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A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA

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Key words
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taxonomic revision
Zygomycota
Zygomycetes

Abstract The basal fungal order Mortierellales constitutes one of the largest orders in the basal lineages. This group consists of one family and six genera. Most species are saprobic soil inhabiting fungi with the ability of diverse biotransformations or the accumulation of unsaturated fatty acids, making them attractive for biotechnological applications. Only few studies exist aiming at the revelation of the evolutionary relationships of this interesting fungal group. This study includes the largest dataset of LSU and ITS sequences for more than 400 specimens containing 63 type or reference strains. Based on a LSU phylogram, fungal groups were defined and evaluated using ITS sequences and morphological features. Traditional morphology-based classification schemes were rejected, because the morphology of the Mortierellales seems to depend on culture conditions, a fact, which makes the identification of synapomorphic characters tedious. This study belongs to the most comprehensive molecular phylogenetic analyses for the Mortierellales up to date and reveals unresolved species and species complexes.

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INTRODUCTION

The order Mortierellales – from historical aspects on morphology and systematics to modern approaches in fungal identification

The Mortierellales are a long known, species rich order of the basal fungi. With nearly 100 described species, the Mortierellales is one of the largest basal fungal orders. However, only 13 genera are described in one family, the Mortierellaceae (Kirk et al. 2008, and Species Fungorum January 2013). Out of these genera six are currently accepted with one potential additional genus recently described (Kirk et al. 2008, Jiang et al. 2011, Table 1). The first species of the type genus was described by Coemans (1863) as Mortierella polycaphala, originally isolated from a mushroom. The name Mortierella was given in tribute to M. Du Mortier, the president of the Société de Botanique de Belgique (Coemans 1863). Nevertheless, the common lifestyle of those fungi is as soil inhabiting saprobic organisms from animal fungal infections (de Hoog et al. 2009). Many mortierelllean species possess the ability to produce polyunsaturated fatty acids or to convert organic compounds, making them highly interesting organisms for biotransformations and other biotechnological applications (Holland 2001, Higashiyama et al. 2002).

As many basal fungal species, the Mortierellales possess a reduced macro- and micromorphology with only few morphological characters available for differentiation. Examples of micromorphological features are shown in Fig. 1 and 2. Overall appearance of the colonies is the typical zonate, rosette-like growth (Fig. 1a) and the often occurring garlic-like odour. Colonies are in general white to light-grey, young mycelium is coenocytic and septate in aged cultures. Asexual spores are produced in sporangia or sporangiola and are passively released (e.g., Fig. 1h, s). The sporangioshores could be widened at the base (e.g., Fig. 10) and variously branched (e.g. Fig. 1h, l). A columella is never protruding into the sporangium. Sexual reproductive structures (zygospores, Fig. 2r) are often surrounded by a hyphal sheath. Variously shaped chlamydospores and stylospores are also possible (Fig. 1w, 2l) (Zycha et al. 1969, Games 1977). Morphological identification based solely on asexual features, leading to the aforementioned traditional classification. Mortierella was furthermore divided into nine sections based on morphology: Actinomortierella, Alpina, Haplosporangium, Hygrophila, Mortierella, Schmuckeri, Simplex, Spinosa and Stylospora (Gams 1977).

Judging from the proposed total number of fungi with 1.5 million species and the current number of described and registered species with 75 000 (Hawksworth 2001) it seems likely that also for the order Mortierellales an unknown percentage of undescribed species may exists, a fact which might influence phylogenetic analyses. Yet, a recent study challenged previous estimations of the potential number of undescribed fungal species and proposed that, at least for Mortierella, nearly all species are most likely described already (Nagy et al. 2011). Based on this knowledge, phylogenetic analyses including sequences of an extensive amount of type and reference strains could reveal the natural evolutionary relationships.

Nevertheless, the phylogenetic position of the Mortierellales is controversial discussed. They are either placed within the subphylum Mucoromycotina (Hibbett et al. 2007) or elevated to an own subphylum, the Mortierellomycotina (Hoffmann et al. 2011). Furthermore, relationships within this order are also
Fig. 1 Typical morphological structures of different isolates of the Mortierellales, which are suitable for species delimitation. a. *M. zychae* CBS 316.52, macroscopic shape of a growing culture with the typical zonate growth; b. *M. hypsicladia* CBS 116202, acrotonous branching of a sporangiophore; c. *M. epicladia* CBS 355.76, sporangiophore and sporangiospores; d. *M. zonata* CBS 228.35, basitonous branched sporangiophore with sporangioles; e. *Gamsiella multdivaricata* CBS 227.78, typical branched sporangiophores; f. *M. elongata* FSU 9721, basitonous branched sporangiophore; g. *M. alpina* FSU 2698, sporangiophore; h. *M. polycephala* FSU 867, sporangiospores with sporangia (arrow) and sporangiospores; i. *Mortierella cf. wolfii* CBS 614.70, sporangiophore with elongated...
poorly understood and were extensively analysed only in few studies until now (Nagy et al. 2011, Petkovits et al. 2011). Our study contributes to the effort to elucidate natural phylogenetic relationships based on one of the largest datasets assembled so far. This study concerns the extension of previous datasets and facilitates an approach to molecular identification of the Mortierellales. We surveyed the diversity of the Mortierellales including a re-evaluation of the morphology based classifications. This study based on the broad sampling of specimens which are maintained at the fungal culture collections CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) and JMRC (Jena Microbial Resource Collection, Jena, Germany).

**MATERIALS AND METHODS**

**Taxon sampling, culture conditions and light microscopic investigations**

For this study, a total of 421 isolates were obtained from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands) and the Jena Microbial Resource Collection (JMRC, Jena, Germany) (Table 2). Strains were cultivated on malt-extract medium (3 % malt extract, 0.5 % yeast extract) for DNA isolation and on oatmeal agar (OA, 3 %), soil extract agar (Gams 1969) or synthetic nutrient deficient agar (SNA, Nirenberg 1981) for morphological examinations. Cultivation was done at 20–37 °C for 7–20 days depending on the requirements of the fungus to sporulate. The light microscopical examinations shown in Fig. 1 and 2 were performed with an Axioshot (Zeiss, Germany). The best method to observe microscopic features is to grow cultures directly on cover slips.

**Preparation of genomic DNA, PCR amplification and DNA sequencing**

Genomic DNA was prepared from mycelia ground to a fine powder in liquid nitrogen followed by purification (Cenis 1992) or living cultures alternatively, using the Jetquick general DNA clean up kit (Genomed) or a high-throughput 96-well plate extraction (Ivanova et al. 2006) following the given protocols. The PCR for the amplification of the ITS1-5.8S-ITS2 nuclear ribosomal DNA region uses ITS5/ITS1 and ITS4 under standard or semi-nested conditions (White et al. 1990, Stielow et al. 2009). PCR for amplifying the partial 28S rDNA (LSU) was done using the standard primers LROR and LRS or the NL-primer (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The primers differ only in their annealing temperature (55 °C or 60 °C). Increasing cycle extension time (90 s/cycle) was done in some cases to improve amplification. PCR products were directly purified using FastAP thermosensitive alkaline phosphatase and shrimp alkaline phosphatase (Fermentas) or using the GeneClean protocol (Vogelstein & Gillespie 1979). The cycle-sequence reaction was set up using ABI big dye terminator v. 3.1, following the manufactures instructions or by using a quarter of the suggested volumes (modified manufactures protocol), followed by bidirectional sequencing with a laboratory capillary electrophoresis system (Life Technologies 3730XL DNA analyser). Sequences were evaluated with Chromas Lite (Technelysium Pty. Ltd.). Sequencing primers were the same as used for PCR. Manually correction and assembling of forward and reverse sequences was done using the Biolomics database (www.bio-aware.com) (Vu et al. 2012) or Seqman (v. 7.2.1). Sequences were deposited at NCBI GenBank (Table 2).

**Alignments and phylogenetic analyses**

A total of 364 sequences of ITS and 213 sequences of LSU were generated in this study. For the extension of the dataset additional sequences were retrieved from GenBank (Table 2). A total of 15 sequences were excluded and 562 were subjected to further analyses (298 ITS and 263 LSU sequences). Alignments were performed with MAFFT v. 6.833 (Katoh 2008) as implemented in EPoS (Griebel et al. 2008). Maximum Likelihood analyses were carried out using RAXML (Stamatakis 2006) provided by the CIPRES Science Gateway v. 3.2 (http://www.phylo.org). RAXML was run under the default settings with the
Fig. 2 Typical morphological structures of different isolates of the Mortierellales, which are suitable for species delimitation. a. *M. verticillata* CBS 315.52, sporangiophore with a sporangiola; b. *M. elongata* FSU 9721, elongated sporangiospores containing central oil droplets; c. *M. wollii* CBS 651.93, cracked sporangia releasing sporangiospores, on acrotonous branched tip of the sporangiophore; d. *M. indohii* CBS 720.71, stylospores; e. *M. schmuckerii* CBS 285.59, sporangiophores alongside a hypha with sporangiola; f. *M. clausenii* CBS 294.59, sporangiophores along a hypha with sporangiola; g. *M. clonocystis* CBS 357.76, typical swollen hyphae; h. *M. zychae* FSU 719, typical swollen hyphae arranged in clusters; i. *M. parvispora* FSU 10759, tip of a sporangiophore,
following adjustments: GTR+GAMMA for bootstrapping and final tree inference with 1,000 bootstrap iterations. The resulting phylogenetic trees which based on the LSU sequences were used to identify clusters of strains. For these clusters MAFFT alignments of the ITS region were computed and RAxML analyses performed. Subsequent alignments are crucial since ITS is in general highly diverse on higher level classification. If a group of sequences contains a high number of a repetitive species not all sequences were included in the ITS tree. Alignments and trees are deposited in TreeBASE under http://purl.org/phylo/treebase/phylows/study/TB2:S13827.

RESULTS AND DISCUSSION

Phylogenetic analyses and relationships within the Mortierellales based on single-locus analyses

According to previous studies (White et al. 2006, Petkovits et al. 2011), the major genus of the Mortierellales, Mortierella, appears as paraphyletic genus since the genera, Dissophora, Gamsiella and Lobosporangium are nested within. Since there is no sequence data or living material available for Aquamortierella and Modicella (White et al. 2006) these genera were not included. Due to lacking species material the newly proposed and described genus Echinochlamydosporium (Jiang et al. 2011) was also excluded from the current analysis. Although the pre-molecular classification schemes defined morphologically well-supported clades (Linnemann 1941, Zycha et al. 1969, Gams 1977) these clades could not be retained in any molecular based analyses (White et al. 2006, Petkovits et al. 2011, this study). The present study extends a previous study by addition of sequence information for 407 specimens. One isolate, Mortierella mutabilis, was excluded due to miss-fitting morphological characteristics. The morphology of M. mutabilis is in contradiction with its original description (Linnemann 1941) and resembles Gamsiella multivariicata in all morphological features as well its molecular data. Since only one isolate is available, we postpone its phylogenetically analysis till additional material is available. Nineteen species were additionally included with a total of 115 sequences. Out of these sequences 57 sequences were generated for ITS, 58 for LSU and 1 ITS sequence was retrieved from GenBank. Out of 421 specimens in total, 213 sequences for LSU and 364 sequences for ITS were generated. The dataset was supplemented with additional sequences form GenBank (69 LSU and 11 ITS sequences) (Table 2).

A first phylogenetic tree based on LSU sequences from 266 taxa was generated to define placement and relationships of all sequences generated in this study (data not shown). A subset of all relevant groups and isolates was taken for the final tree of the LSU dataset (Fig. 3, just for better overview). The final alignment contains 781 characters and 101 taxa. For subsequent deep-level analyses seven artificial subsets out of eight clades of this tree were defined referring to the previously published group delimitations (Petkovits et al. 2011). For each group the ITS1-5.8S rDNA-ITS2 sequences were aligned and analysed with Maximum Likelihood although the backbone of the underlying LSU tree is not resolved (Fig. 3). Groups are mainly located on one branch (‘monophyletic’) except for the under-represented chienii/selenospora-group which was combined and aligned together with the most basal group. Taking these groups as single taxa sets allows alignments providing phylogenetic signals with higher resolution on deep level classification. The alignments of the subsets consists of the following numbers of taxa and characters: subset 1: 58/816 (means 58 taxa and 816 characters, Fig. 4); subset 2: 36/636 (Fig. 5); subset 3: 38/701 (Fig. 6); subset 4: 17/710 (Fig. 7); subset 5: 18/761 (Fig. 8); subset 6: 60/703 (Fig. 9); subset 7: 73/688 (Fig. 10).

Our results do not allow for the revelation of the natural relationships between different species or between groups of species since the clades are poorly supported in the LSU tree. But definition of boundaries between the species/species groups is possible and the presented species groups are in full accordance with the twelve large clades distinguished in a previous study (Petkovits et al. 2011). Because the current dataset is more comprehensive, we will keep, but also extend some of the groups.

Group 1 – selenospora and parvispora (Fig. 4, some morphological features are displayed in Fig. 1j, ii) contains the two most basal groups of the LSU tree (Fig 3). Mortierella selenospora clusters well with M. chienii (Bootstrap support BS = 100 %). Mortierella chienii was not included in the previous study (Petkovits et al. 2011). In cases where the morphological identification does not match the position of the strain in the ITS tree the strains were designated as Mortierella sp. with the epithet in quotation marks. Strains which are very distinct, not part of a clade and consequently might represent undescribed species are highlighted in blue. The selenospora clade also contains the questionable M. wolfii CBS 614.70 which shows different characteristics (e.g. no thermostolerance) to the original M. wolfii strains although the sporangiospores are ellipsoidal to kidney-shaped like those of M. wolfii. A detailed analysis of the morphology and several molecular markers is needed to clarify the status of this particular strain. The other group termed ‘parvispora’ contains also the species M. alliacea, M. basiparvispora, M. fimbricristis, M. jenkinii, M. macrocystis, M. macrocystopsis, M. sossauensis in addition to the previously included species (M. cystojenkinii, M. dichotoma, M. elongatula, M. parvispora, M. pulchella, M. turfolica; Petkovits et al. 2011). Mortierella alliacea, M. chienii, M. cystojenkinii, M. elongatula, M. macrocystis, M. macrocystopsis, M. pulchella and M. sossauensis form well-supported clades and the morphologically defined species boundaries are well reflected in the ITS tree (Fig.4). The parvispora-jenkinii-complex consists predominantly of strains morphologically identified as M. jenkinii or M. parvispora. These two species differ mainly by the shape of their sporangiospores: ellipsoidal for M. jenkinii and globose for M. parvispora. This distinction is not supported by the ITS tree, mixing both types of spores. The strain M. basiparvispora CBS S17.72 is also clustering in this complex, but is differing morphologically from the ex-type strain of this species, which was not included in this study (Gams 1976). A detailed revision of this species in relation to Mortierella will be needed.
| Original name                        | Strain numbers | Microscopic identification | Type status | Locality                          | Substrate | Accession | Accession no. LSU |
|-------------------------------------|----------------|-----------------------------|-------------|-----------------------------------|-----------|-----------|------------------|
| Dissodophora decumbens              | CBS301.87, FSU9780 | -                           | -           | Rhode Island ground-up litter of Quercus-Acer wood, land, incubated at 0°C for 2 months | -         | -         | JX976001         |
| Mortierella ambigua                 | CBS450.88, -    | -                           | -           | Japan soil of salt marsh          | -         | -         | JX976087         |
| Mortierella angusta                 | CBS893.61      | -                           | -           | Northern Victoria Land, Emsondon Point, soil | -         | -         | JX975904         |
| Mortierella antarctica              | CBS194.89      | -                           | -           | Antarctica soil                   | -         | -         | JX976040         |
| Mortierella armillariicola          | CBS105.78      | -                           | -           | Netherlands agricultural soil     | -         | -         | JX976046         |
| Mortierella basiparvispora          | CBS517.72      | -                           | -           | Chile soil, under Fitzroya cupressoides | -         | -         | JX976146         |
| Mortierella beljakovae | CBS102878 | – | Toronto High Park, Ontario | infrabuccal pellet of Camponotus pennsylvanicus (carpenter ant) on Pinus sylvestris | JX976090 | KC018350 |
| – | CBS109594 | – | Toronto, High Park, Ontario | infrabuccal pellet of Camponotus pennsylvanicus, in mature Pinus tree | JX975848 | KC018449 |
| – | CBS109595 | – | Zweifalller Wald near Aachen, Germany | infrabuccal pellet of Formica rufa | JX976129 | KC018358 |
| – | CBS109696 | – | St Andrews, Annesley House, New Brunswick | infrabuccal pellet of Campanotus pennsylvanicus | JX795971 | JX796170 |
| – | CBS109597 | – | Scarborough, Ontario | infrabuccal pellet of Campanotus pennsylvanicus | JX795918 | KC018433 |
| – | CBS109655 | – | Bayerischer Wald, Pfahl bei Veitchach, Germany | infrabuccal pellet of Campanotus herculeanus | JX795869 | JX796171 |
| – | CBS109658 | – | Zweifalller Wald near Aachen, Germany | infrabuccal pellet of Formica rufa | JX976051 | KC018376 |
| – | CBS109659 | – | Utrecht, Lage Vuurche, Netherlands | infrabuccal pellet of Formica rufa | JX975998 | KC018440 |
| – | CBS137.72, FSU9794 | M. beljakovae | Type of Mortierella beljakovae | Rovenis region, Sarnia, Ukraine | JX796126 | HQ667428.1 |
| – | CBS267.71 | – | North Carolina | seedlings, Pinus taeda | JX796072 | KC018446 |
| – | CBS268.71 | – | North Carolina | seedlings, Pinus taeda | JX796043 | KC018723 |
| – | CBS274.71 | – | South Carolina | root, Pinus taeda | JX796011 | KC018887 |
| – | CBS275.71 | – | South Carolina | root, Pinus taeda | JX795913 | KC018401 |
| – | CBS276.71 | – | South Carolina | root, Pinus taeda | JX795937 | KC018442 |
| – | CBS606.68 | – | North Carolina | bark of root, Pinus | JX795897 | KC018397 |
| – | CBS370.95, FSU9795 | M. biramosa | Type of Mortierella wuyishanensis | Wuyi, Fujian, China | JX796094 | HQ667389.1 |
| – | CBS506.81 | – | Odenwald, Oberer Buntsandstein, Germany | decaying fine root, 30 yr old, on acidic loamy soil | JX795963 | KC018407 |
| – | CBS550.80 | – | Odenwald, Germany | rootlet | JX796064 | KC018419 |
| – | CBS145.69 | – | Italy | – | JX795857 | KC018377 |
| – | FSU9675 | M. bisporalis | – | – | JX796024 | – |
| – | CBS109638 | – | Soest, Smickel, Netherlands | thatch of roof | JX795949 | HQ667408.1 |
| – | CBS110640 | M. camargensis | Type of Mortierella camargensis | Camargue, Bois des Rièges, France | JX795923 | JX796163 |
| – | CBS239.96 | – | Naganohara, Gunma, Japan | soil with Armillariella | JX78123 | KC018554 |
| – | CBS659.70 | – | North Carolina | pillbug gut | JX796008 | KC018395 |
| – | CBS237.96 | – | Amakubo, Tsukuba, Ibaraki, Japan | soil under Quercus minuta forest | JX796013 | KC018427 |
| – | CBS292.96 | M. selenospora | – | – | JX795898 | JX796161 |
| – | CBS654.73 | M. selenospora | – | – | JX795912 | KC018381 |
| – | CBS120.34, FSU9799 | M. selenospora | – | – | JX795942 | HQ667430.1 |
| – | CBS29.75 | – | Netherlands | soil | JX795927 | – |
| – | CBS590.85 | – | – | – | JX79612 | JX796159 |
| – | CBS537.76, FSU9801 | M. clonocystis | Type of Mortierella clonocystis | Gran Canaria, Spain | JX795899 | HQ667395.1 |
| – | CBS697.97, FSU9802 | M. clonocystis | Type of Mortierella clonocystis | Gran Canaria, Spain | JX795897 | HQ667395.1 |
| – | CBS327.76, FSU9803 | M. clonocystis | Type of Mortierella clonocystis | Gran Canaria, Spain | JX796017 | HQ667395.1 |
| – | CBS690.82 | – | Nagano, Sanada, Sugadaira, Japan | soil, under Apollonias canariensis | JX796017 | HQ667395.1 |
| – | CBS673.68 | – | – | soil with Armillariella | JX795897 | HQ667395.1 |
| – | CBS456.71, FSU9803 | M. cystojenkinii | Type of Mortierella cystojenkinii | Wageningen, Netherlands | JX796030 | HQ667504.1 |
| – | CBS660.82 | – | Bakkeveen, Netherlands | soil under Quercus robur forest | JX795899 | HQ667395.1 |
| – | CBS873.68 | – | – | soil under Armillariella | JX796017 | HQ667395.1 |
| – | CBS221.35, FSU9804 | M. dichotoma | Type of Mortierella dichotoma | Wageningen, Netherlands | JX796030 | HQ667504.1 |
| – | CBS282.71 | – | Iceland | soil | JX795948 | – |
| – | CBS10517 | – | – | – | JX796042 | KC018348 |
| – | CBS122.71 | – | Alt Mountains, South Africa | – | JX796000 | KC018396 |
| – | CBS126.71, FSU823 | M. elongata | – | – | JX796101 | KC018370 |
| – | CBS208.71 | – | Wageningen, Netherlands | agricultural soil | JX795899 | KC018370 |
| – | CBS330.71 | – | Netherlands | greenhouse soil | JX795899 | KC018370 |
| Original name                  | Strain numbers | Microscopic identification | Type status                      | Locality                      | Substrate                     | Accession no.ITS | Accession no. LSU |
|-------------------------------|----------------|---------------------------|----------------------------------|-------------------------------|-------------------------------|-----------------|------------------|
| **Mortierella elongata**      | CBS488.70, FSU9808 | –                         | Type of Mortierella elongata     | former West-Germany           | municipal waste              | JX975967        | HQ67425.1        |
|                               | CBS611.70       | –                         |                                  |                               |                               |                 |                  |
|                               | CBS355.76, FSU9809 | M. elongata                | Type of Mortierella elongata     | Gran Canaria, Spain           | soil, under Apollonias canariensis | JX976130        | HQ67395.1        |
|                               | CBS356.76       | –                         |                                  | Gran Canaria, Spain           | soil, under Apollonias canariensis | JX975972       | –                |
|                               | CBS555.89       | –                         |                                  | Pará, 200 km SE from Belém, Capítulo Pogo, Brasil |                                 | JX975991        | JX976150         |
| **Mortierella epigama**       | CBS161.76       | M. epigama                | Type of Mortierella epigama      | Exeter, Hatherly Laboratories, England | municipal waste              | JX76109         | JX976158         |
|                               | CBS489.70, FSU9810 | M. epigama                |                                  |                               |                               | JX76607         | HQ67367.1        |
|                               | CBS811.97       | –                         |                                  | Kagoshima, Kamei, Tokunoshima-Island, Japan |                                 | JX76603         | JX981445         |
| **Mortierella exigua**        | CBS358.76       | –                         |                                  | Gran Canaria, Spain           | soil, under Apollonias canariensis | JX976113        | JX981439         |
|                               | CBS510.63       | –                         |                                  | Kiel-Kitzeberg                | agicultural soil              | JX975863        | JX976134         |
|                               | CBS655.68, FSU9811 | M. exigua                  | Type of Mortierella sterilis     | Allahabad, India              | farm soil                     | JX976047        | HQ67406.1        |
| **Mortierella fastihebarestae** | CBS388.71    | –                         |                                  | Kiel-Kitzeberg, Germany       | wheat field soil              | JX976070        | –                |
| **Mortierella limicricystis**  | CBS943.70       | –                         | Type of Mortierella limicricystis| South Patagonia, Puerto Edwards near centre of roa cusion, in very wet bog Beagle Canal, Argentina |                                 |                  |                  |
| **Mortierella fomiczica**     | CBS109589       | –                         |                                  | Brampton, Ontario             | infrabucal pellet of *Cannoponos* pennus/lanicusts, in house (windowsill) | JX975933        | JX976140         |
| **Mortierella gamsii**        | CBS110630       | –                         |                                  | Boekrijk, Belgium             | soil with Porcellio            | JX76106         | JX981410         |
|                               | CBS253.36, FSU9813 | M. gamsii                  | Syntype of Mortierella spinosa   | former West-Germany           | forest soil                   | JX975968        | HQ67415.1        |
|                               | CBS314.52, FSU9814 | M. cf. gamsii              | Syntype of Mortierella spinosa   | former West-Germany           | forest soil                   | JX975892        | HQ67384.1        |
|                               | CBS551.73, FSU9824 | M. gamsii                  |                                  | North Carolina                | pasture soil                  | JX766079        | JX981777         |
|                               | CBS552.73, FSU9825 | M. gamsii                  |                                  | Alleghany County, North Carolina | pasture soil                  | JX975984        | JX981285         |
|                               | CBS749.68, FSU9812 | M. gamsii                  | Type of Mortierella gamsii       | Baam, Maarschalkbos, Netherlands | soil                         | HQ67416.1       |                  |
|                               | FSU2057         | M. gamsii                  |                                  |                                  |                               | JX76118         | JX981287         |
| **Mortierella gemmifera**     | CBS124.72       | –                         | Type of Mortierella gemmifera    | near Nottingham, England      | soil from pine forest         | JX975931        | HQ67371.1        |
|                               | CBS134.45, FSU9815 | M. gemmifera               |                                  | Meerdinkbos near Winterswijk, Netherlands | soil, humus layer             | JX795909        | JX981390         |
| **Mortierella globipina**     | CBS226.78       | –                         | Type of Mortierella globipina    | Kafkijw, Netherlands          | sand dune soil                | JX766006        | JX981600         |
|                               | CBS718.88       | –                         |                                  | Japan                         |                                 | JX76926         | –                |
| **Mortierella globulinera**   | CBS108.68       | –                         |                                  | Schweden                      |                                 | JX75847         | JX981332         |
|                               | CBS746.68       | –                         |                                  | Netherlands                   | agriculture soil              | JX76026         | JX981371         |
|                               | CBS857.70, FSU9826 | M. globulinera             | Naotype of Mortierella globulinera | Sparderswoud near Bussum, Netherlands | soil, in pine forest         | JX76121         | JX976157         |
| **Mortierella histoplasmatoides** | CBS321.78, FSU9819 | –                         | Type of Mortierella histoplasmatoides | near Nottingham, England      | soil from pine forest         | JX975931        | HQ67371.1        |
|                               | CBS355.52, FSU9820 | M. histoplasmatoides       |                                  | Sparderswoud near Bussum, Netherlands | soil, in pine forest         | JX76121         | JX976157         |
| **Mortierella histoplasmatoides** | CBS859.68, FSU9817 | M. histoplasmatoides       |                                  | England                       | decayning needle              | JX79510         | HQ67389.1        |
|                               | CBS867.68       | M. globulinera             | Naotype of Mortierella globulinera | England                       | decayning root                | JX79515         | HQ67386.1        |
| **Mortierella histoplasmatoides** | CBS321.78, FSU9819 | –                         | Type of Mortierella histoplasmatoides | near Nottingham, England      | soil from pine forest         | JX975931        | HQ67371.1        |
| **Mortierella horticola**     | CBS564.76       | –                         |                                  | Tirol, Obergurg, Austria      | alpine raw humus soil         | JX76107         | JX976165         |
| **Mortierella histoplasmatoides** | CBS321.78, FSU9819 | –                         | Type of Mortierella histoplasmatoides | near Nottingham, England      | soil from pine forest         | JX975931        | HQ67371.1        |
| **Mortierella humilis**       | CBS180.72       | –                         |                                  | Schweden                      | agriculture soil              | JX76026         | JX981371         |
|                               | CBS181.72       | –                         |                                  | Netherlands                   | agriculture soil              | JX76026         | JX981371         |
|                               | CBS222.35, FSU9821 | –                         | Syntype of Mortierella humilis   | Mexico                         | soil from Pinus forest        | HQ63025.1       | HQ67401.1        |
|                               | CBS333.95       | –                         | Syntype of Mortierella humilis   | Shennongjia, Hubei, China     | forest soil                   | JX76808         | JX981443         |

Table 2 (cont.)
Mortierella hyalina

- CBS117.74 - Mortierella hyalina
  - Isotype of Hydroporahyalina
  - Boekesteden near s-Graveland, Netherlands
  - JX976083, KC018392

Mortierella humilis

- CBS100563, FSU829 - M. humilis
  - South Carolina bark of stump
  - JX976002, HQ667402

Mortierella hypsicladia

- CBS116202, FSU9825 - M. hypsicladia
  - Kyushu Isl., Kariu Cave, Japan
  - JX975866, HQ667379.1

Mortierella jenkinii

- CBS188.73 - Mortierella jenkinii
  - Nottingham, England
  - turf layer of golf green, received fungicidal treatment for long period
  - JX975999, KC018389

Mortierella longigemmata

- CBS653.93 - Mortierella longigemmata
  - Höglwald, Germany
  - JX976055, JX976162

Mortierella macrocystis

- CBS302.87 - Mortierella macrocystis
  - South Kingstown, Rhode Island
  - soil under Pinus resinosa and Pinus strobus
  - JX975946, JX976164

Mortierella microzygospora

- CBS880.97, FSU9831 - M. microzygospora
  - Shiga, Mabara, Japan
  - soil in hedge
  - JX976027, HQ667394.1

Mortierella minutissima

- CBS226.35 - Mortierella minutissima
  - St. John's, Newfoundland
  - soil
  - JX976078, HQ667348

Mortierella nantahalensis

- CBS610.70, FSU9832 - M. nantahalensis
  - Joyce Kilmer Memorial Forest in the Nantahala National Forest, North Carolina
  - soil
  - JX976022, HQ667388.1

Mortierella oligospora

- CBS101758 - Mortierella oligospora
  - Pennsylvania
  - soil
  - JX976032, KC018327

Mortierella pygmaea

- CBS850.70 - Mortierella pygmaea
  - Wageningen, Netherlands
  - agricultural soil
  - JX976088, KC018422

Mortierella sepedonioides

- CBS314.85 - Mortierella sepedonioides
  - former West-Germany rootlet of gymnosperm
  - JX975974, JX976169

Mortierella sepioides

- CBS313.52, FSU9829 - Mortierella sepioides
  - Type of Mortierella sepedonioides
  - former West-Germany soil under Pinus sylvestris
  - JX976127, HQ667344.1

Mortierella stellata

- CBS850.70 - Mortierella stellata
  - Wageningen, Netherlands
  - agricultural soil
  - JX976088, KC018422

Mortierella varipunctata

- CBS313.52, FSU9829 - Mortierella varipunctata
  - Type of Mortierella sepedonioides
  - former West-Germany soil under Pinus sylvestris
  - JX976127, HQ667344.1
| Original name                  | Strain numbers | Microscopic identification | Type status                                      | Locality                        | Substrate                           | Accession no. ITS | Accession no. LSU |
|-------------------------------|----------------|-----------------------------|--------------------------------------------------|----------------------------------|-------------------------------------|-------------------|-------------------|
| Mortierella paraensis         | CBS381.71      |                             | M. paraensis Type of Mortierella paraensis       | Jaipur, Rambagh Palace Hotel, Rajasthan soil | JX97603 JX976003 | KC018368         |
| Mortierella parasycyae        | CBS343.89      |                             | M. paraensis Type of Mortierella parasycyae      | Pará, Capitópolo Poço, Brazil forest soil, virgin forest | JX975944 JX976003 | KC018329         |
| Mortierella parasycyae        | CBS547.89, FSU9835 |                             | M. paraensis Type of Mortierella parasycyae      | Pará, 200 km SE from Belém, Capitópolo rain forest soil | HJ630353 HJ667429.1 |                |
| Mortierella parvispora        | CBS868.71, FSU9836 |                             | M. parvispora Type of Mortierella parvispora    | Treq near Amersfoort, Netherlands decaying wood, with Botryobasidium subcoronatum | JX975895 HJ667362.1 |                |
| Mortierella parvispora        | CBS304.52, FSU9837 |                             | M. parvispora Syntype of Mortierella parvispora | former West-Germany soil         | JX975859 –                |
| Mortierella parvispora        | CBS311.52, FSU9838 |                             | M. parvispora Syntype of Mortierella parvispora | former West-Germany soil         | JX976076 HJ667373.1 |
| Mortierella parvispora        | CBS315.61, FSU834 |                             | M. parvispora                                    | Cheshire, Delamere Forest, England soil, iron-humus podzol | JX976164 HJ667374.1 |
| Mortierella parvispora        | CBS316.61, FSU835 |                             | M. parvispora                                    | Cheshire, Delamere Forest, England soil, iron-humus podzol | JX976029 HJ667375.1 |
| Mortierella parvispora        | CBS445.68      |                             | M. parvispora                                    | Wageningen, Netherlands best-field soil | JX976049 KC018414 |
| Mortierella polycyphala       | FSU273.35      |                             | M. jenkinii                                       | –                                | JX976093 KC018295 |
| Mortierella polycyphala       | CBS227.35      |                             | M. jenkinii                                       | –                                | JX976096 KC018321 |
| Mortierella polycyphala       | CBS293.34      |                             | M. hyalina                                        | Netherlands                     | JX976050 JX976137 |
| Mortierella polycyphala       | CBS277.72, FSU866 |                             | M. polycyphala                                    | Lincs., Gibraltar Point, England salt-marsh soil under Spartina townsendii | JX976085 JX976175 |
| Mortierella polycyphala       | CBS328.72, FSU867 |                             | M. polycyphala                                    | UK                               | JX976102 KC018296 |
| Mortierella polycyphala       | CBS456.56, FSU759 |                             | M. polycyphala                                    | near Kiev, Ukraine               | JX976024 KC018297 |
| Mortierella polycyphala       | FSU696         |                             | M. polycyphala                                    | –                                | JX976050 JX976137 |
| Mortierella polycyphala       | CBS248.81      |                             | M. polycyphala                                    | Sexibium, Netherlands clay soil under Salanum tuberosum | JX975891 JX976145 |
| Mortierella polycyphala       | CBS865.71, FSU9839 |                             | M. polycyphala                                    | Wageningen, Netherlands agricultural soil | JX975900 HJ667378.1 |
| Mortierella polycyphala       | CBS779.86      |                             | M. polycyphala                                    | Wageningen, Netherlands soil under Quercus-Acer woodland, about sea level, upper 5 cm depth | JX975980 KC018353 |
| Mortierella polycyphala       | CBS780.86      |                             | M. polycyphala                                    | Peace Dale, Hazard Tract, Rhode Island soil, under Pinus strobus and Pinus resinosa woodland, from upper 5 cm depth, soil temp. 2.5°C | JX975880 JX976143 |
| Mortierella pulchella         | CBS205.86      |                             | M. pulchella                                       | Netherlands root                 | JX976031 KC018366 |
| Mortierella pulchella         | CBS312.52, FSU9840 |                             | M. pulchella                                       | former West-Germany root         | JX976104 HJ667427.1 |
| Mortierella reticulata        | CBS675.88      |                             | M. reticulata                                      | Berlin, Gneuwald, Jagen 91, Germany soil, litter layer | JX976092 KC018440 |
| Mortierella reticulata        | CBS110044      |                             | M. reticulata                                      | Lanark near Brancholne, Victoria dung of Perameles gurnii | JX975980 – |
| Mortierella reticulata        | CBS223.29      |                             | M. reticulata                                      | –                                | JX975973 – |
| Mortierella reticulata        | CBS241.33      |                             | M. reticulata                                      | Toronto, Ontario                 | JX976116 JX976133 |
| Mortierella reticulata        | CBS415.81      |                             | M. reticulata                                      | –                                | – |
| Mortierella reticulata        | CBS652.68, FSU9842 |                             | M. reticulata                                      | Rishikesh, India                 | JX976110 HJ667385.1 |
| Mortierella reticulata        | CBS502.70, FSU9844 |                             | M. reticulata                                      | near Bainbridge, Georgia forest soil | JX975885 HJ667436.1 |
| Mortierella reticulata        | CBS122.72, FSU9845 |                             | M. reticulata                                      | near Rovensk region, near Samy, Ukraine soil under Pinus elliott var. eliciatti | JX975987 HJ667300.1 |
| Mortierella reticulata        | CBS156.78      |                             | M. reticulata                                      | Mathya Pradesh and Uttar Pradesh regions, India confereous forest | JX975854 KC018372 |
| Mortierella reticulata        | CBS295.59, FSU9846 |                             | M. reticulata                                      | Queentar, Mexico                 | JX976112 HJ667414.1 |
| Mortierella reticulata        | CBS777.86      |                             | M. reticulata                                      | Shoshone National Forest, Horse Creek soil, under Pseudotsuga menziesii, alt. 2500 m | JX976099 KC018413 |
| Mortierella reticulata        | CBS529.68, FSU9847 |                             | M. reticulata                                      | Shoshone National Forest, Horse Creek soil, under Pseudotsuga menziesii, alt. 2500 m | JX976099 KC018413 |
| Mortierella reticulata        | CBS452.88      |                             | M. reticulata                                      | Crimea, Ukraine                 | JX975988 HJ667387.1 |
| Mortierella reticulata        | CBS811.68, FSU9848 |                             | M. reticulata                                      | Crimea, Ukraine                 | JX976037 KC018429 |
| Mortierella reticulata        | CBS153.76C     |                             | M. reticulata                                      | –                                | JX975875 HJ667419.1 |
| Mortierella reticulata        | CBS295.68, FSU9846 |                             | M. reticulata                                      | Ukraine mushroom compost, together with Entomophthora coronata and Aphanocladium album confereous forest | JX975875 HJ667419.1 |
| Mortierella reticulata        | CBS110.68      |                             | M. reticulata                                      | Wageningen, Netherlands oarf-field soil | JX975982 – |
| Mortierella reticulata        | CBS243.82      |                             | M. reticulata                                      | Baam, C. Dopperlaan 18, Netherlands compost heap | JX975870 JX976156 |
| Mortierella reticulata        | CBS153.76C     |                             | M. reticulata                                      | Schweden forest soil under Picea abies | JX976063 JX976146 |
| Mortierella reticulata        | CBS176.74      |                             | M. reticulata                                      | Athens, Georgia                 | JX975926 KC018428 |
| Mortierella reticulata        | CBS281.71      |                             | M. reticulata                                      | South Carolina                  | JX975911 KC018447 |
| Mortierella reticulata        | CBS800.72      |                             | M. reticulata                                      | Ireland peat soil               | JX975865 KC018386 |
| Mortierella reticulata        | CBS898.68      |                             | M. reticulata                                      | Lincs., Gibraltar Point, England salt-marsh soil | JX976050 KC018374 |
| Mortierella reticulata        | FSU10519       |                             | M. reticulata                                      | Austria                         | JX975899 KC018289 |
| Accession | Strain Name | Origin                  | Description |
|-----------|-------------|-------------------------|-------------|
| FSU10520  | M. alpina   | Austria                 | JX975969    |
| FSU10522  | M. alpina   | Austria                 | JX975930    |
| FSU10523  | M. alpina   | Austria                 | JX976114    |
| FSU10551  | M. alpina   | Austria                 | JX975852    |
| FSU10555  | M. alpina   | Austria                 | JX975996    |
| FSU10558  | M. alpina   | Austria                 | JX975884    |
| FSU10683  | M. alpina   | Austria                 | JX976039    |
| FSU10696  | M. alpina   | Austria                 | JX976108    |
| FSU10706  | M. alpina   | Austria                 | JX976068    |
| FSU10715  | M. alpina   | Austria                 | JX976080    |
| FSU10716  | M. alpina   | Austria                 | JX975879    |
| FSU8712   | M. alpina   | Wehlen, Mosel, Germany  | JX975845    |
| FSU8722   | M. alpina   | Wehlen, Mosel, Germany  | JX975861    |
| FSU8736   | M. alpina   | Wehlen, Mosel, Germany  | JX976119    |
| FSU8737   | M. alpina   | Wehlen, Mosel, Germany  | JX975902    |
| FSU8738   | M. alpina   | Wehlen, Mosel, Germany  | JX976010    |
| FSU10762  | M. alpina   | Wehlen, Mosel, Germany  | JX975861    |
| FSU10767  | M. alpina   | Wehlen, Mosel, Germany  | JX975902    |
| FSU10792  | M. alpina   | Wehlen, Mosel, Germany  | JX976010    |
| FSU10797  | M. alpina   | Wehlen, Mosel, Germany  | JX975902    |
| FSU10541  | M. elongata  | Austria                 | JX975879    |
| FSU10711  | M. elongata  | Austria                 | JX975879    |
| FSU10538  | M. gamsii    | Austria                 | JX975879    |
| FSU10535  | M. humilis   | Austria                 | JX975879    |
| FSU10544  | M. hyalinum  | Austria                 | JX975879    |
| FSU10552  | M. parvispora| Austria                | JX975879    |
| FSU10712  | M. parvispora| Austria                | JX975879    |
| FSU10730  | M. parvispora| Austria                | JX975879    |
| FSU10753  | M. parvispora| Austria                | JX975879    |
| FSU10758  | M. parvispora| Austria                | JX975879    |
| FSU10827  | M. parvispora| Austria                | JX975879    |
| FSU10816  | M. parvispora| Austria                | JX975879    |
| FSU10807  | M. parvispora| Austria                | JX975879    |
| FSU10534  | M. verticillata| Austria               | JX975879    |
| CBS455.67  | M. strangulata| Baarn, Groeneveid, Netherlands | JX975897    |
| FSU9849   | M. strangulata| Baarn, Groeneveid, Netherlands | JX975897    |
| CBS211.32  | M. strombii   | Victoria                | JX975879    |
| FSU9850   | M. strombii   | Victoria                | JX976025    |
| CBS340.76  | M. turficola  | Hesepeper Veen near Coevorden, Netherlands | JX975952    |
| CBS341.76  | M. turficola  | Hesepeper Veen near Coevorden, Netherlands | JX975952    |
| CBS342.76  | M. turficola  | Hesepeper Veen near Coevorden, Netherlands | JX975952    |
| CBS343.76  | M. turficola  | Hesepeper Veen near Coevorden, Netherlands | JX975952    |
| CBS447.76  | M. turficola  | Cauca en Huila, Cordillera Central, Colombia | JX976025    |
| CBS91.80   | M. verticillata| Baarn, Groeneveid, Netherlands | JX975897    |
| CBS130.66  | M. verticillata| Baarn, Groeneveid, Netherlands | JX975897    |
| CBS131.66  | M. verticillata| Baarn, Groeneveid, Netherlands | JX975897    |
| CBS215.35  | M. verticillata| Baarn, Groeneveid, Netherlands | JX975897    |
| CBS279.71  | M. verticillata| Baarn, Groeneveid, Netherlands | JX975897    |

**Mortierella strangulata**
- Neotype of *Mortierella strangulata*
- Neotype of *Mortierella strangulata*
- Neotype of *Mortierella strangulata*

**Mortierella stylospora**
- Type of *Mortierella stylospora*
- Type of *Mortierella stylospora*
- Type of *Mortierella stylospora*

**Mortierella turficola**
- Neotype of *Mortierella turficola*
- Neotype of *Mortierella turficola*
- Neotype of *Mortierella turficola*

**Mortierella verticillata**
- Neotype of *Mortierella verticillata*
- Neotype of *Mortierella verticillata*
- Neotype of *Mortierella verticillata*
| Original name | Strain numbers | Microscopic identification | Type status | Notes |
|---------------|----------------|----------------------------|-------------|-------|
| Mortierella wolfi | CBS280.71, FSU9846 | M. wolfi | M. zonata | cordillera, Central Para Nacional Páramo soil, open vegetation with JX976028, JX976141 |
| Mortierella wolfi | CBS315.52, FSU9856 | M. wolfi | M. zonata | New Zealand decayed hay, HQ630304, HQ667383.1 |
| Mortierella zonata | CBS228.35, FSU9863 | M. zonata | M. zonata | Former West-Germany mushroom casing soil, soil JX975962, KC018421 |
| Mortierella zychae | CBS102879 | M. zychae | M. zychae | Toronto High Park, Ontario pellet of Camponotus pennsylvanicus carpenter ant JX976074 |
| Mortierella zychae | CBS109599 | M. zychae | M. zychae | El Yunque, Rio Blanco Trail, Puerto Rico infrabuccal pellet of ant JX975882 |
| Umbelopsis isabellina | CBS216.72, FSU9868 | M. isabellina | M. isabellina | Wisconsin soil JN943789.1, JN940879.1 |

**Group 2 – verticillata-humilis** (Fig. 5, some morphological features are displayed in Fig. 1c, 2a, g, r) is a group that also contains the genera *M. clonocystis*, *M. epicladia*, *M. epigama*, *M. horticola* and *M. minutissima*. The topology is similar to the one previously published (Petkovits et al. 2011) but includes some morphologically misidentified specimens. Mortierella zonata CBS 863.68 and *M. sossauensis* CBS 898.68 are well separated from any other members of their species. The main cluster of *M. sossauensis* is closely related to the parvispora-jenkinii complex (Fig. 4) while the type strain of *M. zonata* is related to *M. hyalina* and *M. bainieri* (Fig. 10). After a profound morphological revision *M. zonata* CBS 863.68 and *M. sossauensis* CBS 898.68 should be renamed and included in the *M. minutissima*-Hortica complex, which makes this phylogenetic group of *M. minutissima-M. horticola* indistinguishable by ITS sequences although both species could be distinguished by the number of their spores in the sporangiola. While *M. minutissima* develops few-spored sporangiola, *M. horticola* produces single-spored sporangiola. This suggests that the number of spores per sporangium is not strictly fixed in this group and is therefore not of taxonomic relevance. The single specimen CBS 246.75 resembles *M. epicladia* but it clusters distantly from the ex-type material CBS 355.76 which is close to *M. clonocystis* (Fig. 5). Since no other known species group together with CBS 246.75, this might be a so far undescribed species. CBS 226.78 was originally deposited as *M. globulina* and CBS 226.35 as *M. minutissima* but molecular data of both species currently resembles *M. clonocystis*, indicating an original misapplication or a contamination. Morphology of both species was checked twice and both species were finally assigned to *M. clonocystis*. The morphospecies *M. clonocystis, M. epicladia* and *M. epigama* are well recognized by the ITS tree while *M. verticillata and M. humilis* form another species complex. Another apparent cluster, the *M. verticillata-M. humilis* cluster, contains strains including type strains of both species. Based on ITS sequences, a differentiation is not possible. Sequences are similar between 98–100%. Both species are morphologically similar without any significant differences. Consequently both species should be synonymized.

**Group 3 – lignicola** (Fig. 6, some morphological features are displayed in Fig. 1n, y, 2j, l, s, w). This group contains the species *Mortierella beljakovae, M. chlamydospora, M. echinosphaera, M. formicicola, M. gemmifera, M. kuhlmani, M. lignicola* and *M. paraensis*. Several of the morphologically defined species, namely *M. beljakovae, M. chlamydospora, M. echinosphaera, M. formicicola, M. lignicola* and *M. paraensis* are nicely detected by the molecular data. *Mortierella chlamydospora* and *M. echinosphaera* appear to be closely related as they are sister groups (BS = 100%). The species *M. gemmifera* and *M. kuhlmani* are morphologically very similar (complex is supported by BS = 85 %) and differ just gradually by spore shape and chlamydospores. The ex-type strains of both species differ just by 12 different base pairs in the ITS sequences (= 98 %). The original morphological identification of strain CBS 268.71 could not be verified because it did not sporulate under different conditions, but its molecular data places it between the gemmifera-complex, *M. chlamydospora* and *M. echinosphaera*. The strains CBS 109659 and CBS 555.89 were not examined morphologically and assigned as Mortierella sp. since their original descriptions do not correspond with the molecular data.

**Group 4 – mutabilis, globulifera and angusta** (Fig. 7, some morphological features are displayed in Fig. 1e, s, v, x, 2v). This group contains two of the three included non-Mortierella genera: Gamsiella and Dissophora. The genus Gamsiella does not cluster with any other mortierellcean species, although it was reported to be sister with *M. mutabilis* (Petkovits et al. 2011).
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... h an originally different assignment based on morphology. Blue marked strains are potential new species.

Fig. 3 Maximum Likelihood analysis based on 781 aligned nucleotides of the D1/D2 domain of the large subunit (LSU, 28S) rDNA from 101 taxa (100 ingroup taxa of the Mortierellales and 1 outgroup taxon Umbelopsis as member of the Mucorales, Meyer & Gams 2003). The phylogram based on a MAFFT-Alignment (L-InsI). Node supports above 75% is given. The tree defines 7 groups: groups 1–7, which are more profoundly analysed in individual analyses based on the ITS1-5.8S-ITS2 shown in Fig. 4–10. The strains named Mortierella sp. ‘epithet’ are strains with an originally different assignment based on morphology. Blue marked strains are potential new species.
M. globulifera

Fig. 5 Maximum Likelihood analysis based on the ITS1-5.8S-ITS2 dataset for clade 2. The phylogram was constructed from a MAFFT-Alignment of 636 aligned nucleotides of 38 taxa. Node support above 75 % is given. The phylogram is midpoint rooted.

The angusta group is extended by M. simplex and consists of the subclades M. angusta-M. simplex (BS = 88 %) and the subclade Dissophora with D. decumbens and D. ornata (BS = 100 %). Mortierella simplex could not by differentiated from M. angusta by significant features, suggesting an upcoming synonymization of both species. The globulifera group contains exclusively M. globulifera (BS = 94 %). The strain CBS 254.76 formerly identified as M. horticola might represent a new species because of its distinct ITS sequence. The ITS sequences of true M. horticola strains belong to group 2 (Fig. 5) where the ex-syntype of this species is located.

A revision of the morphology revealed different features for M. mutabilis as originally described. Mortierella mutabilis should develop explicitly branched sporangiophores with globose sporangia containing globose to subglobose sporangiospores, for example. But the observed morphology resembles that of Gamsiella. Furthermore, LSU and ITS sequences are similar with 100 and 99.8 %, respectively. Based on these data, we are rejecting the previous group named mutabilis (Petkovits et al. 2011). For the final placement of M. mutabilis, additional strain material is necessary.
Fig. 8 Maximum Likelihood analysis based on the ITS1-5.8S-ITS2 dataset for clade 5. The phylogram was constructed from a MAFFT-Alignment of 761 aligned nucleotides of 18 taxa. Node support above 75 % is given. The phylogram is midpoint rooted.

Fig. 9 Maximum Likelihood analysis based on the ITS1-5.8S-ITS2 dataset for clade 6. The phylogram was constructed from a MAFFT-Alignment of 703 aligned nucleotides of 60 taxa. Node support above 75 % is given. The phylogram is midpoint rooted.

Table 3 Summary of isolates which were revised and assigned to different species within this study.

| Strain number | Original name | Revised name   |
|---------------|---------------|----------------|
| CBS555.81     | M. alpina     | M. kuhlnianii  |
| CBS696.70     | M. alpina     | M. cystogaster |
| CBS272.71     | M. bainieri   | M. kuhlnianii  |
| CBS273.71     | M. bainieri   | M. kuhlnianii  |
| CBS292.96     | M. chienii    | M. selenospora |
| CBS554.73     | M. chienii    | M. selenospora |
| CBS387.91     | M. macrocytops | M. cystogaster  |
| FSU2736       | M. parvispora | M. kuhlnianii  |
| CBS293.34     | M. polyccephala | M. kuhlnianii |
| CBS178.74     | M. sassaensis | M. clonostachys |
Group 5 – strangulata and wolfii (Fig. 8, some morphological features are displayed in Fig. 1q, r, 2c, t) contains only few species, which could all be identified by molecular data. The wolfii group (BS = 100 %) is represented in this study by M. ambiguia (clade support BS = 99 %). Mortierella ambiguia is sister clade (BS = 81 %) to M. capitata (BS = 98 %) and both clades are sister group to M. wolfii (BS = 96 %). The strangulata group is retained, containing M. strangulata and M. rostafinskii (BS = 100 %). Mortierella microyzgospora, M. parazychae and M. pseudozygospora were not assigned to any defined group.

Group 6 – alpina and polycephala (Fig. 9, some morphological features are displayed in Fig. 1b, g, h, k, o, w, 2d, m, n, p). The polycephala group harbours the type species of the whole genus Mortierella: M. polycephala. Therefore, this clade resembles the core group of the genus Mortierella. Related to M. polycephala and well supported in LSU (BS = 99 %) and ITS (BS = 100 %) are the species M. bisporalis, M. hypsicladia, M. indohii, M. oligospora, M. polygonia and M. reticulata. Except for the ex-type strain of M. polygonia CBS 685.71 which clusters within the M. polycephala, all species form well supported clades (Fig. 9). But judging from the different observed morphology of M. polygonia, which is that of M. polycephala instead of that originally described (Gams 1976), this strain should be treated as such. Although the strain is sterile, it shows the typical stylospos of M. polycephala. A second isolate of M. polygonia (CBS 248.81) could not be confirmed as ‘true’ M. polygonia since it does not sporulate, displaying only untypical stylospos and clusters within the alpina-complex (Fig. 9). Therefore the status of this species seems doubtful. Mortierella alpina is one of the major species isolated and identified from our environmental samples collected in Austria. Mortierella alpina forms a heterogeneous cluster with the two species M. antarctica and M. amoeboidae. For M. amoeboidae again is the observed morphology not identical with the described one and resembles the species indicated by molecular data. This justifies M. amoeboidae W. Gams 1976 to be treated as synonym of M. alpina Peyronel 1913. One isolate of M. glob-alpina (CBS 718.88) is placed within the alpina complex and one isolate (CBS 226.78) is located in the M. cononcystis clade (Fig. 5). Verification by inclusion of the type strain is not possible since this particular strain seems to be dead now.

Group 7 – gamsii (Fig. 10, some morphological features are displayed in Fig. 1a, d, f, p, u, 2b, e, f, h, k, o, q, u) is the largest group in this and our previous study containing 73 taxa. The previous dataset (Petkovits et al. 2011) with the species Mortierella acrtona, M. armillaricola, M. biramosa, M. camar­gentis, M. cogitans, M. elongata, M. exigua, M. gamsii, M. his­ttoplasmatoideis, M. hyalina, M. nantahalensis, M. ris­heksha, M. samynesis, M. schmuckeri, M. sclerotiella, M. zonata and M. zychae was extended by M. bainieri, M. clausennii, M. fats·hederae and M. longigemmata. Mortierella armillaricola, M. bainieri, M. fatshe­derae, M. hyalina and M. zychae form monophyletic clades supported by the coherence of several strains (Fig. 10). Mortierella exigua, M. gamsii and M. zonata are polyphyletic. Strains identified as these species appear in different places of the tree. None of the strains of M. exigua clusters together with the ex-type strain. For M. gamsii at least three divided clusters are present. One sequence of an ex-type strain is placed in the elongata-complex. Mortierella schmuckeri forms one monophyletic clade together with M. clausennii and M. camar­gentis (BS = 97 %). Due to a lack of sufficient amounts of strains neither the phylogenetic position nor the species coherence of M. acrtona, M. cogitans, M. histoplasmatoideis, M. longigemmata, M. nantahalensis, M. sclerotiella and M. zonata could be confirmed.

CONCLUSIONS

In order to study and evaluate the monophyly of Mortierella, and to address the phylogenetic relationships of other genera in the Mortierellales, we analysed one of the largest datasets of LSU and ITS sequences for this order. The genera Dissophora, Gamsiella and Lobosporangium are placed within the genus Mortierella. This suggests either a polyphyly of Mortierella with the necessity to establish additional genera or the necessity to reduce the existing genera to one. Although our study contains a comprehensive dataset it is still not possible to elucidate all species and species groups of the Mortierellales. It was already proposed that additional molecular markers are necessary for a profound phylogenetic study (Petkovits et al. 2011). But our study supports existing and reveals new contradictions to the traditional morphology based classifications (Linnenmann 1941, Zycha et al. 1969, Gams 1977). Several species, originally identified as one, appear on different places in the phylogenetic analyses. This might originate either from simple misapplications or from the observed phenomenon of dependency of the phenotype on culture conditions (Petkovits et al. 2011). Furthermore, names of new genera and species published just recently may be superfluous at a nomenclatural level because their respective phylogenetic markers were not compared with the full molecular dataset of the Mortierellales, e.g. Echinocla­mydosporium variabile (Jiang et al. 2011), which may turn out to be a micromorphologically degenerate Mortierella stylospora. Here we present the most comprehensive molecular dataset of the Mortierellales which is available up to date and facilitates revision of existing and validation of upcoming names. Finally, all these actions will lead to several species name changes and synonymizations. Nevertheless, several species or even groups of species seem to be distinguishable by morphological and phylogeny. The monophyletic clade of Mortierella s.str. contains the type species of the genus, M. polycephala Coem. 1863. Whether additional species are related to this group and therefore belonging to the genus Mortierella needs to be evaluated in further studies. Current data (Petkovits et al. 2011) are contradictory with regard to relationships of species and species groups. Due to the lack of suitable morphological criteria the following species and species groups were misapplied and require taxonomic revision, where indicated nomenclatural synonymization. These are: M. angusta, M. basi­parvispora, M. camargensis, M. fimbricystis, M. gamsii, M. gem­mifer, M. globalinpa, M. horticala, M. humillis, M. jenkinsii, M. kuhimanii, M. minutissima, M. parvispora, M. rishikesha, M. schmuckeri, M. simplex, M. sos­saunensis, M. turficola, M. vert­icillata and M. zonata. Underrepresented in this study, but due to the lack of comprehensive additional material, are the species: M. acrtona, M. an­gusta, M. dichtomata, M. epiclada, M. exigua, M. fimbricystis, M. formicola, M. longigemmata, M. microyzgospora, M. nanta­halensis, M. parazychae, M. rishikesha, M. rostafinskii, M. scler­otiella and M. strangulata.

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