Persoonia 30, 2013: 57–76
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

http://dx.doi.org/10.3767/003158513X666259

The family structure of the *Mucorales*: a synoptic revision based on comprehensive multigene-genealogies

K. Hoffmann[1,2], J. Pawłowska[3], G. Walther[1,2,4], M. Wrzosek[3], G.S. de Hoog[4], G.L. Benny[5*], P.M. Kirk[6*], K. Voigt[1,2*]

Key words

Mucorales
families
phylogeny

Abstract The *Mucorales* (*Mucoromycotina*) are one of the most ancient groups of fungi comprising ubiquitous, mostly saprotrophic organisms. The first comprehensive molecular studies 11 yr ago revealed the traditional classification scheme, mainly based on morphology, as highly artificial. Since then only single clades have been investigated in detail but a robust classification of the higher levels based on DNA data has not been published yet. Therefore we provide a classification based on a phylogenetic analysis of four molecular markers including the large and the small subunit of the ribosomal DNA, the partial actin gene and the partial gene for the translation elongation factor 1-alpha. The dataset comprises 201 isolates in 103 species and represents about one half of the currently accepted species in this order. Previous family concepts are reviewed and the family structure inferred from the multilocus phylogeny is introduced and discussed. Main differences between the current classification and preceding concepts affects the existing families *Lichtheimiaceae* and *Cunninghamellaceae*, as well as the genera *Backusella* and *Lentamyces* which recently obtained the status of families along with the *Rhizopodaceae* comprising *Rhizopus*, *Sporodiniella* and *Syzygites*. Compensatory base change analyses in the *Lichtheimiaceae* confirmed the lower level classification of *Lichtheimia* and *Rhizomucor* while genera such as *Circinella* or *Syncephalastrum* completely lacked compensatory base changes.

Article info Received: 29 September 2012; Accepted: 1 January 2013; Published: 13 March 2013.

INTRODUCTION

The fungal order Mucorales – evolutionary position and characterisation

As a member of the *Mucoromycotina*, the *Mucorales* belong to the early diverging, ancient fungi along with the *Kickxellomycotina*, *Zoopagomycotina*, *Entomophthoromycotina*, *Mortiellomycotina*, *Chytridiomycota*, *Neocallimastigomycota*, *Blastocladiomycota*, and *Cryptomycota* and *Microsporidia* with the latter two still highly discussed (Schüßler et al. 2001, James et al. 2006, Hibbett et al. 2007, Hoffmann et al. 2011, Jones et al. 2011a, b, Benny 2012). The *Entomophthoromycotina* were later elevated to the phylum *Entomophthoromycota* (Humber 2012).

Mucorales are characterised by a usually abundant, rapidly growing mycelium as well as anamorph structures usually formed in large quantities. The mycelium is typically unseptate or irregularly septate. Anamorphic sporangiospores are produced in multi-spored sporangia, few-spored sporangiola or merosporangia. Chlamydospores, arthrospores and yeast cells are, in most species, rarely formed. Sporangia are characterised by the inclusion of a variously shaped columella. This well-developed columella counts as a synapomorphic character for the *Mucorales*. Conjugation in homothallic species or between compatible mating types of heterothallic species results in the formation of zygospores. Zygospores often display a specific exospore ornamentation (smooth, rough, warty) and protecting appendages (finger-like, antler-like) born on the supporting cells (suspensors) (Zycha et al. 1969). Some species of the *Mucorales* exhibit dimorphism, possessing the ability to switch between a filamentous, multi-cellular state to a yeast-like state (Bartnicki-Garcia & Nickerson 1962).

Life styles and applications — Fig. 1

Mucoralean fungi are ubiquitous, predominantly saprobic soil organisms on decaying organic material but parasites of plants, fungi and animals also are known. As one of the largest orders in the basal Fungi, the *Mucorales* is also one of the most studied groups in the early diverging fungi. These studies on mucoralean fungi encompass physiology and biochemistry, as well as taxonomy and systematics, and potential applications in industry. In general, mucoralean fungi reproduce anamorphically via non-motile sporangiospores released from variously shaped sporangia. If not homothallic, a compatible mating partner is needed for the formation of the zygospore, where meiosis occurs. The different sexual modality of either homo- or heterothally in the *Mucorales* was discovered more than 100 yr ago, with most species found to be heterothallic (Blakeslee 1904). Volatiles are responsible for the formation of sexual reproductive structures (Burgeff 1924). These volatiles were identified as trisporoids, derivatives of beta-carotene (van den Ende 1967, Gooday 1968). The trisporic acid precursors are mutually processed by the compatible mating partners, resulting in the formation of a mature zygospore (Werkman 1976). Although the composition of the compounds is species specific to allow only intra-species matings (Sutter et al. 1989), inter-species zygospores are also described with some impact on systematics (Blakeslee & Cartledge 1927,

[1] Institute of Microbiology, Department of Microbiology and Molecular Biology, University of Jena, Jena, Germany; corresponding author e-mails: khoff2@freenet.de, kerstin.voigt@hki-jena.de.
[2] Leibniz-Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute, Jena Microbial Resource Collection, Jena, Germany.
[3] Department of Systematics and Plant Geography, University of Warsaw, Warsaw, Poland.
[4] CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.
[5] Department of Plant Pathology, 1453 Fifield Hall, University of Florida, Gainesville Florida 32611-0680, USA.
[6] Royal Botanic Gardens, Mycology Section, Kew, Surrey TW9 3AF, UK.
* Shared senior authors in alphabetical order.

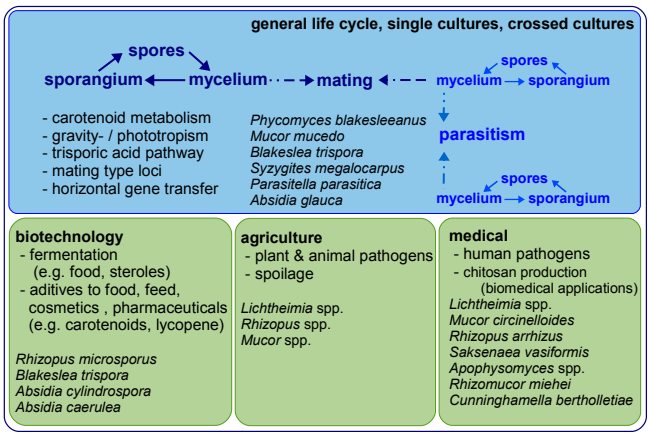


Fig. 1 General life cycle, important fields of scientific research and main applications of *Mucorales*. Exemplary and prominent species are given.

Table 1 Morphological featured observable in mucoralean fungi.

Feature/character	Criteria
Mycelium	Height, colour, rhizoids, arthrospores, chlamydospores, giant cells
Sporangia incl. sporangiophore	Height, origin, branching pattern, size, shape, colour, number of spores, septation, dissolving of the wall, release of spores, response to light
Sporangiospores	Shape, size, ornamentation, colour, appendages
Zygospores	Homo-/heterothallism, air-borne or submerged, relative placement and size of suspensors, shape, size, colour, ornamentation, appendages

Fig. 2 a. *Syzygites megalocarpus* on *Pleurotus ostreatus* (artificially infected); b. *Parasitella parasitica* on *Mucor circinelloides*. Galls (g) and sikyotic cells (s) are marked; c. *Choanephora cucurbitarum* on yellow summer squash. d. *Rhizopus stolonifer* on strawberries. — Scale bar = 20 µm.

Stalpers & Schipper 1980). Combining an order-wide trisporoid profiling with the current knowledge on phylogenetic relationships would most likely reveal the 'languages' of the different clades and their potentials for interspecific mutual recognition. But currently, only profiles for few species are known: e.g. *Phycomyces blakesleeanus* (Miller & Sutter 1984) and *Blakeslea trispora* (Caglioti et al. 1966).

Although a general trisporic acid biosynthesis pathway (Schachtschabel et al. 2005) is widely accepted, the genetic background is resolved only in parts. The synthesis and degradation of beta-carotene is well studied and understood (Almeida & Cerdá-Olmedo 2008, Polaino et al. 2010, Tagua et al. 2012) but most enzymes responsible for trisporic acid production remain undiscovered. So far, only 4-dihydro-methyltrisporate

dehydrogenase and 4-dihydrotrisporin dehydrogenase are verified (Czempinski et al. 1996, Wetzel et al. 2009).

Since an interaction of compatible mating types is essential for matured zygospores to be produced, the information for the mating type is probably genetically coded. The appropriate regions were identified first in *Phycomyces blakesleeanus* (Idnurm et al. 2008) and subsequently discovered in *Rhizopus delemar*, *R. oryzae* (Gryganskyi et al. 2010), *Mucor circinelloides* (Lee et al. 2010) and even in a homothallic species, *Syzygites megalocarpus* (Idnurm 2011). Although heterothallic strains possess only one gene coding for either plus or minus mating type, the phenomenon of rare switches between mating types (Schipper & Stalpers 1980) is not yet explained.

The importance of zygospores for reproduction and distribution compared to the asexual sporangiospores is still unknown, since germination in the natural habitat could not be observed and germination under laboratory conditions has only been described and illustrated for few species (Michailides & Spotts 1988, Yu & Ko 1997). Nevertheless, zygomycetes are reported from the fossil records. The earliest zygomycotan fossil known, exclusive of the *Glomeromycota*, may be *Jimwhitea circumtecta*, possible *Endogonaceae*, from the middle Triassic (Krings et al. 2012). Many fossil zygomycetes have been found in the Carboniferous and later, including *Protoascon missouriensis* and others (Taylor et al. 2005, Kar et al. 2010). Calculations of the diverging time of zygomycetes using molecular data suggest an origin of around 600 mya (Berbee & Taylor 2001).

The zygomycetes are known to be useful for a variety of different applications, including food and food additive production and food preservation. Zygomycetes are used as starter cultures for the fermentation of soybean- or rice-based products in Asia, Africa and South America, e.g. beverages, or the well-known *tempeh* (Henkel 2004, 2005, Hesseltine 1983, 1991, Nout & Kiers 2005, Tamang & Thapa 2006).

Mucorales also are used for diverse biological transformations (Gładkowski et al. 2004, 2011) as well as the production of additives for food, feed, pharmaceuticals (like lycopene) or various applications of chitosan (reviewed by Shahidi et al. 1999), a cell wall component only known to be produced by *Mucorales*. Yet, *Mucorales* also are reported as spoilage agents in stored cereals and other food, especially fruits and vegetables (Martin 1964, Wade & Morris 1982, Ray & Ravi 2005). In addition, some organisms also infect living plants, especially the fruits (e.g., strawberry, yellow summer squash or green beans; Fig. 2c, d) (Dennis 1983). Thus, these fungi play an important role as plant pathogens as well (Shtienberg 1997). Furthermore, some species of the *Mucorales* are facultative parasites of other fungi. They can be biotrophic or necrotrophic parasites with a few species (*Syzygites megalocarpus*, *Dicranophora fulva*, *Spinellus fusiger*) able to infect the fruit body of agarics (Fig. 2a; Zycha et al. 1969), a feature that is thus far not well studied. However, well studied is the biotrophic fusion parasitism (Fig. 2b) between *Absidia glauca* and *Parasitella parasitica*, a model system for studies of horizontal gene transfer and the link between sexual and parasitic interactions (Burgeff 1924, Kellner et al. 1993, Schultze et al. 2005). Trisporic acid and its precursors are also believed to be responsible for recognition of potential hosts for *Chaetocladium* (another parasite) and *Parasitella*, which was assumed from an observed mating-type dependent infection (Burgeff 1924, Schultze et al. 2005). Yet, a strict mating-type dependency was rejected as early as 1926 by a mere tendency which, in addition, seems to be restricted to only few species (Satina & Blakeslee 1926). Currently, an order-wide comprehensive survey of host-ranges for all known biotrophic fusion parasites is lacking. A recent investigation revealed an unstudied mycoparasite, *Lentamyces parricida*, as the most basal with the highest mycoparasitic potential to infect other mucoralean hosts (Hoffmann & Voigt 2009).

Mucoralean fungi are also known as human and animal pathogens. *Mucor corymbifer* (currently *Lichtheimia corymbifera*) was first reported as causative agent of mycosis in a rabbit (Platauf 1885). In the last decades, the reported number of infections caused by members of the *Mucorales* (mucormycoses) has constantly increased. This is probably due to a rising awarness, an improved identification by the use of molecular methods, as well as a permanent worldwide increase of risk factors such as immunosupression, malignancies and diabetes (Roden et al. 2005, Skiada et al. 2011).

The symptoms of infections by *Mucorales* remain unspecific for a long time, making a diagnosis extremely difficult. A fast, proper and effective therapy is required, since these infections can result in death within hours to a few days. Survival rates for mucormycosis are highly dependent on the location of the infection, but they are very low overall at 53 % (Skiada et al. 2011). The large and still increasing numbers of studies pertaining to the susceptibility of *Mucorales* to known and new fungicides indicate a pressing need for an effective therapy. And with the discovery of species-specific susceptibility profiles, it became obvious, that the causative agents should be identified

Table 2 Morphology based family structure of the Mucorales adopted from Zycha et al. 1969, Hesseltine & Ellis 1973, Benjamin 1979, Benny 1982, von Arx 1982.

Family	Main characteristics
Chaetocladiaceae	Unispored sporangiola formed on fertile vesicles, discoid columella, dichotomous branched fertile hyphae, sterile spines, chlamydospores absent, zygospores rough-walled, suspensors opposed
Choanephoraceae	Sporangia and sporangiola, on different sporangiophores, zygospores striate, suspensors apposed or tongs-like
Cunninghamellaceae	(Fig. 3a), unispored sporangiola, sporangia absent, zygospores warty, suspensors opposed
Gilbertellaceae	Sporangiospores appendaged; zygospores rough-walled, suspensors opposed
Mucoraceae	Sporangia present, specialized sporangiola absent, zygospores smooth to warty, variously shaped suspensors: opposed, naked, appendaged, polyphyletic
Mycotyphaceae	Sporangiola on pedicels (Fig. 4k)
Phycomycetaceae	Sporangiophores large and unbranched, zygospores with coiled tongs-like suspensors and branched appendages
Pilobolaceae	Spores are actively liberated, zygospores smooth, suspensors tongs-like or apposed (Fig. 5)
Radiomycetaceae	Sporangia absent, sporophores with a primary vesicle bearing secondary ampullae, sporangiola originating from ampullae, zygospores smooth, suspensors apposed, appendaged
Saksenaeaceae	Provisionally classified together with *Lobosporangium* (currently *Mortierellomycotina*) because of the unusual-shaped sporangia
Syncephalastraceae	Merosporangia, zygospores warty, suspensors opposed (Fig. 4g)
Thamnidiaceae	Sporangiola present, sporangia absent or apically on the sporangiophores, zygospores warty, suspenors opposed (Fig. 4n, o)

correctly to species level (e.g. Vitale et al. 2012). To investigate and to understand mucormycoses, their susceptibility and their evolutionary relationships need to be comparatively investigated. Understanding evolutionary relationships will elucidate approaches to improve existing or to invent new applications in industry, agriculture or medicine.

Morphology-based families

Traditionally, *Mucorales* were classified using their observable characters, for example physiology, biochemistry and, especially, morphology (Table 1). Unfortunately, *Mucorales* display only a small number of distinguishable morphological characters and only a few of them have proven to be useful for distinction between species, genera and families.

Nevertheless, in early mucoralean systematics, clustering of morphologically similar species resulted in well-defined genera and families accepted before the implementation of molecular data in phylogenetic reconstruction (Table 2).

Delimitations of morphology

Traditional approaches used to classify fungi – fossil records, biochemistry and, especially, morphology (e.g., Paterson & Bridge 1994, Benny 1995, Hawksworth et al. 1995) became less important following the emergence of molecular systematics (White et al. 1990). Applying molecular data to phylogenetic analyses has led to the breakdown of the former phylum Zygomycota, combined by the morphological feature 'zygospore' into the subphyla *Mucoromycotina*, *Kickxellomycotina*, *Zoopagomycotina* and *Entomophthoromycotina* (James et al. 2006, Hibbett et al. 2007).

The family structure of the *Mucorales* is still rather unstable, but with the discovery of new, potentially phylogenetic informative characters (molecular data) and with the availability of higher resolution microscopy (e.g., fluorescence, SEM, TEM) it becomes feasible to reveal smaller, presumably monophyletic clades.

The most significant changes have affected the *Thamnidiaceae*, *Mucoraceae*, *Chaetocladiceae* and *Absidiaceae*. The first molecular studies addressing the entire order (O'Donnell et al. 2001, Voigt & Wöstemeyer 2001) showed that species traditionally assigned to *Thamnidiaceae* and *Mucoraceae* were scattered over the entire order. A widely accepted classification predominantly based on morphological traits was published by Benny et al. (2001) and is summarised with the molecular studies in Table 3.

Over the following years, several species and genera were studied in more detail, re-evaluated and revised (for a complete list see Walther et al. 2013 in this issue of Persoonia). In the following only studies that influenced family concepts by the dissection of the genus, the exclusion of a genus from a family or the fusion of families are addressed.

Absidia, *Lichtheimia* and *Lentamyces*

The genus *Absidia* was originally defined by its pyriform, apophysate sporangia (Fig. 3b, c, 4h–j). The first phylogenetic analyses (O'Donnell et al. 2001, Voigt & Wöstemeyer 2001) revealed a paraphyletic origin of this genus, a separation was accomplished later. Mesophilic species were retained in the genus *Absidia* (*Cunninghamellaceae*), whereas thermotolerant species form a separate phylogenetic clade as genus *Lichtheimia* (Hoffmann et al. 2007, 2009). In addition to the thermotolerant species separated from *Absidia*, potential mycoparasitic species were also distinguished in a new genus, *Lentamyces* (Hoffmann & Voigt 2009). This genus harbours two species, *L. parricida* and *L. zychae*. At the same time, two new species were isolated from nature and described as *Siepmannia lariceti* and *S. pineti* (Kwaśna & Nirenberg 2008a, b). This genus also was supposed to include both species of *Lentamyces*. Since molecular data for *Siepmannia* includes only ITS sequences, with no living material accessible, the relationship between the two genera remains unclear.

Choanephora and *Gilbertella*

Although there are morphological differences in zygosporogenesis in the *Gilbertellaceae* and the *Choanephoraceae*, a molecular study combined with ultrastructure supported merging these two families, under the older name, *Choanephoraceae* (Voigt & Olsson 2008).

Pilaira

Due to morphological similarities, this genus was placed traditionally within the *Pilobolaceae* together with *Pilobolus* and *Utharomyces*. But molecular data (O'Donnell et al. 2001, Voigt & Wöstemeyer 2001) revealed a non-relationship of *Pilaira* to both other genera, followed by an assignment to the *Mucoraceae* as published in Index Fungorum. This classification was also suggested on the base of a comprehensive molecular study of the *Pilobolaceae* (Foos et al. 2011).

Table 3 Summary of the family structure of the *Mucorales* based predominantly on morphology (Benny et al. 2001) as well as on combination with molecular data (O'Donnell et al. 2001, Voigt & Wöstemeyer 2001).

Family	Genera
Chaetocladiaceae	*Chaetocladium*, *Dichotomocladium*
Choanephoraceae	*Blakeslea*, *Choanephora*, *Poitrasia*
Cunninghamellaceae	*Cunninghamella*
Gilbertellaceae	*Gilbertella*
Mortierellaceae	*Aquamortierella*, *Dissophora*, *Echinosporangium*, *Modicella*, *Mortierella*, *Umbelopsis*
Mucoraceae	*Absidia*, *Actinomucor*, *Apophysomyces*, *Chlamydoabsidia*, *Circinella*, *Circinomucor*, *Dicranophora*, *Gongronella*, *Halteromyces*, *Hyphomucor*, *Micromucor*, *Mucor*, *Mycocladus*, *Parasitella*, *Protomycocladus*, *Rhizomucor*, *Rhizopodopsis*, *Rhizopus*, *Spinellus*, *Sporodiniella*, *Syzygites*, *Thermomucor*, *Zygorhynchus*
Mycotyphaceae	*Benjaminiella*, *Mycotypha*
Phycomycetaceae	*Phycomyces*
Pilobolaceae	*Pilaira*, *Pilobolus*, *Utharomyces*
Radiomycetaceae	*Hesseltinella*, *Radiomyces*
Saksenaeaceae	*Saksenaea*
Syncephalstraceae	*Syncephalastrum*
Thamnidiaceae	*Backusella*, *Cokeromyces*, *Ellisomyces*, *Fennellomyces*, *Helicostylum*, *Kirkomyces*, *Phascolomyces*, *Pirella*, *Thamnidium*, *Thamnostylum*, *Zychaea*

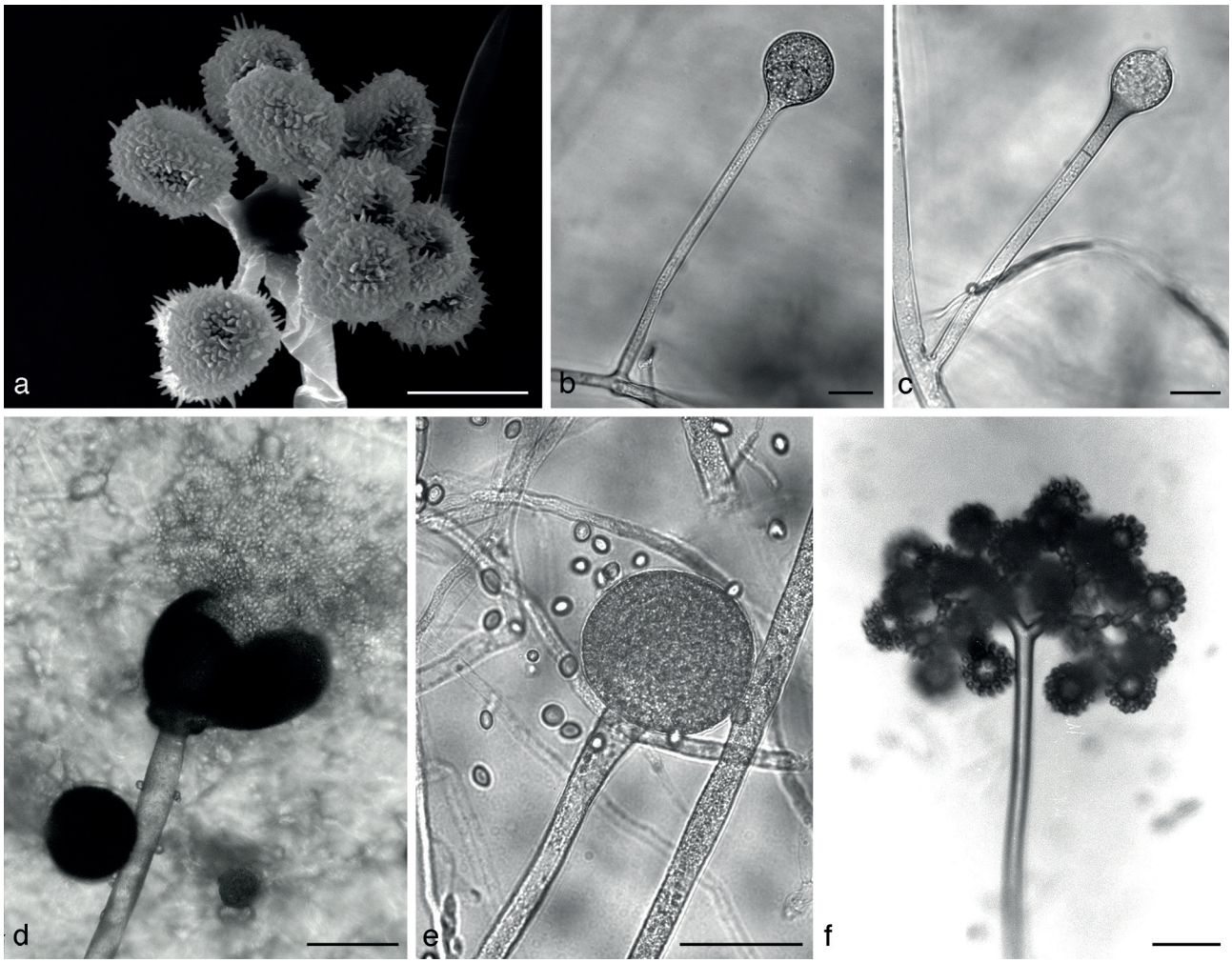


Fig. 3 a. *Cunninghamella* sp. Sporangiophore with apical vesicle and sporangiola on stalks; b. apophysate sporangia of *Absidia* sp.; c. columella of *Absidia* sp. with typical apical projection and subsporangial septae; d, e. sporangium of *Gilbertella persicaria*: d. ruptured sporangial wall and released spores; f. branching sporangiophore of *Blakeslea trispora* with apical vesicles bearing few spored sporangiola. — Scale bars: a = 5 µm; b, c = 20 µm; d–f = 50 µm.

Molecular systematics and implications on Mucorales

Molecular systematics is rapidly developing. Taxon samplings, possibilities to combine data and the number of applicable analytical tools are constantly increasing. In addition, with the ability to sequence whole genomes at relatively moderately cost combined with appropriate annotation software, computing capability and open access, genome-wide phylogeny comes within reach (Fitzpatrick et al. 2006, Kuramae et al. 2006, Huerta-Cepas et al. 2008). However, as only a few mucoralean fungi are fully sequenced, elucidating the phylogenetic relationships within this order is usually based on single genes or the combination of a few genes. Currently (April 2012), 24 genome/transcriptome projects for *Mucorales* are listed in the JGI Genome Online Database (GOLD; Fig. 6), but this includes only four different taxa (*Mucor circinelloides*, *Rhizopus oryzae*, *Rhizopus stolonifer* (each one project), and *Phycomyces blakesleeanus* (21 projects).

There are currently more than 6 000 sequences of zygomycota deposited in GenBank, approximately one-third of these are protein coding sequences. This is the third largest fraction for basal fungi, but still far behind the derived fungi, the *Dikarya* (*Ascomycota* and *Basidiomycota*; Fig. 6). Molecular data for the *Mucorales* have been submitted to GenBank since 1993, with a constantly increasing number, reaching more than 1 000 sequences in 2010 and more then 1 400 last year (Fig. 7). Nevertheless, the submitted sequences are restricted to only a few genera and species, with half of the sequences from the two genera *Mucor* and *Rhizopus* (Table 4). Around 50 species for *Mucor* and nine species for *Rhizopus* are listed in the 10th edition of the Dictionary of the Fungi (Kirk et al. 2008) which is 24 % and 4 %, respectively, of all species accepted in the *Mucorales*.

Studies predominately concerned with molecular phylogenetic aspects of zygomycetes, especially *Mucorales*, are still relatively rare. Searching NCBI and the ISI Web of Science with 'zygomycetes or *Mucorales* AND phylogeny' resulted only in between 40 and 50 analyses including 15 studies where at least 2 loci were applied (April 2012, Fig. 8).

Commonly applied markers for phylogeny are sequences coding for rDNA (especially 18S rDNA for relationship levels of families, orders and above-order as well as ITS1 & 2 for relationships of species and genera). Therefore, the majority of studies are using rDNA sequences for phylogenetic approaches although ITS sequences represent the largest fraction of sequences in GenBank (Fig. 9). Protein coding genes predominantely applied so far are actin and translation elongation factor 1-alpha. Establishment of alternative protein coding markers for the whole order remains difficult. Whereas largest and second-largest subunit of RNA polymerase II (RPB1 & 2), ATPase subunit 6 (ATP6), a DNA replication licensing factor (MCM7), a gene required for rRNA accumulation (TSR1) or cytochrome c oxidase I (COX1) proved to be suitable for other fungal groups mostly belonging to the *Basidiomycota* and *Ascomycota* (Matheny et al. 2002, Reeb et al. 2004, Seifert et al. 2007, Schmitt et al. 2009), these genes have not be successfully amplified for a broad range of *Mucorales* and are still under represented in GenBank (Schoch et al. 2012).

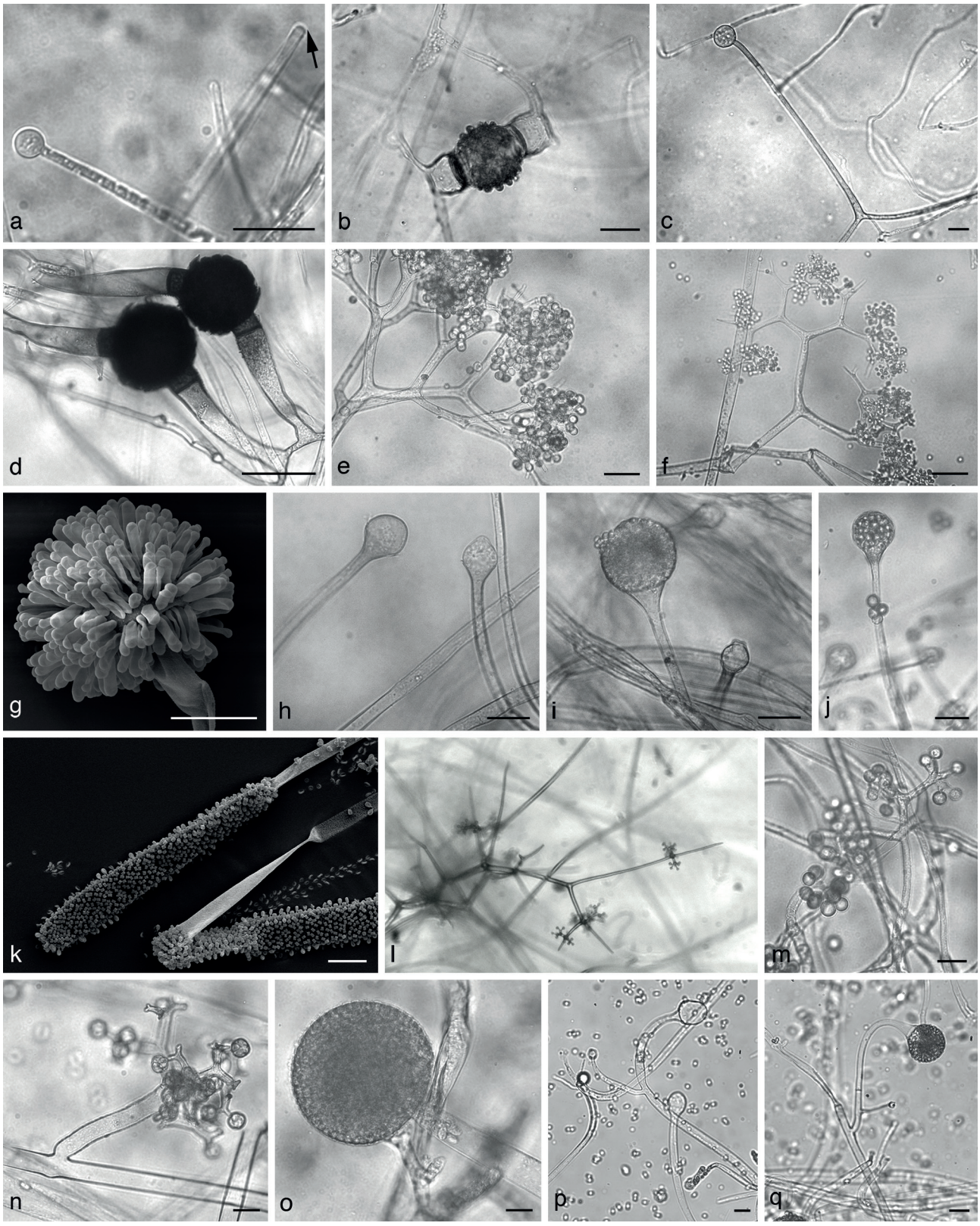


Fig. 4 a. *Umbelopsidaceae*. Sporangium and sporangiophore with the highly reduced columella (arrow). — b, c. *Lentamycetaceae*. b. Warty zygospore, species are homothallic; c. sporangium. — d–f. *Dichotomocladium*. d. Zygospores; e, f. dichotomous branched sporangiophores. — g. *Syncephalastrum racemosum*, merosporangia. — h–j. *Lichtheimia*. h. Columella; i, j. apophysate sporangia. — k. *Mycotypha* sp., cylindrical vesicle covered with sporangiola. — l, m. *Chaetocladium* sp., branched fertile head with sporangiola. Branches often terminate in sterile spines. — n, o. *Thamnidium elegans*. n. Dichotomous branched sporangiophores with sporangiola; o. main multi-spored sporangia. — p, q. Columella and sporangia borne on circinate sporangiophores of *Circinella* sp. — Scale bars: all = 20 µm.

The present study focuses on the family structure of the *Mucorales*. Family boundaries are inferred from a molecular phylogeny based on four markers and including 201 isolates and all currently accepted genera. Historical approaches and changes in recent years are revised, the support of the families by the current data is discussed and the families are characterised morphologically and ecologically. The resulting changes on the higher level nomenclature of the *Mucorales* were already briefly introduced by Voigt (2012). In order to ensure that these changes were based on a stable lower level taxonomy the

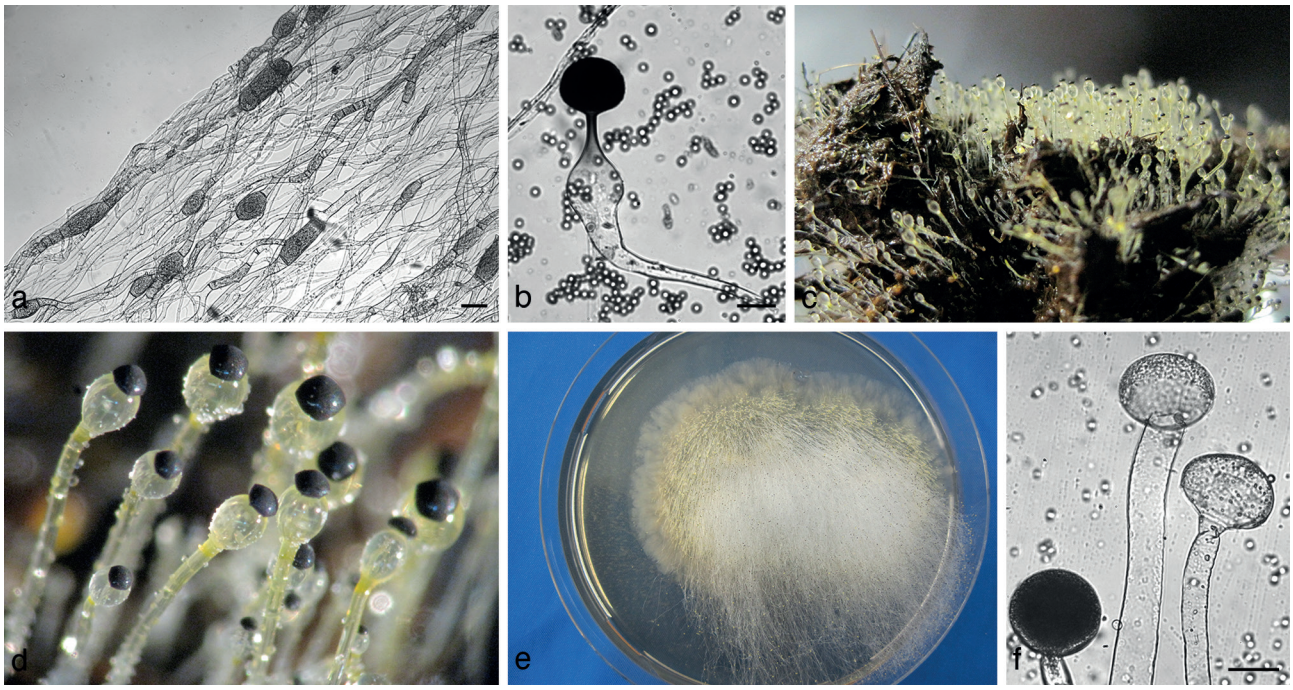


Fig. 5 *Pilobolaceae*. a. Substrate mycelium with trophocysts; b. sporangium of *Utharomyces epallocaulus* with subsporangial swelling; c. colony morphology of *Pilobolus* sp. on horse dung. Sporangia are phototrophic; d. sporangiophores with subsporangial swelling and the black sporangium. Light is focused through the swelling towards carotenoids at the base of the vesicle, the ocellus (orange colour); e. colony morphology of *Pilaira* sp. Sporangiophores are also light sensitive; f. sporangium and columellae of *Pilaira* sp. — Scale bar = 50 µm.

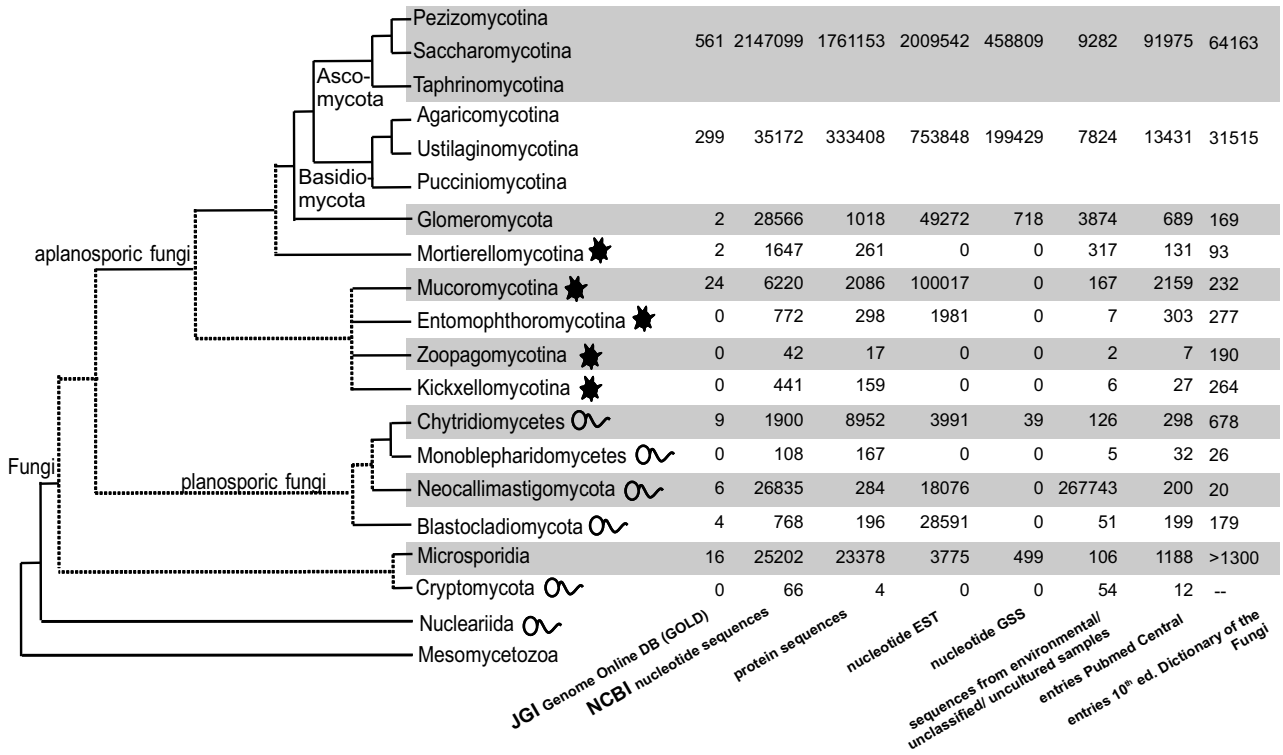


Fig. 6 Schematic fungal tree and important data about the fungal groups. The topology resembles the current understanding of the relationships of the fungal groups according to Hibbett et al. (2007), James et al. (2006) and Schoch et al. (2012) (data retrieved April 2012).

internal transcribed spacer 2 region (ITS2) was analysed for of compensatory base changes (CBCs) as indicators for species boundaries (Müller et al. 2007).

MATERIAL AND METHODS

Strains, DNA isolation, PCR

Strains used for the generation of additional sequences (bold accession numbers in Table 5) were cultivated on 3 % malt extract medium at room temperature. Genomic DNA was extracted as described in Hoffmann et al. (2007). For phylogenetic analyses, sequences of large (LSU) and small (SSU) subunit of ribosomal DNA, ITS (internal transcribed spacer 1 & 2, incl. 5.8 SrDNA), actin (act) and translation elongation factor 1-alpha (tef) were either generated in this study or retrieved from GenBank (www.ncbi.nlm.nih.gov/; Table 5). Primers used for the amplification of LSU were NL1 and NL4 (O'Donnell 1993), NS1 and NS4 for SSU (White et al. 1990), ITS1 and ITS4 for

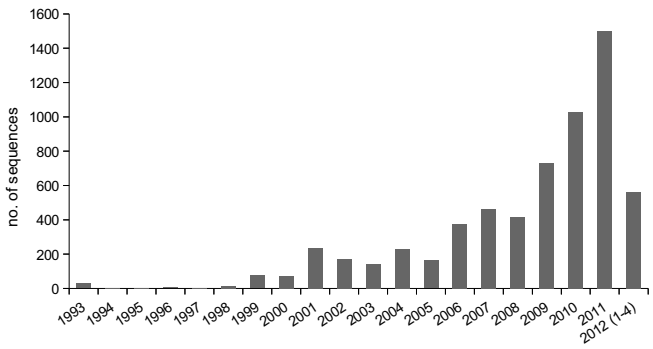


Fig. 7 Chronology of sequences submitted to GenBank since 1993 for the *Mucorales* (data retrieved April 2012).

ITS (White et al. 1990), Act1/Act1b and Act4R/Act4Ra for actin (Voigt & Wöstemeyer 2000) and MEF1 and MEF4/UEF4 for Tef (O'Donnell et al. 2001). PCR fragments were purified using the protocol of Vogelstein & Gillespie (1979) and sequenced on an Applied Biosystems 3730xL DNA Analyzer (ABI, Carlsbad) according to the manufacturer's instructions.

Sequence alignment, phylogeny, distance matrices, CBC

Multiple sequence alignments were generated using MAFFT v. 6.901b (server: mafft.cbrc.jp/alignment/server/) or v. 6.822 as implemented at the CIPRES portal (//www.phylo.org/; Miller et al. 2010). Alignments comprised 201 taxa and 1 586 characters for 18S rDNA, 358 characters for 28S rDNA, 807 characters for actin and 1 092 characters for translation elongation factor 1-alpha. Phylogenetic trees were calculated using RAxML v. 7.3.0 and MrBayes v. 3.1.2 from the CIPRES portal under the default settings with the following adjustments: RAxML was run choosing rapid bootstrapping (GTRCAT) and GTRGAMMA for final tree inference with 1 000 bootstrap iterations. Bayesian inference was run setting the number of substitution types to 6 (GTR), with among-site rate variation set to invgamma. Analysis was run with four chains each in two runs for 5 million generations. 5 001 trees were sampled, and 2 501 trees were analysed discarding the first 50 % of the samples as burnin. Bootstrapping was done with 1 000 iterations. Dataset was partitioned for both analyses. Alignments and phylogenetic trees are deposited in TreeBase2 under TB2:S13469. Distances were calculated using *distMat* from the EMBOSS suite (Rice et al. 2000; http://emboss.sourceforge.net/) with the alignments as input. Distances are expressed as substitutions per 100 bases or amino acids. CBC analyses were done as described previously (Pawłowska et al. In press).

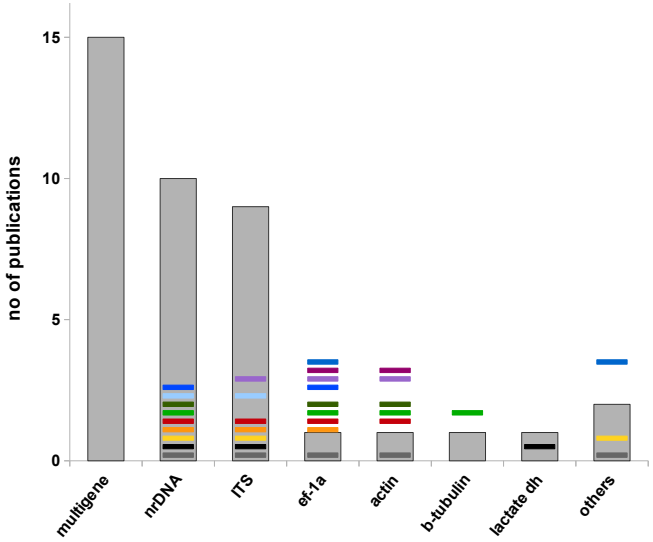


Fig. 8 Number of publications predominantly focused on mucoralean phylogeny retrieved from NCBI and ISI Web of Science by searching '*Zygomycota*/*Mucorales* AND phylogeny'. Publications are separated by the molecular marker applied for phylogeny. Nearly half of all published studies included more than one molecular marker. Published combinations of molecular markers are indicated by different colours (data retrieved April 2012).

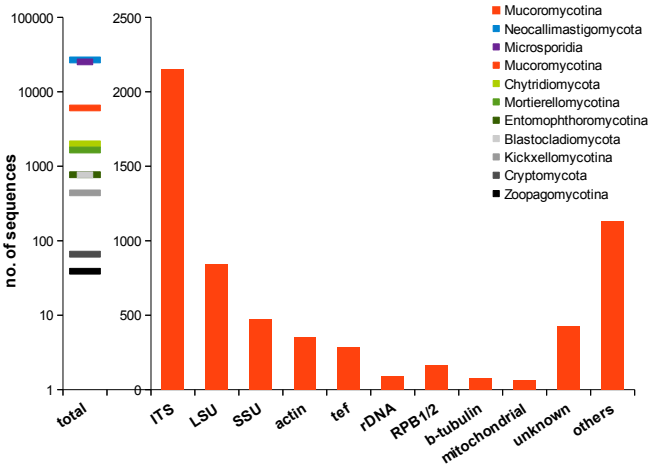


Fig. 9 Distribution of available sequences in GenBank for the *Mucoromycotina*. Also the total number of available sequences for all basal fungal linages are given (data retrieved April 2012).

Table 4 Sequences available at GenBank (April 2012) for mucoralean genera.

Genus	No. of seq.	Genus	No. of seq.	Genus	No. of seq.
Absidia	184	*Gongronella*	31	*Rhizomucor*	200
Actinomucor	68	*Halteromyces*	5	*Rhizopus*	1928
Ambomucor	3	*Helicostylum*	20	*Saksenaea*	50
Amylomyces	163	*Hesseltinella*	4	*Siepmannia*	2
Apophysomyces	69	*Hyphomucor*	4	*Spinellus*	7
Backusella	6	*Kirkomyces*	4	*Sporodiniella*	4
Benjaminiella	12	*Lentamyces*	23	*Syncephalastrum*	64
Blakeslea	93	*Lichtheimia*	679	*Syzygites*	26
Chaetocladium	25	*Mucor*	1501	*Thamnidium*	11
Chlamydoabsidia	6	*Mycotypha*	11	*Thamnostylum*	14
Choanephora	27	*Parasitella*	17	*Thermomucor*	6
Circinella	6	*Phascolomyces*	6	*Umbelopsis*	243
Cokeromyces	16	*Phycomyces*	110	*Utharomyces*	20
Cunninghamella	141	*Pilaira*	59	*Zychaea*	4
Dichotomocladium	29	*Pilobolus*	149		
Dicranophora	4	*Pirella*	5		
Ellisomyces	8	*Poitrasia*	11	environmental/	
Fennellomyces	10	*Protomycocladus*	4	uncultured/	
Gilbertella	16	*Radiomyces*	8	unclassified	108

Table 5 Taxa and sequences used for the phylogenetic analyses. GenBank accession numbers in **bold** are generated within this study.

Internal no.	Species	Isolate	18S rDNA	28S rDNA	Act	Tef
	Ascomycota					
KV5	*Archaeorhizomyces finlayi*	Ny10	JF836020	JF836022	na	JF836025
P248	*Saccharomyces bayanus*	CBS380	X97777	AF113892	na	na
	Basidiomycota					
P249	*Agaricus bisporus*	AFTOL448	AY787216	AY635775	na	na
	Blastocladiomycota					
P251	*Blastocladiella emersonii*	AFTOL302	AY635842	DQ273808	na	na
	Chytridiomycota					
P250	*Batrachochytrium dendrobatidis*	AFTOL21	AH009052	NG_027619	na	na
	Eccrinales					
KV1	*Enterobryus* sp.		AY336711	AY336693	na	na
KV2	*Enteromyces callianassae*	CA12c8	AY336702	AY336696	na	na
KV4	*Palavascia patagonica*	ARGD1c15	AY682845	AY336695	na	na
KV3	*Taeniellopsis* sp.	MA5C17	AY336704	AY336697	na	na
	Endogonales					
P011	*Endogone pisiformis*	AFTOL539	DQ322628	DQ273811	AB609182	DQ282618
	Entomophthoromycotina					
P006	*Basidiobolus ranarum*	AFTOL301	AY635841	DQ273807	na	DQ282610
P021	*Batkoa major*	ARSEF2936	EF392559	EF392401	na	na
P017	*Conidiobolus coronatus*	NRRL28638	NG_017182	NG_027617	HM117709	na
P024	*Entomophaga maimaiga*	ARSEF1400	EF392556	EF392395	na	na
P025	*Entomophthora muscae*	ARSEF3074	NG_017183	NG_027647	na	na
P026	*Erynia radicans*	ATCC60281	JQ014018	JN939182	na	na
P027	*Eryniopsis caroliniana*	ARSEF640	EF392552	EF392387	na	na
P030	*Massospora cicadina*	ARSEF374	EF392548	EF392377	na	na
P033	*Pandora neoaphidis*	ARSEF3240/ARSEF835	EF392560	EF392405	na	na
P007	*Schizangiella serpentis*	ARSEF203	AF368523	EF392428	na	na
P037	*Zoophthora radicans*	ARSEF4784/ARSEF6003	EF392561	EF392406	na	na
	Glomeromycota					
P253	*Glomus intraradices*	AFTOL845	DQ322630	DQ273828	na	na
	Kickxellomycotina					
P053	*Austrosmittium biforme*	32-1-9/ 32-1-8	DQ367462	DQ367494	na	na
P056	*Bojamyces repens*	ME-JL-2	DQ367447	DQ367478	na	na
P057	*Capniomyces stellatus*	mis-21-127	EF396191	EF396194	na	na
P088	*Coemansia reversa*	NRRL1564	NG_017186	NG_027615	AB609183	DQ282615
P091	*Dipsacomyces acuminosporus*	NRRL2925	AF007534	AF031065	na	na
P062	*Furculomyces boomerangus*	AFTOL303	AF007535	DQ273809	na	na
P065	*Genistelloides hibernus*	2-16-2	DQ367448	DQ367479	na	na
P066	*Genistellospora homothallica*	VT-3-W14	DQ367454	DQ367495	na	na
P048	*Harpella melusinae*	NF-15-4b	DQ367514	DQ367518	na	na
P049	*Harpellomyces* sp.	PA-3-1d	EF396192	EF396195	na	na
P092	*Kickxella alabastrina*	NRRL2693	AF007537	AF031064	na	na
P093	*Linderina pennispora*	NRRL3781	AF007538	AF031063	na	na
P095	*Martensiomyces pterosporus*	NRRL2642	AF007539	AF031066	na	na
P097	*Myconymphaea yatsukahoi*	NBRC100467	AB287984	AB287998	na	na
P075	*Pennella simulii*	NY-5-3/ NF-19-8	DQ367515	DQ367502	na	na
P089	*Pinnaticoemansia coronantispora*	NBRC100470	AB287986	AB288000	na	na
P076	*Plecopteromyces* sp.	37-1-2	DQ367445	DQ367476	na	na
P080	*Smittium culisetae*	AFTOL29/IAM14394/BL023	AF007540	DQ273773	HM117719	AB077104
P100	*Spirodactylon aureum*	NRRL2810	AF007541	AF031068	na	na
P087	*Zygopolaris ephemeridarum*	CA-4-W9	DQ367463	DQ367508	na	na
	Mortierellomycotina					
P106	*Dissophora decumbens*	NRRL22416	AF157133	AF157187	AJ287155	AF157247
P107	*Echinosporangium transversale*	NRRL3116	AF113424	AF113462	AJ287156	AF157248
P108	*Gamsiella multidivaricata*	NRRL6456	AF157144	AF157198	AJ287168	AF157260
P111	*Mortierella longicollis*	CBS209.32	JQ040249	JN940876	na	na
P110	*Mortierella verticillata*	CBS374.95	HQ667482	JN940872	na	na
KH001		NRRL6337	AB016017	DQ273794	AJ287170	AF157262
	Mucoromycotina					
P121a	*Absidia caerulea*	NRRL1315	AF113405	AF113443	AJ287133	AF157226
P121f	*Absidia californica*	CBS126.68	EU736273	EU736300	AY944758	EU736246
P121b	*Absidia glauca*	CBS101.48	AF157118	AF157172	AJ287135	X54730
P121e	*Absidia macrospora*	CBS696.68	EU736276	EU736303	AY944760	EU736249
P121d	*Absidia psychrophilia*	CBS128.68	EU736279	EU736306	AY944762	EU736252
P121	*Absidia repens*	NRRL1336	AF113410	AF113448	AJ287136	AF157228
P121c	*Absidia spinosa*	ATCC22755	EU736280	EU736307	EU736227	EU736253
P137	*Actinomucor elegans*	NRRL3104/CBS111559	AF157119	AF157173	AJ287137	AF157229
P190	*Apophysomyces elegans*	NRRL22325	AF113411	FN554250	na	na
P190a		NRRL28632	AF113412	AF113450	AJ287139	AF157231
P140	*Backusella circina*	NRRL2446	AF157121	AF157175	AJ287140	AF157232
kH1		FSU2455	**JX644458**	**JX644491**	na	na
kH9		FSU10121	**JX644459**	**JX644492**	na	na
kH10		FSU10122	**JX644460**	**JX644493**	na	na
kH11		FSU10123	**JX644461**	**JX644494**	na	na
kH12		FSU10124	**JX644462**	**JX644495**	na	na
P169g	*Backusella recurva*	NRRL3247	AF157146	AF157200	AJ287179	AF157270
kH5		FSU10115	**JX644463**	**JX644496**	na	na
kH6		FSU10116	**JX644464**	**JX644497**	na	na

Table 5 (cont.)

Internal no.	Species	Isolate	18S rDNA	28S rDNA	Act	Tef
kH7		FSU10117	**JX644465**	**JX644498**	na	na
kH8		FSU10118	**JX644466**	**JX644499**	na	na
P143	*Benjaminiella poitrasii*	NRRL2845	AF157123	AF157177	AJ287142	AF157234
P114	*Blakeslea trispora*	CBS130.59	AF157124	AF157178	AJ287143	AF157235
P146	*Chaetocladium brefeldii*	CBS136.28	EU736284	EU736311	EU736230	EU736257
P146a		NRRL1349	AF157125	AF157179	AJ287144	AF157236
P146b	*Chaetocladium jonesii*	NRRL2343	AF157126	AF157180	AJ287145	AF157237
P123	*Chlamydoabsidia padenii*	NRRL2977	AF113415	AF113453	AJ287146	AF157238
P115	*Choanephora infundibulifera*	CBS150.51/NRRL2744	AF157127	AF157181	AJ287147	AF157239
P151a	*Circinella* sp.	NRRL13768	**JX644467**	**JX644500**	**JX644524**	**JX644574**
P151b		NRRL13768	**JX644468**	**JX644501**	**JX644525**	**JX644575**
P151	*Circinella umbellata*	NRRL1351	AF157128	AF157182	AJ287148	AF157240
P154	*Cokeromyces recurvatus*	AFTOL627	AY635843	DQ273812	na	na
P154a		NRRL2243	AF113416	AF113454	AJ287150	AF157242
P124a	*Cunninghamella bainierii*	FSU319/NRRL1375	EU736286	EU736313	EU736232	EU736259
P124b	*Cunninghamella bertholletiae*	NRRL6436	AF113421	AF113459	AJ287151	AF157243
P124	*Cunninghamella echinulata*	NRRL1382/CBS156.28	AF157130	AF157184	AJ287152	AF157244
P194	*Dichotomocladium elegans*	NRRL6236	AF157131	AF157185	AJ287153	AF157245
P194a		NRRL2664	JQ775463	JQ775492	EU826394	EU826399
P194e	*Dichotomocladium floridanum*	FSU8694	JQ775462	JQ775491	**JX644526**	**JX644576**
P194b	*Dichotomocladium hesseltinei*	NRRL5912	JQ775464	JQ775493	**JX644527**	**JX644577**
P194c	*Dichotomocladium robustum*	NRRL6234	JQ775465	JQ775494	**JX644528**	**JX644578**
P194d		NRRL6235	JQ775466	JQ775495	**JX644529**	na
P194f	*Dichotomocladium sphaerosporum*	FSU8696	JQ775469	JQ775498	na	**JX644579**
P194g		FSU8697	JQ775467	JQ775496	**JX644530**	**JX644580**
P194h	*Dichotomocladium sphaerosporum* 2	FSU8698	JQ775468	JQ775497	**JX644531**	**JX644581**
P194i	*Dichotomocladium sphaerosporum* 3	FSU8698	**JX644469**	**JX644502**	**JX644532**	**JX644582**
P156	*Dicranophora fulva*	NRRL22204	AF157132	AF157186	AJ287154	AF157246
P157	*Ellisomyces anomalus*	NRRL2749	AF157134	AF157188	AJ287157	AF157249
P195	*Fennellomyces linderi*	NRRL2342	AF157135	AF157189	AJ287158	AF157250
P119	*Gilbertella persicaria*	NRRL2357/CBS442.64	AF157136	AF157190	AJ287159	AF157251
P125	*Gongronella butleri*	NRRL1340/ATCC8989	AF157137	AF157191	AJ287160	AF157252
P126	*Halteromyces radiatus*	NRRL6197	AF157138	AF157192	AJ287161	AF157253
P160	*Helicostylum elegans*	NRRL2568/CBS258.59	AF157139	AF157193	AJ287162	AF157254
P160c	*Helicostylum pulchrum*	CBS639.69	EU736289	EU736316	EU736235	EU736262
P160b		CBS259.68	EU736288	EU736315	EU736234	EU736261
P127	*Hesseltinella vesiculosa*	CBS197.68	AF157140	AF157194	AJ287163	AF157255
P162	*Hyphomucor assamensis*	NRRL22324	AF157141	AF157195	AJ287164	AF157256
P164	*Kirkomyces cordensis*	NRRL22618	AF157142	AF157196	AJ287165	AF157257
P160a		CBS223.63	EU736287	EU736314	EU736233	EU736260
P216a	*Lentamyces zychae*	CBS104.35	EU736282	EU736309	EU736228	EU736255
P134	*Lichtheimia corymbifera*	CBS429.75	JQ014052	GQ342903	GQ342831	FJ719483
P134a		NRRL2982	AF113407	FJ719429	AJ287134	AF157227
P134b	*Lichtheimia hyalospora*	NRRL1304	AF157117	AF157171	AJ287132	AF157225
P134d		NRRL2916	EU826360	EU826368	EF030531	**JX644583**
P134c	*Lichtheimia ramosa*	FSU6197	**JX644470**	**JX644503**	**JX644533**	**JX644584**
P169a	*Mucor amphibiorum*	NRRL28633	AF113426	AF113466	AJ287172	AF157263
P152	*Mucor circinelloides*	NRRL22652	AF157129	AF157183	AJ287149	AF157241
P169	*Mucor circinelloides* f. *circinelloides*	CBS195.68/FSU6169	EU484248	FN650667	na	na
P169h		CBS416.77	EU736294	EU736321	EU736240	EU736267
P169b	*Mucor circinelloides* f. *lusitanicus*	NRRL3631	AF113427	AF113467	AJ287173	AF157264
P141	*Mucor ctenidius*	NRRL6238	AF157122	AF157176	AJ287141	AF157233
kH2		FSU10112	**JX644471**	**JX644504**	na	na
kH3		FSU10113	**JX644472**	**JX644505**	na	na
kH4		FSU10114	**JX644473**	**JX644506**	na	na
P169c	*Mucor hiemalis*	NRRL3624	AF113428	AF113468	AJ287174	AF157265
P169d	*Mucor indicus*	NRRL28634	AF113429	AF113469	AJ287175	AF157266
P135c	*Mucor irregularis*	NRRL28773	AF113435	AF113476	AJ287193	AF157284
P180	*Mucor moelleri*	FSU779/FSU514	EU736298	EU736325	EU736244	EU736271
P180b		FSU531	EU736297	EU736324	EU736243	EU736270
P168	*Mucor mucedo*	CBS144.24	X89434	AF113470	AJ287176	AF157267
P169i	*Mucor plumbeus*	FSU283	EU736295	EU736322	EU736241	EU736268
P169j		FSU289	EU736296	EU736323	EU736242	EU736269
P169e	*Mucor racemosus*	NRRL3640	AF113430	AF113471	AJ287177	AF157268
P169f	*Mucor ramosissimus*	NRRL3042	AF113431	AF113472	AJ287178	AF157269
P182a	*Mycotypha africana*	NRRL2978	AF157147	AF157201	AJ287180	AF157271
P182	*Mycotypha microspora*	NRRL1572/F169	AF157148	AF157202	AJ287181	AF157272
P170	*Parasitella parasitica*	NRRL1461/CBS412.66/NRRL2501	HQ845295	HQ845307	AJ287182	HQ845318
P197	*Phascolomyces articulosus*	NRRL2880	AF157150	AF157204	AJ287183	AF157274
P197a		CBS113.76	**JX644474**	**JX644507**	**JX644534**	na
P183	*Phycomyces blakesleeanus*	NRRL1555	NG_017190	NG_027559	genome[1]	DQ282620
kH20	*Pilaira* sp.	FSU2463	**JX644475**	**JX644508**	na	na
P171	*Pilaira anomala*	NRRL2526	AF157152	AF157206	AJ287185	AF157276
kH19		FSU774	**JX644476**	**JX644509**	**JX644535**	**JX644585**
kH22		NRRL2526	AF157152	na	AJ287185	AF157276
P171a	*Pilaira caucasica*	NRRL6282	**JX644477**	**JX644510**	**JX644536**	**JX644586**
kH14		FSU10081	**JX644478**	**JX644511**	**JX644537**	na
kH16		FSU10083	**JX644479**	**JX644512**	**JX644538**	na
kH17		FSU10084	**JX644480**	**JX644513**	**JX644539**	na

Table 5 (cont.)

Internal no.	Species	Isolate	18S rDNA	28S rDNA	Act	Tef
kH18		FSU10085	**JX644481**	**JX644514**	**JX644540**	na
KH21		FSU6229	EU826363	EU826369	EU826376	EU826385
kH13	*Pilaira* sp.	FSU10080	**JX644482**	**JX644515**	**JX644541**	na
kH15	.	FSU10082	**JX644483**	**JX644516**	**JX644542**	na
kH25	*Pilobolus crystallinus*	FSU6210	**JX644484**	**JX644517**	na	na
kH28	*Pilobolus longipes*	IUE563	EU595654	na	na	na
KH29		IUE409	DQ211054	na	na	na
KH30		IUE340	DQ211053	na	na	na
KH31	*Pilobolus roridus*	IUE415	EU595649	na	na	na
kH23	*Pilobolus* sp.	DSM1343	**JX644485**	**JX644518**	na	**JX644587**
P186	*Pilobolus umbonatus*	NRRL6349	AF157153	AF157207	AJ287186	AF157277
kH24		CBS302.83	**JX644486**	**JX644519**	na	na
kH26		UAMH7297	DQ211050	na	na	na
kH27		NRRL6349	AF157153	na	na	na
KH32		UAMH7298	DQ211051	na	na	na
P172	*Pirella circinans*	NRRL2402/Kh-BI-O	AF157154	AF157208	AJ287187	AF157278
P120	*Poitrasia cicinans*	CBS153.58	AF157155	AF157209	AJ287188	AF157279
P198	*Protomycocladus faisalabadensis*	NRRL22826	AF157156	AF157210	AJ287189	AF157280
P198a		CBS661.86	**JX644487**	**JX644520**	na	na
P191	*Radiomyces spectabilis*	NRRL2753	AF157157	AF157211	AJ287190	AF157281
P135a	*Rhizomucor miehei*	NRRL28774	AF113432	AF113473	AJ287191	AF157282
P135d		CBS182.67	**JX644488**	**JX644521**	**JX644543**	na
P135	*Rhizomucor pusillus*	NRRL3695	HQ845298	HQ845310	na	HQ845321
P135b		NRRL2543	AF113433	AF113474	AJ287192	AF157283
P135e		CBS354.68	**JX644489**	**JX644522**	na	HQ845320
P175	*Rhizopus arrhizus*	CBS112.07	AB250164	AB250187	AB281499	AB281528
P205		CBS438.76	AB250171	AB250194	na	na
P205a		NRRL3139	AF157120	AF157174	AJ287138	AF157230
P176a	*Rhizopus microsporus* var. *azygosporus*	NRRL28627	AF113436	AF113477	AJ287194	AF157285
P176	*Rhizopus microsporus* var. *microsporus*	CBS699.68	AB250155	JN939137	AB512247	AB512270
P176b		NRRL28775	AF113438	AF113479	AJ287195	AF157286
P176c	*Rhizopus microsporus* var. *oligosporus*	NRRL2710	AF157158	AF157212	AJ287197	AF157288
P176d	*Rhizopus microsporus* var. *rhizopodiformis*	NRRL28630	AF113439	AF113480	AJ287196	AF157287
P176e	*Rhizopus stolonifer*	NRRL1477	AF113441	AF113482	AJ287199	AF157290
P193	*Saksenaea vasiformis*	NRRL2443	AF113442	AF113483	AJ287200	AF157291
P184	*Spinellus fusiger*	NRRL22323	AF157159	AF157213	AJ287201	AF157292
P213	*Sporodiniella umbellata*	NRRL20824	AF157160	AF157214	AJ287202	AF157293
P199	*Syncephalastrum monosporum*	NRRL54019/NRRL22812	AF157161	AF157215	AJ287203	AF157294
P199b	*S. monosporum* var. *pluriproliferum*	CBS569.91	**JX644490**	**JX644523**	na	**JX644588**
P199a	*Syncephalastrum racemosum*	NRRL2496	X89437	AF113484	AJ287204	AF157295
P215	*Syzygites megalocarpus*	NRRL6288/xsd08121	AF157162	AF157216	AJ287205	AF157296
P178	*Thamnidium elegans*	NRRL2467/CBS341.55	AF157163	AF157217	AJ287206	AF157297
P200	*Thamnostylum piriforme*	NRRL6240	AF157164	AF157218	AJ287207	AF157298
P136	*Thermomucor indicae-seudaticae*	NRRL6429	AF157165	AF157219	AJ287208	AF157299
P202a	*Umbelopsis isabellina*	NRRL1757	AF157166	AF157220	AJ287209	AF157300
P202c	*Umbelopsis nana*	NRRL22420	AF157167	AF157221	AJ287210	AF157301
P202b	*Umbelopsis ramanniana*	NRRL5844	X89435	AF113463	AJ287166	AF157258
P202	*Umbelopsis* sp.	FSU10157	JQ014049	JN939141	na	na
P189	*Utharomyces epallocaulus*	NRRL3168	AF157168	AF157222	AJ287211	AF157302
P201	*Zychaea mexicana*	NRRL6237	AF157169	AF157223	AJ287212	AF157303
P180a	*Zygorhynchus heterogamus*	NRRL1489	AF157170	AF157224	AJ287213	AF157304
	Neocallimastigomycota					
P252	*Neocallimastix* sp.	AFTOL638	DQ322625	DQ273822	na	na
	Zoopagomycotina					
P230	*Kuzuhaea moniliformis*	NRRL13723	AB016010	DQ273796	na	na
P234	*Piptocephalis corymbifera*	ATCC12665	AB016023	AY546690	na	DQ282619

na = not available; 1 = estExt_Genewise1Plus.C_200172.

RESULTS AND DISCUSSION

Species recognition is an essential step to higher level classification. Yet, morphology and/or mating behaviour played a major role in traditional fungal species concepts. Depending on the experience of the mycologist and on experimental conditions, morphology and mating behaviour could profoundly vary, and today, both methods were shown to be unsuitable to define mucoralean species if they are not combined with DNA data. Additional concepts have been surveyed and evaluated for fungi (Mayden 1997) with the genealogical concordance phylogenetic species recognition (GCPSR, Taylor et al. 2000) being the most likely one to recognize natural species. Phylogenetic species recognition (PSR) already revealed more species within originally identified species using morphological or biological species recognition (e.g., Hibbett et al. 1995, Taylor et al. 1999). The underlying problems of interbreeding and geographic/allopatric speciation were extensively discussed by Taylor et al. (2000). Following the discovery of phylogenetic species, additional biological and morphological characters were revealed that supported those species (reviewed by Taylor et al. 2000).

In *Mucorales*, the application of GCPSR resulted in the detection of several new species (Álvarez et al. 2010a, b, Alastruey-Izquierdo et al. 2010, Hermet et al. 2012) but on the other hand several taxa were synonymized based on comparisons of ITS sequences (Abe et al. 2006, Álvarez et al. 2010a, Walther et al. 2013).

In contrast to the naturally existing species there are no concepts for the recognition of higher or lower taxonomic levels. Traditionally, certain morphological features (Table 2) that were regarded as synapomorphies were used to define families

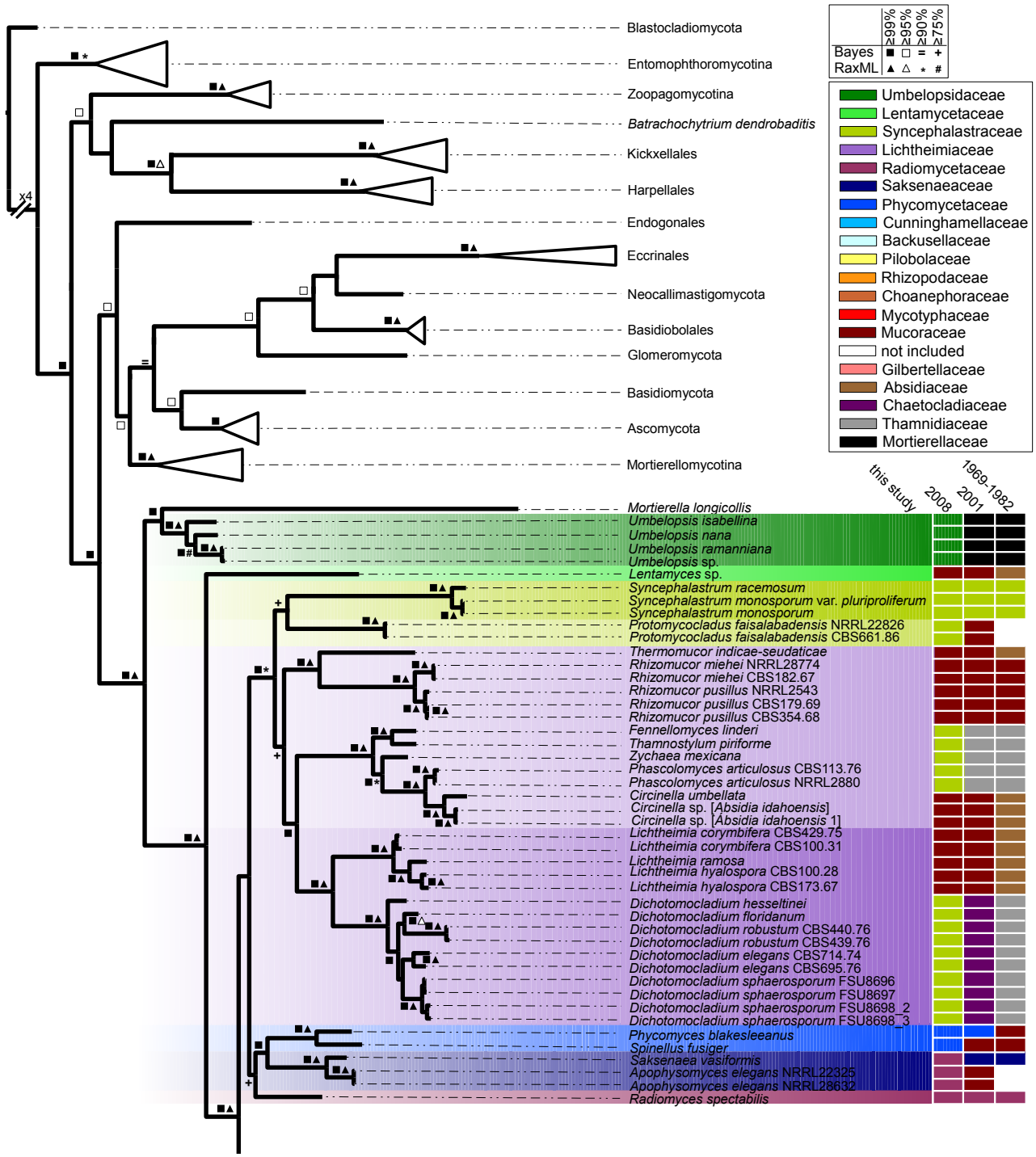


Fig. 10 Bayesian analysis of combined sequences coding for actin, translation elongation factor 1-alpha, 18S rDNA and 28S rDNA. Bootstrap values and posterior probabilities are given for branches supported with equal or higher than 75 % in maximum likelihood (RAxML) and Bayesian analysis (see legend within figure for explanation of the symbols). Strain numbers are given in parts to distinguish different isolates (compare with Table 5). Furthermore, a rough outline about the historical family structures and changes are given on the right site including benchmark studies since 1969 (Zycha et al. 1969, Hesseltine & Ellis 1973, Benjamin 1979, Benny 1982, von Arx 1982, Benny et al. 2001, Voigt & Wöstemeyer 2001, O'Donnell et al. 2001, Kirk et al. 2008). Families accepted here, are colour coded over the whole tree branches.

(Zycha et al. 1969, Hesseltine & Ellis 1973, Benjamin 1979, Benny 1982, von Arx 1982). Later they were adapted based on results of molecular phylogeny. Undoubtedly higher taxa should represent monophyletic groups but the taxonomic rank that a group deserves remains a subjective decision. Genetic distances are helpful in this decision but they cannot be translated directly into higher level taxonomy because of dramatic difference in the phylogenetic age in fungal groups.

Even though studies implementing molecular data are still very rare for *Mucorales* compared to other fungal groups, the number of sequences submitted to GenBank is constantly increasing (Fig. 7). Yet, sequences deposited are predominantly sequences of the rDNA cluster, (Fig. 9). Protein coding sequences are still under represented. This may be due to the lack of appropriate primers which are able to work over a broad range of isolates and an often encountered problem of direct sequencing of the amplificates (Schoch et al. 2012) and the frequent presence of paralogs (Alastruey-Izquierdo et al. 2010). Studies which do apply this kind of molecular data and which are predominantly focused on mucoralean phylogeny count far below 100 if searching ISI Web of Science and NCBI. Furthermore, most of these studies using only one marker for the analyses (Fig. 8). If

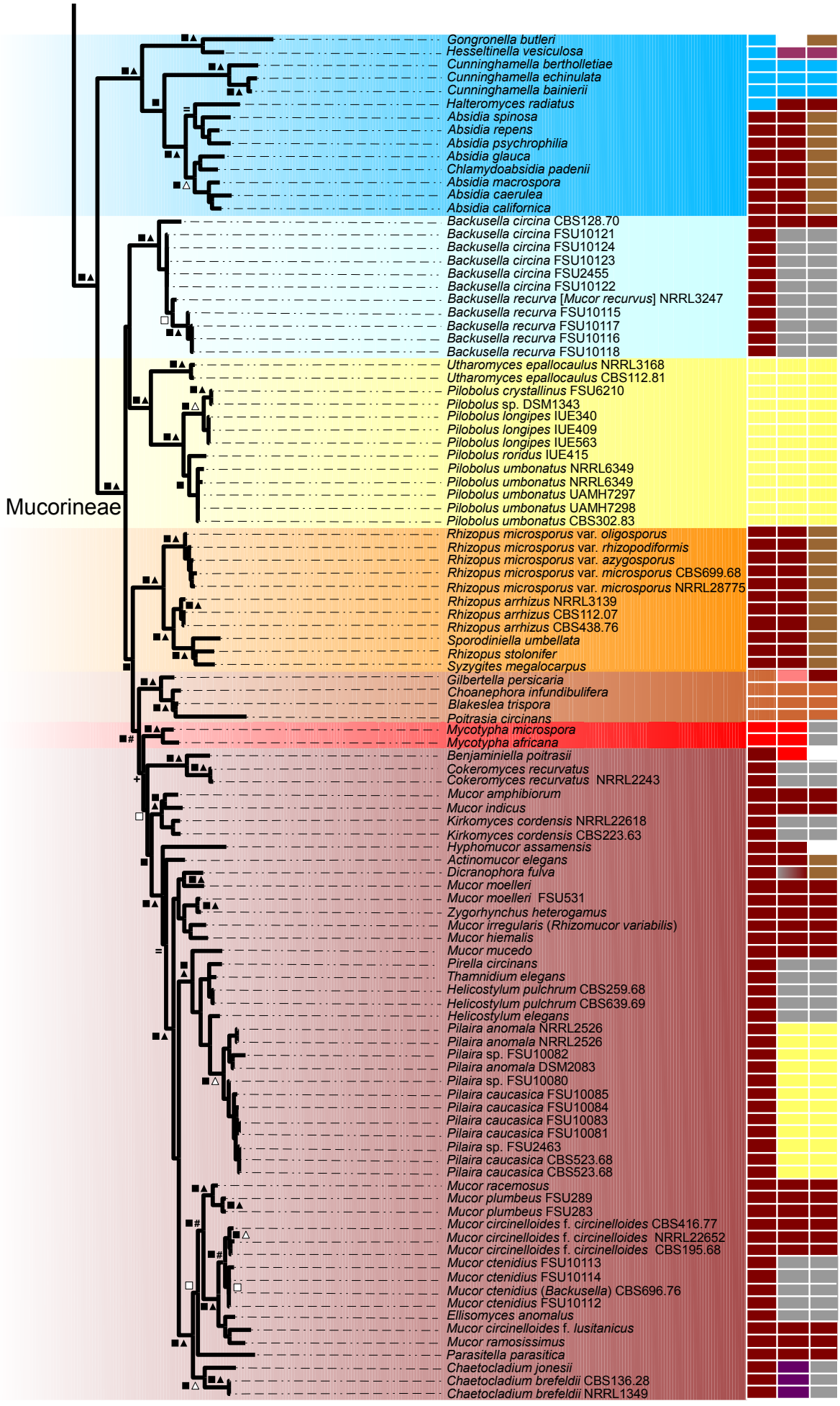

Mucorineae
Gongronella butleri
Hesseltinella vesiculosa
Cunninghamella bertholletiae
Cunninghamella echinulata
Cunninghamella bainierii
Halteromyces radiatus
Absidia spinosa
Absidia repens
Absidia psychrophilia
Absidia glauca
Chlamydoabsidia padenii
Absidia macrospora
Absidia caerulea
Absidia californica
Backusella circina CBS128.70
Backusella circina FSU10121
Backusella circina FSU10124
Backusella circina FSU10123
Backusella circina FSU2455
Backusella circina FSU10122
Backusella recurva [Mucor recurvus] NRRL3247
Backusella recurva FSU10115
Backusella recurva FSU10117
Backusella recurva FSU10116
Backusella recurva FSU10118
Utharomyces epallocaulus NRRL3168
Utharomyces epallocaulus CBS112.81
Pilobolus crystallinus FSU6210
Pilobolus sp. DSM1343
Pilobolus longipes IUE340
Pilobolus longipes IUE409
Pilobolus longipes IUE563
Pilobolus roridus IUE415
Pilobolus umbonatus NRRL6349
Pilobolus umbonatus NRRL6349
Pilobolus umbonatus UAMH7297
Pilobolus umbonatus UAMH7298
Pilobolus umbonatus CBS302.83
Rhizopus microsporus var. oligosporus
Rhizopus microsporus var. rhizopodiformis
Rhizopus microsporus var. azygosporus
Rhizopus microsporus var. microsporus CBS699.68
Rhizopus microsporus var. microsporus NRRL28775
Rhizopus arrhizus NRRL3139
Rhizopus arrhizus CBS112.07
Rhizopus arrhizus CBS438.76
Sporodiniella umbellata
Rhizopus stolonifer
Syzygites megalocarpus
Gilbertella persicaria
Choanephora infundibulifera
Blakeslea trispora
Poitrasia circinans
Mycotypha microspora
Mycotypha africana
Benjaminiella poitrasii
Cokeromyces recurvatus
Cokeromyces recurvatus NRRL2243
Mucor amphibiorum
Mucor indicus
Kirkomyces cordensis NRRL22618
Kirkomyces cordensis CBS223.63
Hyphomucor assamensis
Actinomucor elegans
Dicranophora fulva
Mucor moelleri
Mucor moelleri FSU531
Zygorhynchus heterogamus
Mucor irregularis (Rhizomucor variabilis)
Mucor hiemalis
Mucor mucedo
Pirella circinans
Thamnidium elegans
Helicostylum pulchrum CBS259.68
Helicostylum pulchrum CBS639.69
Helicostylum elegans
Pilaira anomala NRRL2526
Pilaira anomala NRRL2526
Pilaira sp. FSU10082
Pilaira anomala DSM2083
Pilaira sp. FSU10080
Pilaira caucasica FSU10085
Pilaira caucasica FSU10084
Pilaira caucasica FSU10083
Pilaira caucasica FSU10081
Pilaira sp. FSU2463
Pilaira caucasica CBS523.68
Pilaira caucasica CBS523.68
Mucor racemosus
Mucor plumbeus FSU289
Mucor plumbeus FSU283
Mucor circinelloides f. circinelloides CBS416.77
Mucor circinelloides f. circinelloides NRRL22652
Mucor circinelloides f. circinelloides CBS195.68
Mucor ctenidius FSU10113
Mucor ctenidius FSU10114
Mucor ctenidius (Backusella) CBS696.76
Mucor ctenidius FSU10112
Ellisomyces anomalus
Mucor circinelloides f. lusitanicus
Mucor ramosissimus
Parasitella parasitica
Chaetocladium jonesii
Chaetocladium brefeldii CBS136.28
Chaetocladium brefeldii NRRL1349
0.4 substitutions/ site

Fig. 10 (cont.)

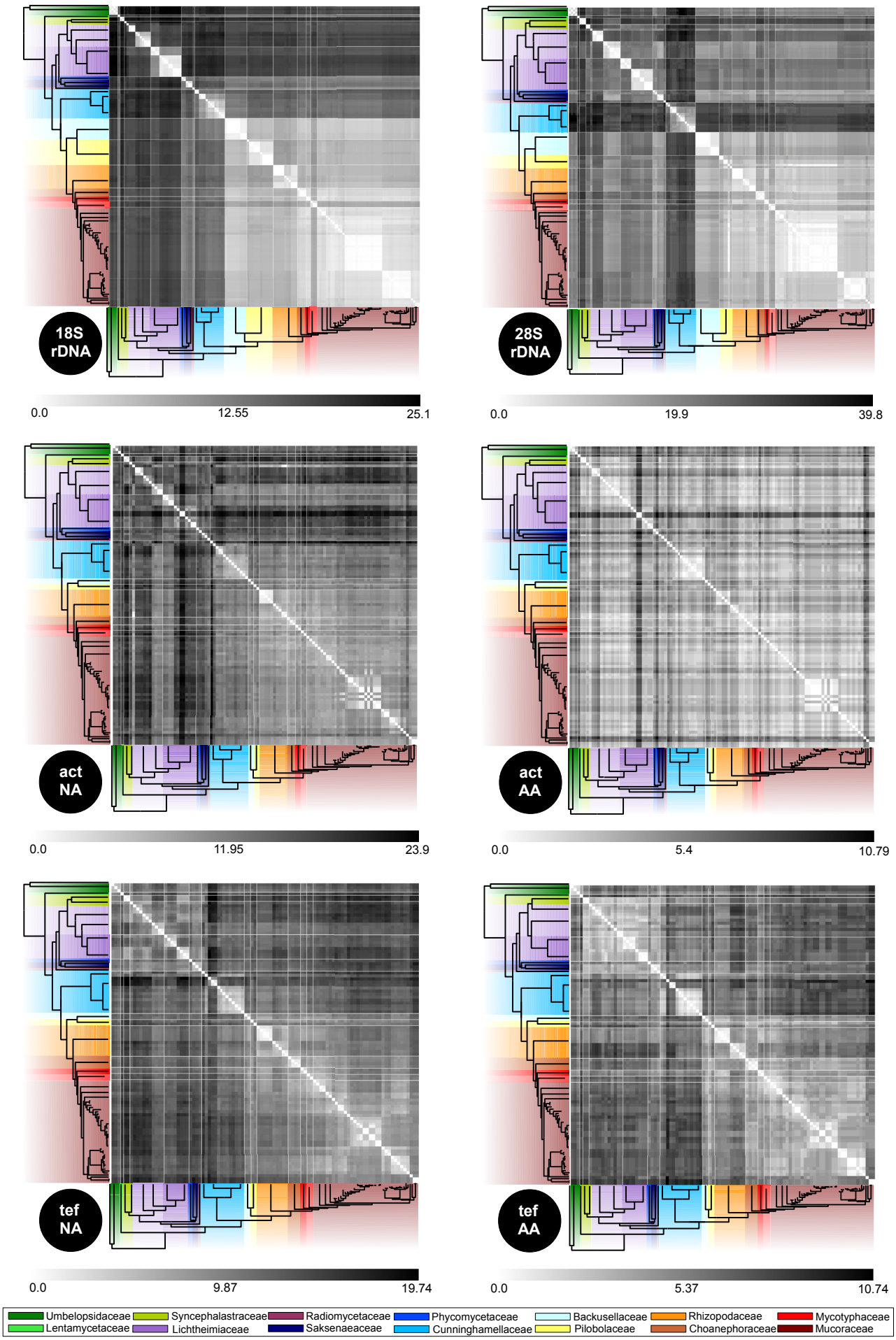


Fig. 11 Distance matrices for all applied loci based on nucleic acid and amino acid sequences. The range of distances is given for each locus. Families are coded according to Fig. 10.

different sequences are combined in an analysis, it is often rDNA and the genomically linked ITS region, but also rDNA combined with protein coding genes (Fig. 8).

The phylogenetic analysis in Fig. 10 consists of combined sequences coding for LSU, SSU, actin and translation elongation factor. At least one member of all accepted genera is included with a total of 201 isolates, 151 belonging to the *Mucorales*, and 103 unique species representing around half of all described species in the order. Species were included if at least two loci were present in the alignment.

A distance matrix was calculated for each locus. The order-wide distance analyses were based solely on the isolates in the illustrated tree (Fig. 10). Species-specific variations for each locus were not considered. The inclusion and analyses of all available sequences from GenBank would constitute a separate research project that goes beyond the scope of this study.

As expected, distance matrices derived from protein coding genes vary less if based on amino acids instead of nucleic acids. Based on the underlying data, amino acid sequences of actin are more conserved within the *Mucorales* with relatively similar distances over the whole order versus the situation for the translation elongation factor. When comparing all distance matrices, three major groups can be distinguished (Fig. 11):

i) Low to moderate distances for the most derived clade of the 'Mucorineae' including the *Mucoraceae*, *Mycotyphaceae*, *Choanephoraceae*, *Pilobolaceae*, *Rhizopodaceae* and *Backusellaceae*. All matrices show the lowest distances for *Mucoraceae* (incl. *Mycotyphaceae*).
   All other groups and clades in the tree show no low distance values to any other group. Shortest distances exist only within each group whereas distances to all other groups are more or less similar. Clades included here are:
ii) the *Cunninghamellaceae*. Within this family, the shortest distances are between the genera *Absidia*, *Halteromyces*, *Chlamydoabsidia* and *Cunninghamella* (except for translation elongation factor, where distances between *Gongronella/Hesseltinella* and *Absidia/Halteromyces/Chlamydoabsidia* are shorter than to the embedded *Cunninghamella*.
iii) *Lichtheimiaceae*/*Syncephalastraceae*/*Lentamycetaceae*/*Umbelopsidiaceae*/*Radiomycetaceae*/*Phycomyceteaceae*. High distance values for the more ancient clades of the phylogenetic tree result from the different evolutionary times of origin which gives the more basal groups more time to evolve separately.

In the following, clades of the phylogenetic tree will be discussed including proposed/necessary changes in nomenclature or family delimitation.

A) Well-established and supported clades:

Aa) *Umbelopsidaceae* W. Gams & W. Mey.

Species of this family were thought to belong most likely to the *Mortierellales*, rather than to the *Mucorales*, mainly because of the highly reduced columella (nearly 'acolumellatae', Fig. 4a) and non-mucoralean colony morphology. The colony mycelium is very dense and velvety as opposed to floccose. And unlike the colonies formed by species of *Mortierella*, those of *Umbelopsis* are reddish, brownish or ochraceous and lack a typical garlic-like odour. This distinction and a relationship to *Mucorales* are surveyed in detail by Meyer & Gams (2003, including a detailed description of the family). With those slight morphological differences compared to all other mucoralean fungi, this group is currently regarded as the most basal in this order. The family is a monogeneric group with a clade support (CS, bootstrap support from the Likelihood analysis and Posterior Probabilities from the Bayesian analysis) of at least 99 %, and a clear distinction from the core *Mucorales* (CS ≥ 99 %) (Fig. 10).

Ab) *Phycomycetaceae* Arx

This clade (CS ≥ 99 %) includes only two genera with different life styles. Species of *Phycomyces* are saprobic, whereas those of *Spinellus* are facultatively parasitic on the basidioma of *Agaricomycotina* (Fig. 2a). Species of *Phycomyces* are model organisms for studies of phototropism and geotropism as well as carotenoide synthesis, carotenoide degradation and zygosporogenesis.

Ac) *Pilobolaceae* Corda

The *Pilobolaceae* is one of the few families recognized from the pre-genomics era with one taxonomic change. The genus *Pilaira* (Fig. 5e, f), thought to be a member of the family due to morphological characteristics, was placed in the *Mucoraceae* and is related most closely to *Helicostylum*, *Thamnidium*, *Pirella* and *Mucor mucedo*.

Main characteristics of this family are the formation of trophocysts (Fig. 5a), the mode of spore release and the growth on dung of herbivores and rodents (Fig. 5c, d). Both included genera possess a vesicle/swelling below the sporangium, which functions in *Pilobolus* during active discharge of the sporangium (Page 1964, Zycha et al. 1969). In *Utharomyces* (Fig. 5b) spores are passively released. *Pilobolus* is especially difficult to cultivate on artificial media over several generations, resulting in changes in morphology and eventually in death of the culture. Based on analyses of molecular data, only the size and shape of the sporangiospores is retained as of relevance in species delimitation since this feature is the only one that correlates with molecular phylogenies (Foos et al. 2011).

Ad) *Choanephoraceae* J. Schröt.

This clade (CS ≥ 99 %) includes species producing only sporangia (*Poitrasia*, *Gilbertella*; Fig. 3d, e), or also sporangiola (*Choanephora*, *Blakeslea*; Fig. 3f). Sporangia and sporangiola are produced on separate sporangiophores. The wall of the sporangium is persistent. At maturity the wall ruptures at preformed sutures to release sporangiospores with hyaline, hair-like polar appendages representing a synapomorphy of this family. The species are saprobes or fruit and vegetable inhabiting parasites, sometimes occurring as major post-harvest pathogens in tropical and subtropical regions (Fig. 2c). The newly introduced subfamilies *Gilbertelloideae* (MycoBank IF550022) and *Choanephoroideae* (MycoBank IF550021) are distinguished by the characters of the zygospore, e.g. suspensors opposed or apposed, zygosporangium ornamented or smooth (Voigt 2012).

Ae) *Cunninghamellaceae* Naumov ex R.K. Benj.

Although this clade is highly supported (CS ≥ 99 %), it is one family that should be studied in more detail. While two recent studies dealt with the genus *Cunninghamella* and incorporated the largest number of isolates studied so far, the sister genera lack such a profound study. The authors evaluated all available information ranging from morphology to growth temperatures, mating experiments and molecular data (Liu et al. 2001, Zheng & Chen 2001). Currently, only *Absidia* and *Cunninghamella* are well sampled; *Gongronella*, and especially *Hesseltinella*, *Halteromyces* and *Chlamydoabsidia*, definitely need more isolates to study. Since *Chlamydoabsidia* is always nested within *Absidia*, its status as a distinct genus should be evaluated; this might also be extended to *Halteromyces*. The distances between sequences are very high in this family representing one of the highest variabilities when compared to other clades (Fig. 11).

Af) *Lentamycetaceae* K. Voigt & P.M. Kirk — MycoBank IF550009

Since the first analyses including species of the genus *Lentamyces* (formerly *Absidia*) it was obvious, that these species should be separated. And since there are no other species of the *Mucorales* closely related to this genus, a separate family is introduced (Voigt 2012). Species of the *Lentamycetaceae* (Fig. 4b, c) are homothallic and mycoparasitic, although the mycoparasitic potential of *L. zychae* was lost during cultivation (Zycha et al. 1969). Kwaśna & Nirenberg (2008a, b) introduced the genus *Siepmannia* that included the two *Lentamyces* species besides the new species *S. pineti* and *S. lariceti*. A correct classification of these taxa is still unclear because only ITS sequences and no living material are available from *S. pineti* and *S. lariceti*. A resampling of strains of *Siepmannia* is necessary to perform multilocus studies and to determine their mycoparasitic potential.

Ag) *Backusellaceae* K. Voigt & P.M. Kirk — MycoBank IF550011

Species included here originally were placed in the *Mucoraceae* or *Thamnidiaceae*. Like other described families once included in the *Mucoraceae* (e.g. *Pilobolaceae*, *Choanephoraceae*), this clade should also be distinguished from the *Mucoraceae*. The monogeneric *Backusellaceae* are characterised by transitorily recurved sporangiophores and the tendency to produce sporangiola in addition to the sporangia. Several *Mucor* species owning these characters were transferred to *Backusella*. Clade support for the *Backusellaceae* is ≥ 99 % (Fig. 10) and it contains three species: *Backusella lamprospora*, *B. circina*, *B. recurva.* The members of the *Backusellaceae* seem to be saprotrophs found e.g. in soil, on wood and fallen leaves (Walther et al. 2013).

Ah) *Rhizopodaceae* K. Voigt & P.M. Kirk — MycoBank IF550010

Like the *Backusellaceae*, the *Rhizopodaceae* forms a well-supported clade, distinct from the *Mucoraceae* (CS ≥ 99 %). Within this clade, a trifurcation is observed (each with a CS ≥ 99 %), with one *Rhizopus microsporus*-clade containing predominantly thermotolerant fungi (growth up to 45 °C), a sub-thermotolerant *R. arrhizus*-group (37–40 °C) and a mesophilic group containing *R. stolonifer*, *Sporodiniella*, and *Syzygites*. This was already observed applying morphology and growth temperatures (Schipper & Stalpers 1984), establishing a classification accepted as standard for many decades. The application of molecular data and biochemistrical features (e.g. production of lactic acids) supported those three major clades, but revealed also new/cryptic species (Abe et al. 2006, 2007). The implementation of GCPSR, including different genetic markers, resulted in the publication of a new, reliable *Rhizopus* classification (Abe et al. 2010). Yet, the final clustering in the *Rhizopodaceae* (Fig. 10) remains unresolved, because some species (*R. caespitosus*, *R. homothallicus*, *R. lyococcus*, *R. schipperae*, *R. sexualis*) were not included because of missing data. But the thermotolerant species *R. caespitosus*, *R. homothallicus* and *R. schipperae*, seem to be closely related to the *R. microsporus* clade (rDNA analysis, Abe et al. 2006). In this study, *R. sexualis* (mesophilic) is related to *R. stolonifer* and *R. lyococcus* (mesophilic) appears as a very basal species (Abe et al. 2006). All species of the *Rhizopodaceae* are reported to be pathogenic to other organisms. Whereas *Syzygites* is a parasite of *Dikarya* (Kovacs & Sundberg 1999), *Sporodiniella* is a parasite of insect larvae (Evans & Samson 1977, Chien & Huang 1997), and species of *Rhizopus* are pathogens of plants and opportunists of animals, including humans.

Ai) *Radiomycetaceae* Hesselt. & J.J. Ellis & ***Saksenaeaceae*** Hesselt. & J.J. Ellis

The *Radiomycetaceae* contains only one genus with three species (Benny & Benjamin 1991). *Radiomyces* is coprophilous and pathogenic to mice (experimental infections, Kitz et al. 1980). The unispored or multispored sporangia are produced on pedicels, which originate from a vesicle. The *Saksenaeaceae* contain two genera, *Saksenaea* and *Apophysomyces* are saprobic in soil and compost. Some species are also known to infect animals and humans (Álvarez et al. 2010a, b).

B) Moderately supported clades:

Ba) *Mycotyphaceae* Benny & R.K. Benj.

The *Mycotyphaceae* currently contains only one genus (Benny & Benjamin 1976). Although the inclusion of adjacent species is proposed (Voigt 2012), the results of molecular phylogenetics are still controversial (Fig. 10). Furthermore, CS is ≥ 99 % for *Mycotyphaceae*, but strong support for the separation from *Mucoraceae* is only given for Bayesian analysis (CS ≥ 90 %). Although molecular distances (Fig. 11) of *Mycotypha* are similar to those of the *Mucoraceae*, the *Mycotyphaceae* is maintained as the sister family to *Mucoraceae* also because of the exceptional sporangiophores bearing terminal, elongate, cylindrical vesicles (Fig. 4k). The unispored sporangiola are of two types, an inner layer that consists of globose spores and an outer layer of spores that are either obovoid or more or less cylindrical.

Bb) *Lichtheimiaceae* Kerst. Hoffm., G. Walther & K. Voigt & ***Syncephalastraceae*** Naumov ex R.K. Benj.

Species of the genus *Absidia* growing well at elevated temperatures were transferred to the genus *Lichtheimia* based on both molecular and physiological data (Hoffmann et al. 2007, 2009). *Lichtheimia* has appeared as a well-supported sister taxon to *Dichotomocladium* in many phylogenetic analysis (e.g. O'Donnell et al. 2001, White et al. 2006) requiring an emendation of the *Lichtheimiaceae*. *Dichotomocladium* has been included in the *Chaetocladiaceae* (Benny & Benjamin 1993) based on morphological structures such as sterile spines, unispored sporangiola and branched, tree-like sporangiophores (Fig. 4d–f, l, m). Molecular data, however, revealed that these morphological features are of no phylogenetic significance. A shared feature of *Lichtheimia* and *Dichotomocladium* is their tolerance of higher temperatures. Species of *Lichtheimia* are consistently able to grow at and above 37 °C (Hoffmann et al. 2007), the species of *Dichotomocladium* tolerate 35 °C and some species, namely *D. hesseltinei*, *D. floridanum* and *D. robustum* are even able to grow at 37 °C (unpubl. data). The subfamilies *Lichtheimioideae* (MycoBank IF550086) and *Dichotomocladioideae* (MycoBank IF550087) are proposed for the *Lichtheimiaceae* (Voigt 2012). Based on a smaller set of sequences a third subclade within the *Lichtheimiaceae* was suggested: namely the *Rhizomucoroideae* (MycoBank IF550085) (Voigt 2012) but this classification could not be verified in this study.

Syncephalastrum (*Syncephalastraceae*) is the only genus in the *Mucorales* producing sporangiola with the spores arranged in a linear series (merosporangia, Fig. 4g). Whether other genera (e.g. *Protomycocladus*) should be included in this family needs to be studied in more detail because of the low phylogenetic branch support (Fig. 10), leaving *Syncephalastrum* the only genus in this family. The final position of *Protomycocladus* could not be resolved unquestionable due to low branch support in this study as well as other publications (e.g. O'Donnell et al. 2001, Voigt & Wöstemeyer 2001, White et al. 2006, Walther et al. 2013).

	FJ345356 *R. miehei*	AF205941 *R. miehei*	AJ278360 *R. miehei*	FJ713069 *R. miehei*	FJ548824 *R. miehei*	JF412011 *R. miehei*	FJ713075 *R. miehei*	GU256738 *R. pusillus*	EU293849 *R. tauricus*	DQ119000 *R. pusillus*	DQ118999 *R. pusillus*	EF540757 *R. pusillus*	AY211270 *R. pusillus*	AF461764 *R. pusillus*	DQ119004 *R. pusillus*	DQ119003 *R. pusillus*	DQ119002 *R. pusillus*	DQ119001 *R. pusillus*	AJ853748 *R. pusillus*	DQ119005 *R. pusillus*
FJ345356	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
AF205941	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
AJ278360	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
FJ713069	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
FJ548824	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
JF412011	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
FJ713075	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
GU256738	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
EU293849	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ119000	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ118999	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
EF540757	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
AY211270	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
AF461764	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ119004	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ119003	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ119002	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ119001	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
AJ853748	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DQ119005	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 12 CBC analyses of ITS2 sequences from the genus *Rhizomucor*. Numbers of detected CBCs are given.

	GQ342879 *L. corymbifera* CBS100.31	GQ342888 *L. corymbifera* CBS102.48	GQ342885 *L. corymbifera* CBS100.17	GQ342878 *L. corymbifera* CBS429.75	GQ342887 *L. corymbifera* CBS115811	GQ342886 *L. corymbifera* CBS100.51	GQ342851 *L. ramosa* CBS100.55	GQ342848 *L. ramosa* CBS124198	GQ342874 *L. ramosa* CBS582.65	GQ342858 *L. ramosa* CBS100.49	GQ342877 *L. ramosa* CBS223.78	GQ342896 *L. hyalospora* CBS100.28	GQ342900 *L. sphaerocystis* CBS420.7	GQ342891 *L. ornata* CBS291.66	GQ342890 *L. ornata* CBS958.68
GQ342879	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GQ342888	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GQ342885	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GQ342878	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GQ342887	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GQ342886	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GQ342851	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
GQ342848	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
GQ342874	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
GQ342858	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
GQ342877	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
GQ342896	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
GQ342900	1	1	1	1	1	1	1	1	1	1	1	1	0	2	2
GQ342891	0	0	0	0	0	0	2	2	2	2	2	1	2	0	0
GQ342890	0	0	0	0	0	0	2	2	2	2	2	1	2	0	0

Fig. 13 CBC analyses of ITS2 sequences from the genus *Lichtheimia*. Numbers of detected CBCs are given.

Fig. 14 CBC analyses of ITS2 sequences from the genus *Dichotomocladium*. Numbers of detected CBCs are given.

	JX644554 *D. sphaerosporum* FSU8698	JQ775416 *D. sphaerosporum* FSU8696	JQ775414 *D. sphaerosporum* FSU8697	JQ775411 *D. hesseltinei* CBS164.61	JQ775412 *D. robustum* CBS440.76	JQ775413 *D. robustum* CBS439.76	JQ775409 *D. floridanum* FSU8694	JQ775410 *D. elegans* CBS714.74
JX644554	0	0	0	0	2	2	1	1
JQ775416	0	0	0	0	2	2	1	1
JQ775414	0	0	0	0	2	2	1	1
JQ775411	0	0	0	0	1	1	1	1
JQ775412	2	2	2	1	0	0	0	1
JQ775413	2	2	2	1	0	0	0	1
JQ775409	1	1	1	1	0	0	0	1
JQ775410	1	1	1	1	1	1	1	0

Closely related to the families, *Lichtheimiaceae* and the *Syncephalastraceae*, are three additional clades: i) *Protomycocladus faisalabadensis*; ii) *Rhizomucor/Thermomucor*; iii) *Fennellomyces/Circinella/Thamnostylum/Zychaea/Phascolomyces*. Clades i) and ii) include thermotolerant species with growth temperature maxima at 45 °C for *Protomycocladus* (Schipper & Samson 1994), and thermophilic species with growth temperature maxima at 55–57 °C for *Rhizomucor* (de Hoog et al. 2000) or above 60 °C for *Thermomucor* (Subrahamanyam et al. 1977). Clade iii) contains species that are predominantly mesophilic, not growing at elevated temperatures. Furthermore, this clade is characterised by circinate (strong or less pronounced) elements in the sporangiophores (Fig. 4p, q).

For a reliable placement of clades i–iii, in relation to the *Lichtheimiaceae* and *Syncephalastraceae*, additional data are needed, since the relationships of the former clades are not significantly supported in any published analyses. Therefore, these clades gain the status *incertae sedis* till their relationships could be solved unambiguously.

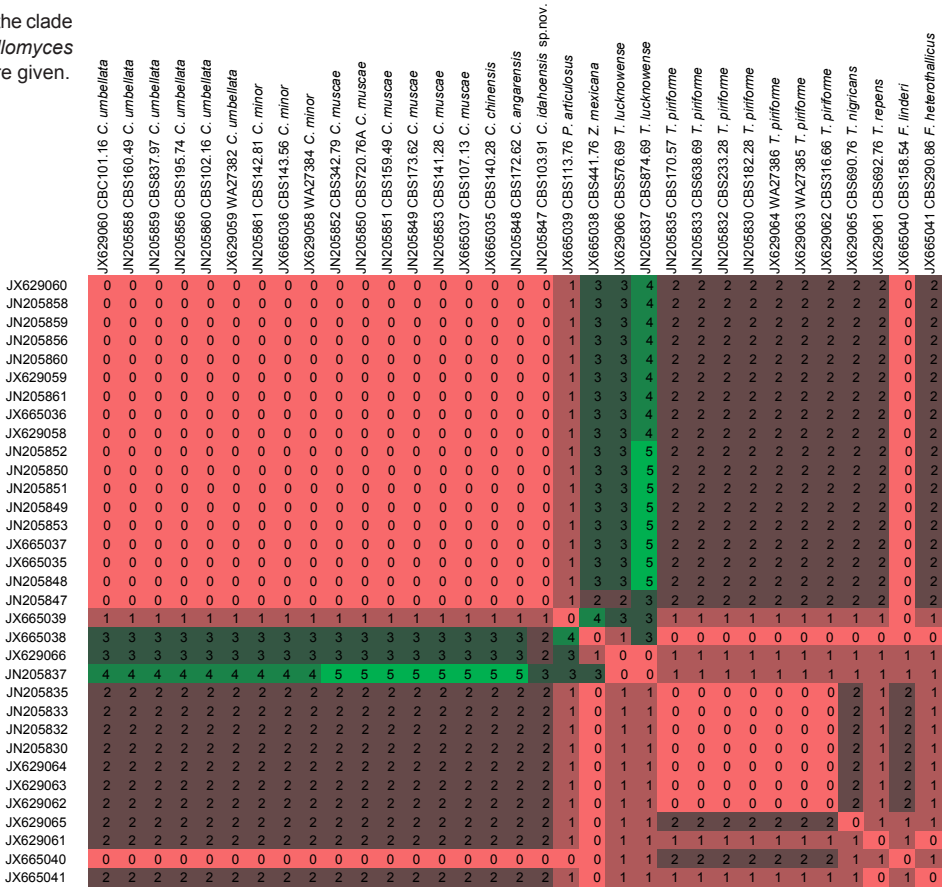
In order to test the taxonomic stability in the newly delimitated *Lichtheimiaceae*, ITS2 sequences of all isolates were searched for compensatory base changes (CBC) as indicators for species boundaries. A comprehensive study on CBC suggests that with a reliability of 93.11 % one CBC is present in two specimens belonging to two different species. But the lack of CBCs does not indicate that two specimens do belong to the same species (Müller et al. 2007). Applying CBC analyses to several clades within the *Lichtheimiaceae*/*Syncephalastraceae*, CBC is widely concordant with species concepts in *Rhizomucor* (Fig. 12), *Lichtheimia* (Fig. 13, except *L. corymbifera* and *L. ornata*), *Dichotomocladium* (Fig. 14), *Zychaea* and *Thamnostylum* (Fig. 15).

There are few species in the analyses which could not be clearly separated from others, which is due to the lack of CBCs (e.g. *Dichotomolcadium hesseltinei* and *D. floridanum*, *Fennellomyces heterothallicus*, *Thamnostylum repens*). However, no CBCs at all were detected in the genera *Syncephalastrum* and *Circinella* showing that CBC analyses cannot be used generally as a tool for species recognition in *Mucorales*. CBC analyses between different genera remains difficult if not impossible (especially in the ancient clades of the *Mucorales*) due to highly diverse ITS2 sequences and thus secondary structure. If differing too much, no comparison of the secondary structure is possible, which results in no detectable CBCs. CBC analyses are in parts suitable for distinguishing species that are highly similar in their morphology (e.g. *Lichtheimia ramosa* and *L. corymbifera*) and could assist in supporting molecular phylogenies.

Bc) *Mucoraceae* Dumort.

The *Mucoraceae* is undoubtly the largest family and presumably the most derived in the *Mucorales* (Fig. 10). Traditionally all species lacking features for classification within any other family where assigned to the *Mucoraceae* making the family polyphyletic. This study has circumscribed a monophyletic *Mucoraceae* with highly diverse features that characterise different species and genera. All species are saprobes except *Dicranophora*, *Parasitella* and *Chaetocladium* which are facultative mycoparasites (*Dicranophora* on *Agaricomycetes*, *Parasitella* and *Chaetocladium* on *Mucorales*). A few species are also described as opportunistic pathogens causing deep and systemic mycoses. Species are either homothallic or heterothallic, the zygospores form a warty to smooth zygosporangial wall with naked (without appendages) opposed suspensors. Sporangia are borne on branched or unbranched, sometimes phototrophic sporangiophores, sporangiola are rare and the sporangia are ± lageniform, ± apophysate and columellate.

Fig. 15 CBC analyses of ITS2 sequences from the clade *Circinella / Phascolomyces / Zychaea / Fennellomyces / Thamnostylum*. Numbers of detected CBCs are given.



SUMMARY AND CONCLUDING REMARKS

Traditional classification in *Mucorales* was done, as in all *Eumycetes*, mainly by using morphological characters. Already eleven years ago large deficiencies in the morphology-based system were revealed by molecular data. The distinctly extended dataset of the current study gives now a clearer picture of the family structure in the *Mucorales*. Our phylogeny based on four markers and contains 14 clades that we interpret as families: 1) *Umbelopsidaceae*; 2) the newly erected monogeneric *Lentamyetaceae*; 3) *Syncephalastraceae* presumably including *Protomycocladus*; 4) *Lichtheimiaceae* containing *Lichtheimia* and *Dichotomocladium*; 5) *Phycomycetaceae*; 6) *Saksenaeaceae*; 7) *Radiomycetaceae*; 8) *Cunninghamellaceae* inclusively *Absidia* s.str.; 9) the newly erected monogeneric *Backusellaceae*; 10) *Pilobolaceae*; 11) the newly erected *Rhizopodaceae* including the genera *Rhizopus*, *Sporodiniella* and *Syzygites*; 12) *Choanephoraceae*; 13) *Mycotyphaceae*; and 14) *Mucoraceae*. Most of these family clades were well supported. Only the delimitation between the *Mucoraceae* and the *Mycotyphaceae* as well as the *Lichtheimiaceae* and the *Syncephalastraceae* could not be defined doubtlessly, few subclades are classified as *incertae sedis*. The *Mucoraceae*, *Mycotyphaceae* and *Cunninghamellaceae* involve several taxonomic deficiencies and a detailed study of the phylogenetic relationships in these families is needed.

Acknowledgements KH and KV thank Dr. H. Vogel and D. Schnabelrauch from the MPI for Chemical Ecology Jena, Germany for their support in sequencing. Financial support was partially provided by the Polish Ministry of Science and Higher Education (MNiSW), grant no. NN303_548839 to JP and MW. We wish to thank the reviewers for critically reviewing and valuable comments on the manuscript.

REFERENCES

Abe A, Asano K, Sone T. 2010. A molecular phylogeny-based taxonomy of the genus Rhizopus. Bioscience, Biotechnology and Biochemistry 74: 1325–1331.

Abe A, Oda Y, Asano K, Sone T. 2006. The molecular phylogeny of the genus Rhizopus based on rDNA sequences. Bioscience, Biotechnology and Biochemistry 70: 2387–2393.

Abe A, Oda Y, Asano K, Sone T. 2007. Rhizopus delemar is the proper name for Rhizopus oryzae fumaric-malic acid producers. Mycologia 99: 714–722.

Alastruey-Izquierdo A, Hoffmann K, Hoog GS de, Rodriquez-Tudela JL, Voigt K, et al. 2010. Species recognition and clinical relevance of the zygomycetous genus Lichtheimia syn. Absidia pro parte, Mycocladus. Journal of Clinical Microbiology 48: 2154–2170.

Almeida ER, Cerdá-Olmedo E. 2008. Gene expression in the regulation of carotene biosynthesis in Phycomyces. Current Genetics 53: 129–137.

Álvarez E, Garcia-Hermoso D, Sutton DA, Cano JF, Stchigel AM, et al. 2010a. Molecular phylogeny and proposal of two new species of the emerging pathogenic fungus Saksenaea. Journal of Clinical Microbiology 48: 4410–4416.

Álvarez E, Stchigel AM, Cano J, Sutton DA, Fothergill AW, et al. 2010b. Molecular phylogenetic diversity of the emerging mucoralean fungus Apophysomyces: Proposal of three new species. Revista Iberoamericana de Micología 27: 80–89.

Arx JA von. 1982 '1984'. On Mucoraceae s.str. and other families of the Mucorales. Sydowia 35: 10–26.

Bartnicki-Garcia S, Nickerson WJ. 1962. Induction of yeast-like development in Mucor by carbon dioxide. Journal of Bacteriology 84: 829–840.

Benjamin RK. 1979. Zygomycetes and their spores. In: Kendrick B (ed), The whole fungus: the sexual-asexual synthesis. Vol. 2: 573–616. National Museums of Canada, Ottawa, Canada.

Benny GL. 1982. Zygomycetes. In: Parker SP (ed), Synopsis and classification of living organisms. Vol. I: 184–195. McGraw-Hill Book Company, Inc., New York.

Benny GL. 1995. Classical morphology in zygomycete taxonomy. Canadian Journal of Botany 73 Suppl. 1: 725–730.

Benny GL. 2012. Current systematics of the zygomycotan fungi with a brief discussion of their biology. In: Misra JK, Tewari JP, Deshmukh SK (eds), Systematics and evolution of fungi. Progress in mycological research: 55–105. Science Publishers, Enfield, New Hampshire, USA.

Benny GL, Benjamin RK. 1976. Observations on Thamnidiaceae (Mucorales). II. Chaetocladium, Cokeromyces, Mycotypha, and Phascolomyces. Aliso 8: 391–424.
Benny GL, Benjamin RK. 1991 '1992'. The Radiomycetaceae (Mycorales; Zygomycetes). III. A new species of Radiomyces, and cladistic analysis and taxonomy of the family; with a discussion of the evolutionary ordinal relationships in Zygomycotina. Mycologia 83: 713–735.
Benny GL, Benjamin RK. 1993. Observations on Thamnidiaceae (Mucorales). VI. Two new species of Dichotomocladium and the zygospores of D. hesseltinei (Chaetocladiaceae). Mycologia 85: 660–671.
Benny GL, Humber RA, Morton JB. 2001. Zygomycota: Zygomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds), The mycota. Vol. VIIA. Systematics and evolution: 113–146. Springer-Verlag, Berlin, Germany.
Berbee ML, Taylor JW. 2001. Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds), The mycota. Vol. VII. Part B. Systematics and evolution: 229–245. Springer Verlag, Berlin, Germany.
Blakeslee AF. 1904. Sexual reproduction in the Mucorineae. Proceedings of the National Academy of Arts and Sciences 40: 205–319.
Blakeslee AF, Cartledge JL. 1927. Sexual dimorphism in Mucorales II. Interspecific reactions. Botanical Gazette 84: 51–57.
Burgeff H. 1924. Untersuchungen über Sexualität und Parasitismus bei Mucorineen I. Botanische Abhandlungen 4: 1–135.
Caglioti L, Cainelli G, Camerino B, Mondelli R, Prieto A, et al. 1966. The structure of trisporic-C acid. Tetrahedron 22, Suppl. 7: 175–187.
Chien C-Y, Huang B-C. 1997. First record of the occurrence of Sporodiniella umbellata Mucorales in Taiwan. Mycoscience 38: 343–346.
Czempinski K, Kruft V, Wöstemeyer J, Burmester A. 1996. 4-Dihydromethyltrisporate dehydrogenase from Mucor mucedo, an enzyme of the sexual hormone pathway: purification, and cloning of the corresponding gene. Microbiology 142: 2647–2654.
Dennis C. 1983. Soft fruits. In: Dennis C (ed), Post-harvest pathology of fruits and vegetables: 23–42. Academic Press, London.
Ende H van den. 1967. Sexual factor of the Mucorales. Nature 215: 211–212.
Evans HC, Samson RA. 1977. Sporodiniella umbellata, an entomogenous fungus of the Mucorales from cocoa farms in Ecuador. Canadian Journal of Botany 55: 2981–2984.
Fitzpatrick DA, Logue ME, Stajich JE, Butler G. 2006. A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evolutionary Biology 6: 99.
Foos KM, May NL, Beach DL, Pomper M, Sheehan KB, Ruch DG. 2011. Phylogeny of Pilobolaceae. Mycologia 103: 36–44.
Gładkowski W, Grabarczyk M, Konopka M, Wawrzeńczyk C. 2004. Lactones 20. Biohydroxylation of saturated bicyclic gamma-lactones with the substituted cyclohexane system. Journal of Molecular Catalysis B: Enzymatic 29: 13–7.
Gładkowski W, Mazur M, Białońska A, Wawrzeńczyk C. 2011. Lactones 35 [1]. Metabolism of iodolactones with cyclohexane ring in Absidia cylindrospora culture. Enzyme and Microbial Technology 48: 326–333.
Gooday GW. 1968. Hormonal control of sexual reproduction in Mucor mucedo. New Phytologist 67: 815–821.
Gryganskyi AP, Lee SC, Litvintseva AP, Smith ME, Bonito G, et al. 2010. Structure, function, and phylogeny of the mating locus in the Rhizopus oryzae complex. PloS ONE 5: e15273.
Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth and Bisby's dictionary of the fungi, 8th ed. International Mycological Institute, Egham, UK.
Henkel TW. 2004. Manufacturing procedures and microbiological aspects of Parakari, a novel fermented beverage of the Wapisiana Amerindians of Guyana. Economic Botany 58: 25–37.
Henkel TW. 2005. Parakari, an indigenous fermented beverage using amylolytic Rhizopus in Guyana. Mycologia 97: 1–11.
Hermet A, Méheust D, Mounier J, Barbier G, Jany JL. 2012. Molecular systematics in the genus Mucor with special regards to species encountered in cheese. Fungal Biology 116: 692–705.
Hesseltine CW. 1983. Microbiology of oriental fermented food. Annual Review of Microbiology 37: 575–601.
Hesseltine CW. 1991. Zygomycetes in food fermentations. Mycologist 5: 162–169.
Hesseltine CW, Ellis JJ. 1973. Mucorales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds), The fungi. Vol. 4b: 187–217. Academic Press, New York.
Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, et al. 2007. A higher-level phylogenetic classification of the Fungi. Mycological Research 111: 509–547.
Hibbett DS, Fukumasa-Nakai Y, Tsuneda A, Donoghue MJ. 1995. Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences. Mycologia 87: 618–638.
Hoffmann K, Discher S, Voigt K. 2007. Revision of the genus Absidia Mucorales, Zygomycetes based on physiological, phylogenetic and morphological characters: Thermotolerant Absidia spp. form a coherent group, Mycocladiaceae fam. nov. Mycological Research 111: 1169–1183.
Hoffmann K, Voigt K. 2009. Absidia parricida plays a predominant role in biotrophic fusion parasitism among mucoralean fungi Zygomycetes: reclassification as Lentamyces parricida gen. nov., comb. nov. Plant Biology 11: 537–554.
Hoffmann K, Voigt K, Kirk PM. 2011. Mortierellomycotina subphyl. nov., based on multi-gene genealogies. Mycotaxon 115: 353–363.
Hoffmann K, Walther G, Voigt K. 2009. Mycocladus vs. Lichtheimia: a correction Lichtheimiaceae fam. nov., Mucorales, Mucoromycotina. Mycological Research 113: 277–278.
Hoog GS de, Guarro J, Gené J, Figueras MJ. 2000. Atlas of clinical fungi, 2nd ed. Utrecht, Reus.
Huerta-Cepas J, Bueno A, Dopazo J, Gabaldón T. 2008. PhylomeDB: a database for genome-wide collections of gene phylogenies. Nucleic Acids Research (database issue): D491-6.
Humber RA. 2012. Entomophthoromycota: a new phylum and reclassification of entomophthoroid fungi. Mycotaxon 120: 477–492.
Idnurm A. 2011. Sex determination in the first-described sexual fungus. Eukaryotic Cell 10: 1485–1491.
Idnurm A, Walton FJ, Floyd A, Heitman J. 2008. Identification of the sex genes in an early diverged fungus. Nature 451: 193–196.
James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, et al. 2006. Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443: 818–822.
Jones MD, Forn I, Gadelha C, Egan MJ, Bass D, et al. 2011a. Discovery of novel intermediate forms redefines the fungal tree of life. Nature 4747350: 200-3.
Jones MD, Richards TA, Hawksworth DL, Bass D. 2011b. Validation and justification of the phylum name Cryptomycota phyl. nov. IMA Fungus 2: 173–175.
Kar R, Mandaokar BD, Kar RK. 2010. Fungal taxa from the Miocene sediments of Mizoram, northeast India. Review of Palaeobotany and Palynology 158: 240–249.
Kellner M, Burmester A, Wöstemeyer A, Wöstemeyer J. 1993. Transfer of genetic information from the mycoparasite Parasitella parasitica to its host Absidia glauca. Current Genetics 23: 334–337.
Kirk PM. 2012. Nomenclatural novelties. Index Fungorum 11: 1.
Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby's Dictionary of the fungi. 10th edn. CAB International, Wallingford, UK.
Kirk PM, Voigt K. 2012. Nomenclatural novelties. Index Fungorum 13: 1.
Kitz DJ, Embree RW, Cazin Jr J. 1980. Radiomyces a genus in the Mucorales pathogenic for mice. Sabouaudia 18: 115–121.
Kovacs RL, Sundberg WJ. 1999. Syzygites megalocarpus Mucorales, Zygomycetes in Illinois. Transactions of the Illinois Academy of Science 92: 181–190.
Krings M, Taylor TN, Dotzler N, Persichini G. 2012. Fossil fungi with suggested affinities to the Endogonaceae from the Middle Triassic of Antarctia. Mycologia 104: 835–844.
Kuramae EE, Robert V, Snel B, Weib M, Boekhout T. 2006. Phylogenomics reveal a robust fungal tree of life. FEMS Yeast Research 6: 1213–1220.
Kwaśna H, Nirenberg HI. 2008a. Siepmannia, a new genus in the Mucoraceae. Mycologia 100: 259–274.
Kwaśna H, Nirenberg HI. 2008b. Validation of the genus Siepmannia Mucoraceae and its four species. Polish Botanical Journal 53: 187–188.
Lee SC, Ni M, Li W, Shertz C, Heitman J. 2010. The evolution of sex: a perspective from the fungal kingdom. Microbiology and Molecular Biology Reviews 74: 298–340.
Liu XY, Huang H, Zheng RY. 2001. Relationships within Cunninghamella based on sequence analysis of ITS rDNA. Mycotaxon 80: 77–95.
Martin WJ. 1964. Effectiveness of fungicides in reducing soft rot in washed, cured sweet potatoes. Plant Disease Reporter 48: 606–607.
Matheny PB, Liu YJJ, Ammirati JF, Hall BD. 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms Inocybe, Agaricales. American Journal of Botany 89: 688–698.
Mayden RL. 1997. A hierarchy of species concepts: The denouement in the saga of the species problem. In: Claridge MF, Dawah HA, Wilson MR (eds), Species: The units of biodiversity: 381–424. Chapman & Hall, London.
Meyer W, Gams W. 2003. Delimitation of Umbelopsis (Mucorales, Umbelopsidaceae fam. nov.) based on ITS sequence and RFLP data. Mycological Research 107: 339–350.
Michailides TJ, Spotts RA. 1988. Germination of zygospores of Mucor piriformis on the life history of Mucor piriformis. Mycologia 80: 837–844.
Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010: 1–8, New Orleans, LA.

Miller ML, Sutter RP. 1984. Methyltrisporate E. A sex pheromone in Phycomyces blakesleeanus. The Journal of Biological Chemistry 259: 6420–6422.
Müller T, Philippi N, Dandekar T, Schultz J, Wolf M. 2007. Distinguishing species. RNA 13: 1469–1472.
Nout MJR, Kiers JL. 2005. Tempe fermentation, innovation and functionality: update into the third millennium. Journal of Applied Microbiology 98: 789–805.
O'Donnell K. 1993. Fusarium and its near relatives. In: Reynolds DR, Taylor JW (eds), The fungal holomorph: Mitotic, Meiotic and Pleomorphic speciation in fungal systematics: 225–233. CAB International, Wallingford, UK.
O'Donnell K, Lutzoni FM, Ward TJ, Benny GL. 2001. Evolutionary relationships among mucoralean fungi Zygomycota: Evidence for family polyphyly on a large scale. Mycologia 932: 286–296.
Page RM. 1964. Sporangium discharge in Pilobolus: a photographic study. Science 146: 925–927.
Paterson RRM, Bridge PD. 1994. Biochemical techniques for filamentous fungi. CAB International, Wallingford, UK.
Pawłowska J, Walther G, Wilk M, Hoog GS de, Wrzosek M. In press. The use of compensatory base changes analysis of ITS2 as a tool in phylogeny of Mucorales illustrated by 'Mucor circinelloides' group. Organisms Diversity & Evolution.
Platauf AP. 1885. Mycosis mucorina. Virchows Archives 102: 543–564.
Polaino S, Herrador MM, Cerdá-Olmedo E, Barrero AF. 2010. Splitting of beta-carotene in the sexual interaction of Phycomyces. Organic and Biomolecular Chemistry 8: 4229–4231.
Ray RC, Ravi V. 2005. Post harvest spoilage of sweetpotato in tropics and control measures. Critical Reviews in Food Science and Nutrition 45: 623–644.
Reeb V, Lutzoni F, Roux C. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes Pezizomycotina, fungi with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Molecular Phylogenetics and Evolution 32: 1036–1060.
Rice P, Longden I, Bleasby A. 2000. EMBOSS: The European Molecular Biology Open Software Suite. Trends Genetics 16: 276–277.
Roden MM, Zautis TE, Buchanan WL, Knudsen TA, Sarkisova TA, et al. 2005. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clinical Infectious Diseases 41: 634–653.
Satina S, Blakeslee AF. 1926. The mucor Parasitella parasitica in relation to sex. Proceedings of the National Academy of Sciences 12: 202–207.
Schachtschabel D, Schimek C, Wöstemeyer J, Boland W. 2005. Biological activity of trisporoids and trisporoid analogues in Mucor mucedo. Phytochemistry 66: 1358–1365.
Schipper MAA, Samson RA. 1994. Miscellaneous notes on Mucoraceae. Mycotaxon 50: 475–491.
Schipper MAA, Stalpers JA. 1980. Various aspects of the mating system in Mucorales. Persoonia 11: 53–63.
Schipper MAA, Stalpers JA. 1984. Revision of the genus Rhizopus. Studies in Mycology 25: 1–34.
Schmitt I, Crespo A, Divakar PK, Fankhauser JD, Herman-Sackett E, et al. 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. Persoonia 23: 35–40.
Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences USA 109: 6241–6246.
Schultze K, Schimek C, Wöstemeyer J, Burmester A. 2005. Sexuality and parasitism share common regulatory pathways in the fungus Parasitella parasitica. Gene 348: 33–44.
Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological Research 105: 1413–1421.
Seifert KA, Samson RA, Dewaard JR, Houbraken J, Lévesque CA, et al. 2007. Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. Proceedings of the National Academy of Sciences USA 104: 3901–3906.
Shahidi F, Arachchi JKV, Jeon Y-J. 1999. Food applications of chitin and chitosan. Trends in Food Science & Technology 10: 73–51.
Shtienberg D. 1997. Rhizopus head rot of confectionery sunflower: effects on yield quantity and quality and implications for disease management. Phytopathology 87: 1226–1232.
Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, et al. 2011. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology ECMM Working Group on Zygomycosis between 2005 and 2007. Clinical Microbiology and Infection 17: 1859–1867.
Stalpers JA, Schipper MAA. 1980. Comparison of zygospore ornamentation in intra- and interspecific matings in some related species of Mucor and Backusella. Persoonia 11: 39–52.
Subrahamanyam A, Mehrotra BS, Thirumalacher MJ. 1977. Thermomucor – a new genus of Mucorales. Georgia Journal of Science 35: 1–4.
Sutter RP, Dadok J, Bothner-By AA, Smith RR, Mishra PK. 1989. Cultures of separated mating types of Blakeslea trispora make D and E forms of trisporic acids. Biochemistry 28: 4060–4066.
Tagua VG, Medina HR, Martín-Domínguez R, Eslava AP, Corrochano LM, et al. 2012. A gene for carotene cleavage required for pheromone biosynthesis and carotene regulation in the fungus Phycomyces blakesleeanus. Fungal Genetics and Biology 49: 398–404.
Tamang JP, Thapa S. 2006. Fermentation dynamics during production of bhaati jaanr, a traditional fermented rice beverage of the eastern Himalayas. Food Biotechnology 20: 251–261.
Taylor JW, Geiser DM, Burt A, Koufopanou V. 1999. The evolutionary biology and population genetics underlying strain-typing. Clinical Microbiology Reviews 12: 126–146.
Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, et al. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21–32.
Taylor TN, Krings M, Klavins SD, Taylor EL. 2005. Protoascon missouriensis, a complex fossil microfungus revisited. Mycologia 97: 725–729.
Vitale RG, Hoog GS de, Schwarz P, Dannaoui E, Deng S, et al. 2012. Antifungal susceptibility and phylogeny of opportunistic members of the order Mucorales. Journal of Clinical Microbiology 50: 66–75.
Vogelstein B, Gillespie D. 1979. Preparative and analytical purification of DNA from agarose. Proceedings of the National Academy of Sciences USA 76: 615–619.
Voigt K. 2012. Zygomycota. In: Frey W (ed), Syllabus of plant families, 13th ed: 130–156. Borntraeger, Stuttgart, Germany.
Voigt K, Olsson L. 2008. Molecular phylogenetic and scanning electron microscopical analyses places the Choanephoraceae and the Gilbertellaceae in a monophyletic group within the Mucorales (Zygomycetes, Fungi). Acta Biologica Hungarica 59: 365–383.
Voigt K, Wöstemeyer J. 2000. Reliable amplification of actin genes facilitates deep-level phylogeny. Microbiological Research 155: 179–195.
Voigt K, Wöstemeyer J. 2001. Phylogeny and origin of 82 zygomycetes from all 54 genera of the Mucorales and Mortierellales based on combined analysis of actin and translation elongation factor EF-1α genes. Gene 270: 113–120.
Wade NL, Morris SC 1982. Causes and control of cantaloupe postharvest wastage in Australia. Plant Disease 66: 549–552.
Walther G, Pawłowska J, Alastruey-Izquierdo A, Wrzosek M, Rodriguez-Tudela JL, et al. 2013. DNA barcoding in Mucorales: an inventory of biodiversity. Persoonia 30: 11–47.
Werkman BA. 1976. Localization and partial characterization of a sex-specific enzyme in homothallic and heterothallic mucorales. Archives of Microbiology 109: 209–213.
Wetzel J, Scheibner O, Burmester A, Schimek C, Wöstemeyer J. 2009. 4-dihydrotrisporin dehydrogenase, an enzyme of the sex hormone pathway of Mucor mucedo: purification, cloning of the corresponding gene, and developmental expression. Eukaryotic Cell 8: 88–95.
White MM, James TY, O'Donnell K, Cafaro MJ, Tanabe Y, Sugiyama J. 2006. Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia 98: 872–884.
White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods and applications: 315–322. Academic Press, San Diego
Yu MQ, Ko WH. 1997. Factors affecting germination and mode of germination of zygospores of Choanephora cucurbitarum. Journal of Phytopathology 145: 357–361.
Zheng RY, Chen GQ. 2001. A monograph of Cunninghamella. Mycotaxon 80: 1–75.
Zycha H, Siepmann R, Linnemann G. 1969. Mucorales. Eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe. Cramer, Lehre.

Note:
New taxa in Voigt 2012 were validated in Kirk 2012 and Kirk & Voigt 2012.