THE SESSILE SPECIES OF *DIDYMIUM*

by

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Thanks also go to the other members of my committee, Dr. John J. Iandolo and Dr. Charles W. Pitts.

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INTRODUCTION

This thesis is presented in the format of two manuscripts, each written in the style acceptable by the mycological journals Mycologia and Mycotaxon, to which they will be submitted for publication. The first paper, "Preparation of Myxomycete Spores for SEM," deals with the scanning electron microscopy technique used in this research, while the second, "The Sessile Species of *Didymium*," is a taxonomic monograph of a portion of a genus of plasmodial Myxomycetes.

The monographic treatment includes only those species of *Didymium* that are never known to produce stalked sporangia. It does not include those that are "normally" stalked, but under certain environmental conditions will develop sessile forms. In choosing a group in need of a taxonomic study, we wanted one that was well delimited and with a number of taxa that could be studied reasonably within the scope and time appropriate for a Master's research program. We also wanted this group to be a part of a larger group into which our interests could be expanded for future research topics. The myxomycete genera which have not recently been monographed contain too many species for a study to be completed within the time available. The alternative was to choose a genus that could be divided into distinct groups. The sessile species of *Didymium* comprise such a group, while the genus is large enough to provide numerous problems for future studies.

PREPARATION OF MYXOMYCETE SPORES FOR SEM[1]

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Spore morphology, including surface ornamentation, spore size, and in some species, the manner in which the dehydrated spore wall collapses are among the more useful diagnostic characters in species identification of Myxomycetes. The ability of the scanning electron microscope (SEM) to resolve minute details has proven superior over other forms of microscopy in visualizing these characteristics. Several procedures ranging from the observation of unfixed spores to those involving the use of fixatives and various drying procedures have been used in the preparation of spores for SEM (Aldrich & Blackwell, 1976; Scheetz & Alexopoulos, 1971; Schoknecht & Small, 1972). After consideration of the alternatives, Aldrich and Blackwell (1976) concluded that the most suitable method of preparing myxomycete spores for SEM would be one in which the image produced most closely resembles that obtained with the light microscope. The method advocated by them involves pretreating the spores to return them to their original globose state and then fixing to keep them in that state while they are subjected to the drying procedure and vacuum of the SEM. However, with this method one encounters the problem of manipulating small amounts of tiny spores through a variety of

1. Contribution No. , Division of Biology, Kansas State Agricultural Experiment Station.

chemicals, requiring repeated centrifugation, and attachement of these spores to stubs for observation.

Mazia and co-workers (1974) have described a technique using poly-D-lysine (HBr 160,000 d, Nutritional Biochemicals Corp.) to affix very minute structures to appropriate surfaces for both scanning and transmission electron microscopy. Using this same adhesive procedure, we have found that fresh or dried spores may be affixed directly to aluminum stubs which can then be transferred through the chemical fixative and drying agents.

The initial step in this technique is to spread the polylysine (1mg/ml) across the surface of the stubs. This may be done using a square coverslip in much the same manner as in the preparation of a blood smear. The stub is then immediately rinsed with distilled water leaving a monolayer coat of polylysine on the surface. This is allowed to dry throughly.

After the polylysine has dried, a drop of Aerosol (Aerosol OT 1%, distilled water 74%, and methyl alcohol 25%) with suspended spores is placed on each stub and allowed to dry. Aerosol is used due to its property of reducing surface tension which aids in dispersing the spores more evenly on the stub.

As the Aerosol evaporates, the spores become affixed to the stubs which then may be transferred through the sequence of chemicals by tying a thread to the peg and submerging the stub into test tubes containing the chemicals. The stub is held suspended in the tube by placing a rubber stopper in the mouth of the tube to hold the thread. If several tubes of each solution are prepared and these are arranged in as many series, then several stubs may be transferred through sequence at the same time.

The sequence of chemical manipulations begins with a 10 minute rinse in distilled water. This is followed by treating with 2% gluteraldehyde in distilled water for two hours to fix the spores and two rinses in distilled

water to assure removal of all of the gluteraldehyde before proceeding to the drying sequence. The spores, affixed to the stubs, are dried using a gradient series of ethanol and critical point drying. Specimens are then stored in a desiccator until ready for use.

LITERATURE CITED

Aldrich, H.C. & M. Blackwell. (1976). Resistant structures in the Myxomycetes. The Fungal Spore. John Wiley and Sons, New York. 413-462 pp.

Mazia, D., W.S. Sale, & G. Schatten. (1974). Polylysine as an adhesive for electron microscopy. J. Cell Biol. Part 2, 63(2):212a.

Scheetz, R.W. & C.J. Alexopoulos. (1971). The spores of *Badhamia gracilis* (Myxomycetes). Trans. Am. Micros. Soc. 90:473-475.

Schoknecht, J.D. & E.B. Small. (1972). Scanning electron microscopy of the acellular slime molds (Mycetozoa=Myxomycetes) and the taxonomic significance of surface morphology of spores and accessory structures. Trans. Am. Micros. Soc. 91(3):380-410.

THE SESSILE SPECIES OF DIDYMIUM

A separate taxonomic treatment of the genus *Didymium*, other than those in manuals such as that of Martin and Alexopoulos (1969), has never been undertaken. However, Nannenga-Bremekamp (1972) has published a treatment of the species of *Didymium* with long stalked sporangia. This study treats only those species that are known to have never produced stalks. In these and other papers concerned with certain species, a number of questions involving species delimitation have been raised. For example, Nannenga-Bremekamp (1966) elevated one variety of *Didymium difforme* (Pers.) S.F. Gray to the rank of species, while Martin and Alexopoulos (1969) conclude that all varieties of the taxon integrade completely. Other problem areas include the taxa which show affinities to *Didymium dubium* Rost. and whether or not *Didymium decipeins* Meylan should be included in a separate genus as proposed by Brooks in his unpublished dissertation. These and other such questions have been considered in this paper. Also, since Martin and Alexopoulos published their treatment of the Myxomycetes, several new sessile species of *Didymium* have been described: *D. rugulosporum* Kowalski, *D. saturnus* Keller, *D. orthonemata* Keller & Brooks, and *D. synsporon* Brooks & Keller. This paper will include keys and descriptions to these species and discuss affinities to the rest of the sessile taxa.

Cultural studies would be very practical in delimiting some taxa in this group, but few species have been grown from spore to spore in the laboratory. Mock and Kowalski (1976) have found that the pH of the culture medium is critical in cultivating certain Myxomycetes. Thus, future research, including experimentation with the affects of light, pH, medium composition, temperature, etc. could lead to the cultivation of many more species of *Didymium* which in turn could be used to gain a greater knowledge of the stability of the diagnostic characteristics of *Didymium*.

Having realized the limitations of being unable to work with an organism

in culture, one must utilize all imaging techniques available. In this paper all photographs have been taken at equivalent magnifications so that quick comparisons may be made between species.

The Herbarium of the University of California (UC), Farlow Herbarium of Harvard University (FH), The New York Botanical Garden (NY), The Herbarium of the State University of Iowa (IA), The National Fungus Collections (BPI), The Herbarium of Kansas State University (KSC), and the private herbarium of Dr. H.W. Keller (HWK) made available their collections of the sessile species of *Didymium* for this study.

KEY TO THE SESSILE SPECIES OF DIDYMIUM

1. Lime crystals aggregated to form a firm, smooth, eggshell-like crust on surface of peridium.....2
1. Lime crystals scattered or loosely aggregated on surface of peridium.....8

2. Spores in clusters.....*D. synsporon*
2. Spores free.....3

3. Spores with encircling ring at equator.....*D. saturnus*
3. Spores without encircling ring at equator.....4

4. Capillitium rigid and resilient.....*D. listeri*
4. Capillitium fragments easily, but not rigid and resilient.....5

5. Spores minutely warted or smooth, pale to brown,usually with a pale swollen area..... *D. difforme*
5. Spores with large spines, uniformly a very dark brown, pale swollen areas absent.....6

6. Spores 9-10 µm diam; white, dendroid vesicular expansions enclosing lime crystals, sometimes interspersed with capillitium.....*D. trachysporum*

6. Spores 12-20 µm diam; no vesicular expansions present.....7

7. Spores 18-20 µm diam with prominent raised ridges; restricted to dung.....*D. rugulosporum*

7. Spores 12-16 µm diam without raised ridges; not found on dung..*D. quitense*

8. Fructification ochraceous to brown.....9

8. Fructification gray to white.....10

9. Spores distinctly tuberculate, 12-14 µm diam.....*D. fulvum*

9. Spores not tuberculate, 7-8 µm diam.....*D. ochroideum*

10. Vesicles present, intermixed with spores and capillitium.....11

10. Vesicles absent.....12

11. Collumella wall-like; vesicles pale grayish brown, free from capillitium; spores 10-13 µm diam.....*D. flexuosum*

11. Collumella absent; vesicles yellow; spores 8.5-11.5 µm diam.....*D. serpula*

12. Trabeculae containing lime crystals and extending from the base of the fructification to the peridium present.....*D. sturgisii*

12. Trabeculae lacking.....13

13. Fructification intricately labyrinthiform, forming a continuous perforated layer.....*D. perforatum*

13. Fructification sporangiate to plasmodiocarpous, but never intricately labyrinthiform.....14

14. Peridium covered by a layer of pure white scales.....*D. nivicolum*

14. Peridium lacking scales.....15

15. Fructification uaually annulate sporangia on a restricted base or plasmodiocarps which form links; dehiscence circumscissile; capillitium slightly rigid; spores not dark, 8-11 µm diam.....*D. anellus*

15. Fructification on a broad base, not annulate; dehiscence irregular; capillitium rigid and resilient; spores greater than 11 µm diam.....16

16. Peridium persistent, continues to encase spores and capillitium after lime crystals have all fallen away; capillitium rigid, abundant, freely anastomosing; spores by transmitted light paler and smoother on one side, 10-14 µm diam; known only from ground sites.....*D. dubium*

16. Peridium ephemeral; capillitium not as rigid, scanty, seldom anastomosing; spores uniform in color, lacking a smoother, paler area, 14-17 µm diam; known only from the bark of living trees.....*D. orthonemata*

Didymium anellus Morgan, Jour. Cinc. Soc. Nat. Hist. 16:148. 1894.

Didymium effusum Link var. *tenue* A. Lister, Jour. Bot. 35:214. 1897

ILLUSTRATIONS: Figs. 1-3.

Fructifications sporangiate or plasmodiocarpous, sessile, pulvinate or flattened, gray or glossy brown from abscence of lime, sporangia centrally depressed, 0.2-0.5 mm wide, plasmodiocarps depressed, slender links, branched, netted or perforated; peridium membranous, colorless or purplish brown, with sparse deposits of small lime crystals, sometimes limeless, dehiscing in a circumscissile manner; columella absent; capillitium abundant, consisting of slender, flexuose, violet brown, freely branching and anastomosing threads; spores dark brown in mass, purplish-gray or purplish-brown by transmitted light, heavily warted, (7-)8-11(-12) µm diam. Plasmodium colorless.

MATERIAL EXAMINED: 28 collections: R.K. Benjamin 20715, north of Urbana, Illinois (BPI); T.E. Brooks 623A, Riley Co., Kansas (IA); Eli Davis 1168, Ontario (IA); Dumont CO1452, Colombia (NY); H.C. Gilbert 236, Salem, Oregon (IA); J.C. Gilman, collection made March 1932, Ames, Iowa (IA); R. Hagelstein 1629, New York (IA); D.T. Kowalski 2209, Chico, California (IA); D.T. Kowalski 2582, Butte Co., California (BPI); D.T. Kowalski 3964, Butte Co., California (NY); D.T. Kowalski 4067, Butte Co., California (IA); E.C. Leonard 17276, Bladensburg, Maryland (IA); G. Lister, collection made on October 24, 1898, Wanstead Park, Essex, England (IA); T.H. Macbride, Iowa? (IA); A.P. Morgan, collection made in 1893 (Probably not type, but is authentic material), Preston, Ohio (IA); A.P. Morgan, collection made in 1894, Preston, Ohio (IA); N.E. Nannenga-Bremekamp 1205, Doorwerth, Netherlands (BPI); N.E. Nannenga-Bremekamp 4846, Netherlands (IA); O.A. Plunkett 176, Rock Creek, California (NY); O.A. Plunkett 301, Griffith Park, California (NY); Shimek and Seaver, Alamagardo, New Mexico (IA); W.C. Sturgis, collection made on September 1, 1906, Boulder, Colorado (BPI); W.C. Sturgis, collection

made in August of 1912, 6,000 feet elevation, Pike View near Colorado Springs, Colorado (IA); W.C. Sturgis, collection made on September 17, 1913, Pike View, Colorado (FH); W.H. Suksdorf 573, Bingen, Washington (IA); I. Tavares 2425a, Berkeley, California (UC); K.S. Thind 86, India (IA); A. Welden, collection made on February 11, 1958, Clarendon Parish, Jamaica (IA).

TYPE LOCALITY: Ohio.

HABITAT: Dead leaves, herbaceous stalks and twigs.

DISTRIBUTION: Bengal, Canada, Ceylon, Colombia, England, India, Jamaica, Netherlands, Philippines, California, Colorado, Illinois, Kansas, Maryland, New Mexico, New York, Ohio, Oregon, and Washington.

The spores and capillitium are notably variable and do not in themselves serve as ideal diagnostic characters. The pulvinate, centrally depressed sporangia which may be in rings or links, and the circumscissile manner of dehiscence are the important diagnostic features.

Didymium difforme (Pers.) S.F. Gray, Nat. Arr. Brit. Pl. 1:571. 1821.

Diderma difforme Pers., Tent. Disp. Fung. 9. 1797.

Licea caesia Schum., Enum. Pl. Saell. 2:219. 1803.

Amphisporium versicolor Link, Ges. Nat. Freunde Berlin Mag. 7:41. 1813.

Licea alba Nees, in Kunze & Schmidt, Myk. Hefte 2:66. 1823.

Lycogala minutum Grev., Scot. Crypt. Fl. pl. 40. 1823.

Reticularia pusilla Fries, Syst. Orbis. Veg. 147. 1825.

Physarum album (Nees) Fries, Syst. Myc. 3:147. 1829.

Physarum caesium (Schum.) Fries, Syst. Myc. 3:147. 1829.

Licea macrospora Schw., Trans. Am. Phil. Soc. II. 4:258. 1832.

Diderma neesii Corda, Ic. Fung. 2:23. 1838.

Diderma libertianum Fresen., Beitr. Mykol. 28. 1850.

Didymium libertianum (Fresen.) de Bary, Mycet. 124. 1864.

Chondrioderma difforme (Pers.) Rost., in Fuckel, Jahrb. Nass. Ver. Nat. 27-28:74. 1873.

Chondrioderma liceoides Rost., Mon. App. 17. 1876.

Diderma persoonii Macbr., N. Am. Slime-Moulds 96. 1899.

Didymium tubulatum Jahn, Ber. Deuts. Bot. Ges. 36:663. 1919.

ILLUSTRATIONS: Figs. 4-7.

Fructifications sporangiate or plasmodiocarpous, smooth, white, sporangia scattered, pulvinate on a broad base, plasmodiocarps 0.3-1.0 mm broad and 0.4-2.0 mm or more in length; peridium of two layers, the outer a thin, eggshell-like crust composed of densely aggregated lime crystals which separates from the iridescent, membranous inner layer; columella lacking, or represented by a purplish membrane with scanty deposits of small lime crystals; capillitium rather scanty, sometimes abundant, of brown or nearly colorless, dichotomously branching threads which are stout below, slender above; spores black in mass, brownish purple by transmitted light, with spines connected by a reticulum, sometimes with a pale swollen area, (7-)10.5-14(-15) µm diam. Plasmodium colorless or yellow.

MATERIAL EXAMINED: 86 collections: Beardslee, collected in 1898, Michigan (IA); E. Bethel, collected in August of 1906, Golden, Colorado (NY); E. Bethel, collection made on September 10, 1906, Denver, Colorado (IA); E. Bethel, collected in September of 1923, Fort Collins, Colorado (NY 13088); G.R. Bisby 3158, Manitoba, Winnipeg (IA); G.R. Bisby 4955, Manitoba, Winnipeg (IA); M. Brandza 40, Roumanie (NY 10070); T.E. Brooks 321, Geary Co., Kansas (IA); T.E. Brooks 609, Kansas (NY 9372); S. Buchet, collected on January 1, 1912, France (IA); E. Davis 759, Komoko, Ontario (NY 8493); Dumont VE3114, Venezuela (NY); Dumont VE3116, Venezuela (NY); Dumont VE3569, Venezuela (NY); Dumont VE3616, Venezuela (NY); Dumont VE5342, Venezuela (NY); Dumont VE5402, Venezuela (NY); V. Duran 823, Berkeley, California (NY); W.T. Elliot, collected in December of 1923, Surrey, England (NY 7183); W.T. Elliot,

collected in April of 1924, Yorkshire, England (NY 7184); W.T. Elliot, collected in September of 1924, N. Wales, England (IA); W.T. Elliot, collection made on January 2, 1925, Warwickshire, Wood End, England (NY); P.S. Enans 10, New Zealand (IA); Fautray, collection made in 1897, France (IA); Gilbert 617, Salem Oregon (IA); Gilbert 778, Salem, Oregon (IA); R. Hagelstein, collection made on September 13, 1925, Plainview, Long Is., New York (NY); R. Hagelstein 763, Mill Neck, Long Island, New York (NY); R. Hagelstein 782, Malverne, Long Island, New York (NY); R. Hagelstein 798, Plainview, Long Island, New York (NY); R. Hagelstein 799, Plainview, Long Island, New York (NY); R. Hagelstein 800, Plainview, Long Island, New York (NY); R. Hagelstein 867, Albertson, Long Island, New York (NY); R. Hagelstein 936, Mill Neck, Long Island, New York (NY); R. Hagelstein 1188 (NY); R. Hagelstein 1628 (NY); R. Hagelstein 1885, Albertson, Long Island, New York (NY); R. Hagelstein 2133, Plainview, Long Island, New York (NY); R. Hagelstein 2134, Plainview, Long Island, New York (NY); R. Hagelstein 2601 (NY); R. Hagelstein 2809, Lords Valley, Pike Co., Pennsylvania (NY); R. Hagelstein 3667, New York (NY); O. Jaap, collected on March 18, 1907 (NY 10167); O. Jaap 11, Germany (IA); O. Jaap 29 (NY 10185); O. Jaap 69 (NY10225); O. Jaap 108 (NY 10264); A. and G. Lister, England (IA); Karsten, Finland (IA); H.W. Keller, collection made on July 4, 1966, Atlanta, Georgia (HWK); H.W. Keller, collection made on September 9, 1969, Michigan (HWK); H.S. Khara 1315, India (BPI); Korf MJ719, Jamaica (NY); D.T. Kowalski 2344, Butte Co., California (NY); D.T. Kowalski 4090 (NY); D.T. Kowalski 4120, Butte Co., California (BPI); D.T. Kowalski 4878, Van Damme Beach St. Pk., Mendocino Co., California (HWK); D.T. Kowalski 4881, Van Damme Beach St. Pk., Mendocino Co., California (HWK); D.T. Kowalski 5406, Sutter Co., California (IA); Lakhanpal 1077, India (IA); A. Lister, collected in 1898, Lyme Regis, England (KSC); Lister 100 NY 13090); Lister 101 (NY 13089); T. Macbride, Iowa? (IA); T. Macbride, Iowa City, Iowa (IA); G.W. Martin,

collected on June 22, 1932, Milford, Iowa (IA); G.W. Martin,collected on April 10, 1971, Coralville, Iowa (IA); G.W. Martin 3553 (IA); G. Massee, Scarboro, England (NY 6634); Ch. Meylan, collected in 1919 (NY 8546); N.E. Nannenga-Bremekamp 1671, Netherlands (IA); N.E. Nannenga-Bremekamp 2352, Netherlands (IA); H.E. Parks 2597, Alameda Co., California (UC); A.P. Morgan, Preston, Ohio (IA); Rex, Pennsylvania (IA); H. Ronn, Hamburg, Germany (NY 7835); H. Ronn, Hannover, Germany (NY 7846); L.E. de Schweinitz, France (IA); C.L. Shear 4509, Duchesnay, Quebec (NY); E.C. Smith, Fort Collins, Colorado (NY); W.C. Sturgis 110 (NY 13092); Sutton 336, Komoko, Ontario (NY 9201); H. and P. Sydow 1799, Germany (IA); I. Tavares 2416 (UC); K.L. Teng 2498, Nanking, Kiangsu, China (BPI); C. Torrend, collected in April of 1908, Portugal (IA); C. Torrend 147 (NY 10339); Wingate, Fairmount Park, Philadelphia, Pennsylvania (NY).

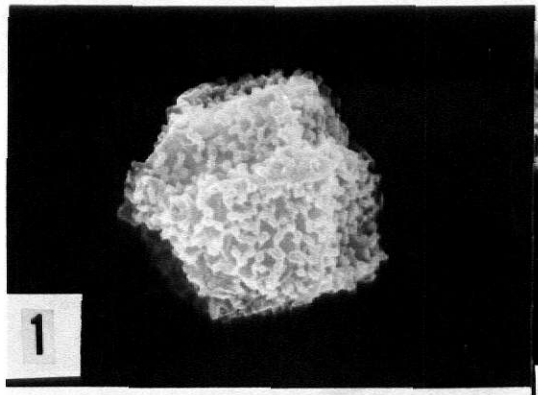
TYPE LOCALITY: Europe.

HABITAT: Dead leaves, herbaceous stalks, and dung.

DISTRIBUTION: Widely distributed in temperate regions of the world.

Several varieties have been reported for this species. Nannenga-Bremekamp raised one variety, *D. difforme* var. *comatum* A. Lister, to the rank of species emphasizing the abundant, fluffy capillitium, and the spores which are brown rather than black in mass. We have examined a specimen collected by Nannenga-Bremekamp, #2352, labeled *Didymium comatum* (A. Lister) Nann.-Brem. Fig. 5 shows a SEM micrograph of this collection which can be compared to Fig. 4 from a collection by D.T. Kosalski of *D. difforme*. Spines which are joined by a reticulum can be seen in either specimen. The spines do appear larger in Fig. 5 than in Fig. 4. After comparing many collections using light microscopy it seems that the color of the spores and the capillitial characteristics of *D. comatum* integrade with those of other collections of *D. difforme*. However, as can be seen in Figs. 4 and 5

Figs. 1-7. 1. Spore of *Didymium anellus*, SEM X4500. 2. Sporangia and plasmodiocarps of *Didymium anellus* on a decaying twig, X10. 3. Spores of *Didymium anellus*, X1400. 4. Spore of *Didymium difforme*, SEM X4500. 5. Spore of *Didymium difforme* (this collection identified as *Didymium comatum* by Nannenga-Bremekamp), SEM X4500. 6. Sporangia and plasmodiocarps of *Didymium difforme* on a decaying leaf, X10. 7. Spores of *Didymium difforme*, X1400.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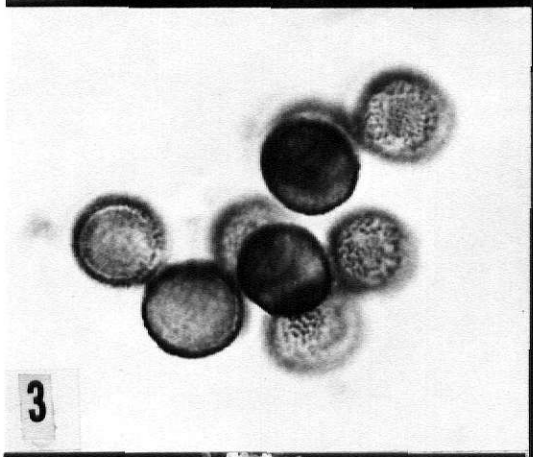
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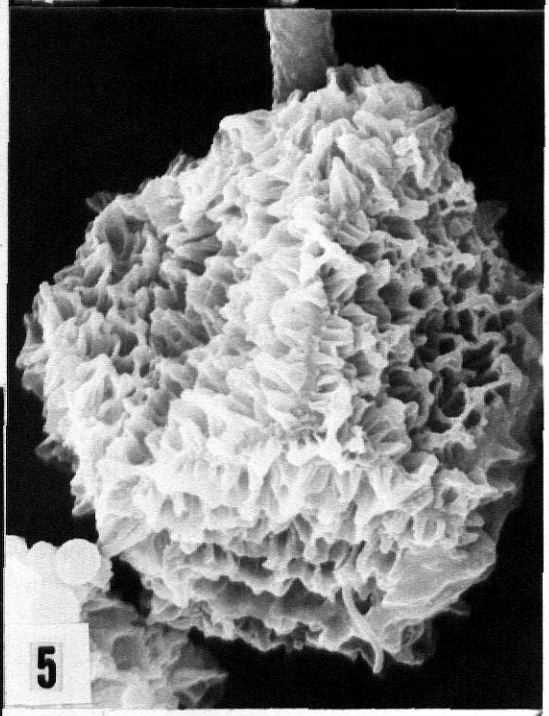
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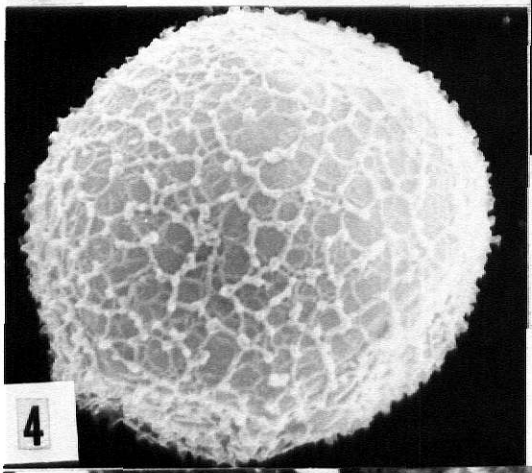
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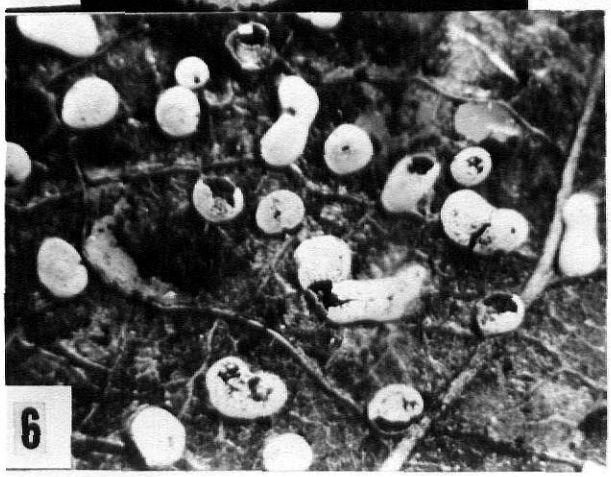
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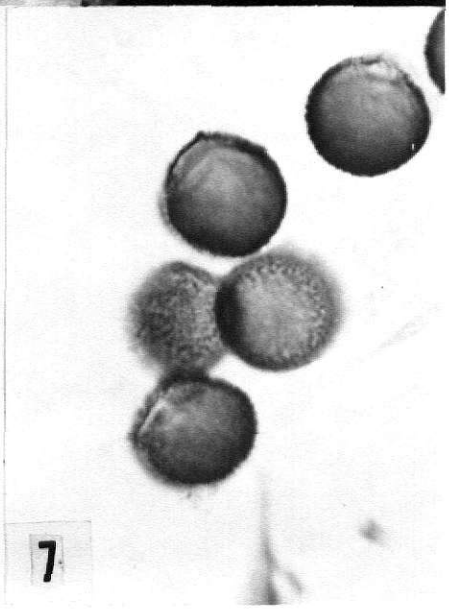
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the spores look quite different when observed using SEM. A more extensive study of many collections of *D. difforme* using SEM would be helpful, but few collections of *D. comatum* exist. It seems best to include *D. comatum* as a synonym of *D. difforme* unless further collections prove otherwise.

Didymium dubium Rost., Mon. 152. 1874.

Didymium wilczekii Meylan, Bull. Soc. Vaud. Sci. Nat. 44:290. 1908.

ILLUSTRATIONS: Figs. 8-10.

Fructifications plasmodiocarpous, pulvinate, elongate, curved, and net like, 1-2 mm wide, 1-30 mm long, 0.3-0.5 mm thick, white to grayish white; peridium membranous, stout, colorless, with some yellow or pale purplish regions, embedded with stellate, rod-shaped, or nodular crystals of lime, sometimes dark when lime not present; columella represented by a thickened base; capillitium abundant, threads pale, brownish purple, more or less connected by transverse bars, and combined to form an elastic network, readily separating from the peridium; spores black in mass, dark purple-brown by transmitted light with a thick layer of spines, (8-)9.5-14(-17) µm diam. Plasmodium gray.

MATERIAL EXAMINED: 35 collections: M. Allen A-662, New Jersey (IA); R.M. Allen C-1640, New Jersey (IA); R.M. Allen H-454, New Jersey (IA); E. Bethel, San Diego, California (IA); E. Bethel, collection made on June 27, 1898, Colorado (IA); T.E. Brooks 589, Riley Co., Kansas (IA); T.E. Brooks 618, Riley Co., Kansas (IA); M.C. Cooke 1597, England (UC 267531); W.B. Cooke 8548A, California (IA); W.B. Cooke 8572, California (IA); W.B. Cooke 8686, California (UC 938724); H.C. Gilbert 642, Oregon (IA); H.C. Gilbert 1054, Oregon (IA); K.A. Hok 38, California (IA); O. Jaap 110, Switzerland (IA); O. Jaap 196, Switzerland (IA); H.S. Jackson 688, Canada (IA); D.T. Kowalski 2574, California (IA); D.T. Kowalski 2654, California (IA); D.T. Kowalski

3959, California (BPI); D.T. Kowalski 4142 (HWK); D.T. Kowalski, California (IA); D.T. Kowalski 4714, Glenn Co., California (NY); D.T. Kowalski 5353, California (HWK); D.T. Kowalski, Hurricane Ridge, Olympic National Park, California (HWK); D.T. Kowalski 9546, Olympic National Park, California (HWK); T.H. Macbride, Carmel, California (IA); K.H. McKnight 12156A, Utah (BPI); K.H. McKnight 12161, Utah (BPI); Ch. Meylan, Jura, Switzerland (BPI 1007); N.E. Nannenga-Bremekamp 2517, Netherlands (BPI); L. Robenhorst 4100, Bavaria (IA); T.E. Savage, collection made on December 4, 1897, Missouri (IA); Whitney, collection made on June 3, 1913, Alaska (IA).

TYPE LOCALITY: Hauenstein, Bohemia.

HABITAT: Dead herbaceous stalks, woody twigs, and decaying leaves.

DISTRIBUTION: Bavaria, Canada, England, Netherlands, Switzerland, Alaska, California, Colorado, Kansas, Missouri, New Jersey, Oregon, and Utah.

The diagnostic features of this species are the flat plasmodiocarps which appear gray due to the dark spores showing through the sparse covering of lime.

Didymium flexuosum Yamashiro, Jour. Sci. Hiroshima Univ. Ser. B, 2. 3:31. 1936.

Didymium parietale Martin and Brooks, Trans. Am. Micr. Soc. 57:320. 1938.

ILLUSTRATIONS: Figs. 11-13.

Fructifications sporangiate or plasmodiocarpous, sessile, white or cinereous, laterally compressed, plasmodiocarps occasionally simple, usually branched or netted, 0.2-0.4 mm wide, and up to 12 mm or more across, borne on a broadly expanded, but colorless and inconspicuous hypothallus; peridium membranous with sparsely distributed deposits of lime in the form of stellate or angular crystals, rarely limeless; columella conspicuous, a thin ridge or plate extending along the plasmodiocarp from the base upward to nearly

the top; capillitium a dense network of slender threads, brownish below, hyaline and more slender at the tips, interspersed with numerous vesicular bodies ranging from 20-24 µm diam; spores black in mass, brown by transmitted light, marked with coarse, scattered spines, (10-)10.5-13.5(-14) µm diam. Plasmodium thought to be white.

MATERIAL EXAMINED: 8 collections: T.E. Brooks 304, Riley Co., Kansas (BPI); T.E. Brooks 366, Geary Co., Kansas (IA); T.E. Brooks 427, Geary, Co., Kansas (NY); T.E. Brooks 453, Geary Co., Kansas (NY); T.E. Brooks 454, Geary Co., Kansas (NY); T.E. Brooks 561, Geary Co., Kansas (NY); T.E. Brooks 619, Geary Co., Kansas (NY); T.E. Brooks 620, Riley Co., Kansas (NY).

TYPE LOCALITY: Kyushu, Japan.

HABITAT: On dead leaves.

DISTRIBUTION: Japan, Iowa, and Kansas.

The thin, plate-like columella which extends down the longitudinal axis of the plasmodiocarp and the vesicular bodies mixed with the spores are the diagnostic characteristics of this species.

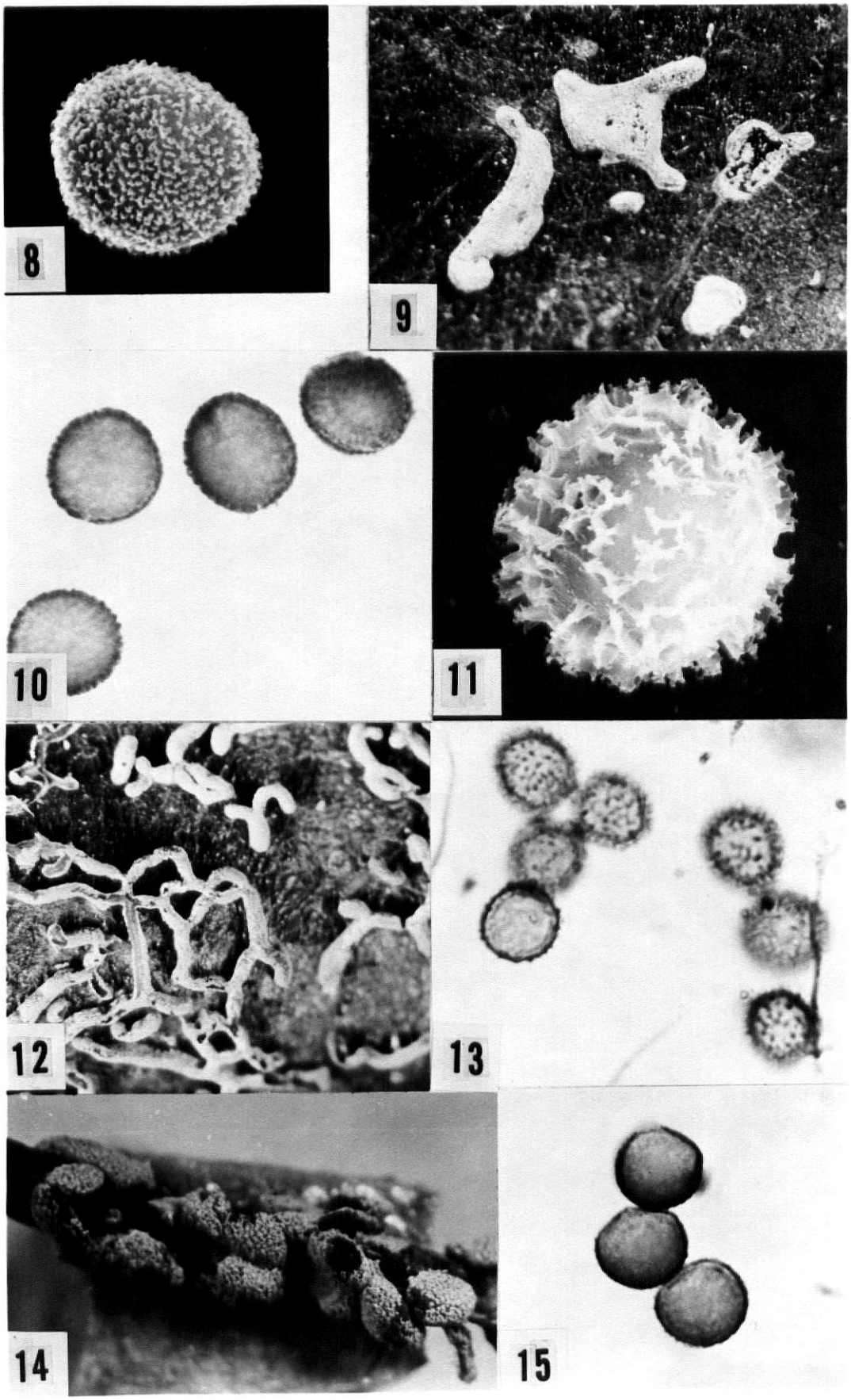
Didymium serpula Fries is closely related to *D. flexuosum* having similar vesicular bodies interspersed with capillitium and spores, but their vesicular bodies are yellow, larger, and connected with the capillitium. Also the columella is lacking and the spores are smaller and paler.

Didymium fulvum Sturgis, Mycologia 9:327. 1917.

ILLUSTRATIONS: Figs. 14-15.

Fructifications sporangiate or plasmodiocarpous, sessile, pulvinate, yellowish brown to tawny, sporangia subglobose, concave beneath, 0.5-0.8 mm diam, plasmodiocarps with a tawny hypothallus which sometimes appears stalk-like with crystalline deposits; peridium membranous with a thick covering of pale yellow, sharp-pointed crystals; columella a convex, thickened base enclosing lime crystals; capillitium an abundant network of delicate threads

Figs. 8-15. 8. Spore of *Didymium dubium*, SEM X4500. 9. Plasmodiocarps of *Didymium dubium* on a decaying leaf, X10. 10. Spores of *Didymium dubium*, X1400. 11. Spore of *Didymium flexuosum*, SEM X4500. 12. Plasmodiocarps of *Didymium flexuosum* on a decaying leaf, X10. 13. Spores of *Didymium flexuosum*, X1400. 14. Sporangia and plasmodiocarps of *Didymium fulvum* on a decaying twig, X10. 15. Spores of *Didymium fulvum*, X1400.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which are pale, purple-brown, becoming almost colorless at tips; spores dark, purplish brown by transmitted light, coarsely tuberculate, paler and smoother on one side, 12-14 µm diam. Plasmodium unknown.

MATERIAL EXAMINED: 1 collection: W.C. Sturgis (TYPE), Colorado (IA).

TYPE LOCALITY: Colorado.

HABITAT: on dead leaves and twigs.

DISTRIBUTION: Colorado and West Pakistan.

For many years this species was only known from Colorado. However, it has recently been found in West Pakistan and will probably be found in other montane regions where it has previously been overlooked.

Didymium listeri Massee, Mon. 244. 1892.

ILLUSTRATIONS: Figs. 16-18.

Fructifications sporangiate or plasmodiocarpous, sessile, pulvinate, white, plasmodiocarps broad and flattened, 2-12 mm or more in length, 0.3-0.5 mm thick; peridium double, outer layer an eggshell-like crust, composed of large stellate crystals of lime tightly compacted, the inner layer delicate and membranous; columella none or possibly represented by a thickened base; capillitium profuse, of rigid, dark, purplish-brown, or pale threads, anastomosing, connected by transverse bars, and attached by slender, colorless branches to the peridium, elasticity not evident; spores blackish-brown in mass, violet-gray to brown by transmitted light, minutely spinulose, 9-12 (-13) µm diam. Plasmodium watery white.

MATERIAL EXAMINED: 12 collections: T.E. Brooks 589, Riley Co., Kansas (NY 9377); T.E. Brooks 590, Geary Co., Kansas (KSC); T.E. Brooks 618, Riley Co., Kansas (NY 9373); A. Lister 3, England (IA); A. Lister 5, England (IA); A. Lister 14, England (IA); G. Lister, collection made on March 17, 1898, Lyme Regis, Dorset, England (NY 10309); T.H. Macbride, California

(IA); T.H. Macbride, Iowa? (IA); T.H. Macbride, collection made in 1892, Iowa (IA); G.A. Rex, England (IA); W.C. Sturgis 217, England (NY 13083).

TYPE LOCALITY: Lyme Regis, England.

HABITAT: On dead leaves and stems.

DISTRIBUTION: Bermuda, England, India, West Pakistan, California, Iowa, and Kansas.

This species, which is similar to *D. dubium*, is distinguished by its lack of capillitial elasticity, the prescence of a firm peridium composed of colsely aggregated crystals, and smaller, paler, and less warty spores.

Didymium nivicolum Meyl., Vull. Soc. Vaud. Sci. Nat. 57:40. 1929

Didymium wilczekii Meyl. forma *pulverulenta* Meyl., Bull. Soc. Vaud. Sci. Nat. 53:454. 1921.

ILLUSTRATIONS: Figs. 19-21.

Fructifications sporangiate or plasmodiocarpous, pulvinate, subglobose, white, sporangiate forms 1-2 mm diam, plasmodiocarps reaching 4 cm in length; peridium delicate, membranous, colorless or somewhat darkened, covered with scales of stellate lime crystals; columella absent or sometimes represented by a thickened base, white to ochraceous, containing stellate lime crystals; capillitium abundant, composed of purple-brown to pale, sparsely branched threads; spores black in mass, purple-brown by transmitted light, thickly spinulose with some reticulation visible, 12-18 µm diam. Plasmodium unknown.

MATERIAL EXAMINED: 2 collections: K.H. McKnight F75, Utah (HWK); Ch. Meylan, collection made in 1929, Jura, Switzerland (NY 7137).

TYPE LOCALITY: Salanfe, Canton Valais, Switzerland, 2000 m.

HABITAT: On decaying plant debris at high elevations near melting snow.

DISTRIBUTION: Switzerland, California, Nevada, Utah, and Washington.

This species was recently studied by Kowalski (1975) in his treatment

of the taxa described by Meylan. The present study supports his concept in which *D. nivicolum* has been separated from *D. dubium* on the basis of its pure white color, peridial crystals which are aggregated into scales, non-elastic capillitium, and strongly warted and larger spores in which some reticulation is apparent.

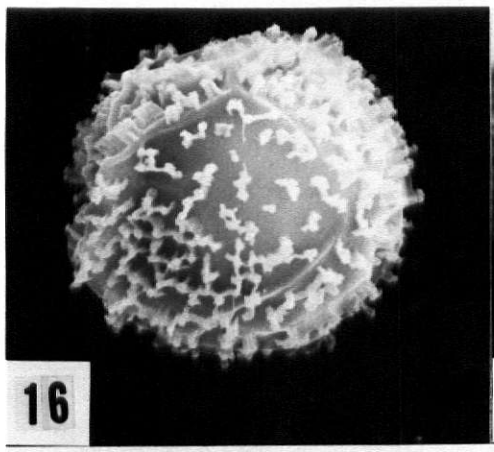
Didymium ochroideum G. Lister, Jour. Bot. 69:297. 1931.

ILLUSTRATIONS: Figs. 22-24.

Fructifications sporangiate or plasmodiocarpous, sessile, pulvinate, pale brown or ochraceous, plasmodiocarps slender, simple or branched; peridium membranous, pale to dull orange-brown, closely covered with small, pale yellow stellate crystals; hypothallus yellow-red, somewhat thickened, lime scanty or lacking; columella lacking or represented by thickened base containing lime; capillitium a loose network of slender, pale purplish, branching and anastomosing threads; spores dark brown in mass, purplish gray or purplish brown by transmitted light, globose, minutely spinulose, 7-8 µm diam. Plasmodium white.

MATERIAL EXAMINED: 20 collections: T.E. Brooks 600, Riley Co., Kansas (NY 9378); T.E. Brooks 601, Geary Co., Kansas (HWK); T.E. Brooks 602, Geary Co., Kansas (NY); E. Davis 1164, Ontario (NY); E. Davis 1450, Ontario (NY); R. Hagelstein 986, Albertson, Long Island, New York (NY); R. Hagelstein 1009, New York (NY); R. Hagelstein 1031, New York (NY); R. Hagelstein 1227, New York (NY); R. Hagelstein 1565, New York (NY); R. Hagelstein 4754, Pennsylvania (NY); T.H. Macbride, Iowa (NY 7148); G.W. Martin, collection made on June 29, 1932, Esterville, Iowa (IA); G.W. Martin, collection made in May of 1936, Iowa City, Iowa (IA); G.W. Martin, collection made in April of 1942, Iowa City, Iowa (IA); G.W. Martin, collection made on February 20, 1946, Iowa City, Iowa (IA); G.W. Martin, collection made in March of

Figs. 16-23. 16. Spore of *Didymium listeri*, SEM X4500. 17. Plasmodiocarps of *Didymium listeri* on a decaying leaf, X10. 18. Spores of *Didymium listeri*, X1400. 19. Spores of *Didymium nivicolum*, SEM X4500. 20. Sporangia and plasmodiocarps of *Didymium nivicolum* on a decaying twig, X10. 21. Spores of *Didymium nivicolum*, X1400. 22. Spore of *Didymium ochroideum*, SEM X4500. 23. Sporangia and plasmodiocarps of *Didymium ochroideum* on decaying wood, X10.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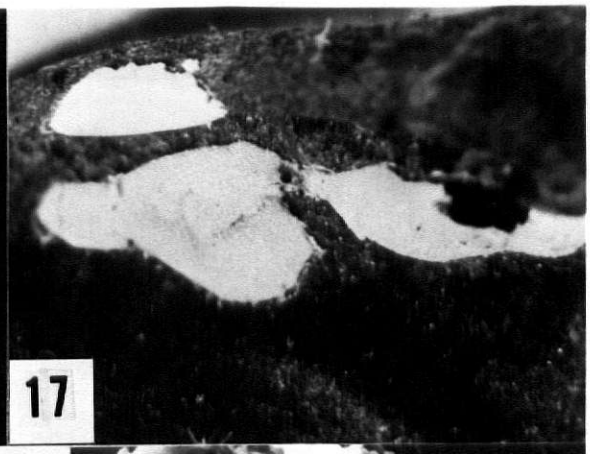
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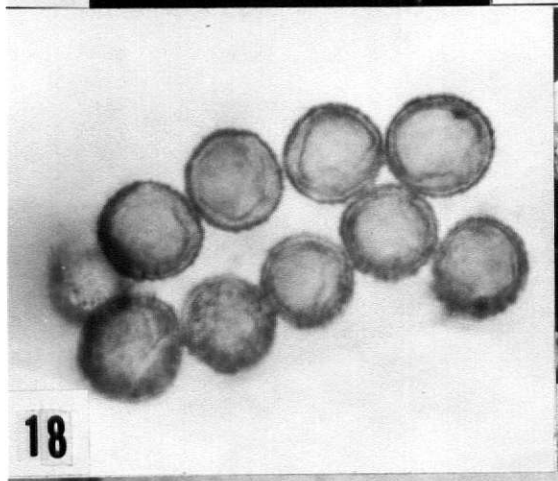
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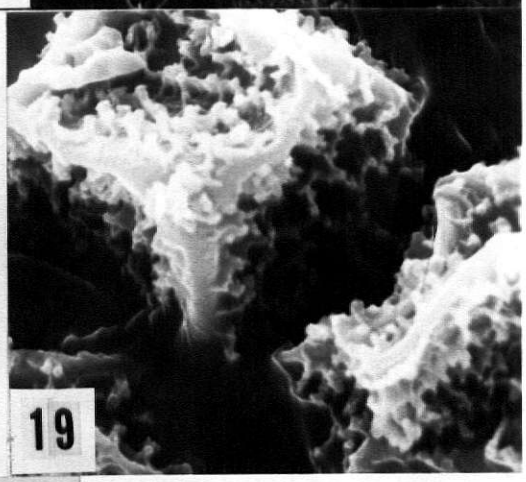
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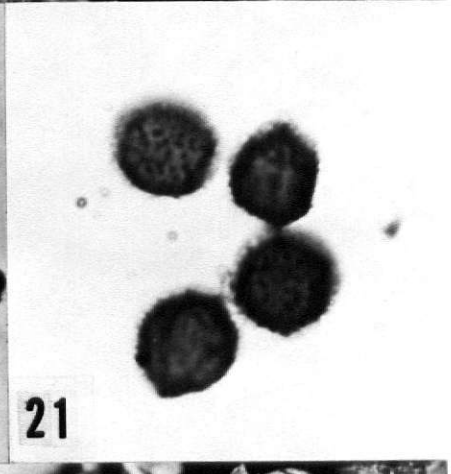
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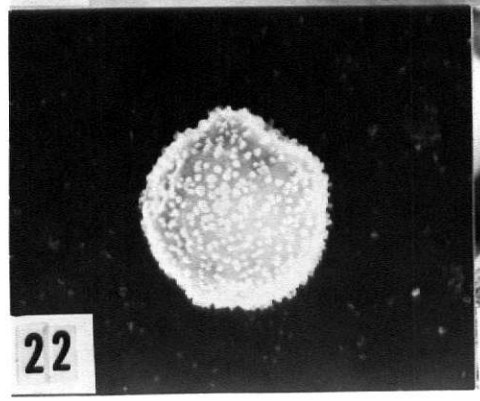
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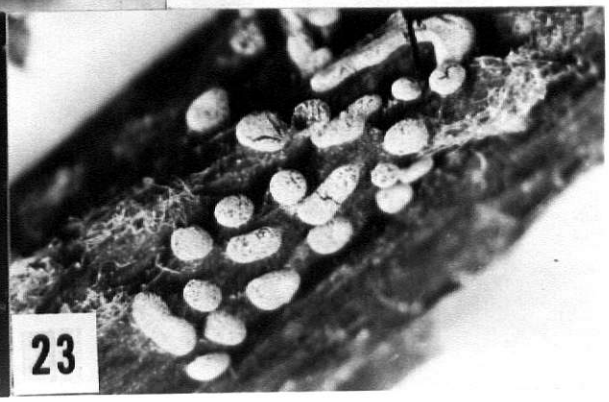
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1946, Iowa City, Iowa (IA); G.W. Martin, collection made in November of 1947, Iowa City, Iowa (IA); G.W. Martin #2, collection made in November of 1957, Iowa City, Iowa (IA); G.W. Martin #3, collection made in November of 1957, Iowa City, Iowa (IA).

TYPE LOCALITY: Tokyo, Japan.

HABITAT: On dead leaves, herbaceous stems, mosses, and dung of herbivouous animals.

DISTRIBUTION: Canada, Japan, India, Iowa, Kansas, New York, and Pennsylvania.

This rarely collected, inconspicuous species is distinguished by the yellow lime crystals and the minutely spinulose spores.

Didymium orthonemata Keller & Brooks, Mycologia 65:290. 1973.

ILLUSTRATIONS: Figs. 25-27.

Fructifications plasmodiocarpous or less frequently sporangiate, sessile, pulvinate, white to gray or darkened when lime is scanty, plasmodiocarps up to 5 mm across, 2 cm in length, and about 0.08-0.2 mm thick; peridium membranous, delicate, either thickly encrusted or sparsely sprinkled with lime; columella lacking or represented by a thickened base which appears limeless; capillitium scanty, hyaline at extremities to dark purple-brown at mid-portions, parallel threads often connected with a short cross thread to form a "H-shaped" structure; spores dark brown in mass, dark violet-brown by transmitted light, uniformly spinulose, 14-17(-18) µm diam. Phaneroplasmodium whitish to sordid brown.

MATERIAL EXAMINED: 1 collection: H.W. Keller 838 (TYPE), Bradford Co., Florida (HWK).

TYPE LOCALITY: Crosby Cemetary, two miles west of Starke along state road #100, Bradford Co., Florida.

HABITAT: Bark of living *Juniperus silicicola* (Small) Bailey.

DISTRIBUTION: Known only from Florida.

This species is most closely related to *D. dubium*. The spores of *D. orthonemata* are larger, though they overlap with the size range of *D. dubium*. *D. dubium* has never been found on the bark of living trees in the areas where it is commonly collected. In contrast, *D. orthonemata* occurs only on living bark. The plasmodiocarps of *D. orthonemata* usually appear much smaller than those of *D. dubium*.

Didymium perforatum Yamashiro, Jour. Sci. Hiroshima Univ. B2. 3. 1936.

Didymium labyrinthiforme Martin, Lodhi, & Khan, Sydowia 14:283. 1961.

ILLUSTRATIONS: Figs. 28-30.

Fructifications plasmodiocarpous, ash-gray, closely reticulate, individual veins about 0.2 mm in width, entire fructification up to 2 cm across; peridium membranous, delicate, iridescent, consisting of a closely compacted layer of pale yellow lime crystals; columella lacking; capillitium of dark, netted, thick threads with many nodose swellings; spores dark brown in mass, violaceous-brown by transmitted light, covered with large spines, (10.5-) 11-14 µm diam. Plasmodium unknown.

MATERIAL EXAMINED: 7 collections: T.E. Brooks 646, Riley Co., Kansas (IA); T.E. Brooks 675, Riley Co., Kansas (IA); T.E. Brooks, Riley Co., Kansas (IA); T.E. Brooks 3175, Kansas (IA); T.E. Brooks 3178, Douglas Co., Kansas (IA); T.E. Brooks 3183, Douglas Co., Kansas (IA); F.L. Lyon 5, Hackberry Glen, Riley Co., Kansas (KSC).

TYPE LOCALITY: Kyushu, Japan.

HABITAT: Dead leaves.

DISTRIBUTION: Japan, West Pakistan, and Kansas.

Although the plasmodiocarps of this species somewhat resemble those of *D. flexuosum*, those of *D. perforatum* lack a columella and vesicular bodies. In addition, the netted capillitium with swollen points of intersection is diagnostic of this species.

Specimens of *D. perforatum* have been collected in this country only from Kansas. Dr. T.E. Brooks made several collections in years past and it has just recently been collected again in Kansas.

Didymium quitense (Pat.) Torrend, Broteria 7:90. 1908.

Chondrioderma quitense Pat., in Pat. and Lagerh., Bull. Soc. Myc. Fr. 11:212. 1895.

ILLUSTRATIONS: Figs. 31-33.

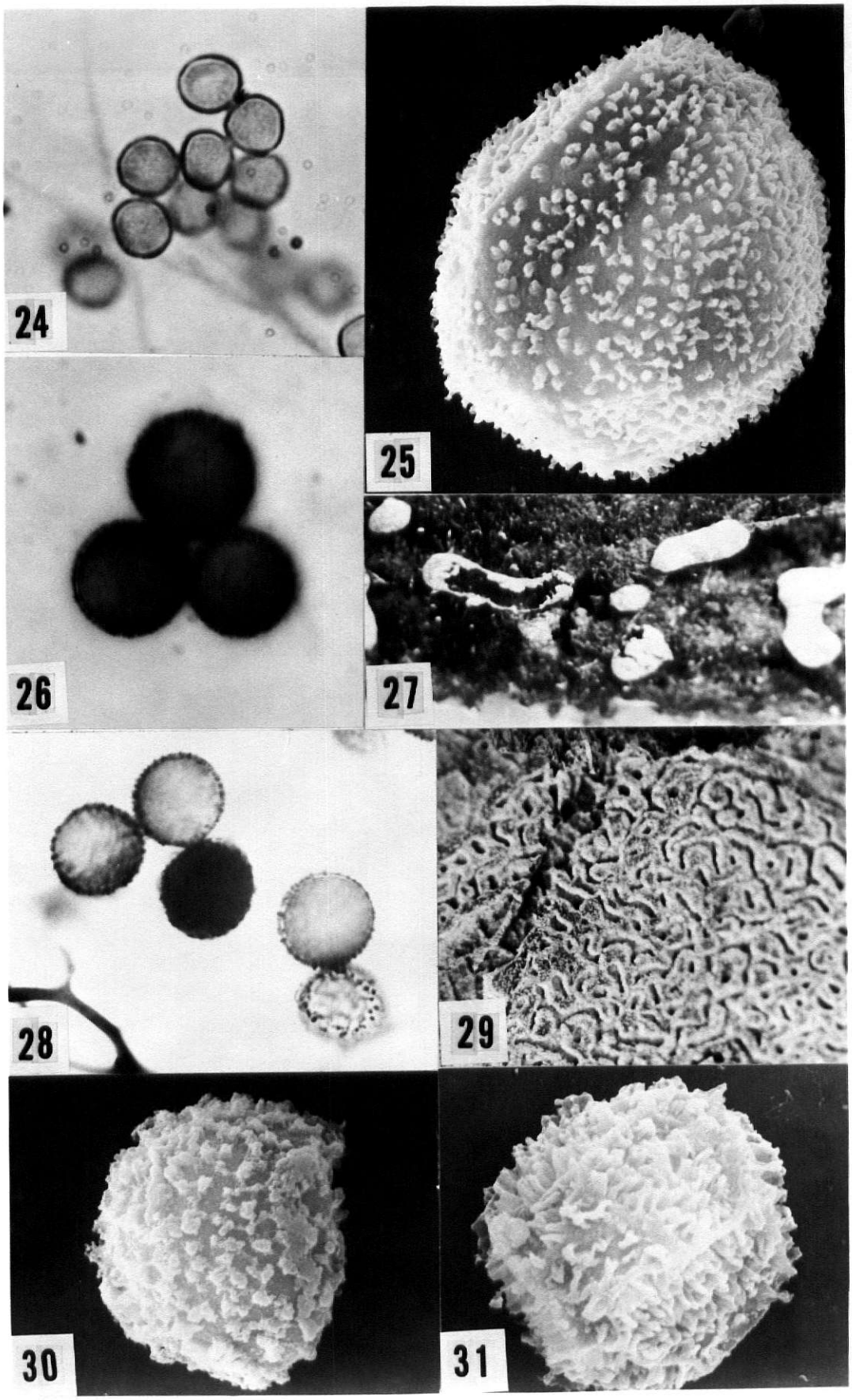
Fructifications sporangiate or plasmodiocarpous, sessile, pulvinate, depressed, smooth, white, sporangia 0.4-1 mm diam; peridium double, outer layer of minute lime crystals combined to form a white, eggshell-like crust which separates from the membranous, pale purplish, iridescent inner layer; capillitium a sparse network of yellow-brown, stout, straight or flexuose threads, equal in thickness throughout; spores black in mass, dark brownish-purple by transmitted light, strongly warted with some reticulation, (11.5-) 12.5-16(-17.5) µm diam. Plasmodium unknown.

MATERIAL EXAMINED: 10 collections: W.B. Cooke 8545A, California (IA); D.T. Kowalski 1235, Chico, California (IA); D.T. Kowalski 2215, Chico, California (IA); D.T. Kowalski 2307, Chico, California (IA); T. Macbride, Colorado (IA); T. Macbride, Carmel, California (IA); W.C. Sturgis, Colorado (NY 13085); W.C. Sturgis, Colorado (NY 13086); W.C. Sturgis 17, Colorado Springs, Colorado (IA); W.C. Sturgis 810, Colorado (NY 13084).

TYPE LOCALITY: Ecuador.

HABITAT: Herbaceous stems and leaves and dead wood.

Figs. 24-31. 24. Spores of *Didymium ochroideum*, X1400. 25. Spore of *Didymium orthonemata*, SEM X4500. 26. Spores of *Didymium orthonemata*, X1400. 27. Sporangia and plasmodiocarps of *Didymium orthonemata* on the bark of *Juniperus silicicola*, X10. 28. Spores of *Didymium perforatum*, X1400. 29. Plasmodiocarp of *Didymium perforatum* on a decaying leaf, X10. 30. Spore of *Didymium perforatum*, SEM X4500. 31. Spore of *Didymium quitense*, SEM X4500.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DISTRIBUTION: Canary Islands, Ecuador, California, Colorado, Montana, and Oregon.

The eggshell-like crust of this species resembles *D. difforme*, but may be distinguished from that species by its more reticulate spores, less crustose outer peridium and a more netted system of capillitium.

Didymium rugulosporum Kosalski, Mycologia 61:636. 1969.

ILLUSTRATIONS: Figs. 34-36.

Fructifications sporangiate, sessile on a wide base, pulvinate, 1-3 mm diam, white, scattered; peridium double, the outer layer smooth and thickened, with an eggshell-like crust which separates from the membranous, delicate, colorless or sometimes iridescent inner layer; columella absent; capillitium dense, readily separating from the peridium, of rather stout, brown threads with colorless points of attachment; spores purple-brown to black in mass, uniformly dark, purple-brown by transmitted light, (16-)18-20(-22) µm diam, warts present and sometimes joined together by a widely meshed reticulum of raised ridges. Plasmodium unknown.

MATERIAL EXAMINED: 1 collection: D.T. Kowalski 5390 (TYPE), Sutter Co., California (IA).

TYPE LOCALITY: Sutter Buttes, 700 ft. elevation, Sutter Co., California, February 18, 1967.

HABITAT: Found only on cow dung.

DISTRIBUTION: Known only from type locality.

The spore and capillitial characteristics of this species make it distinct from all other eggshell-like species of *Didymium*. Its spores are by far the largest when compared to all other species of the genus.

Didymium saturnus Keller, Mycologia 62:1061. 1970.

ILLUSTRATIONS: Figs. 37-39.

Fructifications sporangiate or occasionally plasmodiocarpous, gregarious to scattered, sessile, dull, straw yellow to silvery gray or nut brown; peridium membranous, delicate, and of a single layer with crystals of irregular shapes; columella dull white to yellow, spherical or columnar to rod-like in the plasmodiocarps, sometimes lacking; capillitium abundant, remaining attached to the columella, threads hyaline to pale violet, freely branching and anastomosing; spores black in mass, dark brown by transmitted light, saturnine with a dense covering of applanate spines, 10.5-13(-14) µm diam. Phaneroplasmodium watery white.

MATERIAL EXAMINED: 4 collections: Blackwell, collection made on August 4, 1965, Austin, Texas (IA); H.W. Keller 132 (TYPE), Iowa (HWK); H.W. Keller 150, Stafford Co., Kansas (IA); H.W. Keller 160, Stafford Co., Kansas (IA).

TYPE LOCALITY: Iowa City, Iowa, July 21, 1969.

HABITAT: From oat and wheat straw stacks.

DISTRIBUTION: Iowa, Kansas, and Texas.

This species belongs to the eggshell-like group of the genus *Didymium*. Its unique spore with prominent equatorial ring readily separate it from other spicies of *Didymium*.

Didymium serpula Fries, Syst. Myc. 3:126. 1829.

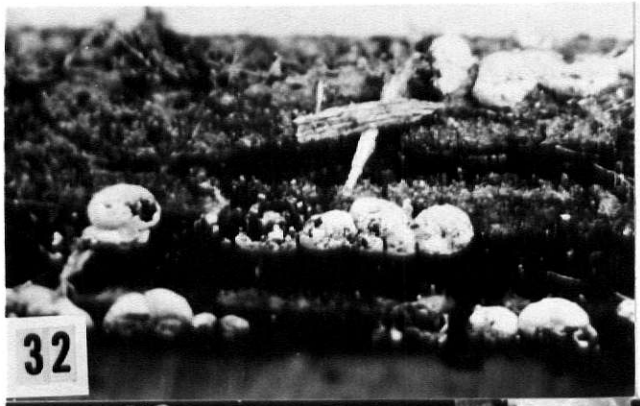
Lycoperdon complanatum Batsch, Elench. Fung. Contin. 1:251. 1786.

Didymium complanatum (Batsch) Rost., Mon. 151. 1874. Not *Didymium complanatum* Schrad., 1797.

ILLUSTRATIONS: Figs. 40-42.

Fructifications plasmodiocarpous, scattered or solitary, depressed, 2-8 mm broad, 0.1-0.15 mm thick, effused, perforated or nearly continuous, dark gray, whitish, or iridescent; peridium membranous, dark gray or iri-

Figs. 32-39. 32. Sporangia and plasmodiocarps of *Didymium quitense* on decaying wood, X10. 33. Spores of *Didymium quitense*, X1400. 34. Spores of *Didymium rugulosporum*, SEM X4500. 35. Sporangia of *Didymium rugulosporum* on dung, X10. 36. Spores of *Didymium rugulosporum*, X1400. 37. Spore of *Didymium saturnus*, SEM X4500. 38. Sporangia and plasmodiocarps of *Didymium saturnus* on decaying oat straw, X10. 39. Spores of *Didymium saturnus*, X1400.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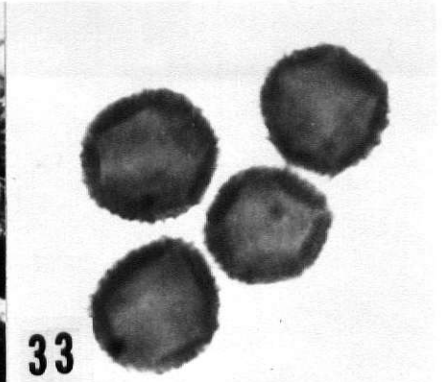
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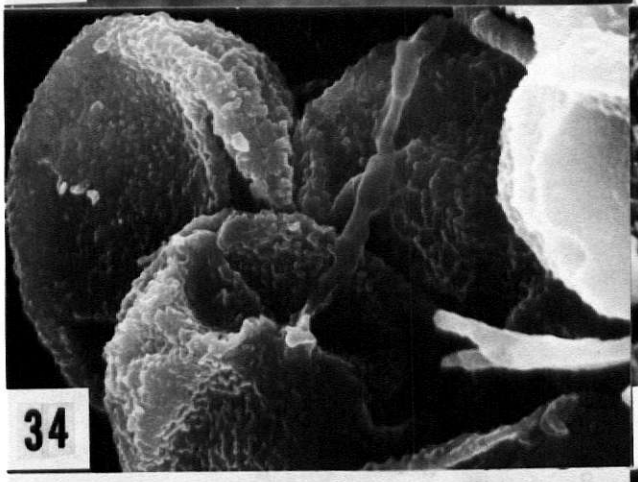
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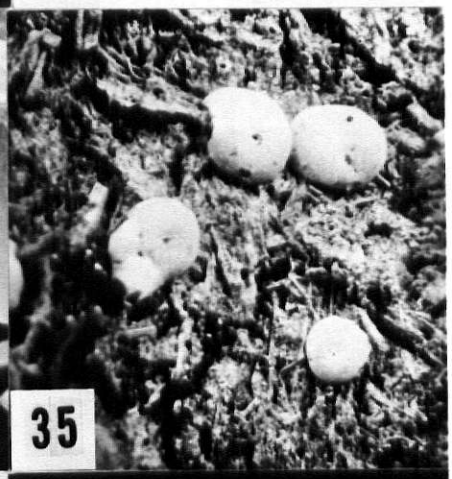
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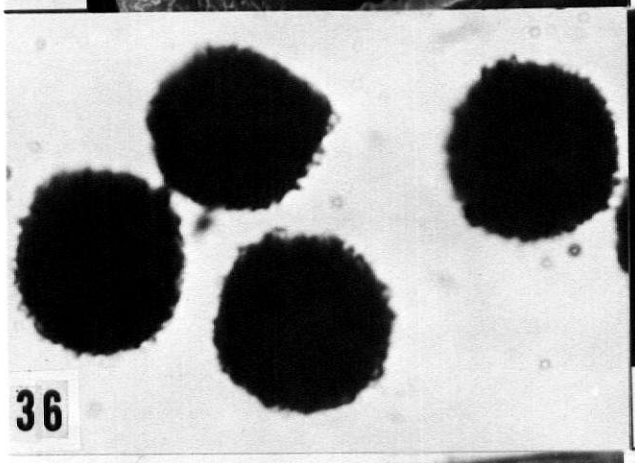
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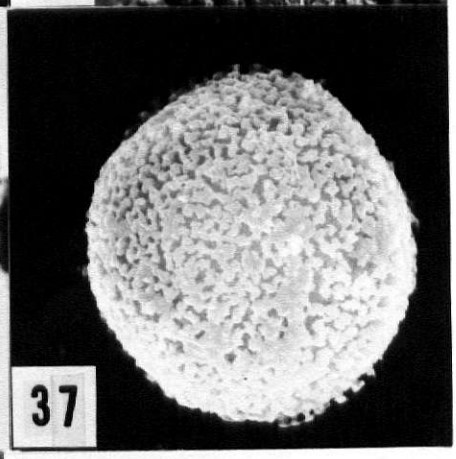
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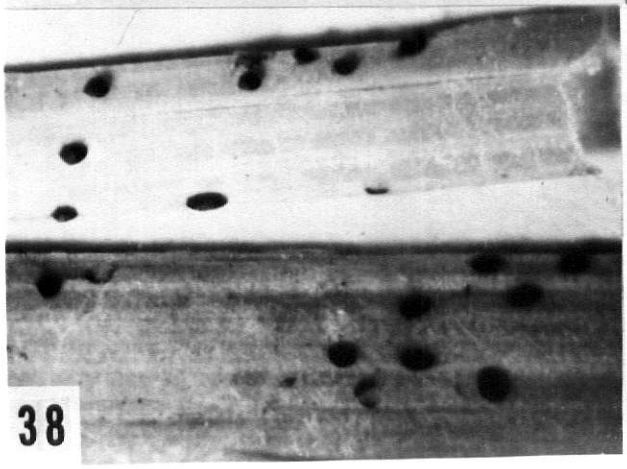
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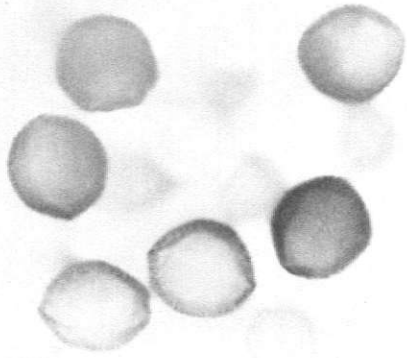
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descent, sparsely or densely covered with white, stellate or irregular lime crystals; columella lacking; capillitium moderately dense, consisting of very slender somewhat branching and anastomosing pale, violet threads, paler and coarse where connected with numerous subglobose vesicles, 24-75 µm diam, filled with yellow, obscurely granular material; spores dull brown in mass, pale, violet-brown by transmitted light, minutely warted, 8.5-11.5 µm diam. Plasmodium yellow.

MATERIAL EXAMINED: 22 collections: H. A.drich 393, Florida (HWK); C.J. Alexopoulos UTMC 1304, Virginia (IA); P. Allison 593, Philadelphia, Pennsylvania (IA); M. Brandza (NY 10013); Aaron Blair UTMC 1317, Virginia (IA); M. Brandza 89,Roumanie (IA); J. Dearness 8641, New York (IA); R. Hagelstein 609, Tennessee (NY); R. Hagelstein 13376, Pennsylvania (IA); Horrard, col lection made on August 21, 1918 (NY 7150); O. Jaap, collection made on July 16, 1916, Germany (IA); O. Jaap 175, Germany (IA); O. Jaap 193, Germany (IA); Lister 299 (NY 13081); T.H. Macbride, Iowa (IA); T.H. Macbride, collection made in 1895, New York (IA); G.W. Martin, collection made on August 20, 1940, Maine (IA); N.E. Nannenga-Bremekamp 5436, Netherlands (HWK); C. Torrend 153 (NY 10350); E. West 10706, Florida (NY 6872); H. Wingate, collection made in 1889, Carpenter Station, Pennsylvania (IA); H. Wingate, collection made in 1890, Carpenter Station, Pennsylvania (IA).

TYPE LOCALITY: Sweden.

HABITAT: Dead leaves and herbaceous stems.

DISTRIBUTION: Europe, West Pakistan, Florida, Iowa, Maine, New York, Pennsylvania, Tennessee, and Virginia.

The distinctive characteristics of this species are the warty, yellow, vesicular bodies attached to the capillitium.

Didymium sturgisii Hagelst., Mycologia 29:397. 1927.

Didymium anomalum Sturgis, Colo. Coll. Pub. Sce. 12:444. 1913.

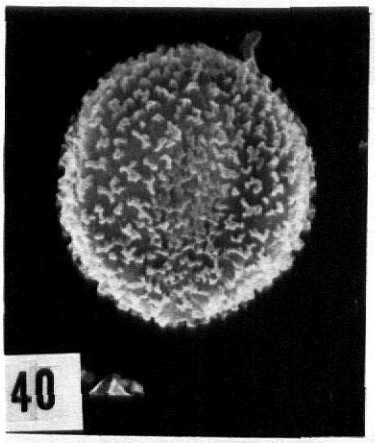
Not *Didymium anomalum* (Rost.) Massee, 1892.

ILLUSTRATIONS: Figs. 43-45.

Fructifications plasmodiocarpous, grayish white, scattered or gregarious, effused, rounded or irregular in outline, 1-10 mm across, 0.1-0.2 mm thick, rough, pitted, or wrinkled; peridium membranous, yellowish, with deposits of white, angular or stellate lime crystals; columella absent, but base with numerous calcareous trabeculae which enclose white crystalline lime and extend to the upper surface where attachement occurs; capillitium lacking or sometimes of slender, hyaline to dark, branched and anastomosing threads attached to the base and peridium; spores black in mass, violet-brown by transmitted light, surface evenly covered by minute spines, (10-)10.5-13.5 (-14.5) µm diam. Plasmodium unknown.

MATERIAL EXAMINED: 22 collections: H. Bilgram, Pennsylvania (IA); T.E. Brooks 1981, Riley Co., Kansas (HWK); T.E. Brooks 2911, Kentucky (IA); R. Hagelstein 2148, Mitchell Field, Long Island, New York (NY); R. Hagelstein 2351, Milford Township, Pike Co., Pennsylvania (NY); R. Hagelstein 2355, Lackawaxen Township, Pike Co., Pennsylvania (NY); R. Hagelstein 2356, Angels, Wayne Co., Pennsylvania (NY); R. Hagelstein 2464, West Fulton, Schoharie Co., New York (NY); R. Hagelstein 2485, West Fulton, Schoharie Co., New York (NY); R. Hagelstein 3472, near High Point, Sussex Co., New Jersey (NY); R. Hagelstein 3473, near Angels, Wayne Co., Pennsylvania (NY); R. Hagelstein 3882, Angels, Wayne Co., Pennsylvania (NY); R. Hagelstein 3886, Angels, Wayne Co., Pennsylvania (NY); R. Hagelstein 3942, Angels, Wayne Co., Pennsylvania (NY); R. Hagelstein 3951, Angels, Wayne Co., Pennsylvania (NY); R. Hagelstein 4216, Angels, Wayne Co., Pennsylvania (NY);

Figs. 40-45. 40. Spore of *Didymium serpula*, SEM X4500. 41. Plasmodiocarp of *Didymium serpula* on a decaying leaf, X10. 42. Spores and vesicles of *Didymium serpula*, X1400. 43. Spore of *Didymium sturgisii*, SEM X4500. 44. Plasmodiocarp of *Didymium sturgisii* on decaying bark, X10. 45. Spores of *Didymium sturgisii*, X1400.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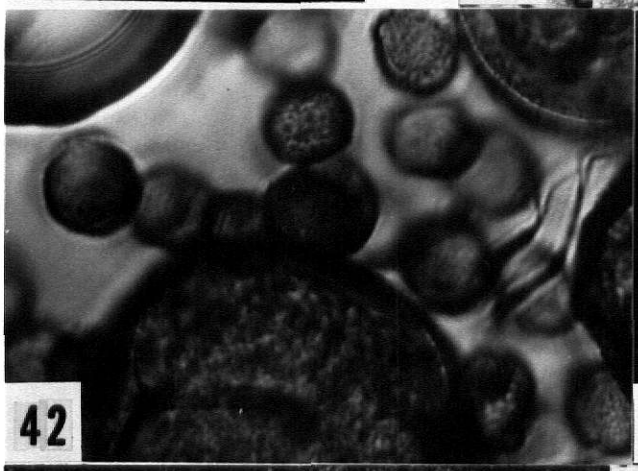
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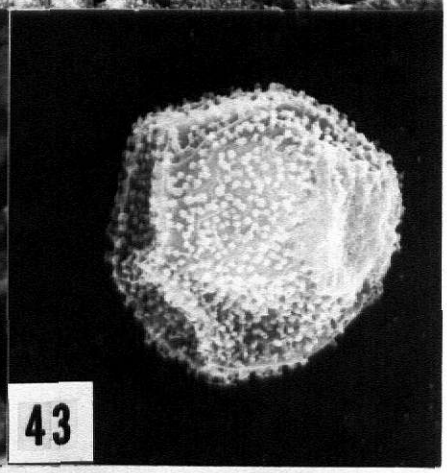
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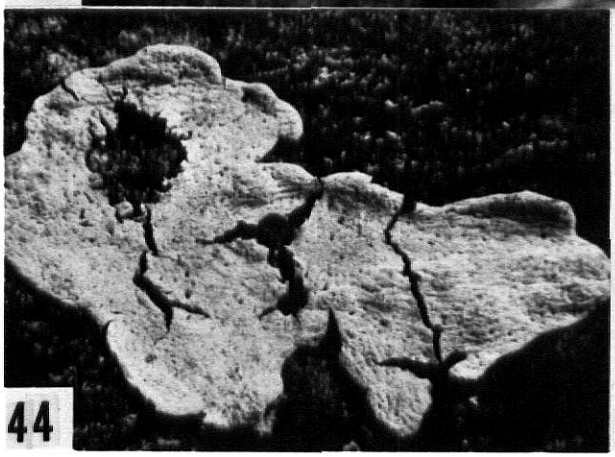
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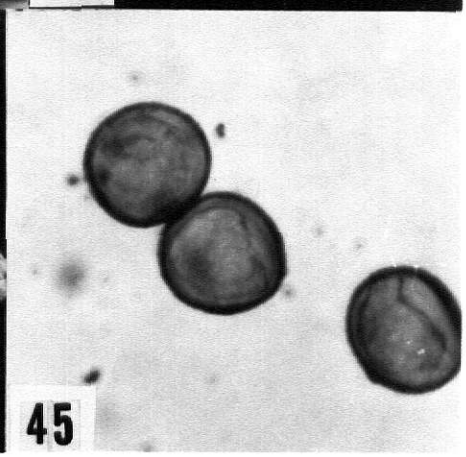
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R. Hagelstein 4845, Blooming Grove Township, Pike Co., Pennsylvania (NY); R. Hagelstein 2151, Minnesota (NY); A.M. Looney, East Okoboji, Iowa (BPI); W.C. Sturgis 717 (TYPE), Holland Dairy, Colorado Springs, Colorado (NY 13109); W.C. Sturgis 1053 (NY 13110); E.A. Walker, Fullerton, Maryland (BPI).

TYPE LOCALITY: Colorado.

HABITAT: Dead wood and bark.

DISTRIBUTION: England, Colorado, Iowa, Kansas, Minnesota, Montana, New Jersey, New York, and Pennsylvania.

The calcareous columns extending from the base of the plasmodiocarp to its upper surface are diagnostic of this species.

Didymium synsporon Brooks & Keller, Mycologia 65:287. 1973.

ILLUSTRATIONS: Figs. 46-49.

Fructifications sporangiate or plasmodiocarpous, sessile, scattered to gregarious, pulvinate to effused, depressed, white to yellowish, plasmodiocarps up to 3 mm in width and 6 mm in length; peridium of two layers, the outer layer smooth and glossy to rough and dull, the inner layer membranous, delicate, pallid, adhering to the outer layer; columella absent; capillitium of sparse, rigid, purplish-brown threads that extend from the base to the upper part of the peridium where they are attached; spores black in mass, violaceous-brown by transmitted light, clusters of 4-25, broadly ovate, 11-12X12-14 µm, large spines on the exposed surface, appearing pale and smooth elsewhere. Plasmodium unknown.

MATERIAL EXAMINED: 3 collections: T.E. Brooks 3718, Cumberland Co., Kentucky (HWK); T.E. Brooks 3735, Cumberland Co., Kentucky (HWK); H.W. Keller 1022, Riley Co., Kansas (HWK).

TYPE LOCALITY: Love Cemetary, north of Alma, Crawford Co., Arkansas on highway US 71, October 27, 1964.

HABITAT: Bark of living *Juniperus virginiana* L.

DISTRIBUTION: Arkansas, Kansas, Kentucky, and Tennessee.

The clustered spores are diagnostic of this species.

Didymium trachysporum G. Lister, Essex Nat. 20:113. 1923.

ILLUSTRATIONS: Figs. 50-52.

Fructifications sporangiate or plasmodiocarpous, sessile, scattered, white or cream-colored, sporangia hemispherical, 0.1-0.6 mm diam, plasmodiocarps slender, simple, curved, branched, or ring-shaped; peridium double, the outer layer eggshell-like, crust smooth or wrinkled and of closely compacted lime crystals, the inner layer membranous, colorless, somewhat iridescent; columella lacking, sometimes with a pale yellow sporangial base that is membranous with a thickened margin, and with scanty, rarely abundant, deposits or lime crystals; capillitium rather scanty, variable, consisting usually of a network of colorless or purplish, stout or slender threads, sometimes with vesicular expansions enclosing lime crystals; spores black in mass, dark, purplish-brown by transmitted light, 9-10 µm diam, with large spines spread irregularly over the surface of the spores or sometimes in lines giving the appearance of a reticulum. Plasmodium colorless.

MATERIAL EXAMINED: 7 collections: P.J. Alexander, England (NY 8963); T.E. Brooks 3428, Riley Co., Kansas (HWK); R.M. Brown UTMC 36 (IA); E. Evans, California (UC); H.C. Gilbert, Salem, Oregon (IA); E. Jahn (NY 8678); D.T. Kowalski 11891, Butte Co., California (HWK).

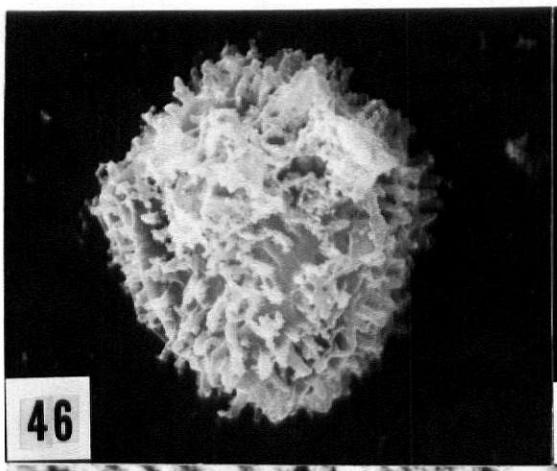
TYPE LOCALITY: England.

HABITAT: Dead leaves, herbaceous stalks, decayed wood, and dung of herbivors.

DISTRIBUTION: England, France, Germany, Austria, California, Kansas, Texas, and Oregon.

This species is apparently rare in the United States, but common in Europe. The small, coarsely warted spores are diagnostic of this species.

Figs. 46-52. 46. Spore of *Didymium synsporon*, SEM X4500. 47. Spores of *Didymium synsporon* grouped into clusters, SEM X400. 48. Spores of *Didymium synsporon* on the bark of *Juniperus virginiana*, X10. 49. Spores of *Didymium synsporon*, X1400. 50. Spore of *Didymium trachysporum*, SEM X4500. 51. Sporangia and plasmodiocarps of *Didymium trachysporum* on raccoon dung, X10. 52. Spores of *Didymium trachysporum*, X1400.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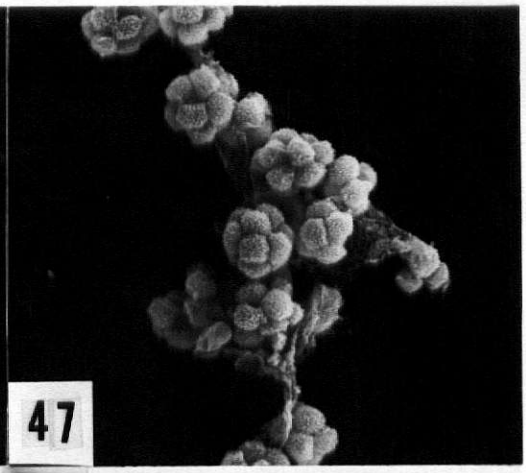
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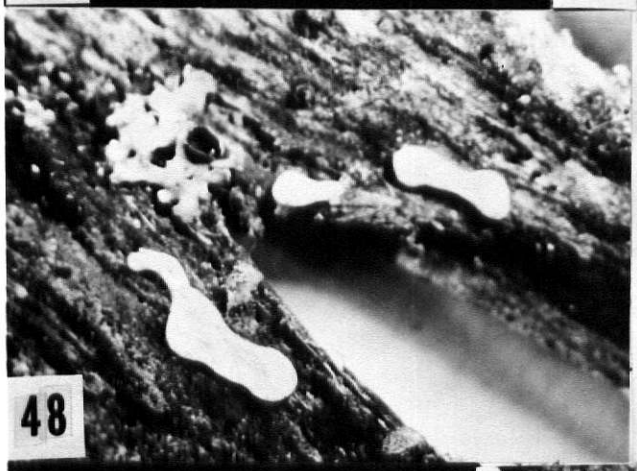
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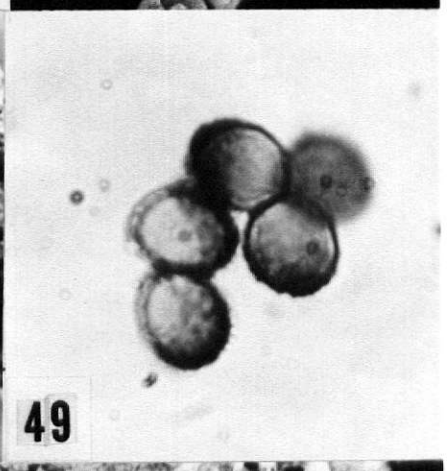
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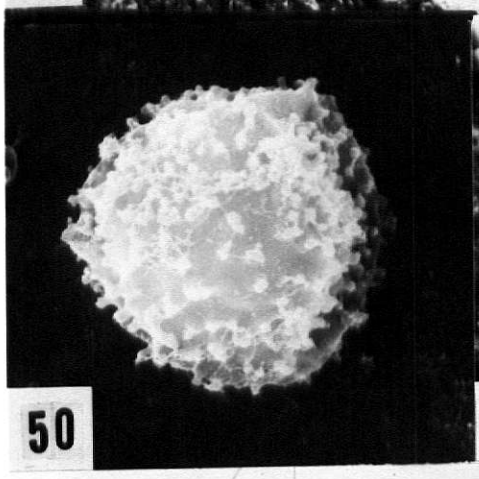
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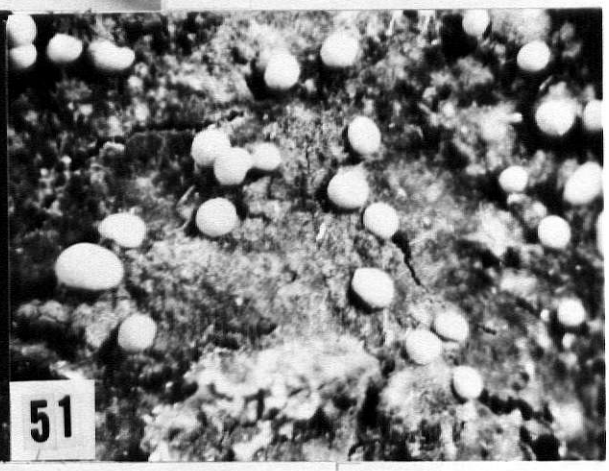
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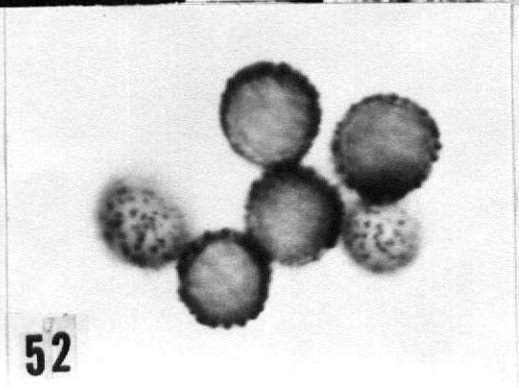
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THE SESSILE SPECIES OF *DIDYMIUM*

by

FRANK LELIN LYON

B.S., Kansas State College of Pittsburg, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

ABSTRACT

This thesis is presented in the format of two manuscripts, each written in the style acceptable by the mycological journals Mycologia and Mycotaxon, to which they will be submitted for publication. The first paper, "Preparation of Myxomycete Spores for SEM," deals with the scanning electron microscopy technique used in this research, while the second, "The Sessile Species of *Didymium*," is a taxonomic monograph of a portion of a genus of plasmodial Myxomycetes.

The SEM paper describes a procedure using poly-D-lysine (HBr 160,000 d, Nutritional Biochemicals Corp.) to affix spores to the surface of stubs for use on the scanning electron microscope. Spores remain securely glued to the stub during transfer through fixatives, drying agents, and critical point drying. The use of a fixative keeps the spores from collapsing so that the image from the SEM resembles that of the light microscope.

The monographic treatment includes only those species of *Didymium* that are never known to produce stalked sporangia. It does not include those that are "normally" stalked, but under certain environmental conditions will develop sessile forms. In choosing a group in need of a taxonomic study, we wanted one that was well delimited and with a number of taxa that could be studied reasonably within the scope and time appropriate for a Master's research program. We also wanted this group to be a part of a larger group into which our interests could be expanded for future research topics. the myxomycete genera which have not recently been monographed contain too many species for a study to be completed within the time available. The alternative was to choose a genus that could be divided into distinct groups. The sessile species of *Didymium* comprise such a group, while the genus is large enough to provide numerous problems for future studies.

A total of 269 collections of sessile *Didymia* were examined. A total of 43 names for this group are reported in this thesis of which only 17

are described as valid.

This treatment differs from that of other recent works by describing *Didymium nivicolum* Meylan as a distinct species and excluding *Didymium decipiens* Meylan from the sessile species of *Didymium* in favor of placing it into a genus of its own.

Seven plates containing 52 figures are included in the thesis. All of the micrographs were taken at equivalent magnifications to allow quick comparisons between species.