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The bark Myxomycetes - their collection, culture and identification

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INTRODUCTION

The purpose of this article is to describe a technique for the isolation of slime moulds from the bark of living trees and to outline some ideas for project work suitable for A-level pupils working in the school laboratory.

Myxomycetes are primarily woodland organisms whose sporangia or fruit bodies occur abundantly during late summer and autumn on damp rotting wood and leaf litter, often in noticeable and colourful colonies. Slime moulds have a remarkable dual life cycle which has led them to be variously classified as Mycetozoa (`fungus animals') and as Myxomycetes ('slime fungi') - see Fry and Fry [1]. The assimilative phase is the plasmodium, a multinucleate acellular mass of protoplasm enclosed in a hyaline sheath, which slowly wanders about feeding holozoically on bacteria, fungi, protozoa and other organic materials. In many of the species belonging to the order Physarales, a large fan-shaped plasmodium develops in which a rhythmic reversible flow of protoplasm occurs. This active back-and-forward streaming movement can easily be seen with a x20 microscope and probably functions to maintain an even distribution of oxygen, nutrients and metabolites throughout the protoplasm as well as contributing to the migration of the plasmodium. In nature, the plasmodium usually develops inside the substrate and finally migrates to the surface to sporulate. The transition from the moist gelatinous feeding stage to an intricate fruiting structure (or group of fruit bodies) is a most spectacular event which involves profound changes in the organism that, once initiated, are irreversible. Sporangia always develop perpendicular to the surface, each species forming a characteristic structure by which it may he identified. The character of the sporangium and spores forms the basis of classification. The sporangia may be stalked or sessile, possess a persistent skin (peridium) or may lose the peridium shortly after it has formed. Typically, the spores are held in a characteristic system of threads which forms the capillitium. In the order Liceales, the capillitium is entirely lacking.

The technique of moist-chamber culture, now well established in studies of the Myxomycetes, was introduced by Gilbert and Martin [2] who wanted to demonstrate the algae that grew on the bark of living trees to their biology classes. Much to their surprise, a number of small and undescribed slime moulds appeared after a short time, along with the epiphytic algae. The Myxomycetes had developed from tiny resting plasmodia (sclerotia), adapted to take immediate advantage of short rainy spells. It has since been found that the bark of living trees provides a unique environment that supports a distinct group of species whose small size has a definite ecological advantage. Besides algae and slime moulds, many varied organisms may appear in bark cultures, including filamentous moulds, rotifers, nematodes, tardigrades, mites and insects such as springtails, psocids, thrips, etc. Some of these occasionally interfere with the development of Myxomycetes in culture.

MOIST CHAMBER TECHNIQUE

1.Collecting bark samples

Thin slivers of bark, together with epiphytic mosses and lichens, are best removed from hard-barked trees with a wood chisel, dropped into a small paper envelope, stuck down and identified as to tree species, locality, grid reference and date. Care should be taken to avoid penetrating to the cambial layer and so allowing the entry of disease organisms into the tree. The inclusion of living tissues in moist chambers often encourages the growth of unwanted filamentous fungi. Bark from certain species such as apple and yew may be collected by simply pulling off loose pieces by hand. Smooth-barked trees such as Holly and Beech usually bear a sparse population of Myxomycetes, though pines with scaling bark often produce good results. Oak, Ash and Sycamore each yield a high density of sporangia and a good species diversity, particularly if the bark is collected from fissures (rather than from the surface of ridges). Cultivated orchard apples are also rich in species and are well worth investigating for their Myxomycete biota.

2.Moist chamber culture

The technique is simple and makes use of standard equipment that is generally available in school laboratories. For each culture, a plastic petri dish is lined with a 9 cm diameter filter-paper disc and moistened with cold boiled tap water. Pieces of bark are then arranged with their cut surfaces facing downwards on the filter paper so that they are touching but not overlapping. They should then be thoroughly wetted with more water sprayed from a wash bottle or other suitable 'squeezy' container (such as those used for chocolate dessert sauces). Any standing water should be tipped off

or pipetted away after allowing the bark to soak overnight. If the bark is not thoroughly wetted, more water should be added. The water used here need not be sterile: boiling is sufficient to destroy 'stray' Myxomycete spores and to drive off the chlorine in tap water. Calcium salts that may he present favour the development of Physarales, which are characterized by their lime content. Plastic petri dishes are sufficiently cleaned for use by being washed in ordinary household detergent solution followed by immersion in clean. hot water. Plastic petri dishes slowly evaporate water from under the lid and the contents should be sprayed at intervals to maintain moist (but not wet) conditions. This slow water loss enables accurate control of the water content, with the filter paper acting as a reservoir. Glass dishes, on the other hand, lose water only very slowly and, if a culture should be made too wet at any time, the experimenter then has difficulty in re-establishing optimum conditions. Culture dishes should be placed near a north-facing window so as to avoid receiving direct sunlight at any time of the day. Light is necessary for the sporulation of species with yellow plasmodia.

3.Observation of cultures

Bark surfaces may be closely examined for sporangia before moistening and it will sometimes be found that fruiting stages are collected with the bark samples. Observation should begin a day or two after setting up the culture and should continue on a daily basis for about ten days. After this time, it is usually sufficient to examine the bark about twice a week. During the first few days, the smallest species will have started to appear and it is necessary to systematically scan the surface of the bark and the exposed areas of filter paper with a x10 to x20 binocular microscope. The lid of the dish needs to be removed for scanning and for the removal of specimens for their subsequent identification. Plasmodia may appear and these may be either allowed to fruit on the bark or they may be transferred to CM/2 Agar (see 8. *Formulae)* in another petri dish where they should continue to feed and grow until fruiting occurs. Plasmodia may be transferred to Water Agar to stimulate the initiation of sporangia. Bark cultures may be allowed to run for up to three or four months: indeed some species of *Licea* which commonly occur on bark often require many weeks before their sporangia appear.

4.Harvesting

Sporangia are most easily removed with fine watch-maker's forceps or, in the case of very small Echinostelia, with a fine entomological pin secured by Araldite to the end of a wooden toothpick. Sporangia are best removed from the bark under a binocular microscope. To make permanent slides, sporangia should be mounted in Lactophenol or, for Physarales which have calcium carbonate in their sporangia, Hoyer's

Medium (see 8. *Formulae).* The Lactophenol mountant may be coloured with a little Cotton Blue to render the fine hyaline capillitium of the Echinostelia more easily visible. Sporangia of bark species should be mounted entire, so that features of the stalk base can be examined. It is essential that spores should be dispersed so as not to obscure details of the capillitial threads or elaters and this can be achieved by either blowing on a dry sporangium held in fine forceps or by repeatedly dipping the specimen in the mountant. Slides may be rendered permanent by the application of clear nail varnish painted around the edges of a square coverslip. Many students at first use too much mounting fluid: if the preparation is to be sealed, then it is important that the mountant should not flow out from beneath the coverslip as this prevents the varnish from sticking to the slide. Lactophenol, or any other dilute acid, may be used to detect the presence of carbonate deposits and so assist in the identification of the Physarales.

5.Protoplasmic streaming

Students interested in the phenomenon of protoplasmic streaming may use the moist chamber technique for the isolation of plasmodia from the environment. Bark samples collected from Apple and from the base of Oak and Ash, particularly if pleurocarpous mosses are present, are reliable sources of physaraceous species with large and active plasmodia. These plasmodia are best transferred, on the bark, to CM/2 Agar. Worthwhile exercises might include investigations to find if Q10 applies, to measure the effects of varying the concentrations of respiratory gases and of ATP, and to measure the speed of movement in different parts of the same active plasmodium. It is important to identify the species under investigation, thus it is necessary to allow part of the plasmodium to sporulate. Plasmodia may be cut into smaller pieces and subcultured on CM/2 Agar. Fruiting may be encouraged by transferring a plasmodium to Water Agar and, in the case of yellow and orange plasmodia, increasing the light intensity. For detailed accounts of Myxomycete physiology, see Gray and Alexopoulos [3] and Olive [41.

6.Identification

Of the 750 or so species of Myxomycetes, some 300 are known to occur in the British Isles [5]. Over 100 species have been reported from the bark of living trees and vines, mainly from Britain and the USA, and many of these are known to occur on other substrates. The slime moulds that feed and fruit on the bark of living trees form a somewhat distinctive biota and these species have been called the Corticolous Myxomycetes [6]. These corticolous species often appear in great numbers and are represented by diverse species from each of the five endosporous

Orders. It is not uncommon to find six or more species fruiting in the same petri dish culture, particularly if the bark is gathered from large mature trees. Monographic treatments for identification include the beautifully illustrated works by Lister [7] which have been out of print for many years, and the more recent monograph by Martin and Alexopoulos [8] which is also well illustrated. Corticolous species have been featured in many short papers in journals, and an article by the author deals with the identification of all known species of the corticolous group [9]. A key to the Orders of Myxomycetes and figures of 26 of the more common British species are given here.

KEY TO THE ORDERS OF MYXOMYCETES

la Spores born externally on individual stalks. CERATIOMYXALES (not found on bark)
1b Spores born inside sporangia of characteristic form. 2

2a Calcium carbonate present in the sporangium. Plasmodium often conspicuous; scarlet, orange, yellow, cream, white or hyaline (but then usually drab-coloured from matter gathered from the substrate). Spore mass violaceous brown, deep purplish brown to black. PHYSARALES.
2b Calcium carbonate entirely absent. Plasmodium not usually evident until immediately prior to the formation of the fruit body. Spore mass white to brightly coloured or brown to fuscous black. 3

3a Spore mass dark reddish-brown or purplish-brown to fuscous black. Capillitium usually abundant, composed of dark threads. Columella (continuation of the stalk into the spore mass) usually present. Stalk never packed with granular. STEMONITALES.
3b Spore mass white or coloured, only rarely dull black, never purple-brown. Capillitium, if present, hyaline or pale-coloured by transmitted light. Columella present or absent. Stalk sometimes packed with granular matter. 4

4a Capillitium absent. Columella absent. Stalk, if present, never hyaline. LICEALES.
4b True capillitium usually present; if absent then columella usually present. Stalk, if present, hyaline or pigmented. 5

5a Sporangia minute, globose, 0.03 to 0.2 mm diameter. Stalked, the base of the stalk usually containing granular refuse matter. Columella absent or present. Capillitium, if present, unsculptured, hyaline or brownish by transmitted light. ECHINOSTELIALES.
5b Sporangia larger, rarely less than 0.2 mm diameter. Stalked or

sessile Columella absent. Capillitium usually distinctly sculptured; hyaline or yellow by transmitted light, rarely pale brown or pink. TRICHIALES.

7. Notes to Figures and short descriptions of common corticolous species

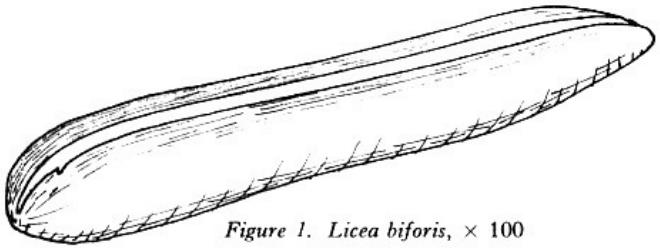


Figure 1. Licea biforis, × 100

Figure 2. Licea kleistobolus, × 100

Figure 3. Licea minima, × 100

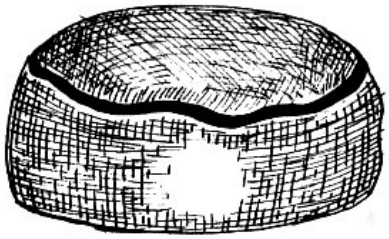


Figure 4. Licea parasitica, × 100

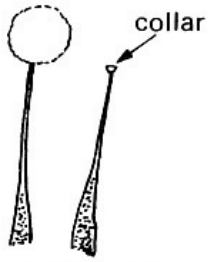


Figure 5. Echinostelium elachiston, × 100

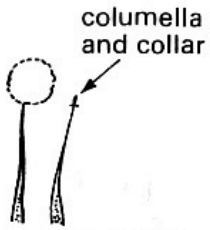


Figure 6. Echinostelium fragile, × 100

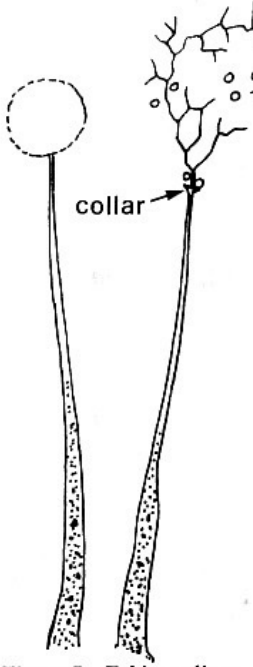


Figure 7. Echinostelium minutum, × 100

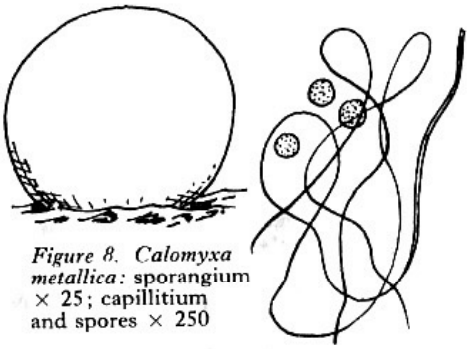


Figure 8. Calomyxa metallica: sporangium × 25; capillitium and spores × 250

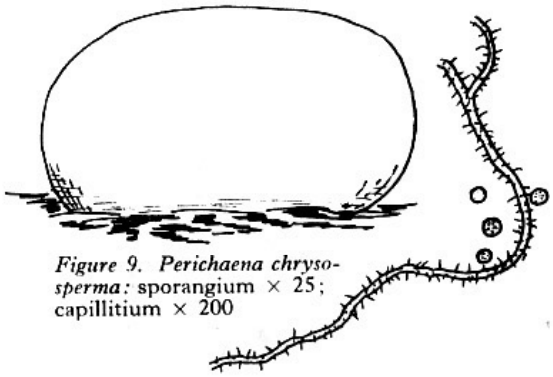


Figure 9. Perichaena chrysosperma: sporangium × 25; capillitium × 200

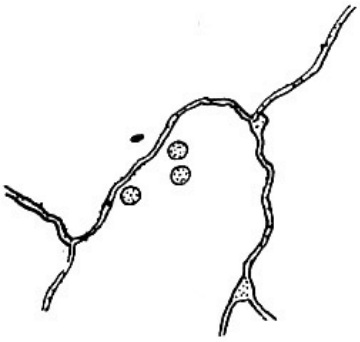


Figure 10. Perichaena corticalis: capillitium and spores × 200

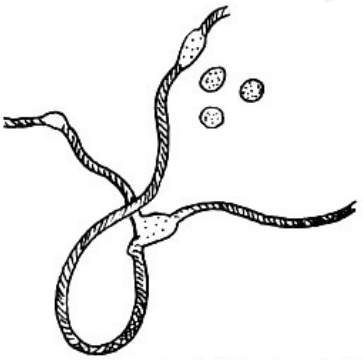
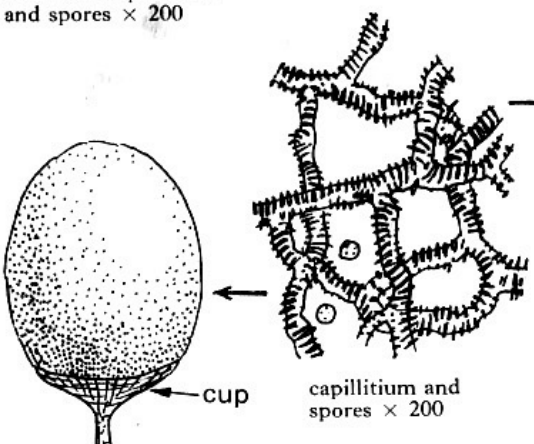
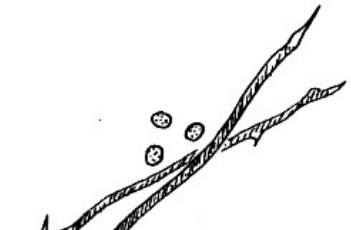


Figure 13. Hemitrichia karstenii: capillitium × 200



capillitium and spores × 200



Liceales

Figure 1 Licea biforis. The elongated, yellow-brown sporangia are commonly found gregariously beneath peeling Apple bark. The longitudinal fissure that divides the peridium into two parts gives each sporangium the appearance of a date stone.
Figure 2. Licea kleistobolus. The small, shining, discoid sporangia are quite common on Apple and Pine bark and usually occur in large numbers. The spores of this sp. measure between 9 and 13 µm diameter and bear groups of warts.
Figure 3. Licea minima consists of brown platelets which lock together by means of platelets which lock together by means of pegs. The pores are red-brown in the mass and appear paler by transmitted light, minutely warted and about 10 to 13 µm diameter. Often taking several weeks to develop in moist chamber, on all kinds of bark.
Figure 4. Licea parasitica. The commonest *Licea* on all kinds of bark, resembling miniature pork pies with a black preformed line of dehiscence. Spores smooth, about 13 µm diameter.

Echinosteliales

Figure 5. Echinostelium elachiston. A recently described species that is common on all kinds of bark. The distinctive character of this species is the presence of a dish-like collar, about 15 µm diameter, that subtends the spore mass.

Figure 6. Echinostelium fragile. The smallest of the more common corticolous species. The spore mass is pink and the sporangia should be sought along the edges of fragments of bark in moist-chamber as they are more easily seen in profile. Sporangia are very difficult to mount and must be removed with a fine needle. The columella has a distinctive fusiform shape and the spores are pink by transmitted light and measure about 14 µm in diameter.
Figure 7. Echinostelium minutum. This little white Myxomycete occurs abundantly on bark as well as on other substrates but is rarely found in the field on account of its small size. Sporangia are best mounted in Cotton Blue in Lactophenol so that the fine hyaline capillitium can be more easily seen. The smooth spores are about 8 µm in diameter and, typically, two or three of them adhere to the columella when the specimen has been mounted.

Trichiales

Figure 8. Calomyxa metallica. The sessile sporangia grow up to 1 mm in diameter and the translucent peridium is lilac and usually iridescent. The capillitium consists of long flexuous threads and the spores are lilac in the mass in bark culture developments.
Figure 9. Perichaena chrysosperma. The yellow or reddish-yellow sporangia are sessile and commonly found on Apple bark. The spores

are bright yellow in the mass and measure 9 to 11 µm in diameter. The capillitial threads usually bear long spines up to about 7 µm in length.
Figure 10. Perichaena corticalis. Very similar to the preceding species except that the spores are usually larger (about 10 to 14 µm in diameter), and the capillitial threads never bear such long spines. Also common on Apple bark and occasionally found on other trees.
Figure 11. Arcyria pomiformis. The spp. of *Arcyria* are distinguished by the profusely branched and anastomosed capillitium which is marked with cog-like thickenings. The sporangia are usually ovate and are coloured yellow buff**.** The base of the sporangium forms a small cup from which the capillitium arises.
Figure 12. Arcyria cinerea. This species is similar to the last except that the sporangia are typically more elongated and are grey in colour. More common than *A. pomiformis* and found on all kinds of bark.
Figure 13. Hemitrichia karstenii. Common on Apple bark and resembling *Perichaena* under the binocular. The capillitium consists of a highly branched system of threads with no free ends and is marked with four to six spiral bands.
Figure 14. Trichia contorta. This species comes very close to the last except that the capillitium is made of short threads (elaters) and, consequently, many free ends are seen when examined with the microscope.

Stemonitales
Figure 15. Colloderma oculatum. One of the few sessile species in this order. The sporangia form within a mucilaginous sheath and often, in bark culture, dry to form iridescent blue sporangia. The capillitium arises from the base of the sporangium and is made of branched and anastomosed threads. The spores are usually about 12 µm in diameter and are strongly spined.
Figure 16. Stemonitis nigrescens. The dark stalked sporangia grow in small groups and up to about 4 mm high. The members of this genus have a long columella which usually reaches to near the top of the sporangium and the capillitium forms a delicate surface net through which the spores are released. The sporangia of *S. nigrescens* have short stalks and the spores are decorated with a reticulate pattern of warts.
Figure 17. Macbrideola cornea. A small dark species that is found more commonly in wetter parts of Britain and is then often associated with acrocarpous mosses. The stalk is hollow and often an air bubble is trapped inside when specimens are mounted in Lactophenol. The base of the stalk often appears red.
Figure 18. Paradiacheopsis fimbriata. Very common on all kinds of bark, especially conifers. The species is found even in the centre of cities on very polluted bark. The fine hair-like stalks and the swollen

ends of the lax capillitium are distinguishing features.
Figure 19. Enerthenema papillatum. A very common and variable species which, like the last, is found on polluted bark from cities. It is the only species which possesses an apical disc. Blown-out sporangia resemble miniature maypoles with the capillitial threads arising from the edge of the disc.
Figure 20. Comatricha elegans. One of the few Stemonitales, with *Colloderma,* that does not possess a columella. The stalk branches at the base of the sporangium into a number of main branches.
Figure 21. Comatricha nigra. An abundant and variable species that is found in cultures of bark of all kinds. This is one of the most abundant species that is regularly found in the field. Typically, the columella reaches to the top of the sporangium but in moist chambers it sometimes falls short.

Physarales

Figure 22. Badhamia foliicola. The sporangia usually occur in groups and are coloured iridescent blue. The lime in the capillitium is colourless. The spores are minutely warted and 11 to 12 µm in diameter. Plasmodium yellow or orange.

Figure 23. Physarum leucophaeum. The sporangia grow up to 1 mm tall and are coated with white or ash-blue lime. The capillitium consists of colourless nodes of calcium carbonate that are connected by fine hyaline threads. These nodes are rounded or angular in outline. Plasmodium hyaline.
Figure 24. Physarum nutans. Very similar to the last species but the nodes are elongated and the stalk relatively longer : the sporangia are typically lens-shaped and nodding. Plasmodium hyaline. Common on all kinds of bark.
Figure 25. Physarum viride. This species is commonly associated with pleurocarpous mosses from near the base of deciduous trees. The sporangia, like *P. nutans,* are lenticular and nodding and the capillitium has elongated (fusiform) nodes of lime. In this species, however, the lime and the plasmodium are yellow. Occasionally sporangia are coloured orange.

Acrasiales

Figure 26. Pocheina rosea. A common cellular slime mould that is more closely related to *Dictyostelium* and not generally regarded as a Myxomycete, this little pink (rarely white) organism is commonly found on bark in moist chamber cultures and is easily mistaken for a true Myxomycete. It is immediately recognized by having a stalk composed of tiers of cells, and possessing no capillitium and no peridium. Specimens may be mounted in Lactophenol and should

be lifted off the bark with a fine needle.

8. *Formulae*

Lactophenol

Phenol (pure crystals)	20 g
2-Hydroxypropanoic acid (lactic acid)	20 g
Propane-1,2,3-triol (glycerol)	40 g
Water	20 g

A little Cotton Blue dye may be added.

Hoyer's Medium.

Soak 10 g of colourless Gum Arabic lumps in 17 cm3 of distilled water for a few days in a covered container. Add

66g of 2,2,2- trichloroethanediol (chloral hydrate) and let the solution stand for a few days until all the material dissolve. Finally, stir in 7 g of glycerol.

Half-strength Cornmeal Agar (CM/2 Agar)

Cornmeal Agar	0.85 g
Bacto Agar	1.25 g
Distilled water	100 cm3

Water Agar Plain Agar 3 g
Distilled water 100 cm3

QUANTITATIVE ECOLOGICAL STUDIES

Papers relating to corticolous species have dealt almost entirely with descriptions of new species, and little ecological data have been published. There are many opportunities for original investigations of micro-distribution of corticolous species that could be undertaken at secondary school level. From casual observation, there appears to be some correlation in the occurrence of certain species with the water-holding capacity of bark. Some species seem to be correlated with high water-holding capacity, which is increased by the presence of mosses and other epiphytic vegetation. The water-holding capacity may be found by soaking bark in water, dabbing the surface dry with filter paper and then drying to constant weight at 100°C. There is evidence that some Myxomycetes have a stratified distribution pattern on the trunks of trees, some species (like *Physarum* spp.) being more common near the ground. Water relations are certainly of considerable importance in distribution. It is probable that vertical zonation is more marked in woodland trees than in phorophytes of the same species growing in open woodland sites. Trees in exposed situations carry a different Myxomycete biota from those in woodland and forest sites. Whichever aspect of

ecological work is undertaken, it is important that the student should limit his investigation to one species of tree or to very few slime mould species. Bark samples should be of similar sized areas and routine statistical tests should be applied if valid quantitative comparisons are to be made.

References

1. Fry, E., and A. Fry, *The Mycetozoa and Some Questions Which They Suggest* (London: 'Knowledge' Office, 1899).
2. Gilbert, H. C., and G. W. Martin, `Myxomycetes found on the bark of living trees', *University of Iowa Studies in Natural History,* 1933, **15,** 3-8.
3. Gray, W. D., and C. J. Alexopoulos, *Biology of the Myxomycetes* (New York: The Ronald Press Co., 1968), pp. 288.
4. Olive, L. S., *The Mycetozoans* (Academic Press, 1975), pp. 293.
5. Ing, B., *A Census Catalogue of British Myxomycetes* (Foray Committee of the British Mycological Society, 1968), pp. 24.
6. Keller, H. W., and T. E. Brooks, Corticolous Myxomycetes I : Two new species of Didymium', *Mycologia,* 1973, 2, LXV, 286-94.
7. Lister, A., *A Monograph of the Mycetozoa:* 2nd edition (1911) revised by G. Lister, pp. 302, pl. 200; 3rd edition (1925) revised by G. Lister, pp. 296, pl. 222 (British Museum, Natural History).
8. (Martin, G. W., and C. J. Alexopoulos, *The Myxomycetes* (Iowa City: University of Iowa Press, 1969), pp. 561, pl. 367.
9. Mitchell, D.W., *A key to the corticolous Myxomycetes*, British Mycological Society Bulletin.