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Phylogeny of the industrial relevant, thermophilic genera *Myceliophthora* and *Corynascus*

Joost van den Brink · Robert A. Samson · Ferry Hagen · Teun Boekhout · Ronald P. de Vries

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Abstract Species of the genus *Myceliophthora* and its teleomorph *Corynascus* have attracted increasing interest due to their potential to produce thermostable enzymes. This study re-assessed the phylogenetic relationship of 49 isolates of nine species belonging to *Myceliophthora* and *Corynascus*. One species, *M. vellerea*, was shown not to belong to the genus *Myceliophthora* and should be placed in the genus *Ctenomyces*. The other species belonged to two phylogenetic clusters: mesophilic fungi with the type species *M. lutea* and *C. sepedonium*, and thermophilic fungi with *M. thermophila, M. hinnulea* and *C. thermophilus*. The phylogenetic data provides no clear separation of the two genera *Corynascus* and *Myceliophthora*. To avoid confusion in future taxonomic studies, it is proposed that all existing *Corynascus* species be renamed to *Myceliophthora*, which is the old name and the one more frequently used. Furthermore, this study identified two groups within the isolates listed as *M. thermophila* and assigned one group (five isolates) to *M. heterothallica* based on AFLP analysis and mating behavior. This study provides new insights into the genetic differences within the genus *Myceliophthora* and will therefore be essential for the interpretation of future genomic and physiological studies of these species.

Keywords *Myceliophthora* · *Corynascus* · Thermophiles · *M. heterothallica* · *M. thermophila* · Thermophilic fungi · Multigene phylogeny · AFLP analysis · Mating behavior

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J. van den Brink · R. A. Samson · F. Hagen · T. Boekhout · R. P. de Vries (✉)
CBS-KNAW Fungal Biodiversity Centre,
P.O. Box 85167, 3508 AD Utrecht, The Netherlands
e-mail: r.devries@cbs.knaw.nl

Introduction

Species of genus *Myceliophthora* and its teleomorph *Corynascus* have attracted increasing interest due to their potential to produce thermostable enzymes. For instance, laccases of *M. thermophila* (basionym: *Sporotrichum thermophilum*) were shown to be thermostable with high activity after expression in different expression hosts (Berka et al. 1997; Bulter et al. 2003; Babot et al. 2011). Due to the potential of *Myceliophthora* to degrade lignocellulolytic plant material, many (hemi-)cellulolytic enzymes of *M. thermophila* are characterized and patented (Bhat and Maheshwari 1987; Roy et al. 1990; Sadhukhan et al. 1992; Badhan et al. 2007; Beeson et al. 2011). The importance of this fungal group has recently been underlined by the sequencing of the genome of *M. thermophila* isolate ATCC42464 (genome.jgi-psf.org/Spoth1).

The first *Myceliophthora* species, *M. lutea*, was described by Constantin and Matruchot in 1894 as a pathogen causing the 'vert de gris' mat disease of cultured mushrooms (Costantin 1892). This species was classified before as a member of the genus *Chrysosporium* (Carmichael 1962), but there after von Arx re-introduced the genus *Myceliophthora* and its type species *M. lutea* (von Arx 1973). Initially, three species were assigned to this genus: *M. fergusii, M. lutea*, and *M. thermophila* (van Oorschot 1980). Another species, *M. vellerea*, was most likely wrongly described as a *Myceliophthora* species based on morphological differences (Sigler et al. 1998). A fourth species, *M. hinnulea*, was assigned to the genus *Myceliophthora* by Awao and Udagawa (1983). The type species of the ascomycete genus *Corynascus, C. sepedonium*, was described by Emmons (1932). This species was originally part of the genus *Thielavia* before von Arx introduced the genus *Corynascus*. This genus can be distinguished from *Thielavia* by the presence of ascospores with two germ pores, one at

each end (von Arx 1973). At that time, the genus *Corynascus* contained the species *C. sepedonium* and *C. novoguineensis* (von Arx 1973). Currently, seven *Corynascus* species are described: *C. heterothallicus*, *C. novoguineensis*, *C. sepedonium*, *C. sexualis*, *C. similis*, *C. thermophilus* and *C. verrucosus* (von Klopotek 1974; Stchigel et al. 2000).

Of all the species of these two genera, *M. thermophila* is most commonly used for applied research (Roy et al. 1990; Berka et al. 1997; Rosgaard et al. 2006; Badhan et al. 2007; Beeson et al. 2011). Several isolates of *M. thermophila* can grow at temperatures up to 50°C on cellulose-rich material and can decompose complex substrates such as birch chips, wood pulp and wheat straw (Bhat and Maheshwari 1987). *M. thermophila* was initially classified in the genus *Sporotrichum* (Fergus and Sinden 1969) before it was assigned to the genus *Chrysosporium* as *C. thermophilum* in 1974 (von Klopotek). Two years later Klopotek described *Thielavia heterothallica* as the teleomorph of *C. thermophilum* (von Klopotek 1976). The current names of these teleomorphs and anamorphs are *Corynascus heterothallica* and *Myceliophthora thermophila*, respectively (van Oorschot 1977; von Arx et al. 1984). A similar re-designation occurred for *C. thermophilus* and *M. fergusii* (Sigler et al. 1998). While the other species of *Myceliophthora* and *Corynascus* were not matched for their teleomorphic or anamorphic counterparts.

Although species within *Myceliophthora* and *Corynascus* are morphologically well described, a study comprising their genetic differences has not yet been performed. Understanding the genetic diversity of these genera is essential for upcoming genomic and applied studies based on the availability of the *M. thermophila* genome sequence. Our study describes the phylogenetic relationships of 49 isolates belonging to the genera *Myceliophthora* and *Corynascus* and investigates in detail the genetic diversity of 11 *M. thermophila* isolates.

Materials and methods

Strains

All strains used in this study are listed in Table 1, and are available from the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (www.cbs.knaw.nl).

DNA extraction, sequencing analysis, and AFLP

Fungal genomic DNA was isolated using the FastDNA® Kit (Bio 101, Carlsbad, USA) according to the manufacturer's instructions. Amplification and sequencing of the ITS region (including internal transcribed spacer regions 1 and 2, and the 5.8S rRNA regions of the nuclear ribosomal RNA gene cluster), and parts of the elongation factor *EF1A* and the subunit of RNA polymerase II *RPB2* genes were performed as described by Houbraken et al. (2007). Fragments containing the ITS region were amplified using primers V9G (TTACGTCCCTGCCCTTTGTA) and RLR3R (GGTCCGTGTTTCAAGAC). Fragments containing the *EF1A* region were amplified using forward primer GCCCCCGGCCATCGTGACTTCAT and reverse primer ATGACACCGACAGCGACGGTCTG. Fragments containing the *RPB2* region were amplified using forward primer GACGACCGTGATCACTTTGG and reverse primer CCCATGGCCTGTTTGCCCAT. Contigs were assembled from the forward and reverse sequences using SeqMan from the Lasergene package (DNASTAR Inc., Madison, WI). The alignments of the sequence datasets using Clustal W and phylogenetic analysis were performed in MEGA version 4 (Tamura et al. 2007). Maximum parsimony analysis was performed for all datasets using the heuristic search option. The robustness of the most parsimonious trees was evaluated with 1000 bootstrap replications (Hillis and Bull 1993). Sequences of *Saccharomyces cerevisiae* S228C were used as outgroup in the analyses of all used loci. Newly generated sequences were deposited in GenBank with accession numbers HQ871703–HQ871841 (Table 1). The generated alignments and the most parsimonious trees were deposited in TreeBase under accession number 11154 (http://purl.org/phylo/treebase/phylows/study/TB2:S11154).

The genotype of each isolate listed as *M. thermophila* was determined using AFLP fingerprint analysis, as described previously by Boekhout et al. (2001).

Mating experiment

The mating experiment was performed on two media: Malt Extract Agar (MEA) and Oatmeal Agar (OA) medium (Samson et al. 2010). A small agar plug containing mycelium (1 mm diameter) from the edge of a vigorously growing 1-day-old colony on MEA medium was transferred to the Petri dishes with OA or MEA media. The initial combination of isolates CBS202.75 and CBS203.75 with one of the nine other *M. thermophila* isolates were incubated in the dark at 35°C (von Klopotek 1974). The combination of isolates CBS117.65, CBS173.70, CBS381.97, CBS669.85, CBS866.85 and ATCC42464 were incubated in the dark at 30°C, 35°C, 40°C or 45°C. The mating experiment was conducted twice for each combination of isolates.

Results

Phylogeny of genera *Corynascus* and *Myceliophthora*

Forty-nine isolates of the genera *Myceliophthora* and *Corynascus* were phylogenetically investigated by compar-

Table 1 *Myceliophthora* and *Corynascus* isolates examined in this study. Type isolates are indicated with [T]

Original species name	Proposed species name	Accession no.	Source and remarks	GenBank no. *ITS1*	GenBank no. *EF1A*	GenBank no. *RPB2*
M. thermophila	*M. thermophila*	CBS 117.65[T]	Dry pasture soil, UK	HQ871764	HQ871705	HQ871803
		CBS 173.70	Wheat straw compost, UK	HQ871765	HQ871706	HQ871804
		CBS 381.97	Man, HIV pos. patient, unknown location	HQ871766	HQ871707	HQ871805
		CBS 669.85	Unknown source; mutant of CBS 866.85	HQ871767	HQ871704	HQ871806
		CBS 866.85	Unknown source	HQ871768	HQ871708	HQ871807
		ATCC 42464	Unknown source	HQ871769	HQ871703	HQ871802
M. thermophila	*M. heterothallica*	CBS 131.65	Birch chips, Sweden	HQ871770	HQ871709	–
		CBS 202.75	Garden soil, Germany; authentic strain of *T. heterothallica*	HQ871771	HQ871710	HQ871798
		CBS 203.75	Soil, Indiana, USA; authentic strain of *T.heterothallica*	HQ871772	HQ871711	HQ871800
		CBS 375.69	Woodpulp, New Brunswick, Canada	HQ871773	HQ871712	HQ871799
		CBS 663.74	Soil under a baobab (*Adansonia digitata)*, Senegal	HQ871774	HQ871713	HQ871801
M. lutea	*M. lutea*	CBS 145.77 [T]	Hay, UK	HQ871775	HQ871722	HQ871816
		CBS 146.50	Mushroom bed, Delaware, USA	HQ871776	HQ871724	HQ871818
		CBS 146.77	Barley (*Hordeum vulgare*), Ireland	HQ871777	HQ871725	HQ871819
		CBS 147.50	Mushroom bed, Pennsylvania, USA	HQ871778	HQ871726	HQ871820
		CBS 147.77	Dust in stable, UK	HQ871779	HQ871728	HQ871821
		CBS 157.51	Mushroom bed, Netherlands	HQ871780	HQ871730	HQ871817
		CBS 157.59	Air in pigsty, Netherlands	HQ871781	HQ871729	HQ871822
		CBS 227.67	Mushroom bed, Netherlands	HQ871782	HQ871721	HQ871823
		CBS 243.75A	Air, Uttar Pradesh, India	HQ871783	HQ871723	HQ871824
		CBS 243.75B	Air, Uttar Pradesh, India	HQ871784	HQ871720	HQ871826
		CBS 379.76	Usar soil, Uttar Pradesh, India	HQ871785	HQ871727	HQ871825
M. hinnulea	*M. hinnulea*	CBS 539.82	Soil from cultivated garden, New Zealand	HQ871786	HQ871714	HQ871808
		CBS 540.82	Soil under Monterey Pine (*Pinus radiata*), New Zealand	HQ871787	HQ871716	HQ871809
		CBS 541.82	Sun-exposed garden soil, New Zealand	HQ871788	HQ871715	HQ871810
		CBS 542.82	Sun-exposed garden soil, New Zealand	HQ871789	HQ871717	HQ871811
		CBS 544.82	Soil, New Zealand	HQ871790	HQ871718	HQ871812
		CBS 597.83 [T]	Cultivated soil, Japan	HQ871791	HQ871719	HQ871813
M. vellerea	*Ctenomyces vellerea*	CBS 478.76	Unknown source, Egypt	HQ871796	HQ871748	–
		CBS 479.76	Unknown source, Egypt	HQ871797	HQ871749	HQ871840
		CBS 715.84	Soil, Alberta, Canada; ex-type of *C. asperatum*	HQ871795	HQ871747	HQ871841
C. thermophilus	*M. fergusii*	CBS 174.70	Wheat straw compost, UK	HQ871792	–	–
		CBS 405.69	Mushroom compost, Pennsylvania, USA; MT +	HQ871793	HQ871731	HQ871814
		CBS 406.69 [T]	Mushroom compost, Pennsylvania, USA; MT −	HQ871794	HQ871732	HQ871815
C. sepedonium	*M. sepedonium*	CBS 111.69 [T]	Soil, Uttar Pradesh, India; ex-type of *T. sepedonium*	HQ871751	HQ871734	HQ871827
		CBS 213.74	Sandy soil, Senegal	HQ871752	HQ871736	HQ871828
		CBS 223.81	Desert soil, Kuwait	HQ871753	HQ871737	HQ871831
		CBS 294.56	Buried cable in soil, Netherlands	HQ871754	HQ871738	HQ871832
		CBS 340.33	Unknown source	HQ871755	HQ871739	HQ871829
		CBS 412.52	Soil, Argentina	–	HQ871740	HQ871833

Table 1 (continued)

Original species name	Proposed species name	Accession no.	Source and remarks	GenBank no. *ITS1*	GenBank no. *EF1A*	GenBank no. *RPB2*
		CBS 415.48	Cotton rope, Uttar Pradesh, India	HQ871756	HQ871741	HQ871834
		CBS 434.96	Soil, Delhi, India	HQ871760	–	–
		CBS 435.96	Soil, Singapore	HQ871761	HQ871745	–
		CBS 438.96	Soil, Uttar Pradesh, India	HQ871757	HQ871742	HQ871835
		CBS 440.51	Soil, UK	HQ871758	HQ871743	HQ871836
		CBS 632.67	Unknown source, Russia; ex-type of *Thielavia lutescens*	HQ871759	HQ871744	HQ871830
		CBS 114383	Barley (*Hordeum vulgare*), Iran	HQ871750	HQ871735	HQ871837
C. novoguineensis	*M. novoguineensis*	CBS 359.72	Soil, Papua New Guinea	HQ871762	HQ871733	HQ871838
Corynascella inaequalis		CBS 284.82	Soil, Tarragona, Spain	HQ871763	HQ871746	HQ871839

ison of sequences (Table 1) of five genomic loci, namely the internal transcribed spacer 1 (*ITS1*), part of elongation factor EF1A, part of the RNA polymerase subunit *RBP2*, the D1/D2 locus of large ribosomal subunit and part of ß-tubulin (*TUBB*). Unfortunately, the sequences of the D1/D2 locus did not have enough variation to perform a phylogenetic analysis. In addition, part of the ß-tubulin locus of *M. lutea* was duplicated on the genome resulting in unclear sequences. Therefore, these two loci were eliminated from the comparison. The constructed phylogenetic trees of the remaining three loci were the results of a bootstrap consensus by maximum parsimony.

The phylogenies obtained from the three loci, *ITS1*, *EF1A* and *RBP2*, gave a clear clustering of the isolates of each species (Figs. 1, 2 and 3). Except for *M. vellerea*, the isolates of *Corynascus* and *Myceliophthora* clustered together and showed a close relation to other isolates of the family *Chaetomiaceae* (e.g. *Chaetomium globosum*, *Corynascella inaequalis* and *Thielavia terrestris)*. Based on the large differences of the *ITS1*, *EF1A* and *RBP2* sequences of *M. vellerea* when compared to those of the *Corynascus* and other *Myceliophthora* species, it is clear that *M. vellerea* has been wrongly placed within the genus *Myceliophthora*. The *ITS1* region of *M. vellerea* was highly similar to *Ctenomyces serratus* (661 of 678 nucleotides identical), suggesting that this species should be placed in the genus *Ctenomyces*.

The *C. sepedonium* isolates and related *Corynascus* species clustered together in all phylogenies. Only 1 of 456 nucleotides of the *ITS1* sequences within this *Corynascus* cluster was found to be parsimony informative. The phylogenies of all three loci showed that *M. lutea* was the closest related species to *C. sepedonium* and related *Corynascus* species. Their close relation was represented by the *ITS1* sequences of *C. sepedonium* and *M. lutea*, where only three nucleotides were parsimony informative.

The isolates of the thermophilic species *M. hinnulea* and *M. thermophila* were closely related in all phylogenies. The *ITS1* sequences of *M. hinnulea* and *M. thermophila* had 12 of 456 parsimony informative nucleotides. Both species clustered with the thermophilic species *C. thermophilus* in the trees of *ITS1* and *RPB2*. Thirty-two of 456 nucleotides of the *ITS1* sequences within this cluster of the three thermophilic fungi were found to be parsimony informative. However, in the *EF1A* tree, *C. thermophilus* clustered separately from all other *Corynascus* and *Myceliophthora* isolates.

Genetic diversity within the thermophilic *Myceliophthora thermophila*

The 11 isolates listed as *M. thermophila* consistently clustered in two groups at all phylogenies (Figs. 1, 2 and 3). This variation between the isolates is also reflected by the relatively high amount of informative sites at the three loci (e.g. 12 informative sites of 456 nucleotides of the ITS1 loci; 2.6%). In comparison, this variation was similar to the sequence variation between the species *M. hinnulea* and *M. thermophila*. The group of 11 isolates of *M. thermophila* clustered into two main groups with the exception of *M. thermophila* CBS663.74. This latter isolate was placed between the two groups of *M. thermophila* in the *ITS1* and *EF1A* trees, but grouped with CBS131.65, CBS202.75, CBS203.75 and CBS375.69 in the *RPB2* tree.

The genetic variation within *M. thermophila* was further investigated by Amplified Fragment Length Polymorphism (AFLP). The banding patterns of the 11*M. thermophila* isolates confirmed the clustering in two groups (Fig. 4). The sequence data and AFLP analysis placed CBS117.65, CBS173.70, CBS381.97, CBS669.85, CBS866.85 and ATCC42464 in one group, while CBS131.65, CBS202.75, CBS203.75 and CBS375.69 were placed in a second group. The AFLP banding pattern of CBS663.74 did not fit with either of the groups, thus confirming the results of the phylogenies of *ITS1* and *EF1A* (Figs. 1 and 2) in which CBS663.74 occurred outside both groups of *M. thermophila*.

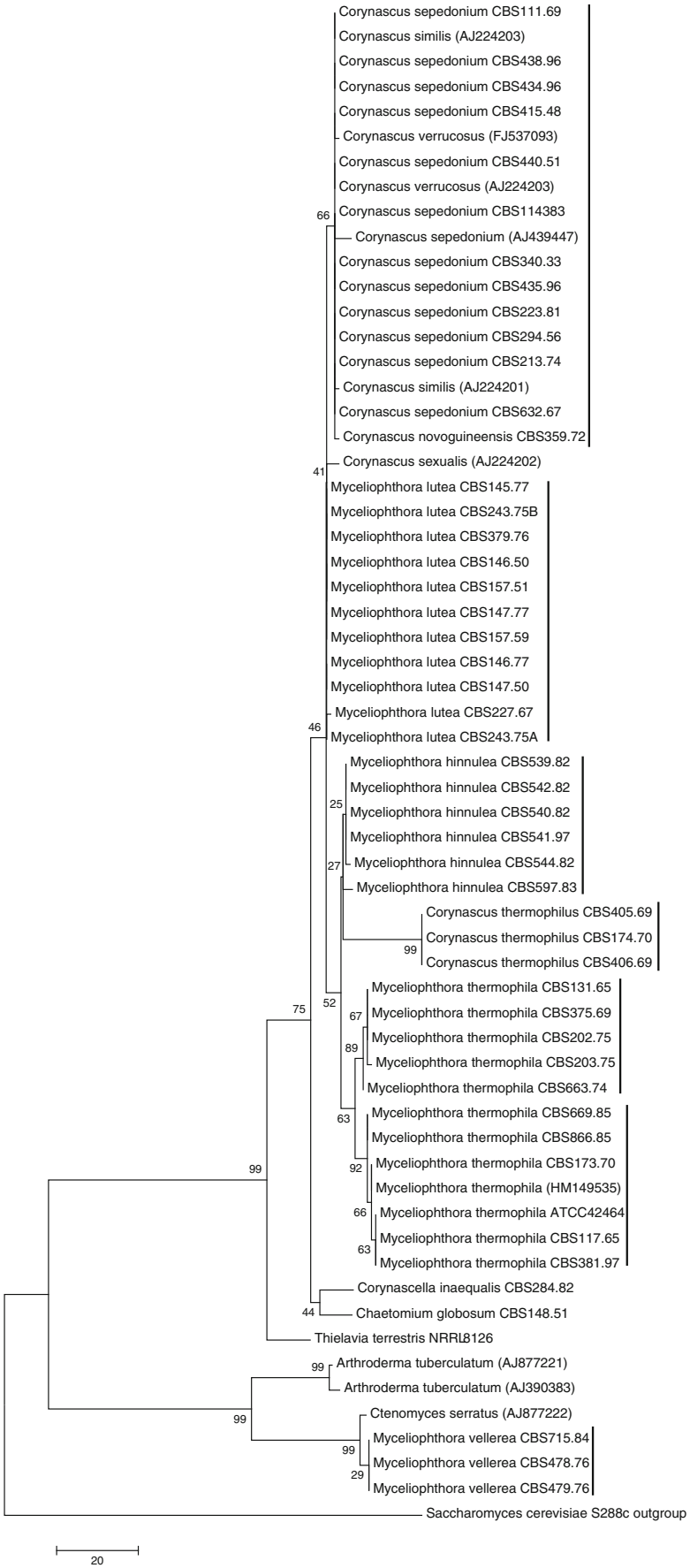

Fig. 1 Parsimonious consensus tree of the analysed *ITS1* region of *Myceliophthora* sp. and *Corynascus* sp. (134 of the 389 nucleotides were parsimony informative). The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches. All positions containing gaps and missing data were eliminated from the dataset

Fig. 2 Parsimonious consensus tree of the analysed elongation factor *EF1A* gene sequences of *Myceliophthora* sp. and *Corynascus* sp. (136 of the 654 nucleotides were parsimony informative). The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches. All positions containing gaps and missing data were eliminated from the dataset

Mating types of *Myceliophthora thermophila* isolates

The mating behavior of each *M. thermophila* isolate was studied by crossing the two mating types CBS202.75 and CBS203.75 with each of the nine other *M. thermophila* isolates. After 3 weeks, all plates had ascomata containing dark brown ascospores at the contact zone between CBS202.75 and CBS203.75 (Fig. 5e–g). The dark colored ascomata were produced in the agar media and were only visible at the reverse of plates (Fig. 5a–d). The mating experiment showed that CBS202.75 and CBS663.74 had the same mating type, while CBS203.75, CBS131.65, and CBS375.69 had the opposite mating type (Table 2). These isolates all belong to one of the *M. thermophila* groups based on the phylogenies described above. The remaining six *M. thermophila* isolates, belonged to the

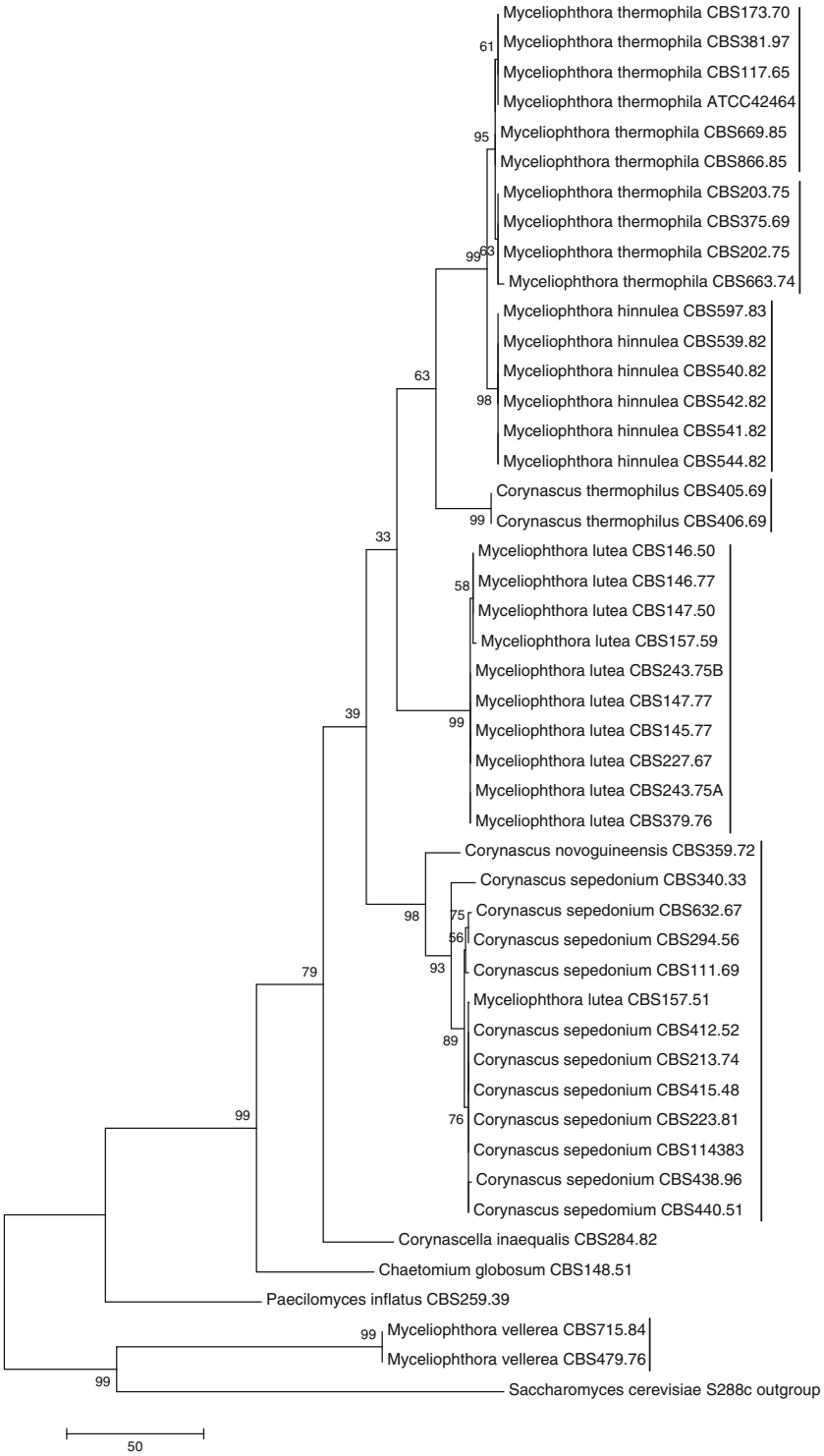

Fig. 3 Parsimonious consensus tree of the analysed partial *RPB2* gene sequences of *Myceliophthora* sp. and *Corynascus* sp. (257 of the 611 nucleotides were parsimony informative). The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches. All positions containing gaps and missing data were eliminated from the dataset

other phylogenetic group, and did not produce fruiting bodies at the contact zone with CBS202.75 or CBS203.75. Moreover, when combined with each other on oatmeal agar plates, isolates CBS117.65, CBS173.70, CBS381.97, CBS669.85, CBS866.85 and ATCC42464 were not able to produce fruiting bodies after 4 weeks at 30°C, 35°C, 40°C or 45°C.

Discussion

Myceliophthora: a single name for species hitherto classified in *Corynascus* and *Myceliophthora*

The molecular phylogeny of *Myceliophthora* and *Corynascus* gave new insights into the taxonomic relationships between

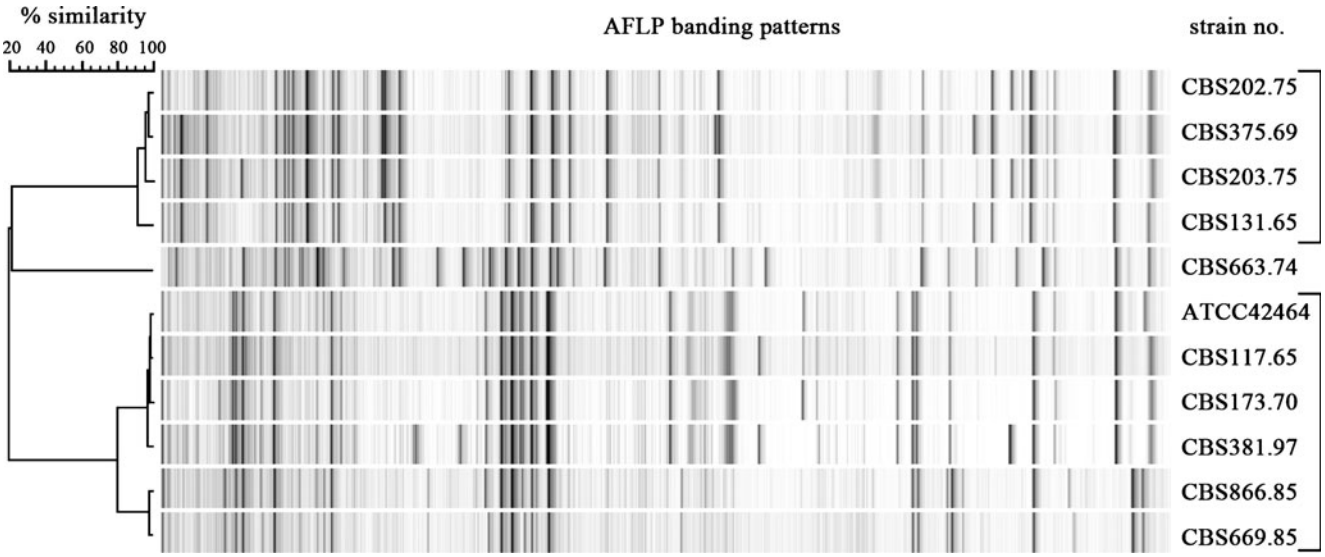

Fig. 4 Clustering of AFLP banding patterns of *Myceliophthora thermophila* isolates. Similarity of the banding patterns is given in percentage

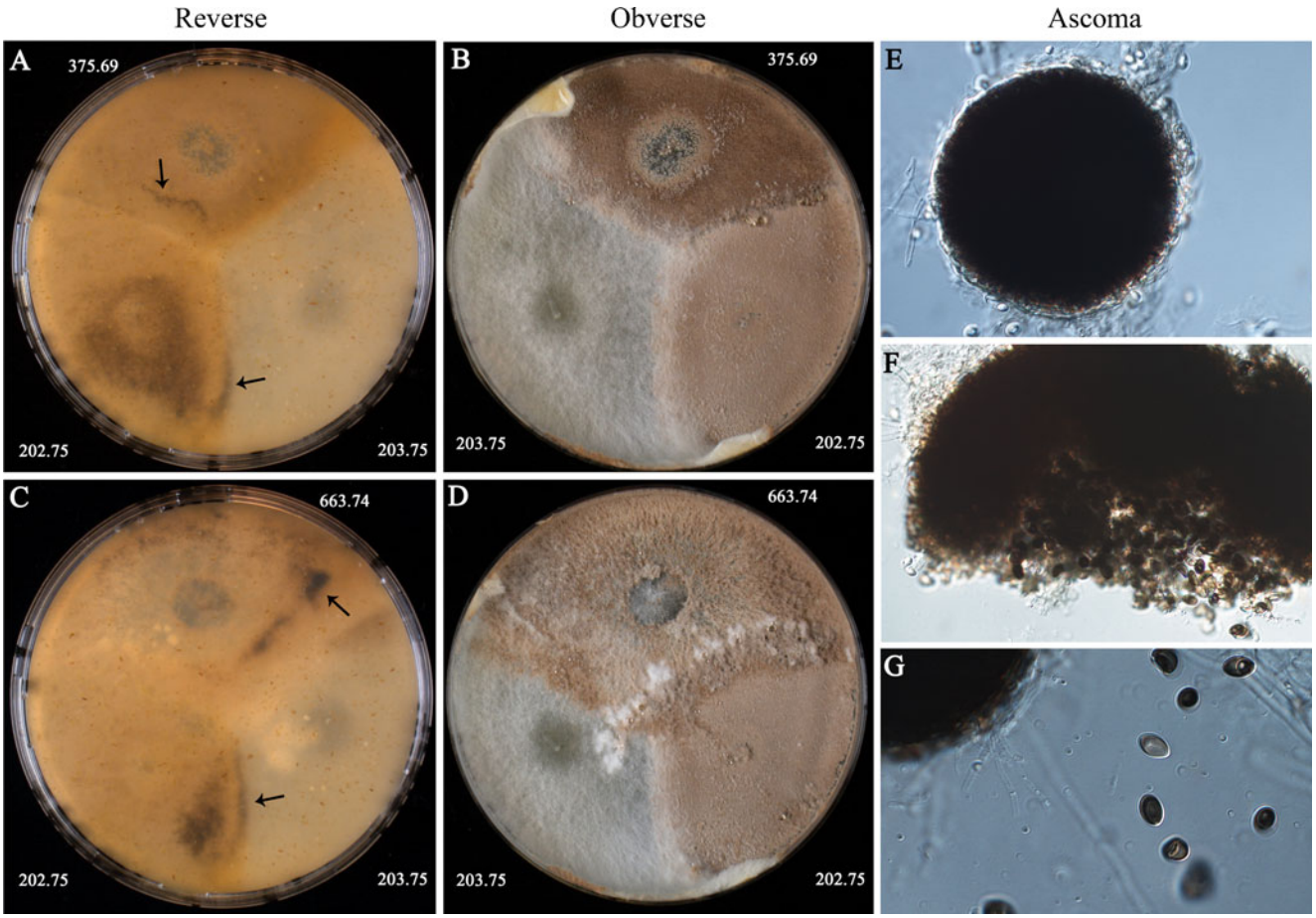

Fig. 5 Plates with different *Myceliophthora thermophila* isolates and microscope pictures of the formed ascoma. Figure **a** and **b** are, respectively, the reverse and obverse of a plate depicting the mating between *M. thermophila* CBS375.69 & CBS202.75 and CBS202.75 & CBS203.75. Figure **c** and **d** are, respectively, the reverse and obverse of a plate depicting the mating between *M. thermophila* CBS663.74 & CBS203.75, and CBS202.75 & CBS203.75. Formed ascoma in figure **a** and **c** are indicated with an arrow. Figure **e**, **f** and **g** are microscope pictures of the produced ascoma and ascospores, respectively, ×100, ×400 and ×1000

Table 2 Mating types of *Myceliophthora thermophila*

Accession no.	Mating type (+, −, absent)
CBS202.75	MT +
CBS663.74	MT +
CBS131.65	MT −
CBS203.75	MT −
CBS375.69	MT −
CBS117.65	absent
CBS173.70	absent
CBS381.97	absent
CBS669.85	absent
CBS866.85	absent
ATCC42464	absent

these two genera. Firstly, the *ITS1* sequences of CBS478.76, CBS479.76 and CBS715.84 confirmed that *M. vellerea* does not belong to *Myceliophthora* and should be classified as *Ctenomyces serratus*. This was already suggested based on morphological characteristics (Guarro et al. 1985).

Another observation was the sequence similarity of many *Corynascus* species. Although morphological differences have been observed, the *ITS1* sequence of *C. sepedonium, C. sexualis*, *C. similis*, *C. novoguineensis* and *C. verrucosus* were more than 99.5% similar. This contrast between morphology and *ITS1* phylogeny for *Corynascus* species has already been reported before (Stchigel et al. 2000). The *EF1A* and *RPB2* sequences of *C. sepedonium* and *C. novoguineensis* showed more diversity and might justify the current classification within *Corynascus*. This shows that analysis of multiple loci (Samson et al. 2007) is useful, especially in the phylogenetic characterization of *Corynascus* species.

The isolates of *C. sepedonium* and *M. lutea* are closely related based on all generated phylogenies. Another common feature of *C. sepedonium* and *M. lutea* is their optimal growth temperature. The isolates of these species prefer to grow below 40°C, while the thermophilic *Corynascus* and *Myceliophthora* species have an optimal growth around 45°C (tested on malt extract agar plates, Supplemental data 1). These results show that fungi within the genera *Corynascus* and *Myceliophthora* can be split into two clusters: i.e., a mesophilic and a thermophilic cluster.

A clear separation of the two genera *Corynascus* and *Myceliophthora* is, however, not apparent from the phylogenetic data. Some species of the genus *Corynascus* have been the associated teleomorph of the anamorphic species classified within *Myceliophthora* (van Oorschot 1980). However, most species have unknown teleomorphs or anamorphs and the phylogenetic data in our study did not clarify this issue. CBS440.51 for instance has been described as an anamorph of *C. sepedonium* (van Oorschot 1980). No differences were observed in the sequence data between the anamorphs and teleomorphs of *C. sepedonium*. The dual name for this single taxon of species belonging to *Myceliophthora* and *Corynascus* should be used carefully. The issue of a single scientific name for fungal species has been increasingly raised, especially since genetic studies have become common practice (Rossman and Samuels 2005; Shenoy et al. 2007; Samson and Varga 2009; Hawksworth 2011). Given that the genus name *Myceliophthora* was described in 1894 and *Myceliophthora* is a common name in publications, we propose to name all *Corynascus* species as *Myceliophthora*. This forms no obstacle for most species of *Corynascus* as their species name is unique for the genus *Myceliophthora*. Only *Corynascus thermophilus* should be renamed under its old anamorph name *M. fergusii* (van Oorschot 1977). For *C. thermophilus*, *C. novoguineensis*, *C. sepedonium*, *C. sexualis*, *C. similis*, and *C. verrucosus* the formal new combinations are listed at the end of the manuscript.

Genetic diversity and mating behavior set *M. heterothallica* apart from *M. thermophila*

The collection of the CBS-KNAW Fungal Biodiversity Centre contains ten isolates listed as *M. thermophila* (basionym: *Sporotrichum thermophilum*). The phylogenetic data revealed clear differences between the isolates and divided these isolates in two groups. One group contained the type isolate of *M. thermophila* and the strain ATCC42464, whose full genomic sequence is available. The other group consisted of five isolates including strains CBS202.75 and CBS203.75, which are authentic isolates of *Thielavia heterothallica* (von Klopotek 1976). Isolates of this later group can mate with each other and their mating types were identified. In light of the phylogenetic and biological species concept, we suggest that this teleomorph group will be named *Myceliophthora heterothallica*. For *Thielavia heterothallica* the formal new combination to the *Myceliophthora* is listed at the end of the manuscript.

According to the sequence data and AFLP analysis, CBS663.74 was different from the other isolates belonging to the *M. thermophila* and *M. heterothallica* group at the genetic level. This strain was also the only one obtained from the African continent, where it was isolated from soil under a baobab tree in Senegal. Nevertheless, the genetic differences did not prevent mating of CBS663.74 with other *M. heterothallica* isolates, suggesting that this isolate fits within the *M. heterothallica* group.

Fungi of the genus *Myceliophthora*, especially *M. thermophila*, are of industrial interest due to their potential to produce thermophilic enzymes (Bhat and Maheshwari 1987; Roy et al. 1990; Sadhukhan et al. 1992; Badhan et al. 2007; Beeson et al. 2011). This study described the genetic diversity amongst different *Myceliophthora* isolates and divided *M. thermophila* isolates in two species *M. thermo-*

phila and *M. heterothallica*. From the applied point of view, it will be of interest to investigate the physiological differences between both thermophilic fungi.

Myceliophthora Costantin 1892, in Cr Hebd Séanc Acad Sci Paris 114; 849–851

Myceliophthora lutea Costantin 1892 (MB232833)—Type species

- Synonym: *Scopulariopsis lutea* (Costantin) Tubaki 1955 (MB305672)
- Synonym: *Chrysosporium luteum* (Costantin) J.W. Carmich. 1962 (MB328210)
- Synonym: *Sporotrichum carthusioviride* J.N. Rai & Mukerji 1962 (MB339566)

Myceliophthora hinnulea Awao and Udagawa 1983 (MB109090)

Myceliophthora thermophila (Apinis) Oorschot 1977 (MB317955)

- Basionym: *Sporotrichum thermophilum* Apinis 1963 (MB344529)
- Synonym: *Chrysosporium thermophilum* (Apinis) Klopotek 1974 (MB311112)

Myceliophthora heterothallica (von Klopotek) van den Brink & Samson, comb. nov. (MB 519538)

- Basionym: *Thielavia heterothallica* von Klopotek 1976 (MB324556)
- Synonym: *Corynascus heterothallicus* (von Klopotek) von Arx, Dreyfuss & Müller 1984 (MB107879)

Myceliophthora fergusii (Klopotek) van Oorschot 1977 (MB317954)

- Synonym: *Thielavia thermophila* Fergus and Sinden 1969 (MB340061)
- Synonym: *Corynascus thermophilus* (Fergus & Sinden) Klopotek 1974 (MB312215)
- Synonym: *Chaetomidium thermophilum* (Fergus & Sinden) Lodha 1978 (MB310883)

Myceliophthora sepedonium (C.W. Emmons) van den Brink & Samson, comb. nov. (MB561525)

- Basionym: *Thielavia sepedonium* C.W. Emmons 1932 (MB277883)
- Synonym: *Corynascus sepedonium* (C.W. Emmons) von Arx 1973 (MB312213)
- Synonym: *Chaetomidium sepedonium* (C.W. Emmons) Lodha 1978 (MB310880)
- Synonym: *Thielavia sepedonium* var. minor Mehrotra & Bhattacharjee 1966 (MB353893)

Myceliophthora novoguineensis (Udagawa & Y. Horie) van den Brink & Samson, comb. nov. (MB561526)

- Basionym: *Corynascus novoguineensis* (Udagawa & Y. Horie) von Arx 1973 (MB312212)

Myceliophthora sexualis (Stchigel, Cano & Guarro) van den Brink & Samson, comb. nov. (MB561527)

- Basionym: *Corynascus sexualis* Stchigel, Cano & Guarro 2000 (MB467480)

Myceliophthora similis (Stchigel, Cano & Guarro) van den Brink & Samson, comb. nov. (MB561528)

- Basionym: *Corynascus similis* Stchigel, Cano & Guarro 2000 (MB467481)

Myceliophthora verrucosa (Stchigel, Cano & Guarro) van den Brink & Samson, comb. nov. (MB561529)

- Basionym: *Corynascus verrucosus* Stchigel, Cano & Guarro 2000 (MB467482)

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