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Analysis of Phylogenetic Relationship of *Cylindrocarpon lichenicola* and *Acremonium falciforme* to the *Fusarium solani* Species Complex and a Review of Similarities in the Spectrum of Opportunistic Infections Caused by These Fungi

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An emerging pattern of similarity in medical case reports led to a project to compare the phylogenetic affinities of two well-known tropical fungal opportunistic pathogens, *Cylindrocarpon lichenicola* and *Acremonium falciforme*, to members of the *Fusarium solani* species complex. *C. lichenicola* and *A. falciforme*, despite their deviating conidial morphologies, were shown via sequencing of the ribosomal large subunit to be well instituted within a clade mainly consisting of typical *F. solani* strains and other species until recently considered variants of *F. solani*. The original name *Fusarium lichenicola* C. B. Massalongo is reestablished, and the new combination *F. falciforme* is made. Recognition of these species as fusaria is necessary for correct interpretation of current and future molecular diagnostic tests. Reevaluation of species morphology in light of the molecular findings showed that certain features, especially elongate filiform conidiophores with integrated terminal phialides, facilitate correct microscopic classification of these atypical *Fusarium* species. There is a strong and underrecognized overlap in the spectra of cases caused by members of the *F. solani* clade, particularly ocular infections, mycetomas, and, in the neutropenic host, disseminated and other serious systemic infections. A novel synthesis of case reports shows that patients from areas with warm climates may develop a distinctive fusarial intertrigo caused by *F. solani*, *Fusarium lichenicola*, or *Fusarium oxysporum*.

The fungal genus *Fusarium* in recent years has been shown to include several well-distinguished major phylogenetic clades (40, 44, 51). In general, each of the clades comprehended within this large anamorph (separately named asexual reproductive form) genus corresponds to one or, exceptionally, two teleomorph (separately named sexual phenotype) genera. At least two of the clades contain medically important members: the clade corresponding to the teleomorph genus *Gibberella* and another unified clade corresponding to two teleomorphs, *Neocosmospora* and the recently delineated *Haematonectria* (51). The first clade, *Gibberella*, includes the important opportunists *Fusarium oxysporum* and *Fusarium verticillioides* (*Fusarium moniliforme*), as well as several infrequently etiologically significant fungi such as *Fusarium proliferatum*, *Fusarium napiforme*, and *Fusarium incarnatum* (*Fusarium semitectum*) (44). It also includes a large number of species not known to be connected with human or animal pathogenicity; many of these have close associations with specific plant hosts. Among members of the second clade, *Neocosmospora vasinfecta* is known rarely to cause opportunistic infections (5), but most medically important isolates in the clade are anamorphic fungi that are referred to as the "*Fusarium solani* species complex" (41). Teleomorphs of these fungi, where known, correspond to the genus *Haematonectria*. In keeping with long-standing tradition, these are all reported under the aggregate name *F. solani*, even though the existence of at least seven separate, noninterbreeding biological species within this group has been known since the early 1960s (34). Fungi named *F. solani* are now known to belong to at least 26 separate phylogenetic species (41), most of which are unnamed or named only as plant-pathogenic formae speciales. How many of these species may be associated with mammalian infection is not known. In addition, *F. solani* isolates belonging to as yet incompletely characterized groups may raise the number of phylogenetic species in this group to more than 50 (51).

While reviewing medical case literature, we noticed that there was a striking correspondence between many of the cases whose causes were attributed to *Cylindrocarpon lichenicola*, a mainly tropical agent of human opportunistic infection, and cases described to be caused by the *F. solani* complex. This led to an investigation to determine whether this apparent correspondence reflected a close phylogenetic relationship or merely an ecological convergence. At the same time, consideration of other human opportunists that might be related to *Fusarium* species led to investigation of the uncommon tropical mycetoma agent *Acremonium falciforme*, a species with curved and sometimes pointed conidia morphologically reminiscent of *Fusarium*. The possible affinity of this species with the genus *Fusarium* had previously been noted by Gams (10). The phylogenetic studies presented here show that both *C. lichenicola* and *A. falciforme* belong to the clade containing *F. solani*, *Haematonectria*, and *Neocosmospora* (collectively referred to hereafter as "the *F. solani* clade"). This recognition improves our overall epidemiological understanding of these fungi and facilitates and clarifies both morphological and molecular laboratory identification.

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MATERIALS AND METHODS

The strains studied were obtained from the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

DNA extraction and amplification. DNA was extracted with a FastDNA kit (Qbiogene, Heidelberg, Germany) from mycelium grown for 3 to 5 days in liquid Complete Medium (46). The large-subunit (LSU) region of ribosomal DNA (rDNA) was amplified with primers V9G (7) and LR5 (57). The components for the PCR were used as described by Schroers (52). The PCR program was 60 s at 94°C (initial denaturation); 35 cycles of 35 s at 94°C (denaturation), 50 s at 55°C (annealing), and 120 s at 72°C (elongation); and 6 min at 72°C (final elongation) followed by chilling to 4°C. The PCR products were purified with a GFX purification kit (Amersham Pharmacia Biotech Inc., Roosendaal, The Netherlands) and visualized on an electrophoresis gel after ethidium bromide staining. The rDNA was sequenced with a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) and analyzed on an ABI Prism 3700 instrument (Applied Biosystems) by using the standard conditions recommended by the vendor. The primers used in the sequence reaction were ITS1 and ITS4 (58), NL1 and NL4 (40), and LR5.

DNA data analysis. Sequence chromatographs were assembled and edited with SeqmanII software (DNAStar, Inc., Madison, Wis.) and aligned with sequences downloaded from GenBank (http://www.ncbi.nlm.nih.gov/), National Center for Biotechnology Information, Bethesda, Md. (Table 1). The alignment was initially performed with the ClustalX program (version 1.8; ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX) and adjusted manually with the Megalign program (DNAStar). The phylogenetic analysis was performed with a part of the LSU rDNA available for all accessions. This part is flanked by positions 116 and 615 in Fig. 1 in the report by Guadet et al. (16), including domain D1 and most of domain D2. A parsimony analysis was performed with the software package PAUP (version 4.0b8) (54). Gaps were coded as missing data; characters were defined as unordered and equally weighted; characters not informative to the parsimony analysis were excluded; heuristic searches of parsimonious trees were performed for all sequences with random sequence addition and 1,000 replicates, using the starting trees from the stepwise addition, tree bisection-reconnection as the swapping algorithm, and all optimal trees for the next swapping round; and branch robustness was tested by use of 100 replications of such searches based on bootstrapped data sets with random sequence addition and 10 replicates per search.

Morphological analysis was conducted with an Olympus BX50 microscope equipped with an Olympus DP10 digital camera unit (Olympus Optical Co. Europe, Hamburg, Germany). Colonies were grown on synthetic nutrient agar (38), oatmeal agar (11), and malt extract agar (Oxoid, Basingstoke, United Kingdom) and were prepared for photography after 7 to 10 days at room temperature (22°C).

RESULTS

Heuristic searches of the shortest trees resulted in 60 trees of equal length. The trees, one of which is shown in Fig. 1, differed by minor changes mainly within the *Gibberella fujikoroi* group. Tree scores are as follows: length = 343; consistency index = 0.434; retention index = 0.794; rescaled consistency index = 0.345; homoplasy index = 0.566. Of the 493 alignment positions chosen, 114 were informative to the parsimony analysis and were included in the analysis. Two multiple gaps were encountered in the data set; both were caused by sequences of the two related taxa used as the outgroup, *Verticillium dahliae* and *Plectosphaerella cucumerina*.

The *F. solani* clade appears in Fig. 1 as a moderately well supported branch, with 67% bootstrap support, compared to 77 and 54% bootstrap supports for the well-established *Cosmospora* and *G. fujikoroi* clades, respectively, in the same data set. Included within the *F. solani* clade, with bootstrap support in the same range, are the opportunistic pathogens *C. lichenicola* and *N. vasinfecta*. The LSU sequences of the ex-type strain of *A. falciforme* and a recently obtained strain were identical, and the two strains were on a branch well separated from other species. The small number of *F. solani* comparison strains from humans with confirmed cases of infection all clustered into "*F. solani* species complex clade 3" of O'Donnell (41); the sequences of two strains (strains CBS 102256 and CBS 109696) were identical to those of members of *Haematonectria haematococca* mating group V, also called *F. solani* var. *petroliphilum*, a group containing pathogens of cucurbits (41) as well as numerous isolates from oily water (22). Confirmatory work on other sequences and characters is necessary, however, before the medical isolates can be fully evaluated as possible representatives of this taxon. *H. haematococca* mating group VI from peas was also closely related to the medical isolates, as was *Haematonectria ipomoeae*, a species isolated from *Passiflora edulis* and various other mostly tropical plants (51). None of the medical isolates were related to the morphologically similar but phylogenetically distinct isolates in the clade formerly referred to as *F. solani* f. sp. *phaseoli*, a group of isolates pathogenic for beans. This clade, which forms part of "*F. solani* species complex clade 2" of O'Donnell (41), has recently been segregated as *Fusarium martiiphaseoli* (22). A typical *Cylindrocarpon* species, the anamorph of *Neonectria radicicola* (32), was strongly phylogenetically distinct from *C. lichenicola* and other members of the *F. solani* clade. Typical *Acremonium* species such as *Acremonium alternatum* and *Acremonium kiliense*, already known mainly to belong to the phylogenetically separate family Bionectriaceae (47, 51) and to be well separated phylogenetically from *Fusarium* species (14), were not included in the dendrogram. A less prototypical *Acremonium* species of nectriaceous affinity, *Acremonium berkeleyanum* (*Acremonium butyri*), is included as the anamorph of *Cosmospora vilior.*

Human-pathogenic *Fusarium* species outside the *F. solani* clade, e.g., *F. oxysporum* and *F. verticillioides*, were clustered in the clade corresponding to the teleomorph genus *Gibberella*. Several other distinct clades containing *Fusarium* anamorphs not known to be associated with human and animal disease, e.g., *Fusarium* sections *Eupionnotes* and *Spicarioides*, as well as *Fusarium buxicola*, were also well distinguished.

Strain CBS 115.40, the ex-type strain of *Cylindrocarpon tonkinense*, a name long considered synonymous with *C. lichenicola* (21), yielded a sequence compatible with that of *F. solani*, not *C. lichenicola*. Morphological investigation showed that, while atypical for *F. solani*, this strain was also completely distinct from typical *C. lichenicola* isolates, with tapering and often curved macroconidia rather than the straight and club-shaped conidia seen in the latter species. It also differed strongly from its own morphological description and illustrations in Bugnicourt's (3) original publication describing *C. tonkinense*, suggesting that the strain currently held as CBS 115.40 may have been derived from a strain transposition error. The matter is being further investigated. Other strains described in literature as *C. tonkinense*, e.g., the strain from a patient with keratitis reported by Matsumoto et al. (33) (available as ATCC 42055 but not examined in the present study), can be clearly recognized from their descriptions and photographs as typical *C. lichenicola* isolates.

Morphological analysis of pathogenic species and strains in the *F. solani* clade showed that members of this group diverged considerably in conidial morphology, although the typical curved macroconidia with foot cells were found in isolates that had been identified as *F. solani*. The main micromorphological factor found in common among the members of the group was

TABLE 1. Taxa of the members of the families Nectriaceae (Hypocreales) and Phyllachoraceae (Phyllachorales) analyzed

Taxon[a]	Natural affiliation (major generic or subgeneric clade level)	GenBank accession no.	Reference for sequence (per GenBank record)	Strain no.[b]	Substratum strain was isolated from (new sequences only)	Geographic origin (new sequences only)
F. napiforme Marasas et al.	*G. fujikuroi* complex	U34541	44	NRRL 13604		
Fusarium brevicatenulatum Nirenberg, O'Donnell, Kroschel et Andrianaivo	*G. fujikuroi* complex	U61649	44	NRRL 25446		
F. verticillioides (Sacc.) Nirenberg	*G. fujikuroi* complex	U34526	44	NRRL 22172		
Fusarium nygamai Burgess et Trimboli	*G. fujikuroi* complex	U34539	42	NRRL 13448		
Fusarium fujikuroi Nirenberg	*G. fujikuroi* complex	U34528	42	NRRL 13566		
Fusarium phyllophilum Nirenberg et O'Donnell	*G. fujikuroi* complex	U34545	42	NRRL 13617		
F. proliferatum (Mats.) Nirenberg ex Gerlach et Nirenberg	*G. fujikuroi* complex	AJ271215	M. Y. Abdalla et al. (unpublished)	Item 2386		
F. oxysporum Schlecht.: Fr.	*Gibberella* clade, *F. oxysporum* complex	AF060383	E. Cigelnik (unpublished)	NRRL 26409		
F. oxysporum		U34542	42	NRRL 13307		
F. oxysporum		U34537	42	NRRL 22902		
Fusarium nisikadoi T. Aoki et Nirenberg	*G. fujikuroi* complex	U61659	44	NRRL 25179		
Fusarium redolens Wollenw.	*Gibberella* clade, *F. oxysporum* complex	U34536	42	NRRL 22901		
Fusarium camptoceras Wollenw. et Reinking	*Gibberella* clade, *Fusarium* section *Fusarium* (formerly section *Arthrosporiella*)	U88102	40	NRRL 13382		
Fusarium culmorum (W. G. Smith) Sacc.	*Gibberella* clade, *Fusarium* section *Fusarium* (formerly section *Arthrosporiella*)	AF006322	43	NRRL 25475		
Fusarium flocciferum Corda	*Gibberella* clade, *Fusarium* section *Fusarium* (formerly section *Arthrosporiella*)	AF006323	43	NRRL 25471		
Fusarium sporotrichioides Sherbakoff	*Gibberella* clade, *Fusarium* section *Fusarium* (formerly section *Arthrosporiella*)	AF006328	43	NRRL 25479		
Albonectria rigidiuscula (Berk. et Br.) Rossman et Samuels	*Albonectria*	U88104	40	NRRL 13412		
Albonectria albosuccinea (Pat.) Rossman & Samuels	*Albonectria*	U34554	40	NRRL 20459		
"*Nectria ventricosa*" C. Booth, anamorph *Fusarium ventricosum* Appel et Wollenw.	*Fusarium* section *Ventricosum*	U88118	40	NRRL 13953		
"*N. ventricosa*"		L36613	40	NRRL 20846		
Cosmospora episphaeria (Tode: Fr.) Rossman et Samuels, anamorph *F. aquaeductuum* (Radlk. et Rabenh.) Lagerh. var. *medium* Wollenw.	*Cosmospora*/*Fusarium* section of *Eupionnotes*	U88100	40	NRRL 20687		
Cosmospora vilior (Starbäck) Rossman et Samuels, anamorph *Acremonium berkeleyanum* (P. Karsten) W. Gams	*Cosmospora*	U57348	A. E. Glenn and C. W. Bacon (unpublished)	ATCC 16217		
Cosmospora flammea (Tul. et C. Tul.) Rossman et Samuels, anamorph *F. coccophilum* (Desm.) Wollenw. et Reink.	*Cosmospora*/*Fusarium* section *Eupionnotes*	U88103	40	NRRL 20441		
Fusarium merismoides Corda var. *violaceum* Gerlach	*Cosmospora*/*Fusarium* section *Eupionnotes*	U88112	40	NRRL 20896		
F. merismoides	*Cosmospora*/*Fusarium* section *Eupionnotes*	U88111	40	NRRL 20895		
F. merismoides var. *crassa* Wollenw.	*Cosmospora*/*Fusarium* section *Eupionnotes*	U88110	40	NRRL 20894		
F. martiiphaseoli Burk (*F. solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. et Hans.)	*Haematonectria*/*Fusarium* section *Martiella*/*F. solani* complex	L36629	45	NRRL 22292		

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TABLE 1—*Continued*

Taxon[a]	Natural affiliation (major generic or subgeneric clade level)	GenBank accession no.	Reference for sequence (per GenBank record)	Strain no.[b]	Substratum strain was isolated from (new sequences only)	Geographic origin (new sequences only)
F. martiiphaseoli		L36632	45	NRRL 22678		
F. solani (Mart.) Sacc.	*Haematonectria/Fusarium* section *Martiella/F. solani* complex	AY097316[c]		CBS 490.63, received as *Cephalosporium keratoplasticum* Morikawa	Human, causing intertrigo	Japan
F. solani		AY097318[c]		CBS 109696	Human, causing eye infection	Ontario, Canada
H. haematococca (Berk. & Br.) Samuels et Nirenberg mating population V, anamorph *F. solani* var. *petroliphilum* Chen (*F. solani* f. sp. *cucurbitae* race 2)	*Haematonectria/Fusarium* section *Martiella/F. solani* complex	L36623	45	NRRL 22141		
H. haematococca mating population VI, anamorph *Fusarium lathyri* Taub. (*F. solani* f. sp. *pisi* (F. R. Jones) Snyd. et Hans.)	*Haematonectria/Fusarium* section *Martiella/F. solani* complex	L36622	45	NRRL 22278		
H. ipomoeae (Halst.) Samuels et Nirenberg, anamorph *F. striatum* Sherb.	*Haematonectria/Fusarium* section *Martiella/Fusarium solani* complex	U88106	40	NRRL 13952		
F. solani (Mart.) Sacc.	*Haematonectria/Fusarium* section *Martiella/F. solani* complex	AY097317[c]		CBS 102256	Human, blood	Germany
F. solani (Mart.) Sacc.		AY097325[d]		CBS 115.40, ex-type strain of *Cylindrocarpon tonkinense* Bugnicourt	*Musa sapientum*	Vietnam
A. falciforme (Carrión) W. Gams	*Haematonectria/Fusarium* section of the *Martiella/F. solani* complex	AY097326[d]		CBS 101427	Human, white-grain mycetoma	United States (patient from India)
A. falciforme		AY097319[c]		CBS 475.67, ex-type strain of *A. falciforme*	Human, white-grain mycetoma	Puerto Rico
N. vasinfecta E. F. Sm.	*Neocosmospora/Fusarium* section *Martiella/F. solani* complex	U47836	J. W. Spatafora (unpublished)	RSA 1898		
N. vasinfecta		U17406	47	JP 963		
C. lichenicola (C. B. Massalongo) D. Hawksw.	*Haematonectria/Fusarium* section *Martiella/F. solani* complex	AY097320[d]		CBS 483.96	Soil	Japan
C. lichenicola		AY097324[d]		CBS 109048 (31)	Human, cornea	Argentina
C. lichenicola		AY097322[d]		CBS 238.58	Soil	Tahiti
C. lichenicola		AY097321[d]		CBS 623.92	Human, necrotic wounds on foot of patient under chemotherapy	Germany
C. lichenicola		AY097323[d]		CBS 279.34	Human, skin	Somalia
F. buxicola Sacc.	*Fusarium* section *Macroconia*	U88125	40	NRRL 20474		
Nectria pseudotrichia Berk. et M. A. Curtis, anamorph *Tuberculariа lateritia* (Berk.) Seif.	*Nectria* sensu stricto	U17410	47	AR 1755		
Nectria cinnabarina (Tode: Fr.) Fr., anamorph *Tuberculariа vulgaris* Tode: Fr.	*Nectia* sensu stricto	U00748	47	GJS[2] 89-107		
Cylindrocladium floridanum Sobers et C. P. Seymour	*Calonectria*	U17408	47	ATCC 22677		
Calonectria morganii Crous, Alfenas et Wingfield	*Calonectria*	U17409	47	ATCC 11614		
Calonectria pyrochroa (Desm.) Sacc., anamorph *Cylindrocladium ilicicola* (Hawley) Boedijn et Reitsma	*Calonectria*	U88097	40	NRRL 13941		
Leuconectria clusiae (Samuels et C. T. Rog.) Rossm. et al., anamorph *Gliocephalotrichum bulbilium*, J. J. Ellis et Hesseltine	*Leuconectria*	U17412	47	AR 2706		
N. radicicola (Gerlach et L. Nilsson) Mantiri et Samuels, anamorph *Cylindrocarpon destructans* (Zins.) Scholt	*Neonectria*	U17415	47	AR 2553		

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TABLE 1—*Continued*

Taxon[a]	Natural affiliation (major generic or subgeneric clade level)	GenBank accession no.	Reference for sequence (per GenBank record)	Strain no.[b]	Substratum strain was isolated from (new sequences only)	Geographic origin (new sequences only)
V. dahliae Klebahn	Sordariomycetes, Phyllachorales, Phyllachoraceae (outgroup)	U17425	47	ATCC 16535		
P. cucumerina (Lindfors) W. Gams, anamorph *Plectosporium tabacinum* (van Beyma) M. E. Palm, W. Gams et Nirenberg	Sordariomycetes, Phyllachorales, Phyllachoraceae (outgroup)	U17399	47	ATCC 96328		

[a] The arrangement of the isolates is as in the dendrogram in Fig. 1.
[b] NRRL, USDA Northern Regional Research Laboratory, Peoria, Ill.; ATCC, American Type Culture Collection, Manassas, Va.; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; other acronyms represent the collection designations of particular researchers or institutions and do not represent public collections.
[c] Newly reported sequence including a partial sequence of the 28S RNA gene.
[d] Newly reported sequence, including the complete sequences of internal transcribed spacer 1, the 5.8S rRNA gene, and internal transcribed spacer 2 and the partial sequence of the 28S rRNA gene.

the production of elongate, filiform, often several-celled conidiophores incorporating terminal monophialides up to 40 μm in length, with a relatively broad, blunt apex. In *F. solani* isolates per se, these elongate conidiophores (Fig. 2) were mainly associated with microconidia; conidiophores forming macroconidia were somewhat shorter. In *C. lichenicola*, however, conidiophores forming macroconidia were typically elongate (Fig. 3). The conidiophores in *A. falciforme* tended to be similar in shape to the conidiophores of other isolates in the *F. solani* clade but were relatively strongly septate (Fig. 4); the terminal phialidic cell was frequently less than 20 μm in length, although longer phialides were also seen. Other species-specific characters were as described previously (2, 8, 10, 12).

DISCUSSION

Sequencing of the LSU of rDNA, a sensitive indicator of major clade relationships within the order Hypocreales (16, 41, 44, 50, 52), indicates that *C. lichenicola* and *A. falciforme* are members of the *F. solani* clade. They are not the first morphologically divergent anamorphs shown to be associated with the *F. solani* clade: O'Donnell (41) showed, using sequencing of the LSU, internal transcribed spacer, and elongation factor 1α sequences, that members of the ascomycetous genus *Neocosmospora*, which have unnamed anamorphs that produce no macroconidia and that otherwise resemble a microconidial state of *Fusarium*, are also nested deeply within the *F. solani* clade. It is clear, especially in the context of the whole range of *Fusarium* species, that the typical *F. solani* morphology is a symplesiomorphy, a shared ancestral character, and that several divergent and reduced forms have evolved from it.

Gams (10) recognized that *A. falciforme* was an atypical addition to the already polymorphous genus *Acremonium*. Its long, septate conidiophores and curved, often two-celled conidia suggested a possible relationship to *Fusarium*. It was not, however, the only recognized *Acremonium* species elaborating some didymoconidia, and its slow growth rate was consistent with placement as an *Acremonium* species. Growth rate was strongly emphasized in the taxonomy of Gams (10), as it was one of the few discrete characters that could unfailingly be used to separate the highly variable fusaria, with their frequent elaboration of *Acremonium*-like, mostly microconidial strains and species, from the even more diverse acremonia. Even the isolation of an *A. falciforme* strain with sharply pointed, very *Fusarium*-like conidia from a patient with mycetoma in Vanuatu (35) did not cause the generic placement of this fungus to be questioned. (Unfortunately, this interesting strain, which was well illustrated and described in the case report, is no longer available for study.) In light of the results of the present study, however, the link between *A. falciforme* and *Acremonium* can no longer be maintained. The type species of *Acremonium*, *A. alternatum*, is a member of the family Bionectriaceae, a group phylogenetically quite distinct from, although related to, the family Nectriaceae, which contains *Fusarium* and its relatives (47, 50, 51). The overall maintenance of biological predictiveness (e.g., for predictions like "all *Fusarium* species will [tend to be] specifically detected by appropriate genus-specific PCR primers") entails that *A. falciforme* be correctly associated with its true biological relatives.

While *A. falciforme* was always an anomaly in its genus, *C. lichenicola* is morphologically typical of the historic concept of the genus *Cylindrocarpon*, as per the monograph of Booth (2). Its relatively long and narrow, several-celled, cylindrical conidia with typical rounded apices deviate only slightly from the macroconidia of other *Cylindrocarpon* species with straight conidia by having a conspicuously protuberant, symmetrical, truncate base. It is now clear that the type species of the genus *Cylindrocarpon*, *Cylindrocarpon cylindroides* (teleomorph *Neonectria neomacrospora*), to which the generic name attaches, is phylogenetically distinct from *C. lichenicola* and members of the genus *Fusarium* (32). The most extensively studied *Cylindrocarpon*, the medically important (60) and plant-pathogenic *Cylindrocarpon destructans* (teleomorph *Neonectria radicicola*), also clusters phylogenetically in a group of organisms well segregated from the *F. solani* and *Gibberella* clades (Fig. 1).

A previous study on the potential of sequencing of the LSU of rDNA for the identification of medically important *Fusarium* species (21) showed that two *C. lichenicola* isolates (recorded as *C. tonkinense*) clustered near *F. solani*; however, this study, as its authors stated, was "not a phylogenetic re-evaluation," and insufficient comparison organisms were tested to

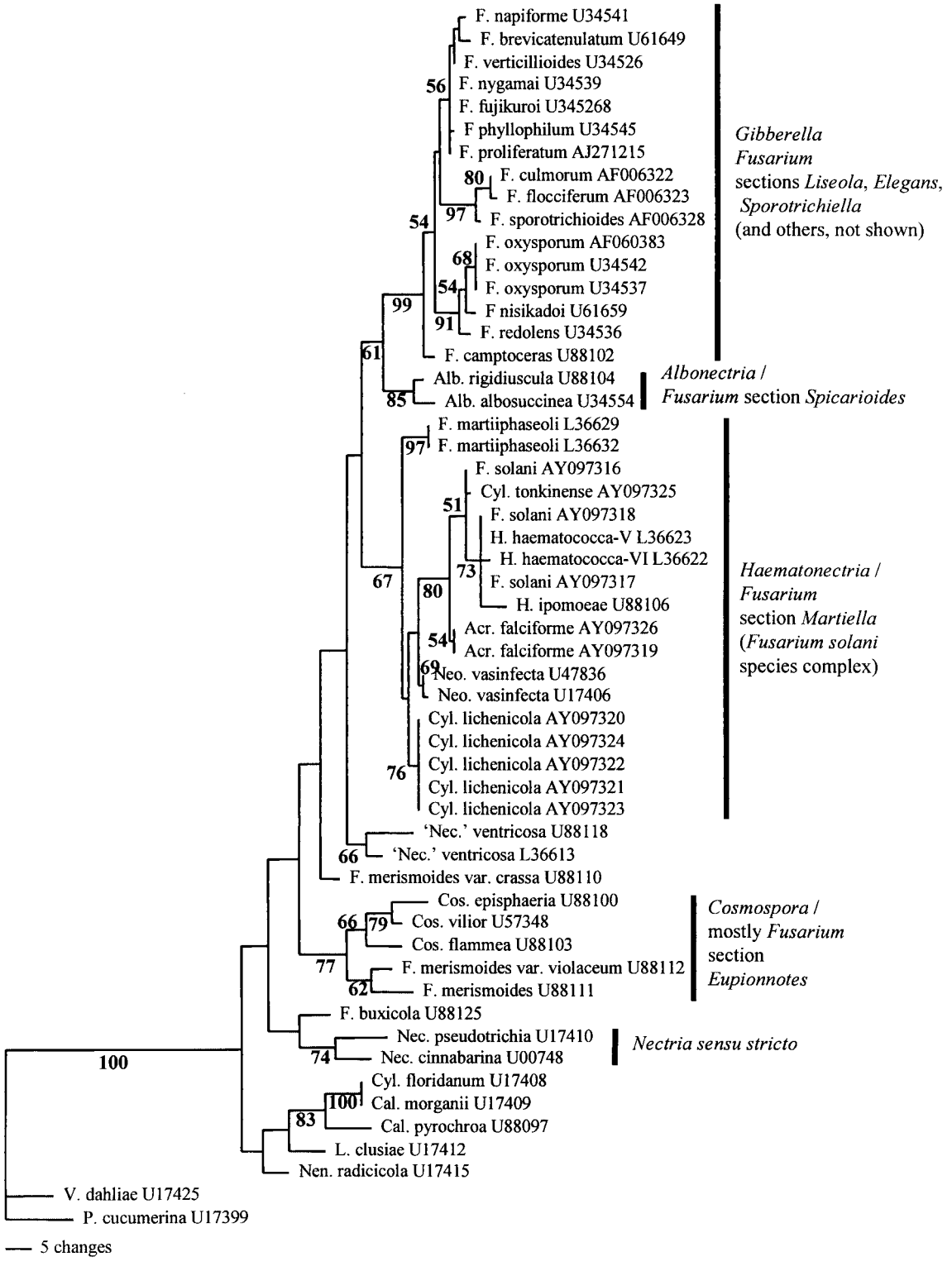

FIG. 1. Phylogenetic relationships within the genus *Fusarium* showing the positions of the members of the *Fusarium solani* clade and its substituents *Acremonium falciforme* and *Cylindrocarpon lichenicola* in relation to those of other pathogenic and nonpathogenic species. One of 60 most parsimonious trees derived from parsimony analysis of positions 116 to 615 of the 28S rDNA is shown. See the text for methods, scores, and other details. Abbreviations for genus names: Alb., *Albonectria*; Acr., *Acremonium*; Cal., *Calonectria*; Ccl., *Cylindrocladium*; Cos., *Cosmospora*; Cyl., *Cylindrocarpon*; F., *Fusarium*; H., *Haematonectria*; L., *Leuconectria*; Nec., *Nectria*; Nen., *Neonectria*; Neo., *Neocosmospora*; P., *Plectosphaerella*; V., *Verticillium*.

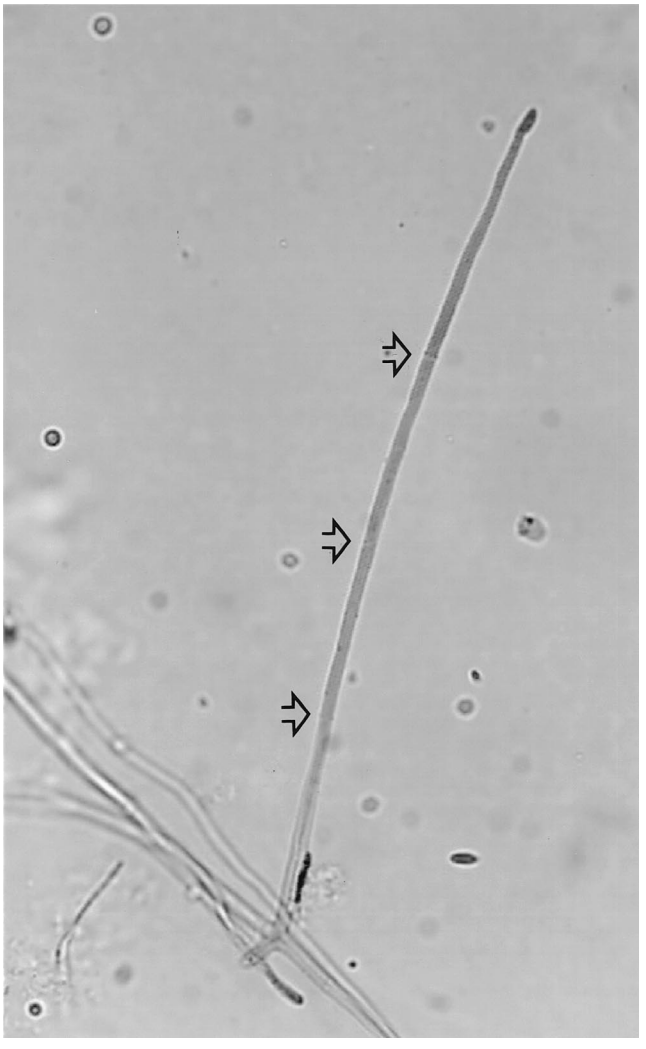

FIG. 2. A typical elongate, microconidium-producing, septate conidiophore of *F. solani*. Septa, which are in slightly different planes of focus, are marked with arrows. Magnification, ×1,400.

determine, for example, that *C. lichenicola* was more closely related to *F. solani* than to other *Cylindrocarpon* species.

Before *A. falciforme* and *C. lichenicola* can comfortably be removed from their current genera and associated with *Fusarium*, some questions about this genus must also be considered. Its validity as currently delimited might be questioned, since *Fusarium* anamorphs are associated with several teleomorph genera, now known roughly to correspond to several related phylogenetic clades. The type species of *Fusarium*, *Fusarium sambucinum* Fuckel, belongs to the clade associated with the teleomorph genus *Gibberella*, making this clade the central group of *Fusarium* species and the one that would bear the name if *Fusarium* were split up. As mentioned previously, this clade contains several medically important species such as *F. oxysporum* and *F. verticillioides* (44). The *F. solani* clade has *Haematonectria* or *Neocosmospora* teleomorphs. Other *Fusarium* species have teleomorphs in the genera *Cosmospora* and *Albonectria* (51). There is therefore considerable temptation for phylogenetic systematists to split *Fusarium* itself into multiple parallel anamorph genera corresponding to the clades or to the teleomorph genera. Although a poorly resolved monophyletic clade appears to encompass all fusaria, some branches bearing disparate anamorphs appear to emerge from within this clade in the dendrogram based on the sequences of the LSU of rDNA (Fig. 1), particularly a branch bearing *Cylindrocladium floridanum* and *N. radicicola*/*C. destructans*. Despite these factors, no move has yet been made to split up *Fusarium*. This may be in part because of the desirability of maintaining as much nomenclatural stability as possible among the widely used, sometimes nearly 150-year-old *Fusarium* species names and in part because there are many morphological and general biological characters that make the historic, unitary generic concept of *Fusarium* useful. Such characters are diverse, e.g., possession of curved, pointed macroconidia with a differentiated foot cell, manifestation of plant-pathogenic ability, and manifestation of a high degree of amphotericin B resistance in opportunistic infections in humans. If *Fusarium* were to be divided, by default the names of the species in the *F. solani* complex would likely be used in combination with the long unused generic name *Lachnidium*, based on a seemingly unnecessary synonym coined for *F. solani* isolates obtained from infected crickets in 1891 (13). To take such a step would be seen by many practical users of fungal anamorph names to be counterproductive. Establishing the link between *Fusarium* and *C. lichenicola*, despite the deviating phragmoconidial morphology of the latter, scarcely attenuates the biological and morphological unity of the *Fusarium* clades. We therefore refrain from sundering the genus *Fusarium* on the basis of our findings.

Even anamorph classifications, while something of an artifice (15), function best if they reflect underlying biological reality to a reasonable degree. In keeping with this practical need, then, we hereby formally reinstate the original name *Fusarium lichenicola* C. B. Massalongo and make the following new formal combination: *Fusarium falciforme* (Carrión) Summerbell et Schroers, comb. nov. Basionym: *Cephalosporium falciforme* Carrión (in *Mycologia* **43:**523, 1951).

The epidemiologic links between *F. lichenicola* and medically implicated isolates of *F. solani* are clearly suggested by a review of case literature pertaining to these fungi. *F. lichenicola*, for example, is most frequently medically significant as an agent of relatively aggressive keratitis subsequent to ocular trauma (1, 29, 31, 33), just as *F. solani* is (18, 19, 26, 30, 59). Other well-documented cases of infection caused by *F. lichenicola* include disseminated infection (25) and local soft tissue infection (24) in leukemia patients and peritonitis related to chronic ambulatory peritoneal dialysis (53). *F. solani* is well known as a more commonly occurring cause of similar infections (8, 18, 37). Most intriguingly, an unusual type of intertrigo, an infection almost never definitively shown to be caused by any fungi other than dermatophytes and *Scytalidium* species (older literature contains many inadequately substantiated case reports in which other species are mentioned, probably based on misattribution of the infection to contaminants isolated when a causal dermatophyte failed to grow), appears to be caused exclusively by opportunistic members of the *F. solani* and *Gibberella* clades. *F. solani* has been reported from two well-documented cases of this intertrigo (6, 49). The cause of a third such case, probably valid but not as rigorously documented (the putative agent was not reisolated on successive

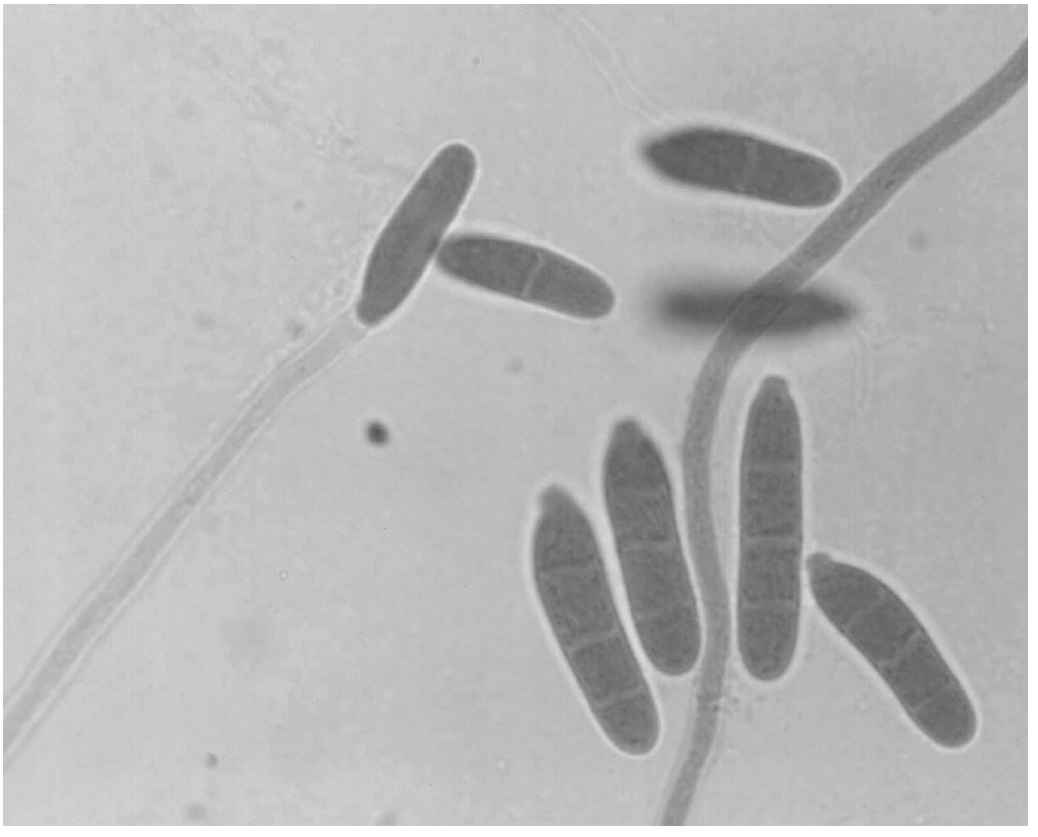

FIG. 3. Macroconidia of *Cylindrocarpon lichenicola* produced from an elongate phialidic conidiophore (not fully shown). Magnification, ×1,400.

occasions in order to rule out dermatophytosis), was ascribed to the invalid name *Cephalosporium keratoplasticum* Morikawa by Harada and Usui (20); the case isolate, preserved as CBS 490.63, is *F. solani* and was sequenced in the present study. Only *F. oxysporum* (48) and a "*Cylindrocarpon* sp." (27) that can be clearly recognized from its published description and photos as *F. lichenicola* have been well documented as causes of similar cases of intertrigo. Three of these fusarial intertrigo cases were described from patients of recent West African origin living in Europe (27, 48, 49), while in a fourth case (6), the patient's geographic history was not discussed, but he was described both as a French resident and as a "practicing Muslim. . .who washes his feet five times per day [prior to] his prayers." The fifth, incompletely confirmed case was from Japan (20). It is possible that, at least in areas with warm climates, the regular exposure of feet for any reason, including hygiene, to water or other materials containing an inoculum of opportunistic *Fusarium* species, including *F. lichenicola*, may rarely be conducive to development of fusarial intertrigo.

F. falciforme has long been known to be a rarely occurring agent of mycetoma in tropical and subtropical areas (17). While *F. solani* is not principally thought of as a cause of mycetoma, it has been reported from at least four such cases worldwide (18); the United Kingdom National Collection of Pathogenic Fungi contains isolates from patients with additional confirmed cases (18). In recent years, there has been evidence that *F. falciforme* is emerging as an agent of localized (28, 36, 56) and disseminated (39) infections in immunocompromised patients, thus overlapping more broadly in pathogenic potential with *F. solani*. An *F. falciforme* endophthalmitis has also been documented (4). The apparent distinction between *F. falciforme* and *F. solani* in terms of their potential to cause opportunistic infections may relate more to their ecological and geographic distributions and the way in which they are contracted by patients than to virulence factors. Nothing is known about the natural habitat of *F. falciforme*, but this species is certainly not a common fungus of food, household plant material, garden soil, and domestic water, as *F. solani* is. By virtue of this factor alone, *F. falciforme* would be less likely than *F. solani* to cause nosocomial infections in temperate-region medical facilities even if both species were identical in virulence.

When *F. lichenicola* and *F. falciforme* are recognized as members of the *F. solani* clade, certain morphological correspondences facilitating recognition in the laboratory become discernible. The long, slender, cylindrical conidiophores and integrated, terminal phialides seen in all these species clearly reflect their biological unity. In contrast, other *Fusarium* groups tend to have strongly different phialides. Many species form shorter, discrete monophialides (phialides with only a single fertile opening) that are subulate (awl or candle shaped, i.e., rigid looking, tapered, and narrow at the apex) or inflated, while some species form polyphialides, in which each phialide has several fertile openings. Monophialides tend to be strongly visibly differentiated with respect to the subtending cells they are attached to; they seldom occur as scarcely differentiated,

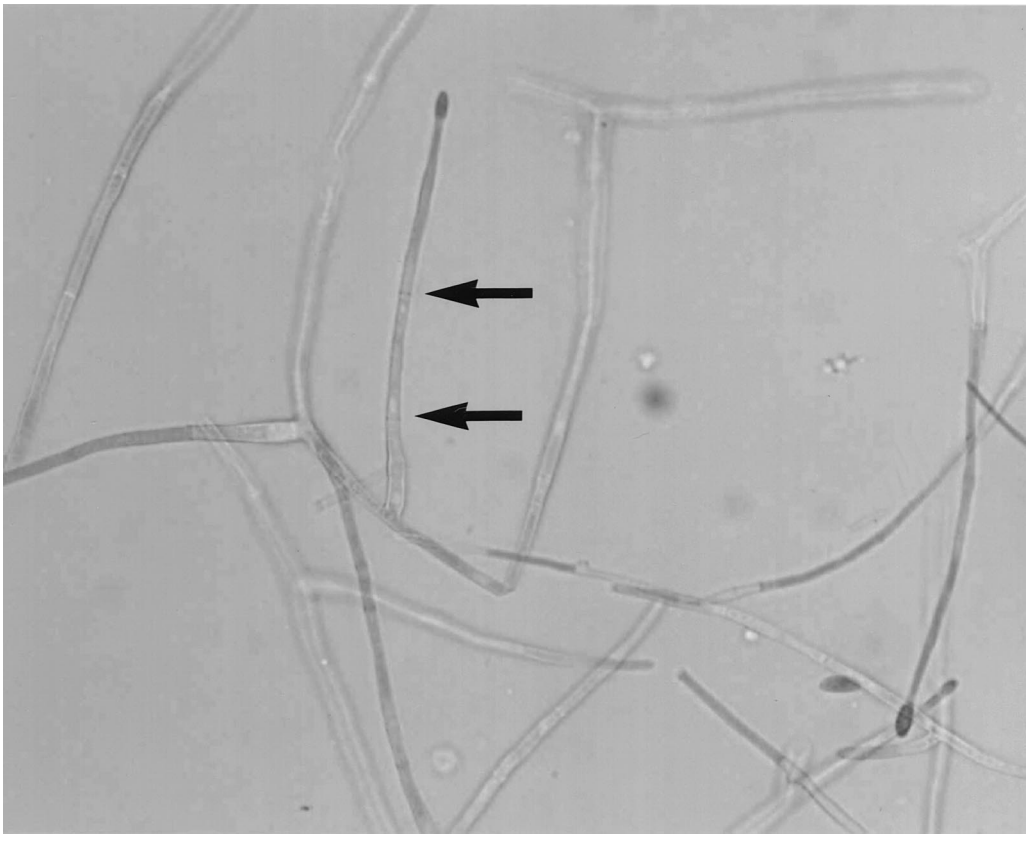

FIG. 4. Conidiophores of *A. falciforme.* The conidiophore at the center has two septa (arrows); comparable structures in typical *Acremonium* species in the family Bionectriaceae (e.g., *Acremonium kiliense*, *Acremonium strictum*, *Acremonium alternatum*) would have no septum or, less commonly, one septum (10). Magnification, ×1,400.

integrated end cells on extended filiform conidiophores, as is common with phialides in the *F. solani* clade. In conidial morphology, *F. solani* shows a classical fusarial macroconidial form (curved phragmoconidia with somewhat pointed apices and basal foot cells), while *F. lichenicola* does not; and *F. falciforme* may vary from strain to strain in this regard. Above and beyond these differences, however, all these species have two- to several-celled conidia that are significantly blunter at the apex and proportionately broader than the macroconidia of fusaria in the *Gibberella* clade. The macroconidia of *F. lichenicola* have lost their curving aspect and their heel-and-toe-shaped fusarial foot cell, but *F. solani* itself is only moderately curved and the foot cells of many isolates are minimally differentiated. While all these species are variable in terms of their colony colorations, similar chestnut red-brown to purplish reverse pigments are seen in many representatives of all three species (2, 8, 10, 12). The violet-purple reverse color that may be seen in *F. falciforme* overlaps with that seen in some *F. solani* isolates.

A similar deep blue-purple color is also seen in *Fusarium coeruleum*, a species formerly called *F. solani* var. *coeruleum* and considered to be closely related to or conspecific with *F. solani.* This species was also once recorded as an agent of mycetoma in Thailand (55). The record, however, is dubious, as molecularly verified isolates of this species are strongly distinct from *F. solani* and are known to have been isolated only from potatoes (22). The description given for the isolate from the patient with mycetoma is also compatible with a strongly purple colored *F. solani* isolate, and it is possible that this case represents yet another case of *F. solani* mycetoma.

For molecular identification, there is a strong need to know which species are and are not true fusaria in order to allow the design of diagnostic primers and probes and to allow meaningful interpretation of related results. For example, the ribosomal primers recently published by Hue et al. (23) are specific for a range of *Fusarium* species spanning both the *Gibberella* and *F. solani* clades and would be strongly predicted to give a positive result with *F. falciforme* and *F. lichenicola*. On the other hand, the finding of those investigators that "*Fusarium nivale*" gave a positive reaction can be deduced as being based on a misidentified isolate, since the species formerly bearing this name, *Microdochium nivale*, is now known to belong to the family Hyponectriaceae, order Xylariales (9), a fungal group much less closely related to *Fusarium* (family Nectriaceae) than most of the species that gave negative reactions with the primer set. As an obligate psychrophile not growing at 35°C, *M. nivale* is almost certainly never pathogenic for mammals. Rationalization of the taxonomy of the fusaria, then, is essential for increasing both diagnostic accuracy and epidemiological understanding of this important group of emerging pathogens.

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