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Acrophialophora, a Poorly Known Fungus with Clinical Significance

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***Acrophialophora fusispora* is an emerging opportunistic fungus capable of causing human infections. The taxonomy of the genus is not yet resolved and, in order to facilitate identification of clinical specimens, we have studied a set of clinical and environmental *Acrophialophora* isolates by morphological and molecular analyses. This set included the available type strains of *Acrophialophora* species and similar fungi, some of which were considered by various authors to be synonyms of *A. fusispora*. Sequence analysis of the large subunit (LSU) and internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA and a fragment of the β-tubulin (*Tub*) gene revealed that *Acrophialophora* belongs in the family *Chaetomiaceae* and comprises three different species, i.e., *A. fusispora*, *Acrophialophora levis*, and *Acrophialophora seudatica*; the latter was previously included in the genus *Ampullifera*. The most prevalent species among clinical isolates was *A. levis* (72.7%), followed by *A. fusispora* (27.3%), both of which were isolated mostly from respiratory specimens (72.7%), as well as subcutaneous and corneal tissue samples. In general, of the eight antifungal drugs tested, voriconazole had the greatest *in vitro* activity, while all other agents showed poor *in vitro* activity against these fungi.**

Acrophialophora is a thermotolerant soil fungus that is widely distributed in temperate and tropical regions. Given its capacity to produce large quantities of cellulases and xylanases, it is also commonly isolated as a decomposer of compost and other self-heating substrates (1, 2).

The genus *Acrophialophora* was erected by Edward (3) with a single species, *Acrophialophora nainiana*. This fungus forms grayish colonies with a black reverse with age. Microscopically, it produces darkly pigmented, straight, septate, unbranched, setae-like conidiophores with thick verrucose walls, which are fertile toward the apex, and flask-shaped, hyaline phialides grouped in verticils. Single flask-shaped phialides are also formed directly from the aerial hyphae. *Acrophialophora* was not fully accepted as a distinct genus, however, until the work of Samson and Mahmood (4), who, after studying a large set of isolates, demonstrated that the aforementioned morphological features were stable, which supported the differentiation of *Acrophialophora* from morphologically similar genera such as *Paecilomyces* and *Masonia*. Those authors accepted three species, based mainly on the size, pigmentation, and ornamentation of their conidia; these species were *A. nainiana* (4 to 10.5 by 2 to 5 μm, hyaline, and finely echinulate), *Acrophialophora fusispora* (5 to 12 by 3 to 6 μm, brown, and finely echinulate, forming spiral bands), which had been described earlier as *Paecilomyces fusisporus* (4), and *Acrophialophora levis* (4.5 to 8 by 2 to 3.5 μm, hyaline, and smooth to slightly roughened). However, while Ellis (5) regarded *A. nainiana* as conspecific with *A. fusispora* and the latter as the type species of the genus, Al-Mohsen et al. (1) considered the three species synonyms and conserved the single species name *A. fusispora*. In this wide concept of the species, other taxa were considered conspecific with *A. fusispora*, i.e., *Masoniella indica* and *Ampullifera seudatica* (4).

Acrophialophora fusispora is currently recognized as an emerging human opportunistic pathogen (6, 7), responsible for cases of keratitis (6, 8, 9), pulmonary colonization and infection (6, 10–12), and devastating cerebral infections requiring intensive antifungal therapy (1, 13–15). Antifungal susceptibility data for *Acrophialophora* are scarce and based mostly on a few clinical reports (1, 15).

The species delimitation for *Acrophialophora*, using a modern phylogenetic approach, has not been properly revised, and the taxonomic position and boundaries of the genus are unknown. Therefore, we carried out a phenotypic and molecular study with a set of clinical and environmental isolates, including all of the available type strains of the species historically included in the genus. In addition, *in vitro* antifungal susceptibility testing was performed with eight clinically available antifungal agents against these isolates.

MATERIALS AND METHODS

Fungal isolates and sequences. A total of 39 isolates were included in this study, i.e., 32 from human clinical samples, 1 from an animal clinical sample, and 6 from environmental sources, including all of the available type strains of the genus (Table 1). Most of the clinical isolates were from the United States and were received by the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA) from different parts of the country. In addition, 39 sequences retrieved from GenBank or the National Institute of Technology and Evaluation Biological Resource Center (NBRC) (Chiba, Japan) database were included in the phylogenetic analyses.

Phenotypic studies. The isolates were grown on malt extract agar (MEA) (30 g of malt extract, 5 g of peptone, 15 g of agar, and 1 liter of distilled water) and oatmeal agar (OA) (30 g of filtered oat flakes, 20 g of agar, and 1 liter of distilled water). Colony features and growth rates were determined at 7 and 14 days of incubation at different temperatures (5, 15, 25, 35, 37, 40, 45, 50, and 52°C). Microscopic features were examined after 14 days of incubation at 25°C on both media, in wet mounts with 85% lactic acid, using light

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TABLE 1 Origin and GenBank accession numbers of the sequences of the *Acrophialophora* spp. included in this study

Species and strain[a]	Origin	GenBank accession no.		
		LSU	ITS	*Tub*
A. levis				
CBS 484.70 (type strain)	Germany, composted domestic waste	KM995840	KM995878	LN624419
FMR 6662 = CBS 120407	Spain, sputum	KM995841	KM995879	LN624420
FMR 12780	Spain, sputum	KM995842	KM995880	LN624421
UTHSCSA DI-13-134	USA, sputum	KM995843	KM995881	LN624422
UTHSCSA DI-13-137	USA, sputum	KM995844	KM995882	LN624423
UTHSCSA DI-13-138	USA, bronchoalveolar lavage fluid	KM995845	KM995883	LN624424
UTHSCSA DI-13-139	USA, bronchoalveolar lavage fluid	KM995846	KM995884	LN624425
UTHSCSA DI-13-142	USA, sputum	KM995847	KM995885	LN624426
UTHSCSA DI-13-144	USA, sputum	KM995848	KM995886	LN624427
UTHSCSA DI-13-145	USA, brain	KM995849	KM995887	LN624428
UTHSCSA DI-13-146	USA, bronchoalveolar lavage fluid	KM995850	KM995888	LN624429
UTHSCSA DI-13-147	USA, bronchoalveolar lavage fluid (canine)	KM995851	KM995889	LN624430
UTHSCSA DI-13-148	USA, lung, right upper lobe	KM995852	KM995890	LN624431
UTHSCSA DI-13-150	USA, sputum	KM995853	KM995891	LN624432
UTHSCSA DI-13-151	USA, sputum	KM995854	KM995892	LN624433
UTHSCSA DI-13-152	USA, leg tissue	KM995855	KM995893	LN624434
UTHSCSA DI-13-153	USA, tissue	KM995856	KM995894	LN624435
UTHSCSA DI-13-154	USA, bronchoalveolar lavage fluid	KM995857	KM995895	LN624436
UTHSCSA DI-13-155	USA, sputum	KM995858	KM995896	LN624437
UTHSCSA DI-13-156	USA, knee tissue	KM995859	KM995897	LN624438
UTHSCSA DI-13-157	USA, bronchoalveolar lavage fluid	KM995860	KM995898	LN624439
UTHSCSA DI-13-158	USA, sputum	KM995861	KM995899	LN624440
UTHSCSA DI-13-159	USA, bronchoalveolar lavage fluid	KM995862	KM995900	LN624441
UTHSCSA DI-13-162	USA, bronchoalveolar lavage fluid	KM995863	KM995901	LN624442
UTHSCSA DI-13-163	USA, bronchoalveolar lavage fluid	KM995864	KM995902	LN624443
A. fusispora				
CBS 100.60 (*A. nainiana* type strain)	India, farm soil	KM995865	KM995903	LN624444
CBS 149.64 (*M. indica* type strain)	India, forest soil	KM995866	KM995904	LN624445
CBS 380.55 (*P. fusisporus* type strain)	India, forest soil	KM995867	KM995905	LN624446
FMR 6258 = CBS 120406	India, soil	KM995868	KM995906	LN624447
FMR 8888 = CBS 120409	India, cornea	KM995869	KM995907	LN624448
UTHSCSA DI-13-135	USA, left sphenoid sinus	KM995870	KM995908	LN624449
UTHSCSA DI-13-136	USA, brain abscess	KM995871	KM995909	LN624450
UTHSCSA DI-13-140	USA, bronchoalveolar lavage fluid	KM995872	KM995910	LN624451
UTHSCSA DI-13-141	USA, sputum	KM995873	KM995911	LN624452
UTHSCSA DI-13-143	USA, chest mass	KM995874	KM995912	LN624453
UTHSCSA DI-13-149	USA, cornea	KM995875	KM995913	LN624454
UTHSCSA DI-13-160	USA, bronchoalveolar lavage fluid	KM995876	KM995914	LN624455
UTHSCSA DI-13-161	USA, sputum	KM995877	KM995915	LN624456
A. seudatica				
CBS 916.79 (*Ampullifera seudatica* type strain)	India, soil	LN736031	LN736030	LN736032

[a] CBS, Fungal Biodiversity Centre (Utrecht, The Netherlands) culture collection; FMR, Facultat de Medicina, Universitat Rovira i Virgili (Reus, Spain); UTHSCSA, Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (San Antonio, TX).

microscopy. All isolates were identified based on the features described by Edward (3), Samson and Mahmood (4), and Ellis (5). Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope, using phase-contrast and Nomarski differential interference optics.

DNA extraction, amplification, and sequencing. FastPrep kits (MP Biomedicals, Santa Ana, CA) were used to extract total genomic DNA from fungal mycelia harvested from colonies grown on potato dextrose agar (PDA) for 7 days at 25°C, according to the manufacturer's protocol. DNA was quantified using a Nanodrop 3000 apparatus (Thermo Scientific, Madrid, Spain).

Three nuclear DNA targets were amplified by PCR and sequenced using the following primer pairs: ITS4 and ITS5 (16) for a region spanning internal transcribed spacer 1 (ITS1) and ITS2 and the 5.8S gene of the ribosomal DNA (rDNA), LR0R and LR5 (17, 18) for a fraction of the 5′ end of the large subunit (LSU) gene of the rDNA, and BT2a and BT2b (19) for a fragment of the β-tubulin (*Tub*) gene. The amplified products were purified with the Diffinity RapidTip purification system (Sigma-Aldrich, St. Louis, MO) and stored at −20°C until sequencing.

Sequencing was performed in both directions, with the same primer pair as used for amplification, at Macrogen Europe (Macrogen Inc., Amsterdam, The Netherlands). The consensus sequences were obtained using SeqMan software (version 7.0.0; DNAStar Lasergene, Madison, WI).

Molecular identification and phylogenetic analysis. In order to assess the taxonomic position of the genus *Acrophialophora*, a first phylogenetic analysis was carried out using partial LSU sequences of the available type strains of *Acrophialophora* species complemented with 15 sequences retrieved from public databases, selected on the basis of BLAST homology searches and representing 8 different genera from the families *Chaetomi-*

aceae and *Sordariaceae*, of the subclass *Sordariomycetidae*. A second phylogenetic analysis directed to assessing the species distribution of *Acrophialophora* was conducted using partial LSU, ITS, and *Tub* sequences and included all of the type strains of *Acrophialophora* species, the type strains of the putative synonyms *Ampullifera seudatica* and *M. indica*, and several clinical and environmental isolates morphologically identified as *Acrophialophora* spp. Multiple sequence alignments were made for each individual locus using Mega version 6.06 (20), with the ClustalW function, and were manually refined when necessary. The nucleotide substitution models for each data set (GTR+G+I for LSU, JC+G for ITS, and T92+G for *Tub*) were calculated using the Find best DNA/protein model tool in Mega 6.06. In order to compare the concordance of the different loci, individual phylogenetic analyses were carried out using the maximum likelihood (ML) algorithm in Mega; the resulting trees were compared visually using a 70% bootstrap cutoff and were complemented with the partition homogeneity test, carried out as implemented in PAUP* software (version 4.0b10; Sinauer, Sunderland, MA). Since no incongruence was found ($P = 0.180$), the three genes were combined into a single data set. The combined phylogenetic analyses were made using maximum likelihood (ML) and Bayesian inference (BI) methods in Mega and MrBayes (version 3.1.2) (21), respectively. For the ML analysis, nearest-neighbor interchange (NNI) was used as the heuristic method for tree inference. Support for the internal branches was assessed by a search of 1,000 bootstrapped sets of data. A bootstrap support (bs) value of ≥70 was considered significant. For BI analysis, two simultaneous runs of 3,000,000 generations were performed, and samples were stored every 100 generations. The 50% majority-rule consensus tree and posterior probability (pp) values were calculated after the first 25% of the samples were discarded. A pp value of ≥0.95 was considered significant.

Antifungal susceptibility testing. Antifungal susceptibility testing was performed according to the methods in the CLSI M38-A2 standard (22). The antifungal drugs tested were amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC), posaconazole (PSC), terbinafine (TRB), anidulafungin (AFG), caspofungin (CFG), and micafungin (MFG). The minimal effective concentration (MEC), defined as the lowest concentration that resulted in short, stubby, abnormally branched hyphae, was determined at 24 h for the echinocandins, and the MIC was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, VRC, ITC, and PSC and 80% reduction in growth for TRB. Geometric mean (GM) MICs were compared using the Mann-Whitney test in GraphPad Prism for Windows (version 6; GraphPad Software, La Jolla CA).

Nucleotide sequence accession numbers. Sequences newly generated in this study were deposited in GenBank under accession numbers KM995840 to KM995877 and LN736031 (LSU), KM995878 to KM995915 and LN736030 (ITS), and LN624419 to LN624456 and LN736032 (*Tub*) (Table 1).

RESULTS

Figure 1 shows the results of the analysis of the LSU sequences (431 bp) of *Acrophialophora* species and related fungi. The type strain of *A. nainiana* (CBS 100.60) clustered with the type strains of *A. levis* (CBS 484.70) and *A. fusispora* (CBS 380.55), being included in a fully supported clade containing several members of the family *Chaetomiaceae* of the order *Sordariales*. The species closest to *Acrophialophora* were members of *Achaetomium*, *Botryotrichum*, *Chaetomidium*, and *Chaetomium*. The latter genus was also found to be polyphyletic.

Figure 2 shows the results of the phylogenetic analysis of the species of the genus *Acrophialophora* using concatenated LSU, ITS, and *Tub* sequences. The final alignment consisted of 1,804 bp (LSU, 843 bp; ITS, 502 bp; *Tub*, 459 bp) of 41 isolates, i.e., one from an environmental source, 33 from clinical origins, and the type strains of *A. fusispora*, *A. levis*, and *A. nainiana* and the putative

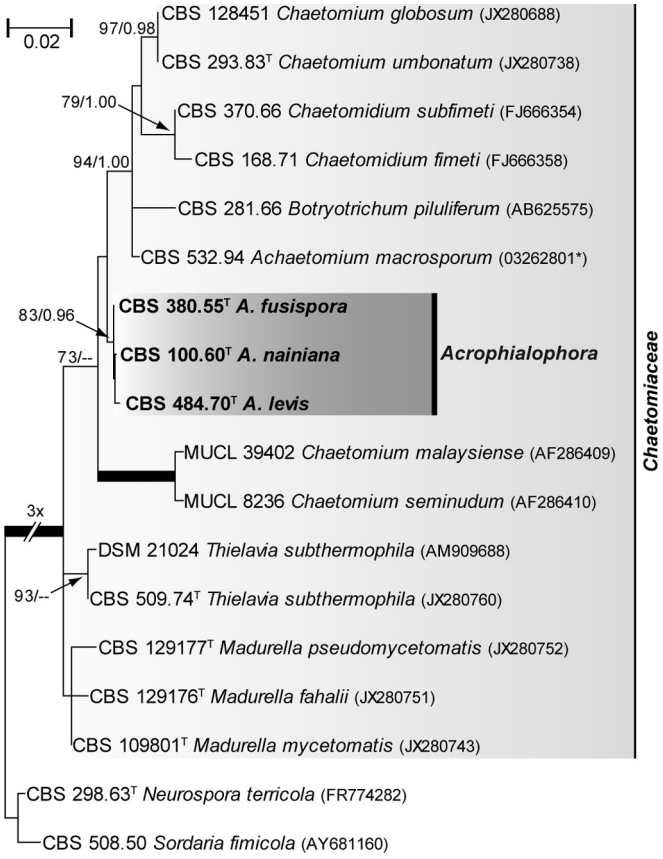

FIG 1 Maximum likelihood (ML) tree constructed with partial LSU sequences (431 bp) of *Acrophialophora* spp. and members of *Chaetomiaceae*. Branch lengths are proportional to the phylogenetic distance. ML bs and Bayesian pp values over 70% and 0.95, respectively, are shown on the nodes. Thickened branches indicate full statistical support. GenBank accession numbers are shown in parentheses. *, sequence retrieved from the National Institute of Technology and Evaluation Biological Resource Center (NBRC) (Chiba, Japan). The tree is rooted with *Neurospora terricola* and *Sordaria fimicola*. T, type strain; CBS, Fungal Biodiversity Centre (Utrecht, The Netherlands) culture collection; DSM, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany); MUCL, Mycothèque de l'Université Catholique de Louvain (Louvain-la-Neuve, Belgium).

synonyms *Ampullifera seudatica* and *M. indica*. *Chaetomium globosum* and *Chaetomium angustispirale* were used to root the tree. The tree showed two fully supported main clades, one of which included the type strain of *A. levis* and the other that of *A. fusispora*. The latter clustered in a fully supported clade with the type strains of *A. nainiana* and *M. indica*, which demonstrated them to be conspecific, since their sequences were practically identical. The type strain of *Ampullifera seudatica* formed a single lineage, basal and distant to the *A. fusispora* clade (98.5% sequence similarity with *A. fusispora* in the combined analysis), and is here considered a different species of the genus named *Acrophialophora seudatica*.

The isolates identified as *A. fusispora* and *A. levis* showed similar macroscopic features on all media tested. The colonies on MEA (Fig. 3a and h) ranged from 30 to 60 mm in diameter after 14 days at 25°C and were flat to slightly umbonate, at first white but soon becoming pale yellow to brownish gray, velvety to felty, with irregular margins and a yellow, brown, or black reverse. *Acrophialophora seudatica* grew more slowly (20 to 25 mm in diameter in 14 days), and its colonies were flat, at first white but rapidly turning

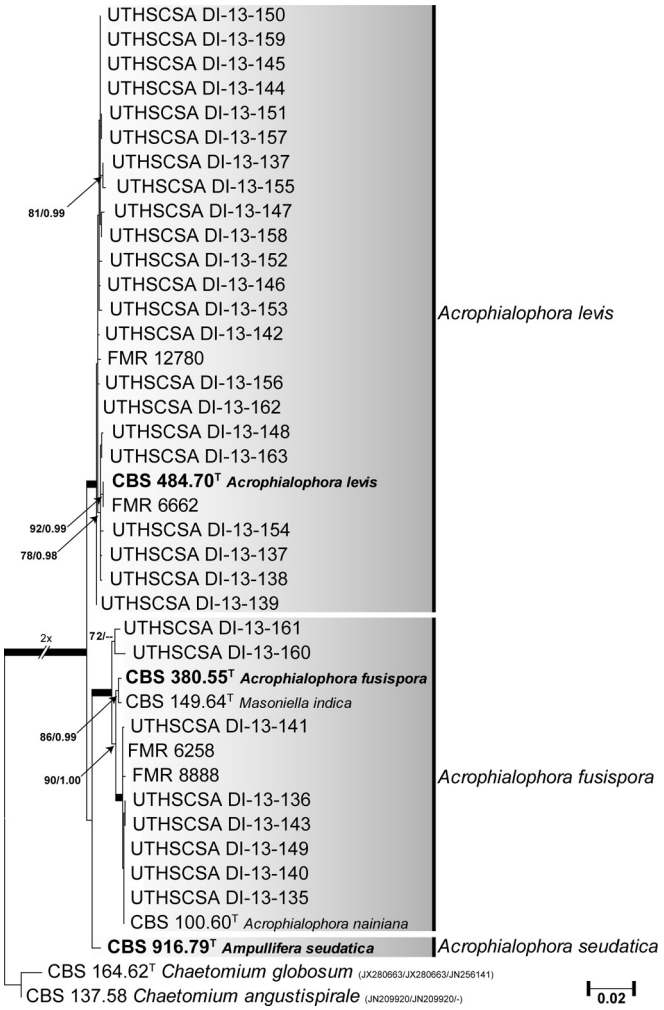

FIG 2 Maximum likelihood (ML) tree constructed with combined LSU (843 bp), ITS (502 bp), and *Tub* (459 bp) sequences of *Acrophialophora* clinical and environmental isolates. ML bs and Bayesian pp values are shown on the nodes. Thickened branches indicate full statistical support. The tree is rooted with *Chaetomium globosum* and *Chaetomium angustispirale*. GenBank accession numbers for LSU, ITS, and *Tub* sequences are shown in parentheses. T, type strain; CBS, Fungal Biodiversity Centre (Utrecht, The Netherlands) culture collection; FMR, Facultat de Medicina, Universitat Rovira i Virgili (Reus, Spain); UTHSCSA, Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (San Antonio, TX).

pale orange, velvety, with a pale orange reverse (Fig. 3p). The optimal temperature for growth was between 35 and 40°C, with a minimum of 15°C and a maximum of 50°C, for all of the species. Microscopically, the isolates of *A. fusispora* were characterized by the abundant production of flask-shaped phialides and polyphialides measuring 5 to 19 by 1.5 to 5 μm (Fig. 3d and e), swollen at the base and tapering abruptly to a narrow neck, mostly formed directly from the aerial hyphae or in the apex of well-differentiated conidiophores, which were erect, unbranched, dark-colored, and with a spiny to warted wall surface (Fig. 3c). Conidia were produced on basipetal chains and were one-celled, subhyaline to brownish, ovoid to fusiform, finely echinulate or forming spiral bands, and measuring 5 to 12 by 2 to 5 μm (Fig. 3d to g). The isolates of *A. levis* also produced abundant flask-shaped phialides and frequently polyphialides, measuring 4 to 13 by 1.5 to 5 μm (Fig. 3i and m), and hyaline to subhyaline ellipsoid to cylindrical conidia, smooth to finely echinulate and measuring 4 to 9 by 2 to 6 μm (Fig. 3m to o). The isolate of *A. seudatica* exhibited flask-shaped phialides measuring 8 to 22 by 2.5 to 4.5 μm, with long necks (Fig. 3r and s), and ovoid to fusiform conidia measuring 6 to 8 by 3 to 4 μm (Fig. 3t and u), with thick and finely verruculose walls, subhyaline or turning pale yellow when mature. This isolate was unable to produce the typical pigmented conidiophores of *Acrophialophora*. Table 2 summarizes the key morphological features that distinguish the three *Acrophialophora* species.

The majority of isolates from clinical sources belonged to *A. levis* (72.7%), while *A. fusispora* accounted for the remaining 34.3% of the isolates. The main source of isolates was the respiratory tract (72.7%), mostly from sputum and bronchoalveolar lavage (BAL) fluid specimens, followed by subcutaneous tissues (9.1%), brain tissue, and corneas (6.1% each). Other sites from which the fungi were cultured included the sphenoid sinus and a chest mass (3% each). No major differences regarding the origins of isolates were observed between *A. levis* and *A. fusispora*.

The antifungal susceptibility results for the isolates belonging to *A. levis* and *A. fusispora* are shown in Table 3. Overall, the highest MIC values were observed for AMB, with geometric mean (GM) MIC and MIC_{90} values of 5.66 μg/ml and 16 μg/ml, respectively. The azole drugs exhibited the best *in vitro* activity, with VRC being the most potent, with overall GM MIC and MIC_{90} values of 0.17 μg/ml and 0.25 μg/ml, respectively, followed by PSC and ITC. The echinocandins exhibited poor *in vitro* activity, with AFG showing the lowest GM MEC and MEC_{90} values (1.86 μg/ml and 4 μg/ml, respectively). TRB showed GM MIC and MIC_{90} values of 0.51 μg/ml and 1 μg/ml, respectively. Although the differences were subtle, the MICs for VRC, ITC, CFG, AFG, MFG, and TRB were significantly lower against *A. levis* than *A. fusispora* ($P < 0.0001$).

TAXONOMY

According to the results of our phylogenetic and morphological analyses, the following new combination is proposed: *Acrophialophora seudatica* (Subrahm.) Sandoval-Denis, Gené & Guarro comb. nov., Mycobank accession number MB811225. Basionym: *Ampullifera seudatica* Subrahmanyam, Nova Hedwigia 31:159 (1979).

DISCUSSION

To our knowledge, this is the first study involving molecular assessment of the fungal genus *Acrophialophora*, a rare opportunistic human and animal pathogen. It also includes the largest set of clinical isolates of the species studied to date. The taxonomy of the genus has been revised and the spectrum of species associated with human disease determined.

According to our results, the genus *Acrophialophora*, belonging to the sordariomycetous family *Chaetomiaceae*, comprises three species, i.e., *A. fusispora*, *A. levis*, and *A. seudatica*. This family includes mostly soilborne cellulose decomposers but also thermotolerant opportunistic pathogens, including neurotropic species such as *Achaetomium strumarium* and *Chaetomium atrobrunneum* (23, 24). Although historically the family *Chaetomiaceae* encompassed mainly ascosporulating fungi, *Acrophialophora* is not the first genus of the family showing strictly asexual reproduction. Recently, de Hoog et al. (24) demonstrated that agents of black-grain mycetomas such as *Madurella* species, which fail to produce fertile sexual morphs, also belong to *Chaetomiaceae*. Ac-

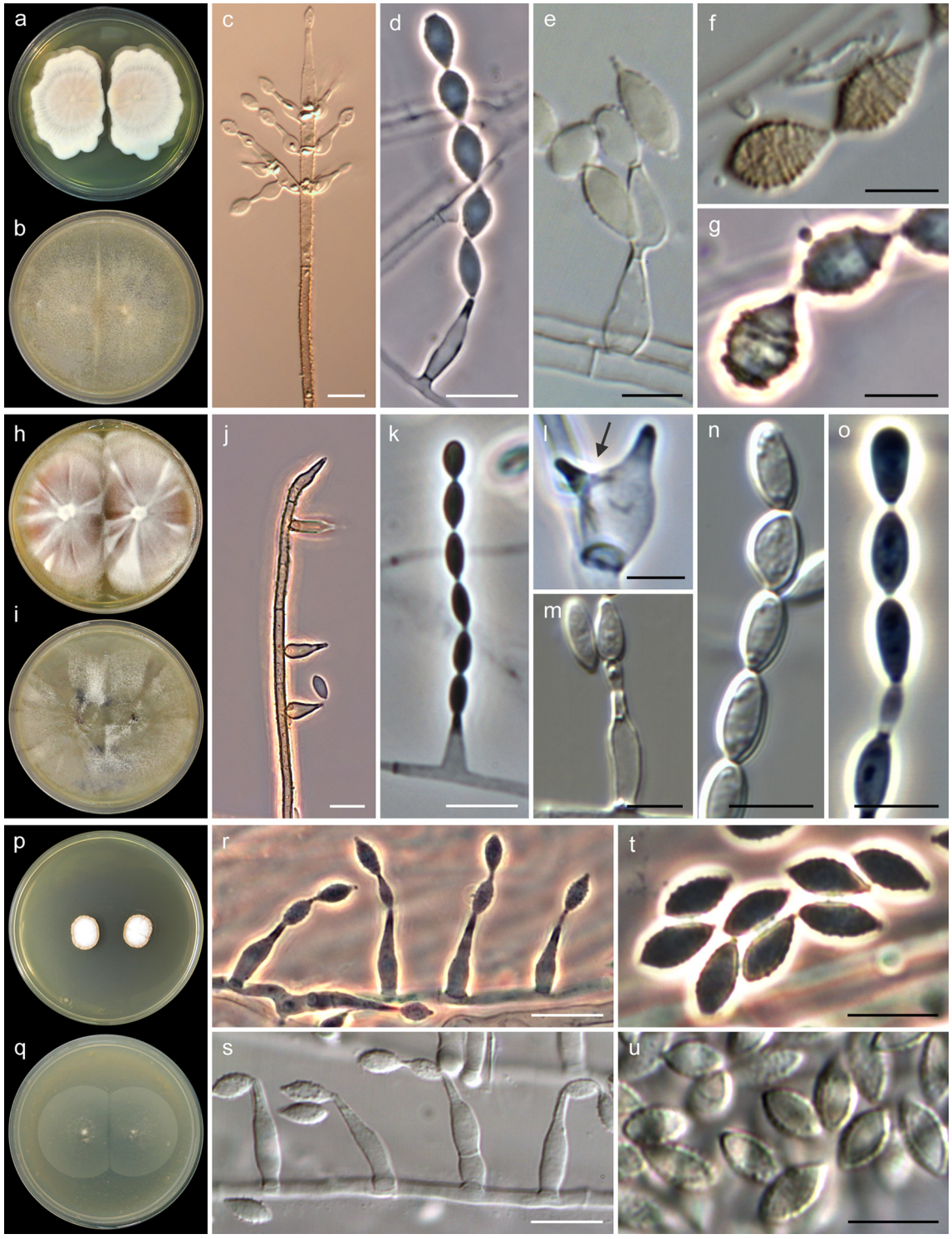

FIG 3 Key morphological features of *Acrophialophora fusispora* (a to g), *A. levis* (h to o), and *A. seudatica* (p to u). (a, h, and p) Colonies on MEA after 14 days at 25°C. (b, i, and q) Colonies on OA after 14 days at 25°C. (c and j) Conidiophores. (d, e, k, l, m, r, and s) Phialides (polyphialides indicated with an arrow). (f, g, n, o, t, and u) Conidia. White bars, 10 μm; black bars, 5 μm.

cording to our LSU phylogeny (Fig. 1), members of the genera *Acrophialophora*, *Chaetomidium*, and *Thielavia* nested unambiguously in highly supported terminal clades, while the positions of the genera *Achaetomium*, *Botryotrichum*, *Chaetomium*, and *Madurella* are unclear, with the genus *Chaetomium* forming two polyphyletic clades. The classifications of the latter genus, however, have been shown to differ significantly when molecular and conventional approaches are compared (24).

TABLE 2 Key differential features of the three species of *Acrophialophora*

Characteristic[a]	*A. fusispora*	*A. levis*	*A. seudatica*
Colony diameter (mm)	30–50	35–60	20–25
Colony color	White, pale yellow, or gray	White, pale yellow, or gray	White to pale orange
Colony texture	Velvety to felty	Velvety to felty	Velvety
Phialide size (μm)	5–19 by 1.5–5	4–13 by 1.5–5	8–22 by 2.5–4.5
Conidial size (μm)	5–12 by 2–5	4–9 by 2–6	6–8 by 3–4
Conidial shape	Ovoid to fusiform	Ellipsoid to cylindrical	Ovoid to fusiform
Conidial ornamentation	Finely echinulate to spiral sculpted	Smooth to finely echinulate	Finely verruculose
Conidial color	Subhyaline to brown	Hyaline to subhyaline	Subhyaline to pale yellow

[a] Characteristics were determined after growth on MEA for 14 days at 25°C.

Because the morphological features used to distinguish the three *Acrophialophora* species recognized by Samson and Mahmood (4) tended to overlap, Al-Mohsen et al. (1) considered them morphological variations of a single species, *A. fusispora*. However, our phylogenetic analysis of the different type strains does not support this conclusion. Our results confirm that *A. fusispora*, *A. nainiana*, and *M. indica* are conspecific, while *A. levis* and *A. seudatica* are two different species. In contrast to the observations of Al-Mohsen et al. (1), our phylogenetic results indicate that subtle morphological findings for these fungi, such as conidial size, shape, color, and ornamentation, show consistent differences to distinguish *A. fusispora*, *A. levis*, and *A. seudatica* (Table 2). The presence (mainly on OA) of erect pigmented conidiophores is typical of cultures of the two clinically relevant species of *Acrophialophora*, i.e., *A. fusispora* and *A. levis*, and can be important for the initial generic diagnosis. However, these pigmented conidiophores are absent in *A. seudatica*. This species was originally described as having simple, hyaline, straight conidiophores (25), a feature that is confirmed here. However, *A. seudatica* is known only from its type specimen isolated from a soil sample from India. Therefore, confirmation of the presence or absence of the typical conidiophores of *Acrophialophora* will be possible only by studying more isolates of this rare species.

The three species of *Acrophialophora* shared very similar LSU sequences (99.9%), but the large differences in their ITS and *Tub* sequences (<96.1% and <96.6% sequence similarity, respectively) show that both loci can discriminate between the three species, making them good candidates for barcoding targets in *Acrophialophora*.

Some authors stated that *Acrophialophora* infections may have been underdiagnosed due to the rarity of these fungi and the potential confusion with similar opportunistic molds, such as *Lomentospora prolificans* and *Scopulariopsis chartarum* (9, 13, 26, 27). Only six well-documented cases of human infections exist in the literature, most of which lacks molecular confirmation of the etiological agent. In only one case was the fungus confirmed as *A. fusispora* by sequencing of the ITS region (15). The rarity is also reflected by the scarcity of reference sequences for comparison in fungal databases, which do not include any type or correctly identified reference strains.

Since the synonymy of the species of *Acrophialophora* was formally proposed by Al-Mohsen et al. (1), *A. fusispora* has remained the only accepted species of the genus and, as such, has been cited as the causative agent in the reported clinical cases (1, 7, 14, 23). According to our results, however, *A. levis* seems to be the most common species isolated from human clinical samples. The identification of some of the isolates included in the study by Guarro et al. (6) was reassessed here by sequence comparison. One clinical isolate (FMR 8888, from a corneal infection) was confirmed as *A. fusispora*, while another (FMR 6662, isolated from sputum) was reidentified here as *A. levis*. The third clinical isolate included in that study (FMR 6404) was not available for analysis, and thus its final identification remains unknown.

Most published cases refer to pulmonary involvement, with or

TABLE 3 Results of *in vitro* antifungal susceptibility testing for the 33 clinical isolates of *Acrophialophora* spp. included in the study

	MIC or MEC (μg/ml)[a]							
Species and parameter	AMB	VRC	PSC	ITC	CFG[b]	AFG[b]	MFG[b]	TRB
A. levis ($n = 24$)								
GM	6.77	0.16	0.50	1.15	3.58	1.65	2.64	0.42
Range	1–32	0.06–05	0.25–1	0.5–4	0.25–32	0.25–8	0.25–32	0.125–4
MIC_{90}	32	0.25	1	2	16	4	32	1
A. fusispora ($n = 9$)								
GM	4.22	0.18	0.50	0.85	11.02	2.61	6.46	0.75
Range	2–32	0.125–0.25	0.25–1	0.125–1	4–32	2–8	0.125–32	0.5–1
MIC_{90}	16	0.25	1	1	16	4	32	1
Overall ($n = 33$)								
GM	5.66	0.17	0.49	1	4.98	1.86	3.21	0.51
Range	1–32	0.06–05	0.25–1	0.125–4	0.25–32	0.25–8	0.125–32	0.125–4
MIC_{90}	16	0.25	1	1	16	4	32	1

[a] AMB, amphotericin B; VRC, voriconazole; PSC, posaconazole; ITC, itraconazole; CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine.
[b] This column contains MEC data.

without systemic dissemination (6, 10–12). Similarly, the majority of our clinical isolates were obtained from respiratory specimens, with one-half of them being from BAL fluid samples. It was not possible, however, to distinguish between true infectious agents, colonizers, and environmental contaminants, given the nature of the samples and the absence of appropriate clinical or histopathological data. The second most common infection in clinical reports is keratitis (6, 9), while in our study it was soft tissue infection, particularly lower extremity tissue infection. Corneal and cerebral samples, in equal proportions, were the third most common sites of isolation. The lack of isolates from the central nervous system (CNS) does not allow us to confirm the potential neurotropism attributed to *Acrophialophora* (14).

The antifungal treatment of *Acrophialophora* infections has been hampered by the paucity of *in vitro* susceptibility data and the lack of specific treatment guidelines. The clinical cases have demonstrated variable results. Arthur et al. (9) reported a favorable outcome with the use of AMB and surgical debridement in a case of keratouveitis. In one report of a pulmonary infection, monotherapy with liposomal AMB (LAMB) was not effective, but the patient responded to combination therapy with LAMB and ITC (1). Guarro et al. (6) reported the use of VRC in two clinical cases, one a case of keratitis that responded favorably to the drug and one a pulmonary infection with a fatal outcome. In addition, Li et al. (15) described a negative outcome using VRC in a case of cerebral infection. The two latter cases, however, were in highly immunocompromised patients with systemic involvement. Our susceptibility results showed that, while AMB and the echinocandins have almost no activity against *Acrophialophora* species, VRC exhibits potent *in vitro* activity. This confirms the observations of Guarro et al. (6), suggesting that VRC may be a potential treatment option for *Acrophialophora* infections.

In conclusion, *Acrophialophora* includes three closely related species, *A. fusispora*, *A. levis*, and *A. seudatica*, that can be accurately identified on the basis of ITS or *Tub* sequencing and detailed morphological study. *Acrophialophora levis* appears to be the most frequent species in clinical samples. VRC shows potent *in vitro* activity against these fungi.

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