Molecular Diagnostics of Arthroconidial Yeasts, Frequent Pulmonary Opportunists

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ABSTRACT *Magnusiomyces capitatus* and *Saprochaete clavata* are members of the clade of arthroconidial yeasts that represent emerging opportunistic pulmonary pathogens in immunocompromised patients. Given that standard ribosomal DNA (rDNA) identification often provides confusing results, in this study, we analyzed 34 isolates with the goal of finding new genetic markers for classification using multilocus sequencing and amplified fragment length polymorphism (AFLP). The interspecific similarity obtained using rDNA markers (the internal transcribed spacer [ITS] and large subunit regions) was in the range of 96 to 99%, whereas that obtained using protein-coding loci (*Rbp2*, *Act*, and *Tef1α*) was lower at 89.4 to 95.2%. Ultimately, *Rbp2* was selected as the best marker for species distinction. On the basis of cloned ITS data, some strains proved to be misidentified in comparison with the identities obtained with phenotypic characters, protein sequences, and AFLP profiles, indicating that different copies of the ribosomal operon were present in a single species. Antifungal susceptibility testing revealed that voriconazole had the lowest MIC against *M. capitatus*, while amphotericin B had the lowest MIC against *S. clavata*. Both species exhibited *in vitro* resistance to fluconazole and micafungin.

KEYWORDS amplified fragment length polymorphism, antifungal, biodiversity, *Geotrichum*, *Magnusiomyces*, *Saprochaete*, *Geotrichum*, *Magnusiomyces*, antifungal susceptibility testing

The arthroconidial yeast-like fungi *Magnusiomyces capitatus* and *Saprochaete clavata* are significant opportunists, causing pulmonary and disseminated infections in patients with acute hematological malignancies and other immune disorders (1–6). Multicenter outbreaks of *S. clavata* associated with high short-term case fatality rates have been reported in Europe (2). These fungi are classified in the family Dipodascaceae, order Saccharomycetales (7, 8), and as such are phylogenetically related to the ascomycetous yeasts, although their thallus is filamentous without budding cells. In culture, both species show relatively expanding, whitish, butyrous colonies (9) with fragmenting hyphae producing arthroconidia and are thermotolerant, with optimum growth occurring at temperatures above 30°C. The environmental niche of these fungi is as yet unknown, but they were recently discovered to be regular colonizers of household dishwashers (10–12) and have occasionally been found in dairy products

Received 2 September 2017 Returned for
modification 2 October 2017 Accepted 18
October 2017

Accepted manuscript posted online 25
October 2017

Citation Kaplan E, Al-Hatmi AMS, Ilkit M, Gerrits
van den Ende AHG, Hagen F, Meis JF, de Hoog
GS. 2018. Molecular diagnostics of
arthroconidial yeasts, frequent pulmonary
opportunists. J Clin Microbiol 56:e01427-17.
<https://doi.org/10.1128/JCM.01427-17>.

Editor David W. Warnock

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(13). The transmission of *S. clavata* through contaminated medical devices has also been suggested (2).

M. capitatus and *S. clavata* are physiologically differentiated by their growth responses to cellobiose, salicin, and arbutin (14) and were reported to show a 4% difference in their ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences (7). Despite these differences, misidentifications between the two species are frequent due to conflicting data sets (15). This sheds doubt on the reliability of rDNA ITS regions as markers for discrimination. Therefore, the aim of the present study was to reclassify 34 clinical and environmental isolates from the *M. capitatus*/*S. clavata* clade using a polyphasic approach. For this purpose, we adopted multilocus sequencing, which has now effectively replaced conventional phenotypic methods for species delimitation. In addition to the rDNA ITS region, we also sequenced the partial large subunit (LSU) rDNA region, as well as the *Tef1 α* , *Rpb2*, and *Act* genes. The topologies of the obtained phylogenetic trees were verified by use of the amplified fragment length polymorphism (AFLP) profiles, and the clinical, phenotypic, and antifungal profiles were compared.

Both *M. capitatus* and *S. clavata* have undergone several name changes since their first identification, which is largely due to repeated changes in the rules of fungal nomenclature. They were classified in different genera, *Saprochaete* and *Magnusiomyces*, upon the discovery of a sexual phase in *M. capitatus*, at the time when separate names were attributed to fungi with sexual and clonal forms of sporulation (16). However, in 2013, the one fungus, one name principle was adopted (17). This principle abandoned the dual naming system for fungi, and molecular phylogeny became the main criterion for genus delimitation. Further, no reevaluation of the current naming systems has been performed for the genera of arthroconidial yeasts in the Dipodascaceae. Therefore, the present study can help to provide a stable nomenclature for this important group of fungi. In addition, we retested the antifungal susceptibility profiles in order to verify whether any systematic differences are evident in this newly defined group.

MATERIALS AND METHODS

Isolates. We used 34 isolates from the *M. capitatus*/*S. clavata* clade recovered from clinical ($n = 19$), environmental ($n = 14$), and unknown ($n = 1$) sources. Detailed strain data are provided in Table 1. Reference strains were obtained from the culture collection of the Centraalbureau voor Schimmelcultures (CBS; housed at the Westerdijk Fungal Biodiversity Institute), Utrecht, the Netherlands. All isolates were subcultured on malt extract agar (MEA; Oxoid, UK) at 25°C prior to the experiments.

Physiology. Physiological tests for the utilization of cellobiose, salicin, and arbutin (all from Acros Organics, Morris, NJ, USA) were performed to distinguish the isolates, with glucose assimilation being used as a control (18, 19). In brief, inocula were prepared in 5 ml yeast nitrogen base (YNB; BD Difco, Sparks, MD, USA). Tubes were incubated at 24°C and shaken for up to 5 days. The effect of temperature on cell growth was verified on MEA at 24°C, 30°C, 37°C, 40°C, and 45°C for up to 1 week.

DNA extraction. Genomic DNA was extracted following the cetyltrimethylammonium bromide (CTAB) protocol (20). The quality of genomic DNA was verified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Samples were stored at -20°C until use.

DNA amplification and sequencing. Five gene regions were amplified from the genomic DNA. The primer pairs used are listed in Table 2. PCR mixtures (final volume, 25 μl) contained 10 $\times$ PCR buffer (2.5 μl), 1 μl of a deoxynucleoside triphosphate mix (10 mM), 1 μl of each primer (10 μM), *Taq* polymerase (5 U/ μl), template DNA (1 μl of 100 ng/ μl), and nuclease-free water. Amplification was performed in an ABI Prism 2720 thermocycler (Applied Biosystems, Foster City, CA, USA), as follows: 95°C for 4 min, followed by 30 cycles of 95°C for 45 s, 45 to 51°C (according to the primer) for 30 s, and 72°C for 1.25 min, with a final incubation at 72°C for 8 min. PCR products were visualized on a 1.0% (wt/vol) agarose gel before sequencing. An ABI PrismH BigDye Terminator (v.3.0) ready reaction cycle sequencing kit (Applied Biosystems) was used for the sequencing reaction. Sequences were determined with an ABI Prism 3100 genetic analyzer (Applied Biosystems) and the primers listed in Table 2, as follows: 1 min at 95°C, followed by 30 cycles of 10 s at 95°C, 5 s at 50°C, and 2 min 60°C. The reaction products were purified on Sephadex G-50 fine columns (GE Healthcare Bio-Sciences, Uppsala, Sweden), and the sequencing was done on an ABI 3730XL automatic sequencer (Applied Biosystems).

Cloning and sequencing of the ITS region. Cloning was carried out to verify the presence of multiple rDNA repeats. The ITS region of randomly selected strains, CBS 132760, CBS 139.32, CBS 561.97, and CBS 576.82, was amplified using primers ITS1 and ITS4 and the PCR conditions described above. The amplicons were cloned into the pJET1.2/blunt cloning vector using a CloneJET PCR cloning kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Plasmids were extracted and purified using a plasmid miniprep kit (Qiagen, Hilden, Germany). pJET1.2 primers were used for the insert sequencing.

TABLE 1 Strains used in this study and GenBank accession numbers of the analyzed loci^a

Species	Collection no.	New name	Substrate of isolation	Isolation location	GenBank accession no.			
					ITS	LSU	Act	Tef1 α
<i>M. capitatus</i>	CBS 311.76		Sputum	Germany	KU301192	KU301158	KU301128	KU301060
	CBS 312.76		Sputum	Borstel, Germany	KU301193	KU301159	KU301129	KU301061
	CBS 162.80		Milk	Weybridge, UK	KU301190	KU301156	KU301126	KU301058
	CBS 571.82		Wood pulp	Stockholm, Sweden	KU301200	KU301166	ND	KU301068
	CBS 572.82		Wood pulp	Stockholm, Sweden	KU301201	KU301167	KU301136	KU301069
	CBS 573.82 ^b		Yeast cake	Unknown	KU301202	KU301168	KU301137	KU301103
	CBS 574.82		Sputum	Oslo, Norway	KU301203	KU301169	KU301138	KU301070
	CBS 575.82 ^c		Tooth	Pretoria, South Africa	KU301204	KU301170	KU301139	KU301071
	CBS 577.82		Sputum	Berlin, Germany	KU301206	KU301172	KU301141	KU301072
	CBS 578.82		Sputum	Unknown	KU301207	KU301173	ND	KU301074
	CBS 579.82		Unknown	Unknown	KU301208	KU301174	KU301142	KU301075
	CBS 580.82		Sputum	Unknown	KU301209	KU301175	KU301143	KU301076
	CBS 207.83		Sputum	New York, NY, USA	KU301191	KU301157	KU301127	KU301077
	CBS 598.83		Oral infection	Unknown	KU301210	KU301176	KU301144	KU301059
	CBS 716.84		Digestive tube, pig	Paris, France	KU301212	KU301178	KU301146	KU301078
	CBS 327.86		Mycosis	Unknown	KU301194	KU301160	KU301130	KU301080
	CBS 623.96		Blood culture	Unknown	KU301211	KU301177	KU301145	KU301062
	CBS 561.97		Blood culture	Denmark	KU301197	KU301163	KU301133	KU301079
	CBS 562.97		Udder, cow	Denmark	KU301198	KU301164	KU301134	KU301065
	CBS 563.97		Udder, cow	Denmark	KU301199	KU301165	KU301135	KU301066
	CBS 129.94		Deep sea	Palmer Peninsula, Antarctica	KU301188	KU301154	KU301122	KU301067
<i>S. clavatus</i>	CBS 132.759	<i>M. clavatus</i>	Dishwasher	Mersin, Turkey	KU301216	KU301182	KU301123	KU301054
	CBS 139.32	<i>M. clavatus</i>	Dishwasher	Ankara, Turkey	KU301189	KU301155	KU301125	KU301055
	CBS 425.71 ^b	<i>M. clavatus</i>	Lung tissue	Baltimore, MD, USA	KU301195	KU301161	KU301131	KU301057
	CBS 576.82	<i>M. clavatus</i>	Sputum	The Netherlands	KU301205	KU301171	KU301140	KU301063
	CBS 758.85	<i>M. clavatus</i>	Cactus rot	Arizona, USA	KU301213	KU301179	KU301147	KU301073
	CBS 969.87	<i>M. clavatus</i>	Blood (leukemia patient)	Paris, France	KU301214	KU301180	KU301148	KU301081
	CBS 970.87	<i>M. clavatus</i>	Moist warm air outlet of bottle washing machine	Belgium	KU301215	KU301181	KU301149	KU301082
	CBS 489.88	<i>M. clavatus</i>	Liver abscess	Paris, France	KU301196	KU301162	KU301132	KU301083
	CBS 132.760 ^d	<i>M. clavatus</i>	Dishwasher	Mersin, Turkey	KU301217	KU301183	KU301124	KU301064
	Dishwasher-2 ^d	<i>M. clavatus</i>	Dishwasher	Eskisehir, Turkey	KU301218	KU301184	KU301150	KU301056
	Dishwasher-4 ^d	<i>M. clavatus</i>	Dishwasher	Istanbul, Turkey	KU301219	KU301185	KU301151	KU301084
	Dishwasher-18 ^d	<i>M. clavatus</i>	Dishwasher	Erzurum, Turkey	ND	KU301186	KU301152	KU301085
	Dishwasher-21 ^d	<i>M. clavatus</i>	Dishwasher	Erzurum, Turkey	KU301220	KU301187	KU301153	KU301086
								KU301087

^aCBS, Centraalbureau voor Schimmelfcultures; ND, not defined.^bType strains.^cUnavailable isolates in AFLP analysis.^dUnavailable isolates in AFLP and AFST analysis.

TABLE 2 PCR primers used for amplification

Locus	Product	Primer	Primer sequence (5'–3')	Annealing temp (°C)	Reference
ITS	ITS1-5.8S rRNA gene-ITS2	ITS1	TCCGTAGGTGAACCTGCGG	48	40
		ITS4	GCTGCGTTCTTCATCGATGC		40
LSU	D1/D2	LR0R	ACCCGCTGAACCTTAAGC	45	41
		LR0S	TCCTGAGGGAAACTTCG		42
<i>Rpb2</i>	RNA polymerase II	Rpb2-6F	TGGGGKWTGGTYTGYCCTGC	51	43
		Rpb2-7R	CCCATWGCYTGCTTMCCCAT		43
<i>Tef1</i> α	Translation elongation factor 1 alpha	YTEF-1G	GGTAAGGGTTCTTCAAGTACGCTTGGG	51	44
		YTEF-6G	CGTTCTTGAGTACACACAGACGTTACCTC		44
<i>Act</i>	Actin	CA14	AACTGGGATGACATGGAGAAGATCTGGC	51	45
		CA5R	GTGAACAATGGATGGACCAGATTCGTCG		45

The purified PCR fragments were sequenced on an ABI Prism 3130xl genetic analyzer at MedSanTek Biotechnologies (Istanbul, Turkey). To eliminate the possibility of contamination, repeat PCRs, dual cloning, and sequencing of both directly obtained and cloned amplicons were carried out.

Sequence alignment and phylogenetic analyses. Consensus sequences were analyzed with SeqMan software from the Lasergene package (DNASTar, Madison, WI, USA) and manually edited. Independent alignments and phylogenetic analyses were performed with BioNumerics (v.4.5) software (Applied Maths, Sint-Martens-Latem, Belgium).

AFLP. High-quality genomic DNA was extracted and analyzed by AFLP fingerprinting, as described previously (21). The raw data generated by fragment analysis with the ABI3500xL genetic analyzer (Applied Biosystems) were imported into BioNumerics (v.7.6.1) software (Applied Maths), and an unweighted pair group method using average linkages (UPGMA)-based dendrogram was generated by use of the Pearson correlation similarity matrix.

Antifungal susceptibility testing. Determination of the broth dilution MICs of antifungal agents was carried out following the Clinical and Laboratory Standards Institute M27-A3 document (22). The concentration ranges were as follows: 0.016 to 16 mg/liter for amphotericin B (Bristol-Myers Squibb, Woerden, the Netherlands), itraconazole (Janssen Research Foundation, Tilburg, the Netherlands), voriconazole (Pfizer Central, Sandwich, UK), posaconazole (Merck, Whitehouse Station, NJ, USA), and isavuconazole (Basilea Pharmaceuticals, Basel, Switzerland); 0.062 to 64 µg/ml for fluconazole (Pfizer); and 0.008 to 8 µg/ml for anidulafungin (Pfizer) and micafungin (Astellas Pharma Inc., Ibaraki, Japan). After 48 h of incubation at 35°C, the MIC and minimum effective concentration (MEC) values were determined visually, using a mirror, by comparing the growth in wells containing the drug with that in a drug-free control well. Quality control was performed for every new batch of MIC plates with the following reference strains: *Aspergillus flavus* ATCC 204304, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 6258. The MICs for all reference strains were within the expected ranges.

RESULTS

Multilocus sequencing. A preliminary analysis based on the nucleotide sequences of the ITS and LSU loci revealed a close match (96 to 99% similarity) between *M. capitatus* and *S. clavata*. Three additional loci, *Rpb2*, *Tef1*α, and *Act*, were sequenced, and a total of 166 new sequences, including 33 ITS, 34 LSU, 32 partial *Tef1*α, 34 partial *Rpb2*, and 33 partial *Act* sequences (Table 1), were added to the previously published 22 ITS and 14 LSU sequences from GenBank or the Centraalbureau voor Schimmelcultures genome database (see Table S1 in the supplemental material). The total lengths of the sequenced ITS, LSU, *Tef1*α, *Rpb2*, and *Act* sites were 348 bp, 597 bp, 628 bp, 708 bp, and 631 bp, respectively. Out of the 2,979-bp concatenated sequences for each isolate, 165 nucleotide sites were found to be variable. In interlocus comparisons, *Rpb2* had the highest number of variable sites ($n = 82$, 11.5%). The variable sites of all loci were predominately present as two variants (Table 3).

Phylogenetic analyses revealed different abilities of the ITS-based tree and concatenated tree containing LSU, *Rpb2*, *Act*, and *Tef1*α loci for distinguishing *M. capitatus* and *S. clavata* (Fig. 1 and 2). The ITS locus was unable to separate the species because some strains assumed positions that conflicted with the remaining data. However, in the concatenated tree excluding ITS, *M. capitatus* and *S. clavata* clearly clustered in two separate groups (Fig. 2), which matched the remaining characteristics analyzed. Consequently, the genetic distance for each locus varied significantly and was the lowest for ITS and LSU and was the highest for *Rpb2*, *Act*, and *Tef1*α. Importantly, *Rpb2* was found to be the best marker for reliable discrimination (Table 3).

The positions of the individual strains differed among the obtained trees. Three reference isolates (CBS 132759, CBS 139.32, and CBS 132760) and four nonreference

TABLE 3 Multilocus sequencing data

Locus	No. of isolates analyzed	No. of sites		Parsimony informative			
		Total	Variable	Total	Two variants	Three variants	Four variants
ITS	33	348	10	5	5	0	0
LSU	34	597	4	4	4	0	0
<i>Tef1</i> α	32	628	32	29	29	0	0
<i>Rpb2</i>	34	708	82	80	79	1	0
<i>Act</i>	33	631	42	40	40	0	0
Concatenated	32	2,979	165	154	153	1	0

isolates (Dishwasher-2, Dishwasher-4, Dishwasher-18, and Dishwasher-21) of *M. capitatus* were placed in the same branch with *S. clavata* strains in the concatenated tree. In contrast, 11 *M. capitatus* strains and 9 strains of *S. clavata*, including the *M. capitatus* and *S. clavata* type strains (CBS 573.82 and CBS 425.71, respectively), showed the same position in the ITS and concatenated trees (Fig. 1 and 2).

Cloning and sequencing of the ITS region. Comparisons of the multiple-sequence alignments of the sequences of the directly sequenced and cloned amplicons of the ITS region revealed some variable nucleotides and insertions/deletions in the cloned strains, especially in ITS2. Two strains, CBS 576.82 and CBS 139.32, changed position at the species level in the ITS tree when the data for the cloned and directly sequenced amplicons were compared (Fig. 1). Such heterogeneity in the ITS region indicated the possibility of different copies of rDNA within the ribosomal operon of selected strains.

AFLP. The AFLP profiles contained approximately 50 to 60 fragments that were 40 to 400 bp long. The AFLP dendrogram comprised two main clusters at the species level (*M. capitatus*, 20 strains; *S. clavata*, 6 strains). The majority of the *M. capitatus* and *S. clavata* strains were divided into two main clusters, which corresponded to their division in the multilocus tree. However, two strains of *M. capitatus* (CBS 139.32 and CBS 132759) were found in the opposite cluster. On the basis of the placement of strains in the clusters, no clear demarcation according to sample origin (e.g., clinical or environmental) or geographic location was evident (Fig. 3).

Physiology. The ability of all strains to assimilate cellobiose, salicin, and arbutin was evaluated. All strains of *S. clavata* could assimilate all of these carbon sources, unlike many *M. capitatus* isolates. However, six isolates of *M. capitatus* (as defined by the ITS sequence data) showed growth on these carbon sources; these were the same isolates that clustered as *S. clavata* strains in the concatenated tree. All *M. capitatus* and *S. clavata* isolates were able to grow at 30°C, 37°C, and 40°C; no growth of *M. capitatus* isolates was observed at 45°C, but the *S. clavata* isolates showed limited growth at this temperature. All *S. clavata* isolates grew robustly after transfer from 45°C to 25°C; however, the *M. capitatus* isolates were not viable when they were incubated at 25°C after preincubation at 45°C (Table 4).

Antifungal susceptibility. The geometric mean (GM) MIC or MEC ranges of eight antifungal drugs are summarized in Table 5. Both tested species showed high MIC or MEC values for fluconazole and micafungin (8 to 32 and 2 to >8 $\mu\text{g/ml}$, respectively). The MIC values for *M. capitatus* were in the range of 0.063 to 1 $\mu\text{g/ml}$ for amphotericin B and isavuconazole; 0.125 to 0.5 $\mu\text{g/ml}$ for itraconazole, voriconazole, and posaconazole; and 2 to 4 $\mu\text{g/ml}$ for anidulafungin. For *S. clavata* isolates, the MICs were in the range of 0.125 to 0.5 $\mu\text{g/ml}$ for amphotericin B, 0.25 to 0.5 $\mu\text{g/ml}$ for itraconazole, 0.063 to 0.5 $\mu\text{g/ml}$ for voriconazole, 0.25 $\mu\text{g/ml}$ for posaconazole, 0.125 to 1 $\mu\text{g/ml}$ for isavuconazole, and 2 $\mu\text{g/ml}$ for anidulafungin (Table S2).

We further evaluated whether the MIC/MEC values correlated among the strains of *M. capitatus* and *S. clavata* (defined by multilocus analysis) using the Mann-Whitney-Wilcoxon test for a skewed distribution. At an alpha value of ≤ 0.05 , for all *M. capitatus* isolates, the lowest GM MIC was observed for voriconazole (0.18 $\mu\text{g/ml}$), followed by posaconazole (0.24 $\mu\text{g/ml}$), amphotericin B (0.32 $\mu\text{g/ml}$), and isavuconazole (0.34

FIG 1 Dendrogram showing the phylogenetic relationships among all 73 isolates. The dendrogram was generated from the ITS regions of *M. capitatus* and *S. clavata* using the maximum-likelihood method. The Kimura 2-parameter model was used as the substitution model with the gamma correction and 1,000 bootstrap replicates. Values show bootstrap support of >70%. Strains that were both cloned and directly sequenced are identified by use of the same symbols in the tree.

$\mu\text{g/ml}$). For all *S. clavata* isolates, the lowest GM MIC was observed for amphotericin B (0.22 $\mu\text{g/ml}$), followed by voriconazole and posaconazole (0.25 $\mu\text{g/ml}$) and then itraconazole (0.27 $\mu\text{g/ml}$). In contrast, the GM MIC of fluconazole was 19 $\mu\text{g/ml}$ for *S. clavata* and 12.28 $\mu\text{g/ml}$ for *M. capitatus*. Both species had high MICs of fluconazole and

FIG 2 Concatenated tree of LSU, *Rbp2*, *Act*, and *Tef1 α* sequences ($n = 34$). The dendrogram was generated using the maximum-likelihood method. The Kimura 2-parameter model was used as the substitution model with the gamma correction and 1,000 bootstrap replicates. Values show bootstrap support of $>70\%$.

micafungin; however, no clear specificity of resistance related to sample origin was observed.

DISCUSSION

The two opportunistic arthroconidial yeasts analyzed in this study were originally described in the genus *Geotrichum* and were distinguished only on the basis of morphological differences: *Geotrichum capitatum* exhibited sympodial conidium production, in addition to arthroconidia, while *G. clavatum* had arthroconidia only. This difference was further supplemented by characterization of physiological parameters (8). However, as larger sets of strains were analyzed, it became clear that classification according to conidial pleomorphism did not always match molecular data. Using D1/D2 LSU data, the species were considered conspecific, as they showed 99% similarity (23), but subsequent ITS sequence analysis revealed that the species were unambiguously different (7). At the

FIG 3 Comparative UPGMA dendrogram of AFLP fingerprints of *M. capitatus* and *S. clavata* strains.

beginning of the ITS1 locus, the species differ in five positions, whereas in ITS2, there were four deletions and one single nucleotide polymorphism observed in *G. capitatum*. These differences between the two species were reconfirmed in most of the strains analyzed with an extended data set in the present study, which allowed the reevaluation of current species delimitations by the incorporation of multilocus sequencing data. Although the ribosomal sequence data showed that the species were 96 to 99% similar, partial sequences of *Rbp2*, *Act*, and *Tef1α* showed significant and consistent similarities of 89.4 to 95.2%. The initial physiological difference between the two species in response to arbutin, cellobiose, and salicin appeared to be completely consistent with these data. In contrast, the absence of sympodial propagation as a diagnostic criterion appeared to have been based on results for too few strains, as this character was found to be randomly distributed among the strains analyzed in the present study.

Some strains assumed a position in the ITS species tree opposite that obtained using physiology and multilocus sequence data. Maintaining the general consensus of a rate of 1% ITS sequence divergence to be considered different species (24), the 4% divergence rate identified in our study cannot be accepted as a reliable threshold for species differentiation for this group. Intraspecies and intragenomic heterogeneities involving the ITS region are based on the presence of multiple intragenomic rDNA copies that show sequence variation. Although this is not uncommon in fungi, when the ratios are close to 50%, amplification results may vary stochastically. A similar situation was documented in another member of the filamentous Saccharomycetales, *Geotrichum candidum* (25). Indels found in multiple-sequence alignment comparisons of direct and cloned amplicons from the rDNA of strains CBS 576.82 and CBS 139.32 were sufficiently different to position them in either *M. capitatus* or *S. clavata*.

The nomenclatural rules and recommendations for fungi have changed several times during the last few decades. In their phylogenetic overview of the Dipodascaceae,

TABLE 4 Carbon assimilation and growth characteristics of *M. capitatus* and *S. clavata* isolates at various temperatures

Species	No. of isolates growing under the following conditions:							
	Carbon source			Temp (°C)				
	Cellobiose	Salicin	Arbutin	30	37	40	45	45 to 25 ^a
<i>M. capitatus</i> (n = 21)	0	0	0	21	21	21	0	0
<i>S. clavata</i> (n = 13)	13	13	13	13	13	13	11	13

^aAll *S. clavata* isolates grew robustly after transfer from 45°C to 25°C.

TABLE 5 GM MIC or MEC and MIC or MEC ranges of amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, anidulafungin, and micafungin for strains of *M. capitatus* and *S. clavata*

Species	MIC ^a (μg/ml)															
	AMB		FLU		ITC		VRC		POS		ISAV		AFG		MFG	
	MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)		MIC (μg/ml)		MEC (μg/ml)		MEC (μg/ml)	
	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM
<i>M. capitatus</i> (n = 21)	0.063–1	0.32	8–32	12.28	0.125–0.5	0.28	0.125–0.5	0.18	0.125–0.5	0.24	0.063–1	0.34	2–4	2.4	2–>8	6.6
<i>S. clavata</i> (n = 8)	0.125–0.5	0.22	16–32	19	0.25–0.5	0.27	0.063–0.5	0.25	0.25	0.25	0.125–1	0.54	2	2	4–8	6.7

^aMICs were determined according to CLSI methods after 48 h of incubation at 35°C. AMB, amphotericin B; FLU, fluconazole; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; ISAV, isavuconazole; AFG, anidulafungin; MFG, micafungin; GM, geometric mean; MEC, minimum effective concentration.

de Hoog and Smith (7) noted that *Geotrichum* was remote as a member of the Endomycetaceae. Indeed, with phylogenetic analysis, the classification of *Geotrichum* could not be maintained for these fungi. At that time, fungi were subjected to a dual nomenclature system in which sexual and nonsexual forms were attributed to separate genera. For that reason, the two former *Geotrichum* species were reclassified in different genera, with one, i.e., *Magnusiomyces*, being defined by the sexual form of propagation, while *Saprochaete* was used when the form of propagation was unknown. In 2013, the principle of dual nomenclature was abandoned (17), with the oldest name for a fungus having priority and covering all forms of propagation of the fungus. Molecular phylogeny was recommended as the main parameter of classification. Judging by the ribosomal phylogenetic data provided by de Hoog and Smith (7) and confirmed by subsequent studies (15), the genera *Magnusiomyces* and *Saprochaete* appear to belong to the same clade, with the caveat that the type species of *Saprochaete*, *Saprochaete saccharophila*, has not yet been sequenced and thus might be unrelated. *Magnusiomyces*, with the type species *Magnusiomyces magnusii*, is the oldest name for the clade containing the two species under study. The name dates back to Zender (1925); however, *Endyllum* Clements (1931), *Saprochaete* Coker & Shanor ex D.T.S. Wagner & Dawes (1970), *Zendera* Redhead & Malloch (1977), *Ascotrichosporon* Kocková-Krachovilová et al. (1977), and *Blastoschizomyces* Salkin et al. (1985) are probably younger synonyms, depending on the final phylogenetic position of their type species. As a consequence, the name *S. clavata* will have to be changed again, as follows (see page 57 in reference 8): *Magnusiomyces clavatus* (de Hoog, M.Th. Smith & Guého) E. Kaplan, comb. nov. Basionym: *Geotrichum clavatum* de Hoog, M.Th. Smith & Guého.

Among deposits in databases that had been identified on the basis of morphology or ITS sequences, several misidentifications were revealed in the present study. Judging from the multilocus sequencing data based on the *Rbp2*, *Act*, and *Tef1α* sequences combined with the ITS sequences, three *M. clavatus* reference isolates (CBS 132759, CBS 139.32, and CBS 132760) were incorrectly attributed to *M. capitatus*. This was also confirmed by physiological tests. *M. capitatus* is consistently unable to grow with arbutin, cellobiose, and salicin and does not tolerate a temperature of 45°C (Table 3). Routine identification of *M. capitatus* with commercially available carbon assimilation tests and automated platforms (e.g., the API 20C AUX, ID 32C, and RapID Yeast Plus systems) is currently not possible because the specific discriminative carbon sources salicin and arbutin are not incorporated in those tests (8, 15). Matrix-assisted laser desorption ionization–time of flight mass spectrometry may aid in diagnosis after further expansion of the fungal mass spectrometry profile database (15, 26).

Intraspecific variability was particularly evident from the AFLP data. Notably, *M. clavatus* clustered in a single AFLP group (Fig. 3), with two main sublineages containing three and four strains. Further, CBS 758.85 was not part of the sublineages in the *M. clavatus* cluster, which is consistent with the concatenated tree (Fig. 2). Despite the small number of *M. clavatus* strains analyzed in the current study, the AFLP pattern represented these previously reported two main sublineages. On the basis of the

multilocus dendrogram, *M. capitatus* was found to be more genetically monomorphic than it was by AFLP analysis. Eleven strains of *M. capitatus* and nine strains of *M. clavatus*, including the type strains, showed the same position in the concatenated tree after multilocus analysis, suggesting a possible clonal relationship; however, lower similarities (80 to 90%) were observed among the strains of *M. capitatus* after AFLP analysis.

Vaux et al. (2) assessed the genomic single nucleotide polymorphisms of 73 *S. clavata* isolates using a mapping approach and obtained two largely homogeneous phylogenetic sublineages. In a recent study, Koç et al. (27) evaluated clinical isolates of *M. capitatus* using repetitive element sequence-based-PCR fingerprinting. On the basis of cluster similarity percentages, five main clones were detected; however, no genetic relationship was observed according to the patients' identity, location, or sample type. In addition, Subramanya Supram et al. (28) investigated seven *M. capitatus* isolates by AFLP genotyping and found ~80% similarity among these isolates, which was consistent with our findings by AFLP analysis.

Although Vaux et al. (2) referred to two subgroups of *M. clavatus* with different virulence, no correspondence of subgroups with the source or transmission route could be identified, which is in agreement with the findings published in the literature (28–30). Notably, *M. capitatus* is the main clinical species, while *M. clavatus* is more frequently encountered in the drawers of household dishwashers. This matches the observed differences in the thermotolerance between the species. It is remarkable that even the slight difference in ecology between these closely related species would lead to such a large clinical difference, given the overall rarity of *M. clavatus* in clinical settings (30). The source and transmission of the main opportunist, *M. capitatus*, are currently unknown and thus need to be clarified.

M. capitatus is mainly known to come from compromised human hosts. The most common risk factor for invasive *Magnusiomyces* infection is prolonged neutropenia with a poor outcome, and the main portal of entrance is the lung (see Table S3 in the supplemental material) (1, 30). A recent cohort study reported a rate of *Magnusiomyces* sp. infection of 3.4 per 100,000 patients with cancer (31), and household dishwashers have been reported to be an environmental habitat (10–12). This environment combines high temperatures and moisture and provides an indoor reservoir for other waterborne opportunists, such as *Candida parapsilosis* and *Exophiala dermatitidis*, which are not commonly isolated from environmental sources. A direct link between susceptible patients and dishwashers has not been established and is perhaps less likely because the immunocompromised host population is mostly hospitalized and thus not directly exposed to household utensils.

Optimal antifungal therapy has not yet been established for infections by *M. capitatus* and *M. clavatus*. Arendrup et al. (32) recommended amphotericin B (with or without flucytosine) or voriconazole for the treatment of *M. capitatus* infections, but no guideline was reported for *M. clavatus*. Durán-Graeff et al. (30) noted that echinocandins are not an option for the treatment of infections caused by these arthroconidial yeasts. This situation highlights an urgent need for the identification of optimal treatment parameters for the filamentous yeasts. The present study presents the results of susceptibility testing for one of the largest series of these species to be tested, with susceptibility to eight antifungals being evaluated. Although clinical breakpoints are not available for *M. capitatus*, antifungal susceptibility testing of new isolates is highly recommended (28). Importantly, *M. capitatus* is considered intrinsically resistant to echinocandins (4, 32, 33). In this study, amphotericin B and triazoles (except for fluconazole) showed potent *in vitro* activity against *M. capitatus*. This confirms the data available in the literature, which show that itraconazole, voriconazole, and posaconazole (MIC ranges, 0.12 to 0.5, 0.25 to 0.5, and 0.03 to 0.5 $\mu\text{g/ml}$, respectively [4, 28, 34–36]) have low MICs against *M. capitatus* but not fluconazole (MIC range, 16 to 32 $\mu\text{g/ml}$ [4, 34, 36, 37]). In contrast, a study from Nepal demonstrated low MIC values for fluconazole (2 $\mu\text{g/ml}$) and high MIC values for amphotericin B (2 $\mu\text{g/ml}$) in seven *M. capitatus* isolates (28).

Amphotericin B is the most active drug against *M. clavatus* isolates, followed by voriconazole, posaconazole, and itraconazole. Consistent with our findings, fluconazole (8 µg/ml) was shown to have poor *in vitro* activity (38). Favre et al. (39) observed high MEC/MIC values for echinocandins (caspofungin and micafungin) and fluconazole (≥ 4 µg/ml and 32 µg/ml, respectively), whereas lower MIC values were observed for amphotericin B (0.25 µg/ml), flucytosine (0.25 µg/ml), and both voriconazole and posaconazole (0.5 µg/ml). A recent study also examined the susceptibility profiles of 11 *M. capitatus* and 4 *M. clavatus* isolates using Etest or the Sensititre YeastOne system (30). Overall, the lowest MICs were obtained for triazoles, except fluconazole (< 1 µg/ml for voriconazole, itraconazole, and posaconazole, respectively), followed by flucytosine and amphotericin B. In general, *Magnusiomyces* species had high MICs for echinocandins, particularly for caspofungin (> 32 µg/ml) (30).

In conclusion, we have provided detailed molecular characteristics of two species of arthroconidial yeasts, thereby greatly expanding the current database beyond rDNA ITS sequence classification and incorporating new identification markers obtained using multilocus sequence typing of various genes, such as *Rbp2*. Moreover, antifungal testing revealed that voriconazole and amphotericin B are the most effective against these potential pathogens. Finally, based on our results, we suggest modifying the nomenclature of *S. clavata* according to updated fungal nomenclature guidelines.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.01427-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

ACKNOWLEDGMENTS

We are grateful to Ilse Curfs-Breuker for technical assistance.

Engin Kaplan, a Ph.D. student at Çukurova University, Turkey, was supported by a grant from the Federation of European Microbiological Societies (FEMS-RG-2015-0049).

The funders had no influence on the study design; collection, analysis, and interpretation of data; preparation of the manuscript; or decision to publish.

J.F.M. received grants from Astellas, Basilea, and Merck. He has been a consultant to Astellas and Merck and received speakers' fees from Gilead Sciences, Merck, Pfizer, and United Medical. None of the other authors have competing interests.

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