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DERMATOPHYTE MORPHOLOGY: A SCANNING ELECTRON MICROSCOPY STUDY

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Abstract

The Dermatophytes are a broad group of fungi belonging to the class Fungii imperfecti that are the causative agents of dermophytosis (ringworm infections). The present work offers an overview of the morphology of these fungi found in cultures according to the scanning electron microscope. The fungi were obtained from cultures left to develop over variable periods of time that would be sufficient for growth. The morphological features of some dermatophytes obtained in artificial cultures are detailed: M. canis, M. gypseum, M. audouini, M. cookei, T. mentagrophytes, T. schouleinii, T. verrucosum, T. ajelloi, T. proliferans, and E. floccosum. In all cases an analysis of the morphology of the reproductive mycelium developed in the culture was made: hyphae, macroconidia, microconidia, and chlamydospores; details that serve to distinguish one fungus from another. In the perfect forms, the morphology of the peridial hyphae and of the ascocarps (cleistotethia) are described.

Key Words: Dermatophytes, Scanning Electron Microscopy, Mycosis, Fungus, Tineas, M. canis, M. gypseum, M. audouini, M. cookei, T. mentagrophytes, T. schouleinii, T. verrucosum, T. ajelloi, T. proliferans, E. floccosum.

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Introduction

The Dermatophytes belong to a broad group of fungi with pathogenic capacity in man and diverse animal species. They are keratinophilic agents; that is, they only parasitize keratin or keratinized structures (the horny layer, skin, and nails) causing dermatological lesions encompassed within the term dermatophytosis (ringworm infections). Speculations have been made about the possibility of Dermatophytes having been derived from a large family of perfect soil fungi (ascomycetes). With the hair bait technique, it has been possible to isolate fungi with morphological features similar to those of the Dermatophytes but that do not induce pathological states in man or in animals.

Although they belong to the class Fungii imperfecti (asexual), some species are also able to develop a perfect state (sexual) when grown on hairs on the surface of soil.

Asexual reproduction is achieved through the formation of spores. Upon finding a suitable medium, a spore germinates and emits one or several tubes that grow distally and branch in the form of filaments known as hyphae. The branchings of the hyphae constitute the mycelium which shows two differentiated portions: the vegetative mycelium, responsible for the maintenance and growth of the fungus, and the reproductor mycelium that contains spore-bearing hyphae (sporophores). The spores are mainly located on lateral projections of the hyphae and have a bud or finger-like aspect; these are the conidia. When the largest axis of the conidium is less than 5 micrometers and is formed of a single cell it is known as a microconidium. If the evagination is larger and is composed of two or more cells, the term macroconidium is used.

Morphological varieties within the hyphae are the chlamydospores and the pectinate hyphae. The former are globose bulges surrounded by a strong wall and localized on the distal portion of the hypha or intercalated on its trajectory. Pectinate hyphae have a comb-like morphology.

In the perfect state, the reproduction of Dermatophytes is sexual and is characterized in that the spores, in this case called ascospores, are the product of fusion of two gametes of opposite sign (conventionally gamete + and gamete -). Out of this union arise more or less spherical formations - the ascocarps (cleistotethia) - surrounded by thick and claw-like hyphae called peridial. In turn, each ascocarp is formed by sacs (asci) in whose interior the

ascospores are located. Essentially, two genera have been described: Mannizzia as the perfect state of the genus Microsporum and Arthroderma as the perfect state of the genus Trichophyton.

The Dermatophytes exhibit different morphologies according to whether they are viewed: a) as pathogens, obtained directly from lesions (they would be seen simply as unicellular and sporulated forms), b) in artificial culture media (where they develop a reproductor mycelium formed of macro- and microconidia, special hyphae, etc., which have served as the basis for their taxonomic classification), and c) when they are grown with the hair bait technique, where some of them are able to develop a perfect stage (for more details, see 1, 2, 5, 6, 9-12, 16, 17, 20, 21, 37, 43, 45, 46, 53).

The present work offers a scanning electron microscope (SEM) analysis of the morphological characteristics of some Dermatophytes belonging to the three existing epidemiological groups (anthropophilic, zoophilic, and geophilic). The morphology described relates to that found in cultures, not in infected integument and/or its appendices.

Materials and Methods

The Dermatophytes analyzed were from three sources: **a)** clinical lesions of patients attending the University Hospital (University of Salamanca, Spain); **b)** from lesions (ringworm) in several animal species (rabbits, calves, rats, etc.); **c)** from different soil samples, according to the techniques proposed by Dwivedi et al. (13) and Vanbreuseghem (44).

The Dermatophytes were cultured in agar containing glucose and neopeptone (Saboureaud's medium); in order to prevent contamination and the presence of saprophytic mold, chloramphenicol and cycloheximide were added (14, 37, and 39).

Identification of Trichophyton verrucosum was carried out by the nutritional tests (adding nutrient substances such as thiamine and inositol) proposed by Georg (18). For differentiating Trichophyton mentagrophytes from T. rubrum the perforating hair tests of Ajello and Georg (3) and the urease test of Philpot (34) were used.

Perfect forms were obtained by crossing isolated strains with two other strains of different signs (37) of the corresponding perfect form, provided by Doctors Ajello (at Communicable Disease Center, Atlanta, Georgia) and Mackenzie (at the London School of Hygiene and Tropical Medicine).

The protocols used for analysis with the scanning electron microscope were as follows: fixation was in 6% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, temperature 4°C over 3-6 hours. The preparations were then washed in 0.1 M phosphate buffer, pH 7.4 plus 0.02 M sucrose for 2 hours. The preparations were then post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, for 2 hours at 4°C and then washed. They were dehydrated in a graded acetone series (30%, 50%, 70%, 80%, 90%, and absolute acetone) and then dried by the critical point method using acetone as the vehicle. A fine layer of gold was sputtered onto the pieces for 2-3 minutes (thickness 20 nm). They were observed with a Philips scanning electron microscope (SEM-500) using 12 and 25 kV as acceleration voltage.

Results

In artificial cultures, the Dermatophytes developed a reproductive mycelium with an enormously rich structure; this permitted their differentiation into three genera: Mycrosporum (M); Trichophyton (t); and Epidermophyton (E). The former two genera include several species that are pathogenic for man and animals.

Since a study of all the species would be beyond the scope of the present work, we selected only some of those most commonly present in our environment (M. canis, M. gypseum, M. audouini, M. cookei, T. mentagrophytes, T. schouleinii, T. verrucosum, E. floccosum) together with those exhibiting striking characteristics (M. audouini), new features (T. proliferans), or representing the three epidemiological groups.

Genus Microsporum

M. Canis: This is a zoophilic fungus, mainly harbored by the cat and, to a lesser extent, the dog. Its perfect or sexual state is unknown. It is the most important causative agent of tinea capitis in our environment (Salamanca, Spain). It has been studied by several authors with light, transmission, and scanning electron microscopes (26, 27, 37, 42, 46, and 50).

Upon examination with the scanning electron microscope a mycelium with the following characteristics was observed: when the colony was young (after a short period of time, about eight days of growth), the hyphae were thin (Figs. 1 and 2), with some swellings, occasionally showing formations resembling smooth surfaced buds (Fig. 1). The macroconidia were elongate, spindle-shaped, with a sharp distal end (Figs. 1, 2, 3); their surface, in the case of young colonies, was almost smooth (Fig. 1), with a

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Fig. 1. Macroconidium (ma) of M. canis belonging to a young colony of short evolution. It is fairly smooth and only exhibits a fine granulation. The relief of a septum can be seen (arrow). The hyphae (hy) shows bud formations with a smooth surface (arrowheads).

Fig. 2. Macroconidium (ma) belonging to an older colony; note that the whole surface is covered with buds.

Fig. 3. Detail of surface of macroconidium shown in Fig. 2; note buds which have cotton-like appearance.

Fig. 4. M. gypseum. Long macroconidia (ma) with few knobs on surface. Lateral microconidia can be seen emerging from hyphae (arrows).

Fig. 5. Macroconidia of M. gypseum belonging to an older culture than those shown in Fig. 4; their surface is covered by numerous knobs of different sizes.

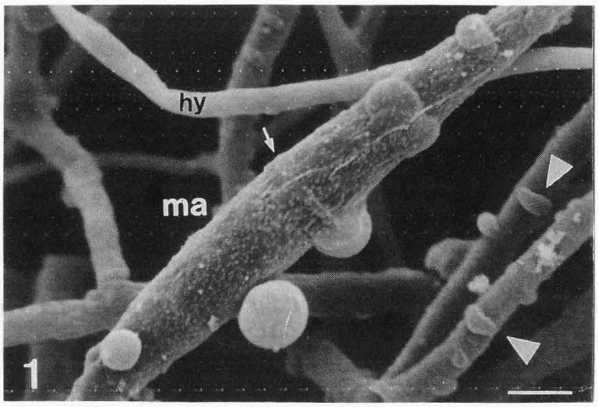
Fig. 6. Macroconidia (ma) of M. cookei. They are ovoid shaped and have abundant knobs.

Fig. 7. Typical terminal chlamydospore (tc) highly characteristic of M. audouini. Lateral microconidia are seen on some hyphae (arrows).

Fig. 8. Pectinate hyphae (ph) of M. audouini. This is a characteristic of the variety rivalieri.

Bar lengths in micrometers:

Figs. 1, 5 and 8	=	5;
Figs. 2, 4, 6 and 7	=	10, and
Fig. 3	=	1.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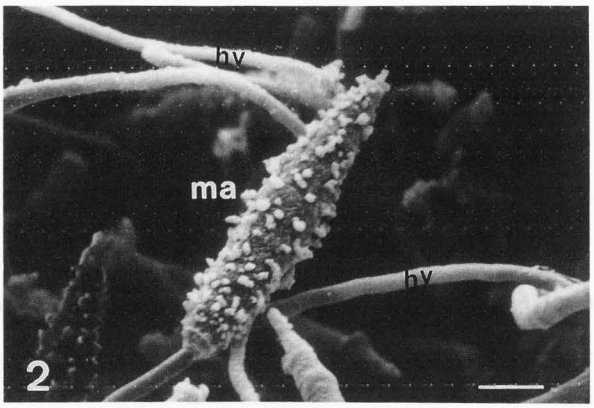
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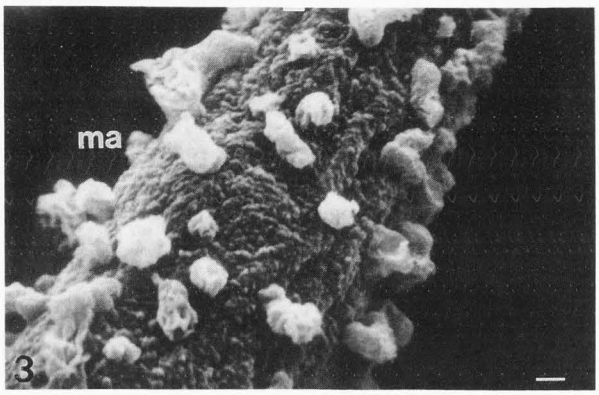
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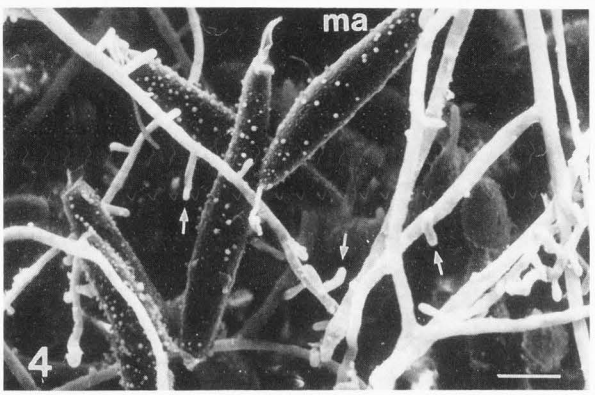
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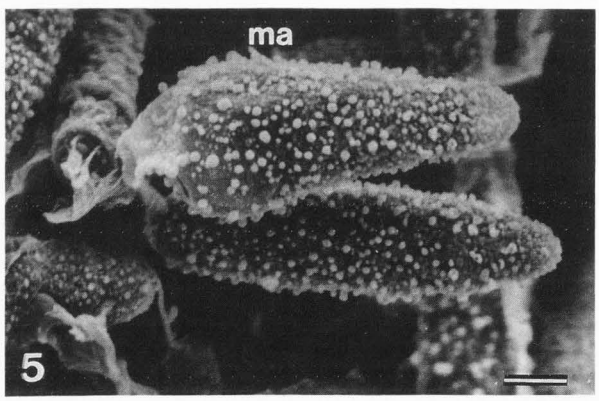
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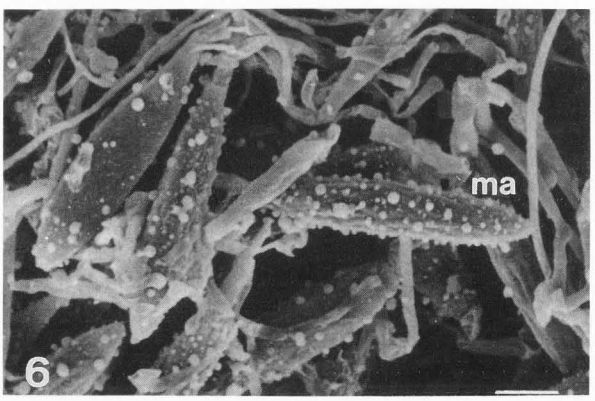
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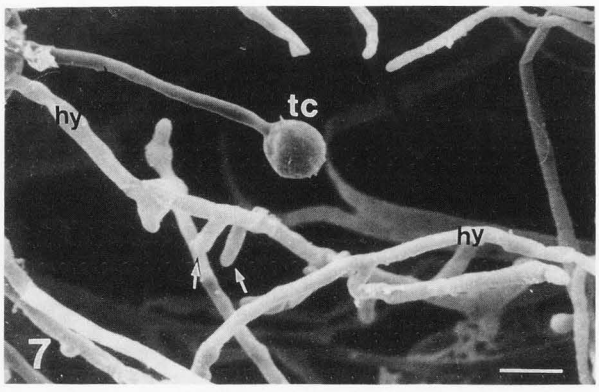
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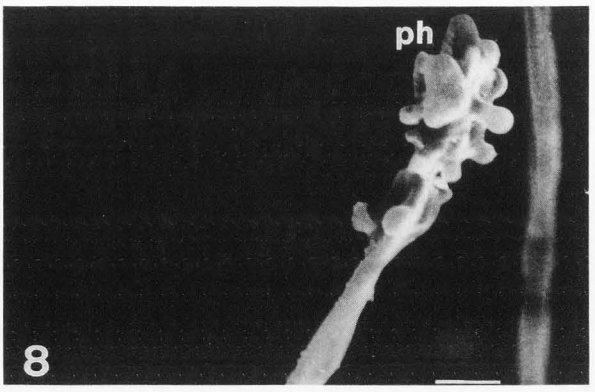
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fine, uniformly distributed granulation. In them the septa were prominent. In older cultures (at 15 days after the start of growth), the surface of the macroconidium exhibited downy knobs starting on the distal end and extending across the whole of the surface of the conidium (Figs. 2 and 3).

M. gypseum: This is a geophilic fungus that is abundant in soils rich in organic matter (gardens, orchards, etc.). It may affect humans, producing circinate herpes and tinae capitis. In this species two perfect forms are known: Nannizzia incurvata and Nannizzia gypsea. The species has been studied by several authors using different techniques (5, 9, 25, 26, 31, 46, 47, 48, 51).

The Mycelia of this species were characterized by their abundant "cigar-shaped" macroconidia (Figs. 4 and 5); they were often found in groups. As the age of the culture progressed, the surfaces of these macroconidia began to show an increasing number of knobs. As an example, Fig. 4 shows macroconidia after a short period of development in culture, there are few knobs; by contrast, in Fig. 5, the macroconidia are older and have more knobs. Unlike the macroconidia, the microconidia were rare and were located laterally on the hyphae (Fig. 4).

M. cookei: This species belongs to the group of geophilic fungi and are mainly found in the soil; the fungus is rarely pathogenic for man and even for animals. Its perfect form is known: Nannizzia cajetani (24, 26, 29, 46).

The species was characterized by the presence of low numbers of hyphae and abundant macroconidia in its mycelium (Fig. 6). Its characteristics were very similar to those of other fungi belonging to the genus Microsporum; they were gherkin-like with spherical buds on the surface. There were few microconidia. They never had characteristics differing from those of other species.

M. audouini: This is an anthropophilic fungus, the causative agent to tinea capitis. It is abundant in Africa and the United States but rare in Europe. It may mutate such that certain varieties are known, e.g., lengeronii and rivalieri (26, 46, 52).

Analyzed with SEM, the species was characterized by exhibiting macroconidia and abundant mycelia, with certain essential features such as the presence of pectinate hyphae and terminal chlamydospores.

The hyphae were thin and abundant with some swelling and septa (Fig. 7). They sometimes displayed lateral microconidia (Fig. 7). The variety rivalieri had pectinate hyphae and was characterized by displaying a zone that broadened on its distal end, emitting globose elongations of different sizes (Fig. 8).

A definitive feature in the characterization of this species was the presence of terminal chlamydospores (Fig. 7), spherical in shape with a smooth surface. Occasionally, it was possible to observe the presence of intermediate chlamydospores like simple swellings of a hypha.

Genus Trichophyton

T. mentagrophytes: There are two varieties: T.M. granulosum, a zoophilic fungus, and T.M. interdigitale, anthropophilic fungi affecting man.

The variety granulosum is the most frequent causative agent of inflammatory tinea (Kerion), and the variety interdigitale produces circinate herpes, especially athlete's foot and onychomycosis. Its known perfect state is Arthroderma Benhamiae. Among other reports, it has been described in references 4, 8, 23, 26, 28, 30, 32, and 35.

The species was characterized by displaying abundant mycelia. The hyphae were thin and regular and contained numerous microconidia grouped in clusters resembling unripe grapes (Fig. 9). At greater magnification, these microconidia were spherical or ovoid and had a smooth surface (Fig. 10).

Although this was the typical arrangement of the microconidia, they were also seen to be arranged laterally; these microconidia had different shapes, either bud like (Fig. 9) or spherical or pyriform, with a short anchoring stalk (Fig. 11).

Another feature was the existence of spiral hyphae arranged in spiral coils (Fig. 12). These formations, although not exclusive to T. mentagrophytes, were very characteristic with regards to their high numbers. Occasionally, it was possible to find terminal and even intermediate chlamydospores. The macroconidia were ovoid or spindle-shaped, with a sharp distal end in which some septa were seen.

T. schouleinii: This fungus is anthropophilic and is the causative agent of tinea favus. Its development is chronic and affects the scalp, where it induces crusty lesions centered around a hair and leading to scar-due alopecia. It may give rise to onychomycosis. Its sexual stage is unknown (26, 33, 37).

The species was characterized by the abundant presence of mycelia, with hyphae varying in thickness. It displayed terminal hyphae, arranged in the form of nail-head hyphae (Fig. 13). Macroconidia were almost absent and microconidia were also uncommon.

T. verrucosum: This is a fungus affecting animals and its principal reservoir is cattle, from where it may pass to man, inducing lesions with an intense inflammatory reaction (circinate herpes, kerion). Its perfect stage is not known (7, 26, 46).

Fig. 9. T. mentagrophytes (variety granulosum). Note lateral microconidia (arrows) and other, more abundant ones, that are spherical or ovoid, with a smooth surface, resembling a cluster of unripe grapes (mi).

Fig. 10. Detail of spherical or ovoid microconidia (mi) of T. mentagrophytes. Note short anchoring stalk.

Fig. 11. Lateral microconidia of T. mentagrophytes (arrows).

Fig. 12. Typical filamentous hyphae in spiral coils (sc) of T. mentagrophytes (variety granulosum).

Fig. 13. Nail-head of T. schouleinii (arrow).

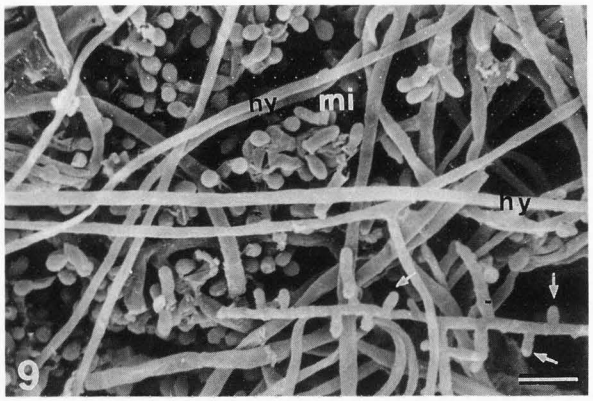
Fig. 14. Mycelium of T. verrucosum. Some of the hyphae have only a few microconidia (arrows). A spherical terminal chlamydospores (tc) is clearly seen. The branching of the hyphae often occurs at right angles (*).

Fig. 15. Intermediate chlamydospores of T. verrucosum arranged in bead-like formations (cl).

Fig. 16. Macroconidia (ma) of T. ajelloi. Their surface is smooth, finely folded, and transversal septa (small arrows) are seen. The long arrows show lateral microconidia.

Bar lengths in micrometers:

Figs. 9, 14, 16	=	10;
Figs. 10, 11	=	1, and
Figs. 12, 13, 15	=	5.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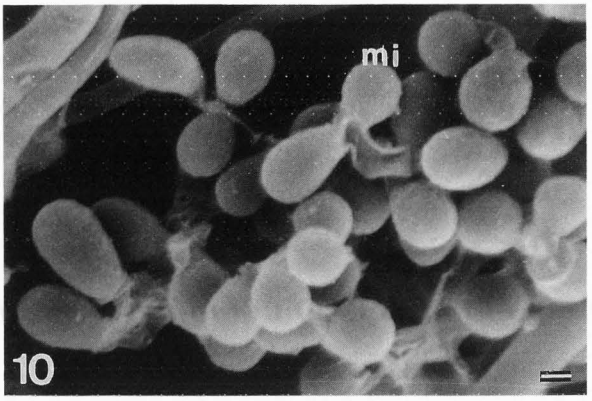
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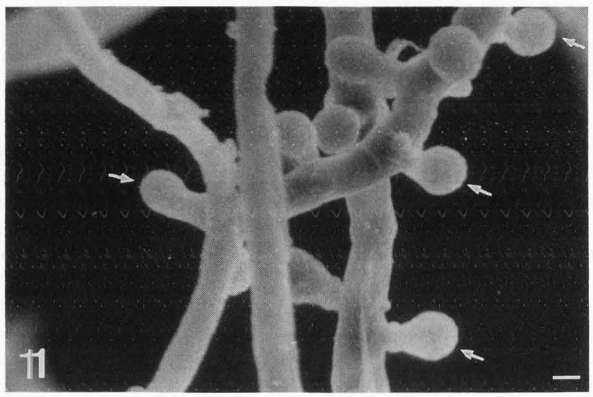
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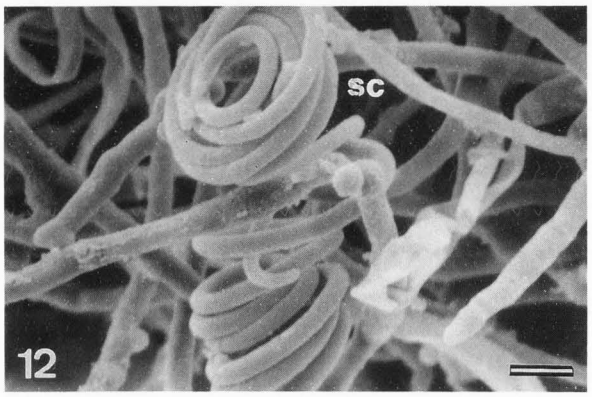
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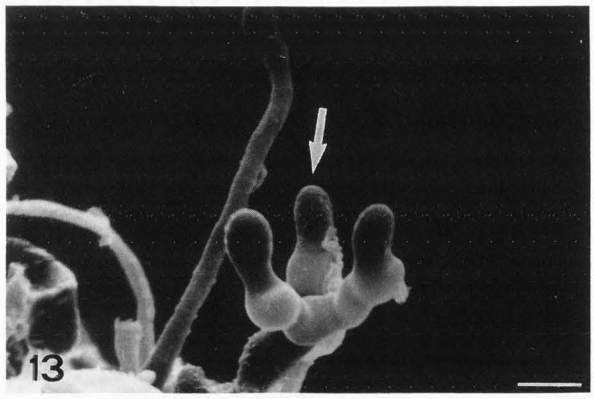
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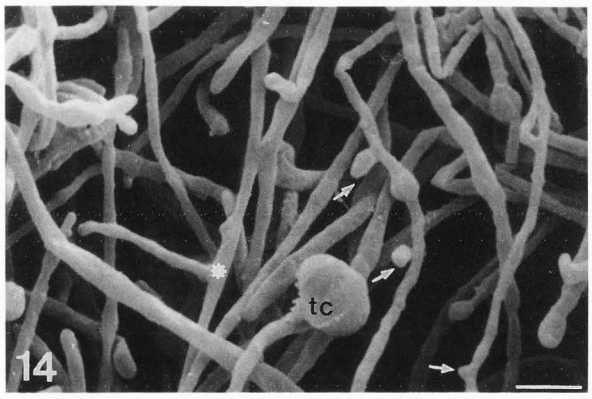
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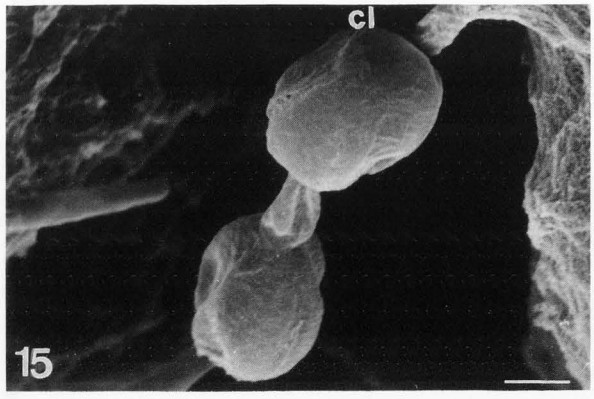
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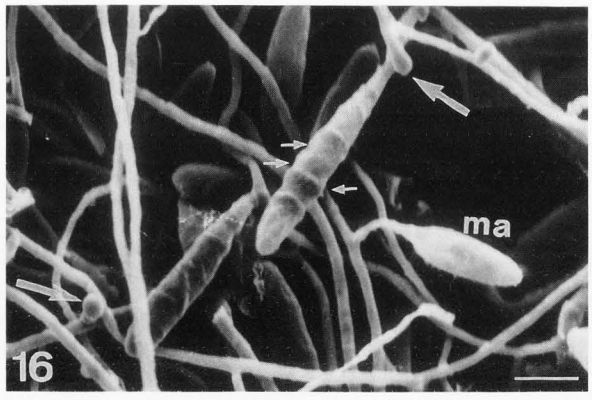
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The essential characteristic of this trichophyton was that it had thick, 90° branched hyphae (Fig. 14), that sometimes adopted an antler-like disposition. Some terminal or intermediate chlamydospores were observed (Fig. 14), occasionally forming chains (Fig. 15); the surface of these was smooth or slightly folded.

Only on some occasions, and then in enriched media, was it possible to observe some macro- and microconidia; the latter are seen leaving the hyphae laterally in Fig. 14.

T. ajelloi: This is a geophilic fungus that is only rarely pathogenic for man. It is found in soil rich in organic matter. Its sexual form, Arthroderma uncinatum, is known (26, 31, 46). The ultrastructural characteristics of this species were: numerous large, elongated fusiform macroconidia with a smooth or slightly surface, and well-defined septa (Fig. 16). Microconidia were sometimes found (Fig. 16).

T. proliferans: Until recently, there has been considerable discussion as to whether this fungus is an autochthonous species or simply a variant of T. mentagrophytes, since until now few species have been isolated (15, 37). Under SEM, its characteristics were elucidated in detail and it was possible to clearly differentiate it from T. mentagrophytes.

The most striking differential feature was the presence on the mycelia of thick hyphae with formations known as propagules; these were seen as dilations on the hyphae (Figs. 17-22) and have never been observed in other Dermatophytes. They varied in size and shape. Sometimes, they were isolated, while others formed chains, as observed in Figs. 17-22. Some were fusiform, while others were more or less spherical, with a smooth surface in some cases (Figs. 17, 19) and a rough surface in others (Fig. 20). They sometimes displayed granulations, as is the case of those shown in Fig. 22. Their mycelia also had macroconidia (Fig. 23) very similar to those corresponding to T. mentagrophytes, with a spherical free end displaying a rough surface. The image was very similar to that of propagules observed in Fig. 22, suggesting that these were developmental stages of a single element.

The macroconidia were joined to the hyphae by a short anchoring stalk, as observed in Fig. 23 and, in greater detail, in Fig. 24, where two concentric layers and a central zone without a clear content can be seen. This Dermatophyte did not have microconidia.

Genus Epidermophyton

E. floccosum: This is the only species of this genus. The fungus affects man and is widespread; in Spain it is most often responsible for tinea cruris (19, 24, 26, 49). Under the scanning electron microscope it displayed numerous macroconidia either laterally or on the distal end of the hypha near it (Fig. 25). These were club shaped with a spherical free end; overall, they constituted the so-called racket-hyphae. Their surface was rough, with irregular folds separated by furrows giving them a brain-like appearance. Annular reliefs, corresponding to the septa, were visible (Fig. 25). The hyphae were thick and septate; in some it was possible to observe small spherical knobs (Fig. 25).

Terminal or intermediate chlamydospores were common (Fig. 26). The surface of these was smooth or finely granulated. Sometimes the chlamydospores were so abundant that they conferred a bead like aspect to the hypha. No microconidia were observed.

Sexual Forms

When some species are cultivated on the surface of the soil, they develop their perfect or sexual form and are characterized by exhibiting more or less spherical formations known as ascocarps (cleistothecia), surrounded by peridial hyphae. Each ascocarp is in turn formed on asci, within which the so-called ascospores are located. Some genera of perfect forms are known: among them those corresponding to T. mentagrophytes (Arthroderma benjamiae) and to M. gypseum (Nannizzia incurvata and Nannizzia gypsea) (3, 8, 26, 31, 32).

Arthroderma benjamiae: The Ascocarp (Fig. 27) was characterized by its spherical shape and was surrounded by peridial hyphae that appeared as spiculated surface elements with periodic, regularly interspersed, strangulations conferring them with a bead-like appearance (Fig. 28). In peripheral zones, it was also possible to see spiral coils that are not true attributes of this form since they also appear in other species.

The asci containing the ascospores, generally 8 in number (26), were easily broken, revealing the ascospores. These were seen as small lentil-shaped formations with a smooth or slightly folded surface (Fig. 29).

Nannizzia incurvata and Nannizzia gypsea: These are perfect forms of M. gypseum. Their ascocarp appeared surrounded by peridial hyphae. The segments were quite long and regular and sometimes branched to form a cactus-like image (compare the difference between Figs. 28 and 30 corresponding to peridial hyphae of the two perfect forms referred to). As in the case of A. benjamiae, there were spiral coils and also some macroconidia located peripherally. The asci were identical to those described (as in Fig. 29).

General Comment

In clinical practice, when there is a suspicion of dermophytosis, confirmation as to whether there is or there is not a fungus causing the condition (pathogenic fungus) can be obtained from samples of the lesions (scales, hairs, etc.), which upon addition of a few drops of 10-20% KOH show the presence of fungus under the light microscope. However, this procedure is rudimentary and for a surer diagnosis it

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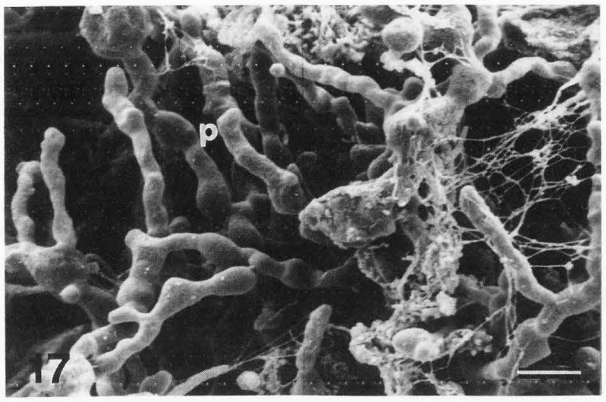
Figs. 17-24. T. proliferans. Note highly developed and irregular hyphae on which there are prominent swellings or "propagules" (p in Figs. 17, 18, 19 and 20). Some times they are isolated (Fig. 20), although they normally form chains (Figs. 17 and 18). Their surface may be smooth (Figs. 17 and 19), slightly rough (Fig. 20), or may show granulations (Figs. 21 and 22). Macroconidia (ma) can be seen, some with a smooth or slightly thicker folded surface (Fig. 23). Fig. 24 shows the surface of the junction between a propagule (arrow) similar to the insertive stalk of the macroconidium shown in Fig. 23 (arrow).

Bar lengths in micrometers:

Figs. 17, 19 and 20	=	10;
Figs. 18, 20, 22 and 24	=	5, and
Fig. 23	=	1.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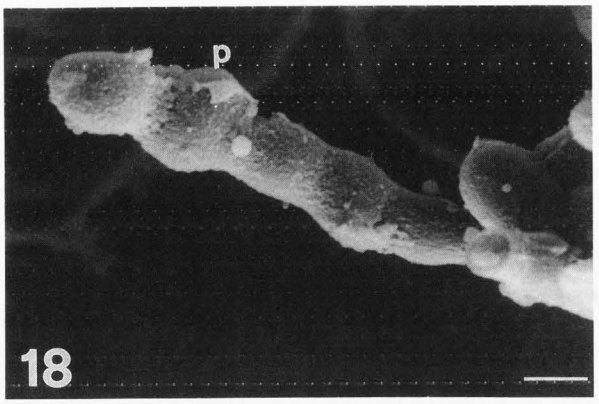
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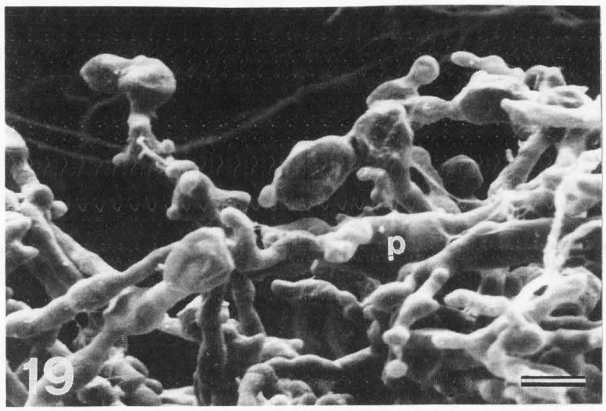
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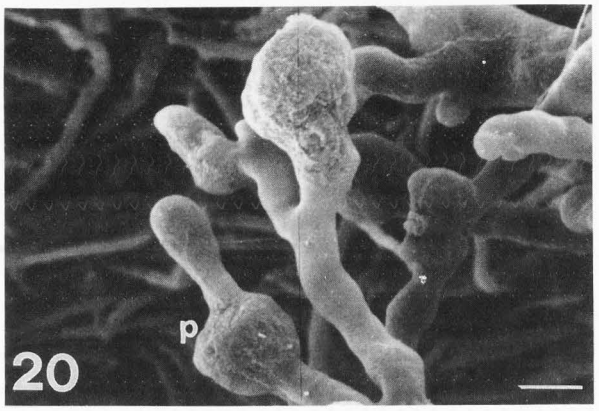
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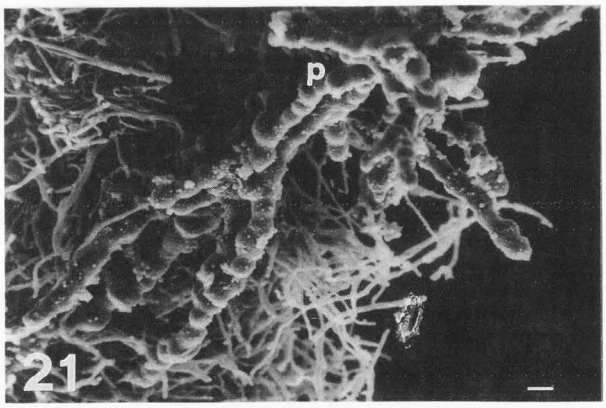
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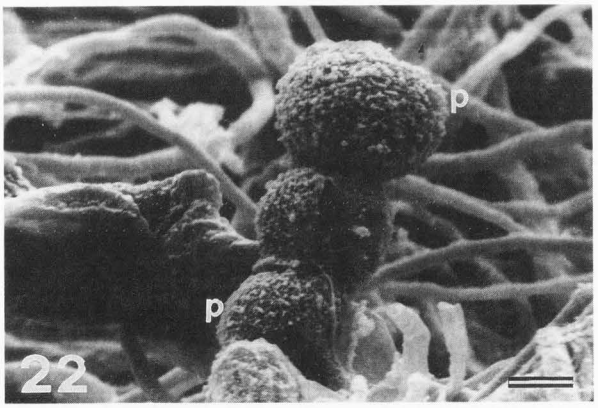
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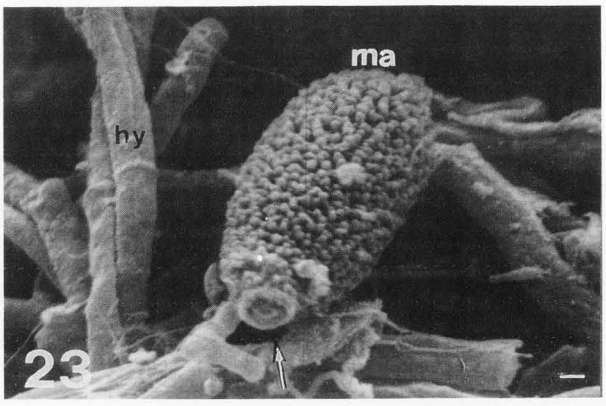
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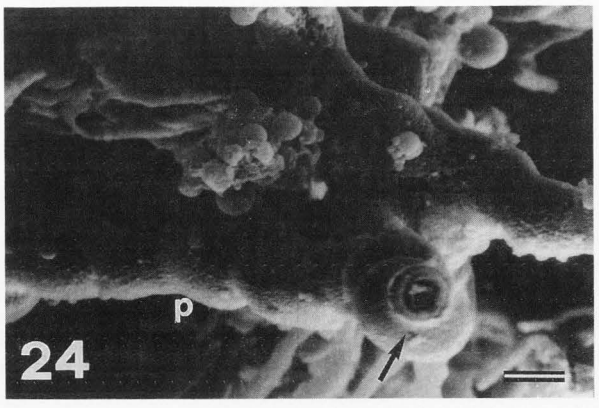
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is necessary to obtain a sample of the lesion and then analyze histological sections with appropriate techniques, among them the Periodic-Acid Schiff reaction (PAS) and the Gomori methenamine-silver nitrate stain (14).

Both in direct visualization and in histological sections, the pathogenic fungus appears either in mycelium form or in sporulated form. However, in no case is it possible to observe morphological characteristics that differentiate one fungus from another and thus reach a diagnosis of the causative agent.

If the scales from the lesion are cultured in artificial media and the culture is examined after some time, it is possible to arrive at a diagnosis of the agent responsible for the condition because it has developed its reproductive mycelium.

Cultured dermatophytes have been studied in several works using different morphological techniques. The macroscopic aspect of the thallus (colony) such as more or less rapid growth, its smooth or rough aspect, consistency, colour, etc., orients diagnosis of the causative agent although several fungi have similar characteristics (37).

Using samples obtained in culture, light microscopy analyzes many details of these fungi and many years ago this permitted their taxonomic classification. Among these details one could cite, for example, the fact that the macroconidia of the genus Microsporum have rough walls, whereas those of the genera Trichophyton and Epidermophyton have smooth walls. Other differential features are whether they have microconidia or not, their form and number; or whether the mycelium has pectinate hyphae (Characteristic of M. audouini), nail head hyphae (typical of T. schouleinii), abundant spherical microconidia (T. mentagrophytes), etc. (14, 37, 40).

Currently, in most cases with such observations it is possible to arrive at an exact diagnosis of the causative agent of ringworm.

Transmission electron microscopy sheds further light on the morphology of the Dermatophytes and affords a better understanding of their walls, septa, and organelles, yielding interesting data for research into these fungi. Descriptions have been offered of the details of the walls of hyphae and macroconidia (22, 36, 50, 52); of the structure of septa (35), of the existence of pores in the septa (52), and of the existence of Woronin bodies (38), etc.

Use of the scanning electron microscope furnishes a detailed analysis of the morphology of the surface of these fungi and helps in their classification. Its resolution and depth of field yields good and easily interpretable images for a better analysis of the surface details of the fungi. In a previous work we undertook a study of the morphology of the Dermatophytes, combining scanning and transmission electron microscopy and compiling an Atlas with the results found (26).

SEM confirms the findings of the light microscope; the surface of the macroconidia corresponding to the genera Trichophyton and Epidermophyton are smooth and those of the species of Microsporum are rough (24, 41, 48). However, the richness of information is much greater owing to the stereoscopic visualization of the fungus, the greater resolution of the microscope, allowing one to clearly define the shape of the rugosities, their number, the way in which the macroconidia are implanted, etc.

The time of evolution of the cultures is important for analyzing the morphological characteristics of the fungus. In cultures of only a few days evolution the mycelium is young and displays certain specific characteristics (macroconidia without the relief of the septa, a smooth surface, fewer verrucosities, etc.); in cultures with a long evolution the characteristics of the mycelium are different (very visible reliefs of the septa, numerous verrucosities, etc.). In this sense, it is necessary to take into account that the different kinds of Dermatophytes have different periods in which the colony begins to develop; this depends on the type of Dermatophyte itself, the culture medium employed, and on the environment in which it develops, among other causes. Once it has begun to develop, the colony is considered to be young during the first 8 days. Between 8 days and the end of the first month the colony is considered to be mature, and thereafter the phenomena of pleomorphism begin to appear, making it difficult to study the mycelium. Naturally, these are only approximate times and are in fact standardized in any mycological laboratory. For us, between 8 and 15 days is the ideal time for microscopic observation of the fungi.

In this sense, for example, Akin and Michaelis (5) have reported that at 8 days of evolution of the culture the surface of the macroconidia of M. gypseum shows polypoid projections that cannot be seen before this time; in a previous work (26) we have observed similar details in several Dermatophytes.

Epidemiologically, Dermatophytes are classified in three groups: anthropophilic, zoophilic, or geophilic; all three can produce lesions in man, although essentially the former two are responsible for these. Lesions caused by zoophilic or geophilic Dermatophytes are more eczematous and inflammatory and less chronic than those produced by anthropophilic species. In this review, we have attended to fungi belonging to three epidemiological groups in a fairly proportional number of species, some of them being more pathogenic than others. Among the geophilic species (less pathogenic), we have chosen two from the genus Microsporum (M. gypseum and M. cookei) and one from the genus Trichophyton (T. ajelloi); the former two because one is pathogenic and the other only rarely pathogenic, and the latter because it is the only geophilic species Trichophyton that we have studied.

Along similar lines, with the SEM it has been possible to determine the morphological characteristics of the Dermatophytes mentioned in the present work: T. proliferans. This species has not received much attention and is thus not very well known. The characteristics of the most significant elements of this fungus, the propagules, have been described in this overview and we are in no doubt that they are exclusive to this Trychophyton and are not present in T. mentagrophytes or in the other species of Trichophyton referred to. For the first time, using the SEM it has been possible to describe the microscopic morphology of T. proliferans, assigning it as an autochthonous species different from T. mentagrophytes.

This kind of insight is exclusive to the scanning electron microscope. However, currently there are few advantages of this (regarding time, costs and material required) compared with less sophisticated methods. It would be difficult to substitute the classical method of diagnosis (light microscopy),

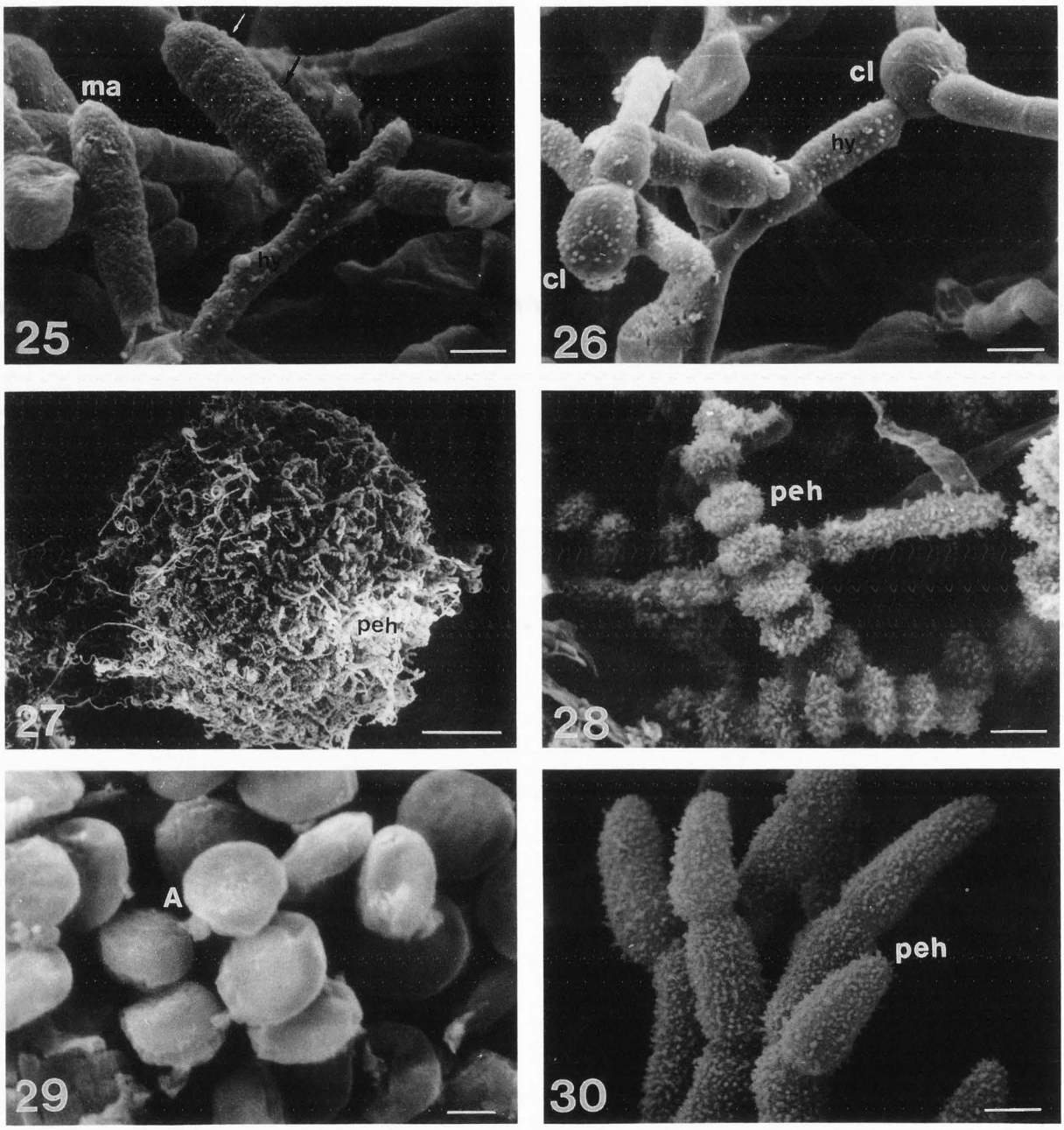

Figure 25. E. floccosum. Thick hyphae (hy), with a smooth and / or rough surface showing spherical knobs. The macroconidia (ma) are uniform in thickness and their distal end is blunt; their surface is smooth, and reliefs of the septa can be seen (arrows).

Figure 26. Clamydospores of E. floccosum (cl).

Figure 27. Ascocarp (cleistothecium) of A. benhamiae at 25 days of culture on hair bait. Peripherally, the peridial hyphae (peh) surround and obscure the asci.

Figure 28. Peridial hyphae (peh) of A. benhamiae. Their surface is spiculated and they exhibit regular strangulations conferring them with a characteristic bead-like aspect.

Figure 29. Ascospores (A) of A. benhamiae. Upon breaking the sac containing them, they are seen as small lentil-shaped bodies, with a smooth or slightly folded surface.

Figure 30. Peridial hyphae (peh) of N. incurvata. They resemble cacti.

Bar lengths: Figs. 25, 26, 28 and 30 = 5; Figs. 27 = 100; Fig. 29 = 1 micrometers.

although the SEM method would be a useful complementary tool and would be of great use in the field of research into the different species of the Dermatophytes.

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Discussion with Reviewers

B. Persky: What is meant by the term "enriched media"? Do enriched media have a direct effect on the production of macro- and microconidia?
Authors: Some dermatophytes require special nutrient media to help in their identification or development. For example, T. verrucosum needs thiamine and inositol to develop macro- and microconidia; T. violaceum and T. tonsurans require thiamine, etc.

B. Persky: Is the age of the fungal colony, i.e., young versus old, important in the diagnosis of the fungal species?
Authors: Yes, it is important. Very young colonies might not develop the macro and/or microconidia. Old colonies may become pleomorphic, making it difficult to identify the structures.

B. Persky: Is there a correlation between the in vitro age of Trichophyton mentagrophytes interdigitale colonies and the in vivo clinical manifestation: athlete's foot?
Authors: We believe there is no correlation since in the lesion the fungus never develops the morphological characteristics observed in the colony.

B. Forslind: In your opinion, how can the TEM and SEM, respectively, be used for analysis of dermatophytes in culture and in infected integument and its appendages?
Authors: As explained in the overview, the samples (scales, hair, etc.) are obtained directly from the lesion; when these are suitably prepared they can be observed with the light microscope, SEM and TEM. This does not permit one to differentiate the causative agent but it does allow one to know whether the lesion has in fact been produced by fungi.

B. Forslind: It appears, at least in M. gypseum, that the development (number) of knobs on macroconidia is related to the age of the culture. Can you describe this in relation to a time scale of culture? Are corresponding phenomena also observed in other pathogenic organisms? Do you see corresponding events in infected skin? Can you comment on the possible important growing condition differences between cultures and actual infected tissue?
Authors: The time of development of a colony of dermatophytes varies depending to several different factors (temperature, the amount of fungi in the culture media, etc.). In the case of M. gypseum, when the colony begins to develop (3-5 days) young macroconidia with few knobs are seen. When the colony is well developed (7-10 days) the number of knobs is much larger. This latter fact can also be seen in other dermatophytes such as M. canis.

When dermatophytes are seen on infected skin (lesions), they do not show any features with which they can be differentiated amongst one another. Their morphology is completely different from that observed in artificial culture media. Thus, on infected skin they do not develop either macro- or microconidia; their reproduction under such circumstances is by spores.

It is important to note that the development of the fungus, its morphology, and form of reproduction are different according to the medium in which it is observed (on infected skin, in artificial culture media

- Sabaureaud's agar - or in the case of some dermatophytes, on hair on soil surfaces).

J.T. Sibley: Do Dermatophytes contain keratinolytic enzymes?
Authors: Yes they do, and that is why they are able to digest keratin.

J.T. Sibley: By what mechanisms do dermatophytes invade the skin?
Authors: As in the case of any other infectious agents, the reasons why a dermatophyte can or cannot invade skin are not well known. An example of this is to be found in the classical cases of Kligman with experimental inoculation of humans with M. canis and other species. In these instances, only in some cases was it possible to achieve infection, regardless of whether the receivers had or had not had previous infections. In the work of this author information was given as to how parasitization occurs but not through which mechanisms.

J.T. Sibley: How do antifungal agents act?
Authors: Antifungal agents may act through two mechanisms: fungistatic or fungicidal. In many cases, such as with griseofulvin, it is difficult to say which of these two mechanisms operates.

J.T. Sibley: Candida albicans is a fungus with keratinolytic ability that infects man. Can you compare the morphology of C. albicans with the morphology of dermatophytes by emphasizing the most salient differences and their functional importance?
Authors: C. Albicans may exhibit two different morphological aspects: 1) the yeast form, when it is found as a saprophyte, and 2) the mycelium form when it acts as a pathogen. These morphological differences in both the saprophyte and pathogenic states are not exclusive to candida albicans; they can also be seen in other yeasts such as Phytosporum orbicularis or obalis (the agent responsible for **versicolor pitiriasis**).

On lesions, dermatophytes can be observed as mycelia, more or less septate, and as spores, or even in both forms; however, they are always seen as pathogens.