available online at www.studiesinmycology.org
doi:10.3114/sim.2008.61.13

STUDIES IN MYCOLOGY 61: 131–136. 2008.

Coniosporium epidermidis sp. nov., a new species from human skin

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Abstract: *Coniosporium epidermidis* sp. nov. is described from a superficial skin lesion with blackish discolouration in an 80-yr-old Chinese patient. The species produces dark, thick-walled, inflated, reluctantly liberating arthroconidia without longitudinal septa. Sequences of the ribosomal operon, as well as of the translation elongation factor 1-α support its novelty. The species is found in a lineage basal to the order *Chaetothyriales*, amidst relatives from rock, but also species repeatedly isolated from human skin and nails and eventually causing mild cutaneous infections. *Coniosporium epidermidis* is consistently found on humans, either asymptomatic or symptomatic. The species indicates a change of life style towards human pathogenicity, which is a recurrent type of ecology in derived *Chaetothyriales*. Superficial and cutaneous infection by melanized fungi is a new category in dermatology.

Key words: Black yeasts, *Coniosporium*, superficial mycosis, taxonomy.
Taxonomic novelties: *Coniosporium epidermidis* D.M. Li, de Hoog, Saunte & X.R. Chen, sp. nov.

INTRODUCTION

In recent years the clinical significance of melanized fungi involved in cutaneous infections has been underlined (Badali *et al.* 2008a). Several of the species concerned, although causing relatively mild infections, are regularly encountered in dermatological specimens, but usually discarded as purported contaminants. Some species, such as *Phialophora europaea* de Hoog *et al.* and *Cyphellophora laciniata* de Vries, however, are recurrently observed on humans, and their environmental niches thus far have remained unknown (de Hoog *et al.* 2000). We here report on a species that originated from the skin of an 80-yr-old male patient who manifested with a 3 yrs history of black bilateral maculae on his feet, with scales, maceration, and fissures. The infection was caused by a *Coniosporium*-like fungus that could not be identified with any of the known species and is therefore introduced here as a new taxon.

The genus *Coniosporum* is considered to comprise environmental fungi forming black spots or patches on plant leaves, bamboo surface, rotten wood, and recently particularly on rock surfaces (Hyde *et al*. 2002, De Leo *et al.* 1999, Sterflinger *et al*. 1997, 2001). Species have black, velvety colonies on the natural substrate, and are characterized microscopically by thick-walled, heavily pigmented arthroconidia with subsequent meristematic development. This report concerns the first human infection caused by a *Coniosporium* species. *Coniosporium* is not among the recognized human pathogens in dermatology. Several melanized fungi have been reported cause mild cutaneous infections, e.g. *Cyphellophora laciniata* de Vries (1962), *Phialophora europaea* de Hoog *et al*. (2000b), and *Cladophialophora saturnica* Badali *et al*. (2009). Such fungi are encountered fairly regularly in samples from human skin and nail (de Hoog *et al*. 2000a). A new dermatological category may be concerned, which will be introduced in this paper.

MATERIALS AND METHODS

Isolation

Clinical specimens were scraped with a scalpel from superficially sterilized blackish skin lesions. A skin biopsy was performed on the black lesion and histological specimens were stained with hematoxylin-eosin. Samples of skin flakes were plated on Sabouraud's glucose agar (SGA) with chloramphenicol and incubated at 27 °C. Strain T22 (= CBS 120353) was isolated from specimens of the first visit of the patient. Another isolate was recovered one year later from the same patient and turned out to be identical by sequence data. Studied strains of the same species included for comparison were isolates encountered during analysis of routine dermatological specimens from Denmark, and an isolate from ant garbage from Brazil. Related strains studied are listed in Table 1.

Morphology

Strains were transferred to malt extract agar (MEA), potato dextrose agar (PDA), cormeal agar (CMA), oatmeal agar (OA) and Czapek agar (CZA) and incubated at 25 °C and 37 °C for at least 4 wk under alternate near-ultraviolet light for growth rate determination and phenetic description of colonies. For study of microscopic morphology strains were point-inoculated on PDA. Blocks of agar of approximately 1 × 1 cm were excised aseptically on sterile microscope slides. Blocks were inoculated, covered with sterile cover slips and incubated in moist chambers for 14 d at 27 °C. Structure and branching pattern of conidiophores were observed at magnifications ×100, ×200 and ×400 in intact slide cultures under the microscope without removing the cover slips from the agar blocks. For higher magnifications, cover slips were removed and mounted in lactic acid with aniline blue.

Sequencing

Approximately 0.1 g of fungal material was transferred to a 2-mL Eppendorf tube containing a 2:1 (w/w) mixture of silica gel and Celite (silica gel H, Merck 7736/Kieselguhr, Celite 545, Machery, Merck, Amsterdam, The Netherlands); DNA was extracted according to methods described previously (Li *et al*. 2008). Amplifications were done with primers ITS1 and ITS4 (for rDNA Internal Transcribed Spacer ITS), NS1, BF83, OLI1, BF963, BF1438 and NS24 (for rDNA Small Subunit nucSSU), D1/D2 (for rDNA Large SubUnit nucLSU), and EF1-728F and EF1-986R (for Translation Elongation Factor 1-α *EF*1α). PCR was performed in 50 µL volumes of a reaction mixture containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2 \cdot 6H_2O$, 0.01 % gelatin, 200 mM of each deoxynucleotide triphosphate, 25 pmol of each primer, 10−100 ng rDNA, and 0.5 U Taq DNA polymerase (Bioline, GC Biotech, Alphen a/d Rijn, The Netherlands), as follows: 95 °C for 4 min, followed by 35 cycles consisting of 94 °C for 45 s, 52 °C for 30 s, and 72 °C for 2 min. Amplicons were cleaned with GFX columns (GE Healthcare, Sweden). Sequence PCR was performed as follows: 95 °C for one min, followed by 30 cycles consisting of 95 °C for 10 s, 50 °C for five s, and 60 °C for two min. DNA was purified with Sephadex G-50 Superfine. Purified amplicons were then sequenced on both strands using the same primers described above. BigDye terminator cycle sequencing Ready Reaction kits (Perkin Elmer Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) were used according to the manufacturer's instructions and DNA was sequenced using a DYE-ET terminator.

Sequence analysis and taxonomy

Sequences were compared in GenBank and using a research database available at the Centraalbureau voor Schimmelcultures Biodiversity Centre (CBS), Utrecht, The Netherlands. Alignment

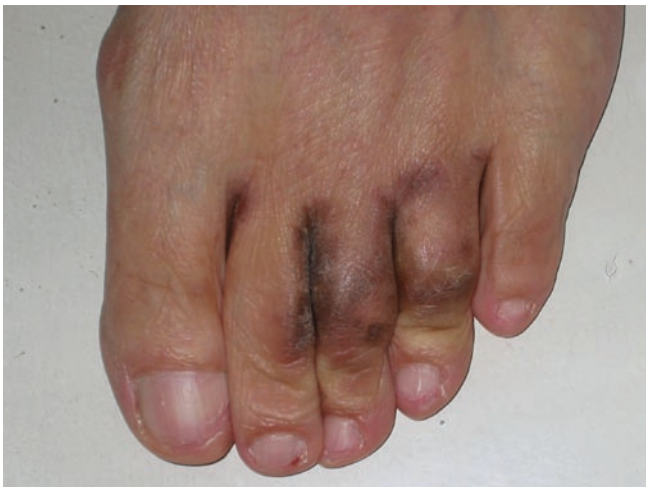

Fig. 1. Blackish discoloured skin of toes and toe webs with scaling.

was done in a database using BioNumerics software v. 4.61 (Applied Maths, Kortrijk, Belgium). SSU sequences were aligned with the ARB beta-package (v. 22-08-2003) developed by Ludwig *et al.* (2004). A distance tree of *Coniosporium epidermidis* and allied black fungi based on the completed ITS 1-2 domain including the 5.8S rDNA gene were reconstructed using neighbor-joining algorithm with Kimura 2 correction with 100 bootstrap replications in Treefinder.

RESULTS

Mycology

A skin biopsy performed on the black lesion (Fig. 1) and histological specimens stained with hematoxylin-eosin (Fig. 2) showed hyperkeratosis and acanthosis. Numerous hyphae and swollen cells were observed in the stratum corneum (Fig. 2). Pigmented hyphae and loose cells were displayed in the entire layers of epidermis, predominated among the low layers. Cells also penetrated the basal membrane to the dermis. Direct examination of skin scrapings with KOH was positive for pigmented arthroconidia and dark-walled hyphae. The disease was considered to be an infection.

At primary isolation, isolate CBS 120353 grew slowly with 8 mm/wk. Colonies on SGA were convex with papillate surface. Obverse and reverse were black, while colonies on MEA, OA, CMA, CZA and PDA were velvety with dark brown to olive obverse. Growth was stimulated under near-UV light; 37 °C was tolerated. Subcultures initially were cream-coloured, smooth and turned black within a wk; this phenomenon disappeared after several transfers. Hyphae were septate, olivaceous-black, forming reluctantly disarticulating arthroconidia with transverse but without longitudinal septa (Fig. 3). Cells were pigmented, thick-walled, and maturated meristematically, the mother cell wall frequently rupturing in an irregular fashion. With time, monilioid conidia (Fig. 3h, i) were predominant and occasional chlamydospores occurred.

Molecular data

1743 bp of the rDNA SSU gene were sequenced of strain CBS 120353 (data not shown). Phylogenetic analysis of aligned sequences revealed close relationship with species in *Cladophialophora, Exophiala, Phialophora, Rhinocladiella, Fonsecaea* and *Capronia*, which all are members of the order *Chaetothyriales*. However,

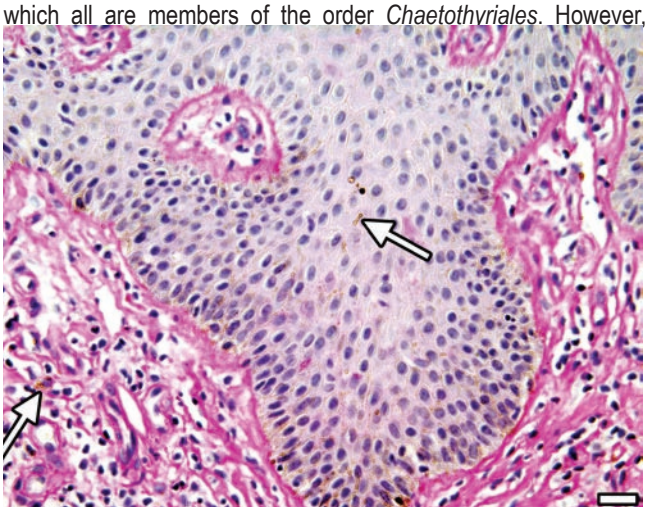

Fig. 2. Skin biopsy stained with HAE; some fungal elements are visible (arrows). Size bar = 5µm.

nearest neighbours were *Coniosporium perforans* Sterflinger (CBS 665.80), and *C. apollinis* Sterflinger (CBS 352.97). Of the LSU domain, 616 bp were sequenced. Nearest neighbour at 97 % similarity was a species published by Crous *et al.* (2007) in a tree as '*Exophiala* sp. 3', CPC 12173 = EU035422.

Length of ITS domain of CBS 120353 was 541 bp. ITS rDNA sequences compared in a dedicated black yeast data base maintained at CBS and containing about 11,000 entries revealed no

Table 1. Isolation data of examined strains.

Name	Accession no.	Country	Source	GenBank
Antarctic black fungus	CCFEE 5323	Antarctic	Thallus of *Lecanora* sp.	FJ392866
	CCFEE 5314	Antarctic	Thallus of *Xanthoria elegans*	FJ392865
	CCFEE 5324	Antarctic	Thallus of *Acarospora flavocordia*	FJ392867
Coniosporium apollinis	CBS 100213	Greece	Rock	AJ244271
	CBS 100218	Greece	Marble	AJ244273
	CBS 109867	Greece	Marble	
	CBS 100216	Spain	Rock	AJ244272
	CBS 109860	Spain	Rock	
	CBS 109865	Italy	Rock	
Coniosporium epidermidis	CBS 123233	Denmark	Hand, female	
	dH 17028	Denmark	Axilla, male	
	CBS 123466	China	Nail with onychomycosis	
	CBS 120353 (T)	China	Skin infection	EU730589
	CBS 120388	Denmark	Toenail, female	
	CBS 123279	Denmark	Toenail, female	
	dH 17006	Denmark	Toenail, female	
	CBS 123261	Denmark	Toenail, male	
	dH 17086	Denmark	Toenail, male	
Coniosporium perforans	dH 17016	Denmark	Axilla, female	
	CBS 109861	Italy	Marble	
	dH 16682	Denmark	Nail, male	
	CBS 885.95 (T)	Greece	Marble	
Coniosporium species	CBS119726	Italy	Stone monument	
	dH 14071	Australia	Cattle	
	dH 16979	France	Chronic nasal oedema	
	dH 14084	Italy	Marble monument	
	dH 14085	Italy	Rock monument	
	CBS 109864	Italy	Rock	
	CBS 109866	Italy	Rock	
	CBS 665.80	Italy	Rock	
Cryptoendolithic fungus	CCFEE 457	Antarctic	University Valley	
Exophiala placitae	CPC 13707	Australia	*Eucalyptus placita*	EU040215.1
Exophiala species	CPC 12172	Canada	*Prunus* sp.	
	CPC 12173	Canada	*Prunus* sp.	
	CPC 12171	Canada	*Prunus* sp.	EU035420
Meristematic fungus	CBS119729	Italy	Stone	
Phaeococcomyces catenatus	Det M175	Netherlands	Nail	
	CBS 650.76 (T)	Switzerland	Air	AF050277
	dH 11392		Unknown	
	dH 14721	Austria	Bloodplasma	
	TRN4	Spain	Rock surface	AY843222
Phialophora europaea	CBS 656.82	France	Nail	FJ489612
	dH 12320	Germany	Nail	
	CBS 101466 (T)	Netherlands	Skin scales	
Sarcinomyces petricola	CBS 726.96 (T)	Guinea	Dung of cow	FJ489613
	CBS 600.93	Greece	Pentelic marble	
	CDC 2008006858	USA	Scalp rash, male	
Uncultured ascomycete	AM901753	Finland	Indoor dust	AM901753

Abbreviations used: CBS = CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CCFEE = Culture Collection of Fungi from Extreme Environments, Viterbo, Italy; CDC = Centers for Disease Control and Prevention, Atlanta, U.S.A.; CPC = culture collection of Pedro Crous, housed at CBS; dH = G.S. de Hoog working collection; TRN = Tino Ruibal working collection.
T = ex-type culture.

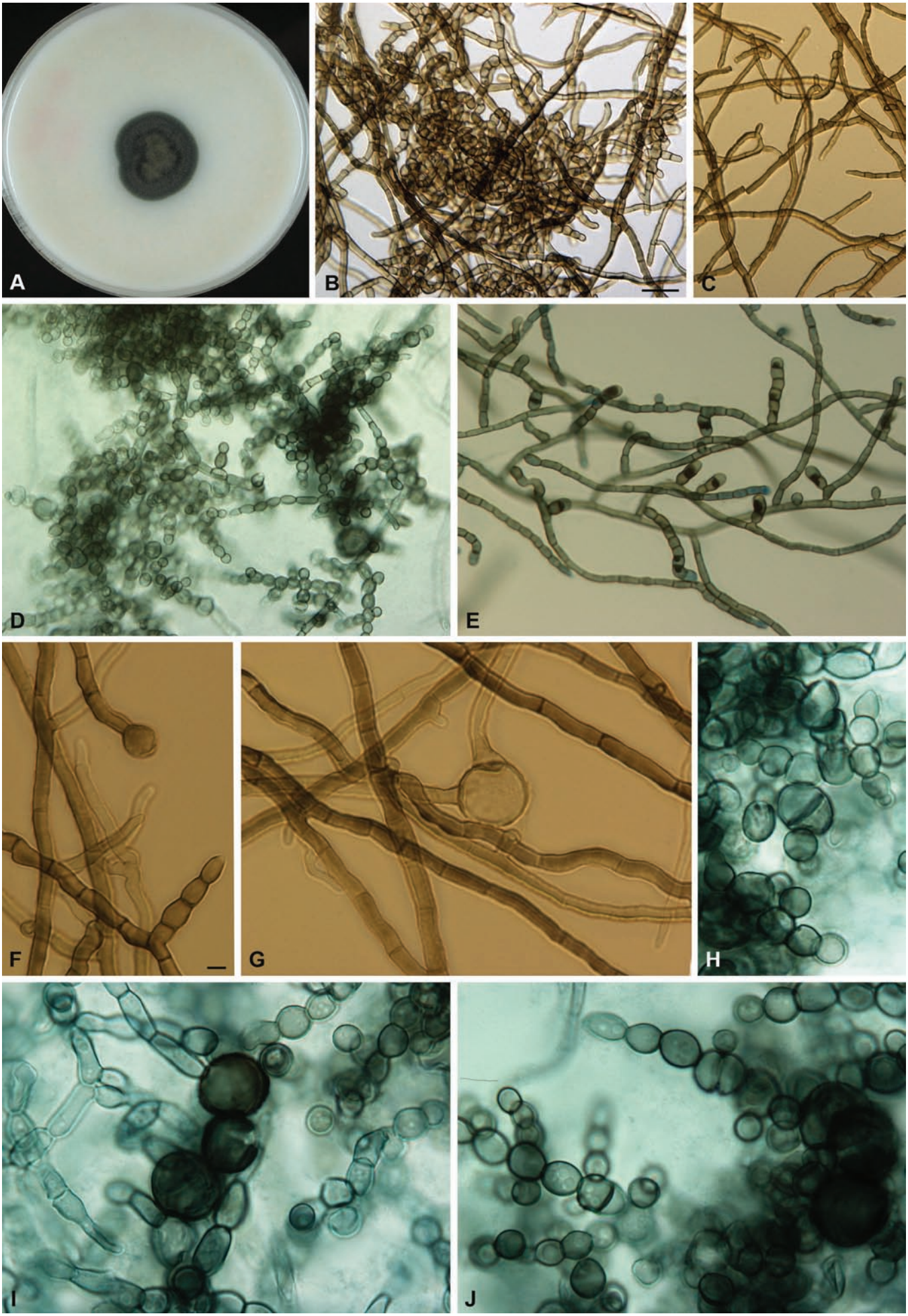

Fig. 3. *Coniosporium epidermidis*, CBS 120353. A. Colony on OA (3 wks). B, C. Elongated and monilioid hyphae, anthroconidia; D, E. Conidial chains; F–J. Mature conidial chains and large conidia with transverse septa. Scale bars 5 µm (B–E); 1 µm (F–J).

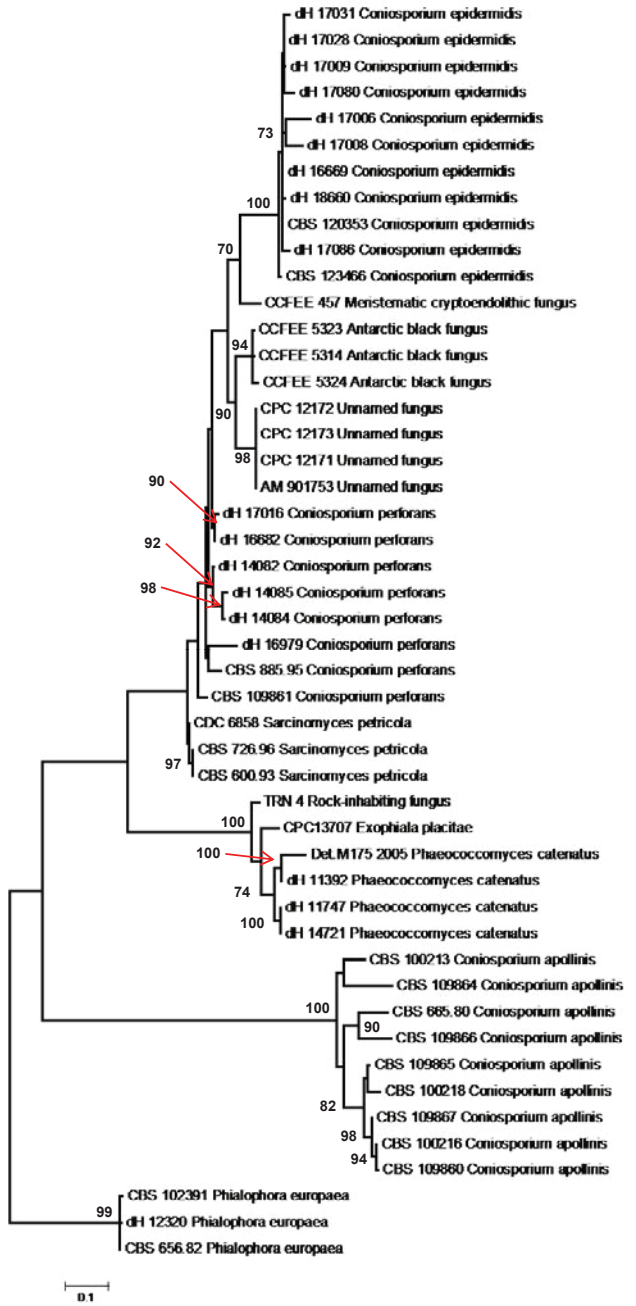

Fig. 4. Phylogenetic tree of *Coniosporium epidermidis* and allied black fungi based on the completed ITS 1−2 domain including the 5.8S rDNA gene, generated with the TREEFINDER package using the Neighbor-joining algorithm and Kimura correction. The tree was subjected to 100 bootstrap replications; *Phialophora europaea*, CBS 656.82 was selected as outgroup.

close match with any known species. Alignment was only partially confident. Among the nearest neighbours were the known rock-inhabiting species *Coniosporium perforans* and *C. apollinis*, as well as a number of undescribed rock-inhabiting species prevalently from the Mediterranean and from the Antarctic (Table 1). '*Exophiala* sp. 3' of Crous *et al.* (2007) was also close; the ITS of this species proved to be identical to a hyperparasitic '*Coniosporium* sp.', AM901753 published by Harutyunyan *et al.* (2008). In addition, strains from human skin and nail samples were involved (Table 1). Identity was found with a group of strains from dermatological skin and nail samples from Denmark, as well as with an environmental sample from Brazil; all these strains either were morphologically identical to CBS 120353, or consisted of sterile melanized hyphae. These strains were therefore regarded to represent a hitherto undescribed taxon, which is introduced below.

Coniosporium epidermidis D.M. Li, de Hoog, Saunte & X.R. Chen, **sp. nov**. – MycoBank MB512506, Figs 3, 5.

Coloniae primum fuscae, effusae, deinde elevatae, velutinae vel cottoneae, griseo-olivaceae; reversum olivaceo-nigrum. Coloniae in agaroso maltoso, agaro PDA, vel agaro farina avenae confecto (OSD) dicto 25 °C 10−15 mm diam post 28 dies. Mycelium immersum vel superficiale, ex hyphis ramosis, septatis compositum, olivaceo-nigrum vel brunneum. Conidiophora vix distinguenda, ramose vel simplicia, terminalia vel intercalaria. Conidia singula, primaria in apice conidiophori, ellipsoidea vel subglobosa, levia, 2-3 µm diam. Mycelium torulosum praesens. Teleomorphosis ignota.

Holotype: dried culture in CBS herbarium (CBS-H-20167); ex-type strain CBS 120353 = T22, isolated from nigramacula, superficial infection of the feet of a 80-yr-old male patient, China, D.M. Li. Additional strains listed in Table 1.

The following description is of CBS 120353 on PDA after 28 ds incubation at 25 °C.

Colonies effuse, becoming raised, attaining 10-15 mm diam, velvety to fluffy, black, blackish-brown to greyish olivaceous; reverse olivaceous-black. Mycelium superficial, regularly and densely septate, profusely branched at nearly right angles, olivaceous-black or dark brown, smooth-, or occasionally rough-walled. Cells gradually swelling at maturation up to 3-8 µm diam; cell walls very thick at maturity. Conidia formed by liberation of arthric cells, swelling to become ellipsoidal or nearly spherical, smooth-walled, mostly 2-3 µm diam, up to 8 µm wide, then frequently the mother cell wall remaining visible on the daughter cell. Truly muriform cells absent. Teleomorph unknown. Cardinal temperatures: optimum 27 °C, maximum 37 °C.

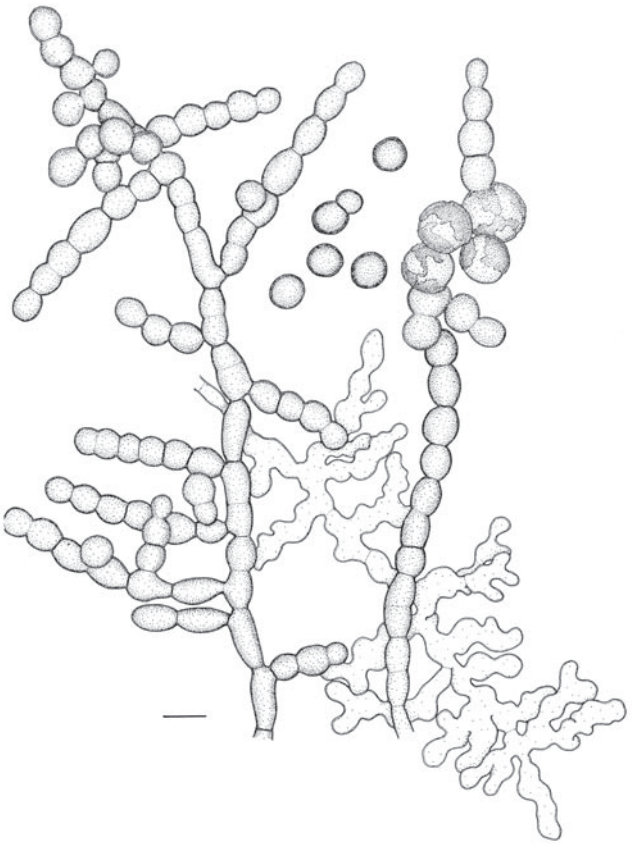

Fig. 5. Microscopic morphology of *Coniosporium epidermidis*, CBS 120353; slide culture on SGA, 28 d.

DISCUSSION

Coniosporium classically includes black, slow-growing anamorphs, most of which are plant colonizers (Hyde *et al.* 2002). Species are characterized by pigmented anthroconidia, and tend to develop meristematically into chains (Ellis 1971). The mother cell wall may eventually rupture during development, leading to irregular cell wall ornamentation (Ellis 1976).

Coniosporium epidermidis was isolated from a tinea nigra-like skin infection on the foot. Tinea nigra, characterized by brown to black superficial macules, is a strictly asymptomatic colonization of dead epidermis (Schwartz 2004, Bonifaz *et al*. 2008) caused by a halophilic member of the order *Capnodiales*, *Hortaea werneckii* (Zalar *et al*. 1999). In contrast, pathological slides of *C. epidermidis* clearly showed that the fungus grew into all layers of the epidermis, and some cells penetrated down to the basal membrane reaching the superficial dermis. Thus the present species causes a real disease process, as was also observed with the tinea nigra-like infection caused by *Cladophialophora saturnica* Badali *et al*. (2008a), another member of *Chaetothyriales*. The optimal growth temperature of *C. epidermidis* at 27 °C (maximum 37 °C) is comparable to that of true pathogens of the skin, the dermatophytes, ranging between 25 and 35 °C (Weitzman & Summerbell 1999). We thus conclude that ordinal relationships predict opportunistic potential. In the course of the present study *Coniosporium epidermidis* was repeatedly isolated from routine dermatological samples in Denmark. It is supposed that the fungus may be common in cutaneous samples, but is generally discarded as a contaminant. We therefore recommend to pay more attention to melanized fungi occurring on skin and nails, and to establish their precise role in pathology.

With SSU rDNA, the black fungus recovered from affected human skin appeared to be an undescribed species, located within a group of species causing mild cutaneous infections, such as *Phialophora europaea* and *Cyphellophora laciniata*. The group was basal to the order *Chaetothyriales,* an order having *Capronia* teleomorphs and being notorious for containing numerous opportunists (Badali *et al*. 2008b). In the corresponding ITS tree (Fig. 4) the group showed considerable diversification among species. The similarity of *C. epidermidis* to the nearest described species, *Coniosporium perforans,* was less than 91 %. This species is, however, a colonizer of rock and monuments in the Mediterranean Basin (Sterflinger *et al*. 1997), as is the case in the majority of taxa in this group. The rock-inhabiting species were attributed to the genus *Coniosporium*, although the generic type species, *C. olivaceum* Link (Ellis 1971), has as yet not been redefined according to modern standards.

Sterflinger *et al.* (1997) supposed that the *Coniosporium*-clade of *Chaetothyriales* was entirely rock-associated. However, with recent additions to this group we notice that it contains several undescribed taxonomic species from human skin and nail samples. In addition, many of the species described as having a rock-inhabiting life-style, such as *Sarcinomyces petricola* Wollenzien & de Hoog, *Coniosporium perforans* and *Phaeococcomyces catenatus* (de Hoog & Hermanides-Nijhof) de Hoog, can also be found on human skin (Table 1). A similar dual ecology was found earlier in the unrelated meristematic fungus *Catenulostroma abietis* (Butin & Pehl) Crous *et al.* (Butin *et al.* 1996). We suppose that these predominantly meristematic skin colonizers are taken up from the environment where they live as oligotrophs on rock, leathery plant leaves and other relatively inert surfaces. They are likely to display a similar oligotrophic character on human skin, and thus probably behave as commensals rather than pathogens. Nevertheless occasional mild infections may occur, such as the ones observed in *Cladophialophora saturnica* (Badali *et al*. 2008a) and the present fungus.

ACKNOWLEDGEMENTS

This work was supported partly by grants from the National Natural Science Foundation of China (C30570003), the China Exchange Programme of the Netherlands Academy of Sciences. D. Attili (Rio Claro, Brazil) is acknowledged for donating a strain from an ant hill. We thank Xin Lian for assistance in sequencing.

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