A) that articulates with apophysis. Spores usually seen discharged and often collapsed on agar surface in PIP. Spores still on stalk often bear a swollen, hydrated sheath (SH in Fig. A and B) that may appear as a lateral droplet or surround the spore completely.

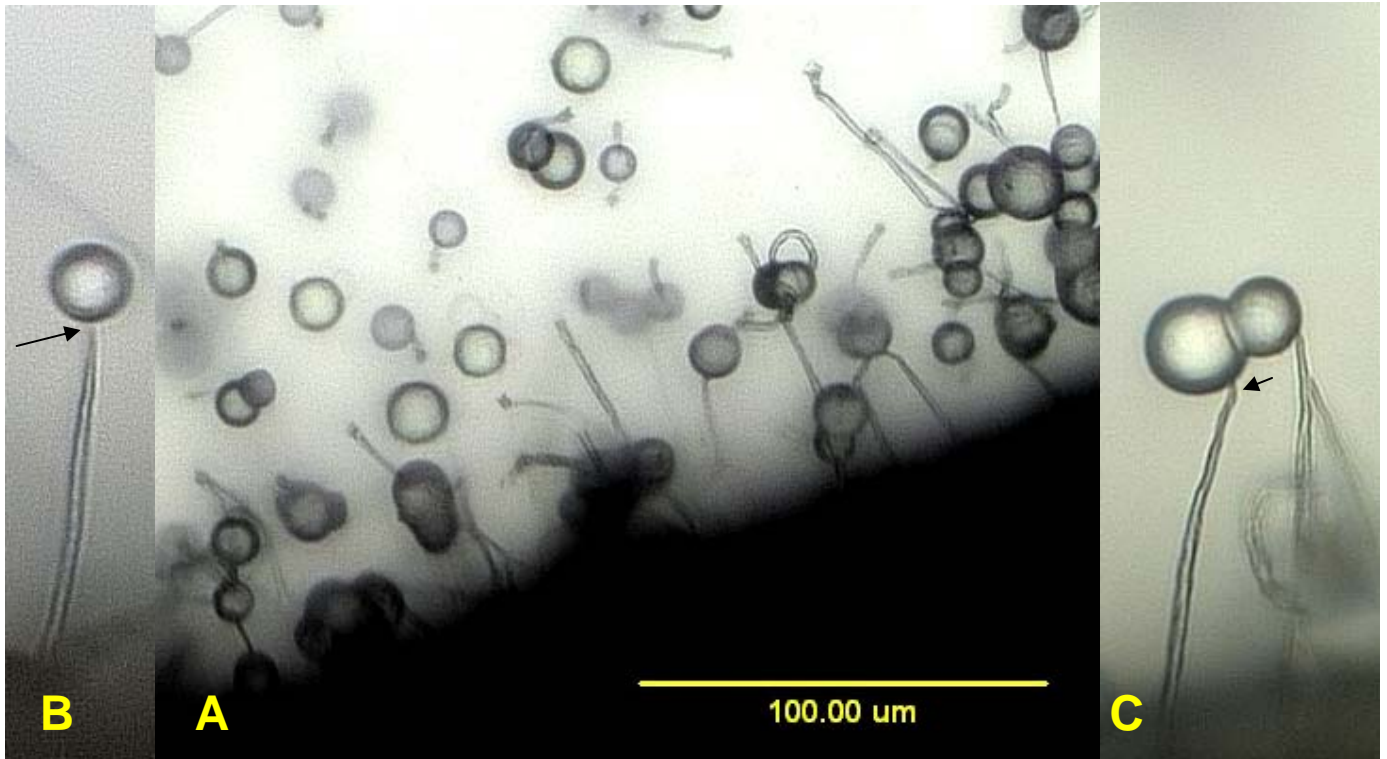
Prespore Cell (PSP): not shown, identical to *Nematostelium* spp.

Scale bar: 100μm, A; 50μm, B

Comments: This is a fairly common species that is most abundant on substrates from standing dead plants in temperate habitats. However, it is not uncommon in the tropics. The swelling of the sheath, originally interpreted as a gas bubble, appears just before spore discharge. Discharge occurs suddenly, and the spore is thrown many micrometers.

Similar species: None. The short stalked species of *Schizoplasmodiopsis* (which see) have thin stalks, and *Cavostelium apophysatum* (which see) has nondeciduous, rough surfaced spores.

Spores single, nondeciduous; stalks long



Tychosporium acutostipes Spiegel, Moore & Feldman

A relatively uncommon species that tends to fruit in very dense stands on pieces of substrate and the surrounding agar in PIP.

Stalk: appearing stiff, relatively thick and only slightly tapered for over 90% of its length, often somewhat undulate (Fig. C), tapering sharply to a fine point at the very upper end (arrows, Figs B and C).

Spore: spherical to broadly turbinate with slightly raised hilum that appears similar to a lunar crater at the junction with the stalk (not shown)

Prespore Cell (PSP): not shown, essentially identical to that of *P. mycophaga*.

Sacle bar: 100um, A, 50um, B, C.

Comments: This species is widespread on decaying primary tissues, especially those on standing plants. It occurs worldwide. One microhabitat where it can be found with some regularity is on decaying emergent aquatic plants such as rushes and cat tails. Although sporocarps are sometimes seen fruiting singly or in small groups, this species tends to fruit in thick stands.

Similar species: This species has much stiffer stalks than *P. mycophaga*, and its spores are nondeciduous. It is much more likely to be mistaken for *Schizoplasmodiopsis vulgare* (which see) with which it frequently co-occurs; however, *S. vulgare* has a stalk that is relatively thick all the way to its junction with the spore, and its spores are relatively irregular in outline because of a reticulum of low ridges that cover the spore wall. This species may also be confused with the very rare *Schizoplasmodiopsis micropunctata* (which see), but that species has a more delicate stalk with a very thin, hairlike tip.

Spores single, nondeciduous; stalks long



Schizoplasmodiopsis micropunctata Olive & Stoianovitch

This is one of the rarest protostelids.

Stalk: proportionally very long, narrowing suddenly to a hairlike tip that may be as long as 25% the diameter of the spore (arrow, Fig. B). This tip may be so thin that the spore almost appears as if it is suspended above the tip of the stalk.

Spore: spherical to broadly turbinate, with minute spines reported to be visible under oil immersion with phase contrast (not shown)

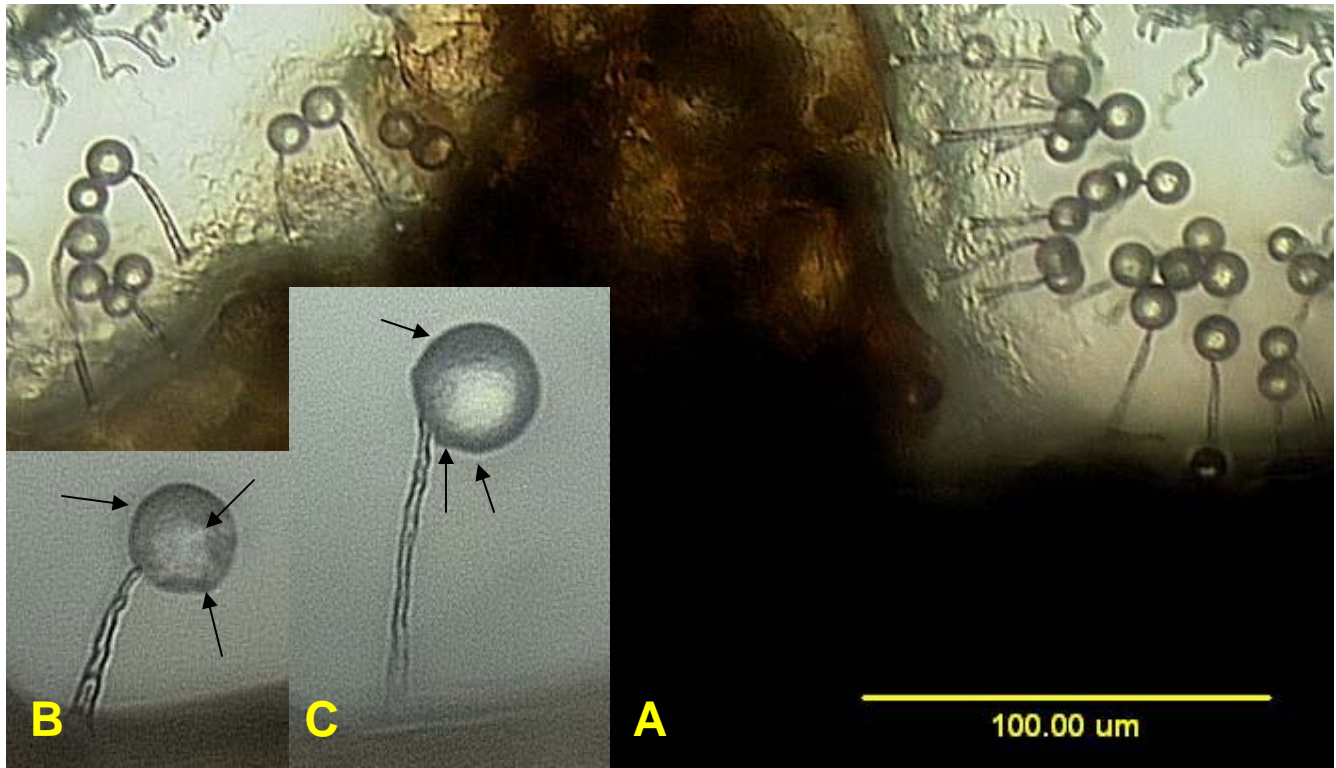
Prespore Cell (PSP): not well described.

Scale bar: 100um, A; 50um, B.

Comments: This species is very rare, but seems to be encountered worldwide in locales where large numbers of samples have been collected. It occurs in usually <0.25% of samples. Because of its rarity, it is difficult to say much about likely microhabitat preferences.

Similar species: It is most similar to *T. acutostipes*, but it is more delicate, and has the distinct hairlike stalk tip.

Spores single, nondeciduous; stalks long



Schizoplasmodiopsis vulgare Olive & Stoianovitch

This is a commonly encountered species that often tends to fruit very heavily. It is certainly the most common long stalked species with nondeciduous spores.

Stalk: relatively thick along its entire length, tapering but never to a sharp point. Length may vary from slightly more than one spore diameter to several spore diameters though most sporocarps in PIP appear as those shown here.

Spore: essentially spherical but coarse looking because of low ridges formed by a reticulum of spore wall thickenings that appear as slight bumps (arrows, Figs. B and C). These may be more or less obvious and are best detected with objectives of 20x or higher. Spore diameter is relatively constant even when stalk length varies.

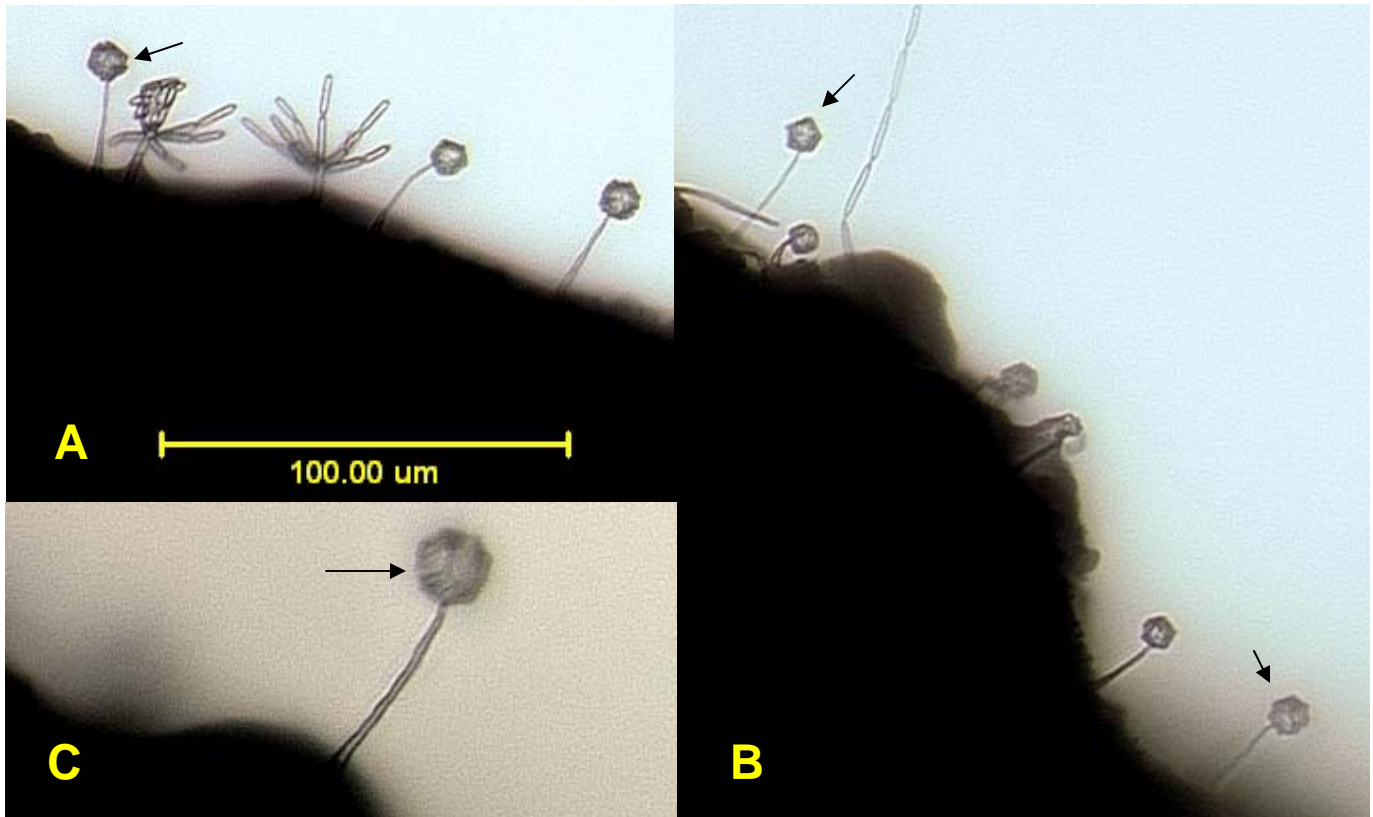
Prespore Cell (PSP): not shown, round in outline.

Scale bar: 100um, A; 50um B, C.

Comments: This is a species that is worldwide in distribution and found on decaying primary tissues about equally on standing plants and in the litter. Though somewhat variable in stalk length, it is usually relatively easy to identify by its coarse appearance. It is another species that is fairly common on decaying emergent aquatic plants.

Similar species: *T. acutostipes* is the most likely species to be confused with *S. vulgare*, but the former has a proportionally thinner stalk which tapers to a fine point. However, one must be careful since the two species will often fruit together with the patches of sporocarps intermixed.

Spores single, nondeciduous; stalks long



Schizoplasmodiopsis reticulata Olive & Stoianovitch

This is a relatively rare species that occurs in situations wherever *S. vulgare* is likely to be found. Like that species, it often fruits very heavily on a substrate. Its distinctive spores make it easy to recognize.

Stalk: relatively longer and usually more gracile and finely tapered than that of *S. vulgare*.

The two sporocarps marked with arrows in Fig. B show stalks whose bases are not blocked by the substrate such that their whole length is obvious.

Spore: essentially spherical with a distinct, raised reticulum of ridges that is easily visible even at low magnifications (arrows, Fig. A-C).

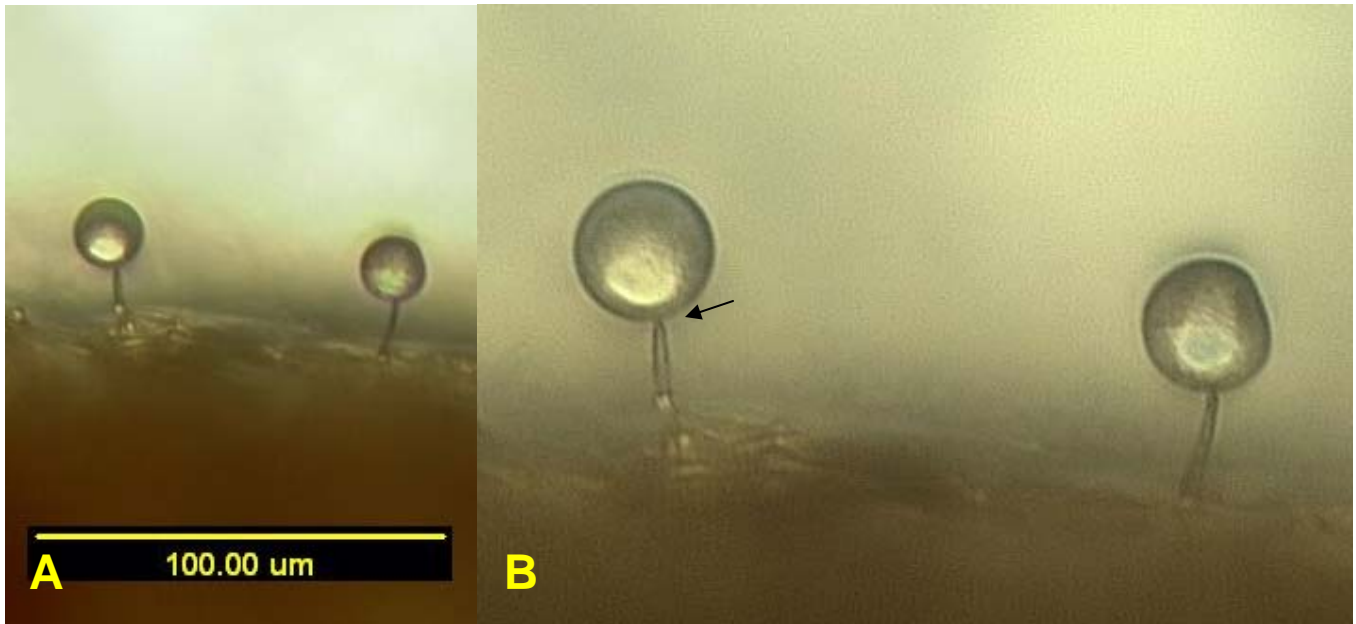
Prespore Cell (PSP): not shown, round in outline.

Scale bar: 100um, A,B: 50um C.

Comments: Though this species is widespread, it is rare. Nonetheless, its reticulate spores are so distinctive that it is almost impossible to mistake for any other species. This particular example was observed on substrates from the Ozarks of Arkansas and has stalks that are on the proportionally shorter end of the range stalk lengths. Some fruitings may have stalks that are up to two spore diameters longer.

Similar species: Only an occasional fruiting of *S. vulgare* that has a coarse reticulum on the spores might be mistaken for *S. reticulata*. It may be the case that the two species are very closely related or conspecific and that spore reticulation is a variable trait.

Spores single, nondeciduous; stalks short



Schizoplasmodiopsis amoeboidea Olive & Whitney

This is a common species which may occur a scattered individual sporocarps in PIP or in groups.

Stalk: short, rarely >1.5 times the diameter of the spore, narrows suddenly to a thinner tip (arrow, Fig. B), often hard to detect in PIP because of irregularities in surface of substrate or because sporocarps on agar are seen directly from above.

Spore: spherical, usually among the largest seen in any protostelids, but may vary in size.

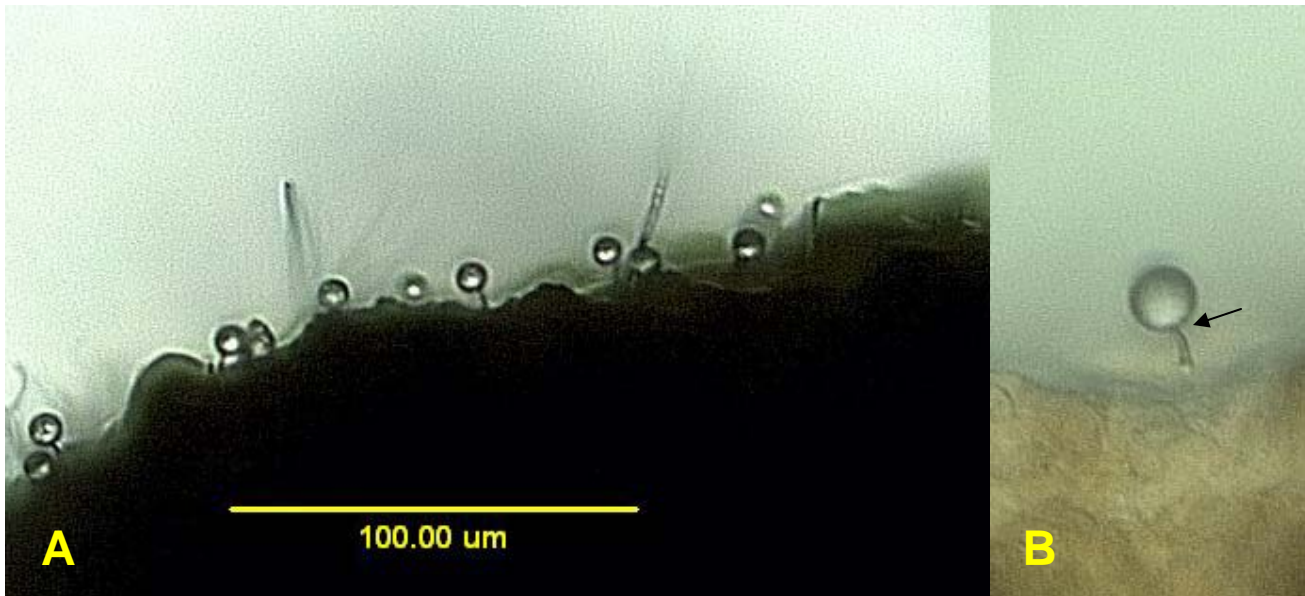
Prespore Cell (PSP): not shown, round in outline.

Scale bar: 100um, A; 50um, B

Comments: This species is found relatively regularly on any kind of substrate on which protostelids may occur.

Similar species: Whenever a short stalked species occurs in a PIP, every effort should be made to see a stalk, even if it means knocking sporocarps over. This species is very often quite difficult to distinguish from *Schizoplasmodiopsis pseudoendospora* because sporocarps at the smaller end of the size range of *S. amoeboidea* overlap in size with those of *S. pseudoendospora*. Two distinguishing characters to look for are that the stalk of this species has the narrow section at its apex while the stalk of *S. pseudoendospora* tapers evenly to its tip, and this species tends to fruit singly or when grouped, the sporocarps are irregularly spaced. However, it is probably the case that one species is occasionally misidentified as the other on PIP.

Spores single, nondeciduous; stalks short



Schizoplasmodiopsis pseudoendospora Olive

This is one of the smallest of protostelids, but also one of the most common.

Stalk: very short, rarely > 1 spore diameter, sometimes, apparently missing, thin, but does not narrow to a point (arrow, Fig. B).

Spore: spherical, small (in most).

Prespore Cell (PSP): not shown, circular in outline, usually many forming simultaneously from fragmentation of plasmodial trophic cell.

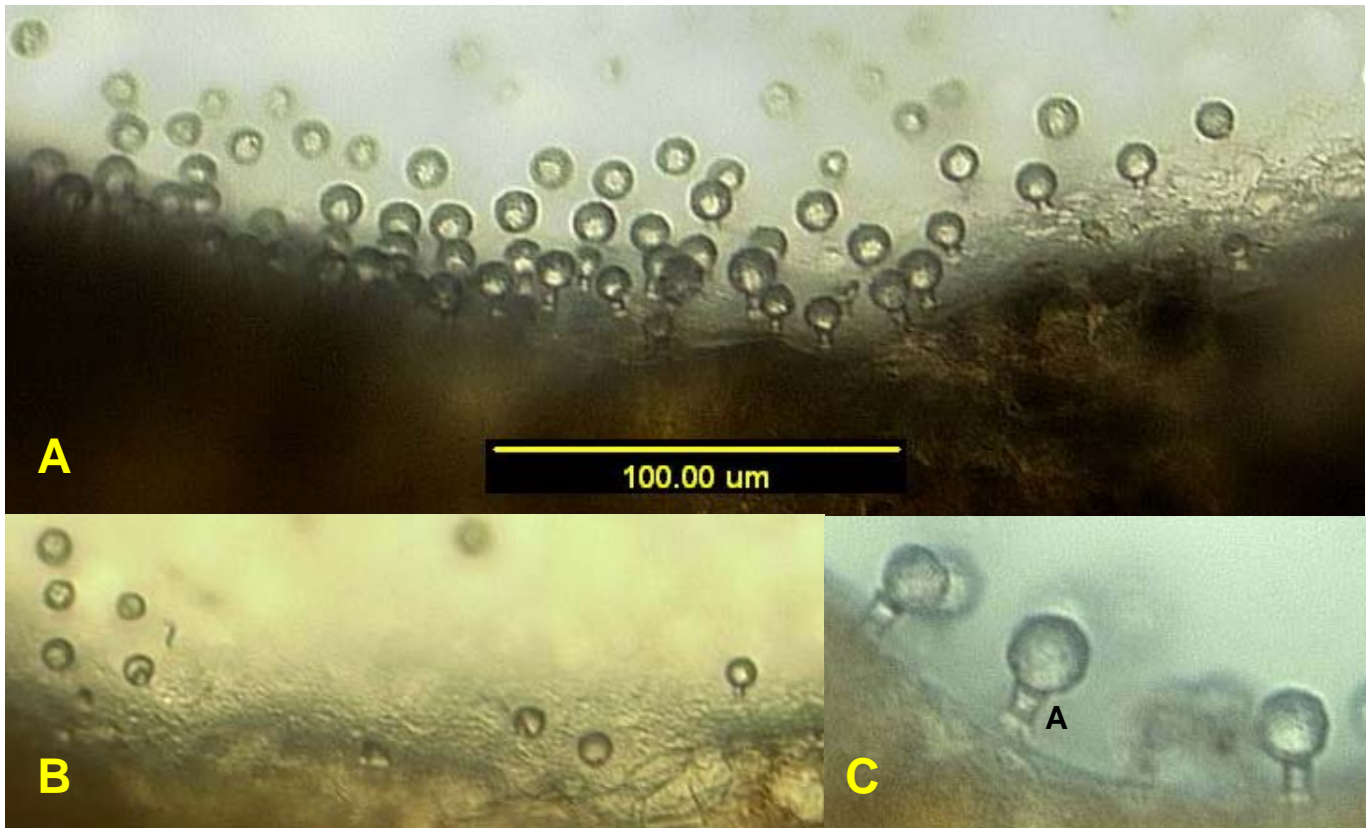
Scale bar: 100um, A; 50um, B.

Comments: This is one of the two or three most common species of protostelids encountered in any survey of protostelids from tropical or temperate regions. It is usually most abundant in the litter. Usually sporocarps are found relatively evenly spaced in large groups since they developed from a single plasmodium. Large plasmodia, and the sporocarps that develop from them may cover patches several millimeters wide. These patches can be seen with a dissecting microscope as dozens to over 100 evenly spaced shiny spots.

Similar species: *S. amoeboides* and this species are hard to distinguish when this species is at the large end of its size range and fruiting singly. Look for stalk tips to distinguish them if it is possible to see the stalks for their full length. See discussion with *S. amoeboides*.

This species is also superficially similar to *Microglomus paxillus* (which see), but that species has two or four spores in a slightly ellipsoid sporangium.

Spores single, nondeciduous; stalks short



Cavostelium apophysatum Olive

This is a fairly common species in the tropics and relatively uncommon in temperate regions. Though it may vary quite a bit in overall size from isolate to isolate, the proportions of the sporocarp are constant (compare Figs. A and B).

Stalk: very short, wide, with a cup-like apophysis that occupies one third to more than one half the total length of the stalk.

Spore: spherical, roughened in appearance by the presence of numerous spines and warts on the spore wall (Fig. C). This spore wall sculpturing often makes the spores appear to be less refractile than those of other protostelids. Some spores may contain two cells in some isolates. Such spores look ellipsoid from above and from the side.

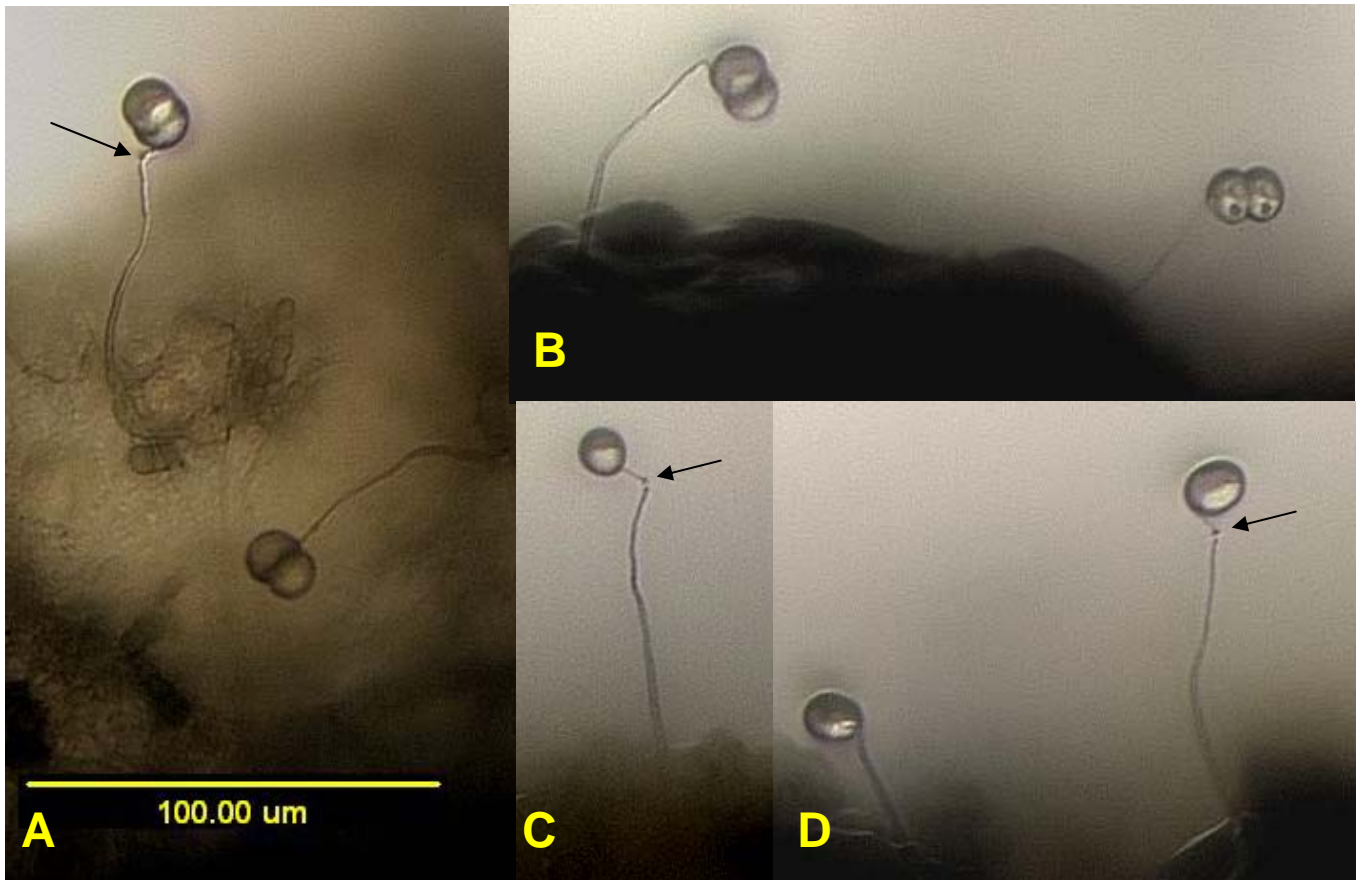
Prespore Cell (PSP): not shown, circular in outline.

Scale bar: 100um, A, B; 50um, C.

Comments: This species is usually found with dense patches of sporocarps. It may be found in litter or on standing dead plants, though it is more found a bit more frequently in the latter. As stated above, it appears to be considerably more common in the tropics.

Similar species: If sporocarps of this species can be viewed from the side, its thick stalk distinguishes it from the short stalked species of *Shizoplasmodiopsis*. Since its spores are nondeciduous and roughened, it is easy to distinguish from *Schizoplasmodium cavostelioides*.

Spores multiple, stalks long



Protosporangium articulatum Olive & Stoianovitch

This is the most commonly encountered of the species of the relatively uncommon genus *Protosporangium*. Most species in this genus have fairly tall fruiting bodies, often exceeding 200um in height. Most species are found most frequently on substrates such as bark and wood.

Stalk: Very long and flexuous, always with a thickened articulation that delimits the uppermost segment of the stalk (arrows in Figs. A, C, and D). This upper segment is often shed with the spores.

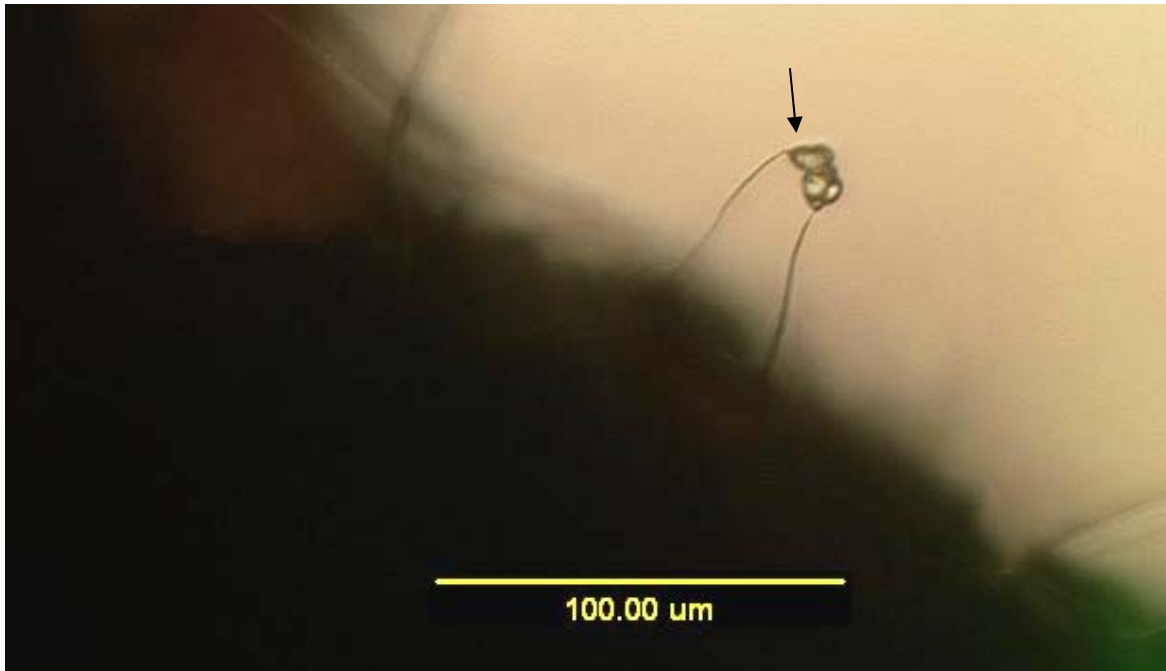
Spores: Usually 2, frequently 4, rarely only one. Nearly spherical, somewhat compressed. The lower spore may be somewhat obconic. Dividing from a single-celled sporogen after stalk elongation is complete.

Prespore cell (PSP): not shown, circular in outline when viewed from above.

Scale bar: 100um, all.

Comments: While this species is found most frequently on bark from living trees, it is about as likely to occur on dead primary tissues in the Hawaiian Islands. It appears to be a species that is often associated with arid habitats, and it occurs at higher elevations (>3000m) than most protostelids.

Spores multiple, stalks long



Protosporangium conicum Bennett

This is the rarest of the members of the genus *Protosporangium*.

Stalk: long, thin, somewhat flexuous, waving readily in air currents.

Spores: Always four per sporocarp, with the one that attaches to the stalk obconic; the other three spherical, arranged at distal end of obconic spore. Usually shed as a unit.

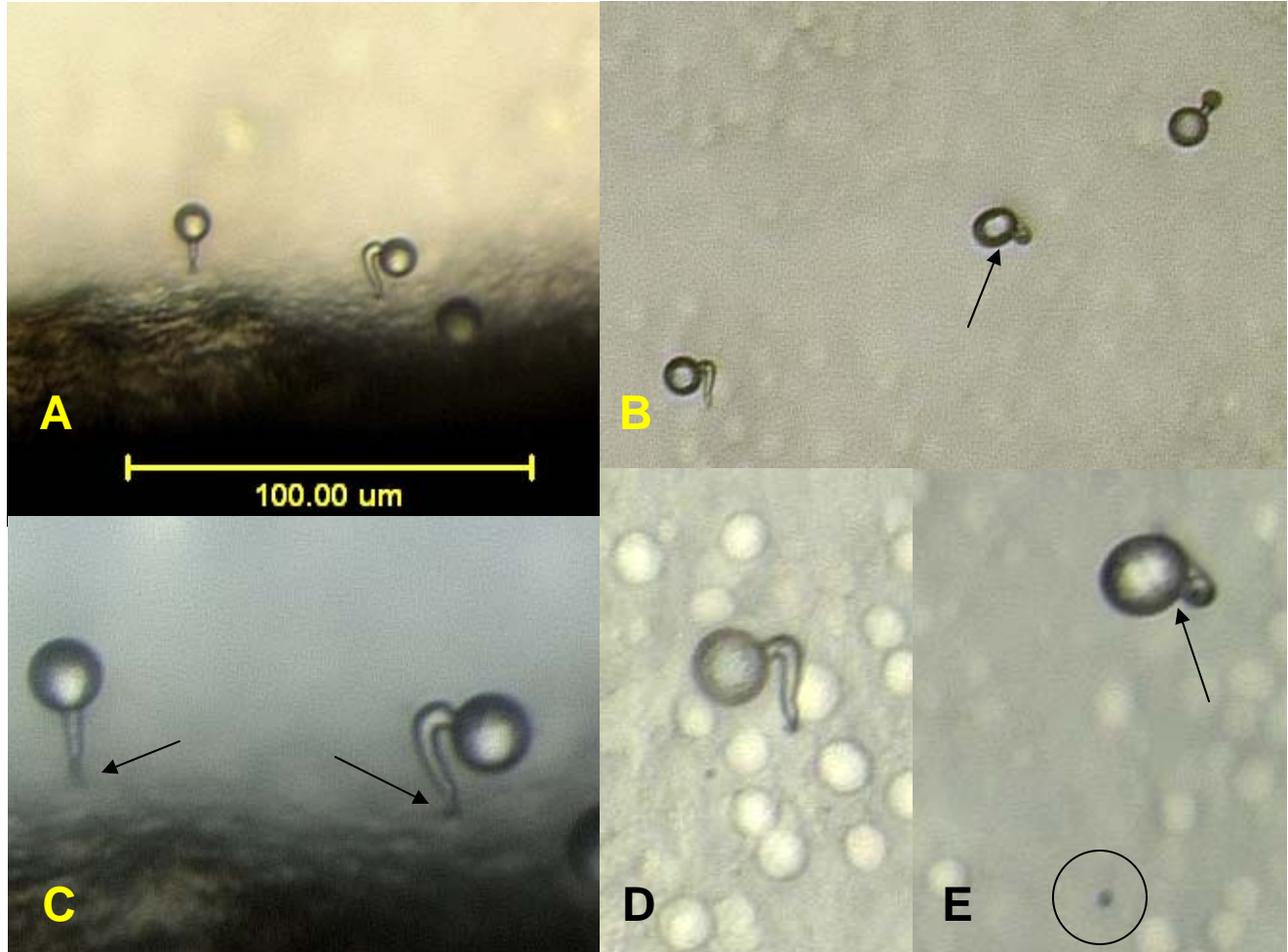
Prespore Cell (PSP): unknown.

Scale bar: 100um

Comments: The few times this species has been encountered, it has always been on bark of living trees. It is the smallest of the four described species of *Protosporangium*. The other species having stalks that are usually twice the length of this species. All these are also found most often on bark or on wood and rarely on decaying primary tissues. To date, all long stalked protostelids with multiple spores have turned out to be species of this genus.

Similar species: None, the obconic spore is absolutely distinctive.

Spores multiple, stalks short



Clastostelium recurvatum Olive & Stoianovitch

This relatively uncommon protostelid is easy to recognize with its recurved stalk and small sporangium of two spores. It is best seen early in the morning because its spores are usually all dispersed by mid morning.

Stalk: bipartite with a short, persistent, apiculate base (arrows in Fig. C) and an inflated, recurved upper portion that bursts to disperse the spores. Stalk bases seen as dark spots from above after spores dispersed (circle in Fig. E)

Spores: smooth and spherical in two spored sporangium, slightly pendulous when stalk is mature. Sporangium articulates to stalk between the two spores (arrows Fig. B,E). Shot off as a unit.

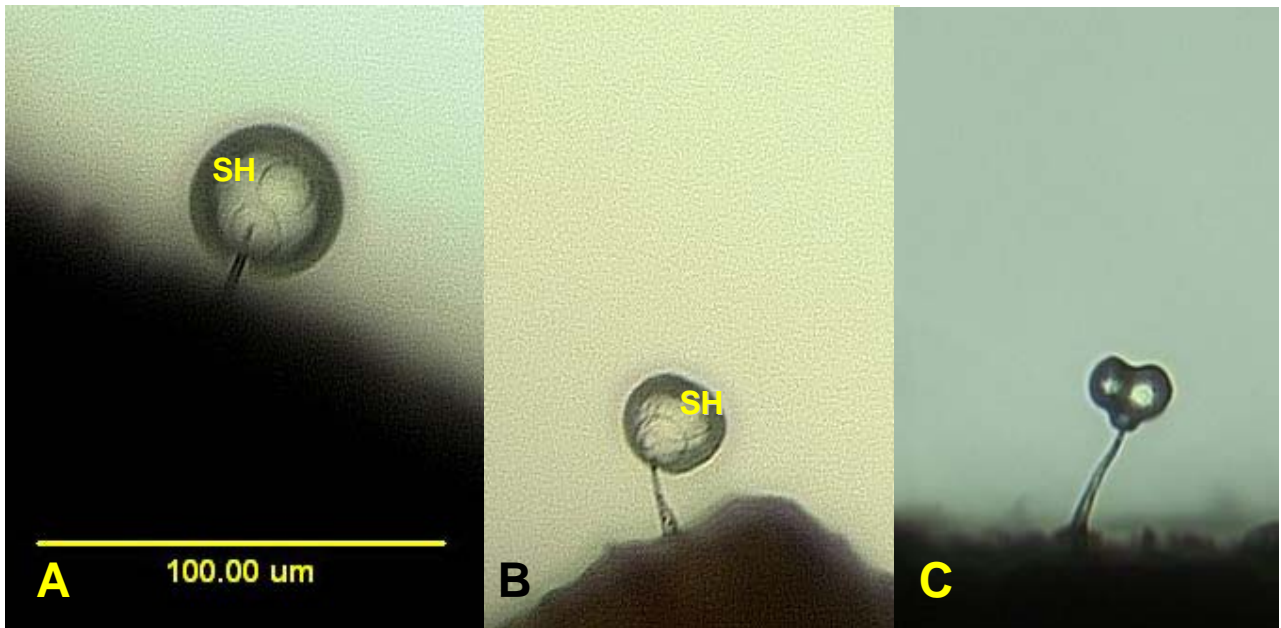
Prespore Cell (PSP): not shown, circular in outline.

Scale bar: 100µm A,B; 50µm C-D.

Comments: Found most often aerial dead plant parts or bark of living trees. Appears to be more common in the tropics than in mid to high latitudes.

Similar species: None of the described species is similar to *c. recurvatum*. However, there is an uncommon undescribed species most often encountered on bark which is superficially resembles it. The undescribed species, for which no trophic state has yet been observed, is the same size and recurved, but it has a single, highly sculptured spore and the stalk is longitudinally ridged and tapers from base to tip. The undescribed species often appears to be yellow to gold under a dissecting microscope.

Spores multiple, stalks short



Echinosteliopsis oligospora Reinhardt & Olive

This common species is treated as short stalked because it is usually first observed on PIP with a hydrated sheath that makes the diameter of the sporangium greater than the length of the stalk.

Stalk: straight to gently curved with a pronounced taper from base to tip.

Spores: 1-8, usually 4, spherical to laterally compressed, usually of different sizes within a single sporangium, spore walls smooth within a hygroscopic sheath that is usually hydrated in the saturated air of a culture plate. When hydrated, spores appear suspended in sheath (Fig. A and B). When dried, one spore at apex of stalk, others arranged around its distal half (Fig. C).

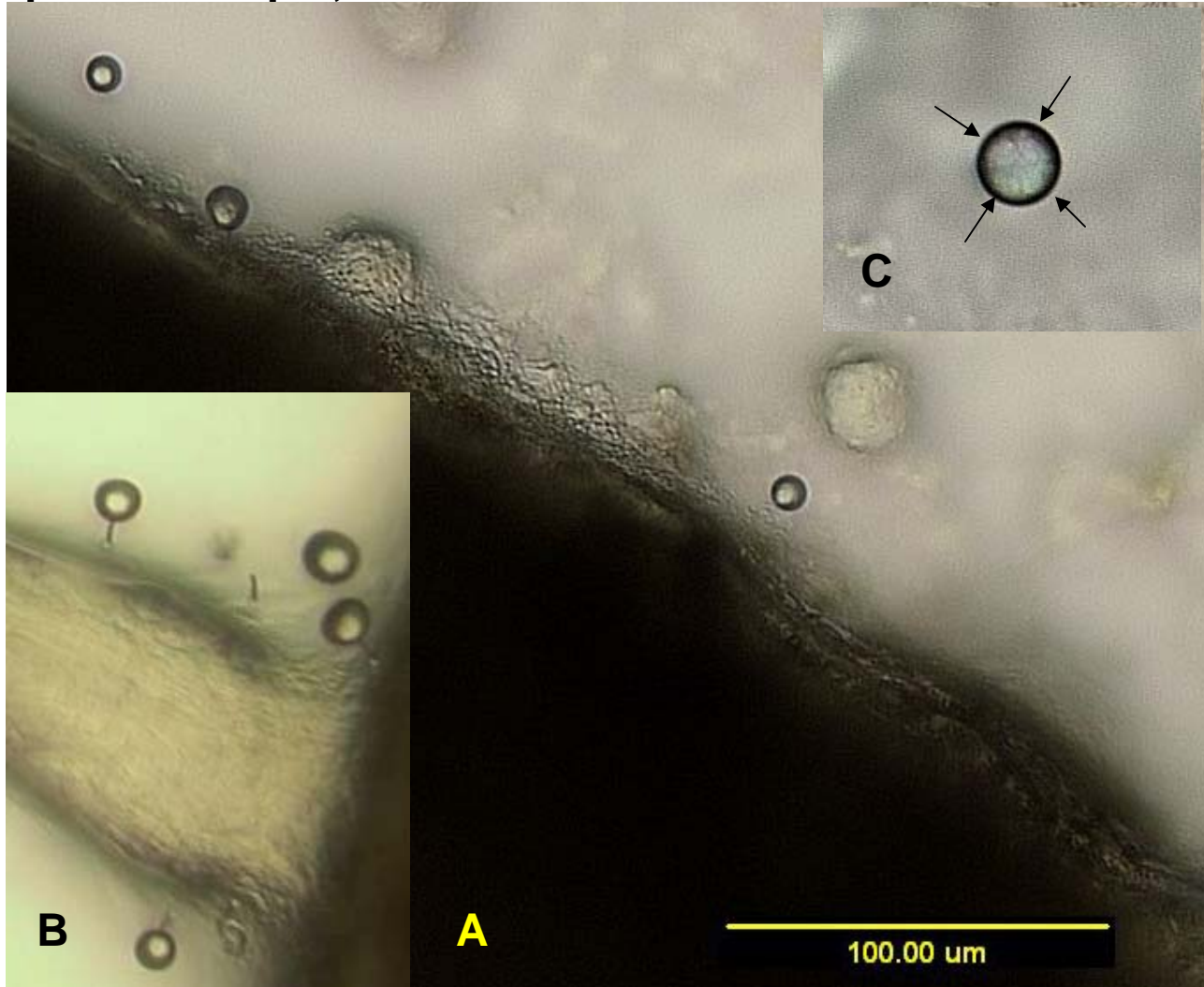
Prespore Cell (PSP): not shown, circular in outline.

Scale bar: 100um, all.

Comments: This species is common worldwide on decaying primary tissues on both standing plants and in the litter. It seems to show patches of local abundance, being very common at some collecting sites and absent at others.

Similar species: This species is distinct from all other protostelids; however, it may at first be mistaken for a small example of the myxomycete genus *Echinostelium*. It lacks both a columella and a capillitium, and the arrangement of spores is unlike any myxomycete when the sheath dries down. In addition the spores usually vary in size within the sporocarp, and they lack any spore wall sculpturing. Spiegel has seen an aggregative ciliate that produces a sorocarp that looks similar to this species on one occasion. Ciliated cells germinate from the undescibe ciliate while amoebae germinate from *E. oligospora*.

Spores multiple, stalks short



Microglomus paxillus Olive & Stoianovitch

This is a relatively rare species of protostelid which is hard to identify if not observed carefully.

Stalk: very short, tapered, narrows to a thin tip.

Spores: two or four compressed against each other to form an ellipsoid (Fig. B) to almost spherical sporangium. Careful focusing, especially with objective of 20x or higher will reveal the individual spores (arrows in Fig. C point to the four spores).

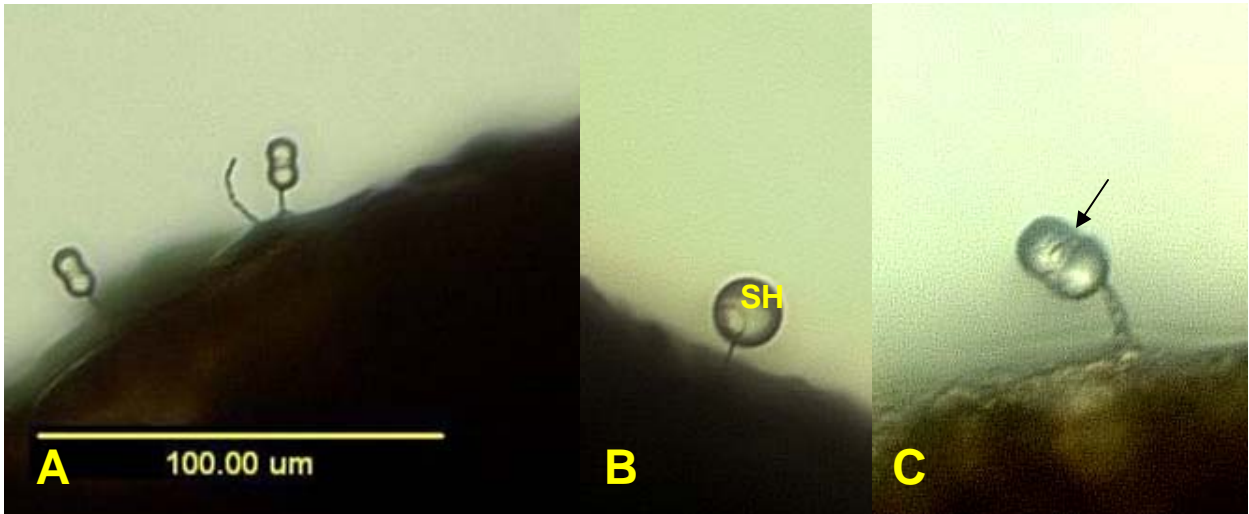
Prespore Cell (PSP): not shown, circular in outline.

Scale bar: 100um, A, B; 50um, C.

Comments: This species is small and easily misidentified if one does not look at it carefully. Any small, short-stalked protostelid whose “spore” looks ellipsoid should be carefully examined to see if it is really a sporangium. This species has been found on primary tissues on both standing plants and in litter and on bark of living trees.

Similar species: Most likely to be misidentified as *s. pseudoendospora*.

Spores multiple, stalks short



Echinostelium bisporum (Olive & Stoian.) Whitney & Olive

Actually the smallest described species of myxomycetes, this relatively common species is most likely to be detected when examining substrates for protostelids.

Stalk: short, stiff with small flange shaped apophysis (not shown).

Spores: always two per sporangium, each spherical to subspherical arranged linearly, distal spore usually slightly smaller. Spore walls minutely spiny with a hilum of fused spines on each where they contact each other (arrow, Fig. C). Often, the hygroscopic sheath is swollen when hydrated when culture plate is first opened (SH in Fig. B).

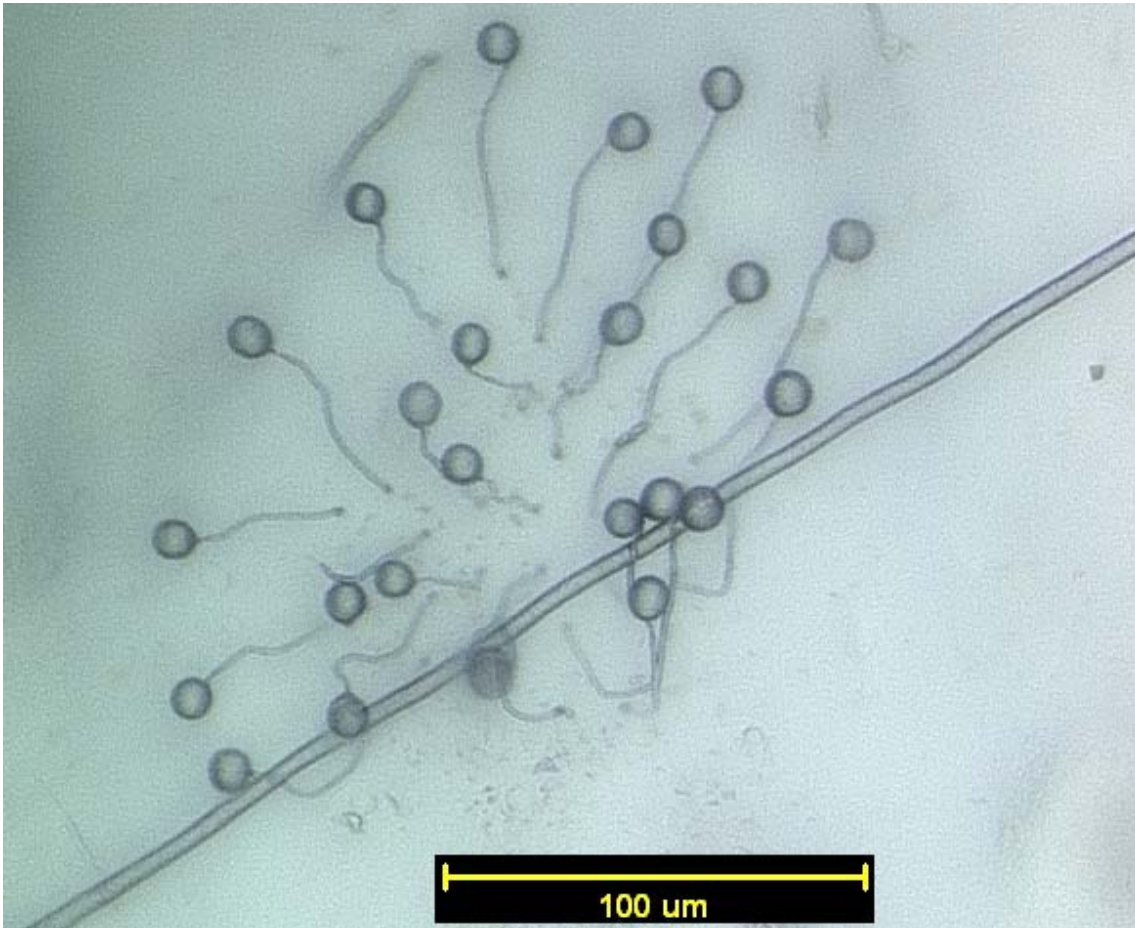
Prespore Cell (PSP): not shown, circular in outline.

Scale bar: 100um, A, B; 50um, C.

Comments: This little myxomycete is found on all kinds of substrates where protostelids are found. It may be a bit more common in the tropics than in temperate areas. Originally described as *Cavostelium bisporum* Olive & Stoian.

Similar species: None, this species is unique and nearly impossible to misidentify.

Sporocarps arranged on a slime column or pad deposited by a plasmodium prior to dividing into prespore cells.



***Ceratiomyxa hemisphaerica* Olive & Stoianovitch**

This rarely found species has been encountered fewer than a dozen times.

Stalk: very long and flexuous, in groups of a few dozen to 100 resting on a small, hemispherical mound of slime deposited by the plasmodium prior to its cleavage into prespore cells. The slime mound in this image does not show well. It is better viewed from the side.

Spore: spherical to ellipsoid, deciduous.

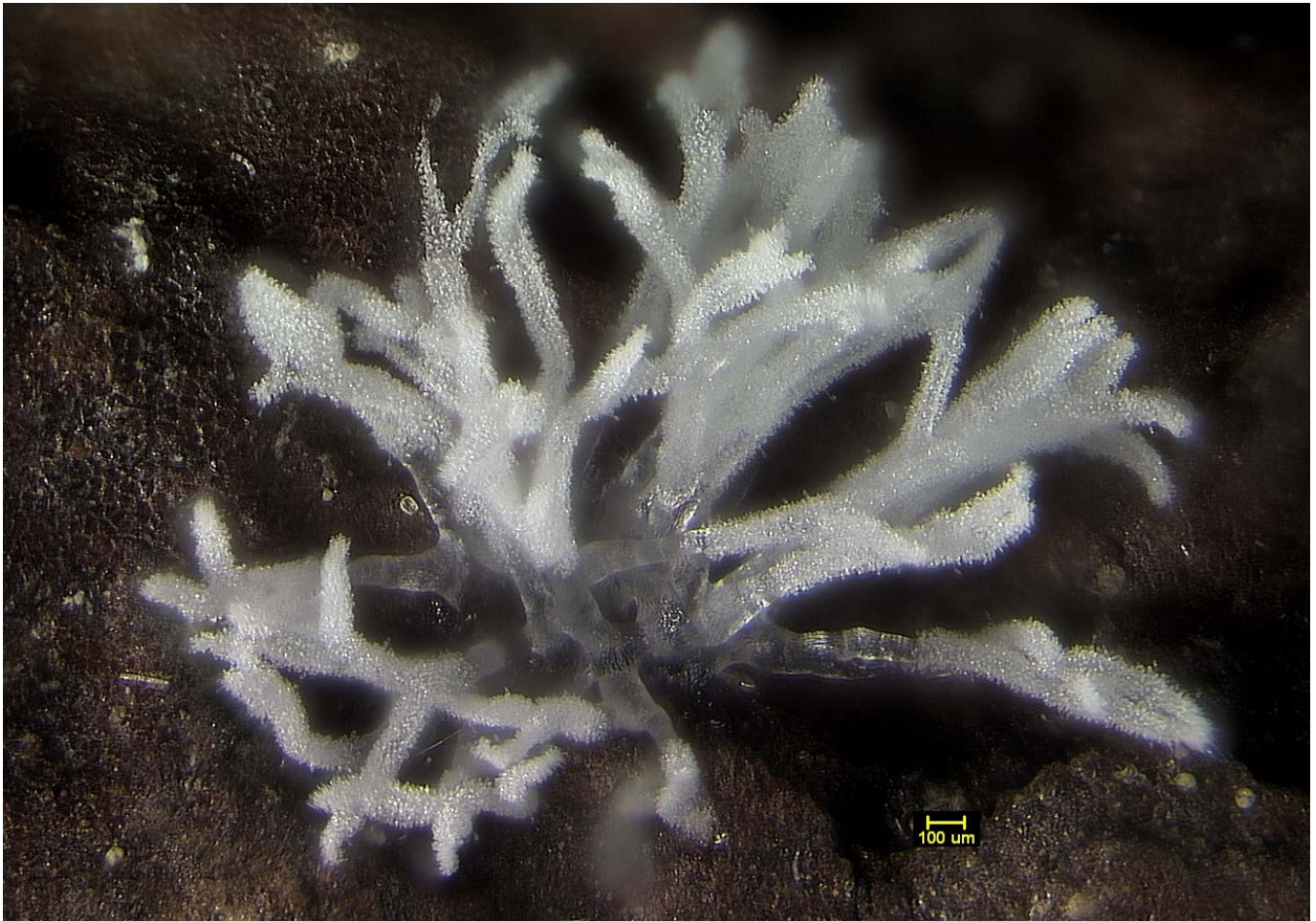
Prespore Cell (PSP): not shown, circular in outline.

Scale bar: 100um

Comments: When this species has been found, it has occurred on rotting wood or fairly substantial primary tissue substrates such as tree fern petioles. It is usually detected by seeing a cluster of sporocarps that radiate outward from a common base.

Similar species: At first glance, an individual sporocarp looks similar to one of *Protosporangium bisporum* (which is not illustrated in this Guide). While that species is often gregarious, it has no common slime mound, and it has two spores instead of one.

Sporocarps arranged on a slime column or pad deposited by a plasmodium prior to dividing into prespore cells.

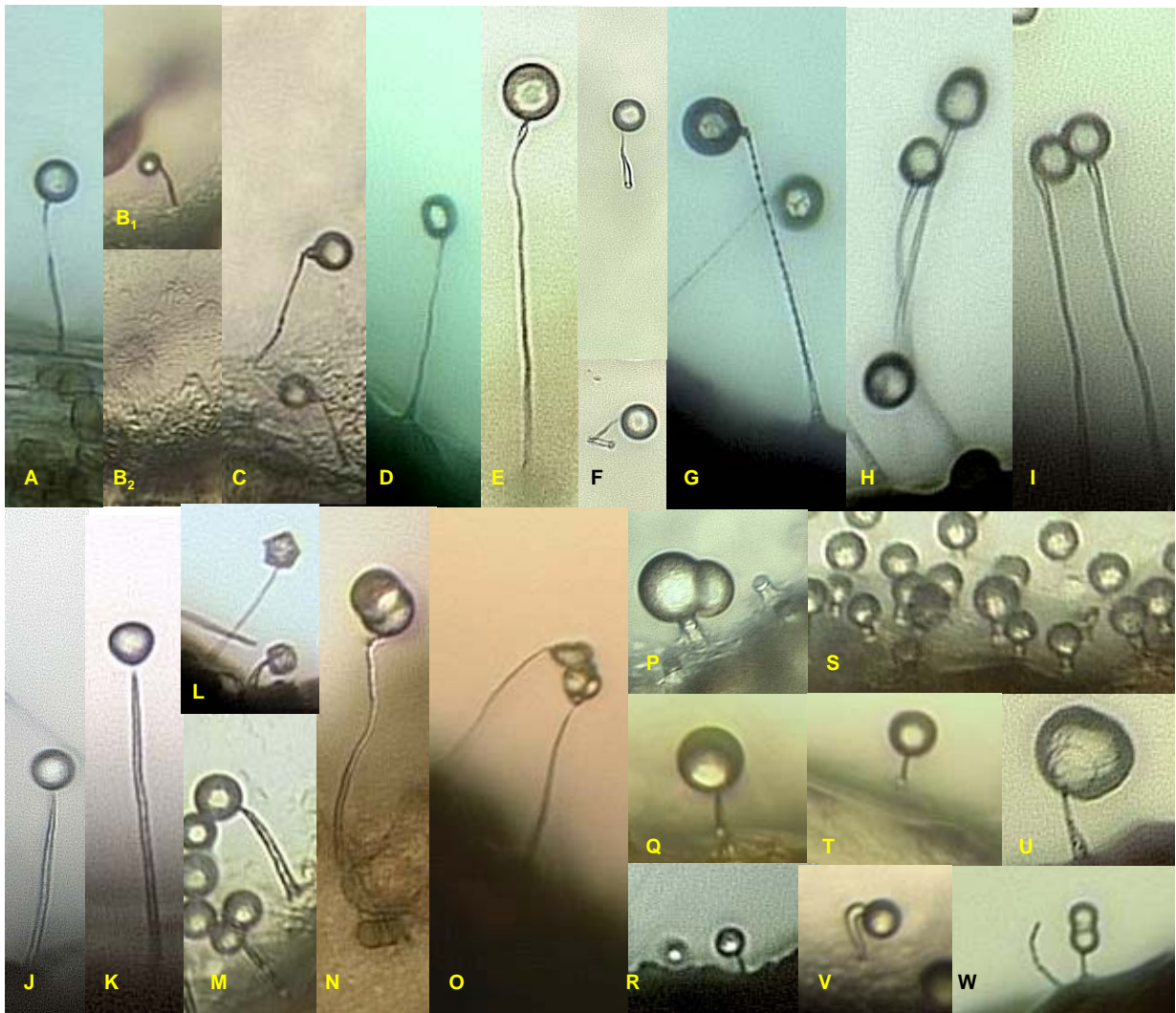


***Ceratiomyxa fruticulosa* (Mull.) Macbride**

This is probably the most commonly encountered of all eumycetozoans, and it is one of the few macroscopic protostelids (even though it is claimed for the myxomycetes in much of the literature). It has slime columns that may show a number of morphologies, and though it is usually white, a fructification may show a whole range of colors, especially early in development. Fructifications may range in size from a few millimeters to over a meter. The species occurs most frequently on rotting wood in mesic to wet forests a day or two after a soaking rain. It is very rarely found in moist chamber cultures for myxomycetes (as was the case with this specimen) and has never been reported in PIP for protostelids. Individual sporocarps have straight stalks about 30-50um long and deciduous, ellipsoidal spores.

Scale bar: 100um

Similar species: *Ceratiomyxa morchella* and *C. sphaerosperma* are much smaller. The former is shaped like a small morel and the latter has a distinct, stalk like base to the slime column with an irregular, spheroid head that supports the sporocarps.



**Same Size Comparison of Protostelids
(Excluding *Ceratiomyxa* spp.)
Described in this Guide**

A. *Protostelium mycophaga*, B. *Protostelium nocturnum* before and after spore discharge, C. *Protostelium pyriformis*, D. *Protostelium arachisporum*, E. *Soliformovum irregularis*, F. *Soliformovum expulsus*, G. *Endostelium zonatum*, H. *Nematostelium ovatum*, I. *Nematostelium gracile*, J. *Tychosporium acutostipes*, K. *Schizoplasmodiopsis micropunctata*, L. *Schizoplasmodiopsis reticulata*, M. *Schizoplasmodiopsis vulgare*, N. *Protosporangium articulatum*, O. *Protosporangium conicum*, P. *Schizoplasmodium cavostelioides*, Q. *Schizoplasmodiopsis amoeboides*, R. *Schizoplasmodiopsis pseudoendospora*, S. *Cavostelium apophysatum*, T. *Microglomus paxillus*, U. *Echinosteliopsis oligospora*, V. *Clastostelium recurvatum*, W. *Echinostelium bisporum*

All the figures on the comparison plate are of sporocarps as they would appear with a 10x objective used for scanning a primary isolation plate. Remember to look for sporocarps on the agar surface surrounding a piece of substrate and fruiting out from the surface of a piece of substrate. With a little practice, one can learn to be fairly efficient at finding protostelids within about a week or two and to be able to identify them to species within a month.

An added bonus to searching for protostelids is the chance to see lots of interesting fungi, other mycetozoans, protists, and small animals. A student in Mycology at the University of Arkansas, taking her first look at the diversity of organisms on a decaying piece of grass exclaimed, "Wow, this is like looking at a coral reef!" We hope that new protostelid hunters will feel the same way.



Find the protostelid in this picture. Hint, it is *Cavostelium apophysatum*.

Suggested Reading:

Olive, L.S. 1975. *The Mycetozoans*. Academic Press, NY.

Spiegel, F.W. 1990. Phylum Plasmodial Slime Molds, Class Protostelia. In L. Margulis, J.O. Corliss, M. Melkonian, and D.J. Chapman (eds.). *Handbook of Protoctista*. Jones and Bartlett, Boston. pp. 484-497.

Spiegel, F.W., S.L. Stephenson, H.W. Keller, D.L. Moore, and J.C. Cavender. 2004. Mycetozoans. In G.M. Mueller, G.F. Bills, and M.S. Foster (eds.). *Biodiversity of Fungi, Inventory and Monitoring Methods*. Elsevier Academic Press, Burlington, MA. pp. 547-576.

The Eumycetozoan Project. <http://slimemold.uark.edu>