JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2011, p. 1209–1216 Vol. 49, No. 4
0095-1137/11/$12.00 doi:10.1128/JCM.01979-10

Molecular and Phenotypic Characterization of *Phialemonium* and *Lecythophora* Isolates from Clinical Samples▽

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Received 30 September 2010/Returned for modification 9 December 2010/Accepted 13 January 2011

Several members of the fungal genera *Phialemonium* and *Lecythophora* are occasional agents of severe human and animal infections. These species are difficult to identify, and relatively little is known about their frequency in the clinical setting. The objective of this study was to characterize morphologically and molecularly, on the basis of the analysis of large-subunit ribosomal DNA sequences, a set of 68 clinical isolates presumed to belong to these genera. A total of 59 isolates were determined to be *Phialemonium* species ($n = 32$) or a related *Cephalotheca* species ($n = 6$) or *Lecythophora* species ($n = 20$) or a related *Coniochaeta* species ($n = 1$). Nine isolates identified to be *Acremonium* spp. or *Phaeoacremonium* spp. were excluded from further study. The most common species were *Phialemonium obovatum* and *Phialemonium curvatum*, followed by *Lecythophora hoffmannii*, *Cephalotheca foveolata*, and *Lecythophora mutabilis*.

A wide range of fungi are able to cause opportunistic infections in immunocompromised patients. Apart from the genera *Aspergillus*, *Scedosporium*, and *Fusarium*, which are the most common filamentous fungal opportunists, many other genera, including *Phialemonium* and *Lecythophora*, are also involved in human infections, often with a fatal outcome (7, 19). *Phialemonium* and *Lecythophora* species are poorly differentiated morphologically, are difficult to identify, and may be confused with poorly sporulating *Fusarium* or *Acremonium* species. With the increasing number of susceptible, immunocompromised hosts, the early recognition of these potentially lethal fungi has become important in providing appropriate patient management (12, 20).

Phialemonium species are widely distributed in the environment, having been isolated from air, soil, industrial water, and sewage (9). Although they show pale colonies *in vitro*, melanin can be demonstrated in their cell wall by Fontana-Masson stain; for this reason, they are considered dematiaceous fungi (11, 33). The majority of *Phialemonium* infections are invasive, affecting both immunocompromised and immunocompetent patients, and the more frequent infections include peritonitis, endocarditis, osteomyelitis, and cutaneous infections of wounds following burns (7, 12, 15, 20, 23).

The genus *Phialemonium* was described by Gams and McGinnis (9) to accommodate filamentous fungi with morphological features between those of *Acremonium* and *Phialophora*. It is characterized by moist colonies with abundant adelophialides (reduced phialides that are not delimited from subtending intercalary hyphal cells by a basal septum) and less common discrete phialides (phialides clearly differentiated from the subtended hyphae and separated by a basal septum), with neither having conspicuous collarettes and with both producing conidia aggregate in slimy heads. On the basis of the conidial shape and the color of the colonies, three species have been described: *Phialemonium obovatum*, *P. curvatum*, and *P. dimorphosporum* (9). *Phialemonium obovatum* has greenish colonies and obovate conidia, *P. curvatum* typically has white to grayish colonies and allantoid conidia, and *P. dimorphosporum* was described as having grayish colonies with obovate or ellipsoidal conidia (7, 9, 15). Subsequently, *P. dimorphosporum*, on the basis of restriction fragment length polymorphism analysis, was reduced to synonymy with *P. curvatum*; the morphologies ascribed to both species apply to *P. curvatum* (12). Recently, the new species *Cephalotheca foveolata* (family Cephalothecaceae), molecularly and morphologically related to *P. obovatum*, was reported to be the cause of a subcutaneous infection in a patient from South Korea (34).

The genus *Lecythophora* is morphologically similar to *Phialemonium*. It also forms adelophialides, but in *Lecythophora*, these conidiogenous cells show conspicuous collarettes, and the colonies usually are pink-salmon to dark brown, although discrete phialides like those of *Acremonium* may be also present (7, 9, 23, 30). Recently, molecular studies confirmed that the six species included in *Lecythophora* are anamorphs of *Coniochaeta*, an ascomycete genus belonging to the family Coniochaetaceae (Sordariomycetes) (6, 13, 31). Although these fungi are saprobes, some species have also been involved in human disease. *Lecythophora hoffmannii* has been associated with cases of subcutaneous infections, keratitis, sinusitis, peritonitis, and canine osteomyelitis (16, 21). *Lecythophora mutabilis* has been described to be the causative agent of human peritonitis, endocarditis, endophthalmitis, and keratitis, among others (2, 7, 8, 22, 28).

Due to the difficulties in the identification of the members of these two genera, their real incidence in human infections and the management of such infections are poorly known.

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▽ Published ahead of print on 26 January 2011.

TABLE 1. *Phialemonium* and *Lecythophora* clinical isolates and types or reference strains of related species included in the study

Isolate[a]	Species	Origin	GenBank accession no. (D1/D2 rDNA)
UTHSC 01-20 (1)	*Lecythophora* sp. 1	Leg wound, MA	
UTHSC 01-20 (2)	*Lecythophora hoffmannii*	Leg wound, MA	FR745935
UTHSC 01-317	*Phialemonium obovatum*	Graft tissue, WA	FR745951
UTHSC 01-590	*Phialemonium obovatum*	Toenail, TX	FR691999
UTHSC 01-1399	*Phialemonium obovatum*	Abscess, MD	FR745945
UTHSC 01-1644	*Lecythophora* sp. 1	Canine bone marrow aspirate, AL	
UTHSC 01-1664	*Lecythophora* sp. 4	Arm wound, CA	
UTHSC 02-294	*Phialemonium obovatum*	Blood, TX	FR745947
UTHSC 02-875	*Phialemonium obovatum*	Blood, TX	FR745948
UTHSC 02-1109	*Lecythophora hoffmannii*	Finger cyst fluid, CO	FR691983
UTHSC 02-1327	*Coniochaeta prunicola*	Canine skin mass, NY	FR691989
UTHSC 02-2050	*Phialemonium curvatum*	Forearm, FL	FR691978
UTHSC 03-1149	*Lecythophora lignicola*	Fingernail, PA	FR745938
UTHSC 03-1890	*Lecythophora hoffmannii*	Canine osteomyelitis, CA	FR745936
UTHSC 03-2258	*Phialemonium obovatum*	Arm, UT	FR745950
UTHSC 03-2653	*Cephalotheca foveolata*	Endocarditis, Singapore	FR745941
UTHSC 03-3574	*Phialemonium obovatum*	Blood, VA	FR692002
UTHSC 03-3588	*Phialemonium obovatum*	Bronchial fluid, TX	FR745946
UTHSC 03-3661	*Phialemonium* sp. 2	Sinus, IL	
UTHSC 04-350	*Phialemonium curvatum*	Synovial fluid, OH	FR691979
UTHSC 04-616	*Phialemonium obovatum*	Arm, TX	FR745944
UTHSC 04-956	*Phialemonium curvatum*	Sinus, MN	FR745926
UTHSC 05-970	*Lecythophora mutabilis*	Aortic valve, MA	FR691992
UTHSC 05-2527	*Phialemonium* sp. 1	Peritoneal dialysis catheter, PA	
UTHSC 05-2926	*Lecythophora* sp. 4	Urine, TX	
UTHSC 05-3214	*Lecythophora hoffmannii*	Vitreous fluid, OR	FR745934
UTHSC 06-733	*Cephalotheca foveolata*	Lymph node, TX	FR745943
UTHSC 06-1277	*Phialemonium obovatum*	Toenail, FL	FR745952
UTHSC 06-1465	*Phialemonium* sp. 1	Shin aspirate, SC	
UTHSC 06-1664	*Lecythophora mutabilis*	Vitreous fluid, MN	FR691991
UTHSC 06-1820	*Phialemonium* sp. 1	Corneal fluid, FL	
UTHSC 06-2147	*Phialemonium curvatum*	Nail, WA	FR745928
UTHSC 06-4324	*Phialemonium curvatum*	Canine pleural fluid, TX	FR691981
UTHSC 07-11	*Phialemonium curvatum*	CSF,[b] MN	FR745929
UTHSC 07-1284	*Phialemonium curvatum*	Toenail, SC	FR691980
UTHSC 07-1556	*Cephalotheca foveolata*	Bronchial fluid, SC	FR691996
UTHSC 07-1957	*Lecythophora* sp. 2	Epidural fluid, MO	
UTHSC 07-2087	*Cephalotheca foveolata*	Lymph node, TX	FR745942
UTHSC 07-2173	*Phialemonium curvatum*	Vitreous fluid, NV	FR745930
UTHSC 07-2959	*Lecythophora mutabilis*	Elbow, AR	FR745940
UTHSC 07-3574	*Cephalotheca foveolata*	Eyes, OH	FR691994
UTHSC 07-3736	*Phialemonium* sp. 1	Left hand, FL	
UTHSC 08-185	*Lecythophora mutabilis*	Nail, PA	FR745939
UTHSC 08-693	*Phialemonium curvatum*	CSF, TX	FR745932
UTHSC 08-851	*Lecythophora hoffmannii*	Eye, MN	FR691984
UTHSC 08-1239	*Lecythophora hoffmannii*	Canine urine, CA	FR745937
UTHSC 08-2292	*Phialemonium curvatum*	Blood, UT	FR745925
UTHSC 08-2569	*Lecythophora hoffmannii*	Bronchial fluid, MI	FR691985
UTHSC 08-2766	*Cephalotheca foveolata*	Eye capsule, NC	FR691995
UTHSC 08-3008	*Lecythophora mutabilis*	Leg, MN	FR691993
UTHSC 08-3010	*Lecythophora hoffmannii*	Bone, CA	FR745933
UTHSC 08-3486	*Phialemonium obovatum*	Canine urine, CA	FR692000
UTHSC 08-3696	*Phialemonium obovatum*	Blood, TX	FR745949
UTHSC 09-597 (2)	*Phialemonium obovatum*	Tissue, MN	FR692001
UTHSC 09-845	*Phialemonium obovatum*	Endocarditis, NC	FR691998
UTHSC 09-1440	*Lecythophora* sp. 3	Respiratory, CA	
UTHSC 09-2358	*Phialemonium* sp. 1	Aspirate cellulitis, MA	
UTHSC R-3447	*Phialemonium curvatum*	Eye, Israel	FR745927
UTHSC R-3448	*Phialemonium curvatum*	Eye, Israel	FR745931
CBS 135.34	*Cephalotheca sulfurea*	Garden cane, United Kingdom	AB189153[c]
CBS 140.41	*Lecythophora hoffmannii*	Water pollution, United Kingdom	AF353600[c]
CBS 153.42[T]	*Lecythophora decumbens*	Fruit, Netherlands	
CBS 157.44[T]	*Lecythophora mutabilis*	River water, Germany	FR691990
CBS 205.38[T]	*Lecythophora fasciculata*	Butter, Switzerland	FR691988
CBS 206.38[T]	*Lecythophora luteoviridis*	Butter, Switzerland	FR691987
CBS 206.73	*Lecythophora mutabilis*	Aorta and mitral valve, USA	AB261979[c]

Continued on following page

TABLE 1—*Continued*

Isolate[a]	Species	Origin	GenBank accession no. (D1/D2 rDNA)
CBS 245.38^T	*Lecythophora hoffmannii*	Butter, Switzerland	FR691982
CBS 267.33^T	*Lecythophora lignicola*	Sweden	FR691986
CBS 279.76^T	*Phialemonium obovatum*	Systemic infection, USA	FR691997
CBS 457.88	*Albertiniella polyporicola*	*Ganoderma applanatum*, Germany	AF096185[c]
CBS 491. 82^T	*Phialemonium dimorphosporum*	Soil, USA	FR691976
CBS 490.82^T	*Phialemonium curvatum*	Skin lesion, USA	FR691977
CBS 508.70^T	*Cryptendoxyla hypophloia*	Wood, Canada	AB191032[c]
CBS 551.75	*Coniochaeta subcorticalis*	Wood, Norway	AF353593[c]
CBS 730.97	*Phialemonium obovatum*	Peritoneal dialysis fluid, USA	
CBS 109872^T	*Coniochaeta rhopalochaeta*	Wood, Argentina	GQ351561[c]
CBS 110467	*Coniochaeta ligniaria*	Wood, Germany	AF353583[c]
CBS 120874	*Coniochaeta velutina*	*Prunus salicina*, South Africa	GQ154604[c]
CBS 120875^T	*Coniochaeta prunicola*	*Prunus armeniaca*, South Africa	GQ1546031[c]
E. W. 95.605	*Coniochaeta ligniaria*	Wood, Germany	AF353584[c]
HKUCC 2984	*Neolinocarpon globosicarpum*		DQ810224[c]
HKUCC 2954	*Linocarpon livistonae*		DQ810206[c]
HKUCC2983	*Neolinocarpon enshiense*		DQ810221[c]
NBRC 100905^T	*Cephalotheca foveolata*	Subcutaneous infection, South Korea	AB178269[c]

[a] CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; E. W., collection Evi Weber; HKUCC, University of Hong Kong Culture Collection; NBRC, NITE Biological Resource Center, Japan; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio. ^{T}, type strain. Numbers in parentheses represent isolate identification numbers of clinical samples.
[b] CSF, cerebrospinal fluid.
[c] Sequences retrieved from GenBank database.

Up to now, studies involving members of these genera isolated from clinical samples have been scarce, and generally, only a few isolates from medical cases have been molecularly or morphologically characterized. To evaluate the potential incidence of the different species of *Phialemonium* and *Lecythophora* in human infections, we have studied a large number of isolates of both genera from clinical samples received in a U.S. fungal reference laboratory during the period from 2001 to 2009. It is important to note that we lack sufficient clinical information to ascertain whether any of these isolates was confirmed to be the causal agent of infection. In order to provide the reliable identification of such isolates, apart from the morphological study, sequences of the D1/D2 domains of the 28S ribosomal DNA (rDNA) were also analyzed. Several molecular studies have demonstrated that this locus is a highly informative phylogenetic marker for these genera and its relatives (1, 6, 31, 34).

MATERIALS AND METHODS

Fungal isolates. A total of 68 clinical isolates, presumably belonging to the genera *Phialemonium* and *Lecythophora*, received in the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSC) for identification and/or antifungal susceptibility determination were included in this study (Table 1). In addition, 10 reference or type strains of different species of these genera, mainly provided by the Centraalbureau voor Schimmelcultures (CBS; Utrecht, Netherlands), were also tested.

Morphological study. Morphological features of the isolates were examined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain), oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 liter distilled water), and water agar with sterilized plant material (small pieces of wood, filter paper, herbaceous leaves) to enhance the formation of ascomata (fruit bodies of the sexual state, or teleomorph) or conidiomata (fruit bodies of the asexual state, or anamorph). Cultures were incubated at room temperature (25°C ± 2°C) for up to 2 months. The isolates were identified using the criteria given by Damm et al. (6), De Hoog et al. (7), Gams and McGinnis (9), Weber (30), and Yaguchi et al. (34). Microscopic features were examined by making direct wet mounts with 85% lactic acid and lactophenol cotton blue, using light microscopy. Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope, using phase contrast and Nomarski differential interference.

Sequencing and analysis. Isolates were grown on yeast extract sucrose (YES; yeast extract, 2%; sucrose, 15%; agar, 2%; water, 1 liter) for 3 to 5 days at 25°C ± 2°C, and DNA was extracted using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA), according to the manufacturer's protocol. The DNA was quantified by use of the GeneQuant*pro* calculator (Amersham Pharmacia Biotech, Cambridge, England). The D1/D2 domains of the 28S rDNA were amplified and sequenced with the primer pair NL1 and NL4, following the protocols described by Cano et al. (4). Sequences of clinical isolates were compared with those of type strains, when available, or reference strains, using the GeneDoc (version 2.6) program (17). A total of 84 sequences, including 15 GenBank sequences of species morphologically similar to some of the clinical isolates previously identified, were analyzed (Table 1). A sequence of *Aureobasidium pullulans* was used as the outgroup. Before this analysis, the sequences were aligned with the ClustalX (version 1.81) computer program (29), followed by manual adjustments with a text editor. The phylogenetic analyses were performed with the software program MEGA, version 4.0 (27). The neighbor-joining method and the Kimura two-parameter algorithm were used to obtain the distance tree. Gaps were treated as pair-wise deletions. Support for internal branches was assessed by a search of 1,000 bootstrapped sets of data.

Antifungal susceptibility. We evaluated the *in vitro* activities of amphotericin B (AMB), anidulafungin (ANID), caspofungin (CAS), fluconazole (FLC), 5-fluorocytosine (5FC), itraconazole (ITC), micafungin (MICA), natamycin (NAT), posaconazole (PSC), terbinafine (TRB), and voriconazole (VRC) against 45 fungal isolates (Table 2) (5). Isolates were grown on PDA slants until they were mature. The inoculum turbidity was standardized spectrophotometrically (530 nm) to yield a final inoculum concentration of 0.4×10^4 to 5×10^4 CFU/ml. Tests were incubated at 35°C, with results being read at both 48- and 72-h endpoints. The MIC and minimum effective concentration (MEC) were defined as described by Perdomo et al. (18).

RESULTS

The 68 clinical isolates were morphologically identified, and their identities were molecularly confirmed to the genus level by a BLAST search. Of these, 32 belonged to *Phialemonium*, 20 to *Lecythophora*, 6 to *Cephalotheca*, 1 to *Coniochaeta*, 5 to *Acremonium*, and 4 to *Phaeoacremonium*. Isolates of the last two genera were excluded from the phylogenetic analysis.

TABLE 2. Results of *in vitro* antifungal susceptibility testing read at 48 and 72 h

Species (no. of isolates)	Drug concn (μg/ml) read at 48/72 h[a]									
	AMB		ITC		PSC		VRC		TRB	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Phialemonium curvatum (12)	1/2	2/4	0.5/0.5	8/1	0.06/0.25	1/0.5	0.25/0.5	2/2	0.25/1	1/1
Phialemonium obovatum (14)	16/>16	>16/—	1/1	1/2	0.5/1	1/2	0.25/1	1/2	0.06/0.06	0.125/0.125
Lecythophora hoffmannii (8)	0.5/1	0.5/2	0.25/0.5	1/0.5	0.125/0.25	0.5/1	0.5/1	0.5/1	0.06/0.25	0.25/0.5
Lecythophora mutabilis (5)	0.125/0.25	0.125/0.5	0.125/0.5	0.125/0.5	0.125/0.25	0.125/0.5	0.25/1	0.25/1	0.125/0.5	0.125/0.5
Cephalotheca foveolata (6)	8/16	16/>16	1/1	1/1	1/1	1/1	1/2	2/2	0.06/0.25	0.25/0.5

[a] —, no reading.

Figure 1 shows the neighbor-joining tree of the D1/D2 domains of 84 sequences, i.e., 59 belonging to the clinical isolates, 10 to type or reference strains, and 15 sequences retrieved from GenBank. The aligned sequence regions consisted of 452 bp. Three main clades (I to III), each supported by a high bootstrap value (bs), were observed in the phylogenetic tree.

Clade I, divided into two subclades, included members of an uncertain higher taxonomic position (*incertae sedis*). In the first subclade (98% bs), 12 clinical isolates morphologically identified to be *P. curvatum* grouped with the type strains of *P. curvatum* (CBS 490.82) and *P. dimorphosporum* (CBS 491.82), which is considered a synonym. These fungi were characterized by smooth or finely floccose, white colonies becoming brown at maturity on OA, short and cylindrical adelophialides, and less commonly, long, tapering discrete phialides. Inconspicuous collarettes could be observed on both types of phialides (Fig. 2A to C). The conidia were hyaline and cylindrical to curved (Fig. 2D). Chlamydospores were not observed. Five of these isolates developed sporodochial conidiomata, which are cushion-shaped structures composed of well-differentiated, straight or flexuous, irregularly branched conidiophores, with stiff-haired setae forming a margin frill and with slimy conidia covering the entire upper surface of the conidioma (Fig. 2E). The second subclade (92% bs) included 5 *Phialemonium* sp. 1 clinical isolates morphologically similar to *P. curvatum*; however, on all culture media tested, they produced blackish, spherical, and closed conidiomata which opened irregularly in the apical part, liberating masses of conidia when they were mature. A BLAST search demonstrated a high degree of similarity of these fungi with the ascomycetes *Linocarpon livistonae* (GenBank accession number DQ810206), *Neolinocarpon enshiense* (GenBank accession number DQ810221), and *Neolinocarpon globosicarpum* (GenBank accession number DQ810224).

Clade II (94% bs) encompassed members of the family Coniochaetaceae, including species of *Coniochaeta* and *Lecythophora* and numerous clinical isolates. Eight clinical isolates and the type strain of *L. hoffmannii* (CBS 245.38) formed a well-supported terminal branch (98% bs). The most distinctive morphological features of these *L. hoffmannii* isolates were the production of slimy, orange to salmon-colored colonies on PDA, short adelophialides with cylindrical collarettes (Fig. 2F), and less frequently, discrete phialides with a wide base and a narrower apical part (ventricose phialides). Some collarettes were formed laterally, directly on the vegetative hyphae (Fig. 2G). The conidia were hyaline and broadly ellipsoidal to cylindrical or allantoid (Fig. 2 H). Two clinical isolates (UTHSC 01-20 [sp. 1] and UTHSC 01-1644) were included in a close terminal branch identified as *Lecythophora* sp. 1. In another terminal branch, isolate UTHSC 03-1149 nested with the type strains of *Lecythophora lignicola* (CBS 267.33) and *Lecythophora luteoviridis* (CBS 206.38). This isolate showed 100% identity with the type strain of *L. lignicola* and was also morphologically very similar in producing discrete ventricose phialides with rather long collarettes (Fig. 2I to K) and lacking chlamydospores. Although *L. luteoviridis* also showed a close genetic relationship with *L. lignicola*, it differed from *L. lignicola* by developing globose, oblong or pear-shaped, and hyaline or faintly brown chlamydospores in young culture. In another terminal branch (98% bs), isolate UTHSC 02-1327 nested with a sequence of the type strain of the recently described ascomycete *Coniochaeta prunicola* (6). The clinical isolate was confirmed to be this species by development of ascomata on water agar with plant material and by development of similar conidial structures, including discrete ampulliform phialides, often constricted at the basal septum, and hyaline, mainly allantoid conidia.

Five clinical isolates were grouped with the type strain of *L. mutabilis* (CBS 157.44) in another terminal branch (95% bs). The most remarkable morphological feature of this group was the production of flat, moist, dark to olivaceous-brown colonies on OA, due to the presence of abundant chlamydospores, which are lateral or terminal, ovoidal to ellipsoidal, thick walled, olivaceous-brown, and never in chains (Fig. 2L to O). The basal subclade of clade II was represented by four clinical isolates (UTHSC 07-1957, UTHSC 09-1440, UTHSC 05-2926, and UTHSC 01-1664), plus sequences of *Coniochaeta rhopalochaeta* and *C. subcorticalis*. These clinical isolates, named *Lecythophora* sp. 2 to 4 in the tree, were morphologically heterogeneous, and their characteristics did not fit with those of any of the known *Lecythophora* species. Common morphological features observed among them were the production of dark colonies on OA and terminal or intercalary, pigmented chlamydospores forming short chains and the absence of ascomata.

Clade III (99% bs) included members of the family Cephalothecaceae. Clinical isolate UTHSC 03-3661, identified to be *Phialemonium* sp. 2, grouped with *Cephalotheca sulfurea* (93% bs), the type species of the genus *Cephalotheca*; however, ascomata were not produced in any of the culture media tested. All six clinical isolates which grouped with the type strain of *C. foveolata* in another subclade (99% bs) developed the teleomorph, characterized by blackish cleistothecial ascomata with a cephalothecoid wall (Fig. 2P), 8-spored asci, and ascospores

TABLE 2—*Continued*

Drug concn (μg/ml) read at 48/72 h[a]											
5-FC		FLC		NAT		MICA		ANID		CAS	
MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MEC_{50}	MEC_{90}	MEC_{50}	MEC_{90}	MEC_{50}	MEC_{90}
>64/—	>64/—	8/8	>64/64	4/4	8/8	0.5/1	>8/—	0.5/1	>16/—	2/>8	>16/>8
>64/—	>64/—	8/16	16/32	4/4	4/4	0.03/0.125	0.125/0.125	0.25/0.25	0.25/0.25	0.25/0.5	0.5/0.5
64/>64	>64/—	8/16	8/16	4/4	4/4	4/>8	>8/—	2/>8	>8/—	2/2	4/>8
2/>64	16/>64	8/16	8/16	4/4	4/4	>8/—	>8/—	2/>8	>8/>8	4/>8	4/>8
>64/—	>64/—	8/16	64/32	4/4	4/4	0.25/0.25	0.5/0.5	0.25/0.25	0.5/0.25	1/1	1/1

that were broadly ellipsoidal to obovate in superficial view and reniform in lateral view with a foveolate wall. The anamorph was characterized by flat, moist, white to yellowish white (later becoming brownish) colonies on OA, with abundant adelophialides and less common discrete phialides, both with a wide base and without collarettes (Fig. 2Q). The conidia were cylindrical and hyaline. These isolates showed globose to ellipsoidal, thick-walled, hyaline, terminal, or intercalary chlamydospores (Fig. 2R). The third subclade (98% bs) included *P. obovatum* and 14 clinical isolates with the typical morphological features of the mentioned species. They were characterized by moist to slightly floccose and pale ochraceous to greenish colonies on OA. The adelophialides had a wide base, were more common than discrete phialides, and lacked visible collarettes (Fig. 2S). The conidia were hyaline and obovate with a truncate base (Fig. 2T). Oval, thin-walled, hyaline chlamydospores were also present (Fig. 2U). Most of these isolates, in old cultures, developed globose and dark structures which were suggestive of immature ascomata, although they never reached maturity (Fig. 2V).

In vitro antifungal susceptibility data varied between the various genera and species (Table 2). As there are no established guidelines for these genera, interpretive comments are based upon achievable serum concentrations using standard dosing regimens and are extrapolated from *Candida* breakpoints. As expected, all isolates displayed high MICs to 5FC and FLC. *Phialemonium* species and *Cephalotheca foveolata* appeared to be resistant to AMB. Most strains exhibited low MICs to PSC, VRC, and TRB, while only *P. obovatum* and *C. foveolata* demonstrated low MECs for the echinocandin agents MICA, ANID, and CAS. ITC data varied by species, and results for NAT, a topical ophthalmic polyene, are at the lower end of the range (2 to 32 μg/ml) typically seen for filamentous fungi (Fungus Testing Laboratory, unpublished data).

DISCUSSION

Some studies have clearly demonstrated the ability of *Phialemonium* and *Lecythophora* species to cause humans infections. However, the scarcity of case reports could be related to the misidentification of clinical isolates due to the morphological similarities of these fungi with other more common genera in human infections, such as *Fusarium* and *Acremonium*. The fact that 59 clinical isolates mainly belonging to *Phialemonium* and *Lecythophora* were received in a reference clinical center over the last 9 years showed that species of both genera are relatively common in clinical samples. However, the most recently published review of *Phialemonium* infections summarized only 20 cases of localized or disseminated infections that have been reported since 1986, of which 5 were caused by *P. obovatum* and 15 by *P. curvatum* (20). The number of clinical cases reporting infections by *Lecythophora* is even lower. Cases are caused by either *L. hoffmannii* or *L. mutabilis* (2, 7, 8, 16, 21, 22, 28). In our study, the above-mentioned two *Phialemonium* species and two *Lecythophora* species were also the predominant species identified. However, it is noteworthy that 6 isolates were identified to be *C. foveolata*, which is a recently described species involved in a case of human infection (25). Although we could not confirm the role of any of these *C. foveolata* isolates as agents of infection, the number found might reflect the possible clinical relevance of this fungus.

Our results confirmed the synonymy of *P. dimorphosporum* with *P. curvatum* (12) and that the two accepted species of *Phialemonium*, *P. curvatum* and *P. obovatum*, are genetically very distant from each other (34). *Phialemonium obovatum*, the type species of the genus, is more closely related to *C. foveolata* of the family Cephalothecaceae, while *P. curvatum* is molecularly related to some species of *Linocarpon* and *Neolinocarpon*, which are *incertae sedis* genera within Sordariomycetes (3).

A relevant feature not originally described in *P. curvatum* was the formation of sporodochial conidiomata, structures that were present in five clinical isolates. Other authors had already reported the presence of these structures (19, 24, 26, 32). Some isolates of *Phialemonium* (*Phialemonium* sp. 1) presented a relatively low level of similarity (97%) with the sequences of the type strain of *P. curvatum*. First, these isolates were identified to be *P. curvatum* due to the presence of adelophialides and the morphology of their conidia. In culture, they developed sporodochial conidiomata similar to those of *P. curvatum*, but a distinctive feature was the production of black, spherical, and closed conidiomata, so far never described in *Phialemonium*. *Phialemonium* sp. 1 was phylogenetically close to some species of the ascomycetous genera *Linocarpon* and *Neolinocarpon*, which have been associated with *Phialophora*-like anamorphs (3, 14). However, further studies are necessary to establish the real relationship between *Phialemonium* and those genera.

Our study showed the association of *Lecythophora* species with *Coniochaeta*, previously demonstrated by others (6, 10, 31). The two species of *Lecythophora* more common in clinical samples, *L. hoffmannii* and *L. mutabilis*, were strongly statistically supported. Although *L. hoffmannii* had been stated to be the anamorph of *C. ligniaria* (6, 7), our study confirmed the

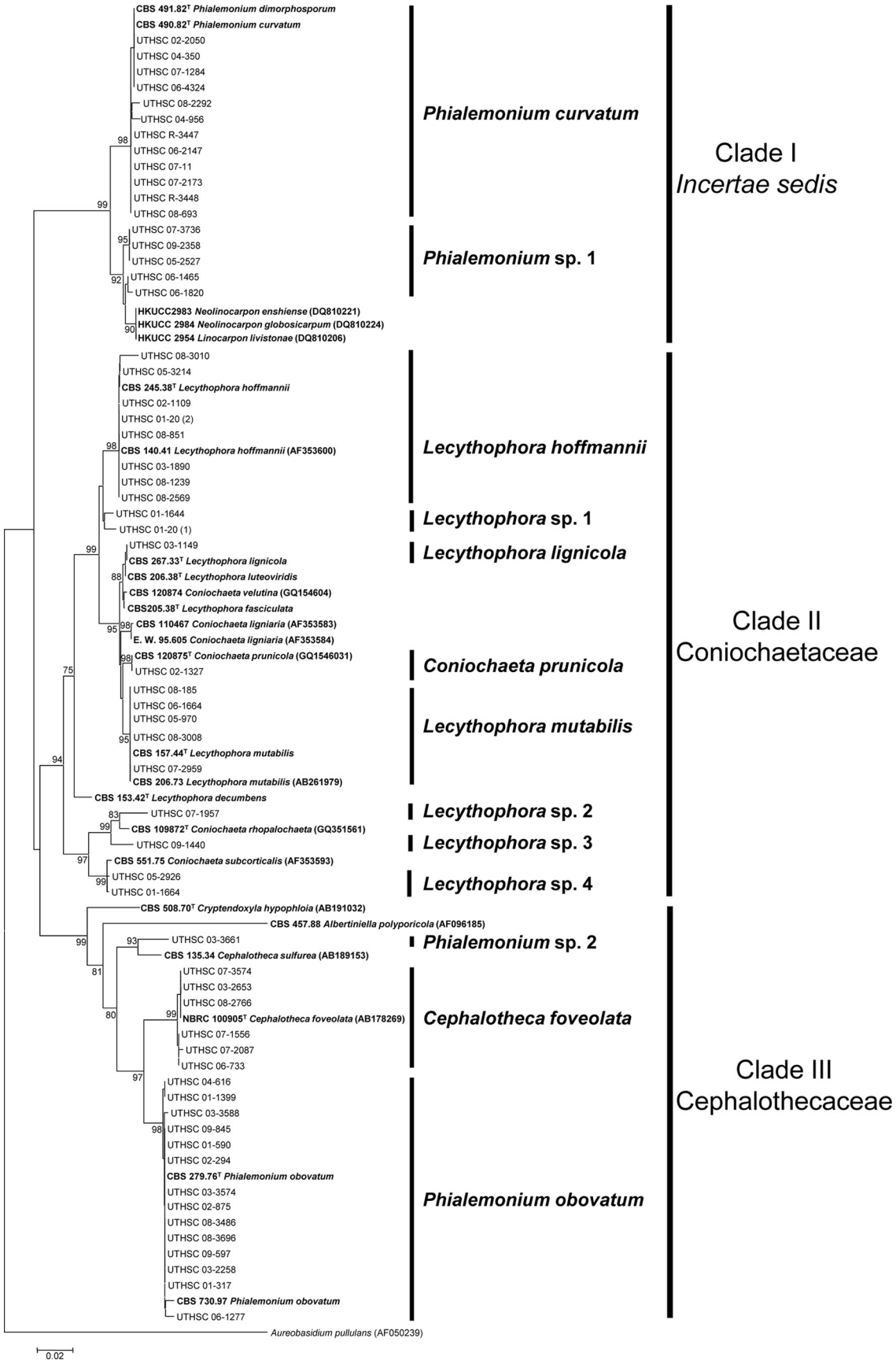

FIG. 1. Neighbor-joining tree among D1/D2 domains of the 28S rDNA sequences of the isolates included in Table 1. Branch lengths are proportional to distance. Sequences not generated in this study and obtained from the GenBank database are indicated in parentheses. Type and reference strains are indicated in boldface.

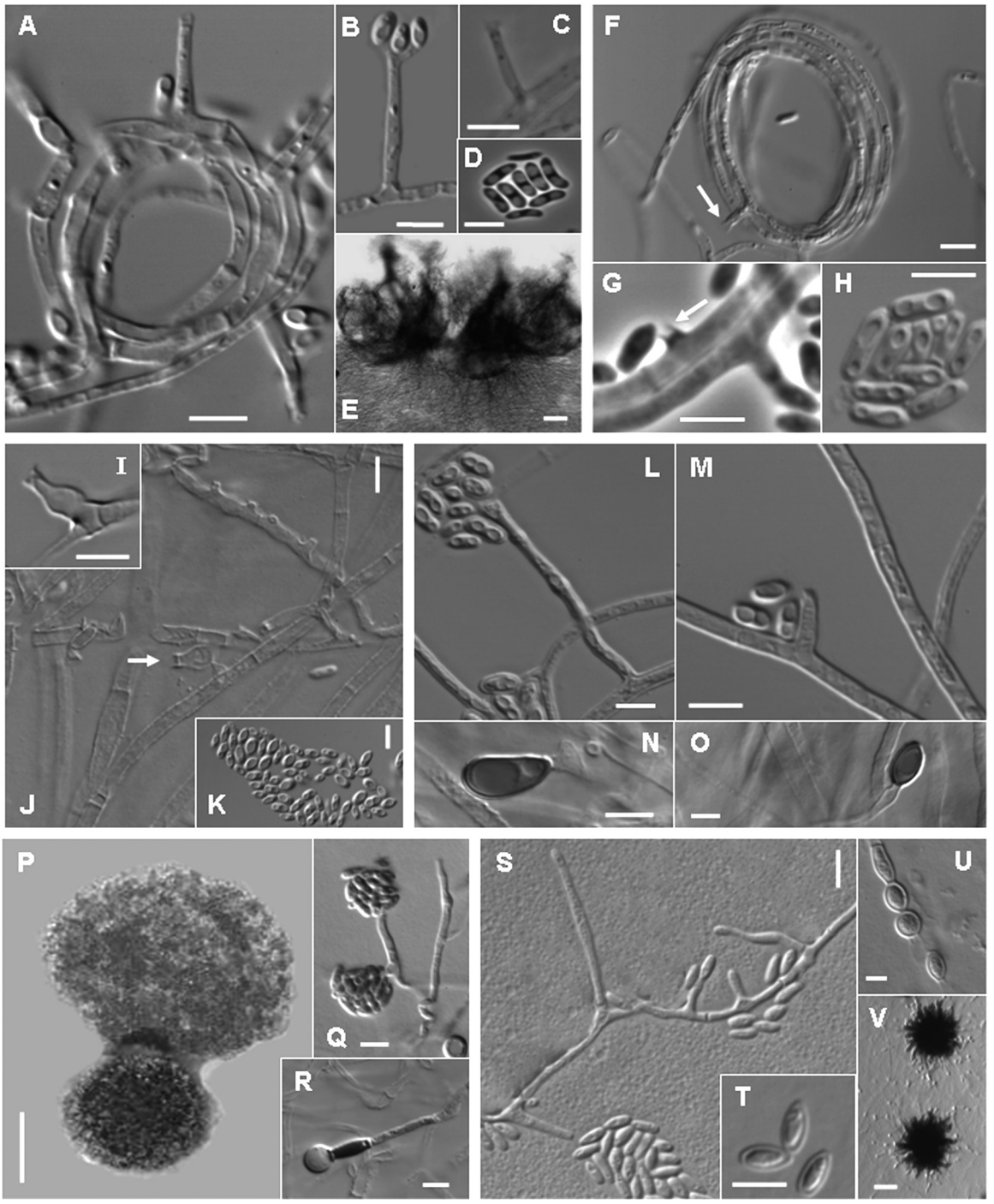

FIG. 2. (A to E) *Phialemonium curvatum* (CBS 490.82^T); (A) adelophialides; (B) conidia; (C) adelophialide with inconspicuous collarette; (D) conidia; (E) sporodochial conidioma. (F to H) *Lecythophora hoffmannii* (CBS 245.38^T); (F) adelophialides with cylindrical collarettes in a hyphal coil; (G) collarette formed directly on the hyphae and conidia; (H) conidia. (I to K) *Lecythophora lignicola* (CBS 267.33^T); (I) ventricose phialide; (J) adelophialide and discrete phialides; (K) conidia. (L to O) *Lecythophora mutabilis* (CBS 157.44^T); (L) phialide; (M) adelophialide; (N and O) chlamydospores. (P to R) *Cephalotheca foveolata* (UTHSC 08-2766); (P) ascoma with ascospores; (Q) adelophialide and conidia; (R) chlamydospore. (S to V) *Phialemonium obovatum* (CBS 279.76^T); (S) discrete phialides and adelophialides; (T) conidia; (U) chlamydospores; (V) inmature ascomata. Scale bars, A to D, F to O, and Q to U = 5 μm; V = 20 μm; E and P = 100 μm.

results of Weber et al. (31), who found that these species were different.

In conclusion, our results suggest that infections by *Phialemonium*, *Lecythophora*, and *Cephalotheca* may be underdiagnosed, since in this study the number of isolates belonging to these genera is considerably higher than that reflected in the literature. In addition, our results confirmed the usefulness of the 28S rDNA gene as a phylogenetic marker for these fungal groups.

ACKNOWLEDGMENTS

We are indebted to the curators of the Centraalbureau voor Schimmelcultures (Utrecht, Netherlands).

This work was supported by the Spanish Ministerio de Educación y Ciencia, grants CGL 2008-04226/BOS and CGL 2009-08698/BOS.

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