Antonie van Leeuwenhoek **83:** 351–360, 2003.

A new meristematic fungus, *Pseudotaeniolina globosa*

F. De Leo[1], C. Urzì[1] and G.S. de Hoog[2,3,*]
[1]*Dipartimento di Scienze Microbiologiche, Genetiche e Molecolari, Università di Messina, Salita Sperone 31, I-98166 Messina, Italy;* [2]*Centraalbureau voor Schimmelcultures, P.O. Box 85167, NL-3508 AD Utrecht, The Netherlands;* [3]*Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands;* [*]*Author for correspondence*

Received 10 July 2002; accepted in revised form 21 October 2002

Key words: Black yeasts, *Coniosporium*, Epiphyte, MCF, Meristematic fungi, Rock-inhabiting fungi, Taxonomy, *Trimmatostroma*

Abstract

A new species of *Pseudotaeniolina*, a genus of anamorphic, melanized fungi with meristematic development, is described. The species is compared to morphologically similar taxa among which are *Trimmatostroma* and *Coniosporium*. Its novelty is supported by SSU (small subunit) and ITS (internal transcribed spacer) rDNA sequence data.

Introduction

Meristematic fungi are characterized by isodiametric cell wall expansion concomitant with ongoing cytokinesis and arbitrarily oriented septation (Zalar et al. 1999a). Additional polarized growth featuring hyphae or budding cells may or may not be present. Bipolar growth is occasionally observed (Butin et al. 1996). Most species grow slowly, have very thick cell walls and are densely melanized. The fungi are often found as epilithic or epiphytic saprobes on exposed surfaces such as desert rock, outdoor statues or leathery plant leaves; or they may thrive in Antarctic rock or in hypersaline coastal ponds. Melanization and meristematic growth are therefore regarded to render the fungus' phenotype suitable for survival under extreme conditions, including low and high temperatures, low water activity and intense solar irradiation (Sterflinger 1998). In the Chaetothyrialean species *Cladophialophora carrionii* (Trejos) de Hoog et al., a meristematic ecotype that occurs inside dry cactus spines (Zeppenfeldt et al. 1994) is also produced when the fungus is traumatically inoculated into human tissue. This tissue form, known as the muriform cell, is the characteristic hallmark of a distinctive skin disease, chromoblastomycosis (Matsumoto et al. 1984). Meristematic growth seems to be suitable for divergent types of environmental stress including those encountered during the infection of warm-blooded animals.

About 25 genera of melanized meristematic, surface-inhabiting fungi have been described. Many of them have few distinctive morphological features, but others exhibit striking features when observed on the natural substrate. Some show a bewildering phenetic polymorphism (Figueras et al. 1996; Yoshida et al. 1996). Over the last decades, several genera of epi-endolithic fungi have been revealed on and in native rock (Staley et al. 1982) and stone monuments, especially in the Mediterranean Basin (Sterflinger et al. 1999; Urzì and Realini 1998; Urzì et al. 2000). These species entirely lack structures enabling identification. *In situ* on rock they mostly consist of extremely small clumps of black cells and have therefore been referred to as microcolonial fungi (MCF; Staley et al. 1982). In culture some are preponderantly single-celled and have been classified in *Sarcinomyces* (Wollenzien et al. 1997), while others produce muriform cells and are assigned to *Trimmatostroma* (Zalar et al. 1999b) if they produce branched conidial chains, or to *Coniosporium* (Sterflinger et al. 1997; De Leo et al. 1999) if they produce unbranched chains (Ellis 1971, 1976). A

superficially similar, monotypic genus, *Pseudotaeniolina*, has been introduced by Crane and Schoknecht (1986) for species from plant material. In these species, meristematic development is followed by arthric secession leading preponderantly to the formation of single cells.

Only very few species of MCF have as yet been cultured. Judging from ribosomal sequence data, the MCF are polyphyletic, being anamorphs of Chaetothyriales and Dothideales (Ascomycetes) (Sterflinger et al. 1997). Currently available ribosomal sequences have revealed that this subdivision does not correspond with morphological generic circumscriptions. Facing the lack of congruity between morphology and phylogeny, we have chosen in the present article to take broad phylogenetic lines into consideration, but to refrain from introducing a new taxonomic system for the meristematic fungi. For *Pseudotaeniolina*, neither cultures nor sequence data are available. The aims of this study are to describe a new species of *Pseudotaeniolina* on the basis of phenetic and genetic data and to compare this entity with existing meristematic taxa.

Material and methods

Strains

Strain MC 769 was isolated from a sandstone sample taken from the outside wall of the church of ''Santa Maria di Mili'' (Mili San Pietro, Messina, Italy; Plate 1). The surface of the stone was characterized by the presence of green patina attributed to the growth of green chlorococcal algae associated with a few sporogenous bacteria and numerous black fungi. Samples were processed according to recommendations of the Italian Normal Commission (Commissione Normal 1990). The rock sample was powdered in a mortar and suspended (1:10) in saline with 0.001% Tween 80 and stirred continuously for 1 h. One ml-volumes of suspension were inoculated in duplicate on Dichloran Rose Bengal Chloramphenicol agar (DRBC; King et al. 1979; Urzì et al. 1992) and incubated at 28 °C for one month. Colonies were transferred to culture plates with Potato Dextrose Agar (PDA, Oxoid) and incubated at 25 °C for one month. Strains for comparison were selected on the basis of morphological and sequence similarities. Strains are listed in Table 1.

Morphology

Cell-morphology was studied using light and phase-contrast microscopy. Daily growth rates and morphology of colonies were recorded on PDA, 2% Malt Extract Agar (MEA, Oxoid), Oatmeal Agar (OA), and Czapek Dox Agar (CzA, Oxoid) in culture plates incubated at 25 °C. Hyphal maturation and conidiogenesis were studied in slide cultures on MEA; slides were mounted in lactophenol with or without cotton blue.

Molecular biology

DNA isolation. DNA extraction was done according to Gerrits van den Ende and De Hoog (1999). In brief, about 1 cm^2 mycelium of 30-day-old cultures was ground and extracted in 200 μl CTAB (cetyltrimethylammonium bromide) buffer with 500 μl chloroform. DNA was precipitated in 96% ethanol at −20 °C. The pellet was washed with cold 70% ethanol. After drying at room temperature, DNA was resuspended in 97.5 μl TE-buffer with 2.5 μl RNAse (20 U ml^{-1}) and incubated for 5 min at 37 °C.

DNA amplification. The internal transcribed spacer (ITS) rDNA was sequenced for each strain; the small subunit (SSU) was sequenced for one strain of each species recognized. PCR was performed in 100 μl volumes of a reaction mixture containing 60 μl distilled water, 10 μl PCR buffer, 20 μl N-buffer, 2 μl of each primer, 2 μl Amplitherm DNA polymerase and 4 μl fungal DNA. Primers NS1, NS24, Oli1, Oli5, Oli9, Oli10, BF951, BF963, Oli2, Oli3, Oli13, Oli14, BF 1419, BF 1438, Oli15, V9G, ITS1, ITS4, ITS5 and LS266 (De Hoog et al. 2000) were employed. Forty amplification cycles were performed: 94 °C, 30 s; 58 °C, 1 min; 72 °C, 30 s, with initial and terminal delay of 1 min in a Biomed thermocycler (type 60).

Alignment and tree reconstruction. Sequences were adjusted using Seqman II of Lasergene software (DNAStar, Madison, U.S.A.). ITS sequences were aligned in BioNumerics v. 2.5 (Applied Maths, Kortrijk, Belgium) and 18S sequences with DCSE (De Rijk and De Wachter 1993). Trees were reconstructed using the TREECON software package (Van de Peer and De Wachter 1994) using the neighbor-joining algorithm with Kimura-2 correction with 100 bootstrap replications and were verified with parsimony in BioNumerics.

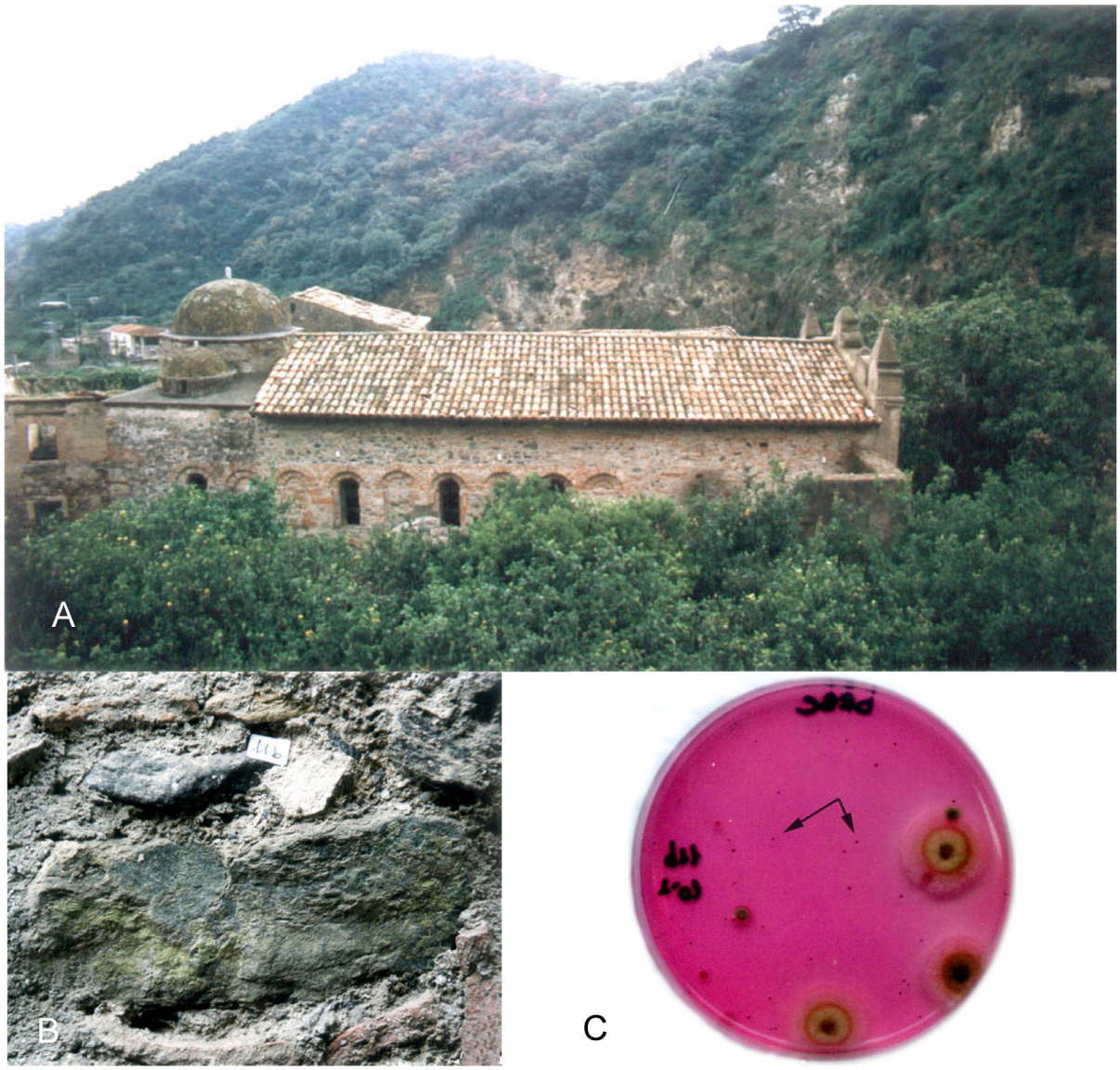

Plate 1. A) View of the church of Santa Maria di Mili (Mili San Pietro, Messina Italy). B) Sandstone sample (11b) from the outside wall of the church from which strain MC 769 was isolated. Algal colonization is visible on the surface. C) Small black colonies isolated from sample 11b growing on DRBC agar after 20 days of incubation at 28 °C.

Results

The near-complete SSU sequence of strain MC 769 was compared with 115 sequences of black yeasts and relatives of the ascomycete orders Chaetothyriales and Dothideales present at CBS (data not shown); nearest neighbours were verified in the public domain using BLASTn. Most of the Chaetothyriales proved only distantly related and were therefore omitted from further analysis. A Dothideales-biased neighbor-joining tree based on 45 near-complete SSU rDNA sequences and, as an outgroup, *Capronia villosa* Samuels (Chaetothyriales) is presented in Figure 1. Strain MC 769 clustered amidst a group of meristematic species. The nearest teleomorph species was *Coccodinium bartschii* Massal. (Coccodiniaceae, Dothideales). *Trimmatostroma salinum* Zalar et al. was found at considerable distance (Figure 1), close

Table 1. List of strains studied.

Strain number	GenBank	Species name	Source	Geography
CBS 116.90 (=ATCC 52681=UAMH 5389)	AJ238471	*Hortaea werneckii*	Fish, eye	Italy
CBS 373.92	AJ238474	*Hortaea werneckii*	Soil, beach	Gran Canaria
CBS 107.67NT	AJ238468	*Hortaea werneckii*	Man, tinea nigra	Portugal
CBS 111.31	AJ238679	*Hortaea werneckii*	Man, tinea nigra	Brazil
CBS 359.66	AJ244249	*Hortaea werneckii*	Man, tinea nigra	Surinam
CBS 117.90 (=UAMH 4978)	AJ238472	*Hortaea werneckii*	Salted fish	Brazil
MZKI B-987		*Hortaea werneckii*	Hypersaline water	Spain
CBS 122.32	AJ238473	*Hortaea werneckii*	Man, tinea nigra	–
CBS 115.90 (=UAMH 4985)	AJ238470	*Hortaea werneckii*	Frog, kidney	Brazil
CBS 100496	AY128703	*Hortaea werneckii*	Seawater-sprayed marble	Greece
CBS 100455 (=MZKI B-675)	AY128704	*Hortaea werneckii*	Coral, seawater	Croatia
CBS 110353 (=DH 12843 =VPCI 176)		*Hortaea werneckii*	Hollow tree	Sudan
DH 12719 (=Onofri 976-21a)		*Trimmatostroma* sp.	Sandstone	Antarctica
CBS 109863 (=MC 662)	AY128701	*Mycocalicium victoriae*	Soil	Italy
CBS 109862 (=MC 633)	AY128702	*Mycocalicium victoriae*	Soil	Italy
Sterflinger NH7-7	AJ312123	*Mycocalicium victoriae*	Limestone	Austria
CBS 214.90T	AJ244238	*Capnobotryella renispora*	*Capnobotrys neesii*, subiculum	Japan
CBS 618.84	AY128696	*Trimmatostroma abietis*	*Ilex* sp.	–
CBS 290.90	AY128698	*Trimmatostroma abietis*	Man	–
CBS 459.93		*Trimmatostroma abietis*	*Abies* sp.	Germany
CBS 300.81	AY128697	*Trimmatostroma abietis*	*Juniperus communis*, needle	Switzerland
CBS 145.97	AY128699	*Trimmatostroma abietis*	Sandstone	Germany
CBS 109889T (=MC 769)	AY128700	*Pseudotaeniolina globosa*	Rock	Italy
CBS 303.84	AJ244268	*Pseudotaeniolina globosa*	Wood	–
CBS 110352 (=DH 12840)		*Pseudotaeniolina globosa*	Human aorta at autopsy	Germany
CBS 486.80	AF362066	*Stenella araguata*	*Paepalanthus columbianus*, dead leaf	Colombia
MZKI B-994	AJ238677	*Phaeotheca triangularis*	Hypersaline water	Spain
MZKI B-950	AJ238674	*Phaeotheca triangularis*	Hypersaline water	Slovenia
CBS 471.90T	AJ244256	*Phaeotheca triangularis*	Humidifier	Belgium
MZKI B-810	AJ238673	*Phaeotheca triangularis*	Hypersaline water	Slovenia
CBS 100458 (=MZKI B-733)	AJ238671	*Phaeotheca triangularis*	Hypersaline water	Slovenia
	AF291707	*Cercospora sorghi*	*Sorghum bicolor*	–
	AF291708	*Cercospora kikuchii*	*Glycine max*	–
	AF291709	*Cercospora zeae-maydis*	*Zea mays*	–
	AF222827	*Cercospora beticola*	–	–
CBS 544.71		*Cercospora dulcamarae*	*Solanum dulcamara*	Romania
CBS 119.25	AF163085	*Cercospora apii*	*Apium graveolens*	Romania
	AF385611	*Coniothyrium zuluense*	*Eucalyptus* sp.	Mexico

Abbreviations used: ATCC = American Type Culture Collection, Manassas, VA, U.S.A.; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DH = G.S. de Hoog private collection; MC = Collection of Istituto di Microbiologia, Messina, Italy; MZKI = Microbiological Culture Collection, National Institute of Chemistry, Ljubljana, Slovenia; UAMH = University of Alberta Microfungus Herbarium and Collection, Edmonton, AB, Canada; VPCI = Vallabhbhai Patel Chest Institute, Delhi, India. NT = ex-neotype strain; T = ex-type strain.

to *Capnodium* and *Scorias* (Capnodiaceae, Dothideales) (based on partial sequences; data not shown). In general the Dothideales proved to be heterogeneous, most branches being unresolved.

Results of sequencing of the ITS rDNA domain are presented in Figure 2. Strains MC 769, CBS 303.84 and dH 12840 were found to be nearly identical. They were clearly separate from the remaining fungi, forming a distinct entity at high bootstrap support. The strains were found to share a main clade with *Trimmatostroma abietis* Butin et al., which showed infraspecific heterogeneity (Figure 2). A strain identified as *T. salicis* Corda, CBS 300.81, clustered amidst *T. abietis* strains. This strain was isolated from *Juniperus* (Table 1), and as *T. abietis* is particularly found on conifers it is likely that a misidentification for that species was concerned. The group was paraphyletic to *Stenella araguata* Syd., CBS 486.80.

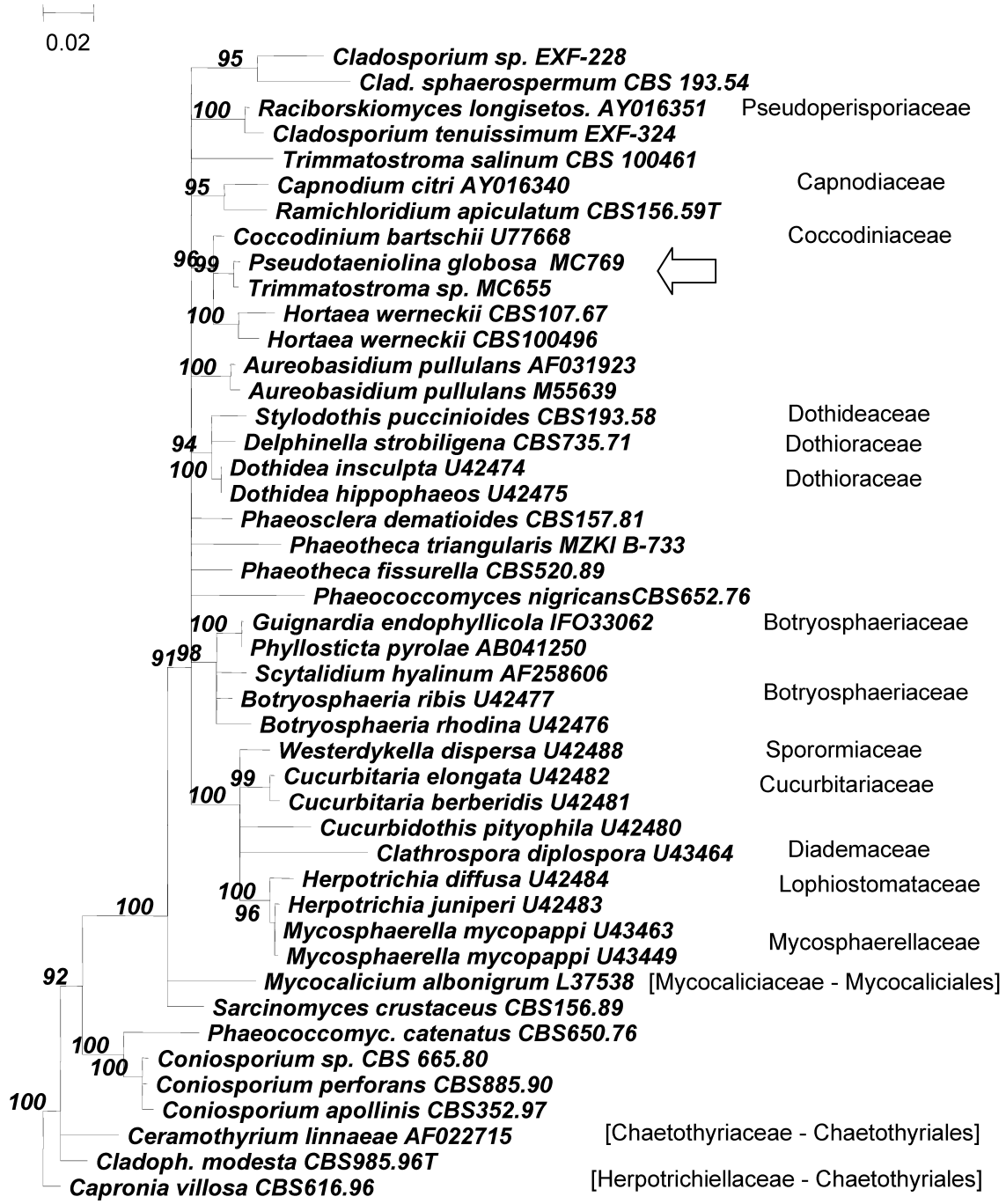

Figure 1. Consensus tree of 45 members of black yeasts and relatives with accent on Dothideales, constructed with the neighbor-joining algorithm in the TREECON package with Kimura (2) correction and 100 bootstrap replicates (values >90 are shown with the branches). Families to which teleomorphs are assigned are listed at the right hand side; species not classified in the Dothideales are in brackets. The new species is indicated with an arrow. *Capronia villosa*, CBS 616.96 is taken as outgroup.

Alignment with species such as *Mycocalicium victoriae* (C. Knight ex F. Wilson) Tibell (Caliciales; Tibell 1987), *Capnobotryella renispora* Sugiyama, *Hortaea werneckii* (Horta) Nishimura and Miyaji and some *Cercospora* species was ambiguous in parts of the ITS1 and 2 domains.

Pseudotaeniolina globosa *De Leo, Urzì and De Hoog,* ***sp. nov.*** *– Plate 2*

Coloniae nigrae, butyreae, 28 diebus 25 mm diametro. Mycelium immersum, ramosum, torulosum, olivaceum, ex cellulis 6–7 × 8–15 μm compositum. Conidia terminalia, meristematica, 1–2 × 0–1 septata, 12–20 μm. Typus: MC 769.

Cultural characteristics at 25 °C

Colonies on MEA black and glistening, buttery, flat,

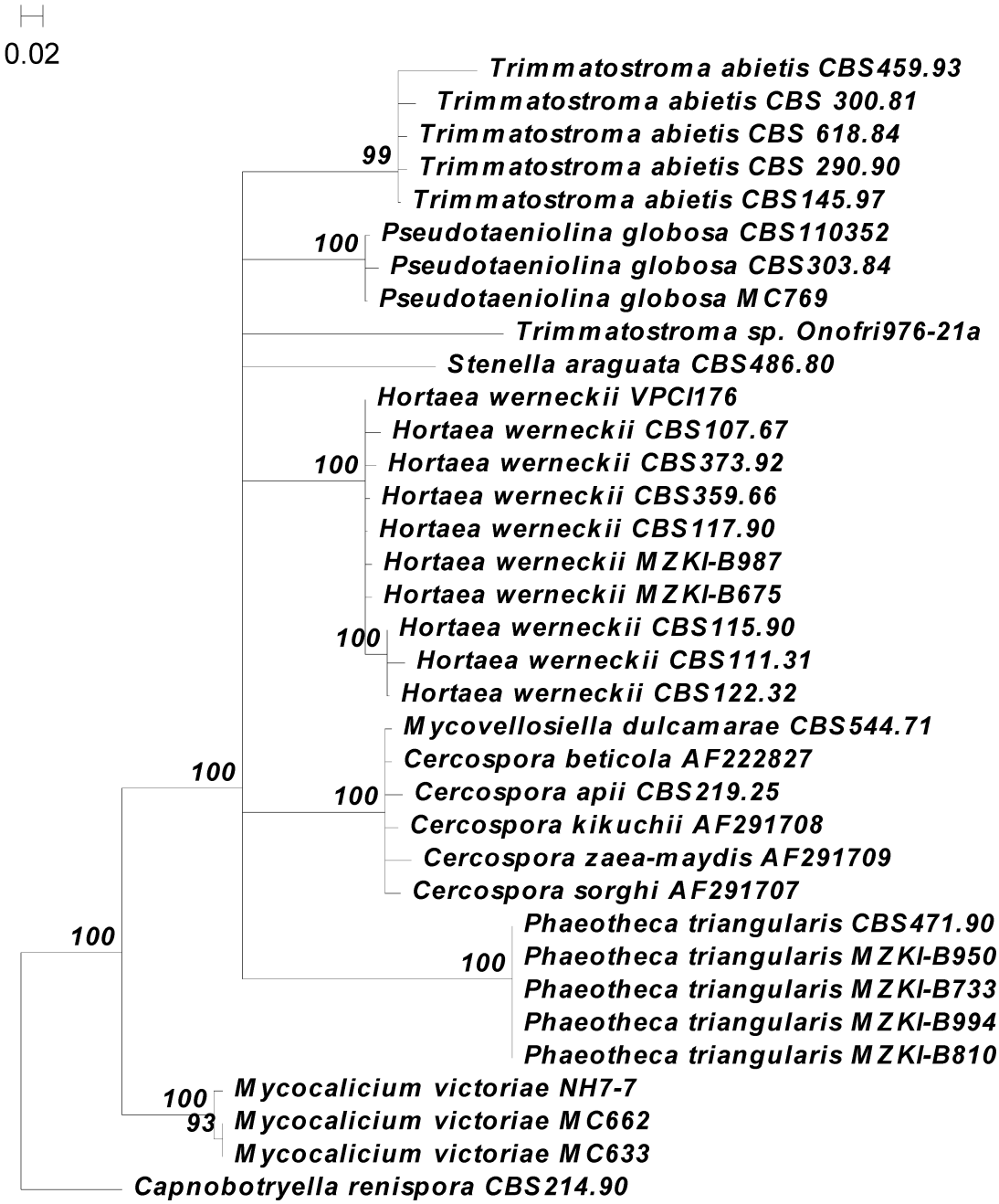

Figure 2. Consensus tree of 35 strains of meristematic melanized fungi with accent on species potentially growing in environments with low water activity, constructed with the neighbor-joining algorithm in the TREECON package with Kimura (2) correction and 100 bootstrap replicates (values >90 are shown with the branches). *Capnobotryella renispora*, CBS 214.90 is taken as outgroup.

slightly raised at the centre, radially folded, attaining up to 25 mm diam in 4 weeks. Aerial mycelium absent. Stroma and setae absent. Colonies on OA flat, with sharp, regular margin, attaining up to 21 mm diam in 4 weeks. Colonies on CzA flat, with fimbriate margin, attaining up to 10 mm diam in 4 weeks. Colonies on PDA flat, with regular margin, slightly raised at the centre, cerebriform, radially folded, attaining up to 28 mm diam in 4 weeks.

Description based on cultures grown on MEA at 25 °C

Mycelium initially consisting of regular, dark olivaceous hyphae, moderately thick- and smooth-walled; hyphal cells 8–15 μm long and 6–7 μm wide. Terminal and lateral, holoblastic budding cells are frequently produced in cultures older than 2 weeks. Sometimes terminal conidia, 10–12 μm diam are observed, these are (sub)spherical and one-celled when young, later becoming septate up to muriform with 1(−2) septa, 12–20 μm diam. Mature hyphae fall apart into separate cells. Older cells at the centre of the colony swell and may become transversely septate, septa often being asymmetrical and cleaving the original cell into a large cell and a very small cell which occasionally has 1(−2) septa. The cells or cell clumps are thick-walled, spherical, 7–17 μm in diam, smooth-walled, verrucose or coarsely punctate; frequently local parts of the outer mother cell wall is shed off. Yeast cells absent. Teleomorph unknown.

Type strain (living and dried): MC 769, ex sandstone of outside wall of the church of ''Santa Maria di Mili'' (Mili San Pietro, Messina, Italy).

Discussion

Pseudotaeniolina globosa is characterized by melanized hyphae that are converted rapidly into chains of spherical cells which after liberation frequently are asymmetrically one-septate (Plate 2). The cells often aggregate in dense clumps. The exact moment of transition is difficult to follow. Strain DH 12840 remained sterile on MEA, showing densely parallel hyphae consisting of short cells. Large multicellular bodies were observed locally on the colony surface; these possibly represented abortive fruit bodies. On PCA the same strain rapidly converted to spherical clumps of cells.

The species fits the genus *Pseudotaeniolina*. *Pseudotaeniolina convolvuli* (Esfandiari) Crane and Schoknecht is the only species known to date in this genus (Crane and Schoknecht 1986). *P. globosa*, like *P. convolvuli*, develops series of arthric, mostly one-celled conidia from meristematically ripening hyphae. The liberated conidia finally swell and take on a yeast-like appearance. Occasionally the spherical cells of *P. convolvuli* have asymmetrical septation, as observed in *P. globosa*. *P. globosa* has larger, more coarsely ornamented cells, and it frequently sheds parts of the outer cell wall. This phenomenon is also known in *Phaeotheca triangularis* De Hoog and Beguin (Zalar et al. 1999a), but the ITS domain of this species was aligned to that of *P. globosa* with difficulty (Figure 2), as holds true for other *Phaeotheca* and also *Hyphospora* species (De Hoog et al. 1999). *P. convolvuli* was described from rotten stems of *Convolvulus*.

Classification of meristematic anamorphs is problematic because of their mostly poorly developed but highly variable morphology. Our species grows with pale olivaceous, branched hyphae which gradually convert into clumps of cells by isodiametric inflation of intercalary and terminal cells. This is observed in many genera of meristematic fungi. Only the subsequent disarticulation into preponderantly one-celled conidia is characteristic for *Pseudotaeniolina*. A large number of meristematic species have been described on the basis of morphology alone (Ellis 1971, 1976). None of these have single cells or aymmetrically septate cells as a final product of conidiogenesis, so in this respect *P. globosa* is unique. *Taeniolella* is also distinctive: while it forms mostly unbranched cells similar to those of *Pseudotaeniolella*, it also shows terminal, holoblastic elongation of chains.

Ellis (1971, 1976) regarded the combination of branched chains of meristematically developing, muriform conidia as diagnostic for the genus *Trimmatostroma*, while *Coniosporium* had unbranched chains. *Taeniolella* had conidia only with transverse septa. Only a limited number of meristematic taxa and sooty moulds have thus far been investigated in SSU rDNA sequence studies (Sterflinger et al. 1997; Reynolds 1998), and thus the mono- or polyphyly of genera like *Trimmatostroma, Coniosporium* or *Taeniolella* can as yet not be specified.

Micromorphologically *P. globosa* is similar to *Sarcinomyces petricola* Wollenzien and De Hoog (Wollenzien et al. 1997), but the cultures of *P. globosa* are slimy and yeast-like after 4–5 d growth, while those of *S. petricola* are dry. In addition, the ITS domains of

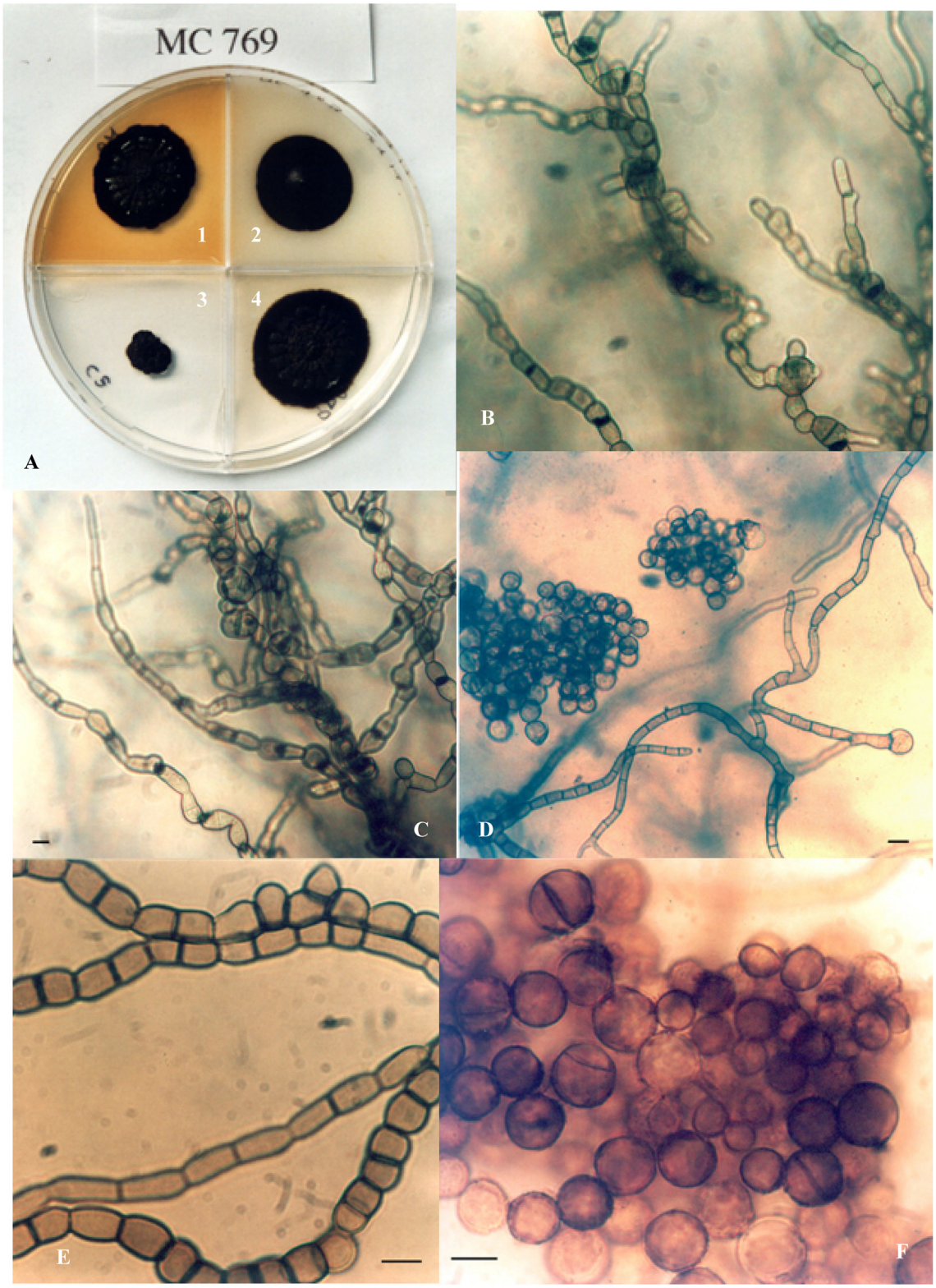

Plate 2. A) Colonies of *P. globosa*, MC 769, after one month incubation. Media tested: 1. MEA; 2. OA; 3. PDA; 4. CzA. B–F) Slide culture on MEA. B and C) Hyphal structure. E) Mature hyphae. D and F) Mature spheerical conidia. Bars represent 10 μm in all micrographs.

the two species differed strongly and could be aligned only with difficulty. Judging from ribosomal sequence data, *S. petricola* is closer to the Chaetothyriales than to Dothideales (data not shown). The same holds true for the stone-inhabiting *Coniosporium* species *Coniosporium perforans* Sterflinger, *C. apollinis* Sterflinger (Sterflinger et al. 1997) and *C. uncinatum* De Leo et al. (De Leo et al. 1999). *C. uncinatum* is morphologically unique in having curved hyphal ends. The similarly rock-inhabiting *C. aeroalgicola* Turian (Turian 1977) is not known to have been preserved; its conidia were multicellular. *Sarcinomyces crustaceus* Lindner forms conidial packets with longitudinal and transverse septation (Sigler et al. 1981) and, judging from rDNA sequence data, is remote from *Trimmatostroma*-like fungi.

Phylogenetically, the taxa related to *P. globosa* are in the Dothideales (Figure 1), but most branches within this order are poorly supported. In the ITS comparison (Figure 2) of taxa with substantial sequence similarity to *P. globosa*, we note that the results reflect an ecological commonality. Most species clustering with *P. globosa* are associated with low water activity, being either epilithic, epiphytic or halophilic. *Trimmatostroma abietis*, the *Trimmatostroma* species with an ITS sequence closest to that of *P. globosa*, is an epiphyte on leathery plant leaves and is occasionally seen on inert surfaces such as rock (Butin et al. 1996). The ITS domain of *T. salinum*, a species inhabiting hypersaline environments (Zalar et al. 1999b) could not be aligned with confidence to any of these species (data not shown). It has muriform rather than one-celled mature conidia. Surprisingly, one of the strains of *P. globosa* was isolated from an aorta of a deceased human patient in which fungal growth was not observed *in vivo* (O. Kurzai, pers. comm.). The isolate was recovered from a male adult who was admitted for aortic aneurysm and died during surgery. The pathogenic potential of *P. globosa* remains unclear. Given that the two other strains of *P. globosa*, as well as some close relatives of the species have a tendency to be oligotrophic, adhering to inert surfaces such as rock, metal, leathery leaves or painted wood, it is reasonable to speculate that the fungus had contaminated a medical device. The strain from aorta proved to be unable to grow at 37 °C. One of the strains of *Trimmatostroma abietis*, with an ecological spectrum similar to that of *P. globosa*, originated from a human (Butin et al. 1996; Table 1), but unfortunately no case report is available of that isolate.

Acknowledgements

K.F. Luijsterburg is thanked for technical assistance. This work was carried out with the financial contribution of the European Community through the Research Project CATS (project nr. EVK4-CT-2000-00028) and the Network COALITION (project nr. EVK4-1999-20001), the Italian C.N.R. (project Nr. 01.00654.PF36) and P.R.A. (Progetti ordinari e giovani ricercatori).

References

Butin H., Pehl L., De Hoog G.S. and Wollenzien U. 1996. *Trimmatostroma abietis* sp. nov. (hyphomycetes) and related species. A. van Leeuw. J. Microb. 69: 203–209.

Commissione Normal 1990. Raccomandazioni Normal: 9/88 Microflora autotrofa ed eterotrofa: tecniche di isolamento in coltura. C.N.R. - I.C.R, Roma.

Crane J.L. and Schoknecht J.D. 1986. Revision of *Torula* and *Hormiscium* species, new names for *Hormiscium undulatum, Torula equina*, and *Torula convolvuli*. Mycologia 78: 86–91.

De Hoog G.S., Guarro J., Gené J. and Figueras M.J. 2000. *Atlas of Clinical Fungi*. 2nd edn. Centraalbureau voor Schimmelcultures / Universitat Rovira i Virgili, Utrecht / Reus, 1126 pp.

De Hoog G.S., Zalar P., Urzì C., De Leo F., Yurlova N.A. and Sterflinger K. 1999. Relationships of dothideaceous black yeasts and meristematic fungi based on 5.8S and ITS2 rDNA sequence comparison. Stud. Mycol. 43: 31–37.

De Leo F., Urzì C. and De Hoog G.S. 1999. Two *Coniosporium* species from rock surfaces. Stud. Mycol. 43: 70–79.

De Rijk P. and De Wachter R. 1993. DCSE, an interactive tool for sequence alignment and secondary structure research. Comput. Appl. Biosci. 9: 735–740.

Ellis M.B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, 608 pp.

Ellis M.B. 1976. *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, 507 pp.

Figueras M.J., De Hoog G.S., Takeo K. and Guarro J. 1996. Stationary phase development of *Trimmatostroma abietis*. A. van Leeuw. J. Microb. 69: 217–222.

Gerrits van den Ende A.H.G. and De Hoog G.S. 1999. Variability and molecular diagnostics of the neurotropic species *Cladophialophora bantiana*. Stud. Mycol. 43: 151–162.

King A.D., Hocking A.D. and Pitt J.I. 1979. Dichloran rose bengal medium for enumeration and isolation of moulds from foods. Appl. Environ. Microbiol. 37: 959–964.

Matsumoto T., Padhye A.A., Ajello L., Standard P.G. and McGinnis M.R. 1984. Critical review of human isolates of *Wangiella dermatitidis*. Mycologia 76: 232–249.

Reynolds D.R. 1998. Capnodiaceous sooty mold phylogeny. Can. J. Bot. 76: 2125–2130.

Sigler L., Tsuneda A. and Carmichael J.W. 1981. *Phaeotheca* and *Phaeosclera*, two new genera of dematiaceous hyphomycetes, and a redescription of *Sarcinomyces* Lindner. Mycotaxon 17: 449–467.

Staley J.T., Palmer F.E. and Adams J.B. 1982. Microcolonial fungi: common inhabitants on desert rocks? Science 215: 1093–1095.

Sterflinger K. 1998. Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. A. van Leeuw. J. Microb. 74: 271–281.

Sterflinger K., De Baere R., De Hoog G.S., De Wachter R., Krumbein W.E. and Haase G. 1997. *Coniosporium perforans* and *C. apollinis*, two new rock inhabiting fungi isolated from marble in the Sanctuary of Delos (Cyclades, Greece). A. van Leeuw. J. Microb. 72: 349–363.

Sterflinger K., De Hoog G.S. and Haase G. 1999. Phylogeny and ecology of meristematic ascomycetes. Stud. Mycol. 43: 5–22.

Tibell L. 1987. Australasian Caliciales. Symb. Bot. Upsal. 27: 1–279.

Turian G. 1977. *Coniosporium aeroalgicolum* sp. nov., moisissure dématiée semi-lichénisante. Ber. Schweiz. Bot. Ges. 87: 19–24.

Urzì C., Lisi S., Criseo G. and Zagari M. 1992. Comparazione di terreni per l'enumerazione e l'isolamento di funghi deteriogeni isolati da materiali naturali. Ann. Microbiol. Enzimol. 42: 185–193.

Urzì C. and Realini M. 1998. Colour changes of Noto's calcareous sandstone as related to its colonisation by microorganisms. Int. Biodet. Biodegr. 42: 45–54.

Urzì C., De Leo F. and Salamone P. 2000. Microbial diversity of Greek quarried marbles associated to specific alteration. In: Monte M. (ed.), Proc. 8th Workshop Eurocare Euromarble EU496. CNR, Rome, pp. 35–42.

Van de Peer Y. and De Wachter R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Appl. Biosci. 10: 569–570.

Yoshida S., Takeo K., De Hoog G.S., Nishimura K. and Miyaji M. 1996. A new type of growth exhibited by *Trimmatostroma abietis*. A. van Leeuw. J. Microb. 69: 211–215.

Wollenzien U., De Hoog G.S., Krumbein W. and Uijthof J.M.J. 1997. *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. A. van Leeuw. J. Microb. 71: 281–288.

Zalar P., De Hoog G.S. and Gunde-Cimerman N. 1999a. Taxonomy of the endoconidial black yeast genera *Phaeotheca* and *Hyphospora*. Stud. Mycol. 43: 49–56.

Zalar P., De Hoog G.S. and Gunde-Cimerman N. 1999b. *Trimmatostroma salinum*, a new species from hypersaline water. Stud. Mycol. 43: 61–67.

Zeppenfeldt G., Richard-Yegres N., Yegres F. and Hernández R. 1994. *Cladosporium carrionii*: hongo dimorfo en cactáceas de la zona endémica para la cromomicosis en Venezuela. Revta Iberoam. Micol. 11: 61–63.