# Ultrastructural aspect of the keratinolytic activity of piedra

**María José Figueras and Josep Guarro**

*Departament de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili, Reus, España*

*Summary*

Piedra are considered asymptomatic superficial mycoses characterised by fungal growth that form nodules on the outside of hair shaft. Two types of piedra exist: white piedra (trichosporosis) caused by the basidiomycetous yeasts *Trichosporon* spp. and black piedra produced by the ascomycete *Piedraia hortae.* While the first produce soft white or light brown nodules formed by closely attached fungal elements (hyphae and arthroconidia), the second produce hard black nodules formed by a compact fungal stroma within which asci and ascospores originated. Nodules of white piedra are less fixed on hair than those of the black variety and can easily be removed.
Classically it has been considered that, although neither fungal infection generally alters or destroys the keratin of hair cortex, they both disrupt the hair cuticle. Recent ultrastructural research on the course of hair infection and keratin destruction has clearly demonstrated that *Piedraia hortae* act as keratinolytic fungi, dissolving the cuticular as well as the cortex keratin.
In this chapter we will highlight recent findings on these rare hair infections and discuss the keratinophilic and keratinolytic activity of these fungi.

*Key words*

White piedra, Black piedra, Keratinolysis, *Piedraia hortae*, *Trichosporon* spp.

Piedra is a fungal infection of the hair characterised by the presence of nodules of different hardness on the surface of hair shafts. Two types of piedra have been described: white piedra caused by the basidiomycetous yeasts *Trichosporon* spp., and black piedra produced by the ascomycete *Piedraia hortae* [1-6]. They are considered superficial mycoses because the hosts do not produce any immunoreactive response [1-3]. Both are rare fungal infections originally confined to the tropics and temperate climates, although descriptions exist of both infections in other geographical regions [1-3]. The low spread of these infections and the harmlessness of the diseases probably explains why so few studies on the keratinolitic capacity of these fungi have been made. In recent years, however, special attention has been paid to the basidiomycetous arthroconidial yeasts *Trichosporon* spp. due to their capacity to act as opportunistic pathogenic fungi that produce severe infections in immunocompromised patients [7-12]. Systemic infections produced by *Trichosporon* spp. have been termed trichosporonosis, while the local infection in the case of white piedra is know as trichosporosis, but the use of these names is not recommended because they can induce confusion. The correct way of referring to these infections is "Pathology X associated to *Trichosporon* sp." [13].

**Corresponding address:**
Dr. María José Figueras
Unitat de Microbiologia, Facultat de Medicina,
Universitat Rovira i Virgili, Carrer Sant Llorenç 21,
43201 Reus, Tarragona, Spain
Tel.: +34 977 759 359; Fax: +34 977 759 322
E-mail: mjfs@astor.urv.es.



## White piedra

White piedra is characterised by white to light brown nodules that may surround the entire hair shaft. Nodules are soft and the fungal mass can easily be detached from the hair. Encapsulated arthroconidia or blastoconidia (2-8 µm on the long axis) with polygonal appearance when crowded together are observed under direct microscopic (10% KOH) examination [1-6]. Beigel was the first to notice the presence of the characteristic white piedra nodules on the hairs of a wig in 1885. The infectious agent was named (*Pleurococcus beigelii*) by Rabenhorst in 1867. In 1890 Behrend gave it the name *Trichosporon ovoides*. Later, in 1902, Wuillemin isolated the fungus from light-coloured nodules on human moustache hair and named it *Trichosporon beigelii* [3-6]. One factor that confuses the taxonomy of these agents is the synonymyzation of *T. beigelii* with *T. cutaneum* made by Diddens and Lodder in 1942. However both names have been used indistinctively in the literature to define this fungal entity. To confuse matters even further, the name *T. beigelii,* used for a long time by medical mycologists is not valid due to the priority of *T. ovoides,* while *T. cutaneum* is considered a valid toxonomic species [14]. For detailed information about the chronology of names see Guého et al. [14]. The taxonomy of the genus has recently suffered many changes, mainly due to the use of molecular biology technique [8,14,15] and several of the species accepted in the genera are now considered responsible for producing white piedra infection. Six species are considered human pathogens: *T. asahii* involved in systemic mycosis, *T. asteroides* and *T. cutaneum* both of which are associated with skin infections, the latter occasionally producing axillary white piedra, *T. inkin* nearly exclusively isolated from human crural area where it causes

white piedra, *T. mucoides* involved in systemic mycosis, onychomycosis and crural white piedra, and *T. ovoides* in capital white piedra and occasionally with superficial mycoses [1,8,9]. Despite these taxonomic changes, many recent papers still use the old name *T. beigelii*, to describe the agent responsible for white piedra, thus confusing the taxonomy of new isolates [10,11]. Identification to the species level is essential for correctly establishing which species are more frequently implicated in white piedra. This can be done by using morphological and biochemical criteria [1,2,8] antisera agglutination [8,16,17] and molecular techniques [14,18,19]. A recent extensive survey of crural white piedra in Equatorial Africa performed using these recent taxonomic criteria reported that the most frequently identified species were *T. inkin*, *T. mucoides* and *T. asahii*, while *T. cutaneum* was never isolated [20]. This peculiar hair infection was believed to occur particularly in tropical areas of South America (Brazil [21], Paraguay and Argentina). However, it has a wide geographic distribution and has also been described in Central and Eastern Europe [22-26], Japan [27], Africa [28], Kuwait [29,30], Thailand and the USA [21,31-33] and is now considered to be a cosmopolitan infection [32].

Scalp and moustache hair were described as the primary sites for white piedra but it was also later described in eyelashes, eyebrows, and axillary and perigenital hair [1,3]. This infection has also been described in horses, monkeys and dogs as well as in soil, water and decaying animal and vegetable matter [1-3,8]. The mode of infection remains unknown but high humidity is said to be a predisposing factor and in the case of genital hair, sexual transmission has been suggested [23,25,34]. The incidence of genital white piedra has been broadly investigated in asymptomatic patients visiting genitourinary clinics and carriage in men varied from 7.4% in heterosexuals in the United Kingdom [35], 23% in homosexual men in Denmark [26], 25.6% in Brazilian young male students [21] to up to 40% in young men with a variety of genital complains in the USA [32]. A 13% and 15.5% anal region carriage in homosexual men has been reported in Denmark and Sweden, respectively [23,26]. Carriage in women has been less investigated; its occurrence varies from 14% in the USA [32] to 18% in Equatorial Africa, where it is considered an autochtonous hair infection [28]. The frequency of isolation has always tended to be higher than the presence of nodules on hair demonstrated by microscopic examination [32]. These fungi may represent a part of the micriobiota of normal perigenital skin in males [36] although, they have also been considered as saprophytic with pathogenic potential [32].

White piedra is described as being commonly associated with bacteria [15,36]. Nodules on hair only produced by bacteria have been denominated trichomycosis axillaris, trichonodosis, trichonocardiosis or trichobacteriosis. The term trichomycosis is a misnomer because the causative agent is not a fungus but bacteria (micrococci) which were earlier considered coryneforms and are now considered nocardia.

Many authors have reported that white piedra only produce a lifting of the cuticular scales without cortex invasion [3,32]. Nevertheless, this capacity has been indicated in some cases [6,31,34,36] but it has never been deeply studied. It has been suggested that the synergistic action between *T. beigelii* and specific coryneform bacteria produces the cortex hair invasion [36]. An association of 11.8% between white piedra and bacteria has been described in a study carried out in Gabon [20].

## Black piedra

Black piedra is characterised by black, firmly adhered nodules that may or may not surround the entire hair and are harder and normally larger than those of white piedra. Crushed nodules mounted with KOH (10-20%) show a tight packed stroma of regularly arranged thick-walled fungal cells, as well as asci containing 2-8 single-celled, fusiform, slightly-curved ascospores (30-45 x 5.5-10 µm) with a single polar filament at each end [2].

Horta clearly differentiated black piedra from the white variety and considered that the fungus causing the first was a species of *Trichosporon* [37], later named *Trichosporon hortai* [38]. When ascospores were discovered in the nodules, the fungus was related to ascomycetes and the present name *Piedraia hortae* was given [39]. A second species, *P. quintanilla*, lacking ascospore appendages has been described from African subprimates but not from humans [1-3]. The infection is normally distributed in tropical climates [1-3,6,40-42] although it has also occasionally been reported in Europe [43]. Black piedra is usually found only on human scalp hair [3] and on the hairs of primates [42]. The source of the fungus and its natural habitat is still under discussion (i.e. water, soil, plants) and the species has been exclusively associated with this disorder [44].

Some authors have established that *P. hortae* is unable to penetrate the cortex of the hair shaft [3-5,40, 45,46], although others consider that it may be able to do so, but without extensive proliferation [6,47]. Until recently [44,48] the keratinolytic activity of this fungus had never been demonstrated, apart from the lifting and disruption of the cuticular layers.

## Differential diagnosis

Direct microscopic examination of infected hair in 10% KOH will enable a clear differential diagnosis of the two types of piedra, and also from eggs of pediculosis and trichomycosis axillaris (trichobacteriosis) with which it is sometimes confused. Hair with trichomycosis fluoresces under UV light but piedra does not. A faint glow on piedra would indicate an additional presence of bacteria [31,36]. White and black piedra can be distinguished from the colour of the modules, which are white to light brown, pale greenish or yellowish in the former and black in the latter, but also by the morphology of the fungal cells and presence or absence of ascospores and asci. However, it is worth mentioning that both types have been described together in mixed infections [3].

## Culture

*Trichosporon* spp. grow well in Sabouraud agar with cloranfenicol but they are inhibited by cycloheximide, which is included in the majority of dermatophyte culture media (Mycosel, DTM) [35,49]. Since ascospore production by *P. hortae* on Sabourand is rare and scant, special techniques using a transplantation and biotine have been described to stimulate the formation of ascospores [50].

## Treatment

The classical and most effective therapy for white and black piedra has been and still is the cutting or shaving of the hairs, a treatment recommended by the

American Academy of Dermatology [51]. Topical antifungals are also recommended and the application of clotrimazole cream alone [29] or after shampooing with ketaconazole proved to be a good therapeutic treatment for white piedra [30]. Nodules of white piedra have been found in cotton fibres of the underwear of a patient with crural white piedra, which indicates the need to disinfect clothes effectively to avoid reinfection [52]. Newer antifungal drugs such as terbinafine has shown promising results in black piedra infections [43,53]. We have suggested that the compact organization of the nodules of black piedra may impair successful treatment and explain why the disease may be so chronic [44,54]. It has been argued that in white piedra the fungus dwells on both the intra- and extrafollicular portions of the hair shaft and therefore shaving and topical antifungals will be inadequate in reaching intrafollicular nidus [32]. Intrafollicular growth, however, has never been demonstrated.

## Original definitions of the role of piedra on hair

Piedra are defined as superficial mycosis because they neither invade living tissue nor provoke an immune response by the host [1,2]. In addition they are considered to be epiphytic to hair substratum since they are thought to colonise hair on a living host only superficially. Piedra can also grow on the hair of dead hosts as well as on loose hair, which classifies them as necrotrophic, and lipophilic because they utilise lipids as their main nutrient source (*Piedraia, Trichosporon*). *Trichosporon*, in particular, has also been considered urophilic, due to its ability to occupy strongly acidic localizations when colonising pubic hair and its capacity to utilise urea and uric acid [1,2].

They do not provoke an inmune response by the host because they never invade the irrigated hair follicle. In our view, the passive role these fungi have on hair infections is not so evident. The fact that they select hair as substratum may be due to their keratinophilic affinity and keratinolytic potential. A review of the concepts that define keratinophilic and keratinolytic fungi are broadly discussed by Kunert in this book. Binding and adherence to the hair substratum is the first step to penetration and invasion. In a recent revision adherence was considered an important step for changing the characteristics of fungi (i.e. *Candida albicans*) from commensalism to pathogenicity [55]. Some authors had considered *T. beigelii* as a saprophytic fungus with pathogenic potential that may peacefully exist on apparently normal integument, as may other opportunistic pathogens such as *C. albicans* and S*taphylococcus aureus* [32].

The keratinolytic capacity of *P. hortae*, for which no other ecological niche has been found, has been fully demonstrated [44,48]. This is not the case for *Trichosporon* spp. due to the fact that the hair infections that they produce have been poorly investigated from this point of view.

## Cortex invasion in white piedra

As commented previously it is normally considered that cortex invasion does not happen in white piedra. An ultrastructural study on white piedra was not able to observe hair destruction [52]. Scrotal hair with concomitant infection by coryneform bacteria and white piedra showed hair cortex invasion, and a mutual synergetic relationship between these microrganisms is suggested [32,36]. Ellner et al. [36], reported that stimulation of bacterial growth in the presence of *T. beigelii* is mediated by the nutrients provided by the fungal metabolic by-products. In addition, proteolytic activity, which is one of the biochemical features of the coryneforms (the primary invader), may then facilitate hair cortex invasion by both microorganisms. To our knowledge this is the only study that illustrates and points out cortex invasion and destruction in white piedra [36]. However, they only show poorly detailed low magnification pictures (x1000) that show elevation of the cuticle by the invading fungus and hair cortex invasion by fungal cells and bacteria [36]. Ellner *et al.* [36] consider that because *T. beigelii* is usually inhibited by cycloheximide and does not grow readily on Mycosel or in dermatophyte culture media, the simultaneous growth of these microorganisms may have been masked on many occasions. That trychobacteriosis may play a prominent part in the genesis of white piedra has again been pointed out due to the relative high frequency of both manifestations [20]. In white piedra we observed nodules infected with bacteria but we could not ascertain whether they were coryneforms because the difficult taxonomy of this group hampered proper identification (Figure 1a-b). The bacteria were always observed at the periphery of the nodules, which suggests that they are not primary invaders (Figure 1b). The ability of corynobacteria to invade hair cortex has been illustrated ultrastructurally [56] although in other cases it could not be demonstrated [57]. From the literature available we should indicate that the ability of *Trichosporon* spp. to

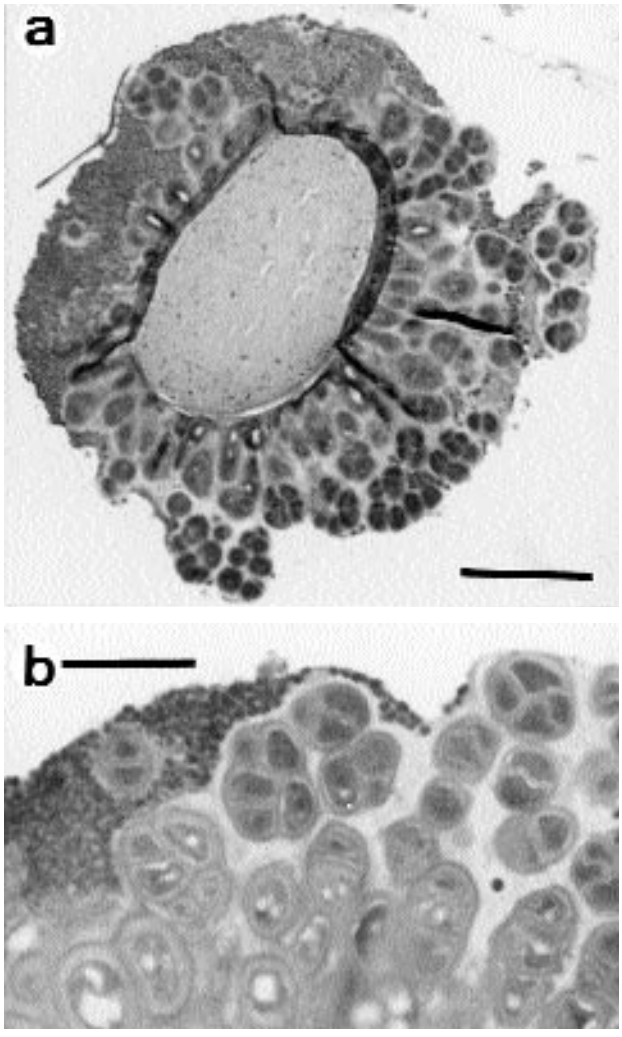


Figure 1 a-b. White piedra. a. Transverse section of a nodule. b. Detail of bacteria observed at the perifery of the nodule. Bars: a, 100 µm; b, 10 µm.

invade and destroy cortex hair keratin in white piedra still has to be fully demonstrated. However, current research in our laboratory on hairs diagnosed with white piedra shows hair cortex invasion in one of the patients (data not published). In addition, a broad study of keratinophilic fungi isolated from sand pits in Turin (Italy) [58] indicated that *Trichosporon* spp. should be considered a keratinophilic fungus according to Majchrowicz and Dominik [59] and Dominik et al. [60] who define them as fungi which are only able to use materials naturally associated with keratin or that result from its breakdown. This definition is further discussed by Filipello Marchisio in this book.

## Ultrastructural aspects of the keratinolytic hair degradation of black piedra

The initial invasion of human hair by *P. hortae* was achieved by producing eroding hyphae which force their way beneath or between the cuticular scales (Figure 2a-c), as described in other fungi [61,62]. However, the presence of perforating organs or fronds, as occurs with some keratinophilic fungi [61-65] has not been observed. In *P. hortae* the disruption produced on the hair cuticle has been attributed to the mechanical pressure produced by the fungal growth [3,5]. Similar effects have been described in other studies [62,66-68]. This was also observed in our case as some mechanical pressure may be suspected when the expansion of the fungus, at the cuticle (Figure 2d) and at the cortex level, continued at a faster rate than the lysis of the surrounding structures. However, the breakdown of keratin by *P. hortae* must result mainly from an enzymatic process, as normally occurs in other fungi [64,67,69-71]. This can be supported by the cytological signs of intense enzymatic activity, such as the presence of abundant mitochondria, observed in the fungal cells of *P. hortae*.

The keratinolytic sequence begins at the cuticle level with digestion of cementing materials, plasmalemma, endocuticle, exocuticle and A layer. Although this digestion scheme is normally followed in *P. hortae*, cuticular scale that appears entrapped within the developing nodule can be totally dissolved irrespective of the degree of keratinization of their structures, as shown in figure 2e-g. This ability has also been described for *Chrysosporium tropicum* and has been attributed to the ability of the fungus to concentrate the secretory enzyma-

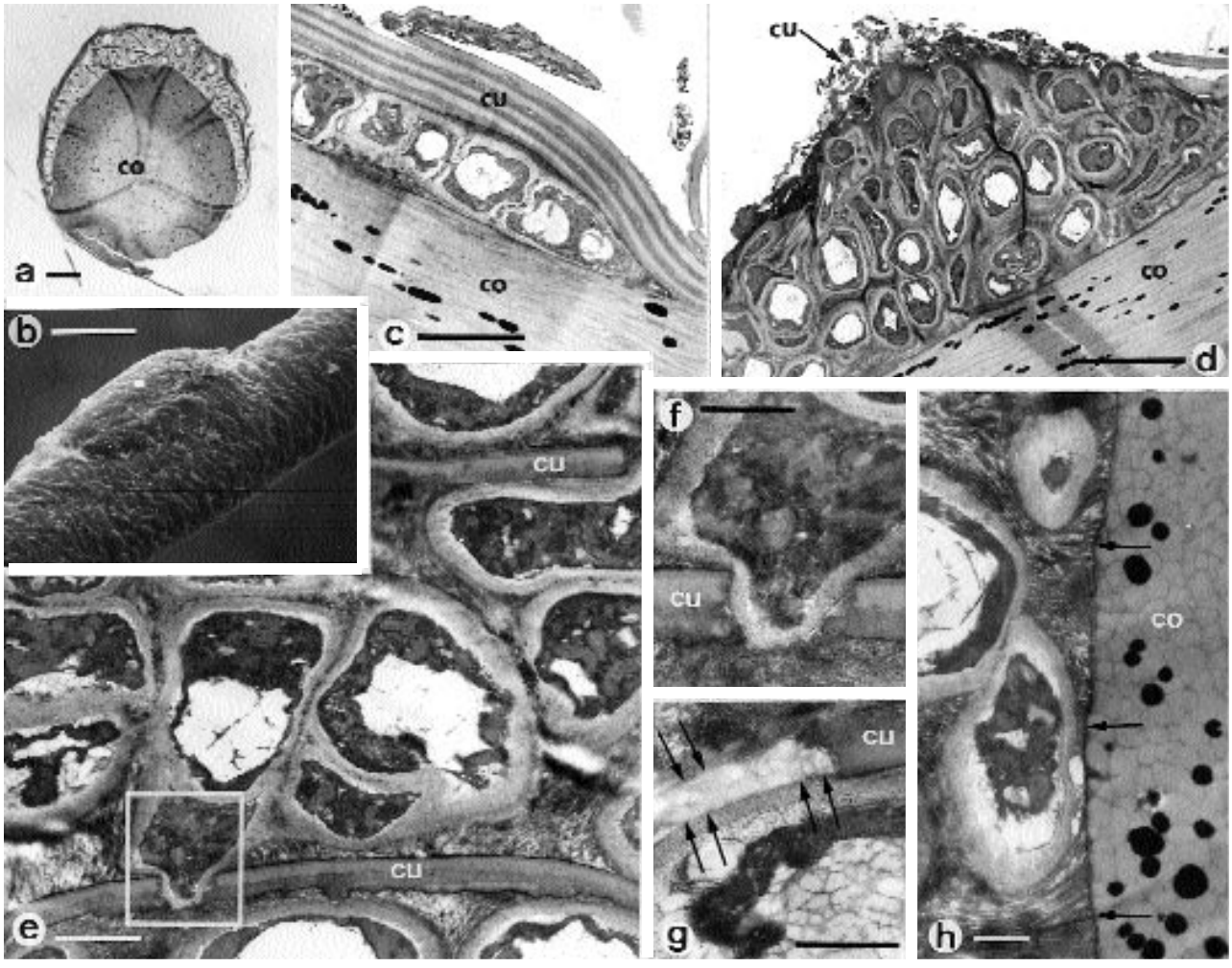


Figure 2 a-h. Initial stages of infection and cuticle degradation in black piedra. a,b. Hair with a young nodule, c. invasive hyphae growing parallel beneath the curticular layers, d. more developed nodule with remains of cuticular scales in the outer region, e. entrapped cuticular cells within the nodule pseudoparenchymatous structure, f. enlargement of indicated region of Fig. 1e; note the capacity of the fungus to penetrate the cuticular cells, g. detail of a disintegrating cuticular cell (arrows), h. contact region of the fungal pseudoparenchyma with the cortex, note the electron-dense border (arrows) and the abundant fibrillar extracellular material. CU, cuticle; CO, cortex; a, light microscopy; b, SEM; c-h, TEM; a,h, cross-section; c-g, longitudinal section. Bars: b, 50 µm; a,d, 10 µm; c, 5 µm; e,h, 1 µm; f,g, 0.5 µm.

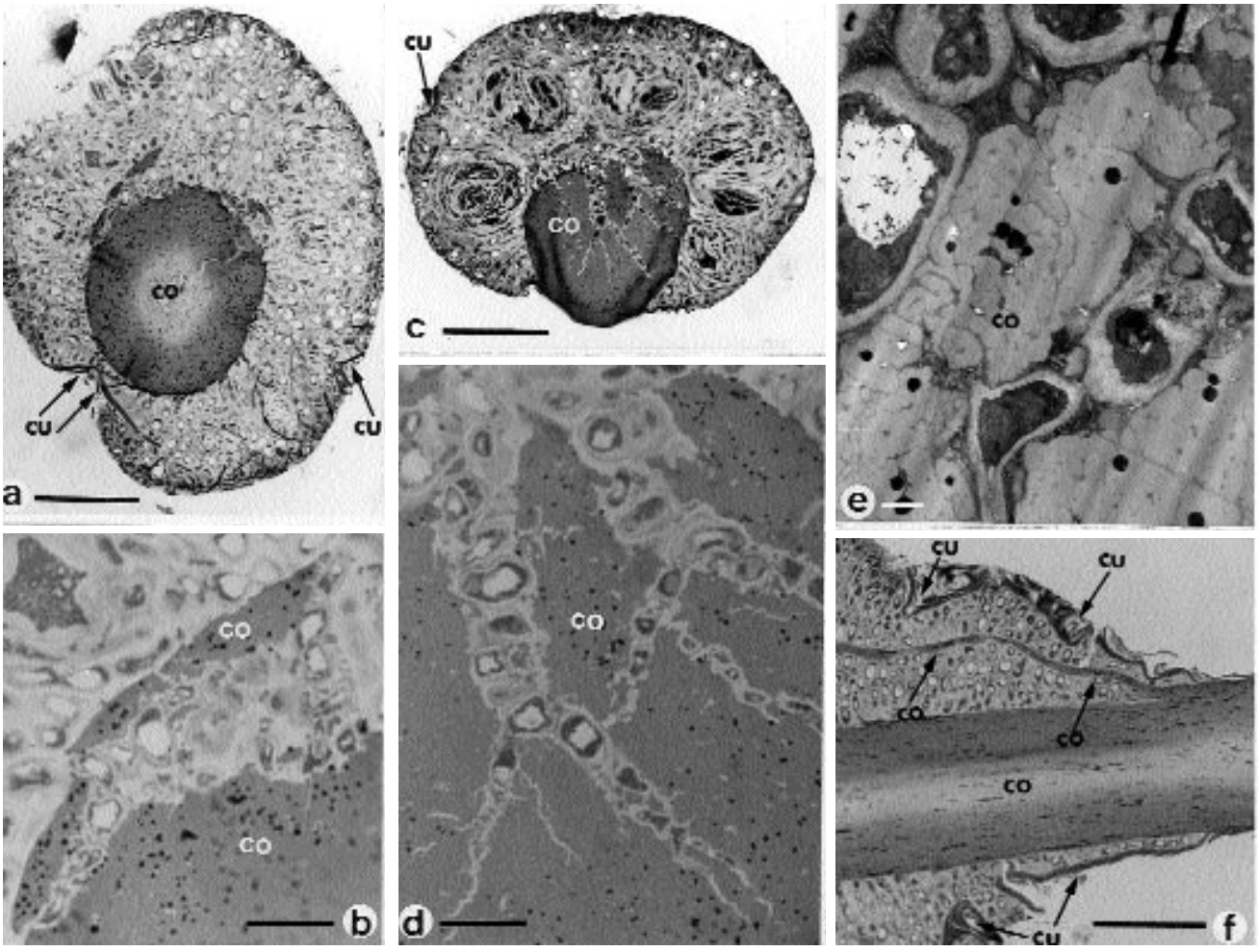


Figure 3 a-f. Patterns of cortex invasion in mature nodules. a. Type one: separation of the external outer cortex, b. detail, c. type two: active boring hyphae penetrating the cortex in several directions; note numerous loculi with asci, d, e. detail of the boring hyphae, f. longitudinal expansion of the fungus growing under the cuticular scales and between the cortex. Note in Figure 3 a,c,f remaining cuticular scales. a-d,f, Light microscopy; e, TEM; a-e, cross-section; f, longitudinal section. Bars: a,c,f, 50 µm; b,d, 10 µm; e, 1 µm.

tic activity at well-defined points [67]. The most resistant part of the cuticle is considered to be the outside layer of the exocuticle and especially the electron-dense layer bordering the external edge of the cell (A layer) because of its high content of cystine [66,72]. At the most advanced decomposition stages this layer forms ribbon-like structures [66,73]. In *P. hortae*, regions with completely disrupted cuticular scales show a total alteration even of this hard structure (Figure 2d-g).

In the cortex, digestion starts with the cementing substances and plasmalemma and follows with materials present between macrofibrils, microfibrils and finally their matrix [66]. Two cortex-disruption patterns were observed in fully developed black piedra nodules (Figure 3a-f). The first was parallel to the axis hair and was produced by fungal cells that grow by separating the external layers of the outer cortex (Figure 3a,b). The second type was produced by active boring hyphae that penetrated, more or less vertically, the axis hair, forming channels which were increased in size as the cortex compounds were slowly digested (Figure 3c-e). This type of invasion seemed to appear on nodules that did not completely surround the infected hair. However, intermediate situations existed, as did infected hairs whose cortex was not affected. No lytic spaces around the invading hyphae were observed, which may indicate the less diffusible activity of the enzymes or that the hyphae were unable to dissolve further all the compounds produced during the keratinolytic breakdown. The natural self-degeneration of the fungal cells involved in the breakdown of hair in the later stages of hair destruction observed with other fungi was not observed in *P. hortae*. This could be due to the stromatic compact organisation of the fungal nodule that may behave as a resistant structure with a slow development, so that small amounts of keratin seem to be sufficient to guarantee the long survival of the fungus. This may also explain why infected primate pelts conserved at museums have surprisingly well preserved fungal structures and why the disease persists. This persistence of the fungus on hair may also be facilitated by the compact organisation of the nodule when it completely encircles the hair or by the development of active boring hyphae that form channels in different directions within the cortex. These hyphae may act as roots that anchor the nodules firmly in the hairs.

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