STUDIES ON CERTAIN KERATINOPHILIC FUNGI

by

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Studies on certain keratinophilic fungi

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Although the pathogenic keratinophilic fungi, or dermatophytes have been known for many years, non-pathogenic species were recognised only after Vanbreuseghem, in 1952, introduced a technique of "baiting" soil with hair. Because only fungi with an affinity for keratin can colonize the hair, this gives a method for selective isolation of such species.

For this thesis, non-pathogenic keratinophilic fungi were isolated from soils, hair and feathers, birds' nests and owl casts; the effect of various environmental conditions on asexual growth and on the development of the sexual state of certain of these keratinophilic species was investigated.

As a result of this work, the most suitable conditions for the primary isolation of keratinophilic fungi by the use of keratinous bait were established. It also became apparent that by altering the temperature of incubation from the normal 24°C certain keratinophilic species could be selectively isolated. The importance of unsterilized soil combined with a suitable keratinous bait (horse hair) and incubation temperature (24°C) was noted for cleistothecial formation by species of Nannizzia and Arthroderma. Horse hair laid on the surface of dung extract agar proved best for obtaining perithecia of keratinophilic species in the family Eurotiaceae.

Keratinophilic fungi were isolated from 171 (72%) of 238

soil samples, 74 (31%) of 234 animals and birds, 7 (80%) of 8 owl casts and 28 (80%) of 33 birds' nests investigated. Birds' nests have not been investigated before for keratinophilic fungi.

Analysis of the isolation results from soil proved that there is a linkage between the keratinophilic fungal flora and animal life. A larger percentage of soil samples from areas with a high animal population yielded keratinophilic fungi; a greater variety of species was isolated and individual fungi were present in quantity.

For the first tiem, an attempt has been made to compare and correlate the keratinophilic species isolated from small animals and soil. Rabbits were shown to carry 2 keratinophilic species and to be responsible for their presence in burrow soil, demonstra in the soil survey. Although no other keratinophilic fungi coul be related to one kind of animal, the evidence suggested that som non-pathogenic species were part of the skin flora of many animal These fungi were isolated more frequently from animals than from soil, although other species which were common in soil, were infrequently recovered from hair samples.

The isolations from birds' nests together with those from feathers suggested that, in the future when more birds have been examined, some species will be proved to be carried on feathers also.

Keratinophilic fungi in the family Eurotiaceae were shown to be regularly present in habitats rich in organic material,

being isolated from soils of this type and from owl casts.

As a result of these studies, species of keratinophilic fungi in the Fungi Imperfecti, Gymnoascaceae and Eurotiaceae have been isolated. Of the 21 species which I isolated, 9 are new species which have been classified and, with one exception, named; 4 of the remaining species, although they have been isolated in other countries, are reported for the first time in Great Britain. Amauroascus verrucosum, of which neither living cultures nor herbarium specimens exist, was re-discovered and is now held in pure culture.

A particular study has been made of the genus Arthroderma, to which, in the course of this work, I have added 5 new species. The generic description, based on the single species A. curreyi, has been expanded to include the 10 species now known and a key for identification, based on cleistothecial characteristics, has been prepared.

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INTRODUCTION

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In 1952, Vanbreuseghem described a simple but highly efficient method for the selective isolation from soil of fungi able to attack natural keratin. As a result of the use of this technique new species of fungi were found (Vanbreuseghem, 1952; Durie & Frey, 1957; Frey, 1959; Dawson, 1963) and it became apparent that the ability to attack and break down natural keratin *in vitro* was not confined to dermatophytes, the fungi which cause the disease ringworm in man and animals.

In an attempt to isolate the equine dermatophyte *Trichophyton equinum* from soil in which there was good reason to expect that it might be present, I substituted horse tail hair for the adult human hair which Vanbreuseghem (personal communication) had used as bait. Although the dermatophyte was not isolated, cleistothecia of *Keratinomyces ajelloi* were found and during the study of this species it became evident that certain environmental conditions were required to enable the perfect state to develop (Dawson & Gentles, 1959). Reports of perfect states of dermatophytes and non-pathogenic keratinophilic fungi followed (Kuehn, 1960; Griffin, 1960; Ajello, 1961; Stockdale, 1961; Dawson & Gentles, 1961; Georg, Ajello, Friedman & Brinkman, 1962) and it became obvious that there was an important and interesting field for mycological investigation in the study of keratinophilic fungi.

For this thesis, studies were undertaken on the effect of environmental conditions on the growth and the development of the perfect states of certain species of keratinophilic fungi. A variety of substrates, in which it seemed possible that non-pathogenic keratinophilic fungi and/or dermatophytes might be present, were investigated by the hair bait and other methods. In addition to soils, nests, owl casts and samples of hair, fur and feathers from dead animals and birds were examined. The aims of the investigation were to isolate keratinophilic fungi, to study the effect of animals on the keratinophilic fungal flora of the soil and to see if any of these fungi could be correlated with a specific type of animal or habitat.

As a result of these studies, a better understanding has been gained regarding the effects of environmental conditions on the growth of keratinophilic fungi and on the development of the perfect states. Methods have been evolved by which certain species can, if necessary, be selectively isolated. Two dermatophytes, Microsporum gypseum and Trichophyton mentagrophytes, and many non-pathogenic keratinophilic fungi were isolated. Several new species of non-pathogenic keratinophilic fungi were discovered, other species which had been reported many years previously and subsequently lost in the living state were recovered and some species were recorded for the first time in

Great Britain. It was possible to correlate 2 non-pathogenic keratinophilic fungi with rabbits and a number of other species were found to be frequently present on the coats of small wild mammals. Evidence suggesting the association of some species with birds was also obtained. Certain fungi were found to have a predilection for soil and other substrates in which there was an unusually high content of organic matter.

HISTORICAL REVIEW

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Keratin, which is a fibrous protein allied to fibrinogen and muscle fibre, is the main component of the non-living outer protective layer of the animal epidermis and its appendages. Skin keratin originates from soluble precursor cells which are situated in the prickle layers of the epidermis. As these cells pass towards the surface the keratin molecule develops, becomes stabilized and assumes the fibrous characteristics of its mature form in the stratum corneum where its toughness and elasticity serve as a protection for the underlying tissues. Nails, hair, feathers and the like also arise from the epidermis and are composed of a hard type of keratin which, chemically, has a higher content of sulphur than is found in that of skin.

Keratinized tissues, especially those composed of hard keratin, are normally relatively impervious to decay and, in nature, are attacked and broken down only by the clothes moth, certain actinomycetes, some chytrids and, in addition, by certain species of fungi (Page, 1950). According to Benedek (1962) the adjective "keratinophilic", which today is used to designate fungi which are capable of degrading keratin, was introduced by Karling (1946). Whereas all keratinophilic fungi can attack keratinized tissues <u>in vitro</u>, only some of them, the dermatophytes, are able to live parasitically and attack

keratin *in vivo* causing the superficial disease ringworm in man and animals.

The ringworm diseases are of considerable antiquity and according to Ainsworth (1953) favus, a form of ringworm in which characteristic cup-shaped crusts or scutula develop, was known to Celsus in the first century A.D.. The term "tinea", which is used today to designate superficial fungal infection of the epidermis or its appendages, might according to Hebra & Kaposi (1880) have been introduced in the 12th century by Stephen Antiochus whilst translating from the work of the Arabian physician Haly Abbas or, alternatively, might have come from Mercurialis in the 17th century from the latin "tinea" meaning a gnawing worm. According to Ainsworth (1953) ringworm of the scalp must have been common in England in Tudor times because licences were granted to permit those suffering from this disease to remain covered in the king's presence.

The first evidence that fungi were the cause of ringworm was found in 1839 when Schoenlein demonstrated the presence of fungal elements in the scutula of favus and, unlike Remak who had noted them several years earlier (1837), appreciated that they were the cause of the condition. It is not possible to mention all the pioneer workers in the field of medical mycology nor their discoveries but the researches of Gruby, whom Sabouraud has described as the "Father of Medical Mycology",

are outstanding. This author, in the years from 1840 to 1845, described fungi from each of the types of ringworm recognised today.

Despite the auspicious beginning, progress in the understanding of the connection of these pathogenic fungi with the disease ringworm was hindered by false premises such as the belief that any fungus was capable of causing infection (Hallier, 1880). However, despite this and other false assumptions medical mycology as a science continued to advance and new species of dermatophytes were discovered. By the turn of the 19th century the time was ripe for a re-appraisal of previous work in the light of new developments in technique. This task was undertaken by the French dermatologist Raimond Sabouraud who, using pure culture techniques, repeated many of the original experiments. In 1910 he published his observations in the monumental "Les Teignes" in which he classified the dermatophytes into 4 genera, Trichophyton, Microsporum, Epidermophyton and Achorion and gave detailed descriptions of the species within each genus.

Among the first to observe the action of the pathogenic keratinophilic fungi in vivo was the British dermatologist Adamson (1895) who reported that within the hair growth of the fungus is downwards into the roots but is stopped short at the living tissue of the bulb. An apparent exception to the fact

that dermatophytes do not invade living tissue is shown by the condition known as Majocci's granuloma and whilst reporting on the treatment of a severe case caused by the dermatophyte Trichophyton rubrum Blank & Smith (1960) suggested that a low dermatophyte inhibitory serum factor in their patient might be the cause of the reduced resistance to the dermatophyte. The inhibitory effect of blood serum was reported by Ayres & Anderson (1934) and by Peck, Rosenfeld & Glick (1940). That it played a part in preventing the invasion of living tissue by fungi was suggested by Lorincz, Priestley and Jacob (1958) and proved by Blank, Sagami, Boyd & Roth (1959). Raubitschek (1962) suggested that the chemical cysteine which is present in living tissues but not in skin, hair or nails might be the factor responsible.

Although the dermatophytes have been described as scarcely more than saprophytes, living and multiplying in what is virtually dead tissue, it has not been possible in vitro to reproduce the simple and restricted morphology of the parasitic state. An approximation to it has been obtained by growing dermatophytes in shake culture in liquid medium (Raubitschek, 1955). Normally when dermatophytes are grown in vitro on nutrient medium or on keratinized tissues strong growth accompanied by the production of various types of asexual spores and vegetative structures develops.

The growth of dermatophytes on keratinized tissues was first studied by Roberts (1894) by laying lengths of hair on pure cultures which were actively growing on liquid malt medium. He found that the hairs were attacked and that the extent of attack varied with the different species of dermatophyte. Penetrating organs, the structures by which most keratinophilic fungi enter hair, were demonstrated by Davidson & Gregory (1934) who believed that penetration was effected by enzymatic digestion. Page (1950) who, in addition to hair, studied the attack of skin, nail and horn by a number of species of dermatophytes, came to the conclusion that digestion could take place enzymatically even when the hyphae were not directly in contact with the keratin. Although subsequent workers have carried out many more investigations in this field and both pathogenic and non-pathogenic keratinophilic fungi have been studied (Vanbreuseghem, 1949, 1952; Daniels, 1953; Barlow & Chattaway, 1955; English, 1963; Chesters & Mathison, 1963) no completely conclusive proof of the presence of a keratin degrading system has been advanced (Chattaway, Ellis & Barlow, 1963).

Nevertheless, the fact remains that both pathogenic and non-pathogenic keratinophilic fungi, given suitable conditions of moisture and temperature, can grow and thrive for long periods on keratinous materials in the absence of any other source of nutrient.

Comparison of the prolific growth produced in artificial culture with the simple and restricted form of dermatophytes in the parasitic state suggests that dermatophytes may exist in nature as saprophytes. This has been suggested by Roberts (1894), Sabouraud (1910a) and Tate (1929) among others. Although the hypothesis is generally believed only a few reports of dermatophytes isolated from sources other than diseased tissues are available despite an intensive search within recent years.

Davidson & Gregory (1934) showed that under laboratory conditions it was possible for small saprophytic colonies of dermatophytes to develop from cast-off fragments of infected material and suggested that, one day, such colonies would be observed under natural conditions. Whilst investigating a shed which had housed ringworm-infected calves Muende & Webb (1937) did discover such saprophytic colonies of Trichophyton mantagrophytes, the dermatophyte which had caused the outbreak, growing on horse droppings.

Szathmary (1936) also believed that dermatophytes were present as saprophytes in soil and by rubbing mud on to the skins of guinea pigs induced a lesion in one animal from which 2 species of fungi were isolated. These he named Trichophyton terrestre primum and T. terrestre secundum and, today, are known as T. terrestre and T. quinckeanum. Szathmary must therefore

be regarded as a pioneer in the field of isolation of dermatophytes from soil. Evolceanu, Alteras & Cojocaru (1961, 1962) have also isolated T. quinckeanum from soil as has Rdzanek (personal communication). T. mentagrophytes has been reported from the atmosphere (Lurie & Way, 1957) and soil (Lurie & Borok, 1955) of caves inhabited by bats and from soil by Evolceanu, Alteras & Cojocaru,(1962a). T. verrucosum, which is the main cause of cattle ringworm has been recovered from a natural source by Walker (1955) but only by removing infected fragments found among the specimen and culturing from them. This author did, however, demonstrate that this fungus was capable of saprophytic growth on sterilized soil and cow dung and stated that, in one instance, the conidial state developed on cow dung.

Only one species of dermatophyte, Microsporum gypseum which attacks both man and animals, has been isolated regularly from soil from which source it was first recovered by Cooke in 1952. The presence of the very characteristic macroconidia of this species in soil was demonstrated by Gordon (1953) and by Lurie & Borok (1955) and this fungus has now been reported as a saprophyte from almost every country in the world. M. gypseum has been shown to be responsible for sporadic outbreaks of ringworm among market gardeners and others working in close contact with earth (Whittle, 1945; Alsop & Prior, 1961; Klokke, 1962).

Among those who believed that many of the ringworm fungi must exist in nature as saprophytes were Emmons (1951) and Vanbreuseghem & Van Brussel (1952) who considered soil to be the obvious substrate. Vanbreuseghem & Van Brussel (1952a) demonstrated that growth on soil or soil agars helped restore to the dermatophytes characteristics which had been lost by their having been held overlong in artificial culture. To recover dermatophytes which had been inoculated into sterilized soil and to investigate samples of natural soils Vanbreuseghem (1952a) devised a technique based on one which Karling (1946) used to isolate keratinophilic chytrids. Keratin, usually in the form of human or animal hair, is spread as "bait" on the surface of moist soil in Petri dishes. Fungi with an affinity for keratin colonize the bait and can then be easily recovered in pure culture. When Vanbreuseghem (1952b) used this technique with samples of natural soil the bait was attacked by a fungus which, although showing morphological similarities to the dermatophytes, proved to be non-pathogenic to both humans and animals. Although some non-pathogenic fungi which, today, we know to be keratinophilic had been discovered many years previously and one of them, Ctenomyces serratus Eidam, had been cultured regularly on old feathers (Eidam, 1880; Dangeard, 1907) the significance of this had not been realized. Not until after Vanbreuseghem's discovery was it fully recognised that the power

of attacking and breaking down keratin *in vitro* was not confined solely to the dermatophytes.

Vanbreuseghem's hair-bait technique opened up a new field for mycological exploration and new species of fungi (Vanbreuseghem, 1952b; Durie & Frey, 1957; Frey, 1959; Dawson, 1963) and the perfect states of fungi already known in the imperfect state were discovered (Dawson & Gentles, 1959; Griffin, 1960; Stockdale, 1961; Dawson & Gentles, 1961; Ajello, 1961; Georg, Ajello, Friedman & Brinkman, 1962). As a result, the systematic position of certain dermatophytes has now been established.

It was noted by Matruchot & Dassonville (1899, 1899a) that dermatophytes and members of the family *Gymnoascaceae* produced similar asexual spores and these authors suggested that a relationship between the groups of fungi was probable. In 1900 Matruchot & Dassonville noted that certain species of the ringworm fungi produced, at times, structures which resembled cleistothecia except that in place of the ascospores conidia were formed. These structures were morphologically akin to the cleistothecia of the family *Gymnoascaceae*.

This family of the class *Ascomycetae* was founded by Baranetsky (1872) and outlined essentially as it is known today by Schroeter in 1893. It is characterized by cleistothecia with a peridium composed of a network of interlaced septate

branching hyphae. The cells of these peridial hyphae are often very characteristic in shape and in certain genera some of them, towards the outside of the peridium, give rise to slender, septate, thin-walled spiral hyphae. It was such spiral hyphae to which Matruchot & Dassonville (1899,1899a) compared the spiral hyphae formed by certain dermatophytes in artificial culture. The non-pathogenic gymnoascaceous fungus which these workers considered as having the most features in common with the dermatophytes was Arthroderma curreyi which, however, was known to them by the name Ctenomyces serratus.

The theory of Matruchot & Dassonville was supported by the Italian worker Nannizzi (1926) who reported (1927) that he had induced a dermatophyte isolated from a human source to form cleistothecia by culturing it on a mixture of old feathers and leather in soil. The perfect state which developed was characteristic of the Gymnoascaceae. Unfortunately, however, the experiment was not completed by culturing from the ascospores and proving the connection between the cleistothecia and the apparently imperfect fungus inoculated and so this proleptic piece of work did not receive the recognition which it deserved. It is ironical that among the most stringent critics of Nannizzi's work were Langeron & Milochevitch (1930, 1930a) who, nevertheless, agreed with the hypothesis of Matruchot & Dassonville and, although the perfect states were not known,

placed one group of the dermatophytes in the genus Ctenomyces Eidam of the Gymnoascaceae. We now know that C. serratus as described by Eidam (1880) is, in fact, 2 separate species and that the species which the dermatophytes resembled was really Arthroderma curreyi (Smith, 1904).

Corroboration of Nannizzi's work was obtained in 1960 by Griffin who, whilst studying the succession of fungi on natural substrates decomposing in contact with soil, noted that cleistothecia had developed on sterilized human hair on one sample. On microscopic examination of these perfect states, which were gymnoascaceous in type, Griffin noted that macroconidia typical of the dermatophyte Microsporum gypseum were produced from the peridial hyphae. In addition, cultures from ascospores gave rise to the asexual form of this dermatophyte on agar medium. Griffin, whilst confirming the basic accuracy of Nannizzi's description, emended it slightly noting the presence of spiral appendages to the peridial hyphae and a difference in shape of the ascospores. Although considering that Nannizzi had erred in naming the fungus Gymnoascus gypseus Griffin retained the name rather than erect a new monotypic genus.

Almost simultaneously with Griffin's discovery other perfect states associated with the asexual state of M. gypseum were reported by Szathmary & Herpay (1960) and by Stockdale (1961).

This latter author erected a new genus Nannizzia, in honour of Nannizzi, and named her strain N. incurvata. Despite the similarity of these fungi in the asexual state, the cleistothecia of each is quite distinctive. As all the species are heterothallic, experiments in crossmating showed that inter-specific crossing did not take place. In a recent paper Stockdale (1963) has shown that it is possible to differentiate between the species in the asexual state and that Szathmary's species, which she named N. fulva, is the perfect form of Microsporum fulvum Uriburu which was originally described by Sabouraud (1910b) and later reduced to synonymy with M. gypseum by Conant (1941). Although N. incurvata and N. gypsea as she has named Nannizzi's strain are distinguishable from each other Stockdale found it impossible to decide which of these species is M. gypseum sensu stricto.

By mating strains of the dermatophyte M. nanum on unsterilized soil with hair bait the perfect state, which proved to belong in the genus Nannizzia, was obtained and named N. obtusa (Dawson & Gentles, 1961). Another new member of this genus was isolated from ringworm lesions on a squirrel and originally described as Keratinomyces ajelloi by Georg, Kaplan, Ajello, Williamson & Tilden (1959). Later this species was re-named M. vanbreuseghemii in the asexual state and N. grubyia in the perfect state (Georg, Ajello, Friedman & Brinkman, 1962).

The fungi discussed so far within the genus Microsporum have been parasitic and capable of causing ringworm in man and animals. However, one species has been described by Ajello (1959) which had been isolated from soil and from small wild animals although none of these showed lesions. Trial inoculations of humans and animals also gave negative results. Ajello (1959) named this fungus M. cookei in the asexual state and N. cajetana in the perfect state (1961). Thus, within the genus Nannizzia one non-pathogenic keratinophilic fungus and a number of pathogenic species are known.

At present, rather the converse holds true for the genus Arthroderma in which, with one exception, no species has irrefutibly been proved to be pathogenic. This genus, founded by Berkeley (1860) for a fungus discovered by Currey in 1854, was for over 100 years monospecific and only after the hair bait method had come into general use were new species added. The second species of Arthroderma was named by Kuehn (1960) who, following a report by Dawson & Gentles (1959) on the discovery of the perfect state of K. ajelloi, re-examined cultures made some years previously from cleistothecia which had developed on robin feathers and an owl pellet kept in the laboratory in a humid atmosphere. In these cultures cleistothecia were found which, according to Kuehn, were very similar to those of A. curreyi but distinguishable from it by the presence of large

tuberculate asexual spores. On account of these spores Kuehn named the species A. tuberculatum.

Prior to the discovery of the perfect state K. ajelloi had been shown to be world-wide in distribution and had been recorded from Britain by Daniels (1954) and Stockdale (1958), from Australia by Durie & Frey (1955) and from America by Ajello (1953) to mention but a few of the reports. Nevertheless, cleistothecia of this species were not observed until horse hair was used as bait instead of adult human hair. In proving the connection of the cleistothecia with K. ajelloi a number of factors influencing sexual reproduction were noted and the importance of the right type of bait in conjunction with unsterilized soil was realized (Dawson & Gentles, 1959, 1961).

Trichophyton terrestre, the second of the non-pathogenic keratinophilic fungi to be discovered by the use of the hair bait technique, was described from Australian soil by Durie & Frey (1957) and shortly afterwards from other countries such as Britain (Stockdale, 1958) and Finland (Lundell, Meinhof & Rieth, 1960). The perfect state A. quadrifidum was reported by Dawson & Gentles (1961) who noted that cleistothecial formation in this species, unlike K. ajelloi, was suppressed by an incubation temperature of 28°C. At this temperature, given the other conditions necessary for sexual reproduction, viz. compatible mating strains, unsterilized soil and a suitable bait, T. terrestre

formed structures morphologically identical to cleistothecia but containing microconidia instead of asci and ascospores. "Conidial nodules" of this type were first noted by Eidam (1880) when describing the fungus which he named Ctenomyces serratus. These structures were figured for T. terrestre by Durie & Frey (1957) and described by Griffin (1960) who, on account of their morphology suggested that when the perfect state should be discovered T. terrestre would be found to be a member of the family Gymnoascaceae.

Similar structures have been described for some of the dermatophytes by Matruchot & Dassonville (1900), by Langeron & Milochevitch (1930, 1930a) and by Balogh (1963). At a meeting in 1959 I gave a demonstration of conidial nodules of the Arthroderma type formed by the dermatophyte Trichophyton simiae. Recently, the perfect state of this species has been discovered and the relationship to Arthroderma confirmed (Stockdale, personal communication). In view of the evidence of this dermatophyte and T. terrestre one may, with reasonable certainty, state that the morphology of conidial nodules is indeed prophetic of that of the perfect state.

Although the majority of the isolations of non-pathogenic keratinophilic fungi have been made from soil a number of these species have been recovered with some frequency from the body surfaces of wild animals. McKeever, Kaplan, Menges & Ajello

(1958) and McKeever, Kaplan & Ajello (1958) investigated the fungi carried by wild mammals and, in addition to the dermatophytes T. mentagrophytes and M. gypseum, frequently recovered the non-pathogenic M. cookei. None of the animals investigated showed clinical signs of ringworm. Marples & Smith (1962) obtained the normal type of T. terrestre from the ventral surfaces of hedgehogs and considered it to be a contaminant from the environment. From the faces and quills of a number of animals they isolated a strain which they considered to be a variety of T. terrestre. Because this variety was not found in the soil and was not isolated from the lower parts of the animals Marples & Smith considered that it must be truly a skin resident. T. terrestre has also been reported from a variety of small mammals by Otčenášek & Dvořák (1962) who believed it to be a contaminant and stated that the species must be common in the soil of the animals' environment.

A. cuniculi and A. multifidum were shown by Dawson (1963) to be present in soil from inhabited rabbit burrows but not in other samples of soil. These species were also isolated from hair samples from rabbits which suggested that they formed a part of the natural flora of the hair and, despite the fact that they were non-pathogenic, were actually residents on the animals. Because rabbit burrow soil is sub-surface soil and this normally contains no keratinophilic fungi, the presence of

A. cuniculi and A. multifidum in the burrows must be attributed to the rabbits.

The effect of the animal population on a keratinophilic fungus in soil was first noted by Ajello (1953) who recovered the dermatophyte M. gypseum more frequently from areas in which animals were present. Durie & Frey (1962), reporting the results of soil surveys in Australia and New Guinea stated that the highest yields of keratinophilic fungi were obtained from areas associated with man or animals. However, no studies designed to prove or disprove this point have yet been undertaken.

MATERIALS & METHODS

MATERIALS AND METHODS

MEDIA

The following agar media distributed into slants or petri dishes were used in the isolation, study and maintenance of strains of keratinophilic fungi during the course of this work.

4% malt extract agar

Extract of malt (Boots)	40g.
Agar (B.D.H. shredded)	20g.
Distilled water	1000ml.

Preparation:- The ingredients are mixed and after heating to dissolve the agar the medium is distributed into containers and sterilized at 15 lbs/sq.in. for 20 minutes. pH unadjusted 5.2.

4% malt extract agar supplemented with actidione (cycloheximide), penicillin and streptomycin

Extract of malt (Boots)	40g.
Agar (B.D.H. shredded)	20g.
Distilled water	900ml.
Actidione (cycloheximide)	0.5g. dissolved in 100ml. Sterile water
Penicillin	20 units/ml.
Streptomycin	40 units/ml.

Preparation:- 4% malt extract agar is prepared and after sterilization is cooled to approximately 50°C and the antibiotics added aseptically before distributing into sterile containers. pH unadjusted 5.2.

Glucose peptone agar

Glucose (dextrose)	40g.
Peptone (Oxoid mycological)	10g.
Glucose (dextrose)	20g.
Peptone (Oxoid mycological)	1000ml.
Agar (B.D.H. shredded)	

Preparation:- As for 4% malt extract agar. pH unadjusted 5.8.

3% peptone agar

Peptone (Oxoid mycological)	30g.
Distilled water	1000ml.

Preparation:- As for 4% malt extract agar. pH unadjusted 6.5.

Czapek Dox agar

Oxoid Czapek Dox agar granules (CM 97)	51.4g.
Distilled water	1000ml.

Preparation:- The granules are dissolved in the water by heating gently and after distribution the medium is autoclaved at 10 lbs/sq. in. for 20 minutes. pH unadjusted 6.8.

Czapek Dox 1% peptone agar

Preparation:- As for Czapek Dox agar with the addition of 1% Oxoid mycological peptone. pH unadjusted 6.7.

2% tap water agar

Agar (B.D.H. shredded)	20g.
Tap water (soft)	1000ml.

Preparation:- As for 4% malt extract agar. pH unadjusted 6.6.

Dung extract agar

Preparation:- Horse droppings are thoroughly mixed with an equal volume of tap water and the mixture filtered, first through several layers of gauze and then through coarse filter paper (Green's hyduro 904). The filtrate is diluted with an equal volume of tap water (soft) and sufficient melted agar added to give a final concentration of 2%. The medium is distributed into containers and autoclaved at 15 lbs/sq. in. for 20 minutes. pH unadjusted 6.4.

Complete medium (Pontecorvo, Roper, Hemmons, MacDonald and Bufton, 1953)

As complete medium was used only infrequently and was uneconomical to prepare in the small amounts required, the prepared medium was obtained when needed by the courtesy of Mr. E. Forbes, Genetics Department, University of Glasgow.

Minimal Medium solution

Sodium nitrate	6g.
Potassium chloride	0.52g.
Magnesium sulphate ($7H_2O$)	0.52g.
Potassium di-hydrogen phosphate	1.52g.
Iron & Zinc	traces
Dextrose	10g.
Distilled water	1000ml.

Preparation:- The pH is adjusted to 6.5 with NaOH before sterilization at 10 lbs/sq.in. for 10 minutes.

B vitamin solution

Riboflavin	10mg.
Nicotinamide	10mg.
P. aminobenzoic acid	1mg.
Pyridoxin-HCl	5mg.
Aneurin-HCl	5mg.
Biotin	0.02mg.
Distilled water	10ml.

Preparation:- The ingredients are weighed, dissolved and the solution Koch sterilized.

Complete medium

Minimal medium solution	1000ml.
B vitamin solution	1ml.
Difco Bacto peptone	2g.
Yeast extract (yeastrel)	1g.
Casein hydrolyzate	5ml.
Acid and alkali hydrolyzates of yeast nucleic acid	3ml.
Agar (Davies powdered)	15g.

Preparation:- The pH is adjusted to 6. The agar is dissolved in the minimal medium solution then the other ingredients are added and the whole filtered through asbestos pulp and sterilized at 10 lbs/sq. in. for 10 minutes.

MOUNTANTS

Lactophenol, and lactophenol with cotton blue or light green added were used in microscopic examination of hairs bearing fungi, of portions of fungal colonies and for mounting slide cultures. Specimens taken from experimentally inoculated animals were examined in Potassium hydroxide solution.

20% Potassium hydroxide solution

Potassium hydroxide	20g.
Tap water (soft)	100ml.

Preparation:- The caustic is carefully dissolved in the water.

Cotton blue in lactophenol

Cotton blue (Gurr, 07885)	0.05g.
Lactophenol	30g.

Preparation:- The cotton blue is added to the lactophenol and left at room temperature for a few days before filtering. This stock solution is further diluted with lactophenol according to the degree of staining required.

Light green in lactophenol

Light green (Gerrard)	0.05g.
Lactophenol	30g.

Preparation:- As for cotton blue in lactophenol.

THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE PERFECT STATES OF KERATINOPHILIC FUNGI

A series of experiments were carried out to investigate the effect of variation of the environmental conditions on the formation of the perfect states of a number of species of keratinophilic fungi. The factors investigated were:-

1. Substrate
2. Sterility of the soil base
3. Nature of keratinous bait
4. Temperature
5. Light
6. pH of substrate

Care was taken to ensure that all environmental factors except the one under test were constant. A substrate of unsterilized soil with horse mane or tail hair bait was used except in experiments involving agar medium, bait type or the effect of sterilization of the soil. Every experiment was run in triplicate using an inoculum of 2 compatible single ascospore strains of each of the keratinophilic species Keratinomyces ajelloi (Arthroderma uncinatum), Trichophyton terrestre (A. quadrifidum) and Microsporum gypseum (Nannizzia incurvata). The strains were conserved on 4% malt extract agar (p.21). For experiments involving agar media 2 point inocula of the compatible single ascospore strains were made $\frac{3}{4}$ to $\frac{1}{2}$ inch apart

on the medium in Petri dishes or test tubes. The media tested were 4% malt extract agar, 3% peptone agar (p.22), glucose peptone agar (p.21), Czapek Dox peptone agar (p.22) and complete medium (p.23).

When soil or other such substrates were used, the inoculum consisted of spore-mycelium suspensions of the single ascospore strains prepared by macerating the growth from an agar culture in sterile water in a tissue grinder. The suspensions were made to such a concentration that they appeared opaque and 1 - 2ml. of each of the compatible strains was distributed with a Pasteur pipette over the soil surface and bait.

Keratinous materials and soils which were to be used unsterilized were tested before use to ensure that they were free from keratinophilic fungi. For soil, this was done by thoroughly mixing the sample before preparing 3 Petri dishes (3½ in. diameter) and baiting. If, after 2 - 3 weeks' incubation, no keratinophilic fungi were seen on the bait the sample was deemed negative for keratinophilic fungi and suitable for use. Keratinous materials were tested for sterility by placing on "negative" soil and incubating for 2 - 3 weeks.

Soil was sterilized by autoclaving at 20 lbs/sq. in. for one hour on each of 3 successive days. In the early trials it was noted that sterilized soil rapidly became contaminated by saprophytic moulds despite all reasonable precautions. To

prevent this, each Petri dish of soil was baited then the part containing soil and bait was tied into a cellophane bag and the lid replaced before autoclaving. The cellophane was left in place throughout the experiment and the spore-mycelial suspensions were introduced by means of a hypodermic syringe. Some samples of sterilized soil were artificially contaminated with saprophytic fungi and/or bacteria several days before the keratinophilic fungi were inoculated.

All keratinous materials were cut into suitably sized pieces before autoclaving at 15 lbs/sq.in. for 20 minutes. Black horse mane or tail hair was used in all experiments other than those in which different baits were investigated. Horse, dog, cat, rabbit, guinea pig and human hair (adult and child) were tested as bait as were feathers, cow horn, nail parings, skin, egg membrane and hoof and horn meal. As well as examining the relative efficiency of the different types of keratinous materials as bait, the effects of sterilization, colour and age of the animal on the efficiency of certain types of hair was also investigated. Horse hair treated by extensive ether extraction was compared with that subjected to water extraction*. Untreated horse hair was used as a control. Growth of the 3 test species on

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* The water extraction was carried out by Mr. M. Baxter, Unit for Research on the Experimental Pathology of the Skin, The Medical School, University of Birmingham.

keratinous materials in the absence of soil was investigated by placing the baits on moist filter paper or on an inert moisture-retaining inert base such as Peralite[x].

To study the effect of light on cleistothecial formation, baited soil plates were inoculated and then enclosed in aluminium foil. When prepared, all plates were placed on a shelf below a strong constant light source and incubated at room temperature. A comparative estimate of the amount of light penetrating each filter was made using an exposure metre.

The effect of temperature on the development of the perfect states was studied by incubation of plates at a range of temperatures from 4 - 37°C in the dark. When the species did not grow or did not form cleistothecia at any of these temperatures the plates were transferred to 24°C after the period of incubation at the original temperature. The incubation temperature for all other experiments was 24°C.

Unsterilized sand in conjunction with buffer solutions was used to study the effect of pH on growth and cleistothecial formation. The buffer solutions were Na_2HPO_4 in M/15 solution and NaH_2PO_4 in M/15 solution and by mixing these a range of

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x Manufactured by The British Gypsum Co., Cocklakes, Carlisle.

buffers of varying pH values were obtained. pH values from 4.6 to 9.5 were tested. Growth on various soil types was investigated using a series of "negative" soils selected for their geological nature.■

■ With the assistance of Dr. Jardine, Department of Geology, University of Glasgow.

COLLECTION AND PROCESSING OF SAMPLES

SOIL SAMPLES

Sources

At first, samples were selected at random but later sampling was more selective and tended to be confined to areas associated with animals such as farm yards and animal burrows. In addition, soils of differing geological types were selected.

Collection

At the time of collection the district, soil type and animal associations of the sample were recorded together with other relevant information such as if the sample were from woodland, arable land etc. Samples which were of the magnitude of a large handful were placed in polythene bags for transport and storage. From animal burrows samples were taken from the mouth of the burrow and from immediately within the tunnel. Signs of use such as the presence of tracks and droppings were noted. A few samples were collected by friends and colleagues who were asked to follow this procedure.

Preparation of samples for incubation

In the laboratory 3 sterile Petri dishes (3.5 in. diameter) were half filled with soil from each sample and, if necessary, sterile water was added to moisten it before baiting. The

keratin bait used as a routine was hair which had been cut into approximately ½ inch lengths and sterilized by autoclaving at 15 lbs/sq.in. for 20 minutes. An experience had shown that horse mane or tail hair was a most satisfactory bait, this was used as a routine although other hair (e.g. guinea pig or child), feathers or horn were, when it was considered advisable, used for comparison in one of the 3 plates. The selected bait was laid on the surface of the soil but was not pressed into it.

Attempts were also made to isolate keratinophilic fungi by placing a thin layer of soil in a Petri dish and covering it with melted, cooled, sterile 2% water agar (p.22) before baiting the surface with sterile horse hair. The method described by Orr (personal communication) which consists of placing small fragments of soil on the surface of 2% water agar in a Petri dish and baiting each fragment with hair was used occasionally. Soil samples were mixed with "negative" sand before baiting. A few samples were also investigated by plating water dilutions on 4% malt extract agar supplemented with antibiotics (p.21).

KERATINOUS MATERIALS

Animal hair, feathers, quills etc. found detached or taken directly from the dead animal or bird were collected in folded paper or in Petri dishes and were investigated by placing on the surface of moistened sand or soil which had previously been

proved free from keratinophilic fungi; by placing them on an inert moisture-retaining substrate such as Peralite and/or by inoculating directly on to agar medium supplemented with antibiotics.

BIRDS' NESTS

The keratinous materials from birds' nests were treated as were samples of hair and feathers but the straw, moss and mud were moistened with sterile water and baited with horse hair.

Before processing, materials other than soil or the like were examined with a plate microscope for the presence of fungi. If any were observed, a portion was mounted and examined microscopically and the characteristics noted. In such cases direct inoculations were made on agar media in addition to the routine treatment.

For all specimens the temperature of incubation used as a routine was 24°C as earlier studies (Dawson & Gentles, 1961) had shown that at higher temperatures cleistothecial formation by certain species might be adversely affected. Towards the end of this work it was found that for certain fungi even this temperature was too high and therefore at least one plate was left at room temperature (12 - 20°C). Occasionally, for a specific reason such as selective isolation, a sample was

incubated at 28°C and 34°C in addition to 24°C.

After 5 - 7 days' incubation all plates were examined with the naked eye and under a plate microscope (X 17.5 & X 35) and thereafter at intervals of several days. At the early examinations the relative amounts of the various fungi present was estimated because, after this time, the more strongly growing species tended to colonize most of the available bait. Baits colonized by fungi were selected under the plate microscope for microscopic examination and for culture. For microscopic examination fragments of bait bearing keratinophilic fungi were transferred with forceps directly to a drop of 95% alcohol on a slide to remove air. The mountant was added carefully, disturbing the growth as little as possible. Semi-permanent mounts were made by ringing with gold size. For culture selected bait fragments were folded into slips of paper and allowed to dry out for at least 24 hours. When inoculating, the material was divided into small fragments (approx. 2mm.long), at least 12 of which were placed on agar media, 4 inocula per slope. The isolation medium was 4% malt extract agar (p.21) and this medium supplemented with actidione, penicillin and streptomycin (p.21). Actidione, at the concentration used has little or no effect on the growth of keratinophilic fungi (Kuehn & Orr, 1962) but is fungistatic to saprophytic moulds. When difficulty in isolation was encountered other media such as glucose peptone (p. 21) and Czapek Dox peptone (p.22) were

used in addition to 4% malt.

The original Petri dishes from all samples were retained for a minimum of 5 weeks and, when it seemed advisable, for as long as 3 months. Before discarding, all plates were given a careful final examination to make sure that no slow-growing fungi or belatedly formed cleistothecia were overlooked.

Some samples of soil were found to be heavily infested with mites and other insects which destroyed the fungi. Such plates were treated by dusting the surface of the soil and bait with Gammexane* which had little or no effect on the growth of the fungi but was lethal for the insects.

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* I.C.I. Pharmaceuticals Division, Wilmslow, Cheshire, England.

MICROMANIPULATOR

When it was desired to make cultures from single ascospores or from single asexual spores a Singer micromanipulator (Barer & Saunders-Singer, 1948) was used with a glass loop (approx. 40 - 50μ diameter) as the micro-instrument. The light source was a point o' light at a distance of approximately 9 inches from the mirror of the microscope. Blue and yellow filters were used to reduce the light intensity because it had been suggested (M. Forbes, personal communication) that over-strong light might render spores inviable.

As shown in fig. 1, a glass ring with about ⅓ of the circumference removed was cemented to a glass slide thus allowing access with the micro-loop to a coverslip placed on the upper surface of the ring. Spore suspensions were prepared in a cavity slide by grinding cleistothecia for ascospores or a fragment of a colony for asexual spores in a few drops of sterile water to which a drop of a 2% sterile solution of the wetting agent Tween 80* had been added. A droplet of this suspension, diluted to give a workable density of spores, was placed on a coverslip and this was set, drop downwards, on the upper surface of the glass ring. The micro-loop was then

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* Honeywill & Steen Ltd., Devonshire House, Mayfair Place, London, W.1.

raised into the suspension and when a spore was trapped within the loop and held there by surface tension the loop was lowered, the coverslip bearing the suspension removed and replaced by one bearing a small drop of agar medium into which the spore was placed. This coverslip was placed over a cavity containing a few drops of water in a slide and the whole put into a Petri dish and incubated. The incubation temperature used as a routine was 28°C but this was varied for spores which proved difficult to germinate. When growth became visible on the agar, which was usually within 4 to 6 days, the agar drop was transferred to an agar slope. For certain fungi, in which spore germination tended to be slow, a refinement based on a technique described by Cox & Bevan (1961) was developed. The agar drop was placed on a small square (approx. 5mm.) of wet sterile cellophane which had previously been placed on the coverslip. After inoculation the cellophane bearing the agar was transferred directly to an agar slope. The media used were 4% malt extract agar or Czapek Dox peptone agar.

STUDY OF KERATINOPHILIC FUNGI

Fungi isolated in the course of this work were studied by some or all of the following methods.

Growth on soil with keratinous bait.

This technique was used with several aims in view.

a) To study fungi in the absence of competition from other keratinophilic fungi on "negative" soil with hair bait.

b) To ascertain if fungi, isolated by methods other than hair bait, were capable of attacking and colonizing the bait.

c) By means of single ascospore isolates to test for homothallism or heterothallism; to reproduce the perfect state and for attempted interspecific matings.

d) In attempts to obtain the perfect state of apparently asexual strains.

e) As a means of maintaining fungi especially those forming cleistothecia.

One simple technique for inoculation of the soil with pure cultures was used for all the above aims and has already been described (p.26). Cultures from single ascospores were tested for homothallism or heterothallism by inoculating suspensions alone and in all possible combinations of pairs. Single ascospore strains of heterothallic fungi were used to attempt to induce cleistothecial formation in strains which had not formed the perfect state in primary culture. Mating

with single ascospore strains was also used for attempted interspecific crossing in those species which appeared to be closely related in the asexual and sexual states. When more than one isolate of a species for which only the imperfect state was known was obtained, these were mated in combinations of pairs on soil with hair bait in an effort to induce formation of the perfect state.

Growth on hair alone

This technique was used to

a) Confirm that a fungus was capable of attacking natural keratin.

b) Study the method of attack.

The technique used was that described by Vanbreuseghem (1949) in which an unsterilized hair, usually human, is stretched vertically across a bent glass rod to which it is sealed with a drop of wax. After inoculation of the hair with a minute fr fragment of growth from an agar culture the glass stand is placed in a covered receptacle containing water to maintain a humid atmosphere and incubated at 24°C. The hairs were mounted and examined microscopically after 10 and 20 days.

Simple variations such as laying the hair or other source of keratin on the surface of 2% water agar (Griffin, 1959), or placing the hair on an inert moisture retaining substrate

such as Peralite or filter paper were also used.

Dung extract medium with hair bait

This was used in preference to baited soil for culture of members of the family Eurotiaceae and for certain species of the family Gymnoascaceae for which the soil hair bait method was not completely satisfactory.

Agar media

Agar media were used to

a) Conserve fungi in pure culture.

b) Study gross colonial characteristics of isolates.

c) Study micromorphology of the asexual state of isolates.

d) Study the effect of temperature on growth.

e) Attempt to induce sexual reproduction in artificial culture.

Cultures were kept on 3% peptone agar (p.22), Czapek Dox peptone agar (p.22) and 4% malt extract agar (p.21) but mainly on the latter 2 media which experience had proved to be more satisfactory. Gross colonial characteristics were studied on a number of different media but those most commonly used were 3% peptone, glucose peptone (p.21) and 4% malt extract agar. Slide cultures were made on these media. The above media and Complete medium (p.23) were used in attempts to obtain perfect states.

ANIMAL EXPERIMENTS

Unless specifically stated otherwise, the animals used in pathogenicity experiments were albino guinea pigs weighing approximately 200g. Each animal was caged separately. The animal was shaved on shoulder or flank, scarified gently with a scalpel blade and the site of scarification moistened with a drop of water before the inoculum was rubbed in. The inoculum consisted of about ¼cm. square cut from a pure culture on agar medium. The culture was chosen at an age when it was sporing heavily. The inoculated site was covered with elastoplast which usually came off naturally within 5 days but, if not, was removed at that time. This method has, in this laboratory, been shown to be very successful in inducing dermatophyte infections in guinea pigs. The animals were examined on the 5th day and at intervals of several days thereafter. At each examination the state of the inoculation site was noted and, if it seemed advisable, scales and hairs were removed for examination. Microscopic examination was carried out after clearing the scales and hairs in 20% potassium hydroxide solution. Because keratinophilic fungi persist for long periods at the site of inoculation retro-cultures were made only when the sample was positive on microscopic examination or a definite lesion was seen.

RESULTS

RESULTS

THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE PERFECT STATES OF KERATINOPHILIC FUNGI

Sexual reproduction by the test species on agar medium was unsatisfactory, cleistothecia forming sporadically or not at all. Results were slightly better when complete medium was used but were still not reliable enough for routine use. It was noted that culture in test tubes gave better results than culture in Petri dishes.

Cleistothecia of the 3 species formed profusely and with regularity on unsterilized soil in conjunction with a suitable bait. On sterilized soil sexual reproduction was inhibited or much reduced. The species most adversely affected was K. ajelloi. The perfect states of T. terrestre and M. gypseum did develop but in much reduced numbers. Cleistothecial production by the test species was considerably improved when an artificial flora of saprophytic fungi and/or bacteria was introduced to the sterilized soil before the inoculation of the keratinophilic fungi and the results almost equalled those obtained when unsterilized "negative" soil was used (plate 1).

No perfect states were obtained although each of the 3 species grew strongly when baits such as horse hair, cow horn

and feathers, which in conjunction with soil gave good cleistothecial formation, were used alone as the growth substrate. Similar negative results were obtained when these keratinous materials were placed on an inert base such as Peralite to simulate the conditions of bait on soil.

The efficiency of different types of keratin as bait varied considerably and was most marked on examination of cleistothecial formation. The most satisfactory hair baits were found to be horse mane or tail hair and the hair of certain children. In efficiency these were followed by hair from guinea pigs and poodle dogs on which reasonable growth and cleistothecial formation was noted for each species of fungus tested. The least satisfactory were rabbit, cat and adult human hair which gave reduced growth and cleistothecial formation. Sterilization did not affect the efficiency of any bait nor did pigmentation. This was shown by the similar results obtained from black and white hair from horse, cat and poodle dog. Horse hair subjected to intensive extraction with water or ether did not differ in efficiency as bait from untreated material. Hairs from horses and guinea pigs of different ages were tested and found to be equally satisfactory but this was not so with human hair. Adult human hair was invariably a most unsatisfactory bait material. Child's hair was, in general, good but hair from some children proved

better than that from others. Because of lack of numbers, no correlation of this difference with hair colour or the age of the child was possible.

Of the baits other than hair, feathers gave good results as did shavings of cow horn and nail parings. It was noted that horn and nail were attacked more slowly than hair. Human skin and hoof and horn meal were unsatisfactory because, apart from being broken down rapidly, they tended to become heavily contaminated by saprophytic fungi and bacteria which made examination and isolation of the keratinophilic fungi difficult. Egg membrane was the least satisfactory type of bait. It often completely resisted attack by keratinophilic fungi and, even when attacked, gave only minimal growth.

The effect of temperature of incubation on growth and sexual reproduction of the species tested is illustrated in table 1 and plate 2. Each species was able to colonize the hair bait over a fairly wide range of temperature although this differed for each species. At 4°C, growth of T. terrestre was evident on the hair bait whilst the growth of the other species was minimal. No reason for the failure of M. gypseum to grow at 10°C can be given because when transferred to 24°C the plates showed good growth. Unfortunately, it was not possible to repeat this experiment. At 34°C, growth of T. terrestre and K. adelloi was inhibited whereas that of M. gypseum was not.

Sexual reproduction paralleled the results for growth but took place over a more restricted temperature range. The maximum temperatures at which the perfect states of M. gypseum, K. ajelloi and T. terrestre developed to maturity were 30°C, 28°C and 24°C respectively. At 30°C K. ajelloi formed abortive cleistothecia consisting of a peridium but lacking ascigerous contents. T. terrestre at 28°C formed conidial nodules (Dawson & Gentles, 1961) and at 30°C only much restricted growth was visible. At the lower temperatures, after 12 weeks' incubation at 4°C only T. terrestre formed the perfect state and these were immature. No perfect states were formed by K. ajelloi at 10°C or by M. gypseum at 15°C.

When plates in which no growth or cleistothecial formation was apparent were transferred to 24°C all species from low temperatures developed well and formed the perfect state. Cultures from 34°C also gave normal growth with cleistothecial formation at 24°C. After incubation for 4 weeks at 37°C M. gypseum grew well and formed cleistothecia at the lower temperature whilst K. ajelloi and T. terrestre did not grow. It was found that exposure to 37°C for approximately 3 weeks was lethal for T. terrestre and from approximately 4 weeks for K. ajelloi. M. gypseum was able to survive this temperature for periods in excess of 7 weeks.

Constant bright light markedly reduced cleistothecial formation and to a lesser extent the growth of the 3 species. Differences in the numbers of cleistothecia were noted when the species were exposed to coloured light. In yellow, orange, red and green light cleistothecia formed as profusely as in darkness but this was not so in white or blue light. Measurements of the amount of light penetrating the coloured filters showed that less light penetrated the blue filters and therefore that the wavelength of the light was the factor inhibiting cleistothecial formation. K. ajelloi proved to be more sensitive to light that M. gypseum or T. terrestre.

The 3 species tolerated a wide pH range; each grew well and formed cleistothecia from pH 5 to 8. This finding was supported by the wide range of geological types of soil from which keratinophilic fungi were isolated or on which they were successfully cultured. Only peat, which is highly acid, was unsatisfactory.

Comment

As a result of these studies the conditions likely to be most suitable for isolating the test and related species and for obtaining their perfect states have been more clearly defined.

The least critical of the environmental factors affecting

the isolation of keratinophilic fungi and the formation of their perfect states in soil hair bait culture are the pH and moisture content of the substrate. It was shown that a wide pH range was well tolerated and, in addition, the test species were successfully isolated from, or cultivated on, geological types of soil ranging from sand to peat. Only peat proved to be unsuitable. In the course of isolation work it was noted that excess water was not detrimental but when the water level was reduced to such a degree that the atmosphere within the Petri dish was no longer highly humid, growth was restricted.

Although strong light reduces the amount of keratinophilic fungi isolated (plate 3), under laboratory conditions this factor is not liable to affect either isolation or the formation of perfect states. However, it seems feasible that light may be partially responsible for the paucity of reports of perfect states of these species having been found in nature. It was shown in the laboratory that light affects A. curreyi in the same way as it does the test species (plate 4). However, cleistothecia of A. curreyi have been found but it is interesting that in 3 instances when the site of discovery was mentioned (Currey, 1854; Salmon, 1900; Marsh, 1924) this was a wood where, in all probability, the light intensity would be low. It might be argued that the reason for the discovery of A. curreyi in the perfect state in nature is because this species is

homothallic. However, heterothallic species are frequently obtained in the perfect state in primary isolation plates so heterothallism is not the complete reason for failure to find these species in nature. Stockdale (personal communication) reported cleistothecia of various species of keratinophilic fungi growing on a dead cat found in a hollow log and the perfect state of T. terrestre was found in an old nest in a shaded position. These facts suggest that in reduced light cleistothecia will form in nature.

Because the type of bait selected may affect growth and cleistothecial formation it is advisable to use a bait of proved efficiency, such as horse or child's hair, for at least one of the 3 Petri dishes used in each attempted isolation. Good bait materials usually give good growth with all species. However, on poor baits individual differences between fungi may be noted. Although A. cuniculi and A. multifidum which were shown to be associated with rabbits (Dawson, 1963) grew equally well on rabbit and horse hair the test species grew poorly on rabbit hair. This fact might be used in selective isolation of A. cuniculi and A. multifidum from soil. No other such correlation of a fungal species with a particular type of bait has been noted although dermatophytes such as M. canis which are generally associated with a particular animal have been cultured on the hair of that animal and on

horse hair.

Hair is the most satisfactory type of bait material because it is easily distributed over the soil surface and lies in close contact with it. When colonized, the removal of selected portions is easy whereas with baits other than hair this may be difficult and good mounts for microscopic examination are almost impossible to prepare. Nail and cow horn, because of their durability under attack by keratinophilic fungi, are useful as baits for long temm conservation of species.

Whatever the factor which controls the efficiency of a bait it is unaffected by colour, sterilization by autoclaving, extensive extraction by water or ether or, with the exception of man, age.

Because different species of keratinophilic fungi grow over slightly different temperature ranges, selective isolation can be carried out. For example, low temperatures are to the advantage of T. terrestre and higher temperatures to the dermatophytes. K. ajelloi, because of its ubiquity and rapidity of growth is often a hindrance to other isolation. It proved possible to isolate M. canis from soil to which it and K. ajelloi had been inoculated. Incubation at 24°C and 28°C yielded only K. ajelloi but when plates were held at 37°C for 7 days before incubation at 24°C the amount of K. ajelloi present was reduced and the dermatophyte was easily isolated.

However, this procedure and that of incubation at 34°C has not yet, with natural soil samples, led to the isolation of any species not obtained from the control at 24°C.

Outbreaks of ringworm caused by the geophilic dermatophyte M. gypseum have been reported among cucumber greenhouse workers by Whittle (1954), Alsop & Prior (1961) and Klokke (1962). Soil from outwith a greenhouse yielded mainly K. ajelloi with some M. gypseum whilst the same soil after a period within the greenhouse gave M. gypseum in virtually pure culture. It seems possible that the factor responsible for the disappearance of K. ajelloi and the corresponding increase in M. gypseum was the temperature within the greenhouse. For growing cucumbers, it is recommended that the temperature should not fall below 21°C and may with safety be allowed to rise to 32°C which is above the optimum for growth of K. ajelloi but well within the range for M. gypseum.

Temperature might also explain why M. gypseum has been infrequently isolated in soil surveys in this country such as was done by Stockdale (1958). In countries in which the temperature reaches higher levels such as Australia and the United States of America many isolations of this species have been made (Durie & Frey, 1955; Ajello, 1953, 1956).

With T. terrestre the effect of temperature is particularly

interesting. At 28°C, compatible mating strains which would form cleistothecia at 24°C, produce instead conidial nodules. For the species in which it has been possible to compare cleistothecia with conidial nodules these have, with the exception of the contents, proved to be morphologically identical to the cleistothecia. Thus, as suggested by Griffin (1960), from them one may well be able to prophesy the form of the perfect state.

The importance of unsterilized soil to cleistothecial formation by K. ajelloi (Dawson & Gentles, 1959, 1961) is re-emphasized despite the fact that reports of perfect states of keratinophilic fungi on sterilized soil (Stockdale, 1961; Ajello, 1961; Georg, Ajello, Friedman & Brinkman, 1962) would appear to contradict this. Not all species are equally exacting in this respect. While many species will form the perfect state on sterilized soil, other species such as K. ajelloi and M. fulvum (Stockdale, personal communication) only rarely do. Soil which has been sterilized provides an ideal substrate for mould growth and rapidly becomes non-sterile even when particular care is taken to keep exposure to a minimum. It has been shown that such re-contaminated soil approadhes unsterilized soil in efficiency as a substrate for cleistothecial formation by certain species of keratinophilic fungi.

ISOLATION OF KERATINOPHILIC FUNGI FROM SOIL

The results from 238 samples of soil investigated for keratinophilic fungi by Vanbreuseghem's hair bait method (1952) are given in tables 2, 3, 4, 5 and 6. The samples have been grouped into 3 main categories based, as accurately as possible, on the degree of association of animal life with the areas in which collections were made. Within each category the samples have been grouped according to the type of animal with which they were associated (e.g. rabbits) or the environment (e.g. farmyard).

In table 2 details are given of the numbers of soil samples investigated within each category of animal association. It will be seen that of the 238 samples investigated 171 (71.8%) yielded one or more species of keratinophilic fungi whilst 67 (28.1%) were negative. There is a progressive decrease from 87.2% positive in samples with well-marked animal associations to 19.1% positive in samples with minimal animal associations. The number of different species of keratinophilic fungi isolated also appears to be related to animal density. Sixteen different species were recovered from areas markedly associated with animals compared to 6 and 4 from samples with moderate and minimal animal associations respectively. When each different keratinophilic fungus from a sample is counted as one isolate

it will be seen that in samples with well-marked and moderate animal associations the figures are considerably higher than those for minimal animal associations.

In table 3 the sites from which soil samples were collected are detailed and the information in table 2 is recorded for each site sampled. The results follow the trend set by the composite results (table 2). In the samples with marked animal associations only those from rabbit burrows show a relatively low proportion of positive results. No keratinophilic fungi were isolated from "woodland & moorland".

An effect of the presence of animal life on the keratinophilic fungal flora of the soil is shown in table 4, in which the number of samples giving rise to one or more species of fungus is recorded. Four keratinophilic species were recovered from each of 4 samples collected from areas with well-marked animal associations. Three species were the maximum obtained from each of 4 samples with moderate animal associations and 2 species from one sample with minimal animal associations. Two and more species were isolated from 43% (30% + 10% + 3%) of the samples with well-marked animal associations compared to 27.7% (19.1% + 8.6%) and 11.1% from samples with moderate and minimal animal associations respectively. This isolation of more than one species of fungus from individual samples is the reason for the high number of isolates recorded from soils associated with

animals (table 2).

The species of keratinophilic fungi isolated are given in table 5 and in detail for individual sites in tables 6a and 6b. In this country Keratinomyces ajelloi is, without doubt, the most ubiquitous of the keratinophilic fungi in soil and was isolated on 128 occasions (49.2% of the total 260 isolates of keratinophilic fungi). Fungi from 2 genera of the family Eurotiaceae were also frequently recovered and have been grouped under the heading"Aleurisma-Anixiopsis". Several species of Anixiopsis other than those at present recognised and the perfect and imperfect state of a species which closely resembles Aleurisma keratinophilum (Frey, 1959) are included in this group. It is not proposed to discuss these species in detail and the numbers have been given primarily to complete the results. Trichophyton terrestre was isolated from each of the 3 categories of animal association and the total of 21 isolations (8% of the total 260 isolates of keratinophilic fungi) placed it after K. ajelloi in frequency of isolation. Arthroderma curreyi is apparently of rather restricted distribution in soil because, although it was recovered 10 times, the isolations were confined to 2 districts. The number of isolates of A. cuniculi and A. multifidum, species associated with rabbits and their burrows, merely reflect on the number of burrows sampled (table 3).

Other species of Arthroderma isolated were the new species A. lomondii, and A. tuberculatum which has not previously been reported from Great Britain.

Microsporum gypseum was found to be rare in soil and of the 5 samples which gave this species 4 were collected from one district. M. cookei, which has not previously been reported from this country, was recovered from 2 sites, each closely associated with animals.

The percentage of isolates of each species which formed the perfect state in primary culture are given in table 5. A. curreyi and A. minutum are homothallic and, as would be expected, each isolate formed cleistothecia. In the heterothallic species it will be seen that the number of isolates forming cleistothecia can be related to the density of the animal population. The proportion of perfect to imperfect isolates of K. ajelloi, A. cuniculi and A. multifidum is high whereas relatively few of the isolates of T. terrestre reproduced sexually in primary culture. A. lomondii formed cleistothecia but A. tuberculatum was recovered only in the imperfect state.

All the isolates of M. gypseum were of one mating strain and not until single ascospore strains became available was it possible to obtain cleistothecia and prove that the isolates were Nannizzia incurvata. No other species in the M. gypseum complex (Stockdale, 1963) was recovered. M. cookei was

isolated in the asexual state.

Other species of fungi isolated included Ctenomyces serratus and Amauroascus verrucosus, neither of which has been reported from Great Britain previously. A fungus which has been provisionally placed in the genus Arachniotus was also obtained. A number of unidentified fungi have been grouped under the heading "small spore" because they are characterized only by the production of microconidia. A species which forms bulbils was also recovered.

Species seen on microscopic examination but which could not be induced to grow on artificial media were not included in the tables. One, which resembled Sepedonium was apparently able to live on hair. Another species was obviously a parasite on the keratinophilic fungi and a third might well have been so.

Comment

It seems worth-while to consider the efficiency of the method of isolation used in this study. In addition to routine hair baiting a number of variations in technique were tested. Soil was placed on the surface of 2% water agar then baited (Orr, personal communication); soil samples were "diluted" with sand known to be free from keratinophilic fungi before baiting; small particles of soil were placed on sand and each baited individually and isolation on agar media was tried. No species was isolated by the use of these rather laborious methods which had not been recovered by orthodox hair baiting. That the hair bait method was consistent was shown when soil samples were re-investigated. The results were identical to those originally obtained.

For isolation, the routine use of an incubation temperature of 24°C with horse hair bait ensured good conditions for growth and that if an isolate were capable of sexual reproduction cleistothecia would, in most cases, develop. After approximately 3 weeks in the incubator all plates were kept at room temperature for several more weeks to make certain that no slow-growing species had been overlooked. In one of the last samples investigated a species, visibly present in the soil, proved difficult to isolate because 24°C was above the optimum temperature for growth. The species was first recovered on

agar plates held at about 5 - 10°C but eventually grew on the isolation plates at room temperature. This suggests that in future studies it would be advisable to put an extra isolation plate at a low temperature. Isolation of all plates at room temperature would, for routine survey use, be too expensive in time and materials. The ideal method for isolation would be to incubate plates from each sample over a range of different temperatures.

Baiting with hair or other keratinous materials is a highly efficient method for the isolation of keratinophilic fungi from soil and, as used in this laboratory, it seems unlikely that species which were present in minimal amounts were missed. The importance of frequent regular examination from the time growth becomes visible on the bait, both with the plate microscope and by microscopic examination of individual hair fragments, must be stressed.

No great difficulty was experienced in isolating keratinophilic fungi in pure culture although certain species of common saprophytic moulds such as Cephalosporium, Penicillium, Gliocladium and Trichoderma were often present as contaminants on the colonized hair. The addition of the antibiotic actidione to the medium aided the isolation of keratinophilic species by retarding the growth of the contaminants. The use of this antibiotic to control contamination by saprophytic moulds was suggested by Philips & Hanel (1950) and in the

isolation of dermatophytes from clinical materials by Georg (1953). Kuehn & Orr (1962) studied the reaction of certain fungi to actidione and noted that gymnoascaceous species showed a high degree of tolerance to it. In the course of my work, it became apparent that keratinophilic species, other than dermatophytes and gymnoascaceous fungi, were not affected by actidione at the concentration used in the medium. Occasionally, colonization of the hair by more than one keratinophilic species led to difficulty in isolation of the less vigorously growing strain but this was usually overcome by repeated inoculations. When the species formed characteristic spores, the micromanipulator was used.

Ajello (1953) noted that the prevalence of the geophilic dermatophyte M. gypseum was greater in soils associated with animals and Durie & Frey (1962) reported increased yields of keratinophilic fungi from soils connected with man and animals. From the results of the survey there can be little doubt that animal life affects the keratinophilic fungal flora. From districts associated with animals, not only were more samples positive for keratinophilic fungi and an increased number of species isolated but also individual species were present in the soil in greater quantity. This latter fact was obvious at the first examinations of isolation plates by the number of colonies developing on the hair and by their rapidity of growth. It seems very probable that the presence of animals is responsible for the rise in the

keratinophilic fungal flora.

No keratinophilic fungi were isolated from "woodland & moorland". The failure to isolate from moorland samples is probably due to the low animal population and the peaty soil which has been shown to be a poor growth substrate. The absence of keratinophilic fungi from woodland soil was also noted by Stockdale (1958) but has not yet been satisfactorly explained.

The samples from rabbit burrows (table 3) show a relatively low proportion of positive results because a number of the burrows investigated were deserted and apparently had been for some considerable time. The fact that A. cuniculi and A. multifidum were common in soil from inhabited burrows and rare in deserted ones suggested that the species might be associated with rabbits.

It was, however, noted that species in the Aleurisma-Anixiopsis group were more frequently recovered from samples in which there was a high content of decaying organic matter. Examples of such soils are those collected from hen runs, near manure heaps and in the vicinity of dead animals. When these species were cultured on ordinary "negative" soil with hair bait they did not grow as well as on the soil from which they were isolated. On dung extract agar with horse hair bait or on baited sand supplemented with liquid dung extract good growth resulted, proving that organically rich substrate aided growth in these species.

ISOLATION OF KERATINOPHILIC FUNGI FROM KERATINOUS MATERIALS

No animal investigated, even those from which the dermatophytes M. gypseum and T. mentagrophytes were isolated, showed clinical symptoms of ringworm. From table 7, it will be seen that of the 234 animals investigated 74 (31.6%) yielded keratinophilic fungi. From the number of animals from which each species of fungus was recovered, it will be seen that T. terrestre (32) was the single species most frequently isolated and was followed by A. currevi (12). Species in the Aleurisma-Anixiopsis group were also recovered frequently. The number of animal species from which keratinophilic fungi were isolated is interesting. A. currevi was recovered from 8 different kinds of animal, Aleurisma-Anixiopsis from 6 and T. terrestre from 5. A. cuniculi and A. multifidum were isolated only from rabbits.

Comment

In this survey, the number of positive specimens is probably lower than it should be due to the technique used in the early stages of the investigation. At first, specimens were divided into small fragments and 12 of these inoculated to agar medium. With this method one could investigate only a

small amount of each specimen and also, despite the addition of antibiotics to the medium, bacteria or saprophytic fungi quite often overgrew the inocula. Later, it was discovered that a much more efficient way to investigate keratinous materials was by spreading them thinly on the surface of moistened sand or soil, previously proved free from keratinophilic fungi. With this method, much larger amounts of each specimen could be processed and contamination by saprophytic fungi or bacteria was reduced to a minimum. Samples from 11 rats were investigated by both methods and it was found that, using the sand technique, 9 were positive whilst when cultured on agar medium only 3 of these 9 yielded keratinophilic fungi.

With the exception of the dermatophyte T. mentagrophytes, each species recovered from the animals was isolated from soil (table 5). The obvious explanation for the presence of keratinophilic fungi on hair and feathers is that they are present as contaminants from the soil of the environment. Small wild mammals, especially those living in burrows, are in constant contact with earth as also are birds, many of which take regular dust baths. Contamination of the body surface with soil fungi is therefore quite possible and has been suggested as the reason for the presence of certain species on animals (Otčenášek & Dvořák, 1962; Marples & Smith, 1962; McKeever, Kaplan & Ajello, 1958; McKeever, Menges, Kaplan & Ajello, 1958).

If non-pathogenic keratinophilic fungi were present on animals as nothing more than contaminants from the environment, one would expect the species most common in soil to be those most frequently isolated from the hair or feathers. However, from table 9 in which the numbers of isolates of species commonly recovered from soil, animals and birds' nests are given, it will be seen that this is not the case. K. ajelloi, by far the most widespread species in soil, was isolated only 7 times from 3 animal species whereas T. terrestre and A. curreyi, which are less frequently present in soil, were recovered 32 times from 5 animal species and 12 times from 8 animal species respectively. It is interesting that A. curreyi and T. terrestre were often the only species isolated from samples of hair and feathers whereas only once were they isolated in virtually pure culture from soil. A. curreyi has been isolated from keratinous materials in districts in which its presence in soil could not be demonstrated.

These facts suggest that A. curreyi and T. terrestre are carried on hair and feathers in some way which is more than normal contamination but is not apparently parasitism. This state might be considered to be analogous to that of the dermatophytes T. mentagrophytes and M. gypseum when they are passively present on the hair of guinea pigs, rats and mice.

Marples & Smith (1962) first suggested that a species of

fungus might be considered to be part of the normal skin flora of an animal. On a number of occasions, they isolated a fungus from hedgehogs which they considered to be a variety of T. terrestre. Because their isolates were relatively common on the animals but could not be demonstrated in the soil of the environment, Marples & Smith believed them to be truly skin residents.

The association of A. cuniculi and A. multifidum with rabbits, suggested by their consistent isolation from inhabited burrows together with their absence from deserted burrows and other soils, was confirmed by the recovery of both species from the hair of rabbits. Recently, a way in which they may spread from animal to animal has been found. The rabbit hair lining of a nest containing young rabbits yielded A. cuniculi in quantity. It seems certain that the fungus would be transferred to the hair of the young animals within the nest.

The evidence from the rabbits suggests also that, rather than the rabbits becoming contaminated from the soil, it is the soil which is inoculated with the fungal species carried by the animals. Burrow soil is sub-surface soil which normally contains no keratinophilic fungi and so the species must be presumed to have come from an outside source. The presence of T. terrestre and A. curreyi in soil may also may be due to inoculation from carrier animals.

ISOLATION OF KERATINOPHILIC FUNGI FROM BIRDS' NESTS

A total of 33 nests built by 9 species of birds were examined. At the time of collection, the nests of 5 hedge sparrows, 2 robins and one blackbird showed fungal growth. The fungi present were Aleurisma-Anixiopsis, Pararachniotus gelicola and A. quadrifidum. Of the 33 nests investigated, 28 (84.8%) yielded keratinophilic fungi whilst 5 (15.1%) did not.

Not only were common species such as K. ajelloi, T. terrestre, A. curreyi and Aleurisma-Anixiopsis recovered but also A. tuberculatum, A. lomondii, C. serratus and the red-pigmenting variety of T. terrestre associated with hedgehogs (Marples & Smith, 1962; English, personal communication). Three new species, A. cocleatus, P. gelicola and Trichophyton globiferum were discovered and named.

In comparison with the isolations of species of keratinophilic fungi from soil and keratinous materials (table 9) it will be seen that T. terrestre, A. curreyi and the Aleurisma-Anixiopsis group were isolated more frequently from nests than from soil. T. terrestre and A. curreyi, however, were more frequent from keratinous materials than from nests. K. ajelloi was more prevalent in soil.

Comment

It seemed desirable to investigate a substrate, other than soil, which was intimately associated with animal life. Ideally, such a substrate should provide a rich source of keratinous material and, if possible, not be in direct contact with the earth. An obvious choice for investigation was birds' nests. Birds are in the nest for long periods when hatching eggs and, if keratinophilic fungi are present on their feathers, it may be presumed that they will be transferred to the nest and perhaps also to the young birds. Many nests are lined with hair and/or feathers. In addition, nests contain keratinous debris from the adult birds and nestlings and therefore might be expected to provide an ideal substrate for the growth of keratinophilic fungi.

Nests did prove to be a fruitful source of keratinophilic fungi; not only were species commonly found in soil recovered but also those rare in soil and a number of new species were discovered. No keratinophilic fungi were isolated from 5 nests. Of these, 3 (2 thrush & 1 blackbird) were old and had been stored for at least a year before coming into my possession. One robin's nest had apparently been deserted before use, which might account for the negative result, but no reason for the failure to isolate keratinophilic fungi from the remaining nest (hedge sparrow) can be given. That the unused robin's nest was

negative suggests the possibility that keratinophilic fungi in other nests may, at least in part, have come from the birds.

Unfortunately, the possibility of definitely relating species isolated from nests with those carried by birds is precluded for the present because only very few dead birds have been investigated. Any conclusions drawn must therefore be tentative and regarded more in the light of a guide for further work.

A number of facts suggest that some of the keratinophilic fungi in nests have originated from the birds. Keratinophilic fungi were isolated each time feathers from dead birds were investigated and fungal species, e.g. A. currevi and T. terrestre, which were not those most frequent in the soil of the birds' environment were isolated. Puga & Mathison (1962) reported the isolation of C. serratus from bird traps and sand dunes in the vicinity of the traps. These authors correlated the frequency of isolation of this species with the large number of feathers present and suggested that it was on the feathers before they were shed. Unfortunately, no record is given of the types of birds found in the traps.

C. serratus has not, in this survey, been recovered from feathers but was isolated from a swallow's nest. It is interesting that this type of nest also yielded A. tuberculatum and the new species A. cocleatus. C. serratus and A. tuberculatum

have been shown to be rare and A. cocleatus has not yet been isolated from soil in this country, suggesting that the birds were the probable source, especially as soil samples in the districts in which the nests were found gave none of these species.

Both A. tuberculatum and C. serratus were isolated from soil samples collected in the cattery (table 6a). Although the high proportion of keratin in this soil may be responsible for the species persisting there the question of how they came to be present arises. The fact that these species were not isolated from soils containing a comparable amount of keratin suggests that the isolations were from a cattery might be of significance. It is possible that cats catching birds within the cattery runs might be the cause of the presence of these species.

The new species T. globiferum has been isolated from 2 districts, each time from a hedge sparrow's nest which suggested an association. However, I received a culture from France which proved to be this species and which had been isolated from soil. P. gelicola although recovered from 4 nests does not appear to be restricted to this habitat because very recently it has been recovered from hair from a rabbit's nest and from a sheep's fleece.

KERATINOPHILIC FUNGI ISOLATED FROM OWL CASTS

In addition to the keratinous materials sampled directly from animals and birds, 8 owl casts were investigated for keratinophilic fungi. One or more species in the "Aleurisma-Anixiopsis" group were isolated from each of 7 casts, 2 also yielded "small spore" species and one Shanorella spirotricha. The only cast from which keratinophilic fungi were not isolated was one which, at the time of collection, was noted as being very old.

Watling (1963) reporting on the fungal succession on kestrel pellets stated that he had obtained Anixiopsis stercoraria. The frequent presence of species in the "Aleurisma-Anixiopsis" group in the pellets of birds of prey is probably due to the fact that they provide an environment rich in decaying organic matter such as has been shown to be suitable for these species. It is just possible, however, that the fungi were present on the hair of small mammals which had been eaten and that they had survived the digestive processes of the bird and thus were present within the cast when it was regurgitated.

ANIMAL INOCULATION

With the exception of the dermatophytes T. mentagrophytes and M. gypseum, no fungal species isolated in the course of this work was shown to be capable of causing ringworm in laboratory animals. All strains were tested on guinea pigs and, in addition, A. cuniculi and A. multifidum were tested on rabbits. In addition to inoculation, these species were rubbed into the hair and A. cuniculi was recovered by culturing from hair samples over a month later, although no lesion developed and no fungus could be seen on microscopic examination.

FUNGI ISOLATED

FUNGI ISOLATED

Because each known species and a number of new species of Arthroderma were recovered and because the majority of the keratinophilic fungi isolated in the surveys were members of this genus it will be discussed in some detail.

History

The genus Arthroderma was created by Berkeley in 1860 for a fungus which had been discovered 6 years previously by Currey. In his description Currey (1854) stated that the specimen, which he had found on dead leaves and twigs in a wood, resembled Trichoderma in having a central mass of spores surrounded by a hairy covering but differed from it in the jointed nature of the peridium and in the brilliant yellow colour of the spores. Currey suggested that, should his specimen merit a new genus, Arthroderma, from the jointed nature of the hairy covering would be an appropriate name. Berkeley followed this suggestion and named the fungus A. curreyi, placing it in the order Trichodermacei of the Hyphomycetes.

Saccardo (1886) also considered A. curreyi to be a Hyphomycete but transferred it to the genus Illosporium as I. curreyi, under which name it is mentioned in Massee's British Fungal Flora (1893). Currey's fungus was re-discovered on dead leaves under trees in the Queen's Cottage Wood, Kew, by Salmon

(1900) who stated that his specimen agreed perfectly with authentic examples of A. curreyi. As the articulated hyphae of Currey's fungus were not found in the genus Illosporium and as the waxy sub-gelatinous sporodochia with the conidia in mucus were not found in Currey's fungus, Salmon (1900) considered that it could not rightly be classified in Illosporium and transferred it back to Arthroderma curreyi in the Hyphomycetes.

In the meantime, a fungus had been reported from Germany by Eidam (1880) who had discovered it growing on decaying feathers. By sub-culturing when necessary to fresh feathers, Eidam was able to study the stages in development of this isolate which he classified in the family Gymnoascaceae and named Ctenomyces serratus. Eidam described sclerotia with unique hooked appendages, asexual spores, cleistothecia and structures consisting of dense globose masses of conidia which, at times, had a peridium of the type found in the perfect state.

Eidam's paper was obviously more widely read than the preceding British publications because fungi were described as C. serratus by a number of authors, none of whom, however, reported finding the sclerotia. From these reports we can now say that the species which they described was, in fact, A. curreyi. In Britain, the perfect state described by Eidam was found by Grove (1922) on feathers buried in soil and by Marsh (1924) from soil from flower pots and also from the

Queen's Cottage grounds at Kew, the place of Salmon's previous isolation. On re-examination of this area 9 months later by Marsh no traces of the fungus were found.

A report by Smith (1903) that Eyre had found C. serratus on decaying beech leaves in Hampshire was followed by 2 papers (Smith, 1904; Smith & Rea, 1904) in which it was stated that preparations of Currey's fungus made by Salmon and a herbarium specimen deposited in Kew by Cooke, had been examined and found to be identical to the Ascomycete C. serratus and that A. curreyi, the name which had many years' priority must, according to rule, take precedence. As Smith had examined Salmon's preparations of A. curreyi and Salmon in his original description had stated that his specimens agreed exactly with authentic specimens of A. curreyi, the perfect state described by Eidam (1880) is directly linked with Currey's original isolate. Höhnel (1905) who had sent a specimen of Currey's fungus to von Rehm as a new species C. xylophilus, distinct from C. serratus because it was discovered on decaying leaves instead of feathers, retracted after Smith's report and stated that he was convinced A. curreyi was equal in all respects to C. xylophilus and C. serratus and that these names should be discarded.

The problem of the resting or sclerotial stage reported by Eidam (1880) and Dangeard (1907) but by no other worker was only resolved recently. Benjamin (1956) examined herbarium

specimens of this resting stage and recognised that they were cleistothecia of another gymnoascaceous fungus. As these cleistothecia were so unlike those of any other member of the family, Benjamin considered that a new genus was necessary and suggested that there would be no confusion in nomenclature if the generic and specific names which had been invalidated by Smith (1904) were revived and this species called Ctenomyces serratus.

It is unfortunate that the mix-up caused by the duplication of names in the last century continues to this day and is not confined solely to fungi which indubitably belong to the genus Arthroderma. Although it was A. curreyi with which Matruchot & Dassonville (1899) compared the dermatophytes, this species was known to them as C. serratus. In 1930, Langeron & Milochevitch transferred the small-spored Trichophyton species to the genus Ctenomyces. Thus today, especially in continental publications, one finds not only Currey's fungus but also a group of the dermatophytes referred to the wrong genus.

As has been stated previously, it was only after the hair bait technique (Vanbreuseghem, 1952) came into general use that other species of Arthroderma were discovered and described. Today, this genus which for over 100 years was represented only by A. curreyi, includes 10 species and is among the largest in the family Gymnoascaceae.

Distribution

The members of the genus Arthroderma are, with the exception of the dermatophyte Trichophyton simiae which has recently been shown to belong in this genus (Stockdale, personal communication), mainly non-pathogenic and probably act as scavengers of keratinous debris in the soil and elsewhere. Most species have been isolated from soil. Some species (A. curreyi, A. cuniculi, A. multifidum and A. quadrifidum) have, in addition, been isolated from the hair of small animals with a certain degree of regularity and have (A. curreyi, A. quadrifidum)also been recovered from feathers and birds' nests. Certain species are now known to be world-wide in distribution. Others, as yet, appear to be more localized. This is probably not a true estimate of the distribution but rather a reflection of the amount of work which has been done in the study of keratinophilic fungi.

The type species of the genus, A. curreyi, has been recorded from England (Currey, 1854; Salmon, 1900; Smith, 1903; Grove, 1922; Marsh, 1924), Scotland (Dawson & Gentles, 1961; Dawson, 1963), Germany (Eidam, 1880), France (Dangeard, 1907) and U.S.A. (Benjamin, 1956). In the course of this work, A. curreyi has been isolated, not only from soil but also from hair samples from wild rodents, from rabbit burrows and from feathers and birds' nests.

A. uncinatum, or its imperfect state K. ajelloi, is, without

doubt, the most ubiquitous soil keratinophilic fungus and has been reported from almost every country in the world as is shown in table 10. This species appears to be primarily a soil inhabitant although it has been isolated occasionally from the hair of small mammals where, in all probability, it is present merely as a contaminant from the environment.

Another species which has been found in many countries is A. quadrifidum or its imperfect state T. terrestre (table 11). In the survey of soil samples this species was shown to be not nearly as common as K. ajelloi. Despite this, T. terrestre was isolated frequently from hair samples from rats, mice and rabbits and from feathers and birds' nests.

The 2 species of fungi proved to be associated with rabbits (A. cuniculi, A. multifidum) and A. minutum which has only been found from the soil from rabbit burrows have yet to be reported from countries other than Scotland. However, as A. cuniculi and A. multifidum were isolated from burrows in scattered districts and from the hair of rabbits, it seems likely that, in the future, they will be reported from other lands. As the rabbit population in Australia came originally from the British Isles it is certainly possible that some or all of these species will be found there.

A. tuberculatum has been reported from the U.S.A. from robin feathers and an owl pellet by Kuehn (1960) and from soil by

Orr (personal communication). The asexual state of this species was isolated from soil from a cattery in England and a second strain, which formed cleistothecia in primary culture, was recovered from a swallow's nest collected near Glasgow. These isolations are new records for Britain.

It is interesting that strains of A. lomondii have been isolated from soil, a mouse and a nest in the Loch Lomond district. Two other strains which closely resemble this species but will not cross mate with it were recovered from duck nests in the Loch Leven district. A. cocleatus is represented by a single strain and therefore no comment on its distribution is possible until further isolations are made.

Pathogenicity

Within the last few years, the perfect state of the dermatophyte T. simiae has been shown to be an Arthroderma and a number of reports have been published indicating that other species may occasionally be the causal agents of disease.

Following the isolation of a strain of K. ajelloi from a squamous erythematous lesion on the leg of an agricultural worker, Vanbreuseghem, Ghislain & Williams (1956) suspected that the species might be pathogenic. However, because attempts to produce experimental lesions with this and another strain of

K. ajelloi were unsuccessful and because K. ajelloi had been isolated from a field in which the patient had worked it was concluded that the fungus was a contaminant in the lesion.

Infection in a malabar squirrel due to K. ajelloi was reported by Georg, Kaplan, Ajello, Williamson & Tilden (1958). Their isolate differed from K. ajelloi as described by Vanbreuseghem (1952a) in that it did not produce a reddish purple diffusible pigment, did form many microconidia and readily caused infection in guinea pigs. Several years later, following the isolation of other strains and the discovery of the perfect state, which was not A. uncinatum but was characteristic of the genus Nannizzia, this pathogenic species was re-named Microsporum vanbreuseghemii in the imperfect state and Nannizzia grubyia in the perfect state (Georg, Ajello, Friedman & Brinkman, 1962).

K. ajelloi as the cause of ringworm in the horse was reported by Rieth & El Fiki (1959) who stated that the lesions on the animal were clinically suggestive of infection by a species of Trichophyton. On microscopic examination, the fungus was shown to be endothrix and to have arthrospores which were broader than long. Circumstantial evidence that K. ajelloi might be the cause of infection in a horse was put forward by Pier & Hughes (1961). One animal showed areas of hair loss and scaling and K. ajelloi was cultured ...

from these areas on several occasions although no evidence of fungal invasion was seen on microscopic examination. Samples from normal areas of this animal and from 5 other horses did not yield K. ajelloi although the presence of this species in the corral soil was demonstrated. The strains of K. ajelloi isolated from horses by Rieth & El Fiki and by Pier & Hughes were reported by Georg, Ajello, Friedman & Brinkman (1962) to have formed cleistothecia which were, in all respects, typical of A. uncinatum, the perfect state of K. ajelloi.

Ehrmann & Thurner (1962) reported the isolation of K. ajelloi from human sources. A lesion on a boy's arm, of about 4cms. in diameter, resembled ringworm and a woman's toe nails were thickened, deformed, brittle and dirty yellow brown in colour. Fungal hyphae were seen on microscopic examination of specimens from these patients and K. ajelloi was cultured. Experimental infection of guinea pigs with each of these strains of K. ajelloi was carried out by Ehrmann & Thurner, who reported that after 2 - 3 weeks mild, scaling, non-inflammatory lesions developed on the guinea pigs. On microscopic examination of material from these lesions dense masses of hyphae were visible in the scales and ectothrix spore formation in the hairs. Cultures from the lesions yielded K. ajelloi.

From the contents of these reports it is certain that, in each case, K. ajelloi was isolated but that this species was

the cause of the condition has, on only one occasion, been demonstrated in such a way as to leave little room for doubt. The only workers who attempted, and were successful in inducing experimental infection in laboratory animals, were Ehrmann & Thurner who noted ectothrix spore formation in the hair. This finding is at variance with that of Rieth & El Fiki for the horse and it is to be regretted that neither these authors nor Pier & Hughes carried out animal experiments with their isolates.

In view of the many strains of K. ajelloi which have been tested for pathogenicity under laboratory conditions and for which the results have been negative (Vanbreuseghem, 1952a; Vanbreuseghem, Ghislain & Williams, 1956; Georg, Ajello, Friedman & Brinkman, 1962) this species must be considered as either of doubtful pathogenicity or as one in which pathogenicity is rare and possibly limited to certain aberrant strains.

As far as I am aware, there have been only 2 reports published which suggest that T. terrestre, the imperfect state of A. quadrifidum, may be pathogenic. Szathmary in 1936 reported that by rubbing mud on to the skins of several guinea pigs, he had induced an inflammatory lesion in one animal. From this lesion he isolated the species which is known today as T. terrestre and another species which is now known to be the dermatophyte T. quinckeanum and in all probability was the

cause of the lesion.

Evolceanu, Alteras & Cojocaru (1962) isolated 11 strains of T. terrestre from samples of soil and, as soon as each was obtained in pure culture, attempted to induce experimental ringworm in mice. Only one strain, which was isolated together with T. quinckeanum, gave any indication of being pathogenic. Of the 6 mice used in the experiment, only one developed a small squamous lesion. On microscopic examination of scrapings from this lesion numerous mycelial filaments were seen in the scales and, in the hairs, growth of the fungus was endothrix. The lesion persisted for about 2 weeks and cultures made from it were the same as the strain which had been inoculated. However, this was the only lesion observed despite the fact that further tests, with this strain and the others, were carried out, not only with mice but also with guinea pigs and man. Because Evolceanu, Alteras & Cojocaru obtained a lesion on one occasion and because all strains which they had isolated produced a trichophytin as active as that of T. mentagrophytes, the authors concluded that T. terrestre was on the way to becoming a pathogen.

Klokke (personal communication) stated that he had isolated T. terrestre from a lesion on an agricultural worker but was not convinced that it was the cause of the condition. This species was twice cultured by myself from urticarial-type

lesions on a horse but because, on careful microscopic examination of hairs and scales from the lesions, no signs of fungi were observed, I came to the conclusion that the species was present as a contaminant from the environment. Support for this conclusion was obtained by the isolation of T. terrestre from the animal's bedding.

It has been reported (Koddie, Shadomy & Barfatani, 1963) that A. tuberculatum was isolated in pure culture from the finger of a man with psoriatic lesions on the scalp, trunk and hand. However, microscopic examination of the skin scrapings was not done and efforts to re-isolate the species from the original lesion and from other sites failed and therefore pathogenicity could not be established.

Although the question of pathogenicity within the genus Arthroderma must remain in doubt until more cases have been studied there is evidence which suggests that certain of the species have a definite predilection for animal hair whilst it is still attached to the animal. A. cuniculi and A. multifidum have been shown to be associated with rabbits while, although it has not been possible to correlate A. curreyi and T. terrestre with particular animals it has been shown that they are present more frequently than can be ascribed to accidental contamination from the environment.

Cleistothecial development and spore dispersal

As the cleistothecia of all members of the genus Arthroderma follow a similar pattern in development, it is not proposed to describe this for each species individually. The cleistothecial initials arise from separate hyphae or may, in homothallic species arise from a single hypha. They appear as small lateral protuberances in close proximity to each other. These are cut off from the parent hypha or hyphae by a wall, grow rapidly and develop into either the clavate septate antheridium or the ascogonium, which is also septate and grows in a tight spiral around the antheridium. Eidam (1880) stated that nuclei from the antheridium passed to the ascogonium. The initials rapidly lose this "text-book" form and become spherical in shape due to an increase in the diameter of the cells and to the production of new cells. Simultaneously with this stage, hyphae, which will eventually form the peridium of the cleistothecium, develop from the base of the antheridium and from the hyphae in close proximity to it. Even before the cells of these hyphae differentiate in appearance from those of the vegetative hyphae, the branching pattern characteristic of the genus becomes visible. At this early stage it is possible to identify A. curreyi by the spiral form of the peridial hyphae, so typical of this species. As the cleistothecium matures, the peridial hyphae develop many

septae and increase in diameter. The cells become dumb-bell shaped due to the ends of the cells increasing in diameter to a greater extent than the central portion and the walls thicken and become echinulate. Appendages to the cleistothecium, such as spiral hyphae and possibly conidia, become apparent. In the final stages of ripening of the cleistothecium, the peridial cells in many species of Arthroderma differentiate further by developing varying numbers of protuberances on the swollen area at each end of the cell. In species in which only a few protuberances are formed per cell these are usually confined to the outer face of the cell whereas in A. multifidum, the species in which differentiation is carried to the highest degree, the protuberances, which may number 8, are formed almost right around the cell.

After the cleistothecial initials become surrounded by the peridial hyphae, it becomes difficult to follow the stages in development of the ascus mass until the time when the cleistothecium contains immature asci which form in grape-like bunches on the branched ascogenous hyphae. It would appear that these hyphae disintegrate when the asci are mature, because no traces of them can then be found. The globose asci, each of which contains 8 ascospores, appear to ripen simultaneously. The walls break down leaving a mass of free ascospores within the peridium.

It seems possible that, in nature, the cleistothecia of species of Arthroderma may serve in dispersal of the fungus in several ways. The morphology of the cleistothecia, with the uncinate branching, echinulate cells and spiral hyphae is such that they will adhere to anything brushing against them. That they do so can be demonstrated by running a camel hair brush across a Petri dish in which cleistothecia are present. It therefore seems reasonable to assume that the cleistothecia will be carried from place to place on the coats of animals or the feathers of birds.

When the cells of the peridial hyphae become dehydrated, they collapse and the hyphae contract longitudinally. On a return to moist conditions the cells absorb water, swell up and return to their original shape. As this happens, a writhing movement of the peridium takes place and the ascospores pass out through the peridium. The range of dispersal will be minimal if the cleistothecium is still in the place in which it was formed. On the other hand, if the cleistothecium has become attached to an animal or bird, the fungus could well be dispersed over a very wide area. Disintegration of the cleistothecium by decay or by the attack of mites or other small insects may also serve in spore dispersal.

It may be, however, that the perfect state is not the main means by which species of Arthroderma are dispersed.

With the exception of A. curreyi which forms relatively large cleistothecia and which, because it is homothallic, can be expected to reproduce sexually whenever conditions are favourable, there have been no published reports of other species found in nature in their perfect state.

Taxonomy

In 1860 Berkeley created the genus Arthroderma for the specimen discovered by Currey in 1854, placed it in the Order Trichodermacei of the Hyphomycetes and described it as follows:-

ARTHRODERMA, Curr.

Peridium spurious, indeterminate, roundish, composed of interwoven, strongly constricted, jointed flocci. Spores collected in the centre.

Other descriptions of this species were given by Saccardo (1886) who renamed it Illosporium curreyi and by Salmon (1900) who replaced it in the genus Arthroderma. However, because of the then unique nature of the peridium and the belief that the species was a Hyphomycete, none of these descriptions is satisfactory.

Much the best of the early reports was that of Eidam (1880) who described what we now know was A. curreyi as the perfect state of C. serratus, classifying it in the Gymnoascaceae. That Eidam's cleistothecium and Currey's isolate were one and the same was noted by Smith (1904) who stated that the name A. curreyi had priority over C. serratus. Two problems remained from Eidam's description, the "conidial nodules" with a peridium similar to that of the cleistothecium and the distinctive sclerotia. The former has yet to be resolved but, in 1956, Benjamin noted that the sclerotia described by Eidam were

cleistothecia of another species of fungus and gave the first accurate definition of the genus Arthroderma in which he stated:-

ARTHRODERMA Berkeley, Outlines of British Fungology, p.357.1860. Cleistothecia whitish to pale yellow, globose. The peridium consisting of a network of branched, anastomosed, nearly hyaline hyphae composed of thick-walled, minutely and densely asperulate, constricted, symmetrical or asymmetrical, dumb-bell shaped cells; a few slender, elongate thin walled, septate, spiral appendages produced terminally or laterally; free ends numerous. Asci globose, 8-spored. Ascospores yellow in mass, small 2.4 - 3.3μ X 2μ, smooth, appearing by phase contrast to possess a slight equatorial groove. Clavate aleuriospores produced in abundance by the vegetative hyphae.

Kuehn in 1958 repeated in essentials Benjamin's description. However, as these descriptions of the genus were based on a single species they are now insufficiently wide and present slight inaccuracies. The following emended generic diagnosis is suggested, based upon that of Benjamin, but altered to cover all the species now incorporated in the genus.

ARTHRODERMA, Berkeley.

Homothallic or heterothallic. Cleistothecia formed singly or in clusters, globose or stelliform, white when young, shades of pale yellow or buff at maturity. Peridium a network of intertwined hyphae differing from vegetative hyphae. Peridial hyphae

septate, branching uncinate with branches recurving towards the centre of the cleistothecium, branches more or less spiral in some species. Peridial cells thick or thin-walled, echinulate to a greater or lesser degree, dumb-bell or modified dumb-bell in shape, symmetrical and asymmetrical, pale yellow, hyaline. The side of the cell towards the inside of the curve of the branch is often flattened with differentiation confined to the outer face. Appendages to the peridium:- 1) Thin, smooth-walled septate spiral hyphae, unbranched or very rarely branched, developed terminally or laterally from the peridial cells, few or many, number of turns and tightness of coiling variable. 2) In certain species, asexual spores arising from the peridial cells. Ascus mass globose. Asci formed in grape-like bunches on branched ascogenous hyphae, globose or sub-globose, thin-walled, evanescent, 8-spored. Ascospores small, lenticular, spherical in surface view, oblong-elliptical in side view, wall smooth or finely echinulate, yellow in mass. Asexual spores conidia, variable in size, shape and number of cells.

Identification

Species of Arthroderma are liable to be confused, at first sight, only with those of one other genus, Nannizzia, which was erected by Stockdale in 1961 for her perfect state of M. gypseum. On close inspection, however, the 2 genera may be seen to differ quite considerably in the type of branching of the peridial hyphae which is, in the main, verticillate in Nannizzia and uncinate in Arthroderma. Another, and in the opinion of Miss Stockdale and myself, important point of difference between the genera is the fact that in Nannizzia the cells of the peridium are, in the main, symmetrical whereas in Arthroderma a certain proportion of the cells are markedly asymmetrical. In their simplest form, the asymmetrical cells resemble dumb-bells with one side, usually that towards the inside of the curve of the branch flattened. Asymmetrical cells are also formed by the development of protuberances from each swollen end of the cell. The protuberances which, in the various species may number from one to 8, generally form on the outer face of the cell leaving the face towards the inside of the curve of the branch less differentiated. No such secondary alteration of the peridial cells takes place in the genus Nannizzia. In this genus some species, e.g. N. incurvata and N. obtusa form long straight appendages which project, often to considerable lengths, outside the peridium. Such appendages are unknown in Arthroderma. Nannizzia species in the imperfect state

are characterized by fusiform, septate, rough-walled macroconidia which are typical of the imperfect genus Microsporum. There is great variation in the types of asexual spores formed by species of Arthroderma. Some species form only microconidia, others only macroconidia and some both. Two species form large globose aleuriospores.

Within the genus Arthroderma, species determination is based primarly on the characteristics of the peridium of the cleistothecium. The type of branching of the peridial hyphae and the type of cells forming these hyphae are the main differentiating factors. Secondary factors which may be taken into consideration include the asexual spores which are often found in close association with the cleistothecia, if not actually formed from the cells of the peridium. In all species of Arthroderma smooth-walled septate spiral hyphae develop terminally or laterally from some of the cells of the peridium. In certain species the spirals tend to be less obvious and to lie within the network of the peridium, having probably been developed from peridial cells within the network. Others, however, are free and very obvious. These spiral hyphae are, to a limited extent, of use in identification but it should be noted that within a species there can be considerable variation, not only in the numbers produced but also in their degree of development. The most characteristic spiral hyphae are those of A. currevi.

Key to the species of the genus Arthroderma*

I Peridial hyphae of mature cleistothecia composed of symmetrical dumb-bell shaped cells; some cells may be flattened on one side.

A Peridial hyphae spiral at ends or over the whole length.

a Peridial hyphae spiral over most of the length; spiral hyphal appendages small (1 - 3 turns) and loosely coiled . . . . . A. curreyi

b Peridial hyphae spiral only at tips; spiral hyphal appendages well-developed (8 - 17 turns) and tightly coiled . . . . . A. cocleatus

B Peridial hyphae showing well-marked uncinate branching.

a Uncinate branching lax; peridial cells large (10 - 27μ X 6.6 - 7μ); many cells flattened on one side. Globose tuberculate conidia associated with the cleistothecium . . . . . A. tuberculatum

b Uncinate branching rather close; cells not large (7 - 11μ X 4 - 7μ); fusiform multiseptate thick-walled macroconidia associated with the cleistothecium . . . . . A. uncinatum

II Peridial hyphae of mature cleistothecia composed of cells with varying numbers of protuberances formed laterally from the swollen ends of the cell.

A Cleistothecia globose.

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* The perfect state of T. simiae has not been included as it has not yet been formally described.

a Peridial cells mainly with 3 protuberances at each end of the cell . . . . . A. cuniculi

b Peridial cells with 4 - 8 protuberances at each end of the cell . . . . . A. multifidum

B Cleistothecia stelliform.

a Cells with one protuberance at each end . . . A. minutum

b Cells mainly with 2 protuberances at each end . . . . . A. quadrifidum

c Cells mainly with 3 protuberances at each end . . . . . A. lomondii

The following species of Arthroderma were isolated in the course of this work:-

Arthroderma curreyi Berkeley

A. tuberculatum Kuehn

A. uncinatum Dawson & Gentles

A. multifidum Dawson

A. cuniculi Dawson

A. cocleatus sp. nov.

A. quadrifidum Dawson & Gentles

A. lomondii sp. nov.

A. minutum sp. nov.

Arthroderma curreyi Berkeley (plates 5, 6)

Homothallic. Cleistothecia globose, white when young, pinkish buff when mature, 450 - 850μ av. 600μ diameter. Peridium 90 - 160μ thick. Peridial hyphae pale yellow, hyaline, septate, spiral, branching. Peridial cells thin-walled (approx. 0.4μ), echinulate, dumb-bell or one-sided dumb-bell in shape with a single central constriction, 8 - 13.5μ X 4.5 - 7.2μ. Appendages to the peridium:- septate smooth-walled spiral hyphae, numerous, small (1 - 4 turns), lateral or terminal. Asci sub-globose, thin-walled, evanescent, 4 - 5μ X 3 - 4μ, 8- spored. Ascospores hyaline, smooth or finely roughened, lenticular (circular in front view, oblong-elliptical in side view), 1.8 - 2.4μ X 1 - 1.5μ,

yellow in mass.

Asexual state:- Microconidia and small thin-walled clavate macroconidia formed "en thyrses" or "en grappe".

Number of strains isolated:- Soil, 10; animals, 12; nests, 4.

Culture on soil with hair bait

The growth, prior to the production of cleistothecia, is white and floccose and rapidly colonizes all available bait. Asexual spores are not formed abundantly and the dominant method of reproduction is by cleistothecia. These first become apparent as very numerous small whitish dots in the mycelium, rapidly increase in size and, as they mature, become pinkish buff in colour. The mature cleistothecia of this species are relatively large and may be formed singly or in clusters.

Culture on agar media

In common with other species of Arthroderma, A. curreyi does not grow well on Czapek Dox agar. Otherwise, growth is good on all common agar media and cleistothecial formation has been observed on 4% malt extract agar, glucose peptone agar, Czapek Dox 1% peptone agar and Complete medium. No cleistothecia have been seen on 3% peptone agar.

On 4% malt extract agar, the edge of the colony is even and slightly sunken. The reverse is smooth and pale cinnamon buff,

shading to white at the edge. In a young colony (10 days at 24°C) the central area is slightly raised, finely floccose and ivory yellow. Outwith this area cleistothecia are developing and are apparent as small white clumps. In a mature colony the surface is very granular and pinkish buff in colour due to the large number of cleistothecia which have developed. On glucose peptone agar the edge of the colony is even and slightly sunken. The reverse is smooth and citron yellow in colour. The surface of the colony is smooth, white and floccose, with no signs of cleistothecia. Growth on 3% peptone agar is restricted. The edge is even and slightly sunken, the reverse is smooth and ochraceous orange in colour. The surface is white and floccose at the centre, surrounded by an area of velvety growth which is pale citron yellow in colour. Cleistothecia do not form on this medium.

Arthroderma tuberculatum Kuehn

The description given by Kuehn (1960) is as follows:- "Cleistothecia spherical to sub-spherical, creamy-white to creamy-yellow, distinct but often clustered into large aggregates, 435 - 970μ diam. appendages included; each ascocarp with one ascigerous mass forming a yellow spherical central region about ⅔ the diam. of the ascocarp. Peridium composed of a network of branched, anastomosed septate, almost hyaline hyphae composed of

thick-walled, densely asperulate, usually dumb-bell shaped cells; cells symmetrical or asymmetrical, elongate, about 4.0μ wide at narrow waist and 6.6 - 7.0μ wide at the enlarged ends, and 10 - 27μ long. Infrequently, peridial hyphae terminating in a slender, short, curved smooth hyphal segment, or terminating in a thin-walled, hyaline, septate, spiral sppendage, coiled in a spring-like manner with 2 - 3 turns measuring about 10μ wide and up to 60μ long; peridial hyphae usually not terminating in appendages. Asci hyaline, ovoid, 3.9 - 4.7μ X 4.4 - 5.5μ, 8-spored, the wall evanescent, ascospores conglomerate for only a short time, then separating. Ascospores bright yellow, smooth, flattened-oblate, often with noticeably flattened sides, 1.1 - 2.0μ X 2.5 - 3.0 (3.5)μ. Imperfect phase represented by cream coloured, lateral or rarely terminal, pedicellate, short-pedicellate or sessile, subglobose, ovoid or rarely elliptical aleuriospores measuring 8.8 - 14.3 X 13.2 - 18.7 (24.2)μ borne singly, covered at maturity with tuberculations or papillations of variable size and shape; pedicels 2.2μ diam., and 0.3 - 12.0μ long; aleuriospores numerous on aerial hyphae. Raquet mycelium present. Vegetative hyphae hyaline, 2.2 - 5.0μ diam."
Number of strains isolated:- Soil, 2; nests, 1.

There are some slight inaccuracies in Kuehn's description of this species. Following Benjamin's description of Arthroderma (1956), Kuehn states that the peridium is composed of a network

of branched anastomosed hyphae. I have not been able, despite a careful search, to find any anastomoses between peridial hyphae. Kuehn has also stated that, although the characteristics of his perfect state seemed identical to those of A. curreyi, they could be differentiated on the basis of the accessory spore phase. That these species would be confused seems unlikely. A. curreyi has peridial hyphae with a very typical spiral growth whereas A. tuberculatum has peridial hyphae which branch uncinately.

Arthroderma uncinatum Dawson & Gentles (plates 7, 8)

Heterothallic. Cleistothecia globose, pale buff, 300 - 900μ, av. 500μ diam., excluding appendages. Peridium 90 - 150μ, av. 125μ thick. Peridial hyphae pale yellow, hyaline, septate, uncinately branched to one side, the outside, of the main hypha. Cells thick-walled, strongly echinulate, symmetrically dumb-bell shaped, 7 - 11μ X 4 - 7μ. Appendages to the peridium:- Septate smooth walled spiral hyphae, varying in number of turns, lateral or terminal: smooth walled multiseptate, fusiform macroconidia, lateral or terminal. Asci sub-globose, thin walled, evanescent, 5.4 - 7.2μ X 4.9 - 6.3μ, 8-spored. Ascospores hyaline, smooth or finely roughened, lenticular (circular in front view, oblong elliptical in side view), 2.3 - 2.7μ X 1.4 - 1.8μ, yellow in mass.

Stat. conid. Keratinomyces ajelloi Vanbreuseghem (1952a).

Number of strains isolated:- Soil, 128; animals, 7; nests, 13.

Culture on soil with hair bait

The appearance of the culture is, to a certain extent, dependent on whether the inoculated strain will, or will not, eventually form cleistothecia. Strains growing asexually on the hair bait form macroconidia in such numbers that the bait is hidden by them and takes on the colour characteristic of the species. Sexual reproduction can often be forecast before initials are visible by the appearance of the culture. Such strains form considerably fewer macroconidia and present a somewhat more floccose appearance. It is in this floccose growth that the cleistothecial initials become apparent. Cleistothecia form abundantly and may be grouped or single.

Culture on agar media

A. uncinatum grows strongly on 4% malt extract agar, glucose peptone agar and 3% peptone agar but on Czapek Dox agar growth is thin. Cleistothecia are not produced except on Complete medium and even then are formed sporadically and are few in number.

On 4% malt extract agar after 10 days at 24°C, the edge of the colony is even and slightly sunken. The reverse of the colony is smooth and dresden brown in colour. The surface is

slightly raised at the point of inoculation but otherwise smooth, finely granular and shading from antimony yellow at the centre to light buff at the edges. On glucose peptone agar the edge of the colony is fern-like. The reverse is smooth and vinaceous purple in colour. The surface is flat and granular and shades from cinnamon buff at the centre to white at the edges. On 3% peptone agar the edge of the colony is even and the reverse smooth and orange buff in colour. The colony surface is granular and slightly raised at the point of inoculation and is cinnamon buff in colour.

Arthroderma multifidum Dawson (plates 9, 10)

Heterothallic. Cleistothecia globose, yellow to yellow buff, 250 - 550μ, av. 390μ diam., excluding appendages. Peridium 50 - 130μ, av. 110μ thick. Peridial hyphae pale yellow, hyaline, septate, uncinately branched, usually to one side, the outside, of the main hypha. Cells dumb-bell shaped at first, 5.8 - 12.5μ X 4.2 - 6.7μ, wall well defined, echinulate; cells when mature with 4 - 8 protuberances around each end of the dumb-bell, protuberances up to 5μ long and 1.6 - 2.5μ broad at the point of contact with the cell, frequently developing to a "T" or shallow "Y" shape. Appendages to the peridium :- septate smooth walled spiral hyphae with 2 - 7 turns, terminal or lateral; sub-globose or obovate unicellular conidia. Asci

sub-globose, thin walled, evanescent, 5 - 6.6μ X 4.5 - 5.8μ, 8-spored. Ascospores hyaline, smooth or very finely roughened, lenticular (circular in front view, oblong elliptical in side view), 2.5 - 3.1μ X 1.5 - 2.3μ, yellow in mass.

Asexual state:- Sub-globose or obovate, unicellular or rarely with 1 or 2 septa, conidia, 8.8 - 19.8μ X 6.6 - 13.2μ, walls smooth, 0.5 - 0.7μ thick. Conidia borne at the ends of the main or short undifferentiated side branches, occasionally sessile along the sides of the hyphae.

Number of strains isolated:- Soil, 11; rabbits, 3.

Culture on hair bait on soil

This species, in the asexual state, forms a creamy, slightly floccose growth around the hair and under low-power magnification the conidia are clearly visible. As is usual fewer conidia are formed when cleistothecia develop. The cleistothecia may be formed singly or in clusters and when mature are obvious.

Culture on agar media

After 10 days at 24°C on 4% malt extract agar, the edge of the colony is even, the reverse smooth, antimony yellow at the centre, shading to white at the edges. The colony surface is white, smooth, slightly raised at the point of inoculation, thinly granular and, at the edge, glabrous. In old cultures the

colony surface is yellow cream in colour. On glucose peptone agar, the edge is finely rayed and slightly sunken. The reverse of the colony is smooth and yellow. The central area of the surface is raised, finely granular and yellow cream in colour and outwith this area the growth is white and finely floccose. Growth on 3% peptone agar is identical to that on glucose peptone agar except that it is rather restricted. A. multifidum does not grow well on Czapek Dox agar.

Arthroderma cuniculi Dawson (plates 11 - 13)

Heterothallic. Cleistothecia globose, pale yellow, 180 - 380µ, av. 281µ diam., excluding appendages. Peridium 57 - 119µ, av. 88µ thick. Peridial hyphae pale yellow, hyaline, septate, uncinately branched, usually to one side, the outside, of the main hypha. Cells dumb-bell shaped at first, 5.8 - 10.8µ X 3.3 - 5.8µ, wall well defined, echinulate. Cells, when mature, with 3 (rarely 2 or 4) protuberances developed around each end of the dumb-bell, up to 3.5µ long and 1.2 - 2.7µ broad at the point of contact with the cell. Appendages to the peridium:- septate, smooth walled spiral hyphae with 4 - 16 turns, terminal or lateral. Asci sub-globose, thin walled, evanescent, 4.1 - 5.4µ X 3.7 - 4.9µ, 8-spored. Ascospores hyaline, smooth or finely roughened, lenticular (circular in front view, oblong elliptical in side view), 2.2 - 2.9µ X 1.4 - 2.0µ, yellow in mass.

Asexual state:- Hyaline conidia, 2.4 - 5.3μ X 1.0 - 2.0μ, unicellular, clavate, smooth walled, borne on short undifferentiated pedicels or sessile, "en grappe" occasionally "en thyrses".
Number of strains isolated:- Soil, 19; rabbits, 4.

Culture on hair bait on soil

In the asexual state this species forms a white, very luppy, granular growth around the hair. Microconidia are produced "en grappe" in dense clusters reminiscent of conidial nodules without a peridium. Young cleistothecia are difficult to discern in this species because they develop among the microconidial masses, but as the cleistothecia mature they become obvious due to their increase in size. The cleistothecia are generally formed in clusters.

Culture on agar media

After 10 days at 24°C on 4% malt extract agar, the colony edge is even and sunken. The reverse is smooth and amber yellow in colour. The surface of the colony is white, finely granular, glabrous at the edge and with sunken rays radiating from the point of inoculation. On glucose peptone agar, the edge of the colony is fern-like and submerged. The surface is rather more floccose than on malt extract agar, raised at the point of inoculation and shading from pale yellow to white at

the edges. The reverse is smooth and yellow. On 3% peptone agar the edge is fern-like and the reverse smooth except for the central area which shows concentric folding. The centre of the reverse is a dark yellow which shades to white at the edges. The surface of the colony is heaped up at the point of inoculation and this point is surrounded by another raised ring, the remainder of the colony is smooth. The central area is cream buff in colour shading to white at the edges. The surface growth in old colonies on all media is fairly heavy, granular and often yellow in colour and the reverse is orange yellow.

Arthroderma cocleatus sp. nov. (plates 14 - 16)

Heterothallic. Cleistothecia globose, buff pink, 256 - 560μ, av. 390μ diam., excluding appendages. Peridium 47 - 104μ thick, av. 75μ. Peridial hyphae pale yellow, hyaline, septate, branching uncinately to the outside of the hyphae. The outermost branches of the peridial hyphae are often coiled into a spiral. Cells, 6.6 - 13.2μ X 3.7 - 6.2μ, thin walled, echinulate, mainly symmetrical, dumb-bell shaped, often rather elongated and occasionally showing a third central swelling. Average breadth of cells at the narrow neck 2μ. Cells with marked globose inclusions which are not fats or nuclei. Appendages to the peridium:- septate, smooth walled spiral hyphae formed terminally, rarely laterally, from 8 - 17 turns, usually tightly

coiled. Asci sub-globose, thin walled, evanescent, 4.1 - 5.3 X 3.7 - 4.9µ, 8-spored. Ascospores hyaline, smooth or finely roughened, lenticular (circular in front view, oblong elliptical in side view), 1.0 - 1.6 X 2.2 - 2.6µ, yellow in mass.

Asexual state:-

Hyaline, smooth-walled, septate, branching hyphae bearing unicellular, thin walled, shortly clavate microconidia, 2.5 - 5.8 X 1.6 - 2.5µ. These are formed terminating short side branches and are formed in dense clusters. Spiral hyphae are also found.

Habitat:- Feathers in a swallow's nest.

Number of strains isolated:- Nest, 1.

This species was named A. coclestus because of the spiral nature of many of the outer branches of the peridium.

Culture on soil with hair bait

A buff-pink, finely granular coating is formed over the hair bait when the fungus is in the asexual state. Growth is more floccose when cleistothecia are going to develop. These are usually formed singly and when mature are buff pink in colour. This colour is very typical of the species.

Culture on agar media

On 4% malt extract agar after 10 days at 24°C, the edge of the colony is even and slightly submerged. The reverse is

smooth, golden yellow at the centre, shading to white at the edges. The surface is white, velvety at the centre to finely granular at the edge. The edge of the colony on glucose peptone agar is finely divided and slightly sunken and the reverse is smooth and reddish tan in colour. The colony surface is finely granular, raised at the point of inoculation and, in colour, buff pink at the centre shading to a paler pink at the edges. On 3% peptone agar the edge is even and sunken. The reverse is smooth and deep reddish tan in colour. The colony surface is smooth and finely granular, glabrous at the edge and raised at the point of inoculation. In colour the surface shades from pinkish buff at the centre to white at the edges. Old cultures on all these media show a heavy, finely granular growth which is evenly pinkish buff in colour.

Arthroderma quadrifidum Dawson & Gentles (plates 17 - 19)

Heterothallic. Cleistothecia stelliform, pale buff, 400 - 700µ, av. 580µ diam., excluding appendages. Peridium 80 - 135µ, av. 103µ thick. Peridial hyphae pale yellow, hyaline, septate, uncinately branched, usually to one side, the outside, of the main hypha. Cells thick walled, strongly echinulate, dumb-bell shaped when young, when mature resembling a short humerus bone with the condyles much accentuated and formed on one face only, 8.0 - 13.0 X 5 - 9µ. Appendages to the peridium:-

septate spiral hyphae which vary considerably in length and number of turns, lateral or terminal. Asci sub-globose, thin walled, evanescent, 4.0 - 6.0 X 3.5 - 5.0µ, 8-spored. Ascospores hyaline, smooth or finely roughened, lenticular, spherical in surface view, oblong elliptical in side view, 1.8 - 2.7 X 0.9 - 1.8µ, yellow in mass.

Stat. conid. Trichophyton terrestre Durie & Frey (1957)

Number of strains isolated:- soil, 21; animals, 32; nests, 9.

Culture on soil with hair bait

In the asexual state, the growth of the fungus around the hair is pure white, very granular and rather lumpy. This is caused by the profuse production of asexual spores. In some strains, the spores are formed in very dense masses resembling conidial nodules but lacking a peridium. Other strains form conidial nodules with a well developed peridium. The nodules often are formed close together and therefore tend to be rather irregular in shape. As with other species, asexual sporing is reduced if cleistothecia are going to develop. These are formed abundantly, either singly or in clusters. Cleistothecia and conidial nodules with a peridium have never been found in the one soil plate.

Culture on agar media

After 10 days at 24°C, on 4% malt extract agar the edge of

the colony is even and slightly sunken and the reverse smooth and buff pink in colour. The surface of the colony is finely floccose but becoming granular at the centre which is slightly raised. In colour, the surface shades from cream at the centre to white at the edges. The edge of the colony on glucose peptone agar is slightly irregular. The reverse is smooth and colourless. The colony surface is granular and, at the centre which is slightly raised, is cream coloured. On 3% peptone agar the edge of the colony is slightly uneven and the reverse smooth and yellow buff in colour. The surface is granular and creamy yellow at the centre and white and floccose at the edges. Old colonies on all the above media show heavy granular growth which is dark cream in colour.

Arthroderma lomondii sp. nov. (plates 20 - 22)

Heterothallic. Cleistothecia stelliform, light buff to cream buff, 330 - 570μ, av. 450μ diam., measured to points of the star, excluding appendages. Peridium 76 - 152μ, av. 107μ thick, measured to a point of the star. Peridial hyphae pale yellow, hyaline, septate, branching uncinately. In the star points the branching is more complicated, some hyphae forming branches to each side, side branches also branching, all branches re-curving towards the centre of the cleistothecium. Cells thin walled, echinulate, dumb-bell shaped when young, when

mature developing 2 or 3, rarely 4, protuberances on the outer face of each end of the cell. Cells 5.3 - 12.5μ, av. 7.2μ in length and 2.5 - 4.9μ, av. 3.8μ in breadth excluding protuberances. Protuberances up to 2.5μ in length and 0.8 - 2.0μ in breadth at the point of contact with the parent cell. Appendages to the peridium:- septate, smooth walled spiral hyphae of 2 - 6 turns, arising terminally or laterally. Asci sub-globose, thin walled, evanescent, 3.3 - 4.9μ X 2.9 - 4.2μ, 8-spored. Ascospores hyaline, smooth or finely roughened, lenticular (circular in front view, oblong elliptical in side view), 0.83 - 1.45μ X 1.8 - 2.6μ, yellow in mass.

Asexual state:- Hyaline, smooth walled, septate, branching hyphae. Macroconidia, 5.8 - 20μ, av. 11μ X 2.0 - 3.7μ, av. 2.7μ, with 1 - 3 septa, clavate. Macroconidia sessile or terminating short side branches, "en thyrses". Microconidia 2.5 - 4.2μ, av. 3.2μ X 1.2 - 2.1μ, av. 1.7μ, smooth walled, clavate, formed on the hyphae "en grappe" and "en thyrses.

Habitat:- Soil, animal hair and bird's nest.

Number of strains isolated:- soil, 1; mouse, 1; nest, 1.

This species was named A. lomondii because the 3 isolates were recovered from the Loch Lomond district.

Culture on soil with hair bait

In the asexual state, a thin very floccose, whitish growth covers the hairs. Very few asexual spores are produced and

these are invariably macroconidia. Microconidia which are formed so abundantly on agar media have not been seen on hair bait on soil. The cleistothecia, which are markedly stelloid, form profusely, generally singly but occasionally in clusters.

Culture on agar media

On 4% malt extract agar after 10 days at 24°C, the edge of the colony is even and slightly sunken. The reverse is smooth, golden yellow at the centre, shading to white at the edges. The surface is flat, slightly raised at the point of inoculation, finely granular and pale cream in colour shading to white at the edges. On glucose peptone agar the colony edge is irregular, fern-like and slightly sunken. The reverse is smooth, shading from golden yellow at the centre to colourless at the edge. The edge of the colony on 3% peptone agar is slightly uneven and sunken. The reverse is smooth and creamy buff in colour. The colony surface is even, very pale cream in colour and floccose. In old cultures on all the above agar media, the growth is granular and creamy yellow in colour.

Arthroderma lomondii variety (plates 23, 24)

Of the 5 isolates originally believed to be A. lomondii, I have separated 2 and, for the present, shall consider them as a variety of this species. The strains of the variety were isolated from duck nests found on an island in Loch Leven. Although the strains of A. lomondii and of the variety will mate freely among themselves, they will not cross-mate with each other. A comparison of the cleistothecia of the variety with those of the species showed them to be almost identical in morphology. There are, however, some slight differences in size which can be seen from table 12. In the imperfect state also, the species and variety show some differences in size and morphology but, again, these are so insignificant that it would be difficult to tell one from the other.

Before it can be decided if these are separate species or varieties of one species, it will be necessary to study and compare perfect and imperfect states of every isolate on a wide variety of substrates.

Arthroderma minutum sp. nov. (plates 25, 26)

Homothallic. Cleistothecia stelliform, cream buff when mature, 313 - 494μ, av. 380μ maximum diameter, excluding appendages. Peridium 66 - 123μ, av. 92μ thick measured to a point of the star. Peridial hyphae pale yellow, hyaline, branching frequently and closely in an uncinate manner. In the star points the branching becomes more complicated. Peridial cells thin walled, echinulate, dumb-bell shaped when young, 5.8 - 9.9μ X 3.3 - 5.3μ, when mature with a single protuberance, up to 5μ long and 1.6 - 3.3μ broad, on the outer face of each end of the cell. Appendages to the peridium:- smooth walled, septate, spiral hyphae, from 1 - 4 turns, loosely coiled, lateral, occasionally terminal, usually formed singly from the cells but occasionally 2 from one cell. Asci sub-globose, thin walled, evanescent, 3.3 - 4.9μ X 3.3 - 4.5μ, 8-spored. Ascospores hyaline, smooth or finely roughened, lenticular (circular in front view, oblong elliptical in side view), 1.7 - 2.5μ X 0.8 - 1.7μ, yellow in mass.

Asexual state:- Hyaline septate, smooth walled hyphae bearing clavate, smooth walled macroconidia, 7.4 - 16.6μ, av. 10.1μ X 2.0 - 3.3μ, av. 2.9μ, with one or 2 septae, sessile. Microconidia 2.5 - 3.7μ, av. 3.0μ X 1.0 - 1.8μ, av. 1.5μ, clavate with slightly roughened walls, formed "en grappe".

Habitat:- soil from rabbit burrows.

Number of strains isolated:- soil, 3.

The fungus was given the specific name minutum because of the

smallness of the cleistothecia.

Culture on soil with hair bait

In the asexual state, growth is whitish and thinly floccose. The species grows very slowly and cleistothecia take over 2 months to reach maturity. There is, apparently, some factor or factors not completely suitable in the soil hair bait conditions supplied in the laboratory. Although each of the primary isolation plates in which this species was found showed only localized growth, each patch contained perfectly formed cleistothecia with a well-developed ascus mass. In laboratory soil hair bait culture, however, although cleistothecia with asci were seen in each plate inoculated, the majority of the cleistothecia contained little or nothing in the way of contents although the peridia were well-formed.

Culture on agar media

After 10 days at 24°C the edge of the colony on 4% malt extract agar is even, slightly sunken and finely divided. The reverse is smooth, old gold at the centre shading to colourless at the edge. The surface of the colony is cream in colour and finely granular, graduating to glabrous at the edge. On glucose peptone agar the edge is irregular and fern-like. The reverse is smooth and old gold in colour at the centre. The surface of the colony

is floccose and shades from yellow cream at the centre to white at the edge. On 3% peptone agar, the edge is even and the reverse smooth and buff at the centre shading to white at the edge. Old cultures on all the above media become granular with a dark buff reverse.

Comment

Although A. curreyi has been described by Benjamin (1956) and Kuehn (1958), the descriptions are rather lacking in detail, probably because the genus was then represented by a single species. Benjamin gives only a generic description and states that the peridial hyphae of the cleistothecium anastomose and that the ascospores appear to possess a slight equatorial groove. Although the isolates from the survey were carefully studied with regard to these 2 points, neither could be confirmed.

Certain features of the cleistothecia of A. curreyi are, to my mind, extremely characteristic of the species. On examination under low magnification, peridial appendages consisting of small spiral hyphae of 1 - 4 turns are noticeable as also is the spiral growth of the peridial hyphae (plate 5a). This growth form, although still visible in mature cleistothecia (plate 5c), is particularly noticeable in immature cleistothecia in which differentiation of the peridial cells is incomplete (plate 5b).

Side branches are formed singly from the peridial hyphae (plate 5c) and usually re-curve towards the centre of the cleistothecium. It seems probable that the branching pattern is basically like that of other species of Arthroderma but that this is masked by the spiral growth of the main hypha. In each cleistothecium, some non-spiral peridial hyphae are present (plate 6c). These are composed of cells in which only one side,

that to the outside of the curve, has differentiated. These hyphae have been compared (Grove, 1922) to the comb-like hyphae formed by the sclerotia described by Eidam (1880) under the name C. serratus. These one-sided hyphae are not, however, typical only of A. curreyi but are found in most species of Arthroderma.

Eidam (1880), for the fungus we now know as A. curreyi, described conidial nodules consisting of dense masses of microconidia formed "en grappe" on much-branched hyphae, surrounded by a peridium of the type found in the cleistothecia. With the exception of Dangeard (1907), no other worker has reported finding these structures in A. curreyi. Attempts, made in this laboratory, to induce them to form by altering the conditions for growth have failed. It is possible, therefore, that Eidam had yet a third species in the complex which he described under the name C. serratus.

A. tuberculatum, in the characteristics of its cleistothecia, most closely resembles A. uncinatum. Each species forms globose cleistothecia with simple uncinate branching, dumb-bell shaped cells and one-sided cells. However, the cleistothecia of A. tuberculatum can, even in the absence of asexual spores, be differentiated from those of A. uncinatum. In A. tuberculatum, the peridial cells are larger, the uncinate branching more lax and a higher proportion of one-sided dumb-bell shaped cells is found. Although it has not yet been proved that asexual spores

of A. tuberculatum are formed by the cells of the peridial hyphae, as are those of A. uncinatum, they are generally closely associated with the cleistothecia and serve as an additional aid in identification.

In culture on agar media, in the absence of cleistothecia, it is possible that A. tuberculatum might be confused with A. multifidum and C. serratus. Each species forms similarly-shaped spores but those of A. multifidum (plate 10f) are smooth walled. The spores of C. serratus, which are rough walled like those of A. tuberculatum, are borne on the hyphae in a different manner. In soil hair bait culture, in the asexual state, only A. tuberculatum and A. multifidum have a similar type of growth. C. serratus grows on the hair in intermittent clumps and the hair, therefore, presents a very characteristic beaded appearance.

In the cleistothecia of A. uncinatum, macroconidia are formed from the cells of the peridial hyphae (plate 8b, 8c). Both immature and mature macroconidia have been seen attached to mature peridial hyphal cells which suggests that, despite their dead appearance and lack of noticeable cell contents, the peridial cells are living. That this is so, is also shown by the ability of peridial cells of all species of Arthroderma to contract on dehydration and when re-hydrated to recover their original shape. Dehydrated peridial cells are shown in plate 28c.

It is not likely that the thick, smooth walled, multiseptate

macroconidia of A. uncinatum (plate 8d) will be confused with those of any other species of Arthroderma. However, they might easily be mistaken for the macroconidia of a species which was first reported as a pathogenic strain of K. ajelloi (Georg, Kaplan, Ajello, Williamson & Tilden, 1959) but was later proved to belong in the genus Nannizzia (Georg, Ajello, Friedman & Brinkman, 1962). The spores of this species, although resembling those of K. ajelloi in size, shape and the manner in which they are borne on the hyphae, are distinguishable by their rough wall. Microconidia are also abundant in cultures of this species but are not, or only rarely, found in K. ajelloi.

A proportion of the strains of K. ajelloi isolated appear to be incapable of sexual reproduction. Cross-mating with single ascospore strains of each mating type and mating in all possible combinations of pairs among themselves gave negative results. Non-mating strains were first noted by Dawson & Gentles (1961) who stated that they differed from Vanbreuseghem's description (1952a) in that they did not form the dark coloured pigment. It was suggested that the ability to produce pigment might be correlated with cleistothecial formation. In general, this statement has proved true, pigmenting strains being able to form cleistothecia while non-pigmenting strains are not. Only one non-pigmenting strain has formed cleistothecia when mated with single ascospore strains.

Another characteristic of the strains which will not form cleistothecia is that they appear to produce rather elongated macroconidia. However, this cannot be relied on as a guide because I have noted that macrospore length can vary considerably in a strain depending on the type of keratinous bait on which it is grown. It may eventually be proved that K. ajelloi is a complex of almost identical species similar to that described for M. gypseum by Stockdale (1963).

Within the genus Arthroderma, the highest degree of differentiation of peridial cells is found in the cleistothecia of A. multifidum. The cells of mature cleistothecia (plate 10c, 10d) develop from 4 to 8 protuberances around each end and the original dumb-bell shape (plate 10b) is completely lost. A similarity to A. curreyi is found in the spiral hyphae (plate 9a, 9b) which are not highly developed. However, because of the highly differentiated peridial cells, the uncinate branching (plate 10a) and the distinctive asexual spores (plate 10f), there is not much likelihood that cleistothecia of A. multifidum will be confused with those of A. curreyi.

Although, like A. multifidum, A. cuniculi has been proved to be associated with rabbits there is no marked morphological similarity between the species. In the young state the cleistothecia of A. cuniculi, which are fairly small, are not obvious because they tend to be embedded in the masses of asexual

spores formed on the hair (plate 13d). However, mature cleistothecia can easily be seen (plate 13e). The peridial cells of the cleistothecia generally differentiate by forming 3 protuberances at each end of the cell (plate 12d). In this respect, the cleistothecia of A. cuniculi resemble those of A. lomondii. The species can be separated, however, because the cleistothecia of A. cuniculi are globose whilst those of A. lomondii are definitely stellate (plate 20a). In addition, the type of growth of these species on soil with hair bait differs considerably; that of A. cuniculi is markedly granular with dense clumps of conidia whereas that of A. lomondii is thin and floccose.

The cleistothecia of A. cocleatus, although distinctive, do show a similarity to those of A. curreyi insofar as the outermost branches of the peridial hyphae are cochleate (plate 14e). However, unlike A. curreyi, there is no spiralling of the inner branches which branch in an uncinate manner (plate 15a). The spiral hyphae formed by the peridial cells are generally extremely well developed and fairly tightly coiled.

The cells of the peridium of A. cocleatus are distinctive (plate 15d-g). Most of the cells are of an elongated dumb-bell shape and many have globose inclusions, the nature of which it has not been possible to determine. They are not nuclear because apart from their large size, they do not stain in acetic orcein

and cannot be fat because no red colour was produced with Sudan III. They did, however, stain deeply with cotton blue and light green and so must be concluded to be cytoplasmic in nature. Inclusions of this type have not been seen in the peridial cells of any other species of Arthroderma.

Although there can be no doubt that A. cocleatus belongs in the genus Arthroderma, in peridial cell structure it resembles species of Nannizzia in that a number of cells are elongated and have a small central swelling such as is often found in cells of N. incurvata. However, the elongated straight projections from the peridium, which are one of the characteristics of the genus Nannizzia, are lacking.

A. quadrifidum was the first species of Arthroderma discovered which forms stellate cleistothecia. The peridial hyphae show close uncinate branching and the cells are distinctive, having 2 protuberances at each end (plate 18b)giving the cells the appearance of a short humerus bone with the condyles much accentuated and formed on one face only.

Conidial nodules (plate 19a) may be formed. These, although somewhat irregular in shape, because they are usually formed in clusters, are identical to the cleistothecia in the morphology of the peridial hyphae and cells. The spores, which are formed in great profusion within the nodules, are microconidia and develop "en grappe" on much-branched hyphae. Although nodules can be

induced to form in strains capable of sexual reproduction, it has not proved possible, with a number of strains forming conidial nodules including the type species, to induce sexual reproduction although these strains have been mated with single ascospore strains and in combinations of pairs among themselves. However, from the morphology of the nodules and by comparison with nodules formed by strains capable of sexual reproduction, it seems unlikely that they can belong to separate species. Possibly reproduction by microconidia from conidial nodules may have replaced reproduction by ascospores.

It was noted (Dawson & Gentles, 1961) that a number of strains of T. terrestre which were apparently asexual in primary isolation plates, were, however, mixed and therefore capable of sexual reproduction. Cleistothecia of A. quadrifidum formed when such strains were mated with plus and with negative single ascospore strains. No reason has yet been found to explain why the perfect state did not form in the primary isolation plates.

In A. lomondii the cleistothecia are stellate and the peridial cells form 3 protuberances at each end (plate 21d). A number of cells, however, form only 2 protuberances (plate 21c). A. lomondii differs from A. quadrifidum so much in culture on hair bait on soil that it is unlikely that the species would be confused. On hair bait, the growth of A. lomondii is thin and very floccose (plate 22a) and only few asexual spores are formed.

A. quadrifidum, on the other hand, shows very granular growth and many asexual spores are produced.

Two strains of A. lomondii were isolated from duck nests in the Loch Leven district. These strains, I believe to be a variety of the species because although morphologically indistinguishable from the strains from the Loch Lomond district they will not cross-mate with them.

The cleistothecia of A. minutum are easily identified by their small size and by the peridial cells which form only one protuberance at each end of the cell (plate 26a). These cells are not likely to be confused with the one-sided dumb-bell shaped cells formed by other species because of the length of the protuberances.

Identification of certain species of Arthroderma, in culture on agar media, is difficult. A. curreyi generally forms cleistothecia but the other species do not. Only A. uncinatum, A. tuberculatum, A. multifidum and A. cocleatus form distinctive asexual spores. A. quadrifidum in culture on 4% malt extract agar shows a typical buff pink colouration of the reverse of the colony. Of the remaining species, only A. cuniculi does not form macroconidia but this type of spore can be rare or lacking in cultures of A. lomondii and A. minutum. The sizes of the spores in these 3 species are also much alike. One can, with practice, separate these species on morphology and the appearance of the colony. It is advisable, however, to confirm the identification

by culture on soil with hair bait. On this substrate each species grows quite differently.

Trichophyton globiferum sp. nov. (plates 27 - 30)

Colonies growing well on agar media. Mycelium composed of septate, hyaline, smooth walled, branching hyphae. Spiral hyphae abundant in old cultures. Short clavate microconidia, 2 - 4.5μ X 1.2 - 2.2μ, with thin smooth walls formed along the hyphae or in grape-like clusters. Multiseptate, 2 - 9 av. 5 septae, clavate macroconidia, 9.1 - 18.2μ, av. 14.8μ X 2.8 - 4.5μ, av. 3.5μ, with thin, smooth or rarely slightly roughened walls, sessile on the hyphae or terminating short side branches. Macroconidial-like structures formed in an intercalary position. Bulbils produced abundantly, sub-globose, 37 - 100μ X 33 - 70μ, white or very pale yellow, composed of irregularly shaped cells with thin smooth walls. In soil hair bait culture globose structures resembling cleistothecia or conidial nodules but lacking contents may form. These consist of a peridium composed of uncinately branching hyphae. The peridial cells are dumb-bell in shape, 6.6 - 10.8μ X 3.3 - 5.4μ, with finely echinulate walls. Well developed, 14 - 28 turns, spiral hyphae are formed laterally or terminally from some of the peridial cells.

Perfect state:- unknown.

Habitat:- birds' nests.

Number of strains isolated:- nests, 2.

The fungus was placed in the genus Trichophyton because of its asexual spores and named globiferum as being descriptive of the

globose bulbils.

Culture on soil with hair bait.

The fungus is definitely keratinophilic, growing on and in the vicinity of the hair. Asexual spores, mainly macroconidia, are formed by the growth on the hair but the bulbils are more often found on soil in the vicinity of the bait. These can be seen with the naked eye as small whitish dots. The presence of these bulbils makes this fungus very easy to identify under low magnification. In some plates after a month a few nodules can be seen developing. Soil plates containing these nodules have been kept for over 6 months but no contents have developed.

Culture on agar media.

T. globiferum grows well on 4% malt extract agar, glucose peptone agar and 3% peptone agar but not on Czapek Dox agar on which growth is thin and spreading. On 4% malt extract agar after 10 days at 24°C, the colony edge is even, the reverse smooth and pale clear yellow in colour. The surface is finely floccose, thin at the edge and very pale yellow in colour. On glucose peptone agar the edge of the colony is irregular and the reverse smooth and very pale yellow. The surface is granular and white in colour. The edge of the colony on 3% peptone agar is irregular. The reverse is smooth and pale yellow tan in colour.

The surface growth, which is slightly raised over the point of inoculation is velvety and pale yellow tan in colour.

Only 2 strains of T. globiferum were recovered. The species was isolated, each time, from the nest of a hedge sparrow. Because the nests were found in different districts, this suggests that T. globiferum may eventually, when more nests and feathers have been investigated, be proved to have some connection with hedge sparrows.

Although no other species of Trichophyton forms bulbils, the morphology of the asexual state of my isolates is such that they belong in this genus. In addition, the nodules formed by the fungus, although lacking contents, are Arthroderma in type and resemble those of T. terrestre (plate 19) and T. mentagrophytes (plate 31).

There can be little doubt that the globose structures are bulbils. They arise from more than a single initial and develop in the manner described by Hotson (1917). Some stages in their development are shown in plate 29. The mature bulbils germinate by producing many germ tubes around the periphery.

Many dermatophyte species in the genus Trichophyton produce structures consisting of a small knot of entangled hyphae. These nodular organs are generally believed to be degenerate cleistothecial initials but, with the discovery of T. globiferum,

it now seems possible that they might equally well be bulbiferous in nature.

Ctenomyces serratus (plates 32 - 36)

C. serratus was the name given by Eidam (1880) to a fungus which he discovered and classified in the family Gymnoascaceae. In addition to the perfect state, Eidam described sclerotia with very characteristic hooked appendages. Benjamin (1956) stated that these sclerotia were the perfect state of another gymnoascaceous fungus and, for it, revived the name C. serratus which had been invalidated by Smith (1904). Frey & Griffin (1961) related the imperfect state to the perfect state.

Cleistothecia of C. serratus were recovered 3 times in the course of the surveys. Single ascospore cultures were made and the species was proved to be heterothallic. Cleistothecia formed freely on "negative" soil with horse hair bait and the stages in their development, which have not previously been described, were studied.

Cleistothecial initials appear first as rather short club-shaped structures which curve slightly around each other (plate 34a). One of these develops into a septate club-shaped antheridium and the other elongates to form the septate ascogonium which coils in a loose spiral around the antheridium (plate 34b). The initials quickly become irregular and enlarge to form a globose amorphous mass in which, until the cleistothecium is almost mature, it is impossible to see any detail (plate 34c). If an almost ripe cleistothecium is crushed, the asci can be seen

forming in bunches on branched ascogenous hyphae.

The peridium begins to develop at the time the initials become irregular in shape. Hyphae grow out from the initials and from the hyphae close to the initials. These hyphae branch, anastomose, increase in diameter and the outer network takes shape (plate 34c). From some of the outer peridial cells elongated club-shaped structures are formed. These become septate and from one side of all but the lower cells hook-like projections develop (plate 35). The distal cell of each appendage often degenerates (plate 36a). The walls of the peridial hyphae and appendages thicken and become roughened and orange-brown in colour. If the transverse septae in the appendages are carefully studied a pore (plate 36b) connecting the cells can be seen.

Fine internal hyphae (plate 36d) give rise to the thick walled outer hyphae (plate 36c). The innermost layer of the peridium is formed of pseudoparenchyma which is more than one cell thick and is composed of irregularly shaped cells (plate 36c, 33b). This layer can be demonstrated by crushing a cleistothecium on agar medium and rolling it around until the ascospores have been removed.

The presence of this pseudoparenchymatous wall, which was not mentioned by Benjamin (1956), means that the perfect state of C. serratus is a perithecium. Because of this, it is exceedingly doubtful if C. serratus can remain in the family Gymnoascaceae.

According to Clements and Shear (1954), the order Perisporiales of the class Ascomycetae is distinguished from the Gymnoascales by having a perithecium with a distinct wall. Within this group, C. serratus might be considered to have resemblances to the family Erysiphaceae as members of this family form perithecia with membranous walls and well developed appendages. However, the asci are formed either in a basal umbel or singly, the perithecial wall is brittle and all the species known are plant pathogens. Therefore, C. serratus cannot be placed in this family.

The family Eurotiaceae in the Perisporiales forms true perithecia but resembles the Gymnoascales because the asci are borne on branched ascogenous hyphae. The only genera in the family Eurotiaceae which form appendages to the peridium are Aphanoascus, Chaetotheca, Cephalotheca, Magnusia, Pleurascus and Arachnomyces. However, the appendages are small and not at all comparable to the massive peridium of C. serratus.

Thus, as C. serratus cannot be satisfactorly be placed in any existing family it would be necessary to create a new family for it which would lie between the family Eurotiaceae of the Perisporiales and the family Gymnoascaceae of the Gymnoascales. However, because only one species is included in the genus Ctenomyces, to do this is, from a practical point of view, not feasible. Therefore, until further species have been discovered,

I believe it would be better to leave C. serratus in the family Gymnoascaceae.

A paper on this subject was published recently (Orr & Kuehn, 1963). I should like to state that, although I have corresponded with these authors on a variety of subjects, my study of C. serratus was independent and the conclusions reached were the result of my own work and thought.

Amauroascus verrucosus Schroeter (plate 37)

Cleistothecia globose, large, 1 - 3mm. in diam., white when young, pale cinnamon brown when mature, formed singly or in clusters. Peridium thin in comparison to the diameter of the ascus mass, composed of branching, interwoven, hyaline or pale straw coloured hyphae which are slightly stouter than the vegetative hyphae but otherwise undifferentiated. Racquet hyphae also present in the peridium. Peridial cells elongated cylindrical, 12 - 26μ X 1.5 - 3.3μ, with thin smooth walls. Appendages to the peridium lacking. Asci globose, thin-walled, evanescent, 18 - 22μ X 16 - 21μ, 8-spored. Ascospores spherical, thick walled (0.75μ), tuberculate, 6.5 - 8.0μ in diameter, cinnamon brown in mass.

Asexual state:- unknown

Vegetative state:- smooth walled septate, branching hyphae and racquet hyphae. Elongated cylindrical chlamydospores formed.

Number of isolates:- soil, 1.

The genus Amauroascus in the family Gymnoascaceae was erected by Schroeter (1893) for the species A. niger and A. verrucosus. Apart from a paper by Dangeard (1907) describing the development of the perfect state of A. verrucosus and a report of the isolation of A. niger from soil in Russia, there is little else in the literature about these fungi.

Neither Benjamin (1956) nor Kuehn (1958) was able to obtain living cultures or herbarium material of either species.

A fungus, identified as A. verrucosus, was isolated, in the course of the soil survey, from one sample of soil. No difficulty was found in obtaining cultures from whole cleistothecia or from vegetative mycelium but the ascospores proved exceptionally difficult to induce to germinate. Although over 150 single ascospores, from cleistothecia of all ages up to a year and from cleistothecia held at low temperatures for periods up to several months, were planted, only 3 grew.

The fungus grows well on all the usual agar media but, in general, does not form cleistothecia. On 4% malt extract agar, however, cleistothecia are formed but only after the cultures are very old. The colony, which is white, thinly floccose and spreading, with a smooth colourless reverse, consists of branching hyphae which may become divided up into elongated chlamydospores with thin walls. Asexual spores were not found although attempts were made to induce their production by culture on natural substrates and on drops of agar on slides.

Culture on soil hair bait was much the best way to obtain cleistothecia. At first, a white, rather floccose growth covers the hairs. Cleistothecial initials become apparent as small white dots which increase rapidly in size. The cleistothecia, which are white when young and become pale cinnamon brown when

mature, due to the colour of the ascospores, are formed singly or in clusters.

Dangeard (1907) conserved A. verrucosus for 3 years in the laboratory by growing it in a mixture of feather debris and dung, and on this base studied the development of the cleistothecia. The descriptions and illustrations of this are very interesting and each stage illustrated has been seen in my isolate. Dangeard states that the asci are 20 - 22μ X 30μ and the ascospores 8 - 10μ with echinulate walls. The measurements I have made for asci are 18 - 22μ X 16 - 21μ and for ascospores 6.5 - 8.0μ in diameter. Otherwise, Dangeard's description tallies with my strain.

Benjamin (1956) has suggested that if, when the species of Amauroascus are re-discovered, no major characters other than spore colour serve to distinguish Amauroascus from Arachniotus, it would seem unnecessary to retain both names. However, such re-evaluation must now await the recovery of A. niger.

Arachniotus ovinus sp. nov. (plates 38 - 40)

Cleistothecia globose, often confluent, large, 1 - 2mm. diam., white when young, pale yellow when mature. Peridium well-developed, composed of hyaline, septate, branching, anastomosing, flexuous hyphae, often with a spiral configuration. Peridial cells 9.0 - 45μ X 2.5 - 4.5μ, thin walled, tuberculate, elongated cylindrical in shape, contorted. Appendages to the peridium:- thin walled, loosely coiled spiral hyphae, 2 - 4 turns, unbranched and branched, formed terminally or laterally. Asci globose, evanescent, 5.0 - 6.6μ X 4.5 - 5.8μ, 8-spored. Ascospores lenticular, lacking ridges or furrows, smooth walled, 2.6 - 3.3μ X 1.0 - 1.6μ, golden yellow in mass.
Asexual state:- smooth walled, septate, branching hyphae bearing obovate conidia, 6.6 - 13.2μ X 3.3 - 6.6μ, with finely echinulate walls sessile on the hyphae or terminally on short side branches.
Habitat:- soil under a sheep's carcass.
Number of strains isolated:- soil, 1.
The specific name ovinus was chosen because the fungus was found in association with a dead sheep.

Culture on soil with hair bait

When inoculated baited soil is held at a suitable termperature (room temperature or lower temperatures) A. ovinus quickly covers the bait with a rather fine white overgrowth. Conidia are

formed abundantly but, as with other species, the production of conidia is reduced when cleistothecia are going to develop. The cleistothecia form in clusters or singly.

Culture on agar media

This species was first obtained in culture on agar medium by the use of a low temperature of incubation (5°C). However, in the time since its original isolation (10 months), it has developed the ability to grow at room temperature. The colonies which are described were grown at room temperature.

A. ovinus grows well on 4% malt extract, glucose peptone and 3% peptone agar. Cleistothecia do not apparently form on agar media. On 4% malt extract agar the edge of the colony is smooth and even. The reverse is smooth and pale yellow in colour. The surface is finely granular, cream at the centre to white at the edges which are glabrous. Growth is heavier on glucose peptone agar. The edge of the colony is even and the reverse smooth and pale yellow in colour. The surface is smooth, cream to white in colour, granular but slightly floccose and raised at the point of inoculation and at the edges. A. ovinus grows more slowly on 3% peptone agar but the colony is little different except that the reverse, which is pale yellow, shows a number of radial furrows which, however, are not apparent on the colony surface. Old cultures on all the above media are

rather more granular and a definite cream in colour.

The only strain of A. ovinus was found in the perfect state in soil under a sheep which had been dead for over a year. Had the fungus not been visible in the sample, it would probably have been missed because, originally, it would not grow at 24°C or even at room temperature. It was obtained in pure culture by placing baited soil samples and inoculated Petri dishes of agar media in the refrigerator at 5°C.

The characteristics of the cleistothecia of A. ovinus agree with those of the genus Arachniotus of the family Gymnoascaceae. Species in this genus form globose cleistothecia with thin walled, uniform, loosely interwoven peridial hyphae; the asci are 8-spored and the ascospores are ovoid, oblate or ellipsoidal, hyaline or light coloured, with smooth or sculptured walls. However, A. ovinus could not be placed in any of the described species. The fact that it has smooth walled ascospores bars it from A. reticulatus, A. aureus and A. trachyspermus and the colour of the cleistothecium from A. ruber and A. dankaliensis. Of the remaining species, A. terrestris produces very large asci (13.5μ diam.) and in A. candidus the peridial hyphae are lacking or scanty with thin smooth walls.

A. ovinus, in the characteristics of the cleistothecium, superficially resembles species of Arthroderma and Nannizzia.

However, it differs from these genera in the very elongated peridial cells with tuberculate walls, the flexuous peridial hyphae and the branched spiral hyphal appendages.

A. ovinus strongly resembles the next species to be described, Pararachniotus gelicola, although it could not be put into the genus Arachniotus because the perfect state is not known.

Pararachniotus gelicola gen. nov., sp. nov. (plates 41 - 44)

Pararachniotus gen. nov.

Colony on agar media growing well. Vegetative hyphae hyaline, septate, branching. Conidia sessile on the hyphae or terminating short side branches. Globose conidial nodules with a peridium of interwoven, branching, flexuous, anastomosing hyphae composed of elongated cylindrical cells with thin tuberculate walls. Outer peridial cells giving rise to thin, smooth walled hyphal appendages, lateral or terminal, branched or unbranched. Peridium enclosing a globose mass of conidia. Type species:- P. gelicola

P. gelicola sp. nov.

Conidial nodules often confluent, globose, white when young buff when mature, 0.5 - 2.0mm. in diameter. Well developed peridium of interwoven, anastomosing, hyaline, septate, flexuous hyphae composed of elongated cylindrical contorted cells, 10 - 42μ X 3.0 - 5.0μ, with thin tuberculate walls. Septate smooth walled spiral hyphae of 1 - 4 turns, branched or unbranched, formed laterally or terminally from outer peridial cells. Conidia within nodule unicellular, obovoid or ellipsoidal, 3.3 - 6.6μ X 1.2 - 2.4μ, formed in dense clusters on branched hyphae. Conidia also on hyphae outwith nodules. Formed along the hyphae, sessile or terminating short side branches, obovoid or ellipsoidal,

4.2 - 8.3μ X 1.2 - 3.3μ, walls thin and finely tuberculate.

Vegetative state:- Hyaline septate, smooth walled, branching hyphae and racquet hyphae.

Number of strains isolated:- nests, 2.

Habitat:- birds' nests.

The fungus was named Pararachniotus because of its resemblance to A. ovinus and gelicola because of its liking for low temperatures.

Culture on soil with hair bait

P. gelicola attacks the hair quickly, forming a thinly floccose whitish spreading type of growth. Asexual spores formed on the hyphae are rarely seen in this type of culture. Racquet hyphae are frequent. Conidial nodules are formed in dense, coalescent clusters on and in the vicinity of the bait. The nodules are buff in colour when mature.

Culture on agar media

P. gelicola grows well on 4% malt extract agar, glucose peptone agar and 3% peptone agar. It does not grow at 24°C and the colonies are described after 10 days at room termperature. On 4% malt extract agar, the edge of the colony is smooth and the reverse is smooth and colourless. The surface is pale yellow in colour. The centre is slightly raised and this area

is surrounded by a zone in which developing nodules are massed together. On glucose peptone agar the edge is smooth and the reverse flat and yellowish in colour. The centre of the colony surface is slightly raised, pale buff fawn in colour and finely floccose. This area is surrounded by a ring of developing nodules and, at the edge, the growth is thin and floccose. The edge of the colony on 3% peptone is even and the reverse smooth and deep orange in colour. The colony surface is velvety to finely floccose, slightly raised at the centre and shading from pale buff to white at the edges. Old cultures on all the above media are very granular due to the abundant production of conidial nodules and are deep buff in colour.

The species was seen growing in birds' nests but could not be obtained in culture until it was realized that 24°C was too high to allow it to grow. P. gelicola actually grows more quickly at 5°C than at room temperature. Two strains were, by the use of low temperatures of incubation, obtained in pure culture.

The 2 strains vary slightly in their rate of growth and in the colour of the reverse of the colony. The conidial nodules, conidia and vegetative hyphae are, however, identical. Very recently a third strain of this species has been recovered from a sample of sheep's wool but this has not yet been studied.

P. gelicola does not resemble any other asexual fungus, but it does closely resemble *A. ovinus*. From the morphology of the peridia of nodule and cleistothecia, it would appear that the 2 species should be placed in the one genus. However, it is not permissible to place an imperfect fungus in a perfect genus and, because *P. gelicola* did not resemble any other described fungi, it was necessary to erect a new genus for it. However, as the conidial nodules of 2 species of *Arthroderma* have been shown to be prophetic of the perfect state, I believe that when the perfect state of this species is found, it will prove to be an *Arachniotus*.

Aleurisma-Anixiopsis group

Within the family Eurotiaceae, the fungi most frequently isolated from soil and keratinous materials have proved to be species of Anixiopsis. These fungi are definitely keratinophilic and, when cultured on hair alone, attack it by forming penetrating organs. These are of the same type as are formed by species in the genera Arthroderma and Nannizzia.

Cultures of A. stercoraria and A. peruvianum were obtained from the culture collection at Baarn, Holland. A. stercoraria was found to be heterothallic and A. peruvianum to be homothallic. Although several homothallic species were isolated, none were identical to A. peruvianum but it has not yet been decided if the differences are sufficiently marked to justify the erection of new species. Heterothallic species were also recovered and a series of cross-mating experiments was undertaken. No isolate could be induced to cross-mate with A. stercoraria. However, several distinct mating groups became apparent among the isolates. No decision has yet been taken as to whether these are varieties or new species, although it seems probable that at least one group will be separated.

A species closely resembling the imperfect Aleurisma keratinophilum, described from soil by Frey (1959) was recovered and, on a number of occasions in primary isolation plates, perithecia of the type found in the family Eurotiaceae were

noted. The perithecia were linked with the asexual state by culturing from single ascospores. Difficulties were encountered in reproducing the perfect state, apparently because it was not possible to reproduce the environmental conditions in which they had formed originally.

As stated previously, the species in the family Eurotiaceae, which were isolated in the course of the survey, showed a predilection for substrates in which the amount of decaying organic matter is high. No such soil has been found free from keratinophilic fungi and growth of Aleurisma on ordinary "negative" soil was unsatisfactory. Although the fungus colonized the hair bait, no perithecia formed. The isolates grew well on horse hair bait on dung extract agar but did not form the perfect state regularly although this technique is most satisfactory for obtaining perithecia of Anixiopsis species. Some success resulted from culture on baited sand enriched with liquid extract of horse dung. However, further studies are required to perfect a technique enabling the perfect states of these isolates to be consistently reproduced when desired.

From the morphology of the asexual spores, ascospores and perithecia there can be little doubt that this species belongs in the genus Anixiopsis of the family Eurotiaceae. That other genera within this family may also prove to be keratinophilic is suggested by the isolation of a species of Samarospora from

soil by the use of hair bait (Borok, personal communication).

Bulbil-forming species (plates 45, 46)

A fungus was found colonizing horse hair bait on 4 separate samples of soil and was obtained, without difficulty, in pure culture. On agar media, the isolates grow rapidly, forming colonies which are white, darkening to cream colour with age, floccose, with a smooth colourless reverse. Neither asexual spores nor chlamydospores are formed on agar media or on hair bait on soil. The only method of reproduction is by the formation of hyaline, pale yellow or colourless, rather irregularly shaped, globose, multicellular structures measuring 15 - 24μ X 13 - 17μ. These are formed singly and terminally on short side branches of the branching septate hyphae. When they are mature the hyphae on which they are formed often degenerate. The structures germinate by forming one or several germ tubes. To decide if these structures are spores, bulbils or sclerotia their development was studied and camera lucida drawings prepared (plate 46).

The tip of a short side branch is cut off by the formation of a septum and the cell formed enlarges and becomes globose in shape. The first cross wall within this cell is generally parallel to the base of the cell. The lower cell does not increase greatly in size but the upper does and repeated cell divisions take place in it. Walls are laid down in all planes until a globose multicellular structure is formed. As the cells mature, the walls become immensely thick.

From the way in which the structures develop, it is certain that they are not sclerotia which are formed by vegetative hyphae massing together. Differentiation between bulbils and spores was difficult but I have decided that the structures are bulbils. That they are spores seemed unlikely because of their rather irregular shape, abnormally thickened walls and size. The only fungal species which form large multicellular spores comprable to the structures are Alternaria, Macrosporium, Sporodesmium and Stemphylium, all of which are classified in the Dematiaceae.

Hotson (1917) defined bulbils in the fungi as reproductive bodies, more or less definite in form and composed of a compact mass of homogeneous or heterogeneous cells which might be few or many in number. In these respects, the structures formed by the isolates from soil agree with the definition. However, Hotson also states that bulbils usually develop from primordia of more than one cell and, in this, the isolates differ. However, the definition does not preclude bulbils being formed from a single initial.

From the key to the described species of bulbiferous fungi given by Hotson, it seems possible that the isolates could be included in the genus Papulaspora except for the fact that bulbils in this genus form from multiple initials. Although I believe the isolates represent a new species, this will not be named until some of the recognised bulbiferous fungi have

been studied.

Non-keratinophilic fungi

A number of fungi which are not keratinophilic were observed, growing either on or near the keratinous bait, in primary isolation plates. Fungi found frequently were Aspergillus fumigatus, Penicillium lilacinum, species of Trichoderma, Gliocladium, Cephalosporium and several different species of Chaetomium.

Uncommon fungi were also seen and obtained in pure culture. These were the phycomycete Coemansia which was growing on a fragment of human skin on soil; a species of Lacellinia which developed on the lining from a sparrow's nest and Penicillium javanicum from hair from a hedge sparrow's nest. A fungus, which I believe to be Actinodendron verticillatum was twice cultured from hair bait on soil.

Some fungi could not be induced to grow on agar media. These were a species of Sepedonium and 2 unidentified species which were apparently growing on keratinophilic fungi which had colonized hair bait on soil.

Short descriptions of the latter 4 species are given.

Actinodendron verticillatum Orr & Kuehn

This species of the family Gymnoascaceae was first described by Smith (1900) under the name Gymnoascus verticillatus and re-named A. verticillatum by Orr & Kuehn (1963a). The peridial hyphae of the cleistothecia form distinctive branches. These branches are large, thick-walled, septate, with a verticel of branchlets arising from each cell.

In the course of this work, A. verticillatum was found on hair bait on 2 samples of soil and, although asci and ascospores were not seen, was identified by the presence of the highly characteristic branches.

Although Orr & Kuehn (1963a) were unable to obtain a culture on agar medium from their specimen which had been sent from Hungary, no difficulty was found in culturing the fungus in this laboratory. In culture on agar medium, the distinctive branches were seen for a few transfers, but no sexual spores could be found. It was, therefore, not possible to prove the connection between the imperfect cultures and the perfect state. However, because on 2 separate occasions the same fungus was obtained in culture from a colony showing the distinctive branches and because these branches were seen in the agar cultures, it is probable that the isolates are indeed those of the imperfect state of this species.

When pure cultures were inoculated to "negative" soil with

hair bait, the fungus did not attack the bait. Because the isolates had originally been obtained from baits carrying other keratinophilic fungi, the pure cultures were inoculated over soil and hair bait already colonized by keratinophilic fungi. After a time, growth of the species became apparent. This suggested that the fungus might be parasitic or that the hair had been broken down to such an extent by the other keratinophilic fungi that A. verticillatum could extract sufficient nourishment for growth. Using this method the 2 isolates were mated but no cleistothecia were detected.

The cultures, which I believe to be the imperfect state of A. verticillatum, grow well on 4% malt extract agar, glucose peptone agar and Czapek Dox 1% peptone agar, but poorly on 3% peptone agar and Czapek Dox agar. An incubation temperature of 28°C was too high to permit growth but the species grew well at 24°C.

The cultures on glucose peptone agar are bright citron yellow and finely granular when young but darken with age to a yellow buff shade and become roughened and lumpy. Occasionally a greenish colouration appears on the surface of the colony.

The morphology is interesting. Very small microconidia are formed terminally on densely clustered short branches. In addition, intercalary, slightly barrel-shaped chlamydospores are formed from alternate cells of the hyphae. In this latter

respect, the isolates closely resemble Coccidioides immitis, the fungus which causes the systemic disease Coccidioidomycosis in man. Because of their low temperature for growth, it is most unlikely that the isolates believed to be A. verticillatum will prove to be pathogenic.

A species which resembles my isolates has been described by Carmichael (1962) under the name Chrysosporium merdarium, but they differ slightly in spore size. Until I can study Carmichael's isolate, it is impossible to say if this difference in size is significant.

Sepedonium species

A fungus with the spherical, bright yellow, tuberculate chlamydospores of Sepedonium has been observed on 2 occasions on hair bait on soil. Despite repeated attempts, it has not been obtained in culture on agar medium which is unusual because Sepedonium generally grows well on most common agar media. Although it does not break down hair as do the keratinophilic fungi, it is apparently capable of limited growth on it because the strains were kept for some time by transferring a hair bearing the species to a freshly baited soil plate.

Parasitic species

In the course of isolation of keratinophilic fungi from soil, 2 species of fungus which I believe to be parasites of other fungi were observed; neither species could be induced to grow on agar media. The fungi are described and illustrated but have not been named.

Parasitic species 1 (plates 47,48)

This fungus was found growing over keratinophilic fungi on bait on 11 separate soil samples. It appears as a faint greyish-white floccose overgrowth on the keratinophilic fungi on the bait. The hyphae are branching, septate, smooth-walled and hyaline. The spores, which are formed abundantly, are hyaline, spherical (6.6 - 8.8μ diam.) with a thick (1 - 1.5μ) smooth wall and are formed singly at the tips of short side branches.

There is no direct evidence, such as the presence of haustoria, to prove that this species is parasitic but several facts suggest that it may be so. The fungus appears only after the bait on soil has been colonized by keratinophilic fungi, it has not been seen on hair in the absence of keratinophilic fungi and will not grow on agar media. Hyphae and spores of keratinophilic fungi in the presence of this species often appear collapsed and unhealthy. By transferring infected hairs to soil plates with bait already colonized by

keratinophilic fungi, it was possible to keep the species for some time.

Parasitic species 2 (plates 47, 48)

This fungus was observed growing on the hyphae of K. adelloi on hair bait in primary isolation plates of one sample of soil. It was not obtained in culture on agar medium.

As will be seen from the illustrations, the morphology of the species is simple but beautifully adapted for parasitic life. The thallus is small, measuring from 19 - 33μ, av. 25μ in length, hyaline and with smooth walls. The basal portion consists of 2 swollen lobes (8 - 11μ X 4 - 6.6μ), joined at the top. These lobes straddle the host hypha. From the apex of each lobe a single, unbranched, non-septate, smooth-walled conidiophore, 11 - 15μ, av. 12μ in length is produced. Around the upper end of each conidiophore 6 - 10 clavate, unicellular, smooth-walled, sessile spores 2.9 - 4.1μ X 0.8 - 1.2μ are formed.

DISCUSSION

DISCUSSION

The first perfect state of a keratinophilic fungus, found by the use of Vanbreuseghem's hair bait technique, was that of K. ajelloi. The cleistothecia of this species formed on horse hair which I had used to bait a sample of soil instead of adult human hair. While studying K. ajelloi in soil hair bait culture, it became apparent that cleistothecial production was affected by a number of external factors.

There is little reported in the literature about the effect of environmental conditions on the development of perfect states and on asexual growth of keratinophilic fungi cultured on soil with keratinous bait. This was therefore investigated, using pure cultures of the non-pathogenic species K. ajelloi and T. terrestre and the geophilic dermatophyte M. gypseum. Although other species of keratinophilic fungi were not formally investigated, any facts relevant to the investigation were noted.

When these pure cultures were grown on unsterilized soil with horse hair as bait, cleistothecia formed abundantly and quickly, reaching maturity within 3 weeks at an incubation temperature of 24°C. However, when horse hair was used with sterilized soil few, if any, cleistothecia developed. The effect of sterilization of the soil was particularly noticeable with K. ajelloi. Obviously, sterilization altered the soil, either by producing some substance deleterious to the keratinophilic fungi or by destroying some

beneficial factor.

Cleistothecial production was improved by inoculating the baited sterilized soil with fungi or bacteria taken from soil, before adding the keratinophilic fungi. Such "re-contaminated" soil almost equalled unsterilized soil in efficiency as a substrate for cleistothecial formation. Because this improvement was brought about by adding fungi or bacteria, it can be assumed that some factor, destroyed by sterilization, was replaced by the contaminating organisms. Because K. ajelloi and T. terrestre can form cleistothecia on a complete nutrient medium (p. 23), it seems probable that the factor missing in sterilized soil is nutritional in character. That this factor is required only in minimal amounts and can be stored, is suggested by the fact that some species can, for a time, after their isolation from nature form cleistothecia on agar media.

The wide pH range tolerated by K. ajelloi, T. terrestre and M. gypseum under experimental conditions was reflected by the variety of soil types, ranging from sand to peat, from which keratinophilic fungi were isolated, or on which they were successfully cultured. Only peat, which is highly acid, was a poor substrate for growth. This could explain the absence of keratinophilic fungi from samples of moorland soil.

Hair is a better bait to work with than materials such as cow horn, feathers, skin and nails, because it is easily handled

and very suitable for mounting for microscopic examination and preparing for culture of keratinophilic fungi. Skin is unsatisfactory as bait because it is broken down rapidly and becomes heavily contaminated by common soil fungi and bacteria which make examination and isolation of keratinophilic fungi difficult. Feathers, cow horn and nail parings are, apart from their mechanical disadvantages, good baits. Horn and nail are destroyed more slowly than hair by keratinophilic fungi and, therefore, when keratinophilic fungi were to be kept in soil bait culture for a long time, these hard keratins were used.

The various kinds of bait differed in efficiency, particularly in their ability to support cleistothecial formation. Good baits were, in general, good for isolating all species of keratinophilic fungi and the converse held also. Rabbit hair, which is a poor bait, is an exception to the rule. On this bait, only A. cuniculi and A. multifidum grew as well as on a good bait such as horse hair and, therefore had an advantage over the other fungi. It seems possible that because they are carried in vivo on rabbit hair, A. cuniculi and A. multifidum have become adapted to this substrate. No other correlation of a keratinophilic species with bait from a particular type of animal or bird was noted.

Whatever makes a bait good or bad, appears to be stable because the efficiency of a bait is not altered by pigmentation, sterilization by autoclaving, extraction with water or ether or,

except for human hair, by the age of the donor animal. Adult human hair is a poor bait; child's hair is good. Scalp infections with the dermatophyte M. audouini are confined to children and spontaneous remission of the infection usually occurs at puberty. The changes which are responsible for this remission could well be those which make adult hair unsuitable as bait. Some variation in the efficiency of child's hair as bait was noted but could not be related to age or hair colour. This variation might be explained by the fact that in epidemics of scalp ringworm, certain children are more resistant to infection than others; the hair of such resistant children might well be a poorer bait.

The inhibition of cleistothecial formation by white and blue light, although unimportant in the laboratory, can explain why only the perfect state of A. curreyi has been found in nature. This species is homothallic, but there must be some other explanation for its discovery, because compatible strains of heterothallic species are often found together in soil (table 5). Most of the discoveries of A. curreyi have been made in woods where the light intensity is below normal. That low light intensity is the explanation is supported by my discovery of A. quadrifidum in a bird's nest in a shaded position and by a colleague's discovery of a dead cat in a hollow tree. This cat was completely covered by a mat of various species of keratinophilic fungi, many of which had formed cleistothecia.

Experiments involving temperature of incubation gave interesting results with the possibility of practical application. The species tested grew and formed cleistothecia over differing temperature ranges which are given in table 1. Low temperatures (5 - 10°C) favoured T. terrestre and higher (30 - 34°C) the dermatophyte M. gypseum. Although the bait was not attacked by any keratinophilic species at 37°C, the time they remained viable varied. M. gypseum survived for over 7 weeks and K. ajelloi and T. terrestre for shorter periods.

That high soil temperatures could cause an increase in M. gypseum, was suggested by studies in this laboratory of soil before and after a period in a greenhouse. M. gypseum, which was present only in small amounts before the soil was transferred to the greenhouse, became dominant and was the only species isolated after exposure to the higher temperatures. It appears that M. gypseum is more prevalent in soil in hot countries than in Britain. Five of 238 soil samples in Britain yielded this species whereas in America 126 of 438 and in Australia 12 of 96 soil samples gave M. gypseum (Ajello, 1956; Durie & Frey, 1956). It would be interesting to compare the keratinophilic species isolated from soil in various countries with the soil temperature.

The majority of reports of dermatophytes, other than M. gypseum, from soil have come from countries with hot summers such as Roumania and Hungary (Evolceanu, Alteras & Cojocaru, 1961, 1962;

Szathmary, personal communication). An explanation for this could be that, at the higher temperatures, the amount of non-pathogenic keratinophilic fungi in the soil is reduced enabling the dermatophytes to survive. Experiments in which I inoculated K. ajelloi and M. canis together into soil, showed that at 24°C and 28°C the dermatophyte could not be recovered by the use of hair bait.

Agar media were, on the whole, poor substrates for cleistothecial formation by K. ajelloi, T. terrestre and M. gypseum. Of the 15 species in the genera Arthroderma and Nannizzia only A. curreyi forms cleistothecia regularly on agar media. Other species such as A. lomondii and A. cocleatus can do so for one or 2 transfers after isolation from nature but soon lose this ability.

When cleistothecia of Arthroderma species are formed on agar media, the peridium is less well developed than in cleistothecia formed under natural conditions. This fact is important because the branching of the peridial hyphae and the shape of the peridial cells play a large part in identification of species in this genus.

Since the discovery of pure culture, there has been a tendency to neglect the study of micro-organisms under natural conditions, as was so ably done by previous generations of mycologists. It is my opinion that, because of over-emphasis on pure culture, we do not always get the complete picture.

For example, in Currey's original description of A. curreyi (1854), he compared his isolate to Trichoderma. On agar medium Trichoderma is unlike A. curreyi but in soil Trichoderma grows in globose clumps and the possibility of Currey's comparison becomes obvious. Although pure culture studies are essential, it should always be remembered that no artificial medium can wholly reproduce natural conditions. By studying fungi and other organisms in pure culture and under natural conditions, I believe that much additional information may be gained.

The knowledge obtained from the studies on soil-keratinous bait culture was applied to isolation of keratinophilic fungi from natural soil samples. It was realized that a soil bait method of culture could also be used for isolating keratinophilic fungi from keratinous materials such as feathers and hair and from non-keratinous materials such as moss and straw. Isolation of keratinophilic fungi from such materials by culture on agar medium was unsatisfactory. Only a small amount of each sample could be investigated, contamination by common moulds was high and the keratinophilic fungi were obtained in the asexual state. However, when the material to be investigated was laid on the surface of "negative" sand or soil and, if necessary, extra keratin added, keratinophilic fungi were isolated and their perfect states obtained in the one procedure. This method allows much larger amounts of each specimen to be investigated and the

results show that it is more efficient than culture on agar medium.

The temperature of incubation can be used in several ways to isolate certain species of keratinophilic fungi from others. By incubating at different temperatures, the amounts of the keratinophilic species isolated from a sample can be altered. For example, low temperatures (5 - 10°C) are good for T. terrestre, A. ovinus and P. gelicola whereas higher temperatures such as 34°C favour M. gypseum, other dermatophytes and the non-pathogenic species C. serratus and A. keratinophilum.

There is another way in which temperature can be used to select certain species of keratinophilic fungi from soil. If unbaited soil is held at 37°C for a time before baiting and incubating at 24°C, species of fungi such as T. terrestre and K. ajelloi, which do not survive for long at 37°C, are killed off or the amount present considerably reduced, leaving dermatophytes and those non-pathogenic keratinophilic species which tolerate 37°C well, as the dominant organisms.

In this way, M. canis was easily recovered from a mixture of M. canis and K. ajelloi inoculated to soil. After incubation at 24°C, following 7 days at 37°C, the dermatophyte colonized the bait strongly but only very little K. ajelloi could be found. As stated previously, by direct inoculation of these soil samples at 24°C and 28°C only K. ajelloi was recovered.

This method was used in addition to routine methods to investigate samples of soil from a cattery (table 6a) in which there was an epidemic of ringworm caused by M. canis. The dermatophyte was, however, not recovered. From the results of the laboratory experiments on M. canis and K. ajelloi, one could expect to recover the dermatophyte had it been present in the soil. That M. canis must have been inoculated to the soil from the infected animals seems certain. It would appear, therefore, that M. canis is not capable of competition, under natural conditions, with the many other species of keratinophilic fungi which were shown to be present. It has already been suggested (p. 160) that higher temperatures in soil are necessary for dermatophytes to compete successfully with non-pathogenic keratinophilic fungi.

Keratinophilic fungi were isolated from natural sources, using the hair bait technique for soil samples and an adaptation of this technique (p. 161) for materials other than soil. In all, 238 samples of soil, 234 samples of hair and feathers, 33 birds' nests and 8 owl casts were investigated. Many species of keratinophilic fungi were isolated. Nine new species of keratinophilic fungi were discovered, classified and, with one exception, named. Four species, A. tuberculatum, C. serratus, M. cookei and Shanorella spirotricha, were found for the first time in Great Britain. A. verrucosus, which has been lost

in the living state for many years, is now held in pure culture. In addition, a contribution to the ecology of keratinophilic fungi has been made.

It was noted that certain fungi in the family Eurotiaceae were very frequently isolated from soil samples in which the amount of decaying organic matter was high. Confirmation of this predilection of members of the family Eurotiaceae for organically rich substrates, was obtained when they were isolated from every owl cast which yielded keratinophilic fungi.

When the results of the soil survey were analysed, with regard to degree of association of the samples with animals, it became apparent that there was a correlation between the density of the animal population and the amount and variety of keratinophilic fungi isolated. From soil samples closely associated with animals, more species of keratinophilic fungi were recovered, individual species were present in increased amounts and the proportion of samples yielding keratinophilic fungi was higher.

The extra keratinous debris in soils associated with animals can explain the greater amount of individual keratinophilic fungi recovered but not the increase in the number of species, unless these lie dormant until stimulated to growth by keratin. This is unlikely and the only feasible explanation for the increased flora is that animals introduce the fungi to their

environment. A. cuniculi and A. multifidum were isolated only from soil from rabbit burrows. No other keratinophilic species could, however, be proved to be associated with a particular kind of animal.

Comparison of the frequency of isolation of common keratinophilic species from animals and soil (table 9) showed that A. curreyi and T. terrestre were more often recovered from animals. These species were, on occasion, isolated from animals from districts in which the presence of these fungi in soil could not be demonstrated. K. ajelloi which is ubiquitous in soil was, however, seldom isolated from animals.

If contamination were the reason for the presence of keratinophilic fungi on animals, one would expect to find the fungi in quantities corresponding to their distribution in soil. This is not the case. Therefore, A. curreyi and T. terrestre cannot be true contaminants and must be considered as a part of the skin flora.

A. cuniculi and A. multifidum were isolated only from the hair of rabbits, confirming the association suggested by their recovery from rabbit burrows. The demonstration of A. cuniculi in hair taken from a wild rabbit's nest showed that transfer of a fungus from animal to animal takes place. The young in this nest would certainly have been in such close contact with the fungus that it must have found its way to their hair.

Soil in rabbit burrows is sub-surface soil which does not normally contain keratinophilic fungi. Recovery of A. uniculi and A. multifidum from the burrows means, therefore, that the species must have been inoculated to the soil by the rabbits. It seems probable that A. curreyi and T. terrestre in soil also originate from animals although no definite proof is yet available.

Mackenzie (1961) demonstrated the amount of fungus which could be spread by an animal. He allowed a mouse, which was an a-symptomatic carrier of T. mentagrophytes, to walk over a large dish of nutrient agar for some minutes. After incubation, the surface of the medium was almost covered by colonies of the dermatophyte.

For the first time, birds' nests were examined for keratinophilic fungi. Nests proved to be a good source and many keratinophilic fungi, including 2 new species were obtained. That birds carry keratinophilic fungi such as A. curreyi, A. tuberculatum, T. terrestre and C. serratus is suggested by the results of isolation from nests and feathers. Proof, however, must await the examination of large numbers of birds. A. curreyi and T. terrestre were recovered from feathers and, from nests, were isolated more frequently than from soil. K. ajelloi, on the other hand, has not yet been isolated from feathers and only infrequently from nests.

A. tuberculatum and C. serratus, which are rare in soil in this country, were isolated from swallows' nests. That 3 nests of this kind each yielded a fungus rare in soil, implies that the fungi came from the birds and, because swallows are migrants, that they were brought into this country on the feathers.

Pugh & Mathison (1962) reported the frequent isolation of C. serratus from soil in the vicinity of bird traps but, unfortunately, did not specify the type of birds. The only site from which A. tuberculatum and C. serratus were isolated from soil was a cattery. It seems possible that cats catching birds within the compound could be the explanation for their presence.

Keratinophilic fungi, belonging to species in the Fungi Imperfecti, Gymnoascaceae and Eurotiaceae, were isolated during my investigations. Nine new species were discovered; 4 species which have been found in other countries were isolated for the first time in Britain; certain named fungi were shown to be keratinophilic and a number of unusual non-keratinophilic species were obtained.

In addition to the isolation of each described species of Arthroderma, 5 new species were discovered. These are A. multifidum, A. cuniculi, A. cochleatus, A. minutum and A. lomondii. The main morphological differences between the perfect states of species of Arthroderma are apparent from the

key devised for their identification (p. 91).

The genus is divided into 2 groups depending on whether the peridial cells remain dumb-bell or one-sided dumb-bell in shape, or differentiate further as they mature. In A. uncinatum, A. tuberculatum, A. curreyi and the new species A. cocleatus, the cells remain dumb-bell in shape. In this group, A. cocleatus is similar to A. curreyi and A. uncinatum to A. tuberculatum. In A. cocleatus, the spiral growth of the peridial hyphae is confined to the tips and normal uncinate branching is seen, whereas in A. curreyi the peridial hyphae grow in a spiral over their whole length. A. uncinatum and A. tuberculatum can be differentiated by peridial cell size, by the proportion of dumb-bell shaped cells to one-sided dumb-bell shaped cells and by the presence of asexual spores in association with the cleistothecia.

The second major group, in which the cells of the peridial hyphae differentiate by forming protuberances as they mature, contains A. quadrifidum and the new species A. minutum, A. lomondii A. cuniculi and A. multifidum. This group is sub-divided into species with globose cleistothecia (A. cuniculi and A. multifidum) and those with stelliform cleistothecia (A. minutum, A. lomondii and A. quadrifidum). This difference in cleistothecial shape is apparent even under low magnification such as a hand lens.

Within these 2 sub-groups, the species are separated by the number of protuberances formed by the peridial cells.

A. cuniculi forms 3 protuberances at each end of the cell whereas A. multifidum forms up to 8. The number of protuberances formed by the peridial cells of A. minutum, A. quadrifidum and A. lomondii are 1, 2 and 3 respectively, at each end of the cell.

Identification of species of Arthroderma on agar medium can be difficult. Normally, only A. curreyi forms cleistothecia. A. cocleatus, A. multifidum, A. tuberculatum and A. uncinatum form distinctive spores and are easy to identify. The colonies of the remaining species are similar. A. cuniculi forms no macroconidia. Each of the remaining species forms macroconidia which are alike in shape although differing somewhat in size and frequency. A. quadrifidum, when grown on 4% malt extract agar, has a pinkish reverse to the colony; A. lomondii forms a somewhat floccose colony and that of A. minutum is rather granular. However, given an asexual culture of any one of these latter 4 species, or A. curreyi, it is extremely difficult to decide what it is. It would be of great value if a medium could be devised to simplify identification and I believe that this might be possible.

Culture on soil with hair bait is a help in identification. A. curreyi and A. minutum, being homothallic, form cleistothecia. A. lomondii develops a very thin floccose growth on the hair bait while A. quadrifidum and A. cuniculi are granular. However, on hair it is most unlikely that the one would be mistaken for

the other.

Each of the new heterothallic species of Arthroderma was crossed with other species in a series of experiments. Cleistothecia formed only when compatible ascospore strains of individual species were mated. Such experiments were particularly necessary to confirm that species which showed resemblances in the sexual and asexual states were, in fact, separate species.

A. lomondii was isolated from 2 districts but when single ascospore strains from each district were crossed, cleistothecia did not form, although strains from the same district mated freely among themselves. Because of this and because no marked differences are apparent in cleistothecial morphology, the strains from the second district are, for the present, being considered as a variety. The variety differs slightly from the species in rate of growth and in the proportion of microconidia to macroconidia. However, as the variety was found only recently this has not yet been fully investigated.

Some strains of K. ajelloi and T. terrestre cannot be induced to reproduce sexually even by crossing with each of a pair of compatible single ascospore strains or by mating with other isolates which do not form cleistothecia. Such strains may have lost the ability to reproduce sexually. Alternatively, the imperfect states of K. ajelloi and T. terrestre

may be more than one species. This was the case with M. gypseum which for a time had 3 perfect states to one asexual state. Stockdale (1963) has now shown that it is possible to differentiate between the 3 species in the asexual state.

Certain species of Arthroderma can form conidial nodules. These structures were first described by Eidam (1880) as being associated with the cleistothecia of the fungus we now know as A. curreyi. Because conidial nodules were not seen in any of the 26 strains isolated and because experimental attempts to induce them to form failed for 6 strains, it appears that either Eidam's isolate was a-typical or that there was yet another species in the mixed fungi which he described under the name C. serratus.

Strains of T. terrestre, capable of sexual reproduction, can be induced to form conidial nodules by culture on soil with hair bait at 28°C which, for this species, inhibits the formation of cleistothecia. Except for the contents, the morphology of the conidial nodule is identical to that of the cleistothecium. Griffin (1960) stated that the conidial nodules would be prophetic of the morphology of the perfect state of T. terrestre. Griffin's statement has proved true for T. terrestre and also for the dermatophyte T. simiae.

On 2 occasions hedge sparrow nests gave a new species of keratinophilic fungus which, in morphology, was typical of the genus Trichophyton and was named T. globiferum. The specific

name globiferum was chosen because, in addition to spores, the species forms globose bulbils. In culture on soil with hair bait, after several months, the strains of this species form nodules with a peridium typical of Arthroderma. Contents, however, are lacking.

A number of dermatophytes, such as T. mentagrophytes and T. quinckeanum can form conidial nodules with the morphology of Arthroderma, and I believe that this fact could be used to simplify the classification of dermatophytes in the genus Trichophyton. It would be useful to divide the species of Trichophyton for which the perfect state is not yet known into those which cannot form conidial nodules and those which can. If a descriptive term such as Pararthroderma were placed before the name of the species as, for example, Pararthroderma T. mentagrophytes, this would show that the species could form conidial nodules and that these are of the Arthroderma type. Except for workers interested in this problem, such information is not easy to obtain.

Species in the genus Microsporum were found to be rare in soil in Britain. From 238 soil samples, M. gypseum was isolated 5 times and M. cookei twice. It is interesting that all the isolates of M. gypseum were of the one mating strain and the perfect state, N. incurvata, could not be obtained until single ascospore strains became available. No other species in the

M. gypseum complex was recovered. The identification of M. cookei was confirmed by mating with single ascospore strains when the perfect state N. cajetana developed.

Mature cleistothecia of a hitherto never isolated keratinophilic species were found on soil underneath a dead sheep. If the cleistothecia had not been seen, the fungus would probably have been missed because it would not grow at 24°C or room temperature. The cleistothecia were Arachniotus in type, with the cells of the peridial hyphae not highly differentiated. The isolate could not be fitted into any of the known species and was therefore named A. ovinus because of its association with the sheep.

When pure cultures were obtained by the use of low termperature incubation (5°C) they were inoculated to soil baited with horse hair. The fungus colonized the bait and formed cleistothecia freely. As far as I am aware, a species of Arachniotus has not previously been reported to be keratinophilic.

From 2 birds' nests another new asexual species was seen and recovered. In culture on soil with hair bait and on agar medium, the growth consists mainly of conidial nodules with a well developed peridium which, in cell shape, type of hyphae and appendages closely resembles A. ovinus. As can be seen from plates 38 - 44, the similarity is striking but because there was no perfect state, the fungus could not be placed in

Arachniotus. A new genus was created and because of the resemblance to Arachniotus, named Pararachniotus, with the specific name gelicola to denote the preference of this fungus for low temperatures. Should the perfect state of P. gelicola be discovered, there can be little doubt from the morphology of the conidial nodules that it will prove to be an Arachniotus.

Because of the discovery of species such as A. ovinus and P. gelicola which have a predilection for low temperatures, this factor must be taken into consideration in future isolation work. I would like to investigate soil samples and other materials by the use of hair bait and low incubation temperatures to see how frequently cold-loving species were missed in the survey and to determine their frequency in nature. The results of such work might well prove to be very interesting.

In the months which have elapsed since A. ovinus and P. gelicola were first recovered, both species have accustomed themselves to higher temperatures and now grow at 24°C. This conditioning of fungi to higher temperatures may perhaps prove useful in the study of non-pathogenic keratinophilic fungi related to the dermatophytes.

When a new species of Arthroderma or Nannizzia is found it is impossible to decide from its morphology or from the way in which it attacks hair in vitro, if it will cause ringworm in man or animals or be non-pathogenic. However, a study of the

temperatures at which it will grow provides a fairly accurate guide; dermatophytes, in general, grow at higher temperatures than the non-pathogenic species.

It would be interesting, in a long-term series of experiments, to attempt to condition non-pathogenic species related to the dermatophytes to higher temperatures and, if this could be done, to test the conditioned strains for pathogenicity. The converse of conditioning dermatophytes to low temperatures to find if they would cease to be pathogenic would also be worth doing.

C. serratus can grow and colonize hair bait at 34°C, as do the dermatophytes, but none of the strains which I have tested caused ringworm in experimental animals. This species has been classified in the family Gymnoascaceae. However, examination of the perfect states of the strains isolated from soil and nests showed that the innermost layer of the peridium was composed of pseudoparenchyma. The perfect state is therefore a perithecium and the species should not remain in the Gymnoascaceae. The outer hyphal peridium and appendages preclude transferring it to any other family. However, from a practical point of view it is not feasible to create a new family for a single species and, therefore, for the present, the taxonomic position of C. serratus must remain unsettled.

Species in the genus Anixiopsis of the family Eurotiaceae have been shown, in the course of this work, to be keratinophilic

and to attack hair by forming penetrating organs. Anixiopsis species were isolated from soil, animals, nests and owl casts but these have not yet been studied in detail. However, from the morphological studies which have been done and from the results of mating experiments, it seems apparent that there are a number of new species among the isolates, none of which proved to be identical to the 2 named species.

Aleurisma keratinophilum was described and named by Frey (1959) who isolated it from soil by the use of hair bait. The perfect state of a fungus which, in the imperfect state closely resembles A. keratinophilum, has been found. The perfect state shows the morphology of Anixiopsis. Culture of single ascospores has proved the connection between the perfect and imperfect states but a technique to induce perithecia to form freely and regularly in the laboratory has not yet been discovered. The best results so far have been obtained using horse hair bait on "negative" sand moistened with a liquid extract of horse dung.

A fungus which forms bulbils was isolated from 4 samples of soil by the use of hair bait which it attacked strongly. Apart from the way in which the bulbils are formed, this species might well be placed in the genus Papulaspora. However, the particularly interesting point about the isolation of this species is that it is so unlike any other species which has been proved to be keratinophilic and suggests the possibility

that another group of keratinophilic fungi may be found among the bulbiferous fungi.

A number of other imperfect keratinophilic fungi have been isolated but as only microconidia are formed and there is nothing distinctive about the colonies these have not yet been identified. No perfect states have been discovered although cross-mating experiments on soil with hair bait have been undertaken.

Non-keratinophilic fungi were also recovered in the course of the investigations. Among these were the 2 parasitic species and A. verticillatum which, although they could not attack the bait, were apparently dependent on the presence of keratinophilic fungi to enable them to grow in soil hair bait culture. Other unusual species which were found growing on or near the bait were Lacellinia, Coemansia and P. javanicum. A number of common fungal species were regularly recovered from isolation plates; these included Chaetomium, Gliocladium, Cephalosporium, Penicillium, particularly P. lilacinum, and species of Actinomycetes.

These findings emphasize the importance of direct investigation of natural substrates in the laboratory. The hair bait technique is a classical example of the way in which this can be done. It seems probable that a similar method, using other materials as bait, could be a useful tool for selective isolation of other kinds of fungi. For example, it

was noted that Phycomycetes frequently attacked skin when it was used as bait on soil.

Fungi in many genera have the ability to attack and break down keratin. Although all the evidence suggests that keratinophilic fungi must produce keratinolytic enzymes, these have not yet been proved to exist (Chattaway, Ellis & Barlow, 1963). Not all keratinophilic fungi can be isolated from soil by the use of the hair bait technique. Non-pathogenic species and some dermatophytes such as T. mentagrophytes, M. canis and E. floccosum are easily isolated in this way but dermatophytes such as T. schoenleini and M. audouini cannot be recovered by the use of keratinous bait. It is interesting that the majority of species in the latter group attack hair in vivo whereas other species which, in vivo, only invade skin attack the bait strongly.

Any definition of keratinophilic fungi must be sufficiently wide to encompass all the variations found in this rather heterogeneous group and the following is suggested:-

Keratinophilic fungi are fungi which have the ability to attack and degrade keratinous tissues in vitro and/or in vivo.

In nature, the function of keratinophilic fungi is probably to break down keratinous debris which, with the exception of skin, is extremely resistant to decay. It has been suggested (Vanbreuseghem, 1961) that "dermatophytes could probably continue to thrive in the soil even without the

continuous fungal enrichment from human and animal origin. Even more, they would not care very much about the extinction of animal life on the surface of the earth, although we must admit that keratin looks like a very precious nutrient for their development". I believe that all keratinophilic fungi would be unable to exist for very long in soil in the complete absence of keratin. That they do die out when keratin is lacking is shown by the results from investigation of rabbit burrows (Dawson, 1963). To a certain degree it seems that there is a symbiotic relationship between keratinophilic fungi and animals. The fungi benefit by the deposition of keratin and the animals by the removal of keratinous debris from their environment.

In the few years which have elapsed since the hair bait technique was reported (Vanbreuseghem, 1952), great advances have been made in the knowledge of keratinophilic fungi. Not only have many new species been discovered but the perfect states of a number of known species, including dermatophytes, have been found and/or studied by the use of this technique. Perhaps most important of all is that it has given mycologists a tool to search for keratinophilic fungi and because of this ecological studies have become possible.

From the results of the work carried out for this thesis, it is obvious that much more remains to be done in the field of keratinophilic fungi. Although new species and many known

species have been isolated and a contribution made to the ecology of keratinophilic fungi, as the work progressed it became more and more apparent that there is a need for further research on many aspects of the keratinophilic fungi.

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STUDIES ON CERTAIN KERATINOPHILIC FUNGI

by

Christine O. Dawson

TABLES & ILLUSTRATIONS

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Table 1. The effect of the temperature of incubation on the growth and sexual reproduction of compatible mating strains of Trichophyton terrestre, Keratinomyces ajelloi and Microsporum gypseum cultured on horse hair baited unsterilized soil.

Incubation temperature in °C	Compatible mating strains					
	T. terrestre		K. ajelloi		M. gypseum	
4	C[1]	+++	-	+	-	+
10	C	+++	-	++	-	-
15	C	++++	C	++++	-	+++
22	C	++++	C	++++	C	++++
24	C	++++	C	++++	C	++++
28	-[2]	++	C	++++	C	++++
30	-	-	C[1]	+++	C	++++
34	-	-	-	-	-	+++
37	-	-	-	-	-	-

C Cleistothecia present

\+ Minimal vegetative growth

++ Moderate vegetative growth

+++ ++++ Profuse vegetative growth

1 Immature cleistothecia

2 Conidial nodules

Table 2. General details of the numbers of soil samples investigated from areas associated with animal life, together with the number of different species and the total number of isolates of keratinophilic fungi recovered.

Degree of association of soil samples with animals	Soil samples			Number of different species of kerat. fungi	Number of isolates of kerat. fungi ■
	number investigated	kerat. fungi present	kerat. fungi absent		
Well-marked	133	116 (87.2%)	17 (12.7%)	16	187
Moderate	58	46 (79.3%)	12 (20.6%)	6	63
Minimal	47	9 (19.1%)	38 (80.8%)	4	10
Totals	238	171 (71.8%)	67 (28.1%)	-	260

■ Each different species obtained from a soil sample is counted as one isolate.

Table 3. Details of the sites from which soil samples were collected with the numbers of different species and isolates of keratinophilic fungi recovered.

Degree of association of soil samples with animals	Sites sampled	Number of soil samples		No. of species of kerat. fungi isolated	Total no. of isolates
		investigated	yielding kerat. fungi		
Well-marked	Farmyards	24	24	7	42
	Gardens & greenhouses	28	26	5	38
	Cattery	24	24	9	41
	Rabbit burrows	41	29	6	44
	Rodent runs	11	9	5	16
	Hens & birds	5	4	4	6
Moderate	Open farmland	58	46	6	63
Minimal	Woodlands & moorland	22	-	-	-
	Geological soil types	25	9	4	10
Totals		238	171		260

Table 4. The effect of the presence of animal life on the keratinophilic fungal flora of soil as shown by the frequency with which one or more species of keratinophilic fungi were isolated from soil samples.

Degree of association of soil samples with animals	Number of soil samples yielding kerat. fungi ■	Number of soil samples giving			
		1 species	2 species	3 species	4 species
Well-marked	116	65 (56%)	35 (30%)	12 (10%)	4 (3%)
Moderate	46	33 (71%)	9 (19.1%)	4 (8.6%)	-
Minimal	9	8 (88.8%)	1 (11.1%)	-	-
Totals	171	106 (61.9%)	45 (26.3%)	16 (9.3%)	4 (2.3%)

■ See table 2, column 2.

Table 5 The species of keratinophilic fungi isolated from soil. The number of samples yielding each species and the percentage of samples in which the perfect state developed are given.

Species of fungus	Degree of animal association and no. of samples yielding each species of fungus			Total number of isolates
	well-marked (116 samples positive)	moderate (46 samples positive)	minimal (9 samples positive)	
Keratinomyces ajelloi	81 (44.4%)	42 (21.4%)	5	128 (35.0%)
Trichophyton terrestre	17 (17.6%)	2	2	21 (14.2%)
Arthroderma curreyi	5 (100%)	5 (100%)	-	10 (100%)
Arthroderma cuniculi	19 (84.2%)	-	-	19 (84.2%)
Arthroderma multifidum	11 (72.7%)	-	-	11 (72.7%)
Arthroderma minutum	3 (100%)	-	-	3 (100%)
Arthroderma tuberculatum	2	-	-	2
Arthroderma lomondii	1 (100%)	-	-	1 (100%)
Microsporum gypseum	5	-	-	5
Microsporum cookei	2	-	-	2
Ctenomyces serratus	2 (50%)	-	-	2 (50%)
Amauroascus verrucosus	1 (100%)	-	-	1 (100%)
Alcurisma & Anixiopsis	30 (30%)	12 (25%)	2 (100%)	44 (31.8%)
Arachniotus ovinus	1 (100%)	-	-	1 (100%)
Bulbil type	3	1	-	4
Unidentified	4	1	1	6

Imperfect name used because not all isolates formed cleistothecia.

Table 6. Details of the species of keratinophilic fungi isolated from the different types of samples within each category of animal associations.

a) Samples with well-marked animal associations

Species of fungus	No. of samples yielding each species of fungus					
	Farmyard	Garden & green-house	Cattery	Rabbit burrow	Rodent runs	Hens & birds
K. ajelloi	20	22	24	8	6	1
T. terrestre	4	7	3	-	2	1
A. curreyi	1	-	-	4	-	-
A. cuniculi	-	-	-	18	-	1
A. multifidum	-	-	-	10	1	-
A. minutum	-	-	-	3	-	-
A. tuberculatum	-	-	2	-	-	-
A. lomondii	-	-	-	-	1	-
M. gypseum	-	4	1	-	-	-
M. cookei	1	-	1	-	-	-
C. serratus	-	-	2	-	-	-
A. verrucosus	-	-	1	-	-	-
Al. & Anix.	13	4	4	-	6	3
A. ovinus	1	-	-	-	-	-
Bulbil type	-	-	3	-	-	-
Unidentified	2	1	-	1	-	-

b) Samples with moderate and minimal animal associations

Species of fungus	No. of samples yielding each species of fungus		
	Moderate animal associations	Minimal animal associations	
	Open farmland	Woodland & moorland	Geological soil types
K. ajelloi	42	-	5
T. terrestre	2	-	2
A. curreyi	5	-	-
Al. & Anix.	12	-	2
Bulbil type	1	-	-
Unidentified	1	-	1

Table 7

Species of keratinophilic fungi isolate
materials taken directly from anim

Animal species		Rats	House mice	Field mice	Voles	Shrews	Rabbits	Hares	
No. of animals examined		158	24	8	18	2	11	2	
No. yielding kerat. fungi		41	4	5	5	2	8	1	
SPECIES ISOLATED	K. ajelloi	4	-	-	-	-	2	-	-
	T. terrestre	22	2	2	-	-	5	-	-
	A. curreyi	-	-	2	2	1	2	1	
	A. cuniculi	-	-	-	-	-	4	-	-
	A. multifidum	-	-	-	-	-	3	-	-
	A. lemondii	-	-	1	-	-	-	-	-
	Aleurisma-Anixiopsis	17	1	1	-	-	3	-	
	T. mentagrophytes	1	1	-	-	-	-	-	-
	M. gypseum	1	-	-	-	-	-	-	-
	Unidentified	-	-	2	3	1	-	-	-

Table 8. Details of the types of birds' nests investigated and the species of keratinophilic fungi isolated.

Type of nest		Thrush	Blackbird	Hedge sparrow	Chaffinch	Sparrow	Duck and Eider duck	Swallow	Robin	Totals
No. investigated		2	5	10	3	3	2	3	5	33
No. yielding kerat. fungi		-	4	9	3	3	2	3	4	28
FUNGAL SPECIES ISOLATED	K. ajelloi	-	3	4	1	-	-	2	3	13
	T. terrestre	-	1	3	2	1	1	1	-	9
	A. curreyi	-	1	-	-	-	-	3	-	4
	A. cocleatus	-	-	-	-	-	-	1	-	1
	A. tuberculatum	-	-	-	-	-	-	1	-	1
	A. lomondii	-	1	-	-	-	2*	-	-	3
	Aleurisma-Anixiopsis	-	2	7	1	1	1	4	2	18
	C. serratus	-	-	-	-	-	-	1	-	1
	Pararachniotus gelicola	-	-	3	-	-	-	-	1	4
	Trichophyton globiferum	-	-	2	-	-	-	-	-	2
	T. terrestre (red var.)	-	-	-	-	-	1	-	-	1
	T. terrestre type	-	-	1	-	-	-	-	1	2
	Gymnoascus sp.	-	-	1	-	-	-	-	-	1
	Unid. s.s.	-	-	-	-	1	-	-	-	1
	Total isolates	-	8	21	4	3	5	13	7	61

*Variety of A. lomondii

Table 9. Comparison of the frequency of isolation of common species of keratinophilic fungi from soil, hair and feathers, birds' nests and owl casts. The number of samples yielding each species is given and is also expressed as a percentage of the positive samples within each group.

Species of fungus	No. of samples yielding each species of fungus			
	Soil (171 samples positive)	Hair and feathers (74 samples positive)	Birds' nests (28 samples positive)	Owl casts (7 samples positive)
Keratinomyces ajelloi	128 (74.8%)	7 (9.4%)	13 (46.4%)	-
Trichophyton terrestre	21 (12.2%)	32 (43.2%)	9 (32.1%)	-
Arthroderma curreyi	10 (5.8%)	12 (16.2%)	4 (14.2%)	-
Aleurisma & *Anixiopsis*	44 (25.7%)	24 (32.4%)	18 (64.2%)	7 (100%)

Table 10. Distribution of *Keratinomyces ajelloi*.

AUSTRALIA	1955	Durie & Frey	Nature(Lond.), 176, 936.
	1956	Frey & Durie	Aust.J.exp.Biol.Med.Sci.34,199.
	1960	Griffin	Trans.Brit.myc.Soc.,43, 583.
BELGIUM	1952	Vanbreuseghem	Bull.Acad.Belg.Cl.Sci.,38,1068.
BRITAIN	1954	Daniels	Nature(Lond),174, 224.
	1958	Stockdale	Nature(Lond),182, 1754.
	1959	Dawson & Gentles	Nature(Lond),183, 1345.
FINLAND	1960	Lundell	Mykosen, 3, 136.
GERMANY	1960	Meinhof et al	Arch.klin.exp.Derm.,212, 30.
	1960	Schönfeld et al	Arch.klin.exp.Derm.,212,78.
	1961	Rieth	Arch.klin.exp.Derm.,213,662.
HUNGARY	1959	Banhegyi	Ann.Univ.Sci.Budapest Rolando Rötvös, 2, 37.
INDIA	1962	Padhye & Thirumalachar	Curr.Sci.,31, 100.
JAPAN	1957	Kominami	Tojoku J.exp.med.,66, 233.
	1961	Ito et al	Bull.pharm.Res.Inst.Osaka,31,7.
NEW GUINEA	1958	Report 1956/7	Inst.of med.Research,Royal North Shore Hospital,Sydney, p.33.
NEW ZEALAND	1961	Marples	Trans.roy.Soc.Trop.Med.Hyg.55,216.
NORWAY	1960	Lindqvist	Nord. Veterinaermed.,12, 21.
	1961	Lindqvist	Acta path.microbiol.Scand.51,381.
POLAND	1963	Prochacki & Bielunska	Acta Microbiol.Polon. 12, 143.
ROUMANIA	1959	Evolceanu & Alteras	Mycopathologia(Den Haag),11,196.
	1961	Evolceanu et al	Derm.Vener.Bucuresti,5, 335.
	1962	Evolceanu et al	Sabouraudia, 2, 14.
SOUTH AMERICAN COUNTRIES	1959	Mata & Mata	Rev.Biol.trop.(S.Jose),7,119.
	1960	Londero & Ramos	Rev.Inst.med.trop.Sao Paulo,3,75.
	1960	Yarzabal et al	Arch.Soc.Biol.Montevideo,25,74.
	1961	Yarzabal	An.Fac.Med.Montevideo, 46,41.
U. S. A.	1953	Ajello	J.invest.Derm. 21, 157.

Table 11. Distribution of Trichophyton terrestre.

AUSTRALIA	1957	Durie & Frey	Mycologia, 49, 401.
	1958	Report 1956/7	Inst. of med. Research, Royal North Shore Hospital, Sydney, p. 33.
	1960	Griffin	Trans.Brit.myc.Soc.,43, 583.
BOHEMIA	1962	Otčenášek & Dvořák	Sabouraudia, 2, 111.
BRITAIN	1958	Stockdale	Nature(Lond.) 182, 1754.
	1961	Dawson & Gentles	Sabouraudia, 1, 49.
FINLAND	1962	Lundell	Personal communication
GERMANY	1960	Meinhof et al	Arch.klin.exp.Derm. 212, 30.
	1960	Schönfeld et al	Arch.klin.exp.Derm. 212, 78.
	1961	Rieth	Arch.klin.exp.Derm. 213, 662.
HUNGARY	1936	Szathmary	Magy.belorv.Arch. 37, 1.
NEW GUINEA	1958	Report 1956/7	Inst. of med. Research, Royal North Shore Hospital, Sydney, p. 33.
NEW ZEALAND	1961	Marples	Trans.roy.Soc.Trop.Med.Hyg.55,216.
	1962	Marples & Smith	Sabouraudia, 2, 100.
POLAND	1963	Prochacki & Bielunska	Acta Microbiol.Polon. 12, 143.
ROUMANIA	1962	Evolceanu et al	Sabouraudia, 2, 14.
	1962	Evolceanu et al	Mycopathologia(Den Haag),16,35.

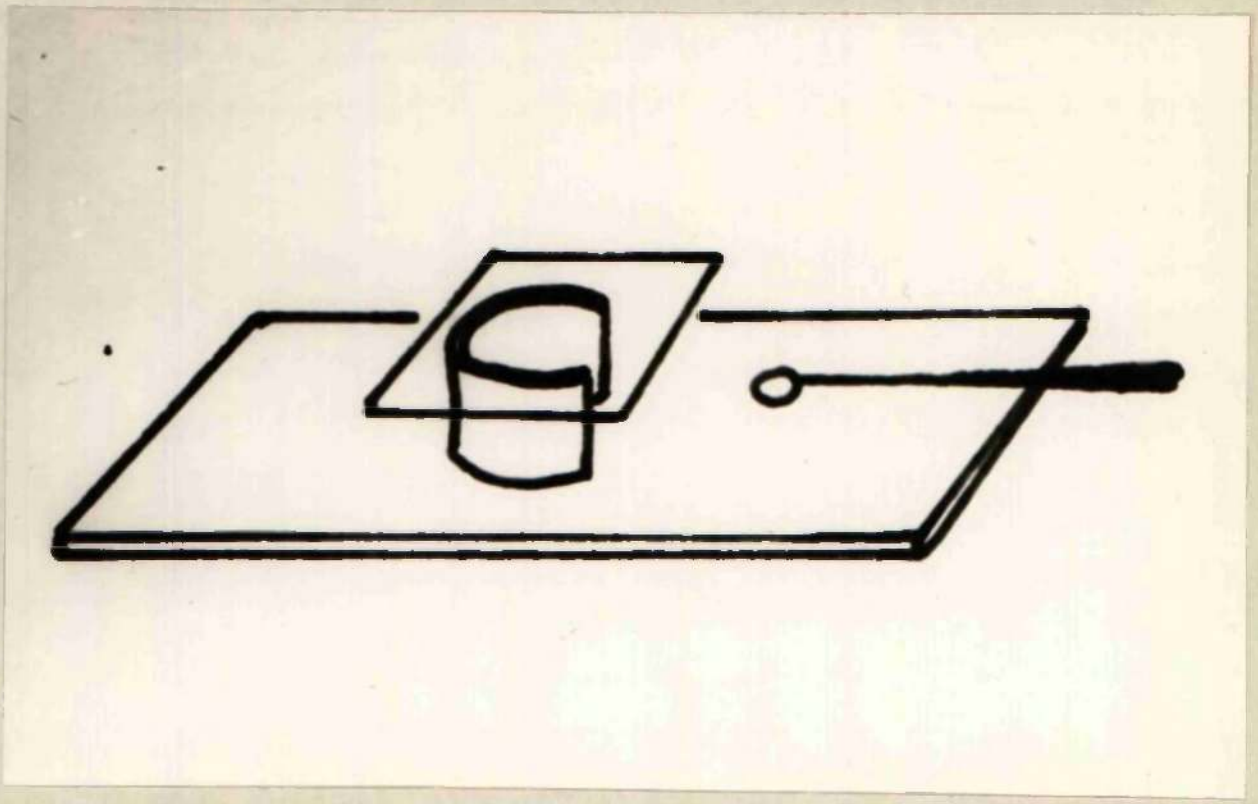
Table 12. Comparison of the measurements of the perfect and imperfect states of Arthroderma lomondii and its variety.

	A. lomondii	A. lomondii variety
Diameter of cleistothecium	330 - 570µ, av. 450µ	380 - 608µ, av. 480µ
Thickness of peridium	76 - 152µ, av. 107µ	90 - 160µ, av. 123µ
Peridial cells length	5.3 - 12.5µ, av. 7.2µ	5.8 - 9.1µ, av. 7.3µ
Peridial cells breadth, excluding protuberances	2.5 - 4.9µ, av. 3.8µ	2.9 - 4.9µ, av. 3.7µ
Protuberances length	up to 2.5µ	up to 3.3µ
Protuberances breadth	0.8 - 2.0µ	1.2 - 2.4µ
Spiral hyphae number of turns	2 - 6	2 - 11
Asci length	3.3 - 4.9µ	4.1 - 4.9µ
Asci breadth	2.9 - 4.2µ	3.3 - 4.2µ
Ascospores length	1.8 - 2.6µ	1.8 - 2.5µ
Ascospores breadth	0.8 - 1.5µ	0.8 - 1.6µ
Macroconidia length	5.8 - 20µ	5.8 - 13.2µ
Macroconidia breadth	2.0 - 3.7µ	1.5 - 2.3µ
Microconidia length	2.5 - 4.2µ	2.0 - 3.3µ
Microconidia breadth	1.2 - 2.1µ	0.8 - 1.6µ

ILLUSTRATIONS

Figure 1.

Diagram showing the partially enclosed chamber formed by slide, broken ring and coverslip, in which extraction and inoculation of spores with the micro-loop is carried out.



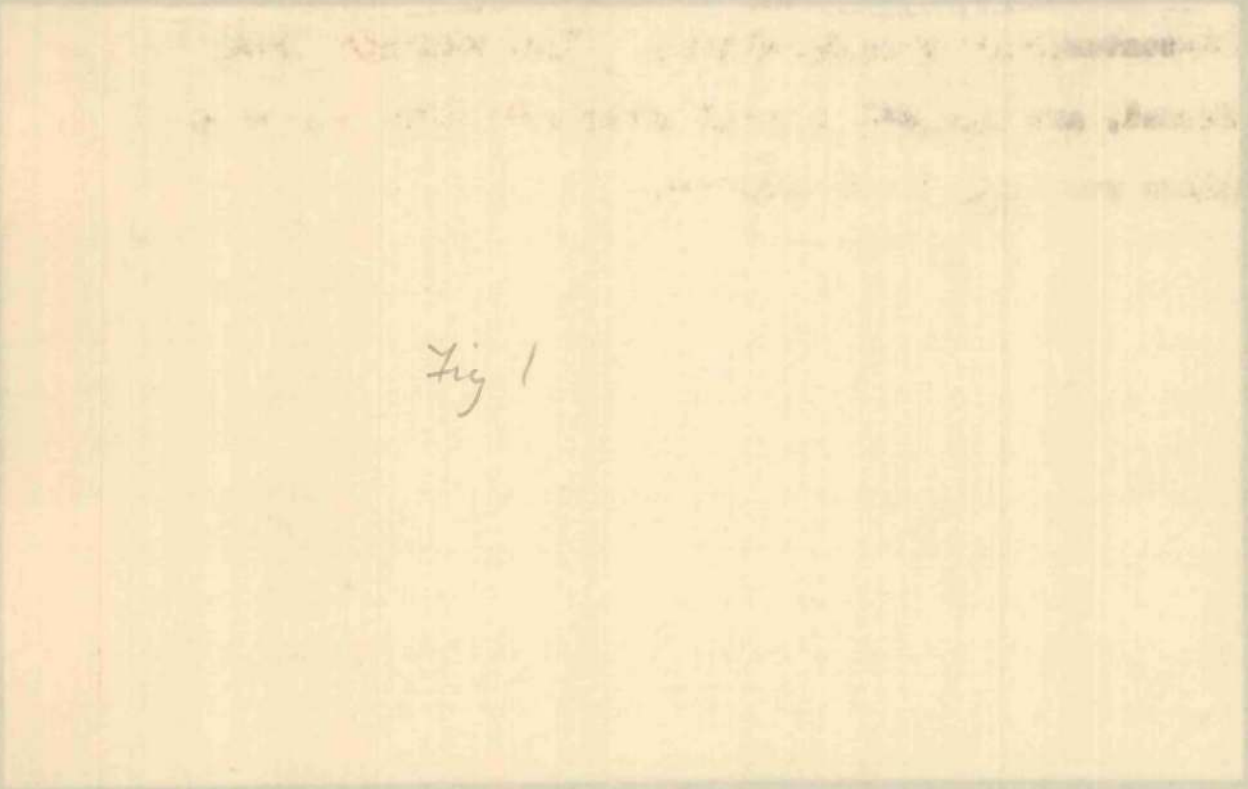


Fig 1

Plate 1.

The growth and formation of the perfect states of Trichophyton terrestre, Keratinomyces ajelloi and Microsporum gypseum on unsterilized, sterilized and re-contaminated sterilized soil. Cleistothecia, when formed, are apparent as small round structures on, or in close proximity to, the hair bait.

Trichophyton terrestre

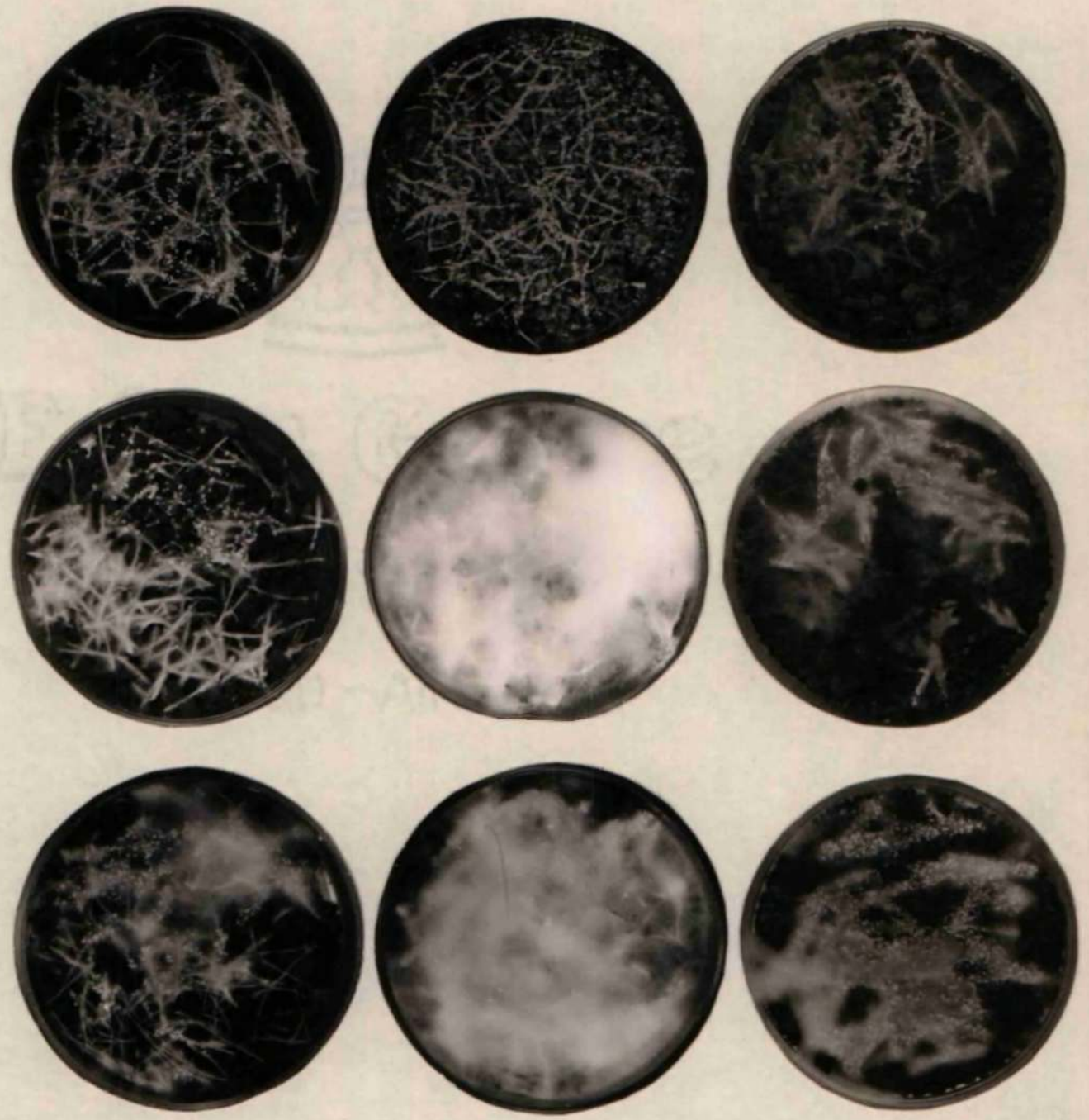
Unsterilized soil	Sterilized soil	Recontaminated sterilized soil

Keratinomyces ajelloi

Unsterilized soil	Sterilized soil	Recontaminated sterilized soil

Microsporum gypseum

Unsterilized soil	Sterilized soil	Recontaminated sterilized soil



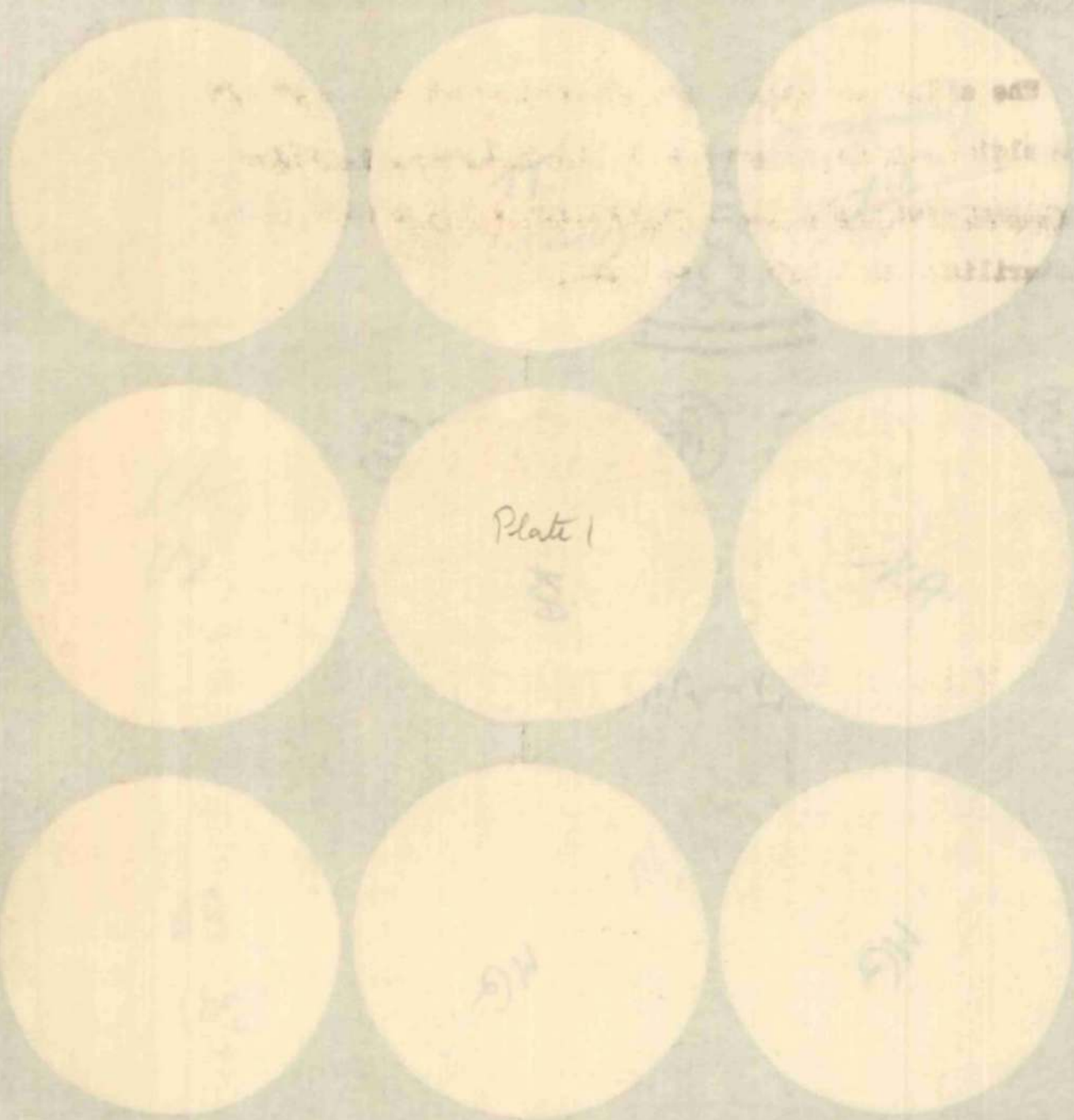


Plate 1

Plate 2.

The effect of temperature of incubation on the growth and cleistothecial formation of *Trichophyton terrestre*, *Keratinomyces ajelloi* and *Microsporum gypseum* cultured on unsterilized soil with horse hair bait.

Trichophyton terrestre

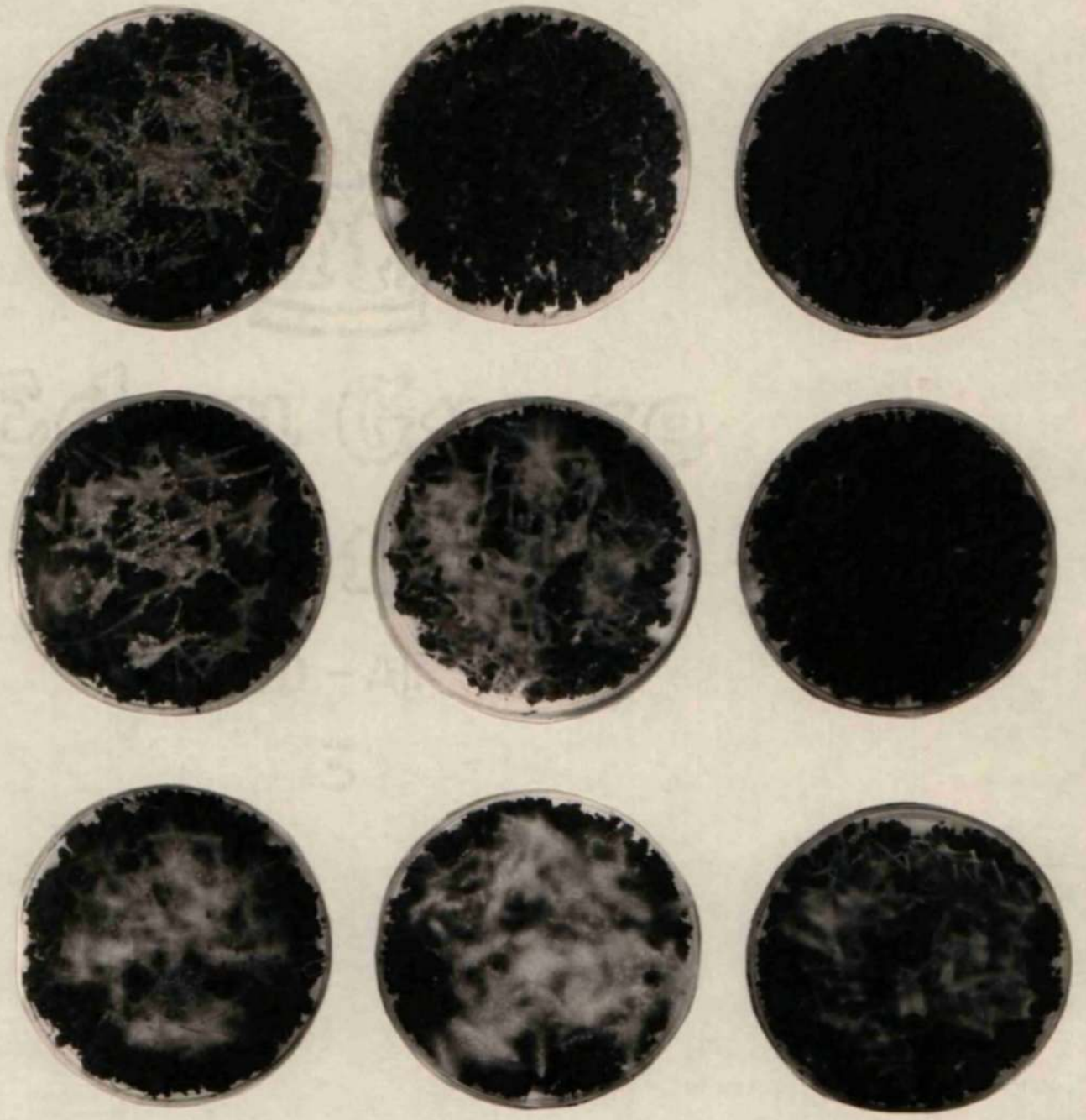
24°C 30°C 34°C

Keratinomyces ajelloi

24°C 30°C 34°C

Microsporum gypseum

24°C 30°C 34°C



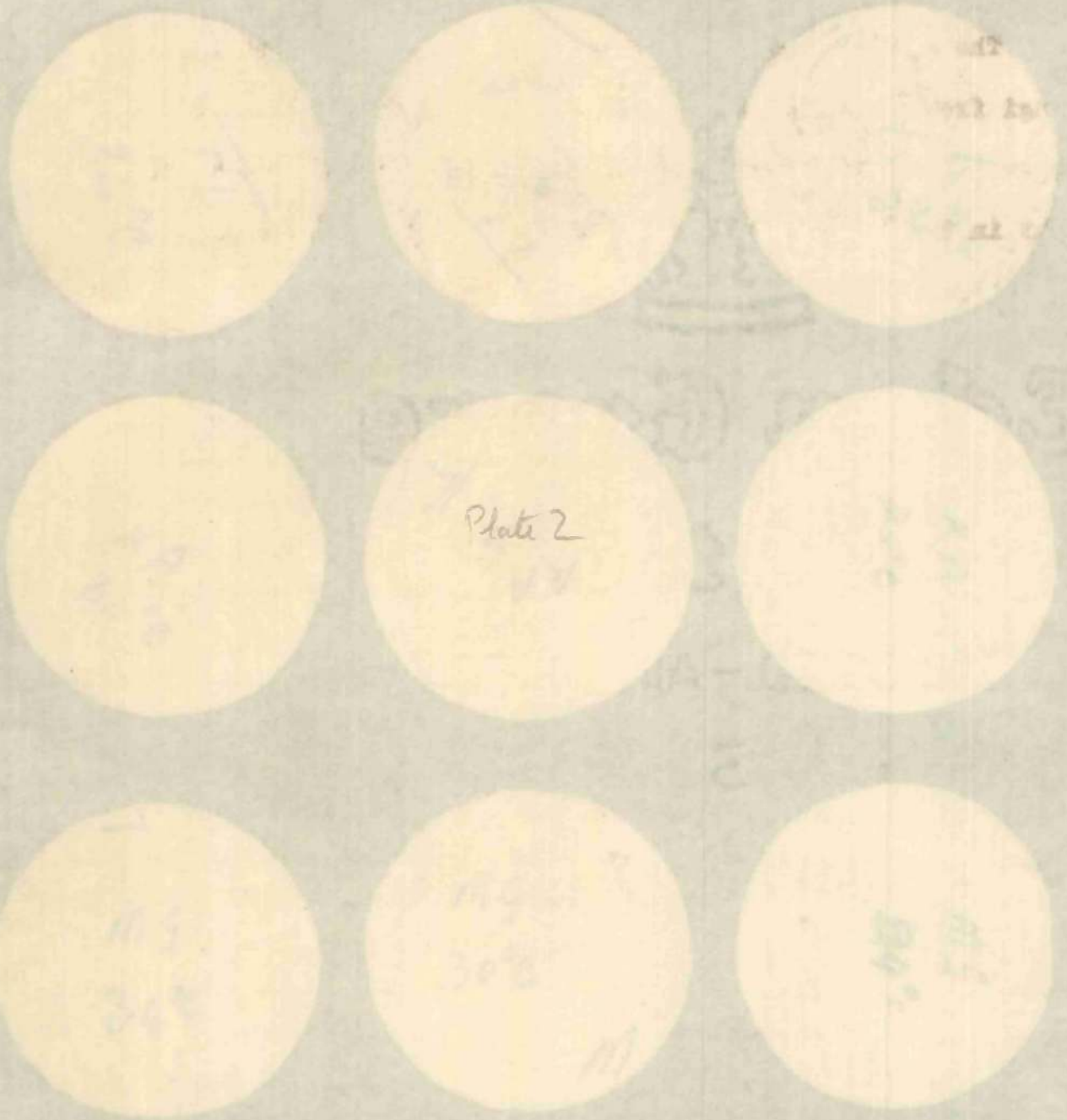


Plate 2

Plate 3.

The effect of light on the isolation of keratinophilic fungi from a sample of natural soil. The sample in the upper photograph was incubated in constant bright light and that in the lower photograph was incubated in darkness.

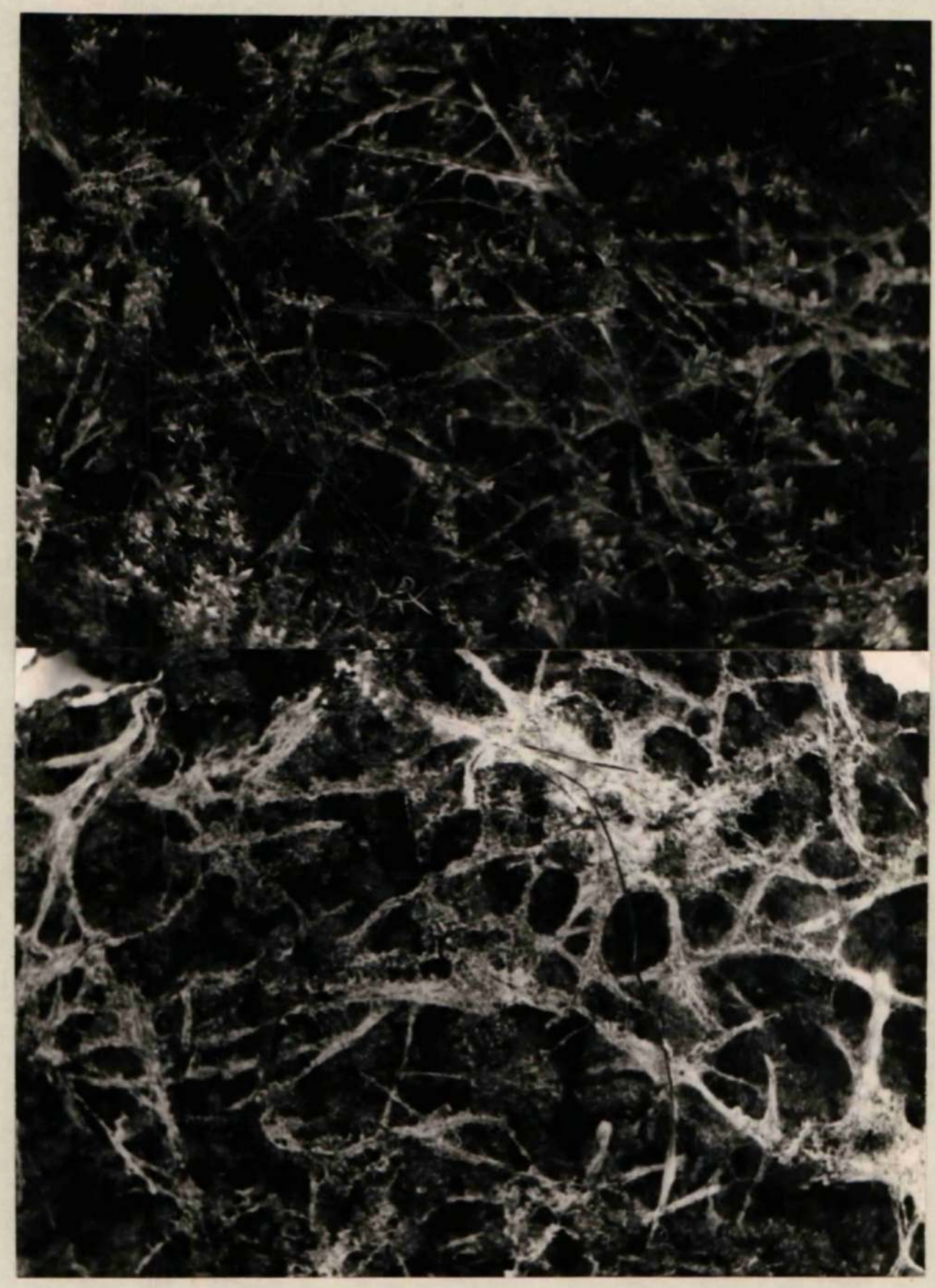


Plate 3

Plate 4.

The effect of light on the growth of *Arthroderma curreyi* cultured on negative soil with horse hair bait. The sample in the upper photograph was incubated in constant bright light and that in the lower photograph in darkness.

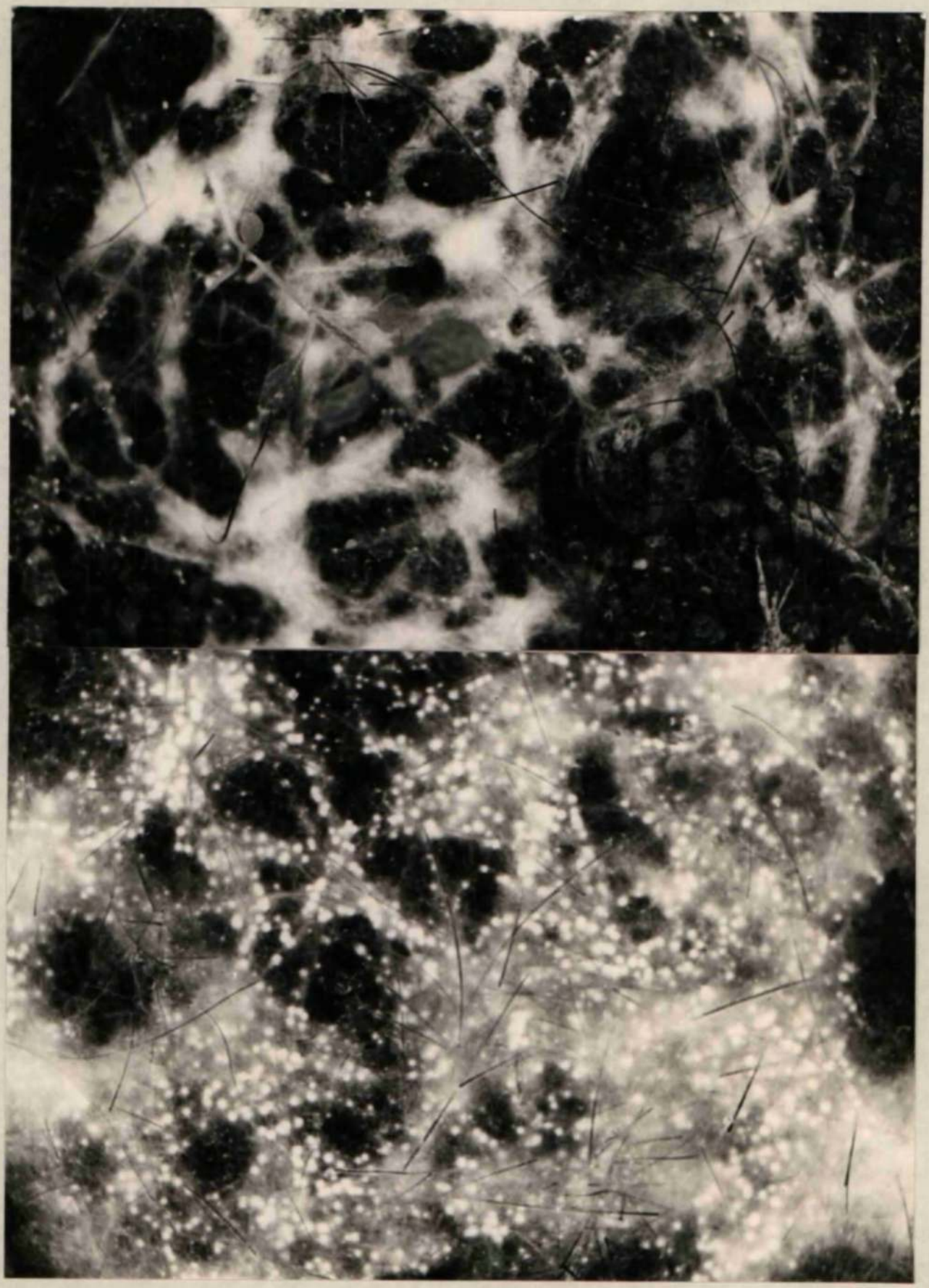


Plate 4

Plate 5.

Arthroderma curreyi

a) Mature cleistothecium, X 200.

b) Sector of the peridium of an immature cleistothecium showing the spiral growth of the peridial hyphae, X 800.

c) Sector of the peridium of a mature cleistothecium showing the spiral growth of the peridial hyphae, branching and dumb-bell shaped cells, X 800.

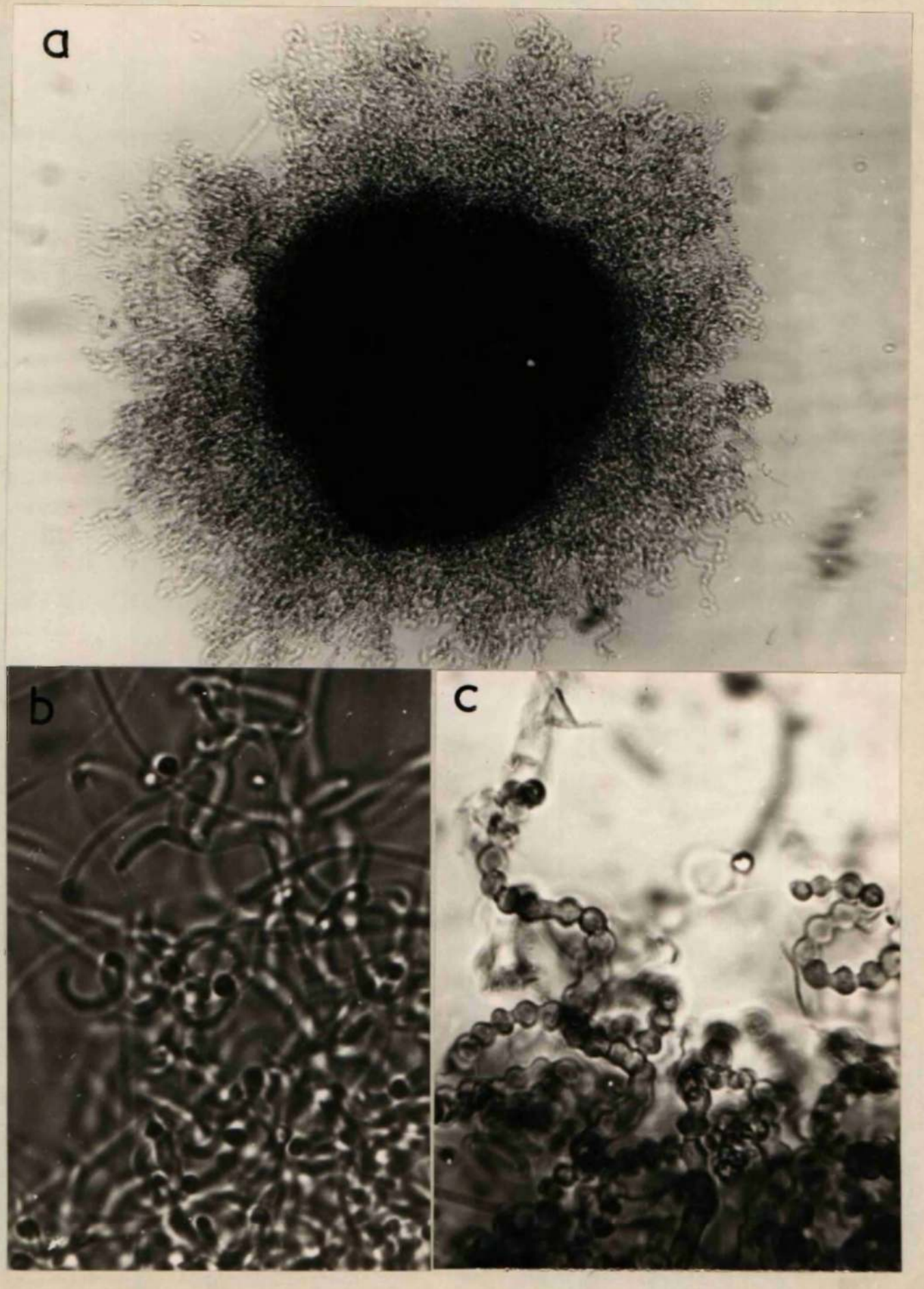

a
b
c

Plate 5

Plate 6.

Arthroderma curreyi

a) Peridial cells and spiral hypha, X 2000.

b) Ascus and ascospores, X 2000.

c) Immature asci in grape-like clusters on branched ascogenous hyphae. Peridial hyphae composed of one-sided dumb-bell shaped cells, X 800.

d) Asexual spores, X 800.

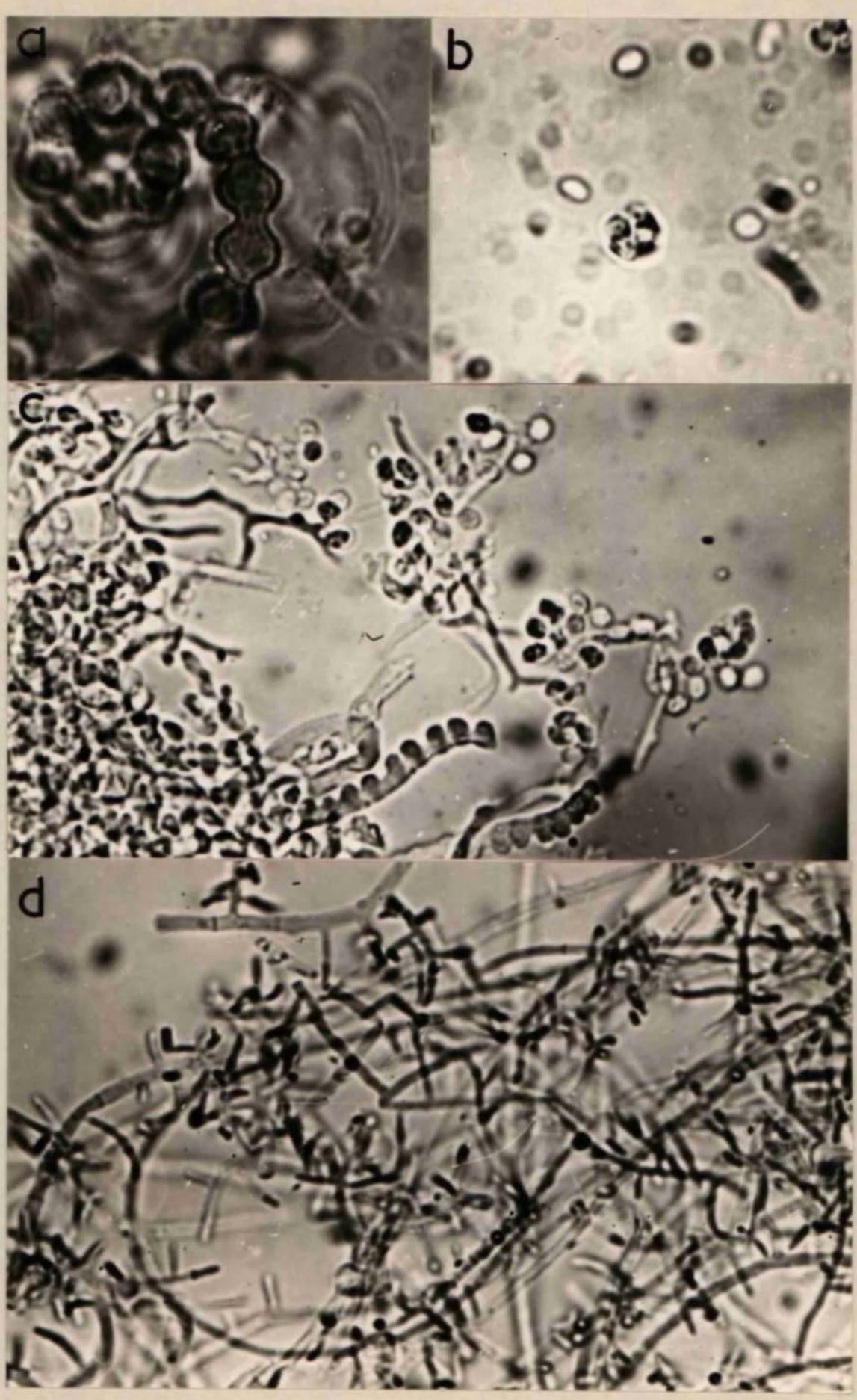

a
b
c
d

Plate 6

Plate 7.

Arthroderma uncinatum

a) Mature cleistothecium, X 200.

b) Sector of peridium of a mature cleistothecium showing spiral hyphae and uncinate branching, X 800.

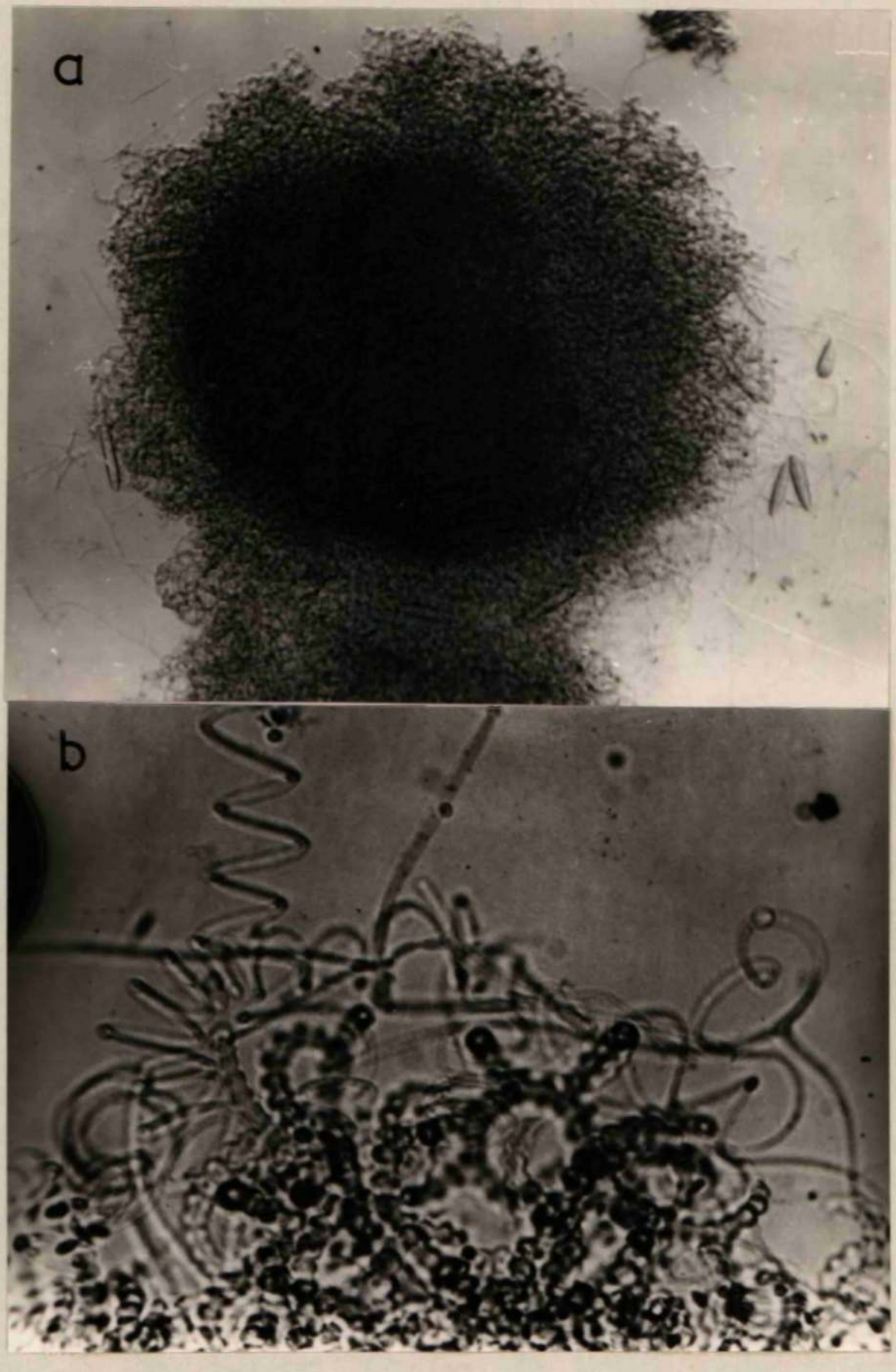

a
b

Plate 7

Plate 8.

Arthroderma uncinatum

a) Dumb-bell shaped peridial cells, X 2000.

b) Immature macroconidium developing from a cell of a peridial hypha, X 800.

c) Mature macroconidium which has developed from a cell of a peridial hypha, X 800.

d) Thick walled multiseptate macroconidia developed in clusters from vegetative hyphae, X 800.

e) Ascus and ascospores, X 2000.

f) Racquet hyphae, X 800.

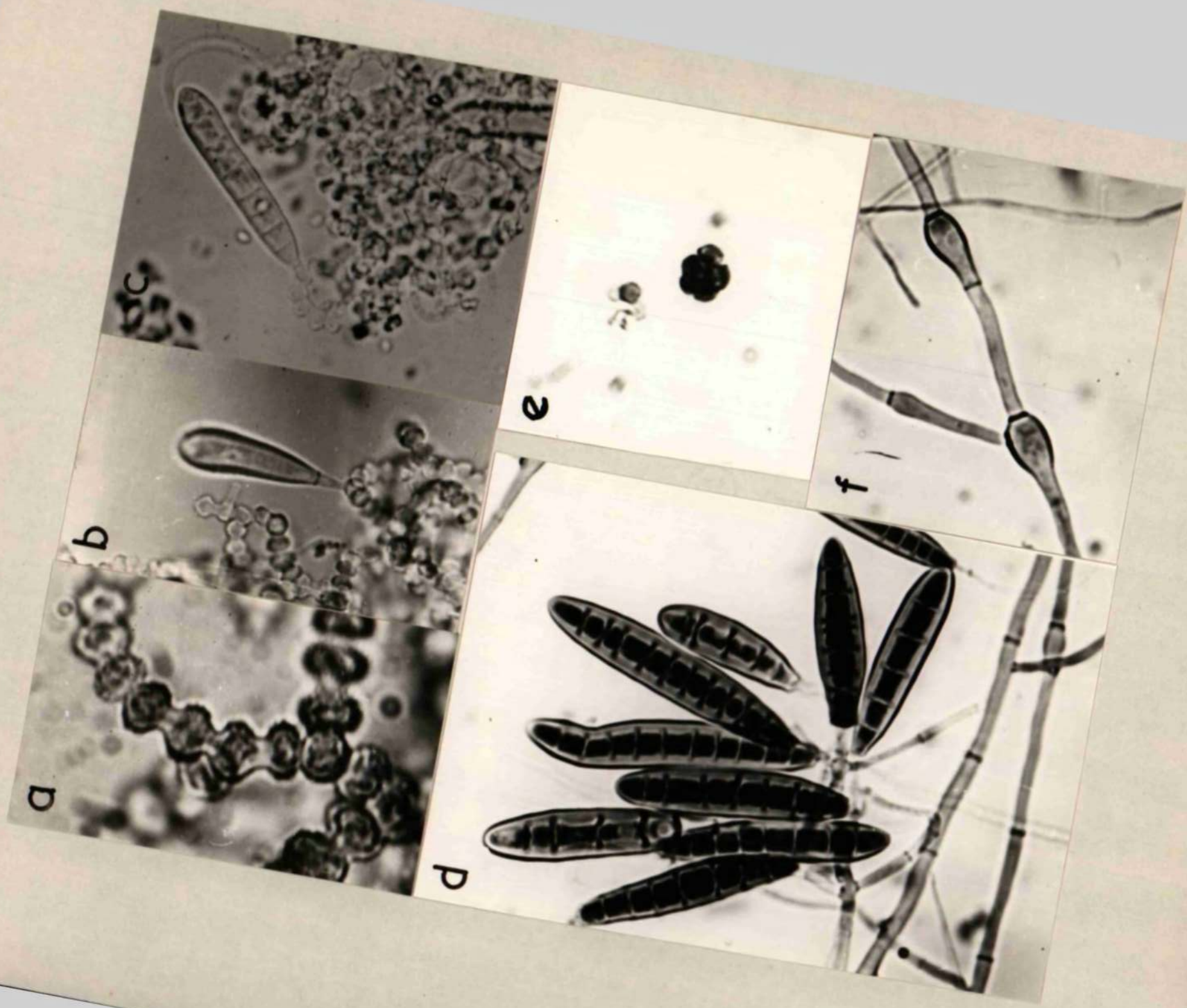

c
a
b
e
f
d

Plate 8

Plate 9.

Arthroderma multifidum

a) Mature cleistothecium, X 200.

b) Sector of the peridium of a mature cleistothecium showing branching, peridial cells and a spiral hypha, X 800.

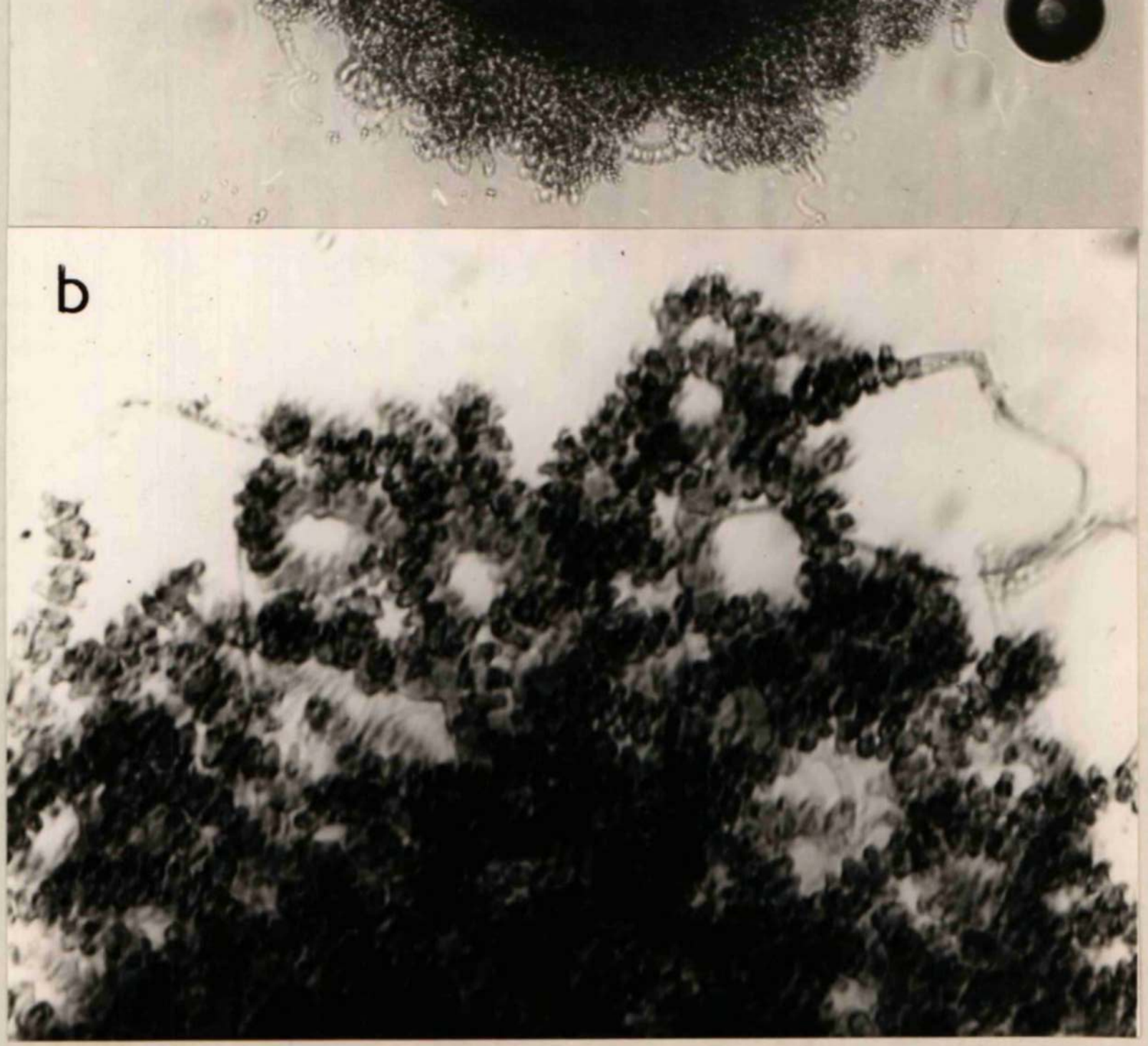

b

Plate 9

Plate 10.

Arthroderma multifidum

a) Peridial hypha showing uncinate branching, X 1500.

b) Dumb-bell shaped cells found in immature cleistothecia, X 2000.

c) Spiral hypha and fully differentiated peridial cells, X 2000.

d) Fully differentiated peridial cells showing the various shapes which the protuberances can assume, X 2000.

e) Ascus, X 3500 approx.

f) Sub-globose conidium, X 2000.

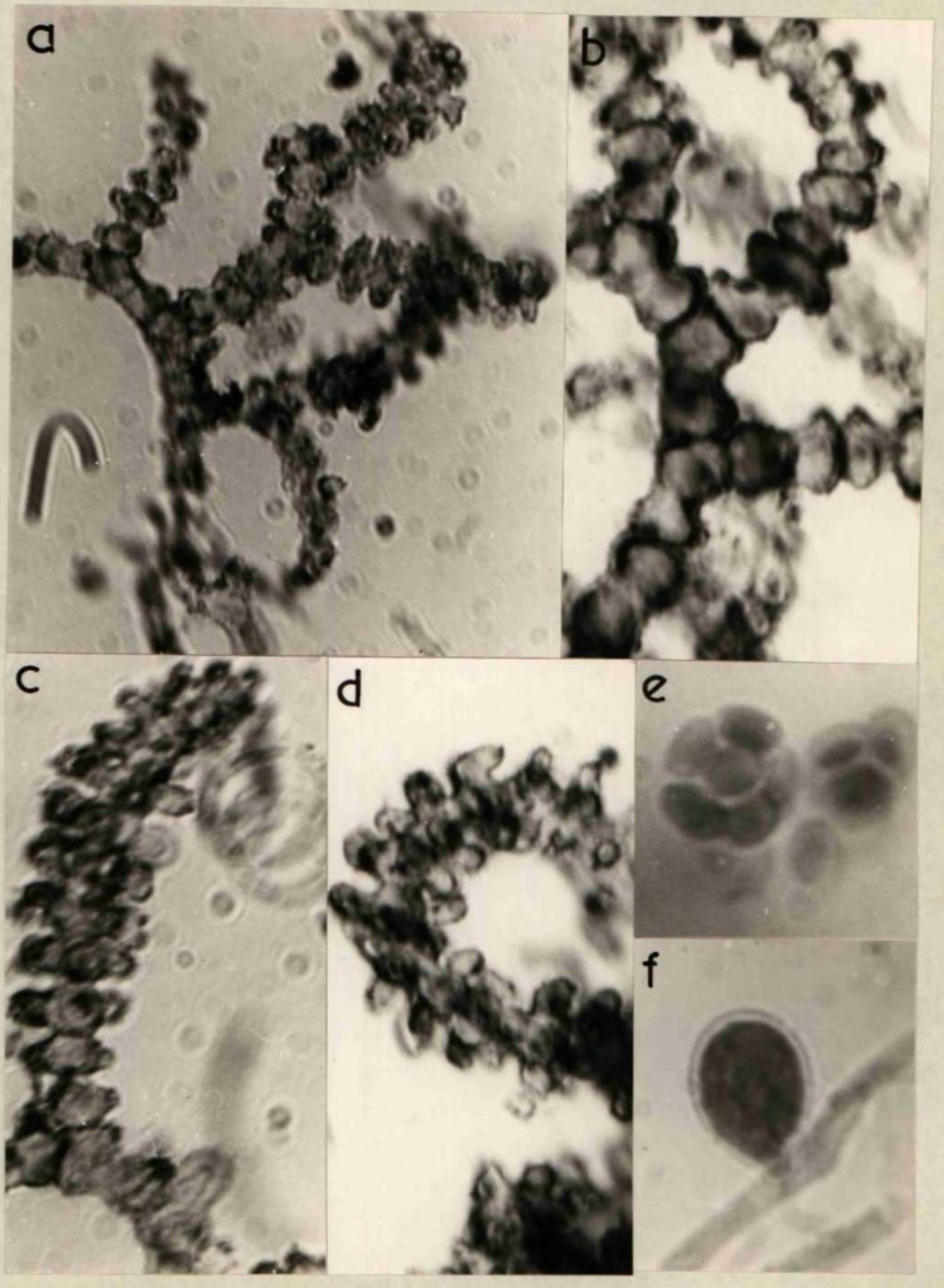

a
b
c
d
e
f

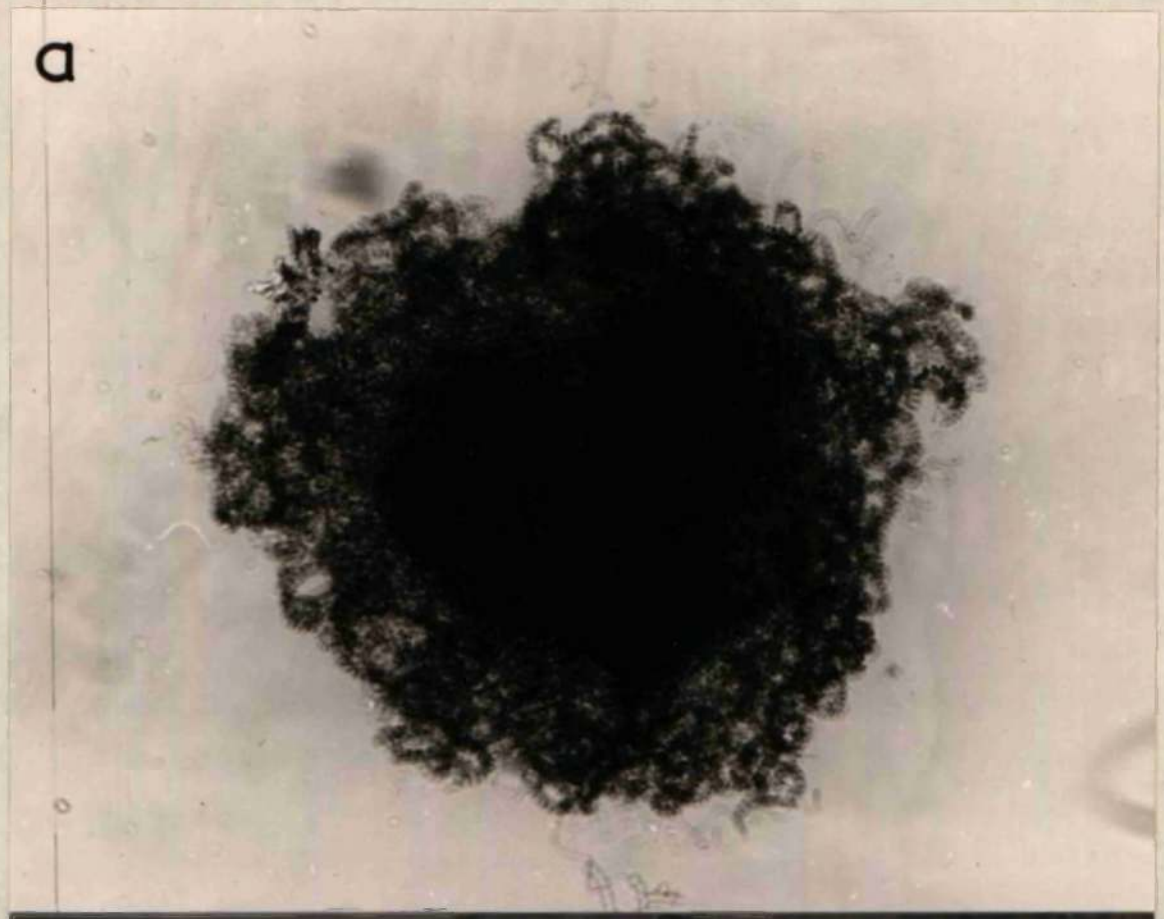
Plate 10

Plate 11.

Arthroderma cuniculi

a) Mature cleistothecium, X 200.

b) Sector of the peridium of a mature cleistothecium showing peridial cells and a spiral hypha, X 800.


a


b

Plate II

Plate 12.

Arthroderma cuniculi

a) Peridial hypha showing uncinate branching and dumb-bell shaped cells, X 800.

b) Spiral hypha developing from a cell of a peridial hypha, X 800.

c) Undifferentiated dumb-bell shaped peridial cells, X 2000.

d) Fully differentiated peridial cells showing the 3 protuberances at each end, X 2000.

e) Ascus, X 2000.

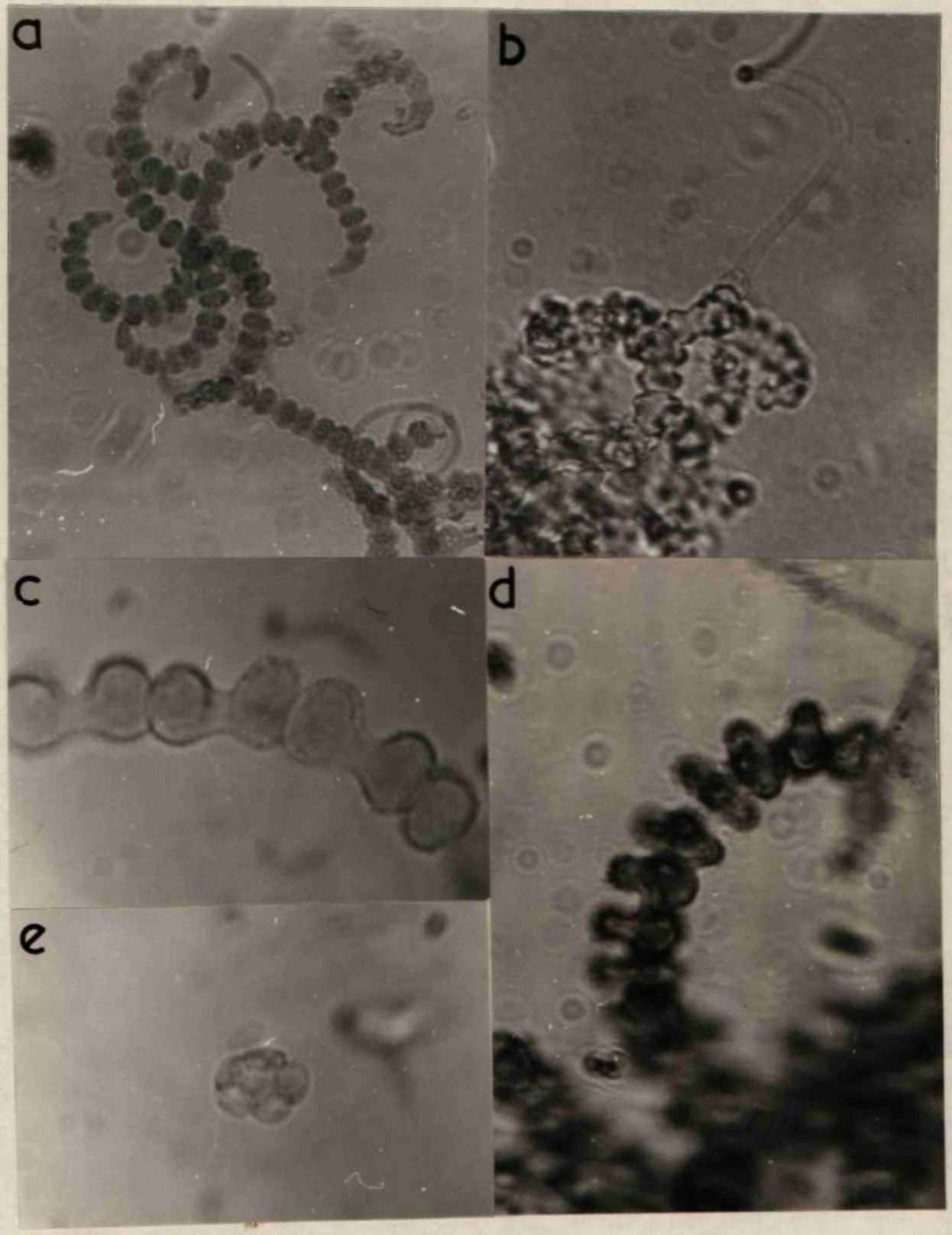

a
b
c
d
e

Plate 12

Plate 13.

Arthroderma cuniculi

a) Spiral hypha formed in culture on agar medium, X 800.

b) Microconidia formed "en grappe", X 800.

c) Microconidia, X 2000.

d) Asexual growth on hair bait on soil.

e) Cleistothecia on hair bait on soil.

a
b
c

Plate 13

Plate 14.

Arthroderma cocleatus

a) Mature cleistothecium, X 200.

b) Young cleistothecial initial, X 2000.

c) Older cleistothecial initial which has lost its well-defined shape, X 2000.

d) Sector of the peridium of a mature cleistothecium showing peridial cells and spiral hyphae, X 800.

e) Sector of the peridium of a mature cleistothecium showing the spiral coiling of the outermost branches, X 800.

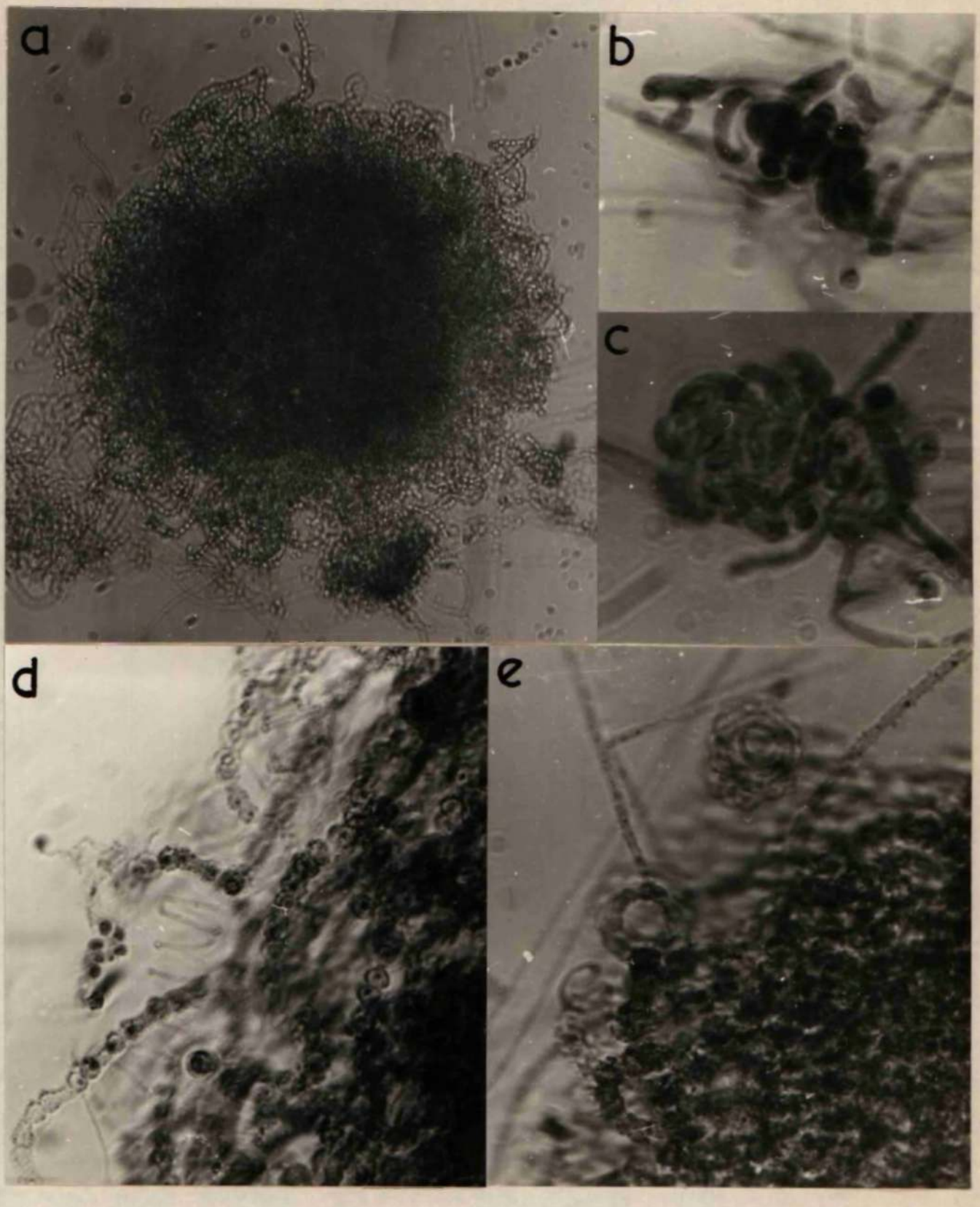

a
b
c
d
e

Plate 14

Plate 15.

Arthroderma cocleatus

a) Peridial hypha showing uncinate branching, X 800.

b) Spiral hypha developed from a peridial hypha, X 800.

c) Spiral hypha developed from a peridial hypha, X 2000.

d) Dumb-bell shaped peridial cells with large globules, X 2000.

e) Elongated slightly asymmetrical peridial cells, X 2000.

f) Very elongated peridial cells, X 2000.

g) Peridial cells with globules stained with cotton blue, X 2000.

h) Ascus and ascospores, X 2000.

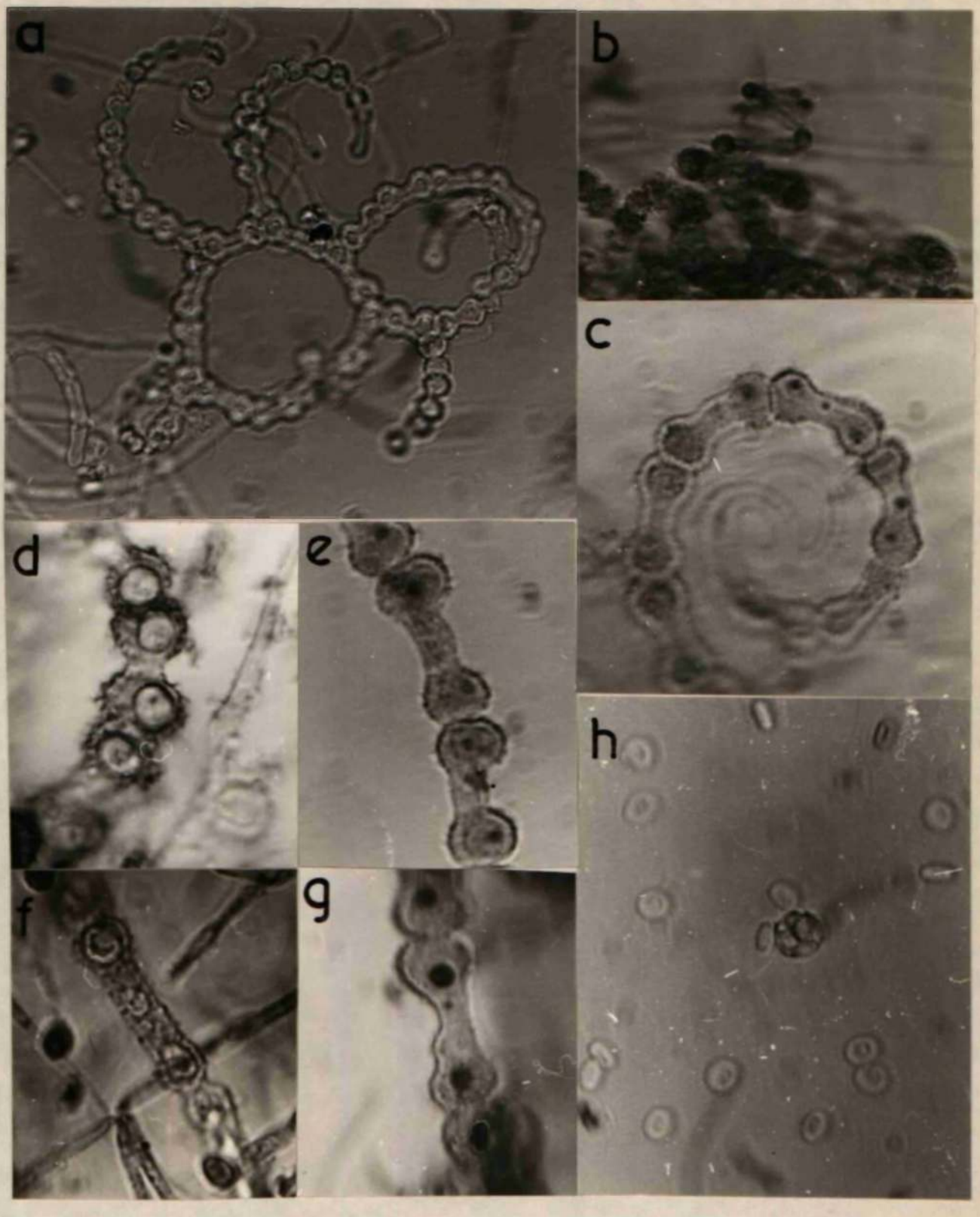

a
b
c
d
e
f
g
h

Plate 15

Plate 16.

Arthroderma cocleatus

a) Conidia in clusters, X 800.

b) Conidia, X 2000.

c) Conidia "en thyrses", X 800.

d) Spiral hyphae formed in agar culture, X 800.

e) Pectinate hyphae formed in agar culture, X 800.

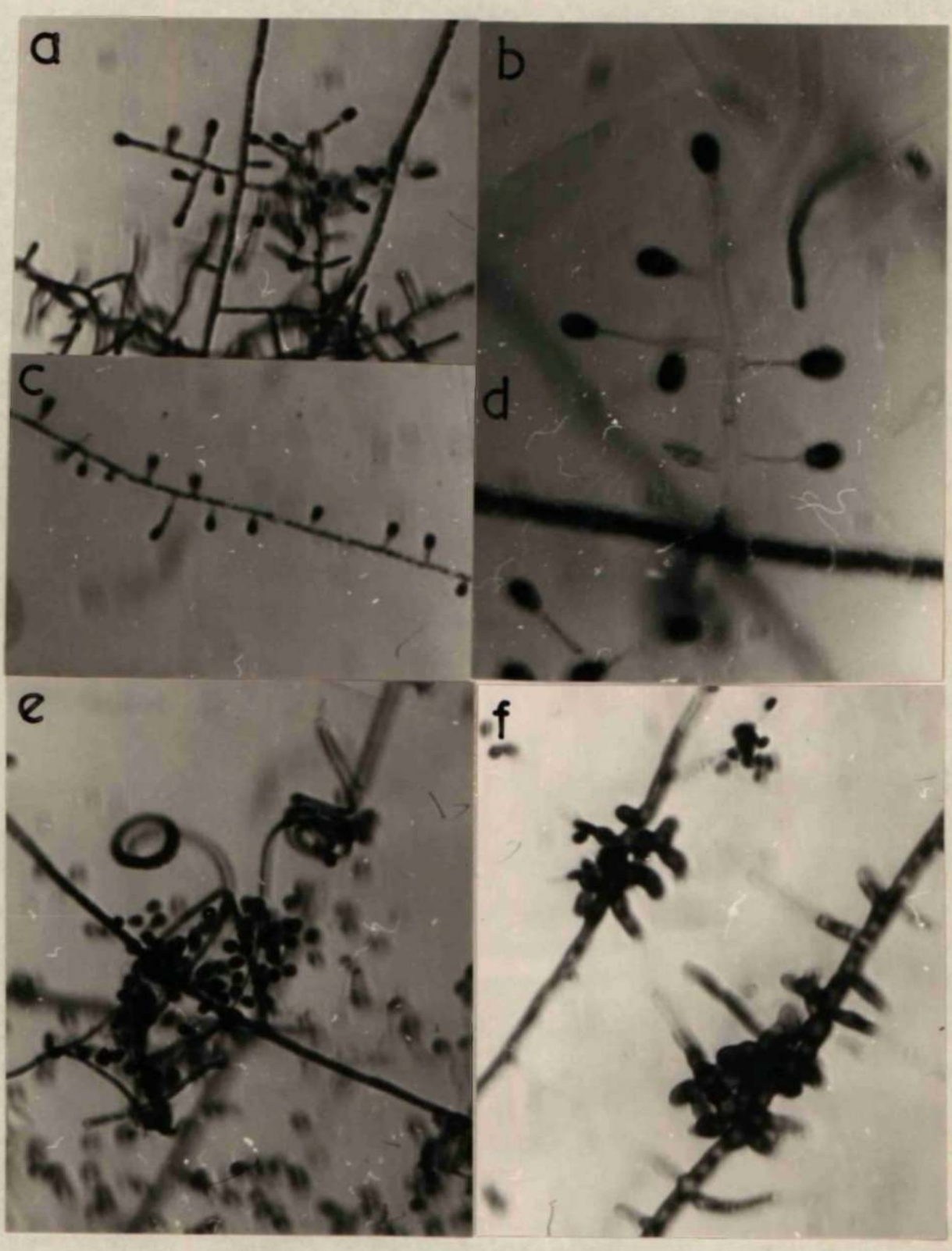

a
b
c
d
e
f

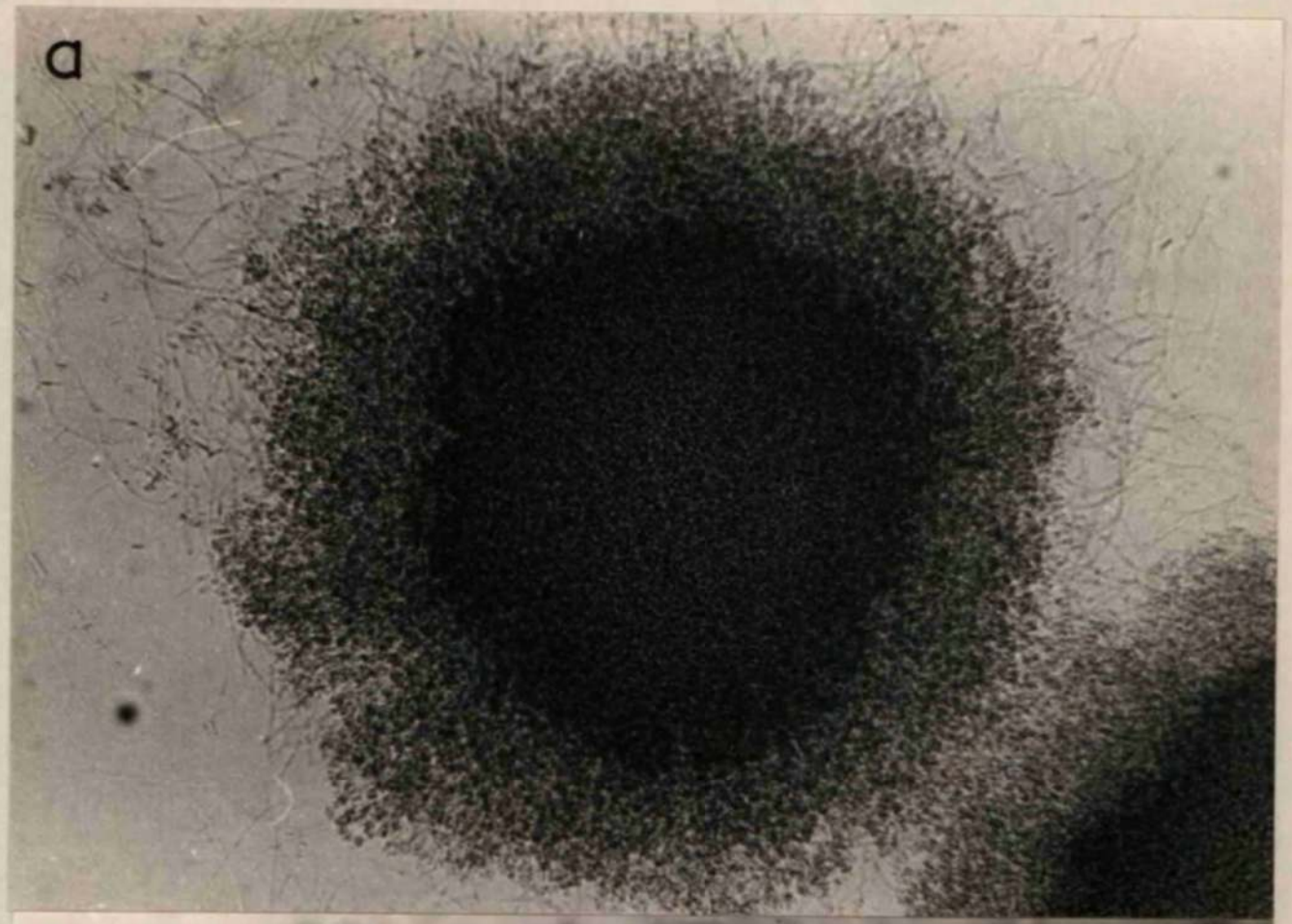
Plate 16

Plate 17.

Arthroderma quadrifidum

a) Mature cleistothecium, X 200.

b) Sector of the peridium of a mature cleistothecium showing peridial cells and uncinate branching, X 800.


a

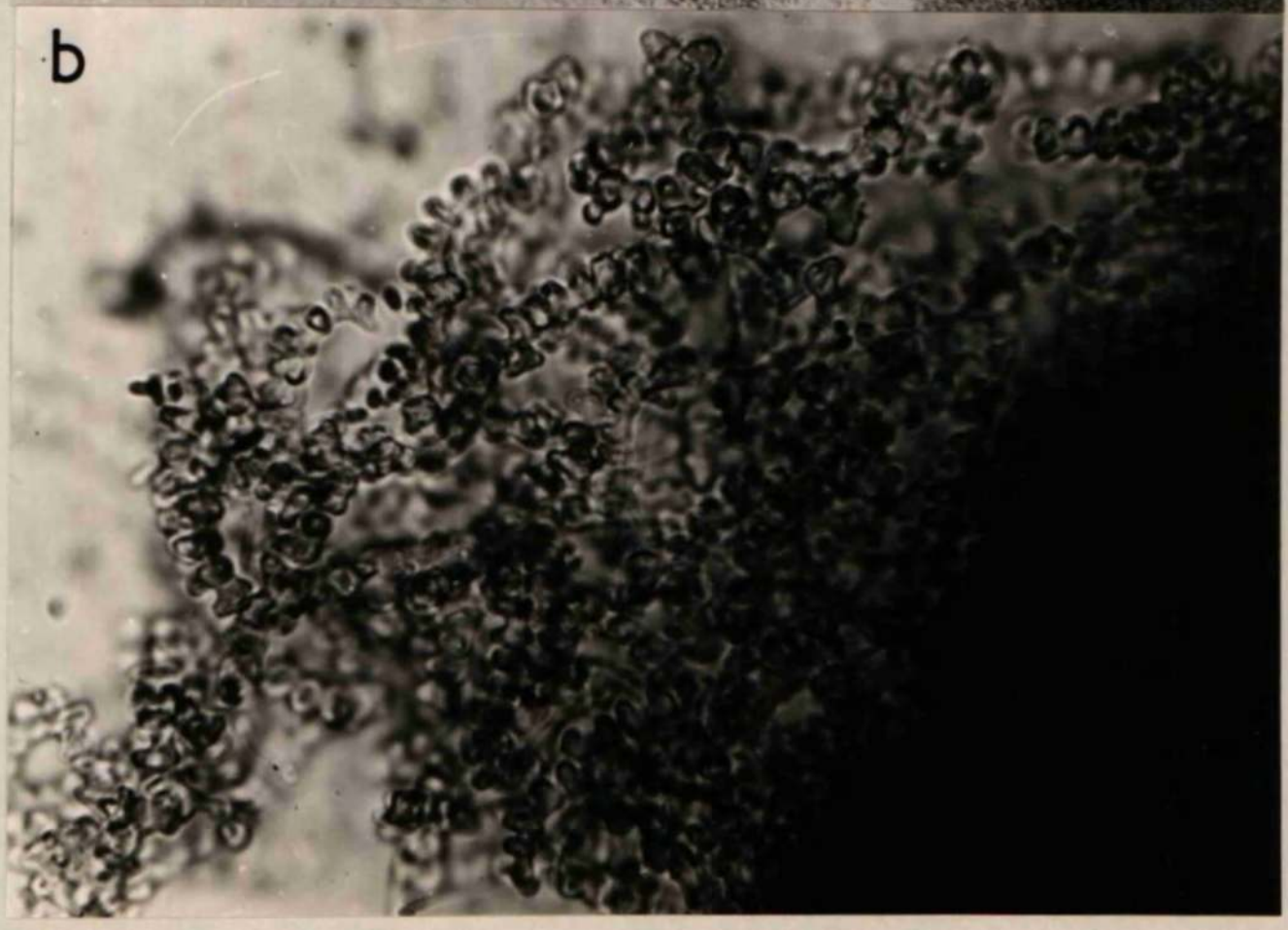

b

Plate 14

Plate 18.

Arthroderma quadrifidum

a) Peridial hypha showing uncinate branching, X 800.

b) Mature peridial cells with 2 protuberances at each end of the cell, X 2000.

c) Ascus and ascospores, X 2000.

d) Spiral hyphae formed in agar culture, X 800.

e) Macroconidium and microconidia, X 2000.

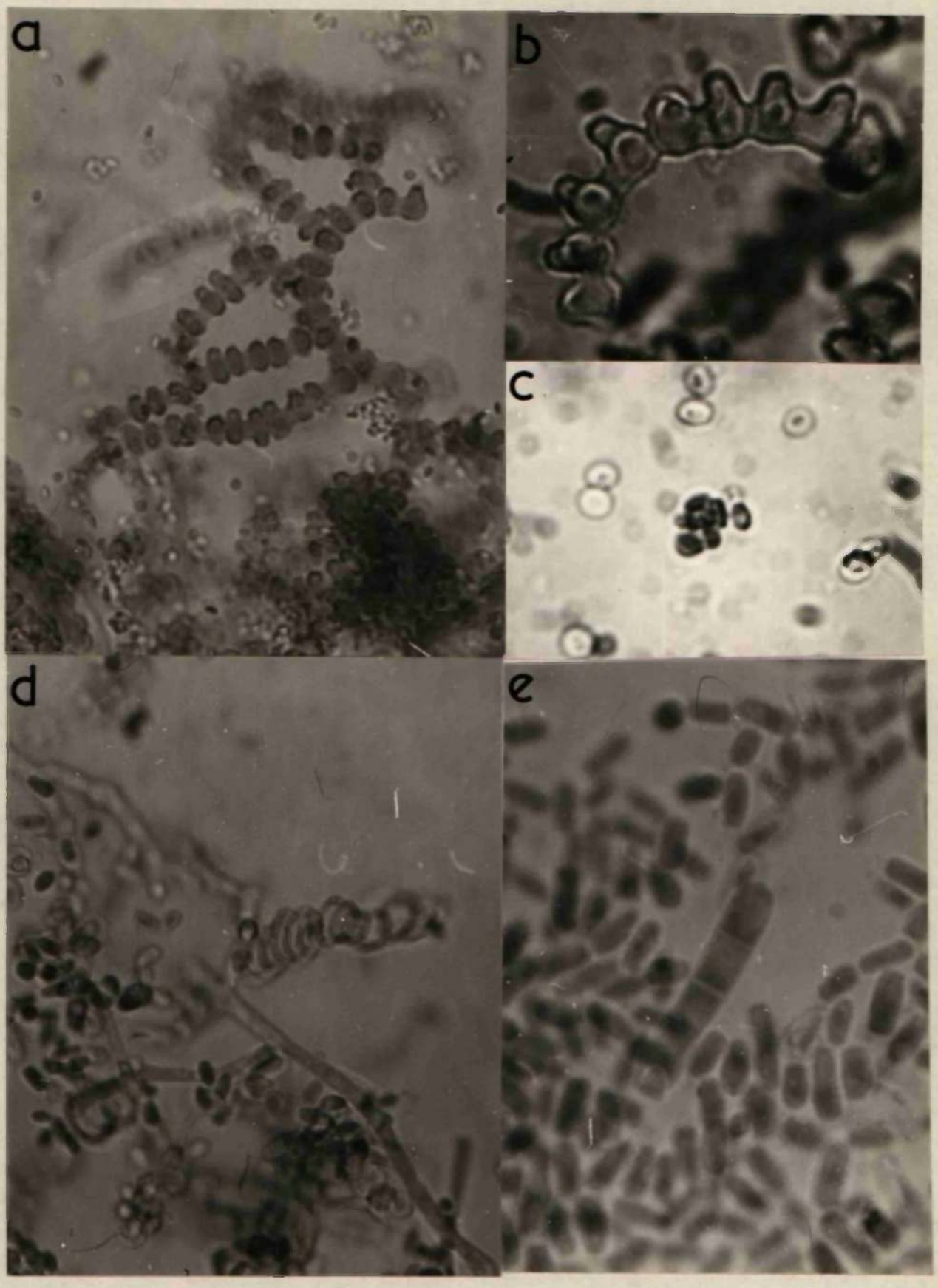

a
b
c
d
e

Plate 18

Plate 19.

Arthroderma quadrifidum

a) Mature conidial nodule, X 200.

b) Young conidial nodule, X 800.

c) Sector of the peridium of a mature conidial nodule showing spiral hyphae and peridial cells, X 800.

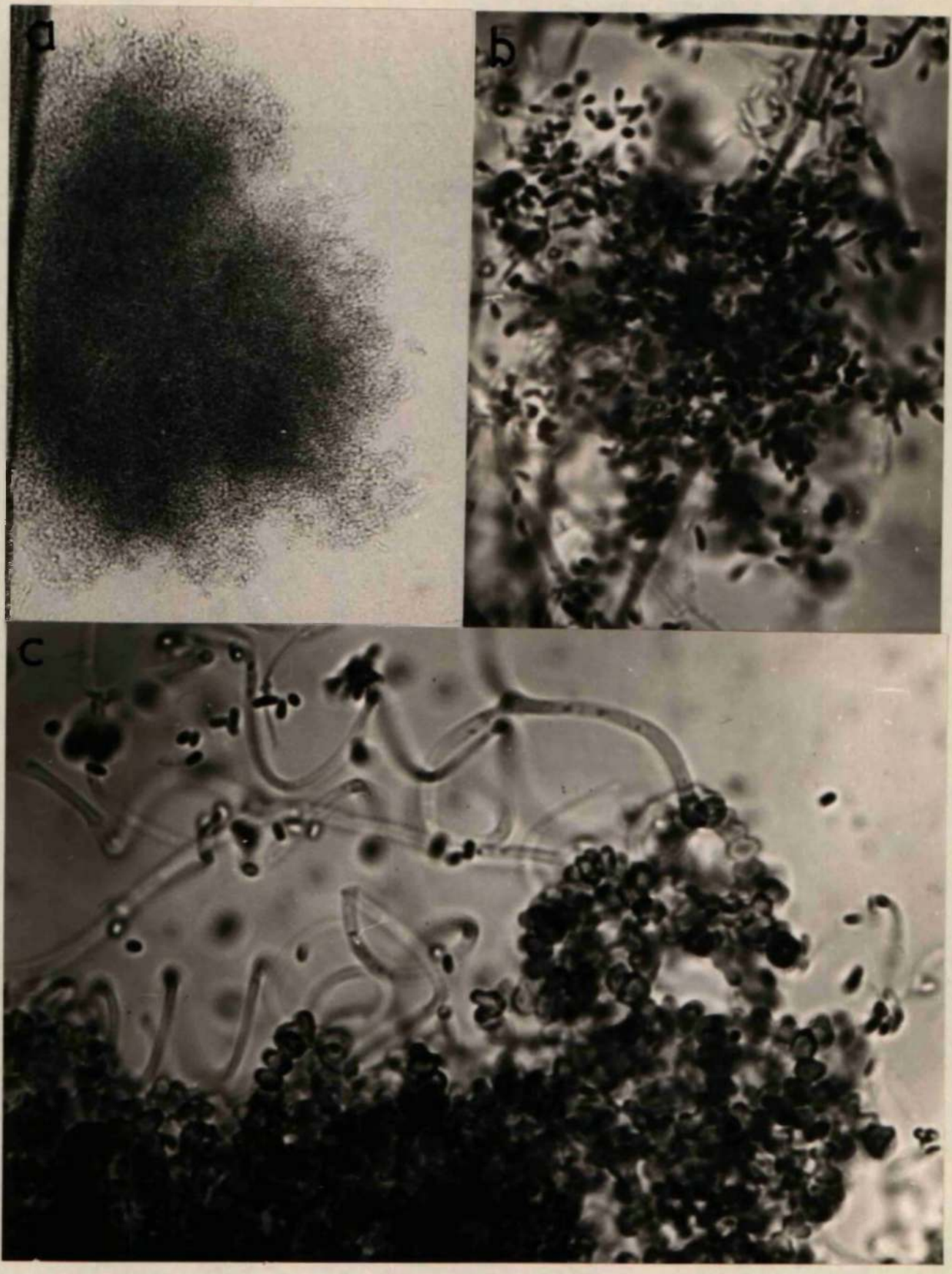

a
b
c

Plate 19

Plate 20.

Arthroderma lomondii

a) Mature cleistothecium, 200.

b) Sector of the peridium of a mature cleistothecium showing branching, peridial cells and a spiral hypha, X 800.

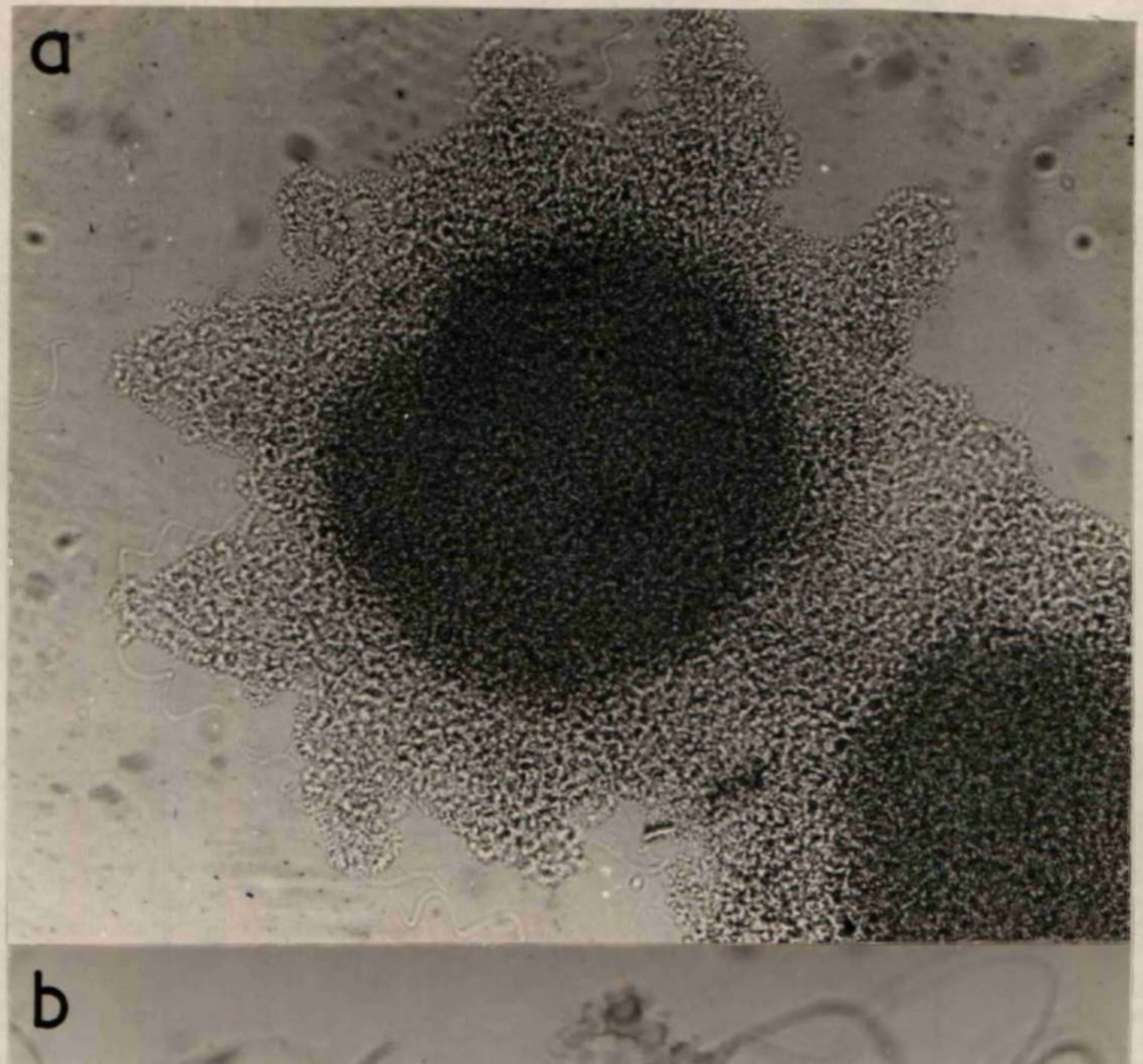

a
b

Plate 20

Plate 21.

Arthroderma lomondii

a) Peridial hyphae showing uncinate branching, X 800.

b) Dumb-bell shaped peridial cells, X 2000.

c) Asymmetrical peridial cells, X 2000.

d) Fully differentiated peridial cells with 3 protuberances at each end of the cell, X 2000.

e) Cleistothecial initial, X 2000.

f) Ascus, X 2000.

g) Ascospores, X 2000.

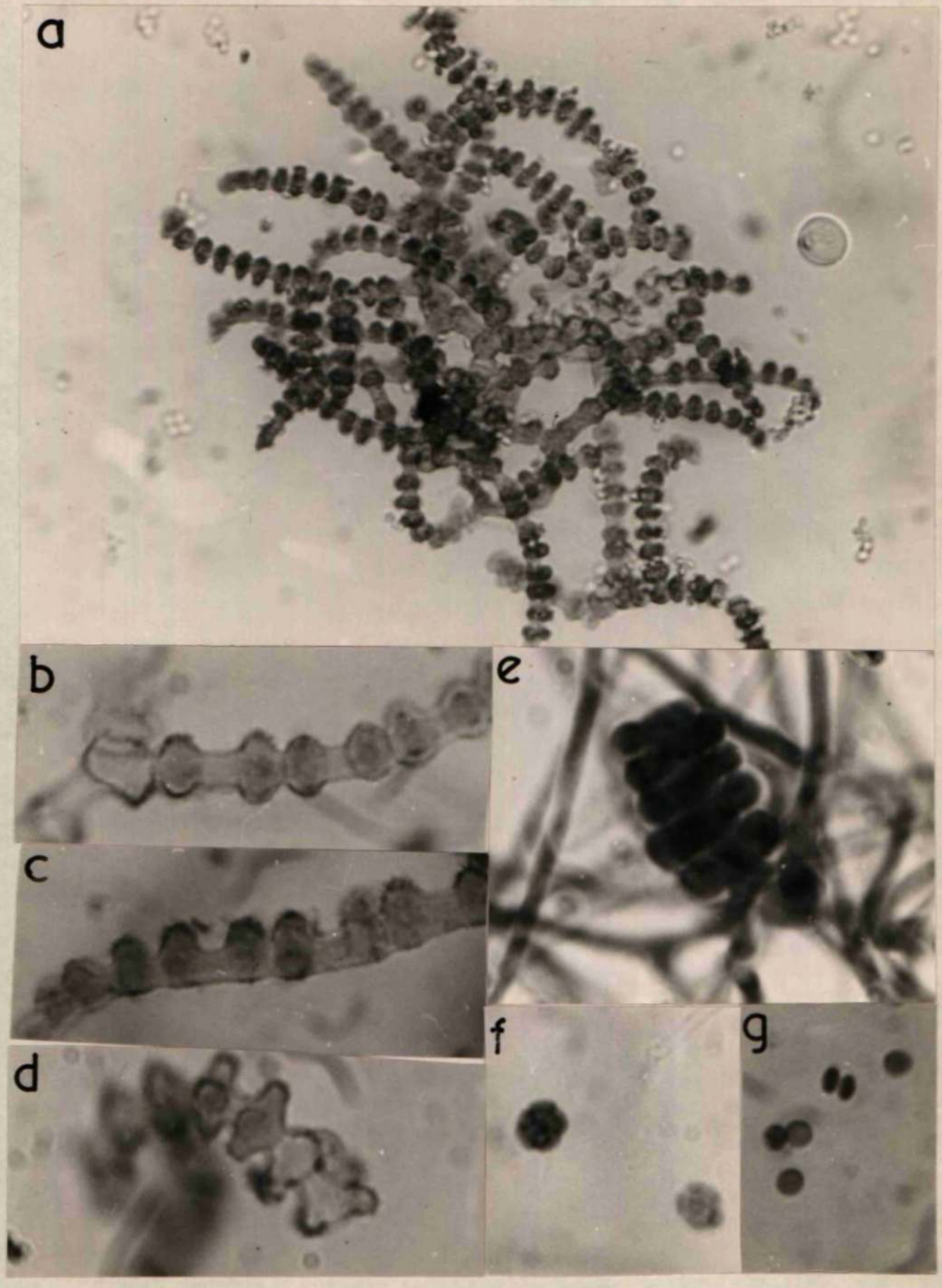

a
b
c
d
e
f
g

Plate 21

Plate 22.

Arthroderma lomondii

a) Single ascospore strain growing on hair bait on soil.

b) Compatible mating strains growing on hair bait on soil.

c) Microconidia in clusters, X 800.

d) Microconidia "en thyrses", X 800.

e) Macroconidia, X 800.

f) g) h) i) Macroconidia, X 2000.

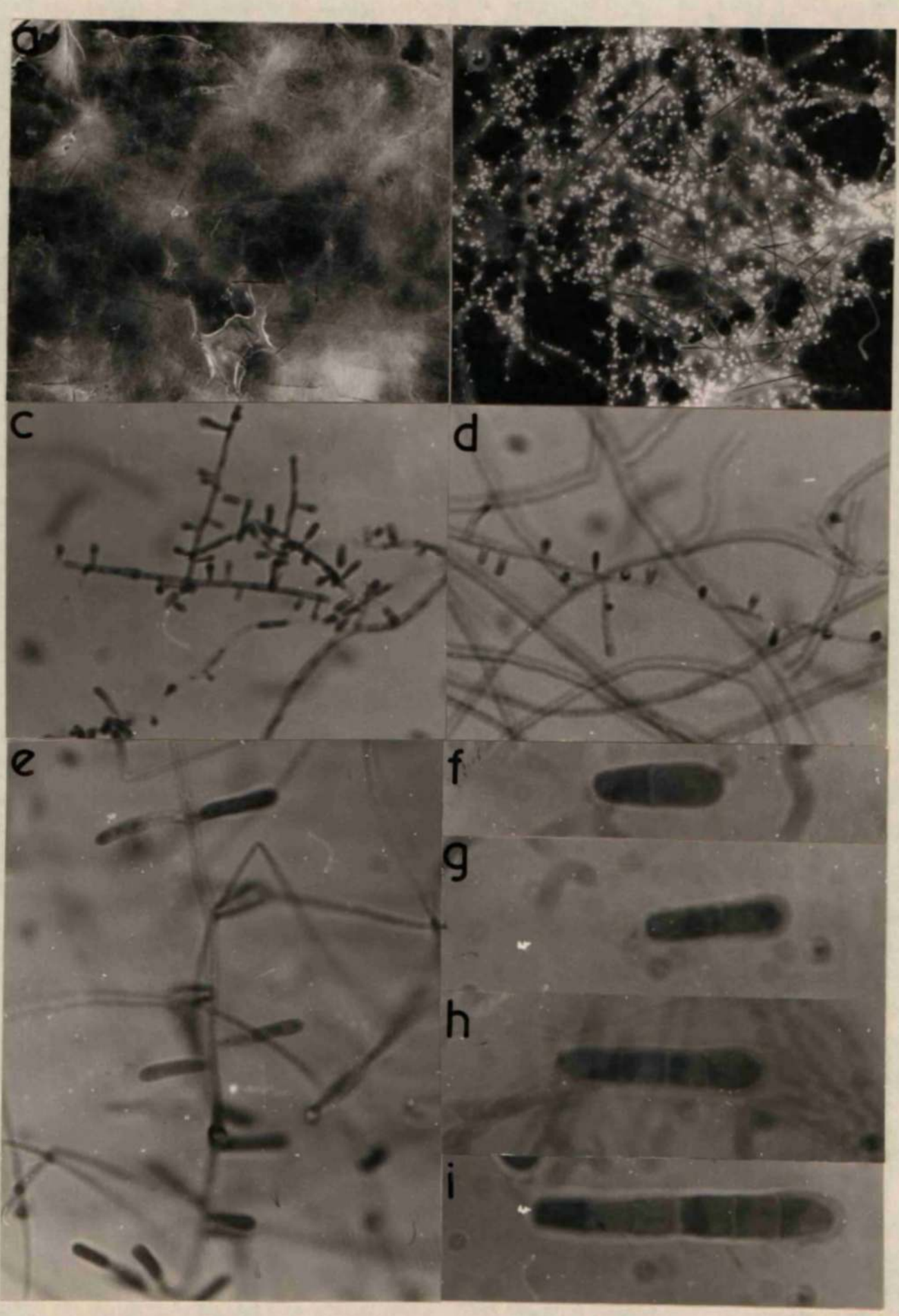

a
c
d
e
f
g
h
i

Plate 22

Plate 23.

Arthroderma lomondii variety

a) Mature cleistothecium, X 200

b) Sector of the peridium of a mature cleistothecium, X 200.

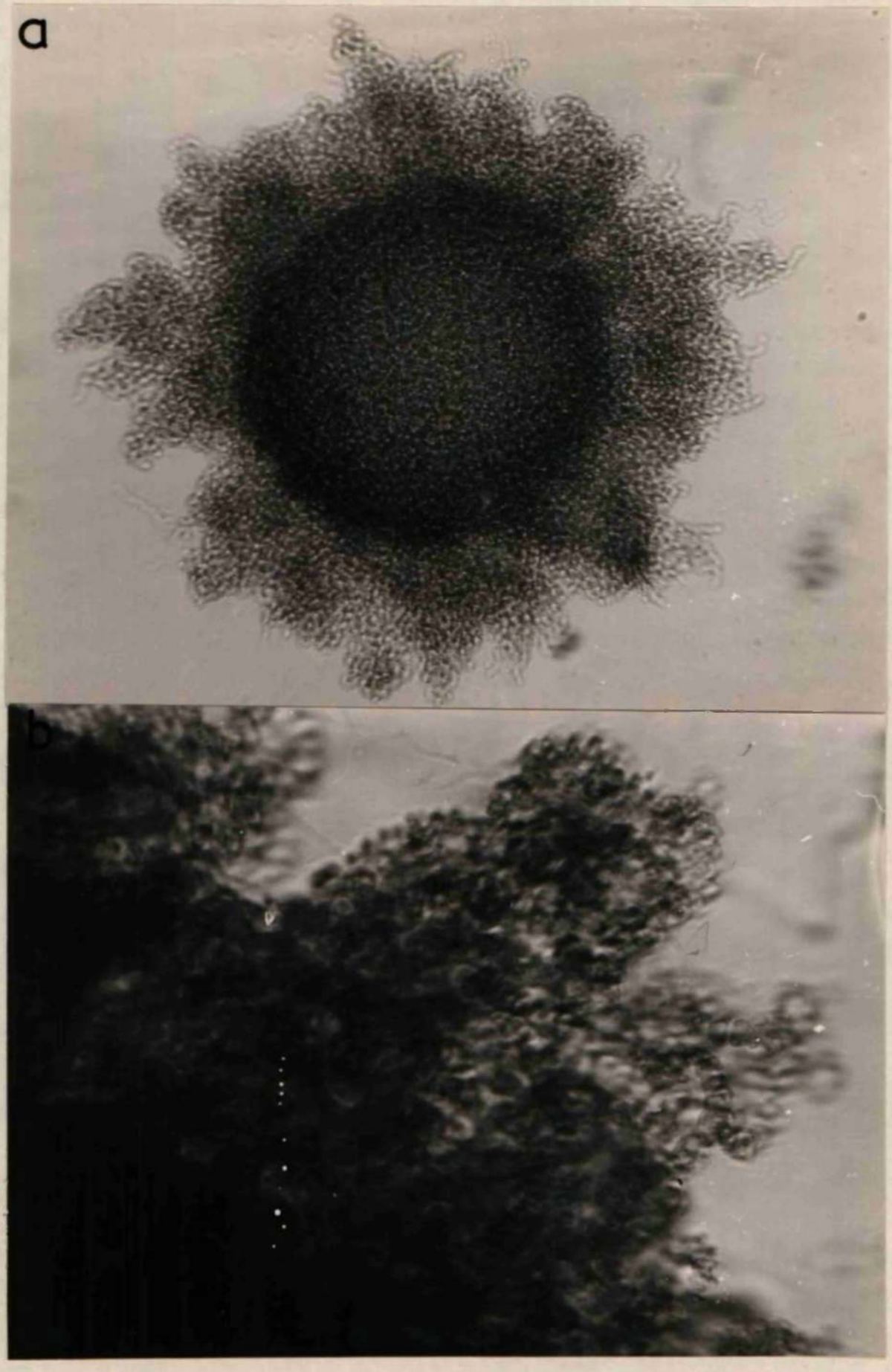

a
b

Plate 23

Plate 24.

Arthrodefma lomondii variety

a) Peridial hypha showing uncinate branching, X 800.

b) Ascospores and dumb-bell shaped peridial cells, X 2000.

c) Peridial cells showing 2 protuberances at each end of the cell, X 2000.

d) Peridial cells showing 3 protuberances at each end of the cell, X 2000.

e) Asexual spores, X 200.

f) Macroconidia, X 800.

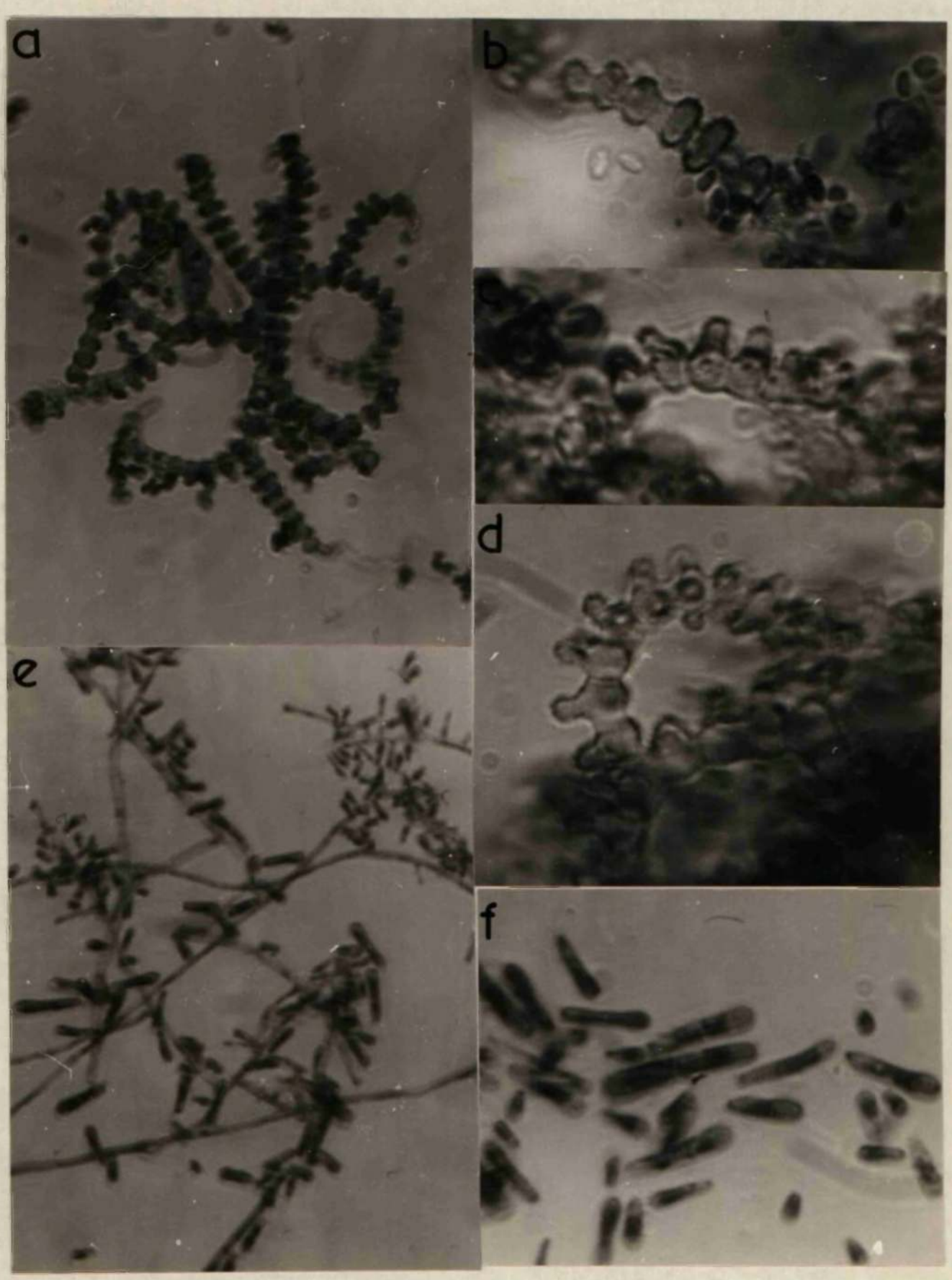

a
b
c
d
e
f

Plate 24

Plate 25.

Arthroderma minutum

a) Mature cleistothecium, X 200.

b) Sector of peridium of mature cleistothecium showing peridial cells and a spiral hypha, X 800.

Plate 26.

Arthroderma minutum

a) Fully differentiated peridial cells, X 2000.

b) Undifferentiated peridial cells, X 2000.

c) Ascus, X 2000.

d) Spiral hypha, microconidia "en grappe" and chlamydospores, X 2000.

e) Macroconidium, X 2000.

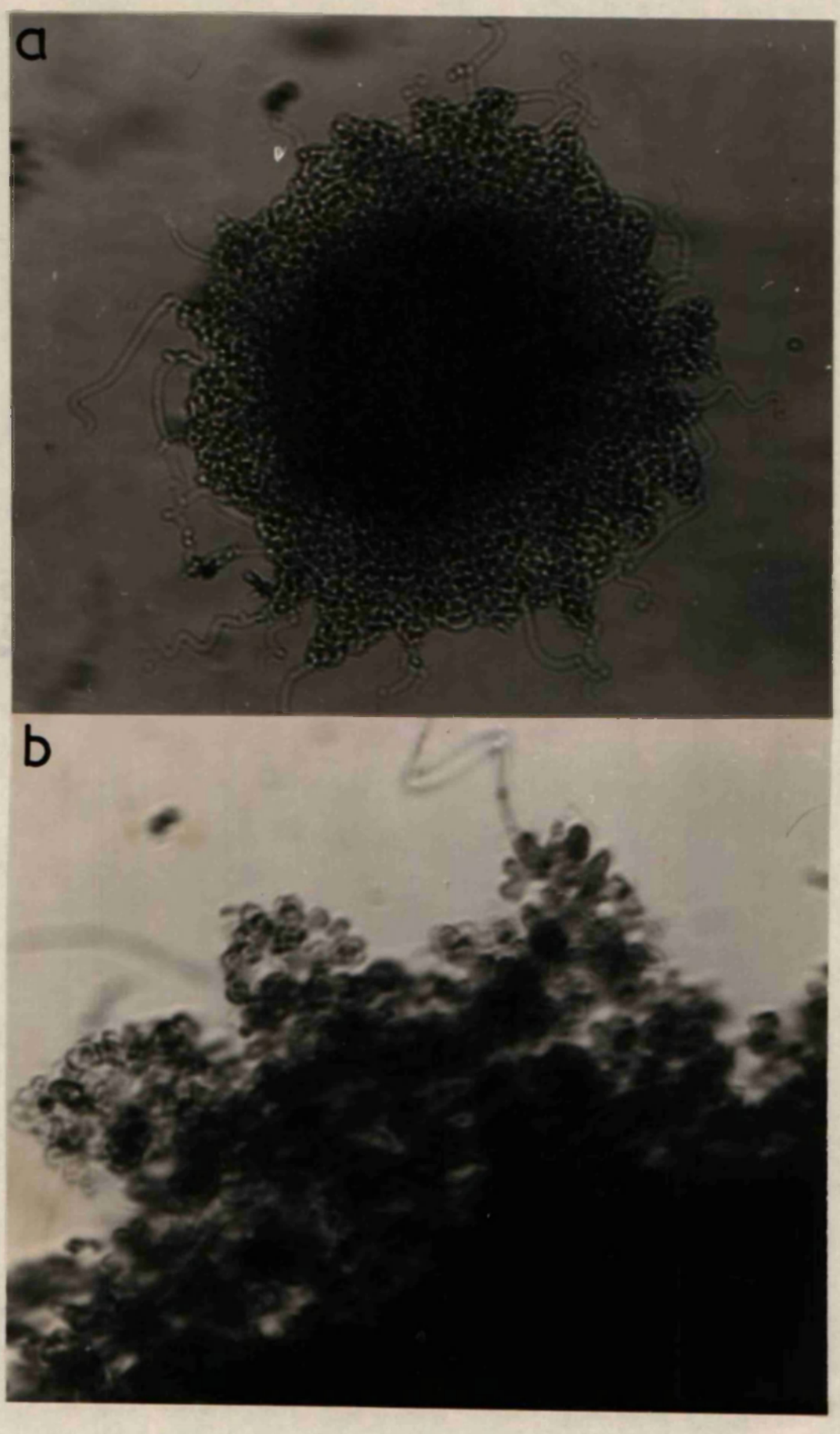

a
b

Plate 25

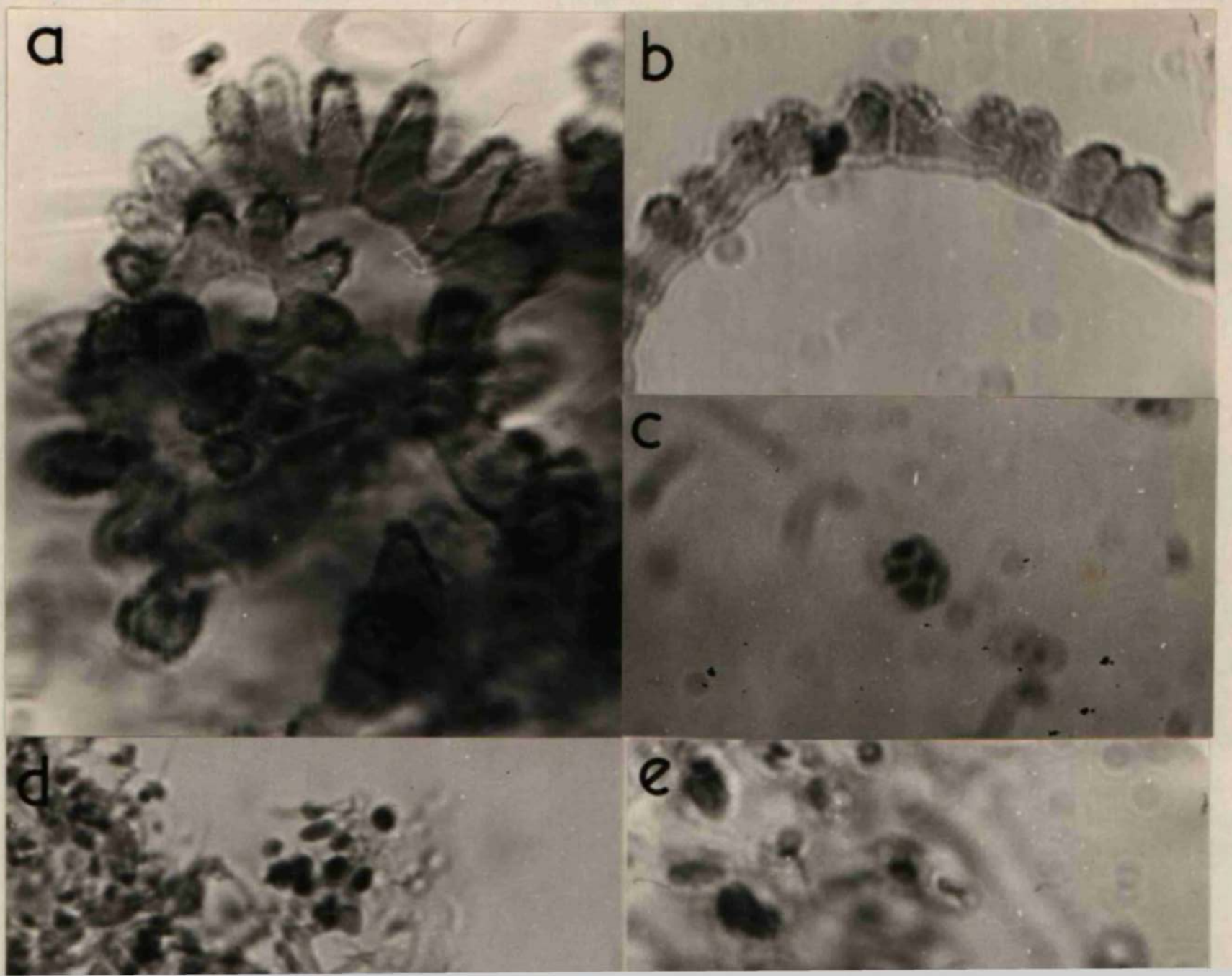

a
b
c
d
e

Plate 26

Plate 27.

Trichophyton globiferum

a) Nodule, X 200.

b) Sector of the peridium of a mature nodule showing spiral hyphal appendages and dumb-bell shaped cells, X 800.

Hair bait technique
Contaminants. Other than keratinophilic fungi. Multiple growths. use of micro-manipulator.
actidione action.

Gymnoascaceous

T. mentagrophytes & M. gypseum are generally present on the hair of guinea pigs into Chance

67. Cattery?
Allescheria - Cunninghamella.
Cow casts

67 Russia into a hair.

Arthroderma curreyi
uncinatum
quadrifidum

K. ajelloi — Squamous ulcers of leg but fixed not unsuccessful
imperfect Terrestre.

imperfect T. simiae

Difference between Keratinophilic & dermatophytes?
Majocchi's granuloma
Persistence of keratinophilic fungi.

pour : coat.

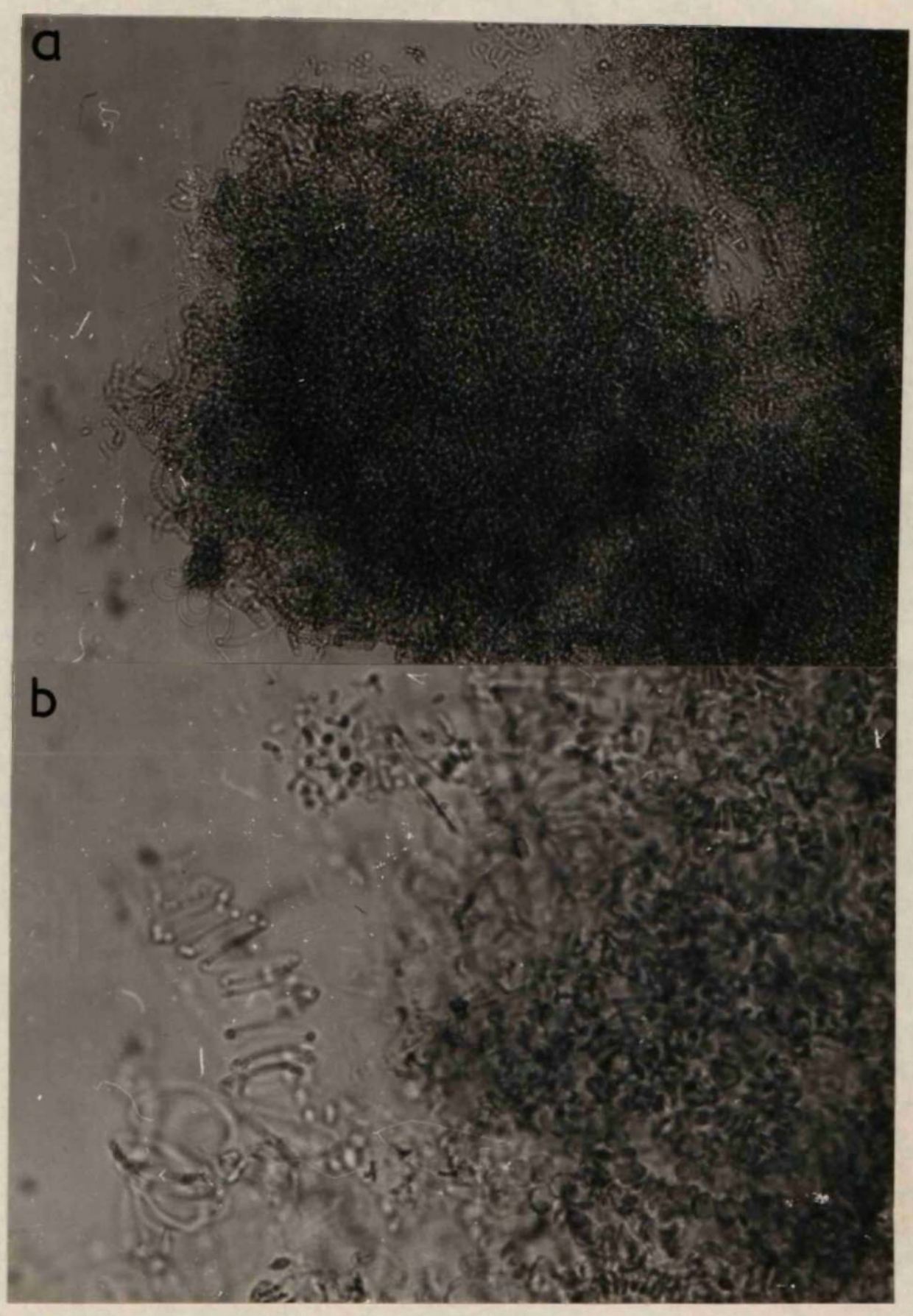

a
b

Plate 27

Plate 28.

Trichophyton globiferum

a) Peridial hypha of nodule showing uncinate branching, X 800.

b) Dumb-bell shaped peridial cells; The terminal one giving rise to a spiral hypha, X 2000.

c) Peridial cells which have collapsed in concertina fashion because of dehydration, X 2000.

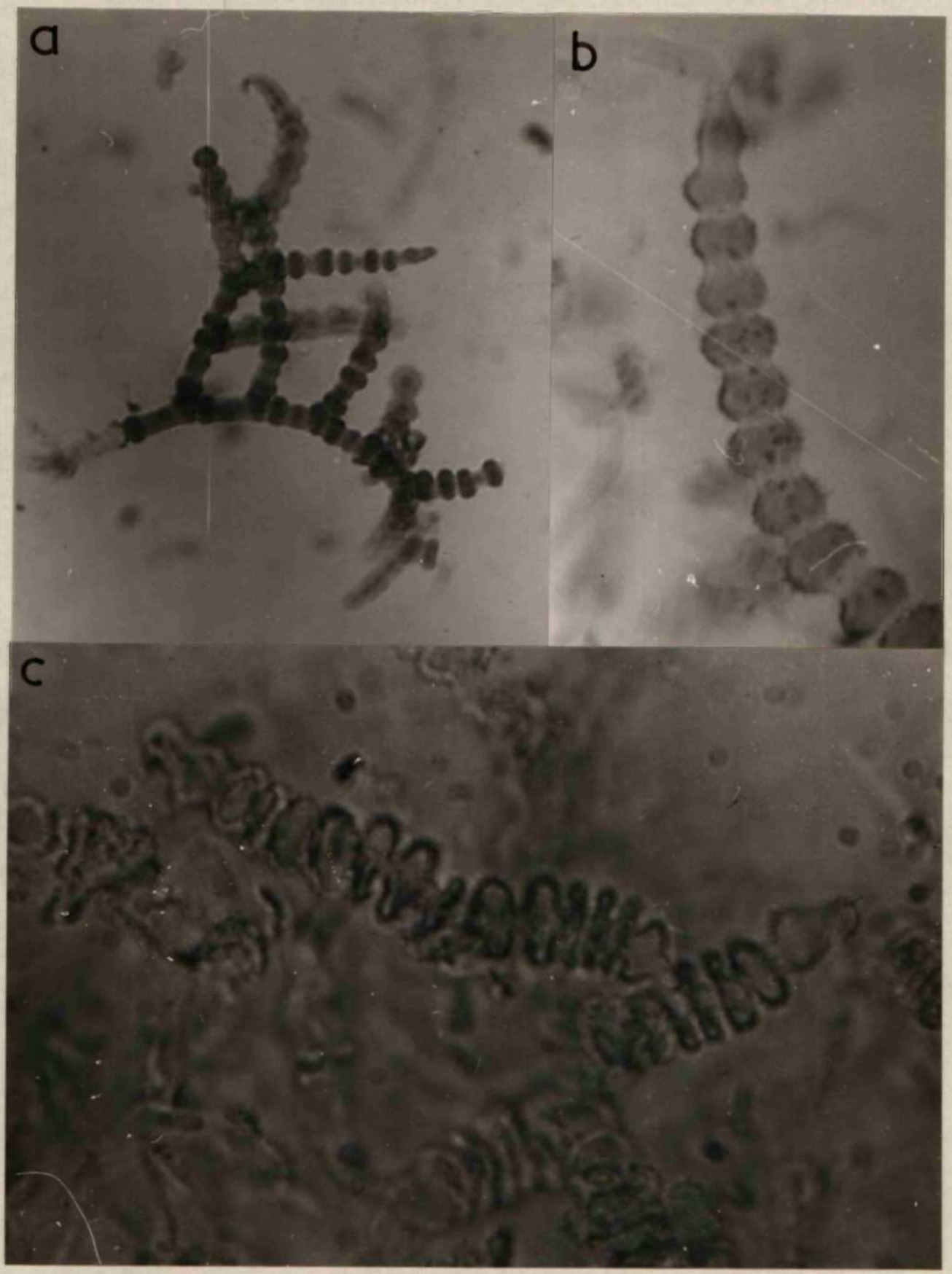

a
b
c

Plate 28

Plate 29.

Trichophyton globiferum

a) Early stage in the development of a bulbil, X 800.

b) Later stage in the development of a bulbil, X 2000.

c) Mature bulbils, X 800.

d) Germinating bulbil, X 800.

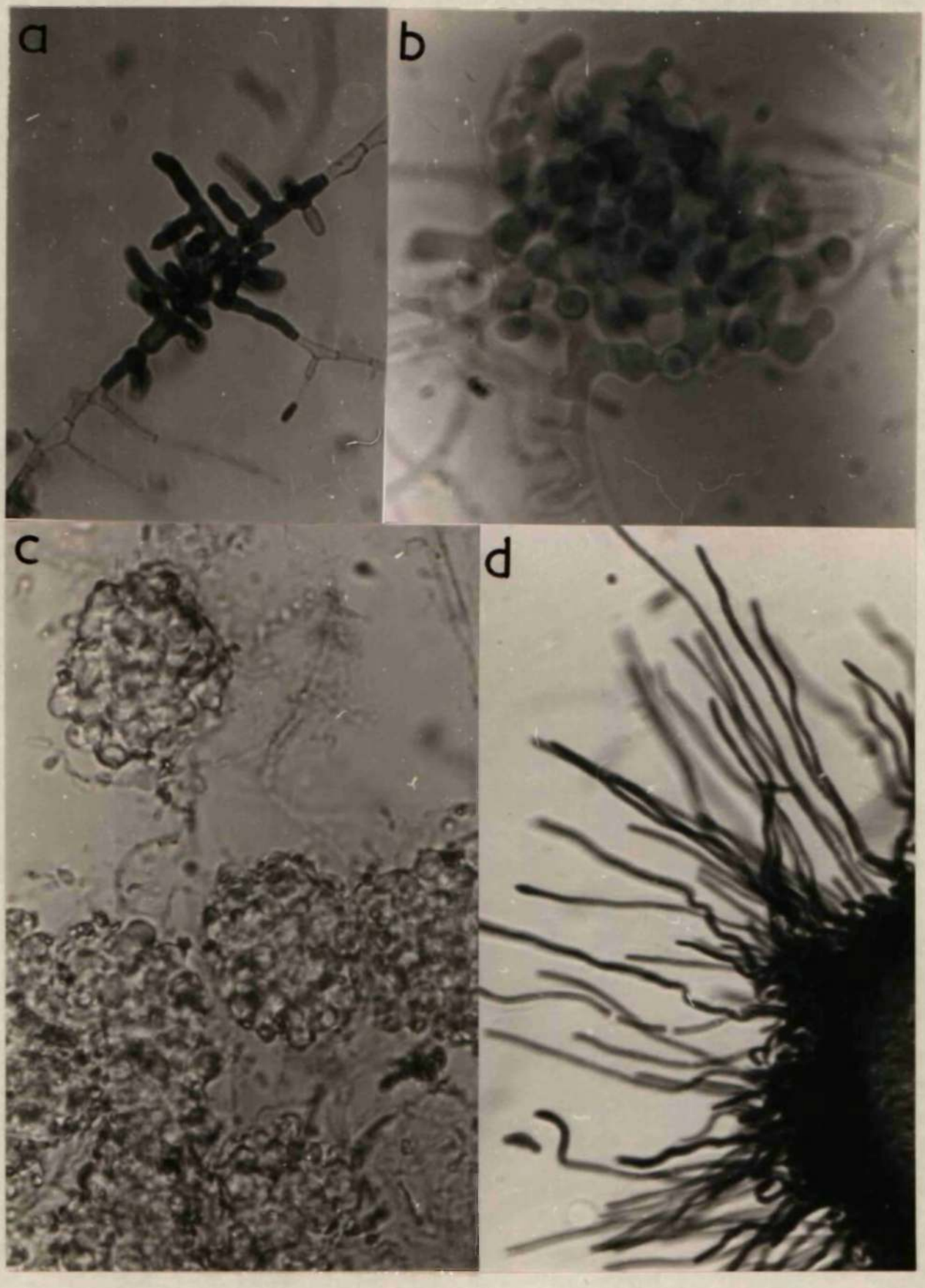

a
b
c
d

Plate 29

Plate 30.

Trichophyton globiferum

a) Microconidia "en thyrses", X 800.

b) Microconidia "en grappe", X 800.

c) Macroconidia "en thyrses", X 800.

d) Macroconidium, X 2000.

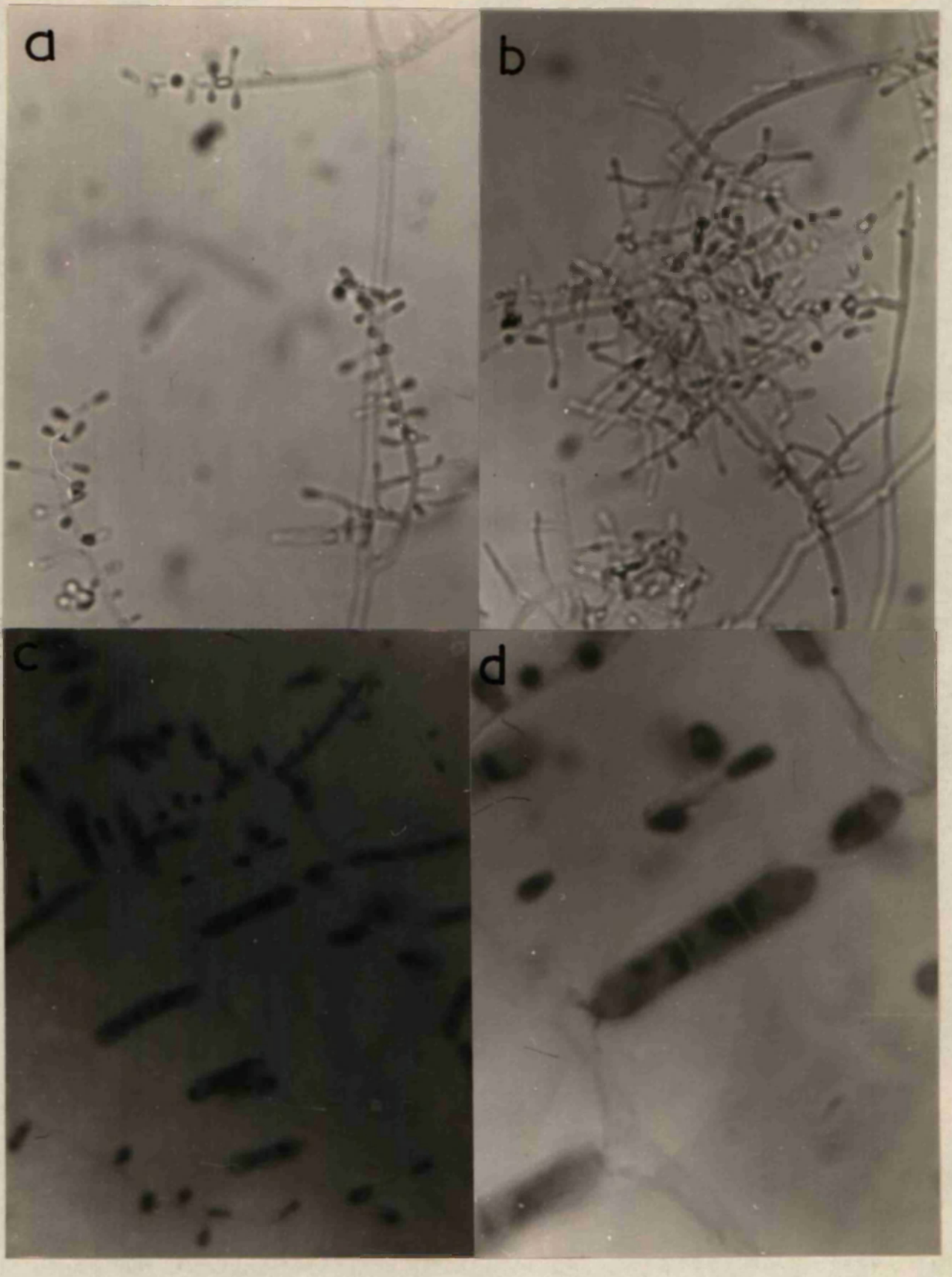

a
b
c
d

Plate 30

Plate 31.

Trichophyton mentagrophytes

a) Mature conidial nodule, X 200.

b) Sector of the peridium of a mature conidial nodule showing dumb-bell shaped cells and uncinate branching, X 800.

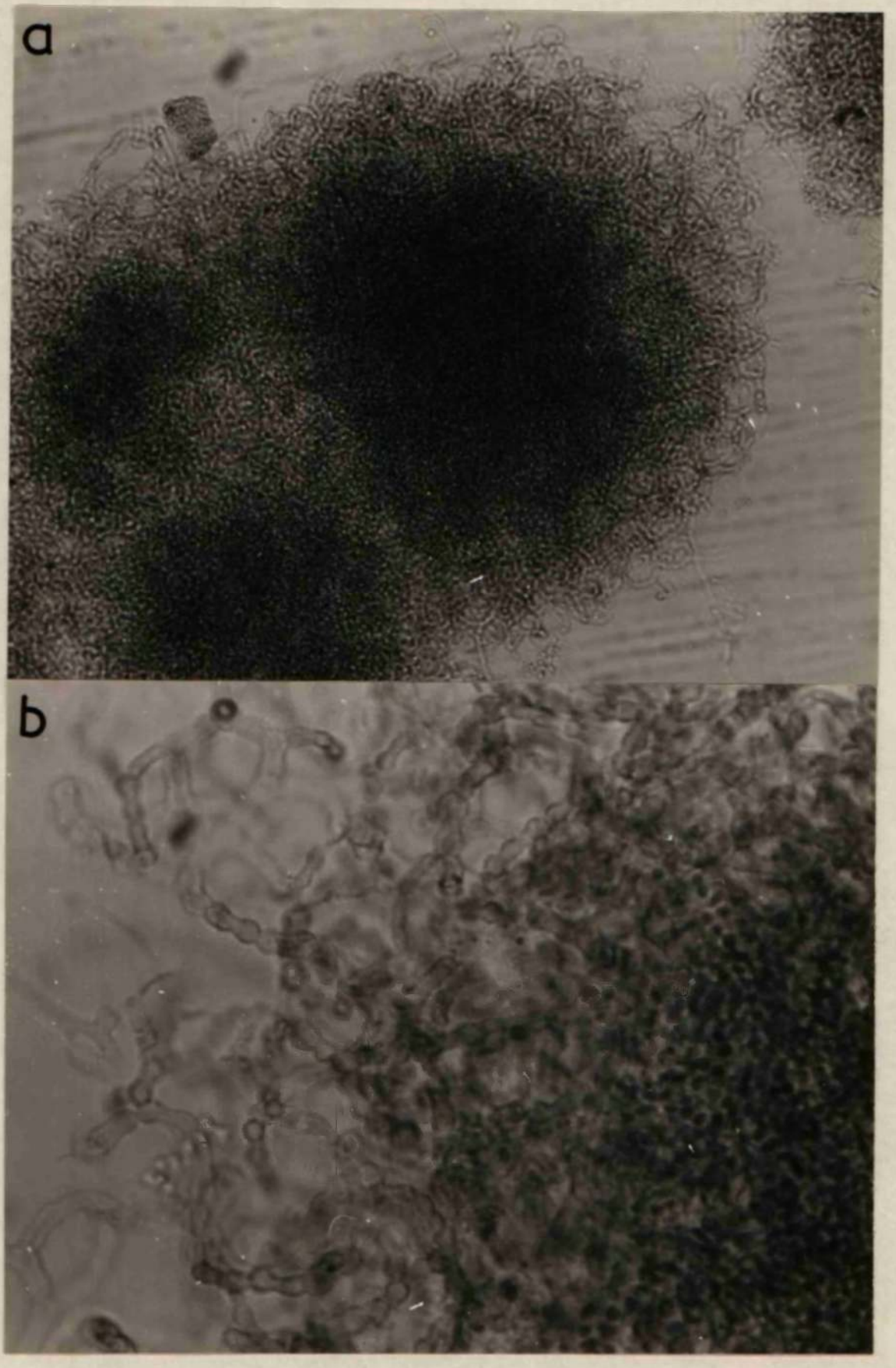

a
b

Plate 31

Plate 32.

Ctenomyces serratus

a) Mature perithecia, X 200.

b) Portion of the peridial wall of a mature perithecium showing pseudoparenchyma, thick-walled peridial hyphae and the large hooked appendages, X 800.

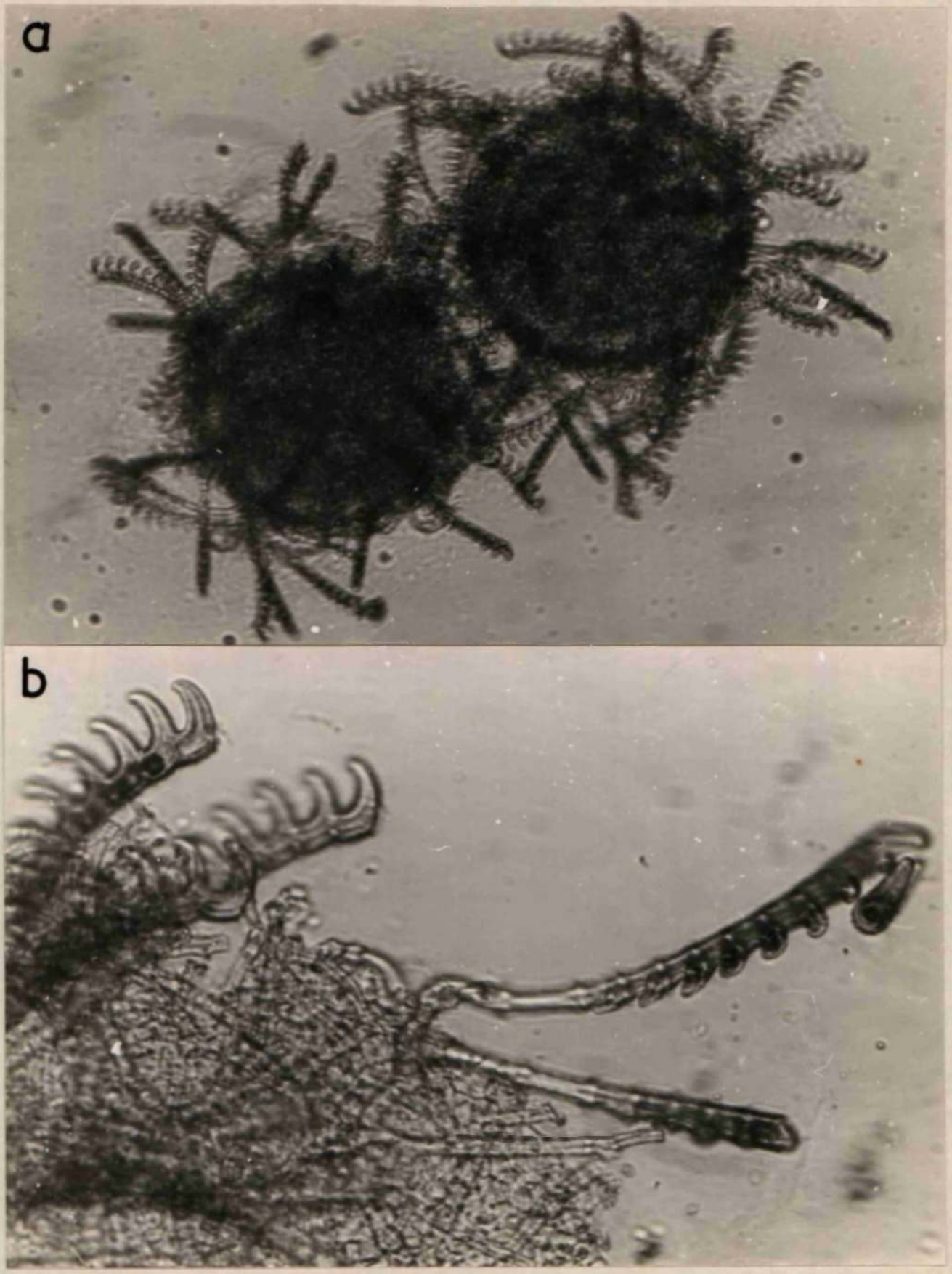

a
b

Plate 32

Plate 33.

Ctenomyces serratus

a) Surface view of the peridium of the perithecium showing branching, anastomosing peridial hyphae and hooked appendages, X 800.

b) Pseudoparenchymatous layer of the peridium, X 2000.

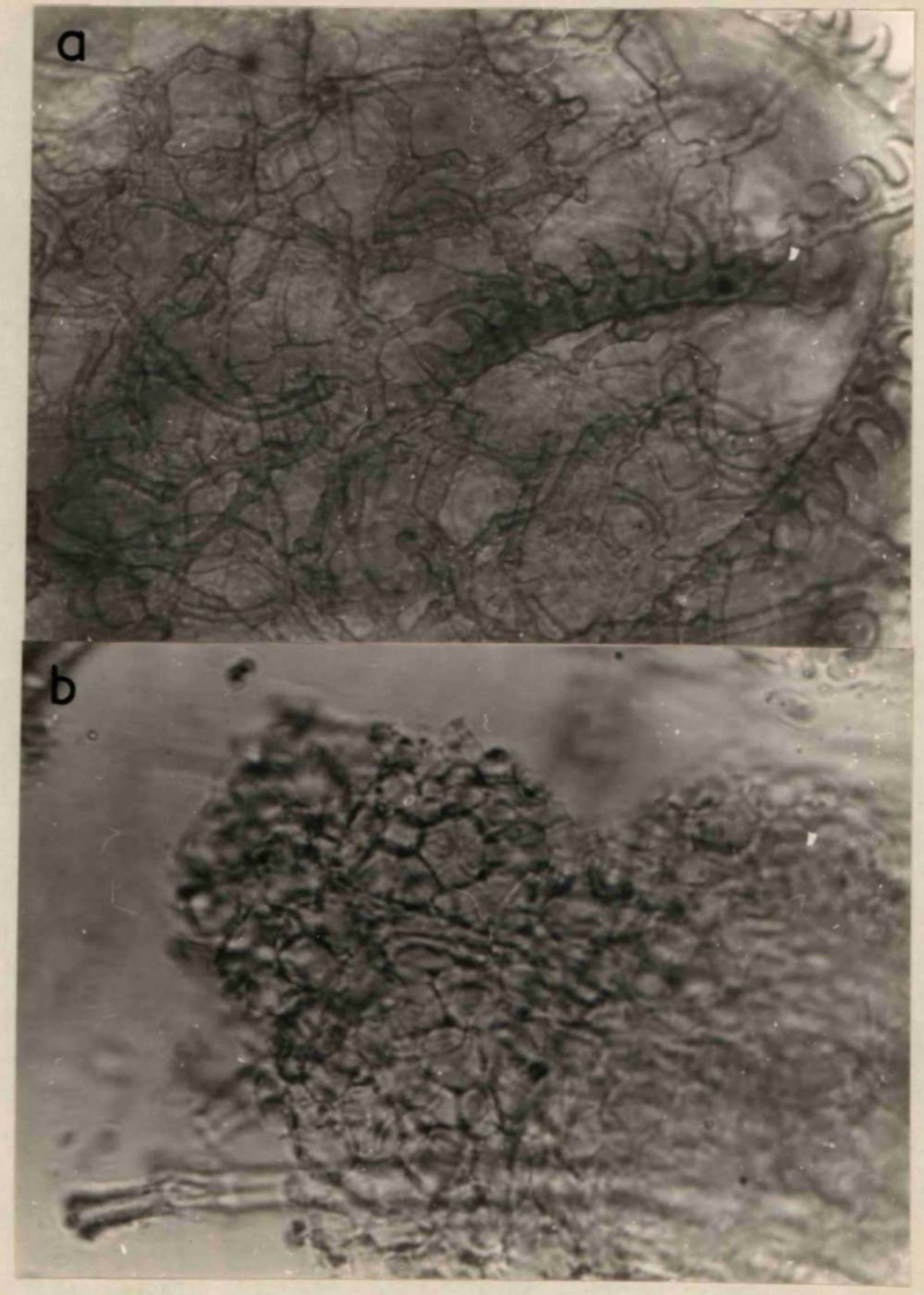

a
b

Plate 33

Plate 34.

Ctenomyces serratus

Camera lucida drawings

a) Very young stages in the development of cleistothecial initials.

b) Cleistothecial initial.

c) Developing peridial hyphae.

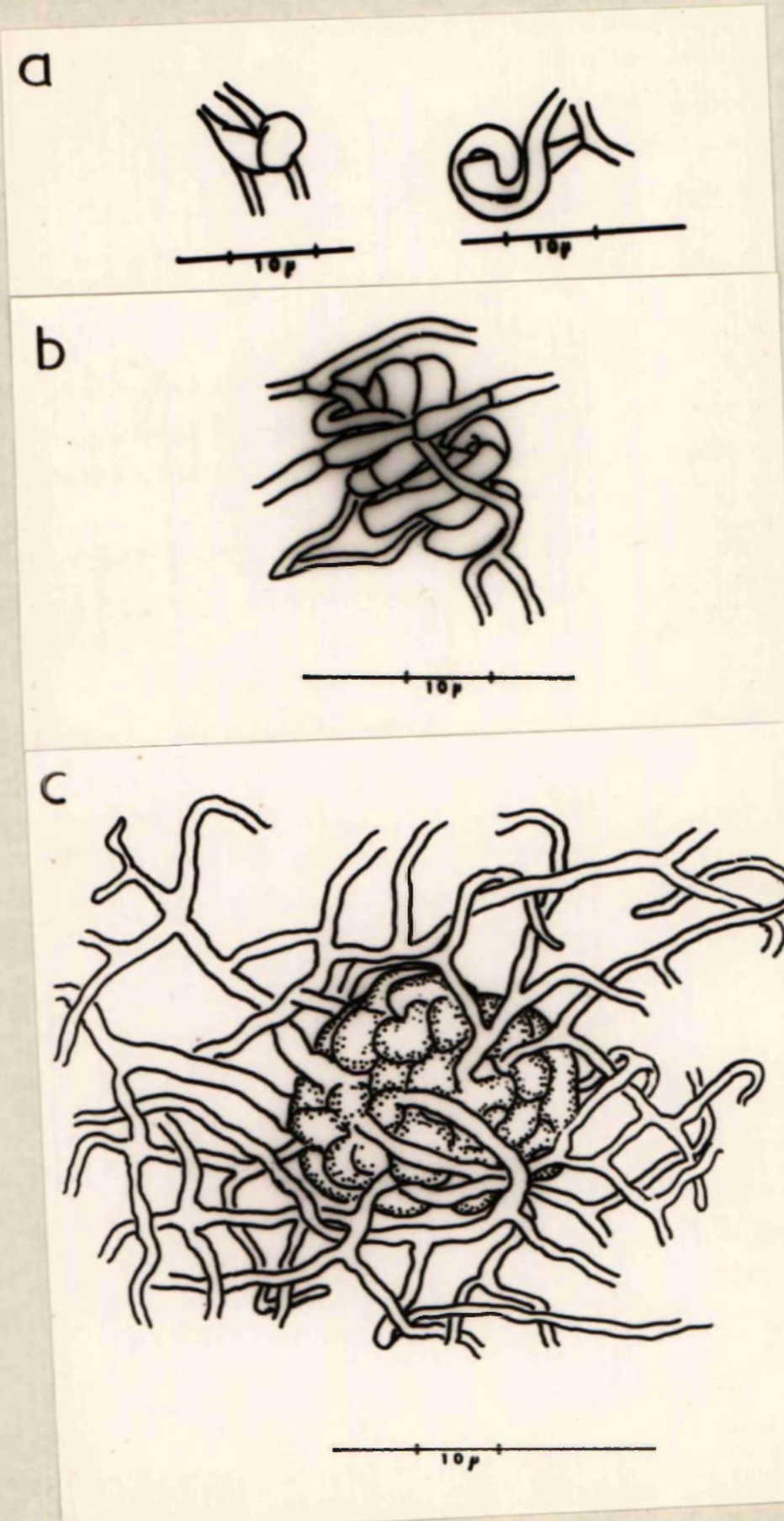

a
10μ
10μ
b
10μ
c
10μ

Plate 34

Plate 35.

Ctenomyces serratus

Camera lucida drawings

a) Elongated club-shaped structure which will develop into a hooked appendage.

b) Multiseptate club-shaped structure.

c) Hook-like projections developing.

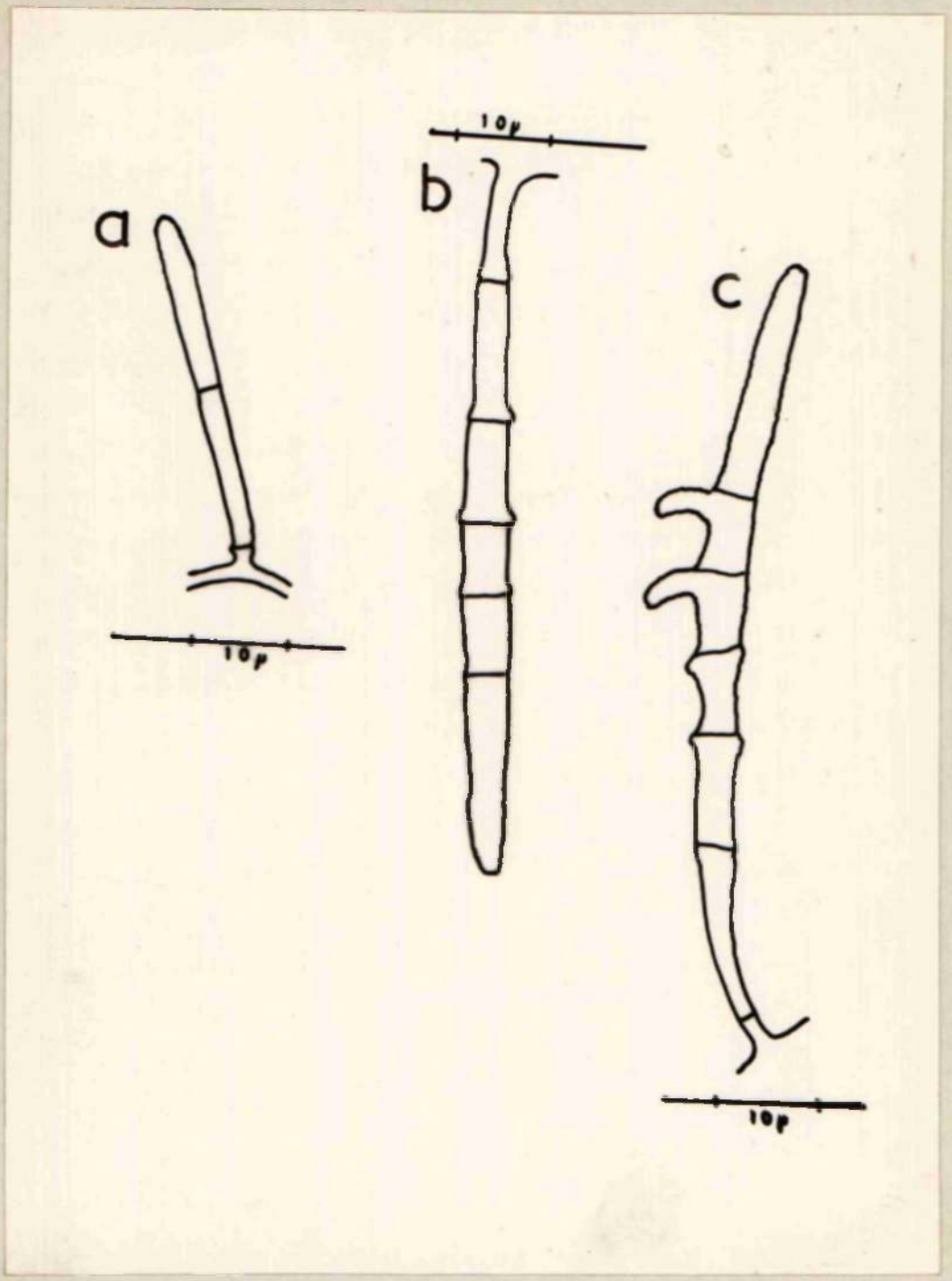

a
10μ
b
10μ
c
10μ

Plate 35

Plate 36.

Ctenomyces serratus

Camera lucida drawings

a) Hooked appendage to peridium of perithecium.

b) Pore connecting cells of hooked appendage.

c) Thick-walled anastomosing outer peridial hyphae and underlying pseudoparenchyma.

d) Thin-walled inner peridial hyphae arising from pseudoparenchyma.

e) Ascospores.

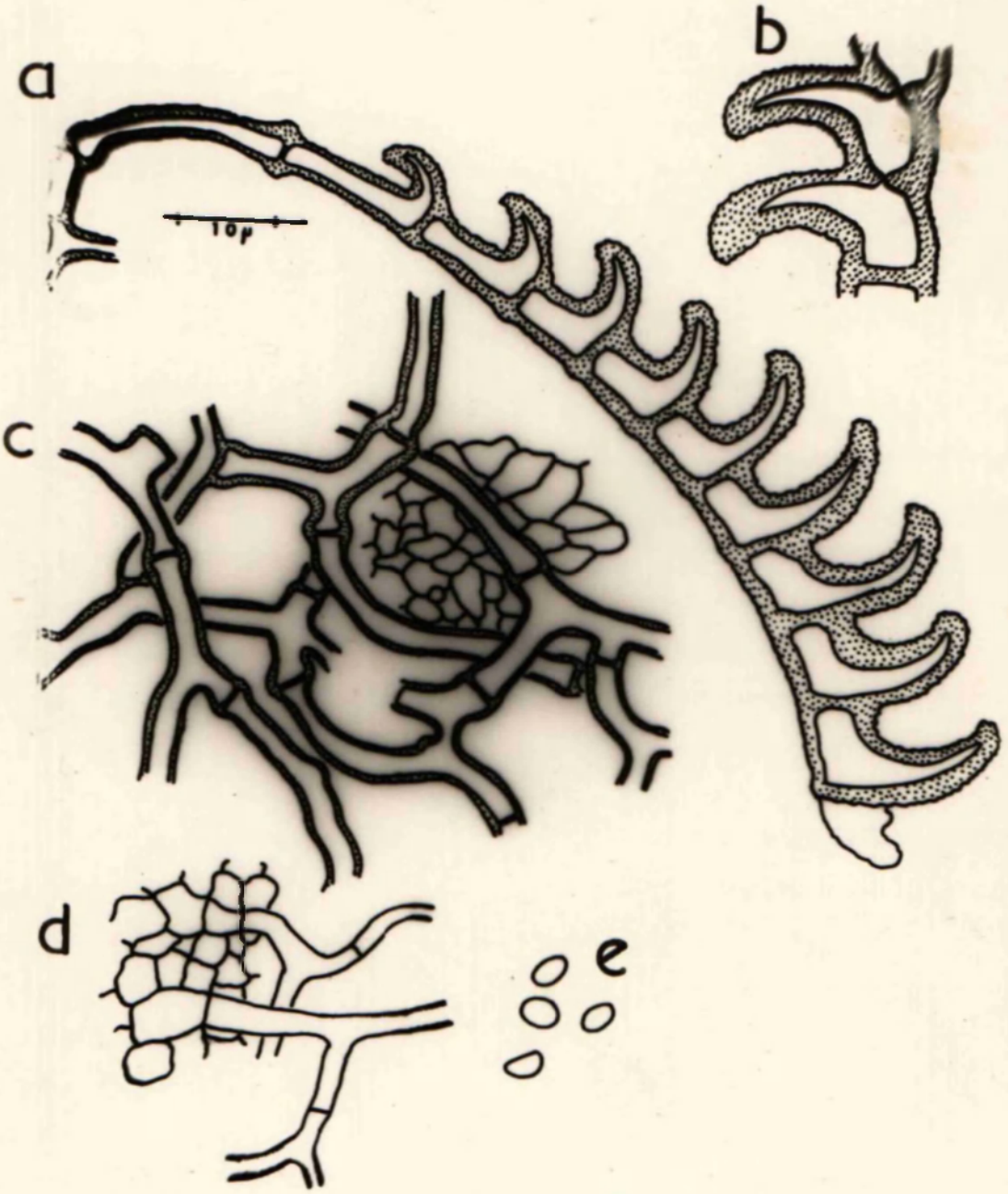

a
b
10μ
c
d
e

Plate 36

Plate 37.

Amauroascus verrucosus

a) Sector of the peridium of a mature cleistothecium showing undifferentiated peridial hyphae, X 800.

b) Asci, X 2000.

c) Asexual growth and cleistothecia on horse hair bait on soil.

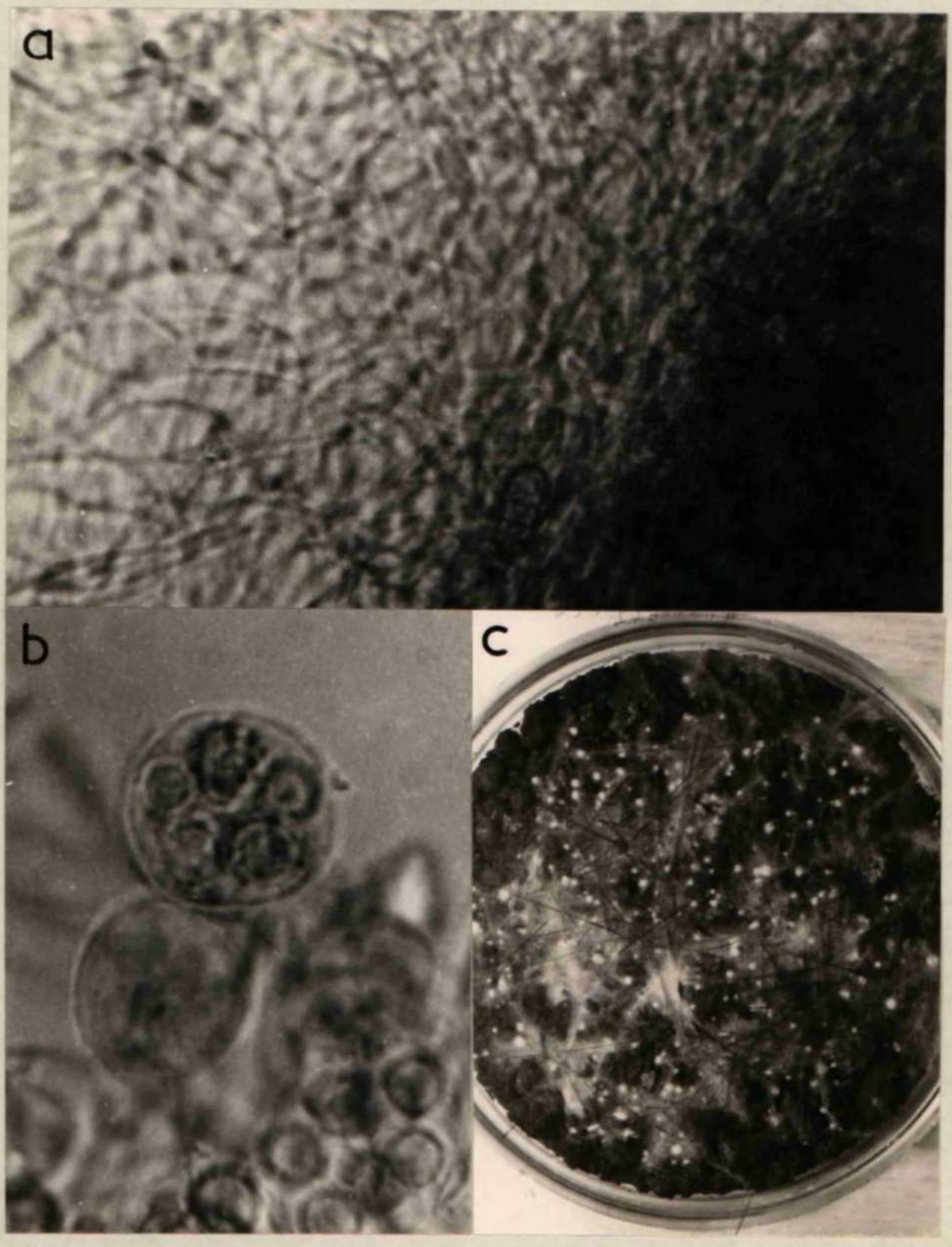

a
b
c

Plate 37

Plate 38.

Arachniotus ovinus

a) Mature cleistothecium, X 200.

b) Sector of the peridium of a mature cleistothecium showing peridial hyphae and asci, X 800.

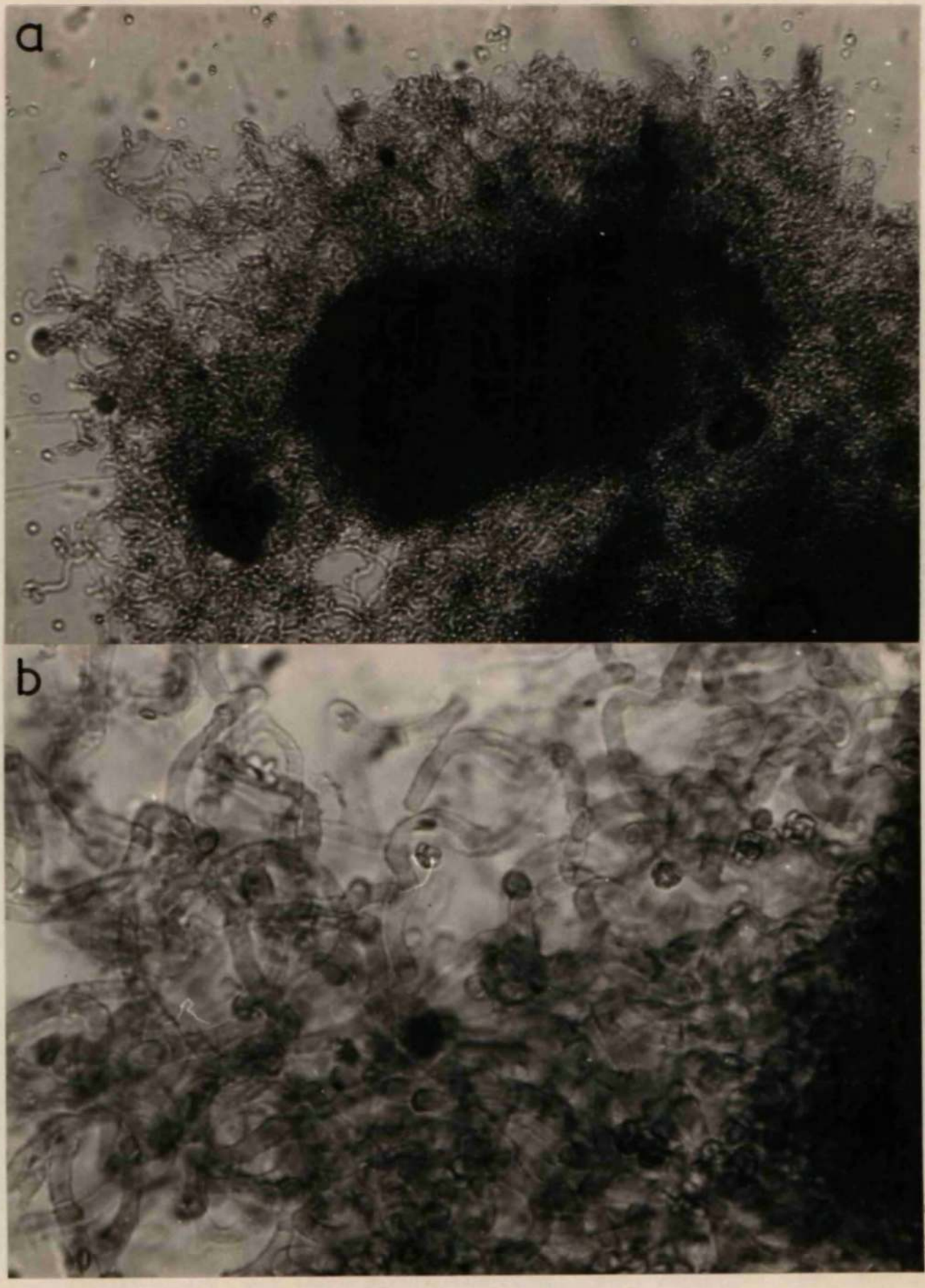

a
b

Plate 38

Plate 39.

Arachniotus ovinus

a) Peridial hyphae and spiral hyphal appendages, X 800.

b) Cells of peridial hyphae, X 2000.

c) Asci and ascospores, X 2000.

d) Conidia, X 800.

e) Conidia, X 2000.

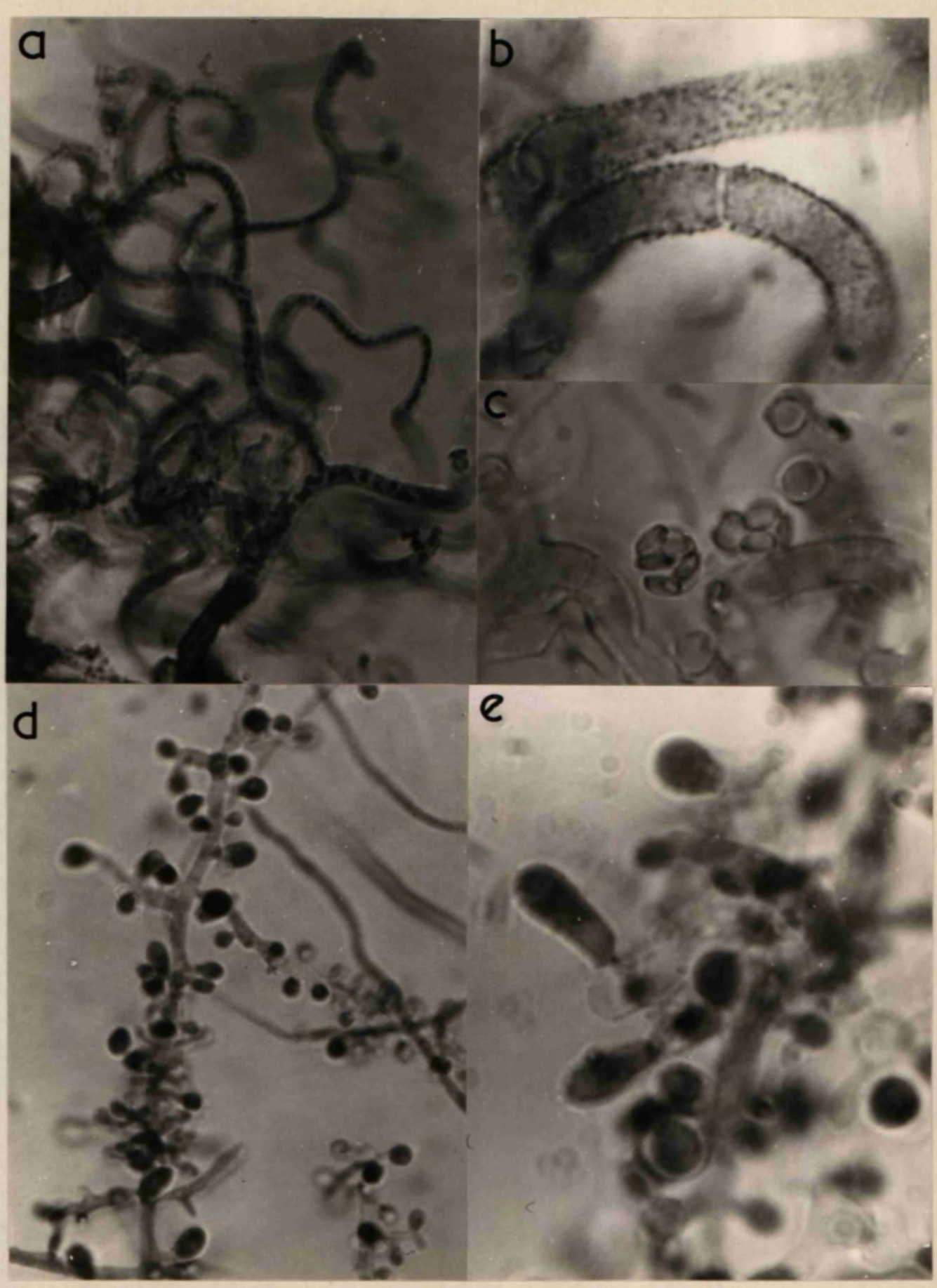

a
b
c
d
e

Plate 39

Plate 40.

Arachniotus ovinus

Camera lucida drawings

a) Ascus and ascospores

b) Peridial hyphae and spiral hyphal appendages.

c) Anastomosing cells of peridial hyphae.

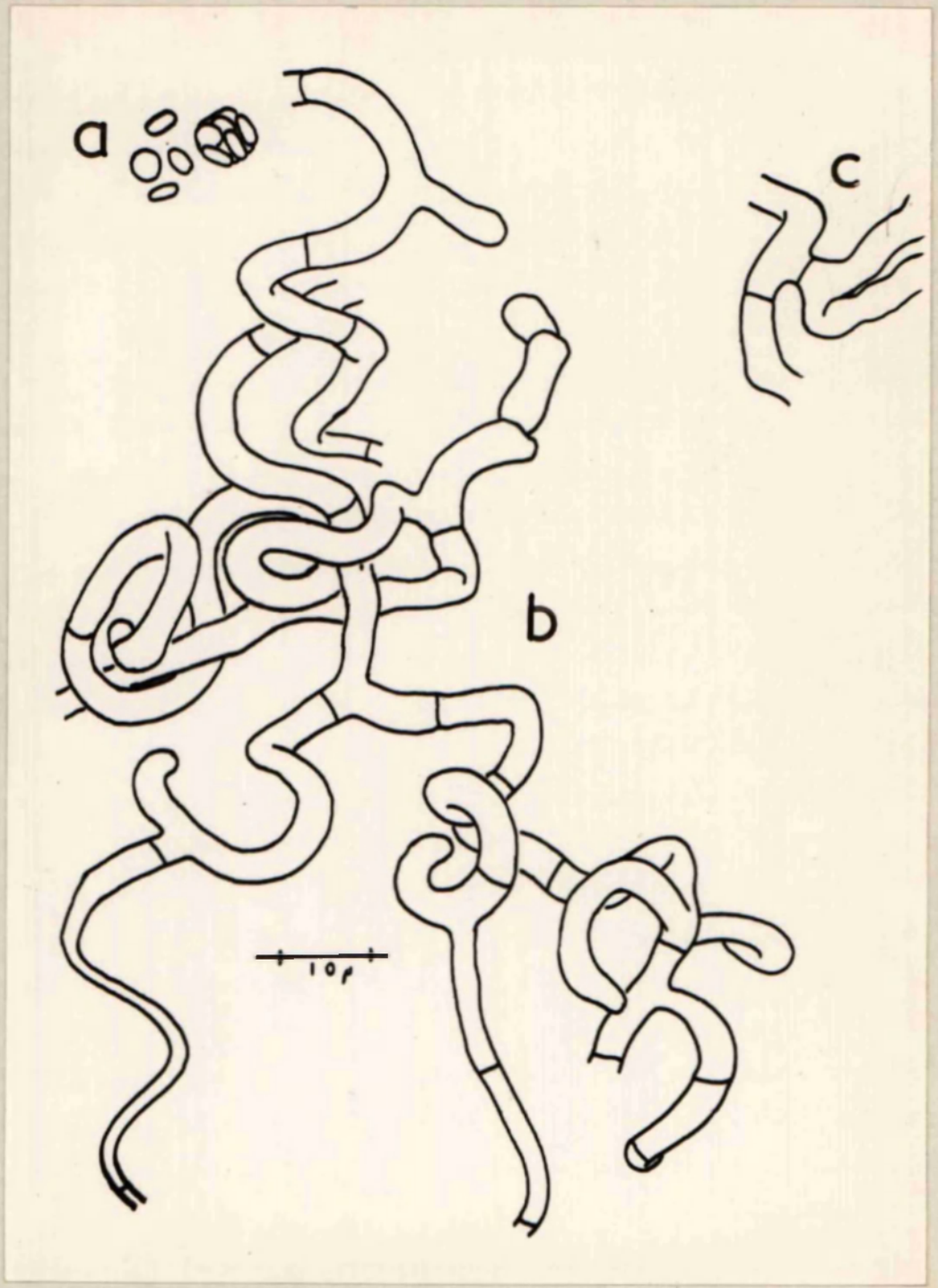

a
b
c
15μ

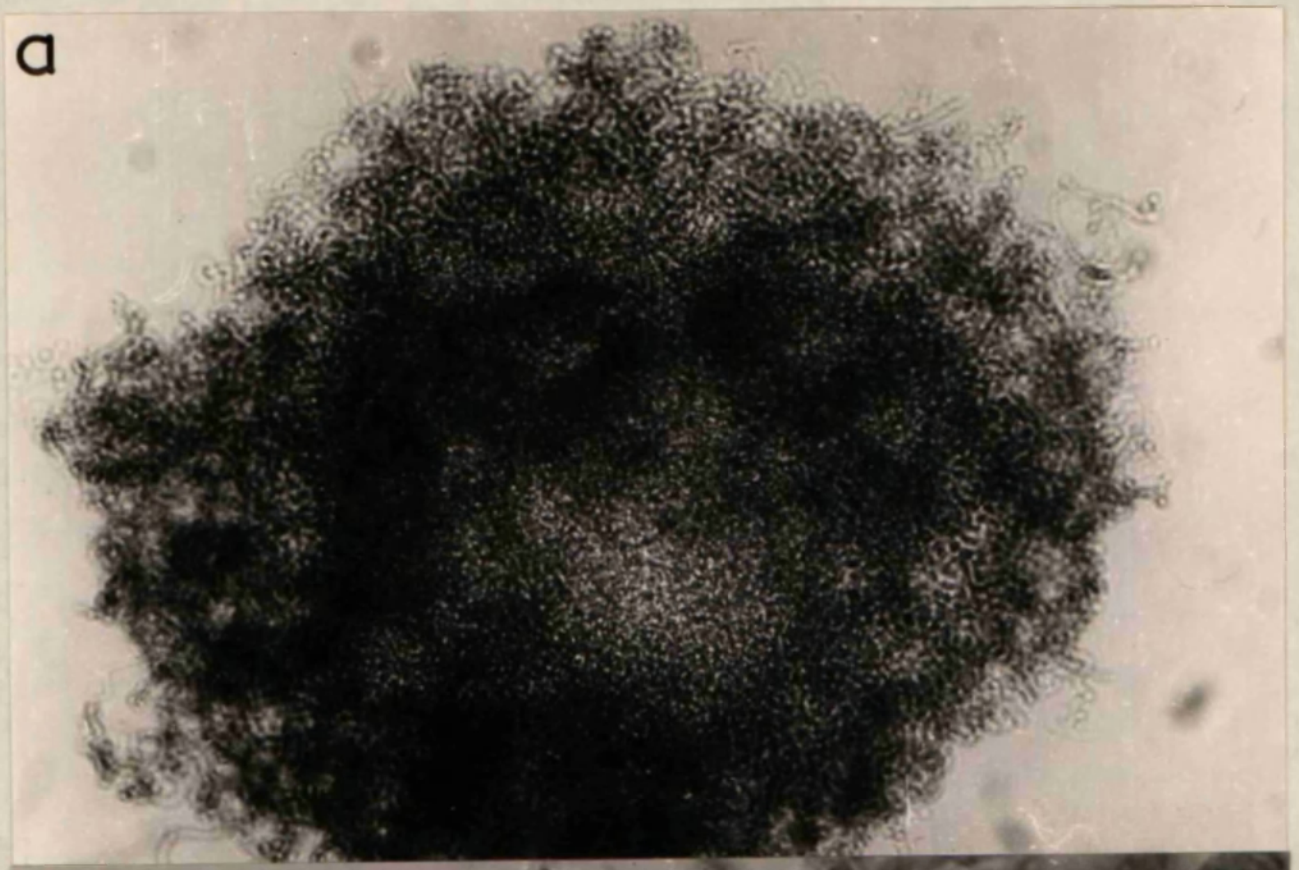
Planche 40

Plate 41.

Pararachniotus gelicola

a) Mature conidial nodule, X 200.

b) Sector of the peridium of a mature conidial nodule showing peridial hyphae and spiral hyphal appendages, X 800.


a

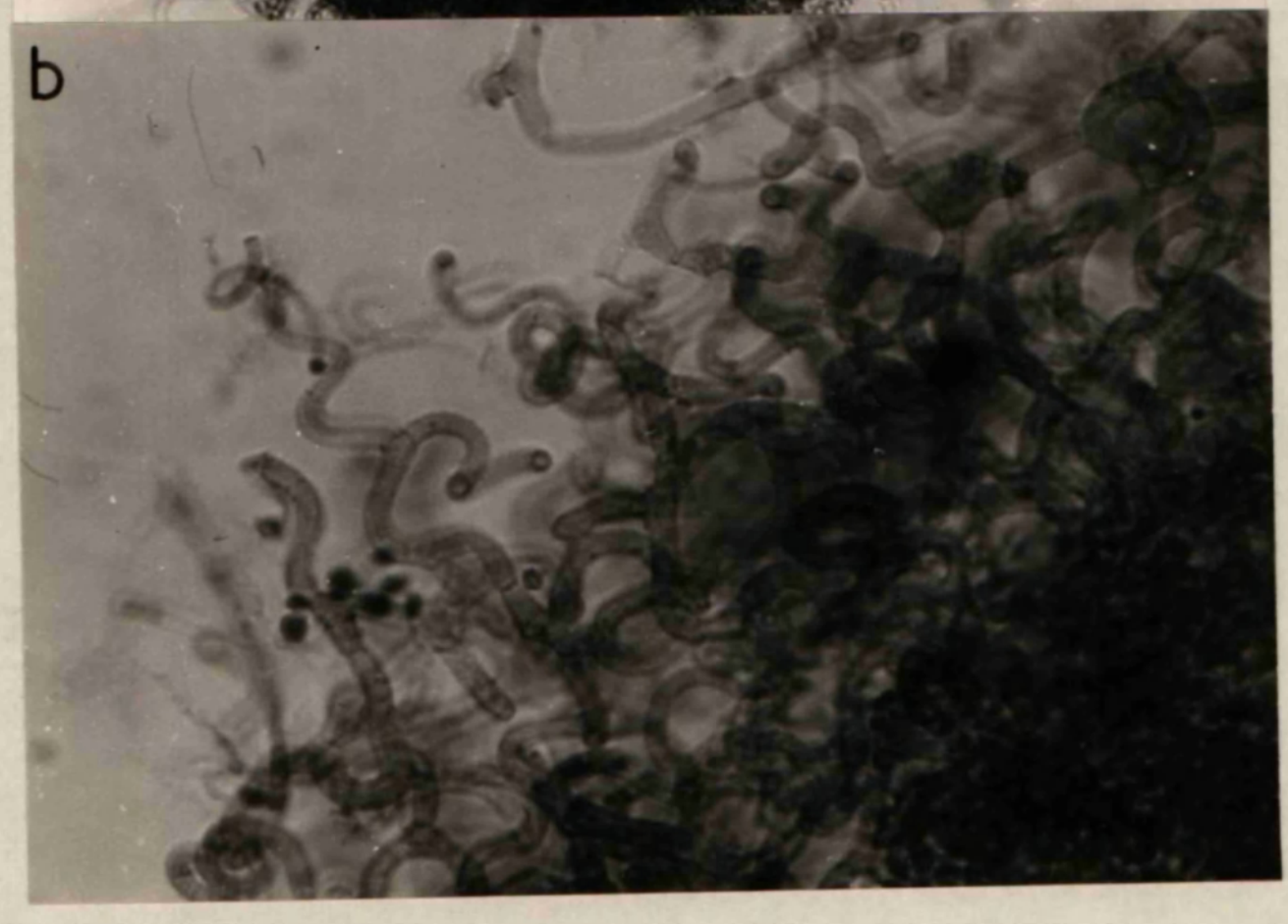

b

Plate 41

Plate 42.

Pararachniotus gelicola

a) Peridial hyphae and conidia from within the nodule, X 2000.

b) Anastomosing peridial cells, X 2000.

c) Conidial nodules on horse hair bait on soil.

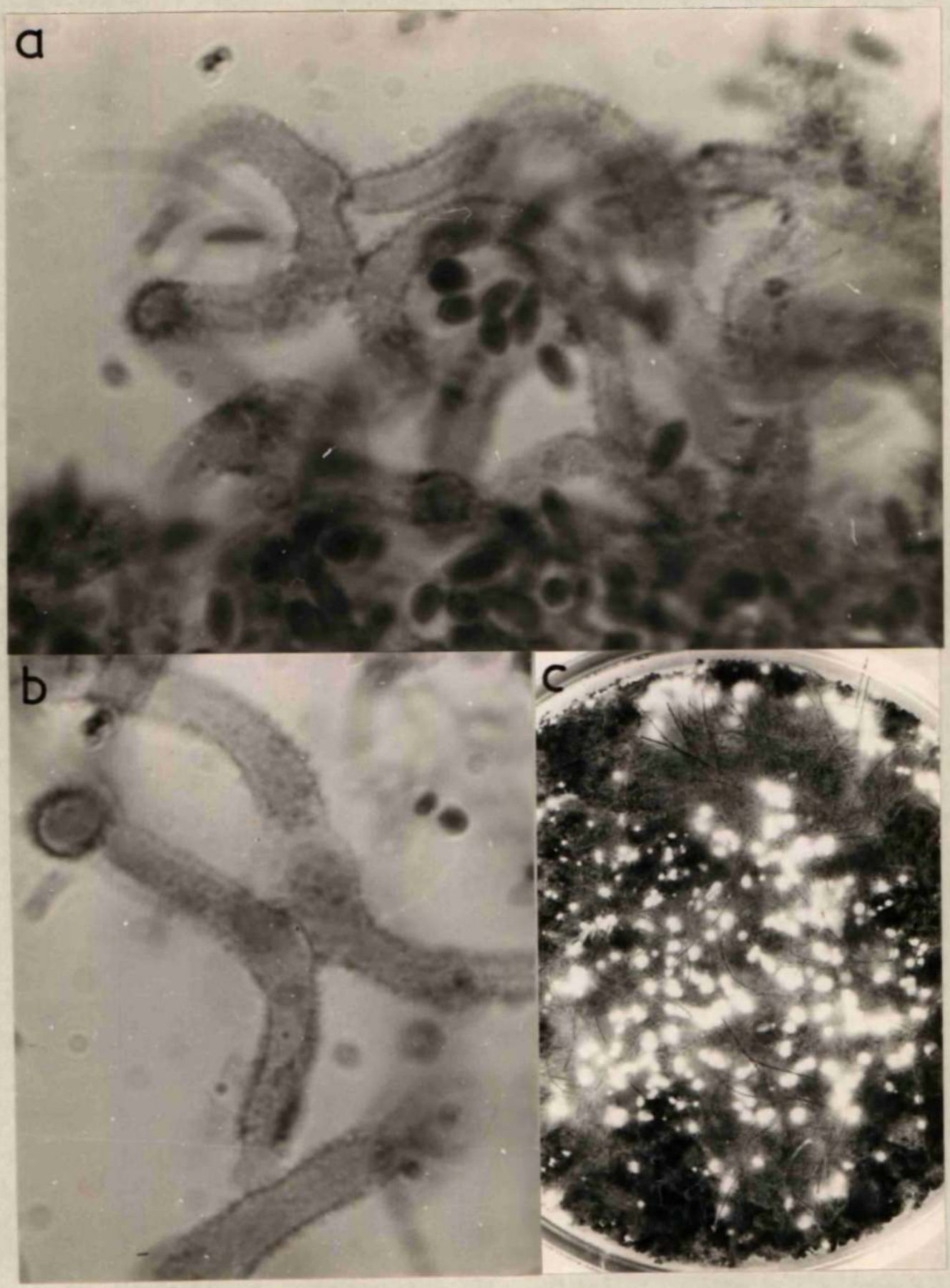

a
b
c

Plate 42

Plate 43.

Pararachniotus gelicola

a) Conidia from a crushed conidial nodule, X 800.

b) Hyphae and racquet hyphae, X 800.

c) Conidia formed on a hypha in the normal manner, X 2000.

d) Conidia from within a conidial nodule, X 2000.

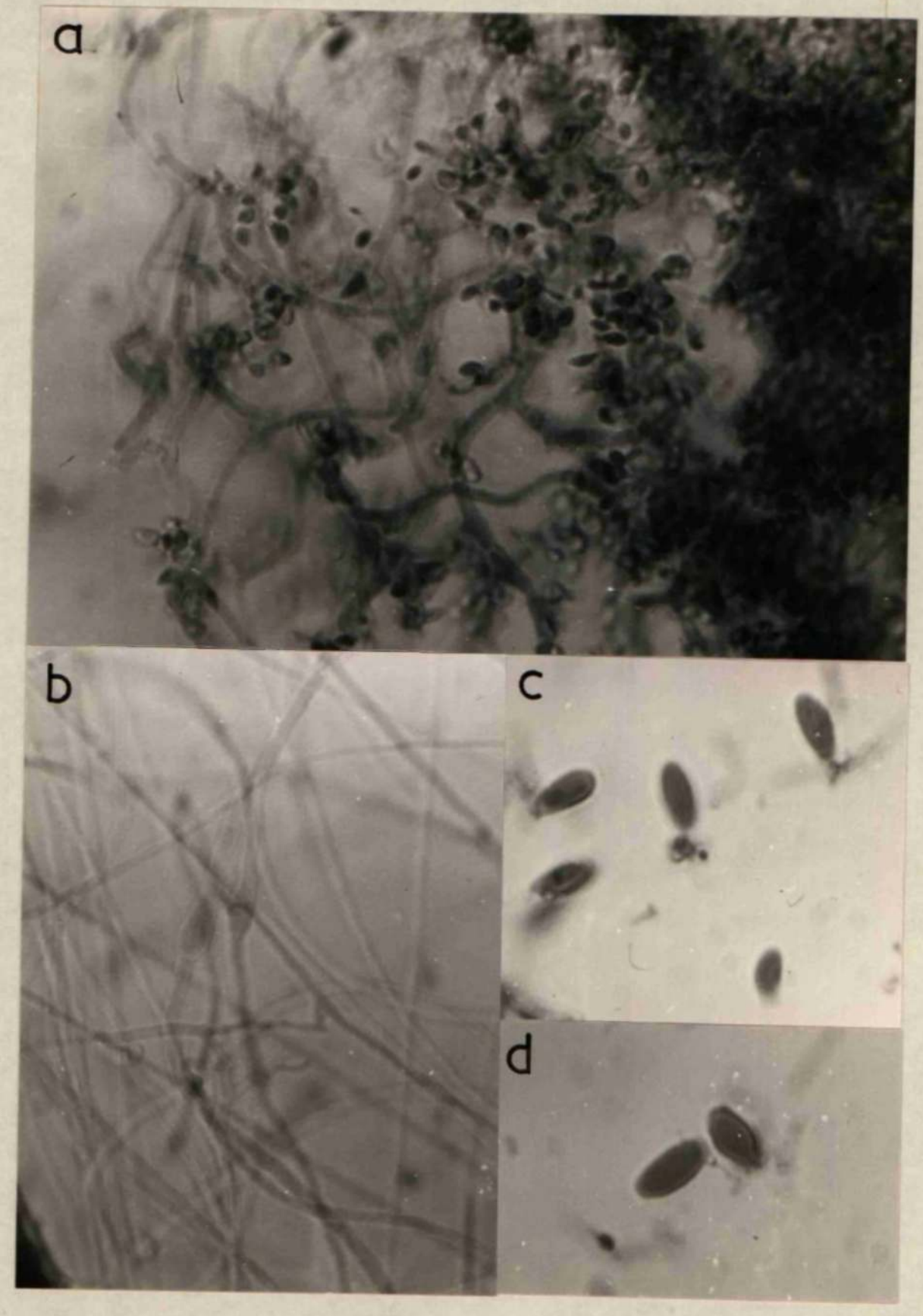

a
b
c
d

Plate 43

Plate 44.

Pararachniotus gelicola

Camera lucida drawings

a) Conidia from a conidial nodule.

b) Cells of the peridium of a conidial nodule.

c) Anastomosing peridial cells.

d) Peridial hyphae.

e) Spiral hyphal appendages.

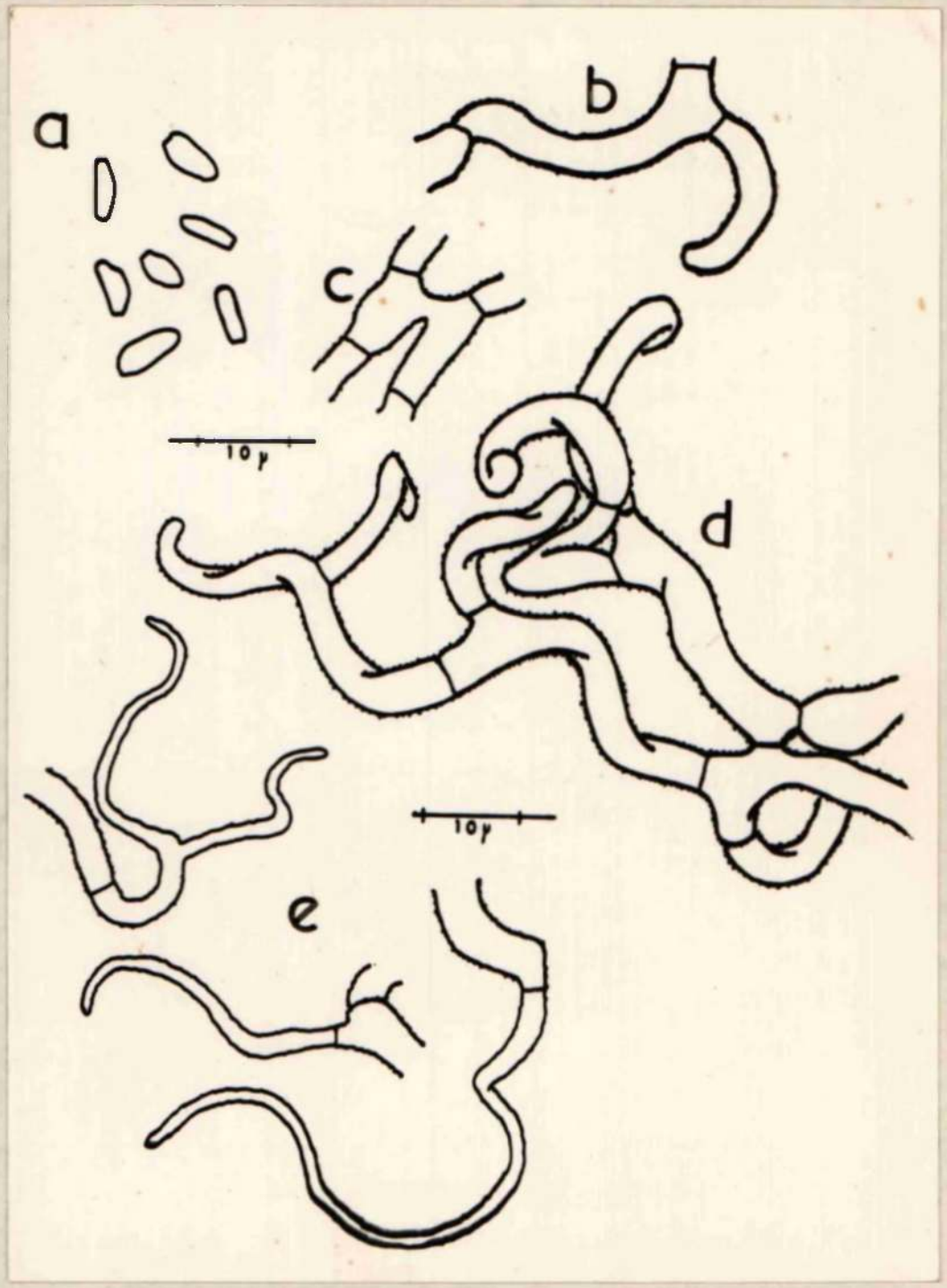

a
b
c
10μ
d
10μ
e

Plate 44

Plate 45.

Bulbil-forming species

a) Early stage in development of a bulbil; swollen hyphal tip cut off by the formation of a septum, X 2000.

b) First division transversely across the cell, X 2000.

c) Second and third divisions, X 2000.

d) Mature bulbils, X 2000.

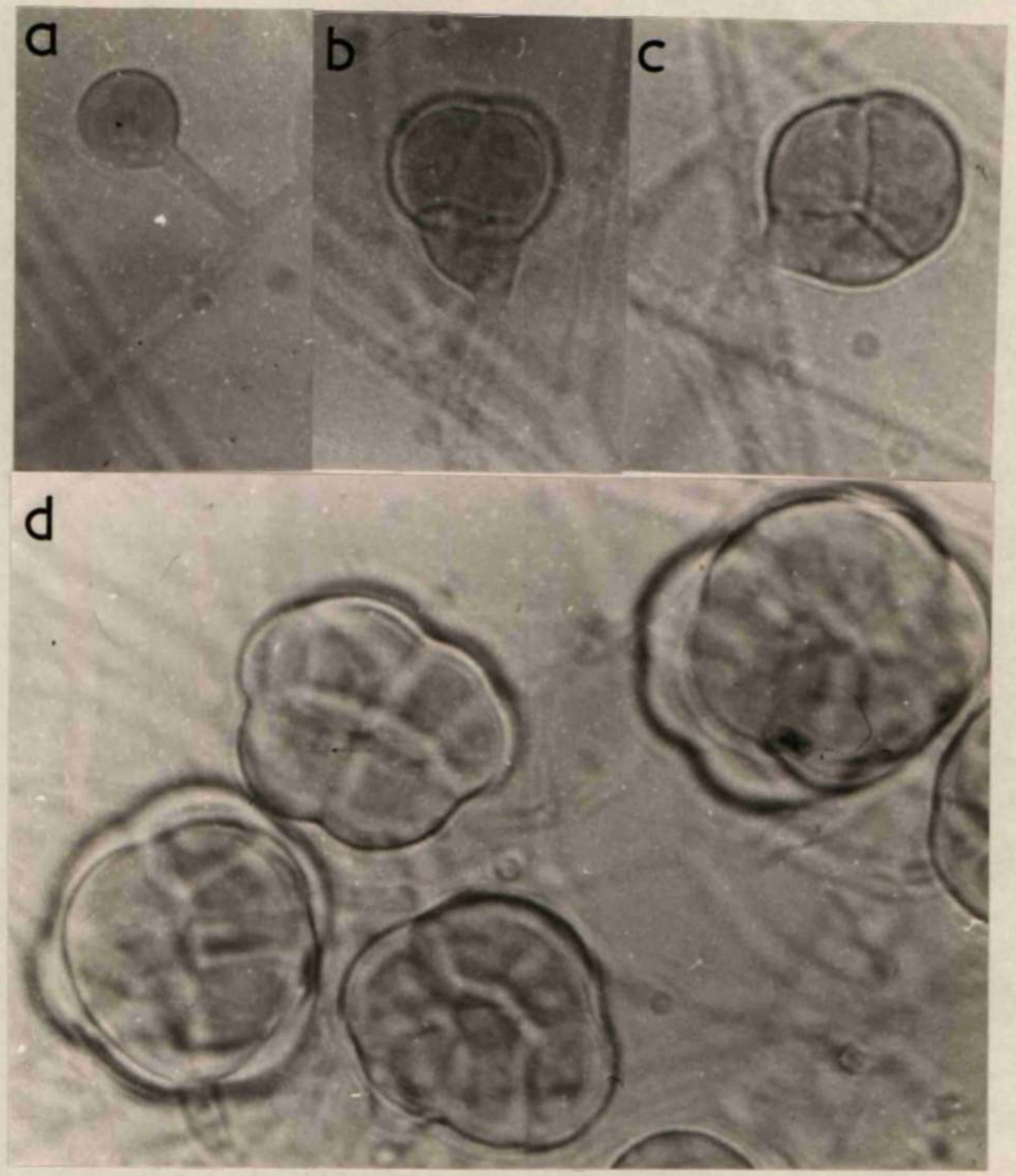

a
b
c
d

Plate 45

Plate 46.

Bulbil-forming species

Camera lucida drawings

Stages in development of the bulbils.

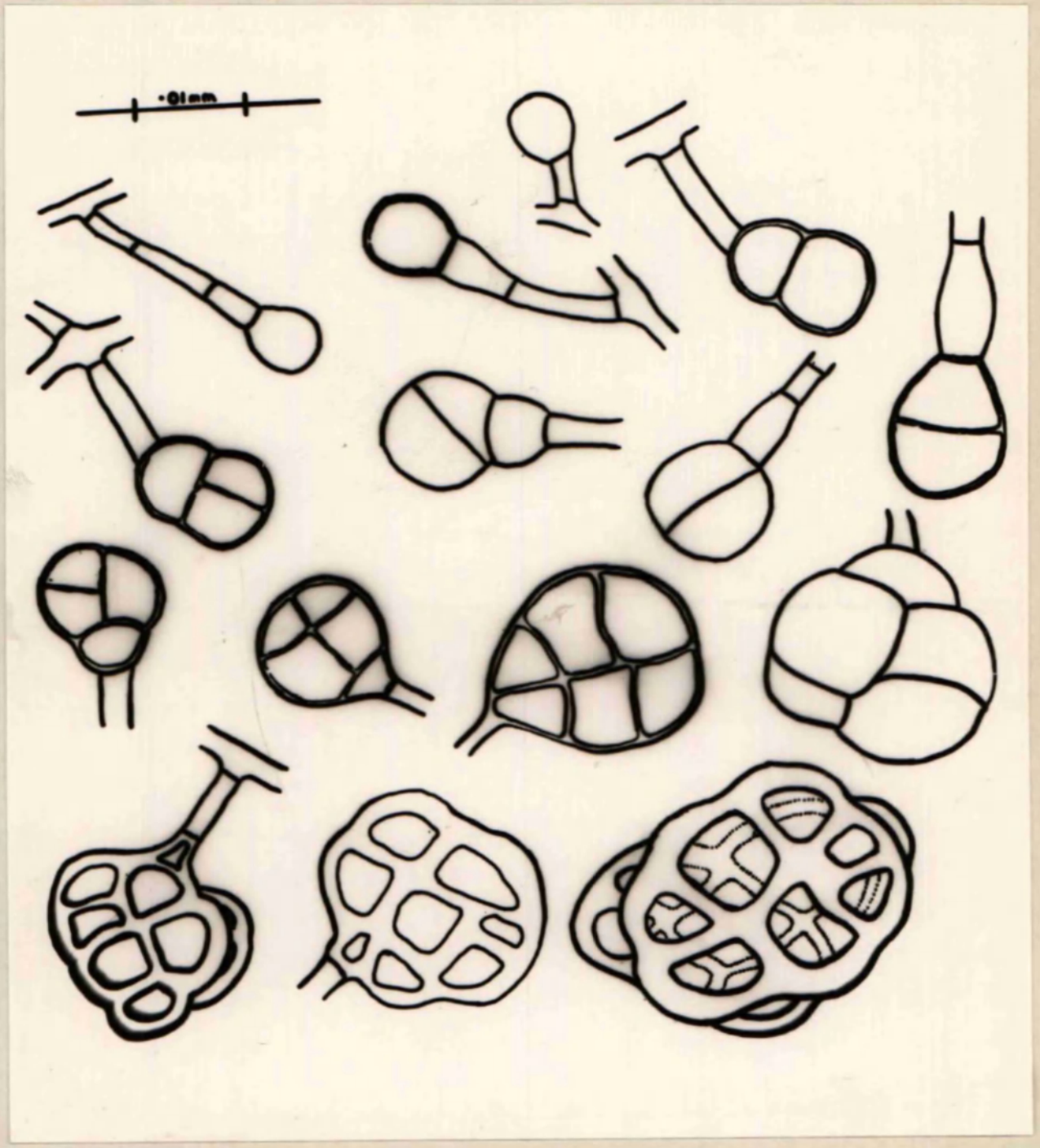

·01mm

Plate 46

Plate 47.

Parasitic species

a) Horse hair bait on which can be seen macroconidia of Keratinomyces ajelloi and the round spores of parasitic species 1, X 800.

b) Spore and sporophore of parasitic species 1, X 2000.

c) Thallus of parasitic species 2 consisting of 2 basal lobes which give rise to 2 conidiophores bearing conidia, X 2000.

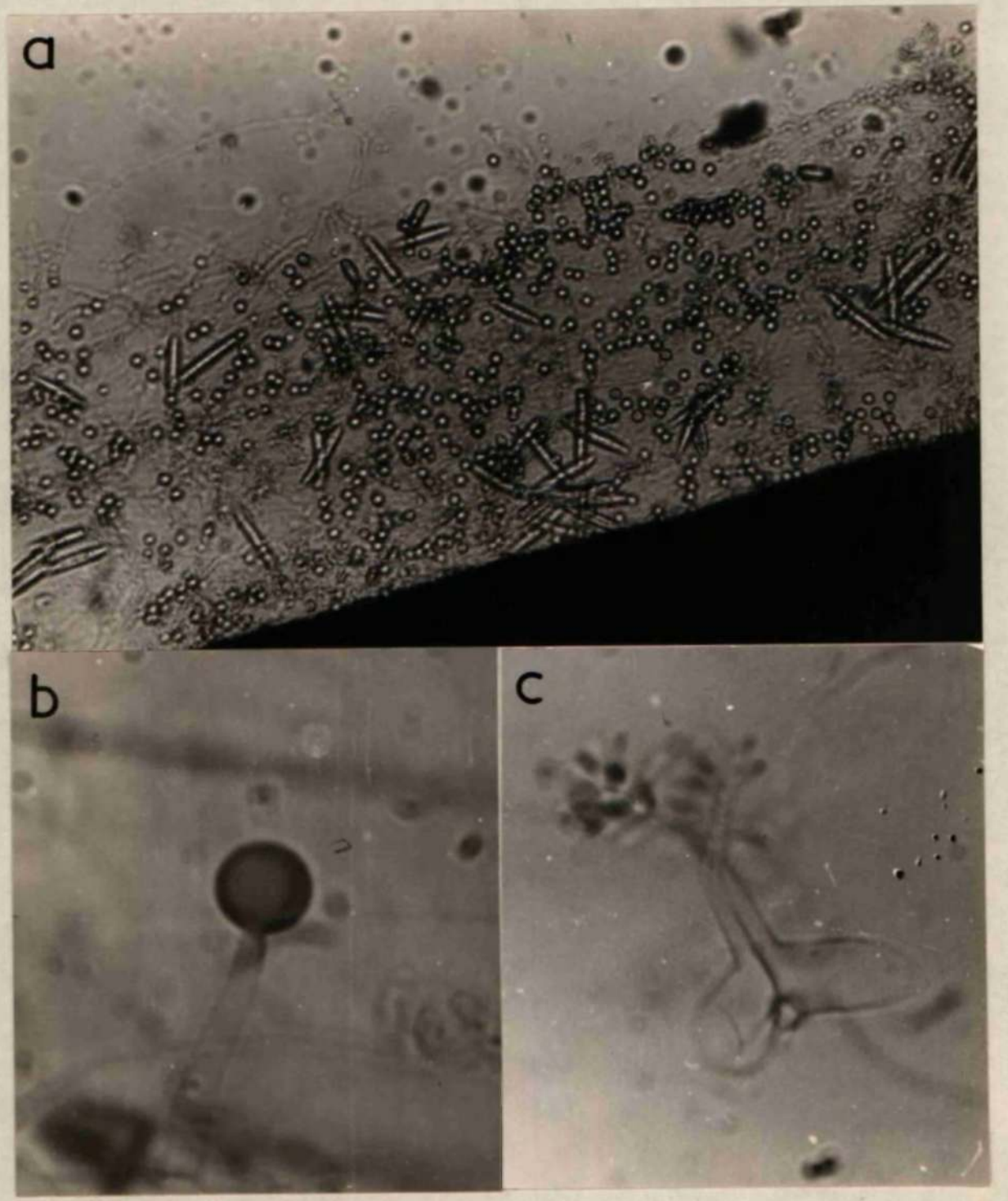

a
b
c

Plate 47

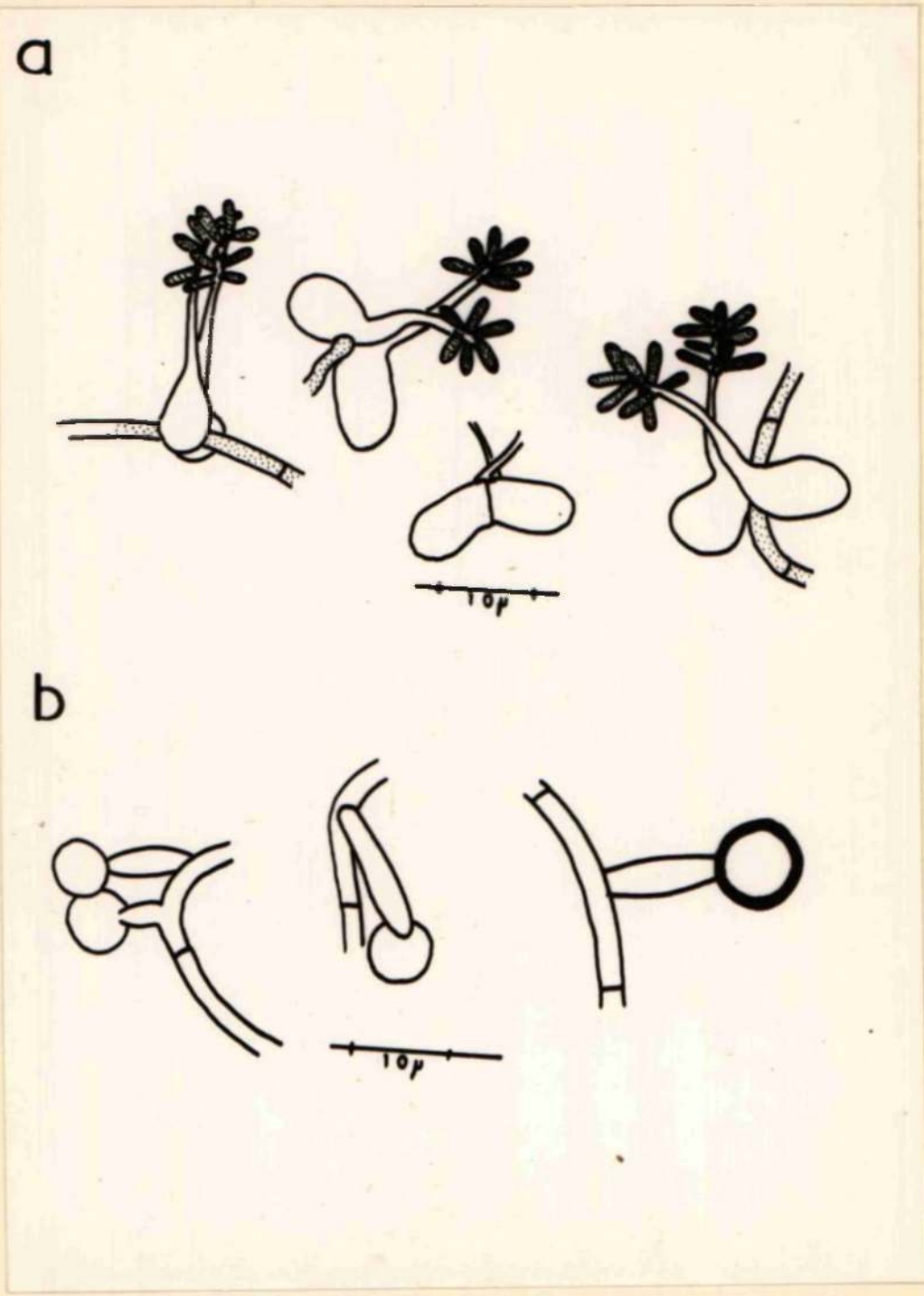
Plate 48.

Parasitic species

Camera lucida drawings

a) Parasitic species 2.

b) Parasitic species 1.


a
10μ
b
10μ