

Delimitation of *Umbelopsis* (*Mucorales*, *Umbelopsidaceae* fam. nov.) based on ITS sequence and RFLP data*

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In a continuation of studies started by de Ruiter *et al.* (1993), all known species of the *Mortierella isabellina*-group (*Micromucor*/*Umbelopsis* clade of O'Donnell *et al.* 2001) and a few other *Mucorales* and species of *Mortierella* were investigated by RFLP (including ITS1, 5.8S, ITS2 and the 5' end of the large subunit rDNA gene) and ITS1 sequence analyses. This monophyletic group is unrelated to *Mortierella* and is only distantly related to the core group of the *Mucoraceae*. *M. longicollis* falls outside the *Umbelopsis* clade. Molecular data resolved two subclades within the *M. isabellina*-group; however, they are not correlated with any differences in sporangial wall and shape, spore pigmentation and shape, or sporangiophore branching. Therefore we subsume all taxa in one genus, *Umbelopsis*. The new family *Umbelopsidaceae* and the new combinations *U. isabellina*, *U. ramanniana*, and *U. autotrophica* are proposed.

INTRODUCTION

The relation of the '*Mortierella isabellina*-group' to the remaining species of *Mortierella* and its position within the *Mucorales* has long been a matter of debate. The *Mortierellaceae* (genera *Mortierella*, *Dissophora*, *Echinosporangium*, *Actinomortierella*, *Modicella*, and probably *Aquamortierella*, according to Benny & Benjamin 1993, Kirk *et al.* 2001) differ from the remaining families of the *Mucorales* morphologically in sporangial and zygosporangium structures, and in growth habit with mainly dichotomously branching hyphae. Physiologically and ecologically they are distinct in being particularly prone to an oligotrophic mode of life. Their colonies also often have a garlic-like odour. This indicates a remote taxonomic affinity with the remaining families of the *Mucorales* (Benjamin 1979). Molecular studies have shown that the *Mortierellaceae* are more closely related to the *Endogonales* than to the *Mucorales* (Yotsumoto & Mikawa 1993, Gehrig, Schüßler & Kluge 1996, Tanabe *et al.* 2000, O'Donnell *et al.* 2001). The order *Mortierellales* was introduced with a minimal diagnosis by Cavalier-Smith (1998), but the

justification of its distinction from the *Endogonales* is uncertain.

The '*M. isabellina*-group', or subgenus *Micromucor*, though generally classified in *Mortierella*, with *M. isabellina* and *M. ramanniana* as the best known species, deviates from the remaining taxa of the genus by velvety colonies and a dense layer of erect sporangiophores, in most species pinkish or ochraceous spores, sporangia sometimes with a small columella. Its colonies lack a garlic-like odour (Turner 1963). Three taxa of this group produce angular spores. This shape is due to pressure of the rigid sporangial wall (Tokumasu *et al.* 1990). Isolates identified as *Mortierella ramanniana* var. *angulispora* (nom. inval.) are probably the commonest of these species; they also form copious chlamydo-spores and a pronounced columella, just like that found in *M. ramanniana* var. *ramanniana*. *M. vinacea*, also having angular spores, produces fewer chlamydo-spores and slightly darker, less abundant sporangia containing only a minimal columella, while a third such species, *M. longicollis*, has longer sporangiophores and often nodding sporangia. Linnemann (1941) classified this group as *Mortierella* sect. *Pusilla* (nom. inval., based on a confused interpretation of *M. pusilla*, a species that according to Oudemans's original drawing in Leiden (L) does not belong to this group); Linnemann (1970) called it sect. *Isabellina* (nom. inval.). Mil'ko (1974) retained *M. ramannianus* and the

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associated varieties (at species rank) in *Mucor* sect. *Ramannianus* (nom. inval.), thus widely separated from *Mortierella vinacea* and *M. isabellina* in sect. *Isabellina* (not validly published). Gams (1977) introduced *Mortierella* subgen. *Micromucor* for the group, distinguishing it from all remaining sections left in the genus as subgen. *Mortierella*. Section *Micromucor* (Naumov 1935, 1939; nom. inval. Art. 36) was also introduced in *Mucor* for the same group of fungi.

Linnemann (1941) described *Mortierella nana* with single-spored sporangia in section *Pusilla*, thus establishing the zygomycete affinities of this unusual fungus. The first to recognize the zygomycete affinity of *Umbelopsis versiformis* Amos & H. L. Barnett (1966) and its synonymy with *M. nana* was Martha Christensen in 1981 (pers. comm.). Von Arx (1984) somewhat arbitrarily distributed the species of the '*M. isabellina*-group' over the genera *Micromucor* (W. Gams) von Arx 1984 and *Umbelopsis* Amos & H. L. Barnett 1966, after Watanabe (1977) had described an isolate as *Umbelopsis multispora* without recognizing its zygomycete affinity because he saw some septa in the hyphae. This species (represented by CBS 236.82) turned out to be the same as *Mortierella vinacea* and the latter was recombined in *Umbelopsis* by von Arx (1984). The great morphological variability of *U. nana* was described in detail by Kendrick *et al.* (1994). Decisive morphological features (in particular zygospores) that would be indicative of family relationship with either the *Mortierellaceae* or the *Mucoraceae* are lacking in *Umbelopsis* and *Micromucor*. Some of the species had originally been placed in *Mucor*, but the texture of the low colonies is atypical of this genus and the columella of the sporangia is absent or small. In addition, reddish sporangiospores are otherwise unknown in *Mucor*.

De Ruiter *et al.* (1993) started a series of multifaceted comparative studies on this group, using extracellular polysaccharides as a taxonomic criterion. Results of these studies also support a sharp delimitation of the *M. isabellina*-group from the *Mortierellaceae*. The present detailed molecular study was conducted to further examine the relationship of these taxa.

The GC content of nuclear DNA did not help resolve whether the *M. isabellina*-group should be separated from other species of *Mortierella* (Zhou, Huang & Zheng 1992).

Using 18S rRNA sequences, Yotsumoto & Mikawa (1993) produced cladograms in which *Mortierella s. str.* and red species of the *M. isabellina*-group form two very distinct clades. A full account of this work has not been published. Tanabe *et al.* (2000) obtained similar results. Gehrig *et al.* (1996) in a more inclusive phylogenetic analysis also showed that the *Mortierellaceae* are either a sister-group to the *Endogonales* or possibly members of this order. O'Donnell *et al.* (2001) analysed representatives of all genera of the *Mucorales*, using sequences of the large and small subunits of

the rDNA gene cluster and the translation elongation factor-1 α gene exons. They convincingly showed that the very distinct *Micromucor-Umbelopsis* clade, although traditionally included in the *Mortierellaceae*, was a basal sister-group to all other genera of *Mucorales*, while species of the unrelated genus *Mortierella* could be used as outgroup to root the parsimony tree. The *Micromucor-Umbelopsis* clade is therefore sufficiently distinct from the *Mucoraceae* and deserves family rank.

In this study, all recognized species and varieties of the *M. isabellina*-group were investigated to examine their relationship with the *Mucoraceae*, the *Mortierellaceae*, and other members of the same group. Several molecular techniques were used to answer some questions that arose from morphological observations. For example, at which rank should the varieties of *Mortierella ramanniana* be classified? Some isolates of uncertain position in this complex still remain to be placed. Are the Australian taxa, *U. westae* and *U. swartii*, really distinct species? Are these taxa linked to *M. ramanniana* by new Japanese isolates with a unilateral wall thickening (described by Sugiyama *et al.* 2003 as *U. gibberispora*)? What significance is to be attributed to the variations in colony pigmentation of *M. isabellina*? Is the distinction of *U. roseonana* from *U. nana*, based on a minor difference in pigmentation, justified? Sugiyama *et al.* (2003) used sequences of the 28S subunit of rDNA in order to classify their new species *U. gibberispora*. They also included the ex-type strain CBS 222.29 (Naumov's original isolate designated ex-neotype by Váňová (1991)) of *Micromucor ramannianus* var. *angulisporus* Naumov ex Váňová 1991 in their analysis and found it closely associated with *U. vinacea*. As the name *angulisporus* has only been valid at varietal rank since 1991, it will not displace the well-known *U. vinacea*. However, a new species, *U. angularis* W. Gams & M. Sugiy. (Sugiyama *et al.* 2003), had to be introduced for the above-mentioned isolates that had generally been misidentified as *M. ramanniana* var. *angulispora*.

From our results and those of our predecessors, we shall draw the necessary nomenclatural consequences at the end of this paper, introducing the family *Umbelopsidaceae* for the single genus *Umbelopsis*, and raising the former varieties to species rank. A detailed morphological survey of the species of *Umbelopsis* and a further analysis of the remaining questions will be provided in a third paper in this series.

MATERIALS AND METHODS

Fungal strains and culture conditions

Representative isolates of all known species and varieties and some deviating isolates were obtained from the CBS culture collection. The strains examined are listed in Table 1. Cultures were grown in malt broth to obtain mycelium for DNA extraction.

DNA techniques

DNA extraction

The harvested mycelium was ground in a mortar with a pestle in liquid nitrogen. Genomic DNA was isolated using a previously described protocol (Meyer & Mitchell 1995), dissolved in deionized water, quantified spectrophotometrically and stored at 4 °C or at –20 °C.

rDNA amplification

The internal transcribed spacer regions of the rDNA gene cluster, consisting of ITS-1, the 5.8S rDNA gene and ITS-2, were amplified with primers, homologous to conservative sequences within the small subunit (SSU) rDNA gene (primer SR6R: 5' AAGTATAAGTCG-TAACAAGG 3') and the large subunit (LSU) rDNA gene (primer LR1: 5' GGTTGGTTTCTTTCCT 3'). To amplify the ITS1 region alone, the primer SR6R in combination with a primer located in the 5.8S rDNA gene (primer 5.8S: 5' CGCTGCGTTCTTCATCG 3') were used. For the RFLP analysis, a fragment of the rDNA gene cluster, including the ITS1, 5.8S gene, ITS2 regions as well as the 5' end of the LSU gene, were amplified as a single fragment with the primers SR6R and LR7 (5' TACTACCACCAAGATCT 3') located 3 bp upstream to the D2 region of the LSU gene (all LSU primers developed by Rytas Vilgalys (Duke University, Durham, NC; www.biology.duke.edu/fungi/mycolab/primer.htm). Amplification of the rDNA gene fragments was performed as described previously (Vilgalys & Hesters 1990, Gams & Meyer 1998).

RFLP analysis

The approximately 2300 bp fragment extending from ITS-1 through the 5' end of the LSU rDNA gene, as detailed above, was amplified using the primers SR6R and LR7. For restriction fragment length polymorphism (RFLP) analysis, aliquots of 15 µL of each amplicon were digested with 20 U of the restriction endonucleases *Hha*I, *Ava*I, *Msp*I, *Eco*RI, *Hae*III, *Sau*3A (New England Biolabs, Beverly, MA), respectively, at 37 °C for 3 h or overnight. *Sau*3A and *Hae*III gave the most informative RFLP-profiles, which were subsequently used for all strains investigated. Restriction digests were separated by electrophoresis in agarose gels (2.0% agarose supplemented with 1.0% FMC agarose) for 6 h at 6 V cm⁻¹ in 1 × TBE buffer and detected by ethidium bromide staining.

Sequence analysis of the ITS1 region

Amplicons of the ITS1 region were purified over 'Magic PCR Columns' (Promega, Madison, WI) according to the manufacturer's protocol. The PCR products were sequenced directly on an 373A ABI automated DNA sequencer, using 70 ng DNA and

10 pmol of either the primer SR6R or 5.8S to sequence the forward or reverse strands, respectively. Sequences reported in this paper have been deposited at EMBL under the accession numbers given in Table 1.

Sequence data analysis

Sequence alignment was initially done by using the program Pileup of the GCG computer package (Genetic Computer Group, Madison, WI) and the alignment was manually optimized using the program SeqPup v.0.6 (iubio.bio.Indiana.edu/soft/molbio/seqpup). Maximum parsimony analysis was performed using sequences of the ITS-1 region from 27 different strains, 3 *Mucoraceae*, 2 *Mortierellaceae*, and 22 taxa of *Umberopsidaceae* with the program PAUP* 4.06.10 (PPC/Altevec) for a Macintosh (Swofford 2002). The search for the most parsimonious trees was performed using the heuristic search option of PAUP* 4.06.10. Bootstrap analysis (Felsenstein 1985) with 1000 bootstrap replications using the heuristic search option of the program PAUP* 4.06.10 was carried out to test the robustness of the internal branches.

RESULTS

The Mortierella isabellina-group is unrelated to Mortierella

RFLP of the ITS-LSU regions

Among all restriction enzymes tested, only *Hae*III and *Sau*3A generated informative RFLP patterns (H = *Hae*III patterns and S = *Sau*3A patterns). The RFLP patterns obtained were distinctive and in most cases species-specific. Seven major RFLP profiles were identified (see Fig. 1A–B and Table 2). Patterns HE/SE–HH1/SH1 were specific for the *Mortierellaceae*, patterns HA/SA–HD/SD for the *Mucoraceae*, and patterns HH2/SH2–H1/S1 for the *M. isabellina*-group, i.e. the *Umberopsidaceae*. The RFLP patterns clearly separate *Umberopsis* (RFLP patterns HH2/SH2 to HH5/SH4 and H11/S11 to H16/S13) from the subgenus *Mortierella* (RFLP patterns HE/SE to HG/SG and HH1/SH1), with the RFLP profiles generated from the subgenus *Mortierella* showing greater diversity.

Sequences of the ITS1 region

Parsimony analysis of the aligned 248 bp DNA sequences of the ITS-1 region (Fig. 2) yielded 21 most parsimonious trees. Bootstrap values are given below the branches. The 21 trees differ only in the position of the four groups of red *Umberopsis* species, i.e. (1) CBS 914.85 *U. angularis* and CBS 212.72 *U. autotrophica*, (2) CBS 478.63 *U. ramanniana*, (3) CBS 101226 and 101744 *Umberopsis* sp., and (4) CBS 101745 and 109328 *U. gibberispora*, to each other, but this does not change the general topology of the tree. Phylogenetic analysis

Table 1. Strains studied.

Name	Strain number	Origin and depositor	EMBL accession no.
Endogonales^a			
Mortierellaceae			
<i>Mortierella hyalina</i> (Harz) W. Gams 1969	CBS 654.68 = NRRL A-12620	Deer dung, India, <i>B. S. Mehrotra</i>	AJ495420
<i>Mortierella longicollis</i> Dixon-Stew. 1932 (T) ^b	CBS 209.32	Sandy loam, Australia, Victoria, <i>E. McLennan</i>	AJ495419
<i>Mortierella polycephala</i> Coemans 1863	CBS 327.72	Salt-marsh soil under <i>Spartina townsendii</i> , UK, England, Lincs., Gibraltar Point, <i>M. Turner</i>	
<i>Mortierella reticulata</i> Tiegh. & G. Le Monn. 1873	CBS 452.74	Soil, Chile, <i>J. Grinbergs</i>	
Mucorales			
Mucoraceae			
<i>Absidia corymbifera</i> (Cohn) Sacc. & A. Trotter 1912 (NT) ^b	CBS 429.75 = ATCC 46771 = NRRL 2981	Soil, Afghanistan, <i>J. J. Curtis</i>	
<i>Mucor circinelloides</i> f. <i>janssenii</i> (Lendn.) Schipper 1976	CBS 762.74	Milk powder, Netherlands, RIVM	AJ495422
<i>Mucor racemosus</i> Fresen. 1850 f. <i>racemosus</i>	CBS 222.81	Nut of <i>Juglans regia</i> , Netherlands, Zuid Limburg, Reymerstok, <i>M. D. Northolt</i>	AJ495421
<i>Rhizomucor pusillus</i> (Lindt) Schipper 1978	CBS 432.78	Denmark, Novo Industri	AJ495423
Umbelopsidaceae			
<i>Umbelopsis angularis</i> W. Gams & M. Sugiy. 2003	CBS 914.85	Root of <i>Picea abies</i> , Germany, <i>E. Falk</i>	AJ495435
<i>Umbelopsis autotrophica</i> (E. H. Evans 1971) W. Gams 2003	CBS 212.72	Forest soil, Sweden, Skogshögskolan Stockholm	AJ495436
<i>Umbelopsis fusiformis</i> H.-Y. Yip 1986 (T)	CBS 385.85 = DAR 51585	Soil from forest of <i>Eucalyptus regnans</i> , Australia, Victoria; near Melbourne, <i>H.-Y. Yip</i>	AJ495445
<i>Umbelopsis gibberispora</i> M. Sugiy. <i>et al.</i> 2003	CBS 101745 = P6073	Leaf litter of <i>Pinus luchuensis</i> , Japan, Okinawa Pref., <i>S. Tokumasu</i>	AJ495441
<i>Umbelopsis gibberispora</i> (T)	CBS 109328 = SFC 00116	Leaf litter of <i>Fagus crenata</i> , Miyama City, Kyoto, <i>T. Ohsono</i>	AJ495442
<i>Umbelopsis isabellina</i> (Oudem. 1902) W. Gams 2003	CBS 208.32	Sandy loam, Australia, Victoria, authentic for <i>M. isabellina</i> var. <i>ramifica</i> D. Dixon-Stew. 1932	AJ495428
<i>Umbelopsis isabellina</i>	CBS 224.35 = ATCC 44853 = IFO 7874	Germany, isol. Bortels, dep. <i>H. Zycha</i>	AJ495427
<i>Umbelopsis isabellina</i>	CBS 560.63	Soil from <i>Larix</i> forest, Germany, near Hann.-Münden, <i>G. Linnemann</i>	AJ495426
<i>Umbelopsis nana</i> (Linnem.) Arx 1982	CBS 373.67 = IFO 9128	Forest soil, former USSR, Caucasus, near Lake Riza, <i>K. Cejp</i> , received from O. Fassatiová as <i>Pseudogymnoascus caucasicus</i>	AJ495431
<i>Umbelopsis nana</i>	CBS 858.68	T of <i>Mortierella alba</i> Mańka & Gierczak, forest soil, Poland, <i>K. Mańka</i>	AJ495432
<i>Umbelopsis nana</i>	CBS 730.70	Forest soil, Netherlands, Winterswijk, <i>J. C. Went</i>	AJ495430
<i>Umbelopsis ovata</i> (H.-Y. Yip) H.-Y. Yip 1986 (T)	CBS 499.82 = IMI 262028	Rhizoplane of <i>Isopogon ceratophyllus</i> , Australia, Victoria, Brisbane Ranges, <i>H.-Y. Yip</i>	AJ495429
<i>Umbelopsis ramanniana</i> (A. Möller 1903) W. Gams 2003	CBS 219.47	<i>Lactarius deliciosus</i> , Netherlands, <i>A. L. van Beverwijk</i>	AJ495438
<i>Umbelopsis ramanniana</i>	CBS 478.63	<i>Amanita</i> sp., Netherlands, <i>H. A. van der Aa</i>	AJ495437

<i>Umbelopsis ramanniana</i>	CBS 243.58	Human skin, between toes, Netherlands, <i>H. M. van Praag</i>	AJ495439
<i>Umbelopsis</i> sp.	CBS 112.08 = IMI 029661	Soil, UK, Scotland, <i>E. Dale</i>	AJ495440
<i>Umbelopsis</i> sp.	CBS 852.72 = INII 81	Portugal, <i>T. Amaral</i>	AJ495444
<i>Umbelopsis</i> sp.	CBS 101226	Soil and debris associated with colony of <i>Reticulitermes</i> <i>hesperus</i> , USA, California, <i>T. R. Gordon</i>	AJ495434
<i>Umbelopsis</i> sp.	CBS 101744 = P5068	Leaf litter of <i>Pinus thunbergii</i> , Japan, Akira Pref., <i>S. Tokumasi</i>	AJ495433
<i>Umbelopsis swartii</i> H.-Y. Yip 1986 (T)	CBS 868.85	Soil under <i>Eucalyptus regnans</i> , Australia, Victoria, Wallaby Creek, H.-Y. Yip	AJ495424
<i>Umbelopsis versiformis</i> Amos & H. L. Barnett 1966 (T)	CBS 150.81 = ATCC 18060 = IMI 114459	Root of <i>Quercus borealis</i> , USA, Virginia, <i>H. L. Barnett</i>	AJ495425
<i>Umbelopsis versiformis</i>	CBS 473.74	T of <i>U. roseonana</i> (W. Gams & Gleeson) Arx 1984; poor quality soil, Australia, Victoria, Parkville, Univ. of Melbourne, <i>P. Gleeson</i>	AJ495443
<i>Umbelopsis vinacea</i> (Dixon-Stew.) Arx 1984	CBS 561.63	Swamp soil, under <i>Cladonia</i> , Sweden, Abisco, <i>G. Linnemam</i>	AJ495443
<i>Umbelopsis vinacea</i>	CBS 236.82 = ATCC 38089	T of <i>Umbelopsis multispora</i> T. Watanabe 1977, root of <i>Fragaria</i> sp., Japan, Shimane Pref., <i>T. Watanabe</i>	
<i>Umbelopsis westeae</i> H.-Y. Yip 1986 (T)	CBS 870.85	Soil from acid heathland, Australia, Victoria, Frankston, <i>S. Ducker</i> , 1953, <i>H.-Y. Yip</i>	

revealed three major well-supported clades corresponding to the *Mortierellaceae* (81% bootstrap), *Mucoraceae* (86%) and *Umbelopsidaceae* (85%). The well-supported *Umbelopsidaceae* clade contains all but one of the species of the *M. isabellina*-group and the genus *Umbelopsis*. This clade is distinct from the remaining species of *Mortierella* and more closely related to species of the *Mucorales*.

Mortierella longicollis

This species, of which only one strain (CBS 209.32) is available, is morphologically close to *U. vinacea* with red sporangia and angular spores, but it has longer sporangiophores and the sporangia are often nodding; *M. longicollis* is distantly related to other taxa of the genus *Umbelopsis* and it may be more closely related to *Mortierella*. In the cladogram it appears close to *Mortierella hyalina*. But its real affinity will require further studies.

A major subdivision of the Umbelopsidaceae

RFLP

Two major groups of RFLP patterns, HH/SH and HI/SI, are characteristic for this group. The first group with patterns HH and SH comprises *Umbelopsis vinacea*, *U. isabellina*, *U. ovata*, *U. nana*, and *U. versiformis*. The second distinct RFLP group with patterns HI and SI comprises *U. angularis*, *U. autotrophica*, *U. ramanniana*, *Umbelopsis* sp., *U. gibberispora*, *U. westeae*, *U. swartii*, and *U. fusiformis*. In the first group, *HaeIII* generated five distinct profiles and *Sau3A* four, with *U. nana* and *U. versiformis/roseonana* sharing the same *Sau3A* profile SH3, indicating a closer phylogenetic relationship between these two species than to the other species of this subclade. The RFLP group HI/SI is characterized by six *HaeIII* profiles and three *Sau3A* profiles. *HaeIII* clearly separates *U. angularis*, *U. autotrophica*, *U. ramanniana/Umbelopsis* sp./*U. gibberispora*, *U. westeae*, *U. swartii*, and *U. fusiformis* from each other. However, the RFLP profiles obtained with *Sau3A* suggest that these species are more closely related than the other species analysed (see Fig. 1A–B and Table 2).

Sequences

The *Umbelopsis* clade is divided into two well-supported subclades, which coincide with the two major RFLP patterns. The first subclade, with 100% bootstrap support, contains *U. isabellina* (split into two groups: CBS 560.63, and CBS 224.35/CBS 208.32), *U. ovata*, *U. vinacea*, *U. nana*, and *U. versiformis* (=the strain CBS 150.81, identified till now as *U. nana*). The second subclade, with 100% bootstrap support, comprises the red species related to *U. ramanniana* (*U. angularis*, *U. autotrophica*, *U. ramanniana*, *Umbelopsis* sp.,

^a Classification of the *Mortierellaceae* in this order is provisional.

^b T, ex-type culture; NT, ex-neotype culture.

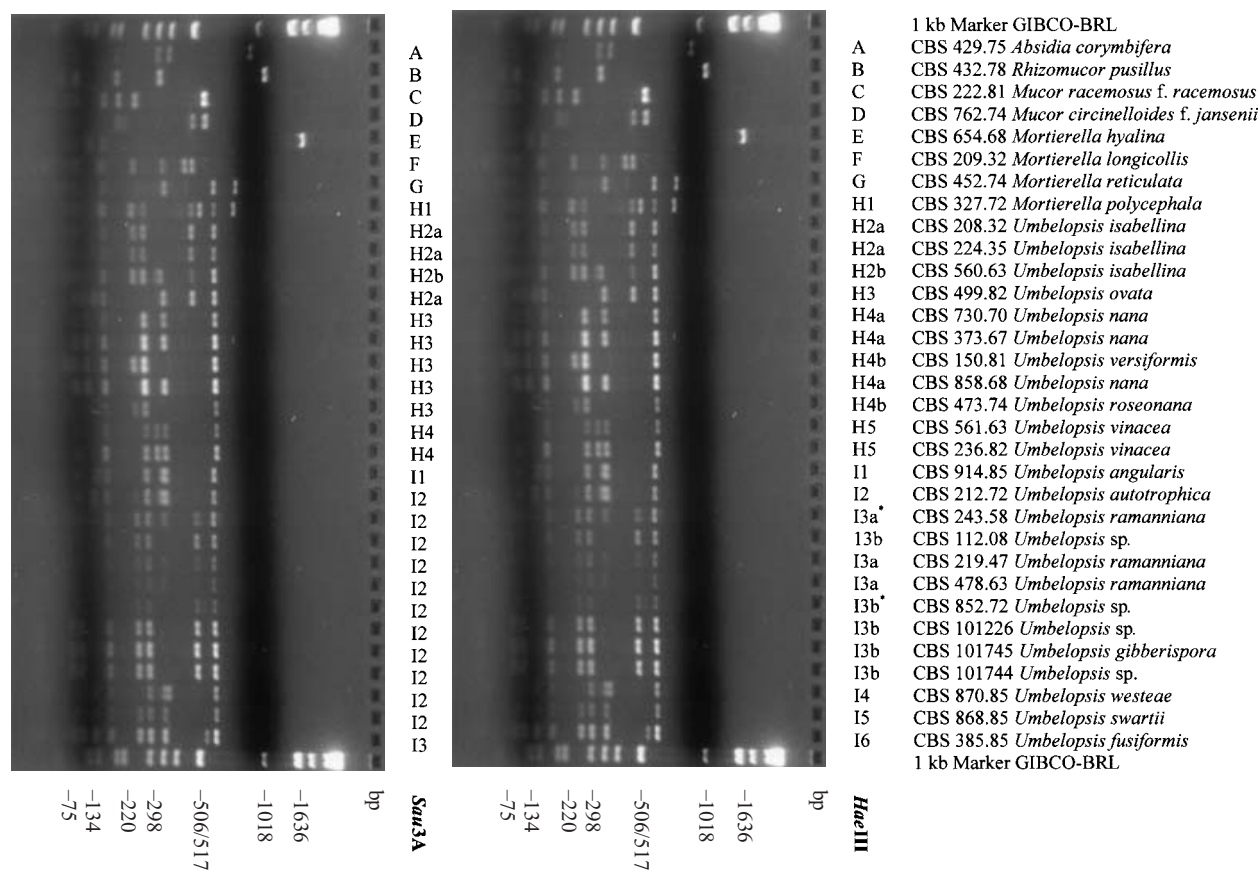


Fig. 1. RFLP patterns of the ITS region in *Umbelopsis* and some other zygomycetes, generated with *HaeIII* and *Sau3A*. *, partial digest.

and *U. gibberispora*) and also *U. westeae*, *U. swartii*, and the ochraceous *U. fusiformis*.

Distinction of critical species; status of the former varieties of *Mortierella ramanniana* and the Japanese *Umbelopsis gibberispora*

Two varieties have been distinguished in the past from typical *Mortierella ramanniana*, which typically has ellipsoidal spores and is thiamin-dependent. Var. *autotrophica* differs by having globose spores and being thiamin-autotrophic (Evans 1971). Naumov (1935, 1939) initially invalidly described *Mucor angulisporus* as a separate species; it was reduced to varietal rank by Linnemann (1941) and validated as such by Váňová (1991). Two taxa with angular spores are commonly found. The meagre original diagnosis by Naumov could apply to either the taxon that is close to *U. ramanniana* with a rather pronounced columella and copious chlamyospores, which Linnemann understood under this name, or to *Mortierella vinacea* (Dixon-Stewart 1932), which has fewer chlamyospores and sporulates more scantily in a slightly darker purple shade. These two taxa fall into each of the two subclades of *Umbelopsis*. *Micromucor ramannianus* var. *angulisporus* was typified by Váňová (1991) with CBS 222.29, Naumov's original isolate. Molecular work by Sugiyama *et al.* (2003) has revealed that this strain is

very close to *M. angulispora* and unrelated to var. *angulispora sensu* Linnemann. Therefore, Linnemann's taxon with angular spores was formally introduced as *U. angularis* W. Gams & M. Sugiy. by Sugiyama *et al.* (2003). *U. angularis* (CBS 914.85) is sufficiently distinct from *U. ramanniana* (CBS 478.63) to be recognized at species level. *U. angularis* and *U. autotrophica* are also closely related but have two distinct *HaeIII* RFLP profiles HI1 and HI2 (Fig. 1A, Table 2) and 13 bp (5.2%) sequence difference in the ITS1 region. A further group of isolates has been identified as *Mortierella ramanniana* but is designated here as *Umbelopsis* sp. (e.g. CBS 112.08). *U. ramanniana* has a distinct *HaeIII* RFLP profile HI3a, *Umbelopsis* sp. and also *U. gibberispora* being characterized by the profile HI3b (Fig. 1A, Table 2). The sequence of *U. ramanniana* differs by 7 bp (2.8%) from that of *Umbelopsis* sp. *Umbelopsis ramanniana* is characterized by ellipsoidal spores; in isolates of *Umbelopsis* sp. spores are ellipsoidal or subglobose. This second, morphologically only slightly different group, referred to here as *Umbelopsis* sp., deserves species rank. Typification of *U. ramanniana* has not yet been settled and either taxon would qualify to bear that name. Our choice for the clade of CBS 478.63 as emblematic of the name is still arbitrary.

U. ramanniana and *U. gibberispora* also are closely related, differing by 12 bp (4.8%) within the ITS

Table 2. Results of the RFLP analysis of the ITS1, 5.8S and ITS2 regions with the restriction enzymes *Hae*III or *Sau*3a.

CBS No.	Name ^a	<i>Hae</i> III fragments (bp)	<i>Hae</i> III RFLP type	<i>Sau</i> 3a fragments (bp)	<i>Sau</i> 3a RFLP type
CBS 429.75	<i>Absidia corymbifera</i>	943, 382, 345, 213	HA	1218, 348, 218, 192, 154	SA
CBS 432.78	<i>Rhizomucor pusillus</i>	1152, 350, 213, 124, 87	HB	775, 428, 348, 129	SB
CBS 222.81	<i>Mucor racemosus</i> f. <i>racemosus</i>	598, 269, 213, 180, 124, 116, 71	HC	804, 602, 360, 316, 223, 154, 116	SC
CBS 762.74	<i>Mucor circinelloides</i> f. <i>jansenii</i>	598, 514, 213, 124, 116	HD	804, 602, 360, 316, 223, 154	SD
CBS 654.68	<i>Mortierella hyalina</i>	1981, 180, 143, 124	HE	1393, 348, 316, 154	SE
CBS 209.32	<i>Mortierella longicollis</i>	514, 465, 359, 255, 180, 124, 68	HF	1100, 548, 348, 316, 154	SF
CBS 452.74	<i>Mortierella reticulata</i>	795, 705, 465, 371, 180, 124	HG	700, 685, 348, 316, 154, 90	SG
CBS 327.72	<i>Mortierella polycephala</i>	793, 705, 575, 514, 300, 255, 180, 124, 116	HH1	1110, 578, 435, 348, 316, 267, 247, 154, 109	SH1
CBS 208.32	<i>Umbelopsis isabellina</i>	705, 514, 300, 255, 180, 124, 116	HH2a	1110, 348, 316, 154	SH2a
CBS 224.35	<i>Umbelopsis isabellina</i>	705, 514, 300, 255, 180, 124, 116	HH2a	1110, 348, 316, 154	SH2a
CBS 560.63	<i>Umbelopsis isabellina</i>	705, 514, 340*, 300, 255, 180, 124*, 116* ^b	HH2b	1274*, 1110, 348, 316, 164*, 154	SH2b
CBS 499.82	<i>Umbelopsis ovata</i>	705, 514, 371, 180, 150, 132, 124	HH3	1110, 348, 316, 154	SH2a
CBS 730.70	<i>Umbelopsis nana</i>	705, 371, 300, 180, 141, 124	HH4a	857, 348, 316, 294, 154, 148	SH3
CBS 373.67	<i>Umbelopsis nana</i>	705, 371, 300, 180, 141, 124	HH4a	857, 348, 316, 294, 154, 148	SH3
CBS 858.68	<i>Umbelopsis nana</i>	705, 371, 300, 180, 141, 124	HH4a	857, 348, 316, 294, 154, 148	SH3
CBS 150.81	<i>Umbelopsis versiformis</i>	705, 300, 255, 180, 141, 124, 116	HH4b	857, 348, 316, 294, 154, 148	SH3
CBS 473.74	<i>Umbelopsis roseonana</i> = <i>versiformis</i>	705, 300, 255, 180, 141, 124, 116	HH4b	857, 348, 316, 294, 154, 148	SH3
CBS 561.63	<i>Umbelopsis vinacea</i>	705, 371, 332, 300, 180, 124	HH5	1110, 348, 316, 174, 154	SH4
CBS 236.82	<i>Umbelopsis vinacea</i>	705, 371, 332, 300, 180, 124	HH5	1110, 348, 316, 174, 154	SH4
CBS 914.85	<i>Umbelopsis angularis</i>	662, 368, 300, 180, 150, 124	HI1	753, 633, 348, 316, 154	SI1
CBS 212.72	<i>Umbelopsis autotrophica</i>	662, 368, 300, 255, 180, 143, 124	HI2	1274, 348, 316, 154	SI2
CBS 243.58	<i>Umbelopsis ramanniana</i>	720*, 662, 560, 368, 300*, 255, 180*, 124*, 116*	HI3a*	1274, 348, 316, 154	SI2
CBS 219.47	<i>Umbelopsis ramanniana</i>	662, 560, 368, 300, 255, 180, 124, 116	HI3a	1274, 348, 316, 154	SI2
CBS 478.63	<i>Umbelopsis ramanniana</i>	662, 560, 368, 300, 255, 180, 124, 116	HI3a	1274, 348, 316, 154	SI2
CBS 112.08	<i>Umbelopsis</i> sp.	662, 560, 300, 255, 180, 124, 116	HI3b	1274, 348, 316, 154	SI2
CBS 852.72	<i>Umbelopsis</i> sp.	732*, 662, 608*, 560, 300, 255, 180, 124*, 116*	HI3b*	1274, 348, 316, 154	SI2
CBS 101226	<i>Umbelopsis</i> sp.	662, 560, 300, 255, 180, 124, 116	HI3b	1274, 348, 316, 154	SI2
CBS 101744	<i>Umbelopsis</i> sp.	662, 560, 300, 255, 180, 124, 116	HI3b	1274, 348, 316, 154	SI2
CBS 101745	<i>Umbelopsis gibberispora</i>	662, 560, 300, 255, 180, 124, 116	HI3b	1274, 348, 316, 154	SI2
CBS 870.85	<i>Umbelopsis westeae</i>	662, 371, 368, 300, 180, 141, 124, 116	HI4	1274, 348, 316, 154	SI2
CBS 868.85	<i>Umbelopsis swartii</i>	662, 368, 300, 255, 180, 141, 124, 116	HI5	1274, 348, 316, 154	SI2
CBS 385.85	<i>Umbelopsis fusiformis</i>	662, 615, 368, 300, 255, 180, 141, 124, 116	HI6	1274, 548, 348, 316, 154	SI3

^a The order in Table 2 corresponds with the sequence of patterns shown in Fig. 1.

^b *, partial digestion.

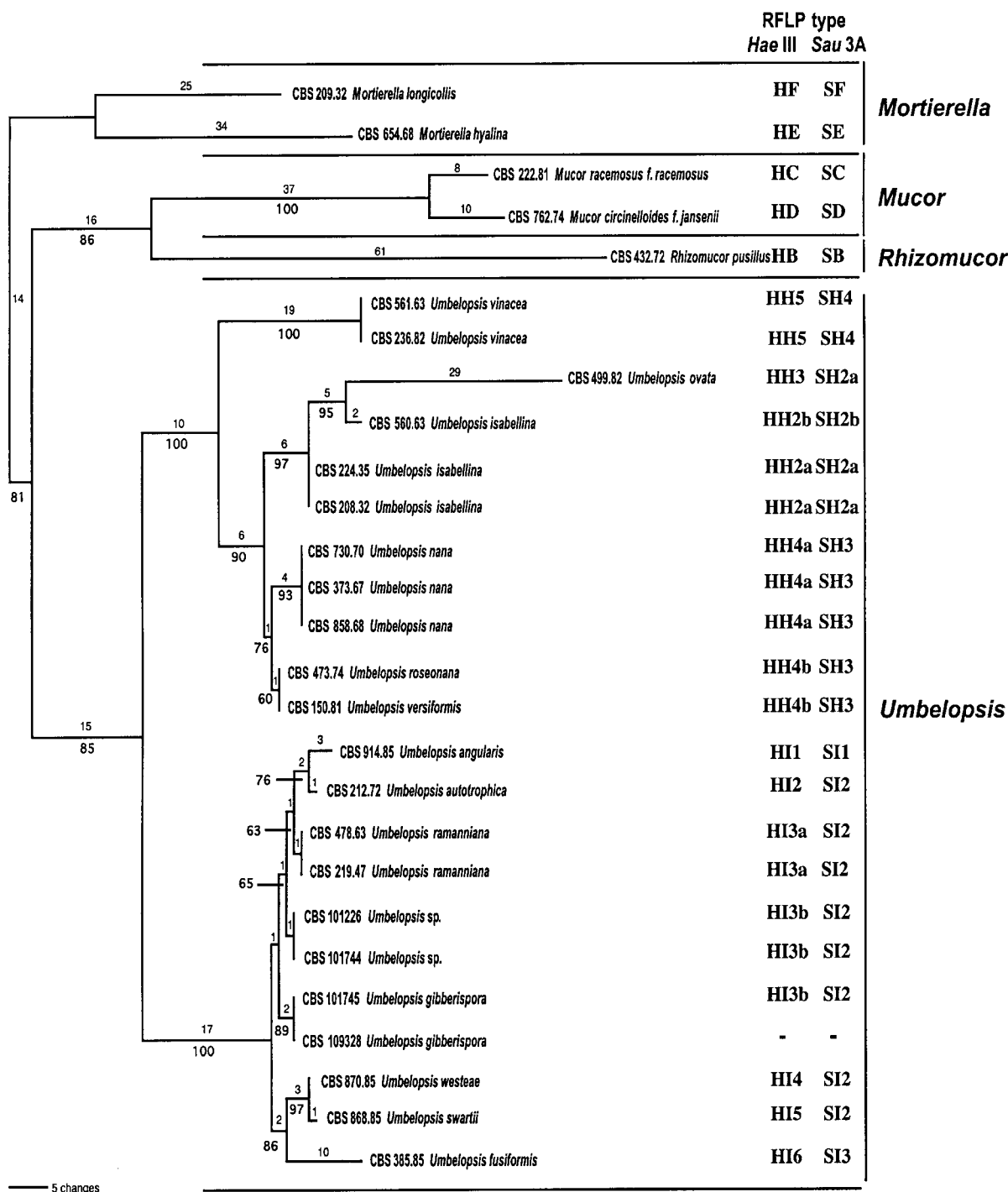


Fig. 2. Phylogenetic reconstruction of *Umbelopsis* and some other zygomycetes. One of 21 most parsimonious trees based on ITS1 sequences, tree length = 349, CI = 0.7822, HI = 0.2178, RI = 0.8544, including 27 taxa and 138 informative positions in the aligned 248 bp sequences. Bootstrap values above 50% are given below the branches. The branch length is given above the branches.

region. Sequences of *Umbelopsis sp.* and *U. gibberispora* differ by 8 bp (3.2%) for CBS 101744 or by 10 bp (4%) for CBS 101226 from each other. These sequence differences are similar to those generally found between other accepted species of the genus, which suggests that these groups also represent distinct species. The newly described Japanese *U. gibberispora* (Sugiyama *et al.* 2003) is well-supported (89% bootstrap).

Umbelopsis westeae* and *U. swartii

Umbelopsis westeae and *U. swartii* (Yip 1986) are closely related though distinct species. They differ morphologically only in the length of the spore appendage and sporangiophore size. These two species form a well-supported clade (92% bootstrap). They are characterized by distinct *Hae*III RFLP profiles HI4 and HI5 (Fig. 1A, Table 2). Their ITS1 sequences differ by 1 bp

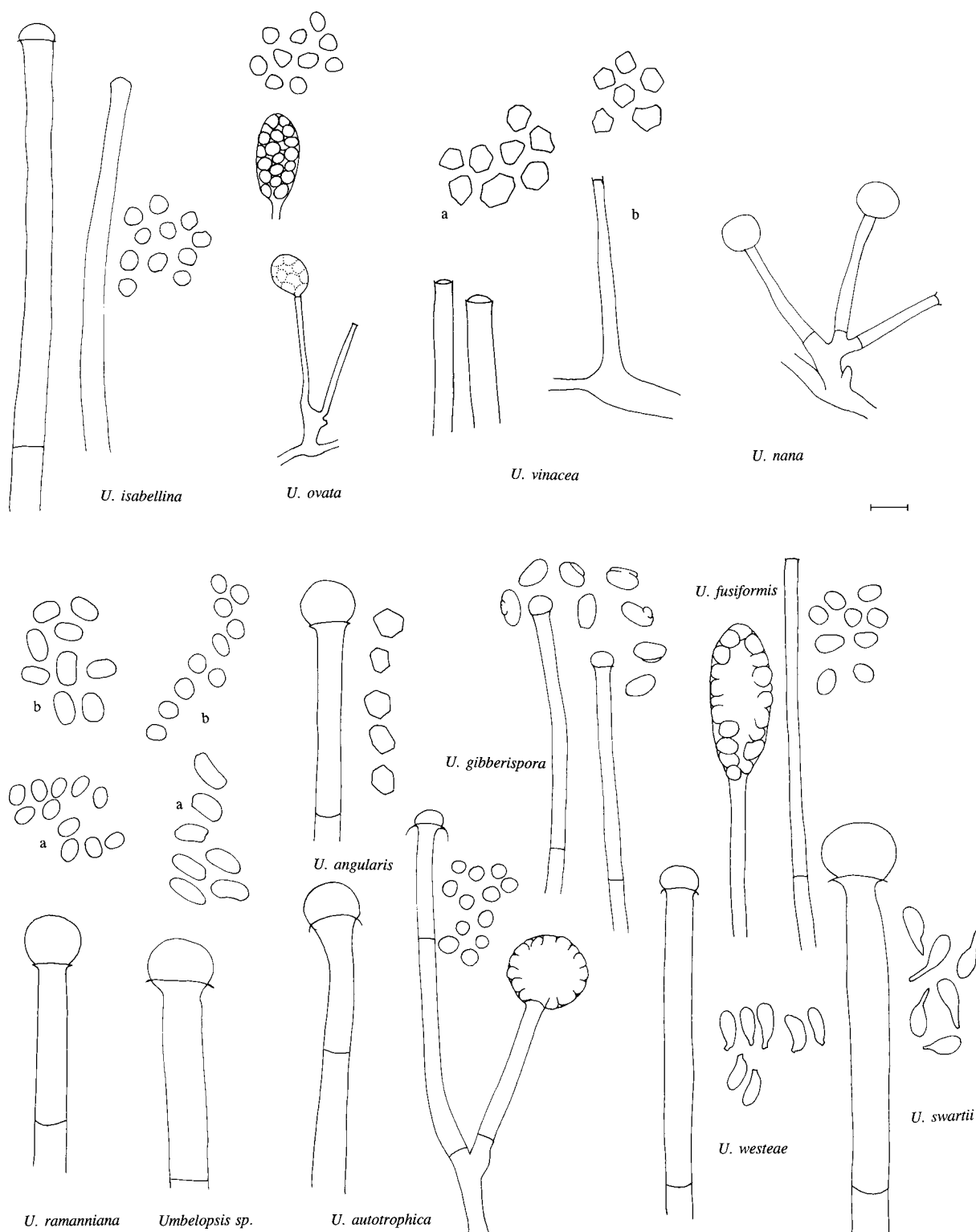


Fig. 3. *Umbelopsis* spp. Synopsis of sporangiophore tips, spores and some sporangia. Material illustrated: Top row: *U. isabellina* (CBS 110512), *U. ovata* (CBS 499.82), *U. vinacea* (a, CBS 236.82; b, CBS 222.29), and *U. nana* (CBS 444.68). Bottom row: *U. ramanniana*, (a, CBS 243.58; b, CBS 219.47), *Umbelopsis* sp. (a, CBS 101744; b, CBS 101224), *U. autotrophica* (CBS 310.93), *U. angularis* (CBS 367.95), *U. gibberispora* (CBS 109328), *U. westeae* (CBS 870.85), *U. swartii* (CBS 868.85), and *U. fusiformis* (CBS 385.85). Bar = 10 µm.

(0.4%). An intermediate position of *U. gibberispora* between *U. ramanniana* and *U. westeae/U. swartii* was expected on morphological grounds because of its unilateral wall thickening of the spores, which could be

regarded as a precursor of the polar spore appendages in *U. westeae* and *U. swartii*. This hypothesis finds some support in the molecular findings of the ITS-1 sequence (Fig. 2).

Umbelopsis isabellina

The strain CBS 560.63 has one additional band of 340 bp in the *Hae*III profile HH2b and two additional bands 1274 and 164 bp in the *Sau*3A profile SH2b. CBS 208.32 and CBS 224.35 lack these bands (Fig. 1A–B, Table 2). These differences seen in the RFLP patterns could be correlated with either the paler (CBS 208.32) or the darker ochraceous-grey strains (CBS 224.35) so far distinguished as varieties of this species. Strain CBS 560.63 with intermediate pigmentation and a tendency to sectoring differs in its ITS1 sequence from CBS 208.32 and CBS 224.35 in 15 bp (8% difference). Other possible morphological differences among a larger set of isolates of this group require further study to establish a reliable distinction of species.

Umbelopsis roseonana and *U. nana*

*Hae*III RFLP analysis differentiated *Umbelopsis nana* (RFLP pattern HH4a) and *U. roseonana* (RFLP pattern HH4b) (Fig. 1A, Table 2). Sequences of three of the four isolates of *U. nana* from very diverse origins were found to have identical ITS-1 sequences, but *U. roseonana* (CBS 473.74) and one strain of *U. nana* (CBS 150.81) differ from the former in 6 bp out of 248 bp (3.2% difference). Thus the separation of *U. roseonana* from *U. nana* is supported by all molecular data. This separation was originally made because of the pigmentation of *U. roseonana*, which could thus form a link to *U. vinacea* with multispored sporangia. Our finding that CBS 150.81, ex-type of *U. versiformis*, has an identical ITS sequence as *U. roseonana*, incited a reexamination of the culture and a pale pink pigmentation was observed. This suggests that these taxa are conspecific and *U. versiformis* has priority over the synonymous *U. roseonana*.

DISCUSSION AND CONCLUSIONS

Our findings again underline the distant relatedness of the *Mortierella isabellina*-group with the *Mortierellaceae*. The *Mortierellaceae* deviate so strongly from the remaining *Mucorales* that they require classification in a separate order. Morphological criteria reflecting their divergence include their fine, mostly dichotomously branched vegetative mycelium, their colonies often exhibiting a garlic-like odour and their optimal sporulation on meagre media such as soil extract or potato-carrot agars. The oligotrophic life-style (but strong chitinolytic potential) of *Mortierella* distinguishes the genus/family from the mesotrophic *Mucorales*. When present, zygospores are hyaline and smooth-walled or slightly indented and supported by unequal suspensors. *Umbelopsis* is not known to form sexual structures and thus its position in relation to the *Mucorales* could not be assessed in morphological systematics. It is not closely related to other genera of the *Mucoraceae*, no matter how broadly this family is conceived, and takes a basal position among the *Mucorales*,

contrary to the suggestion by von Arx (1984). The recognition of a separate family, *Umbelopsidaceae*, is justified and strongly supported by the findings of O'Donnell *et al.* (2001) and our data.

The *M. isabellina*-group is monophyletic. The generic name *Umbelopsis*, originally understood as a genus of hyphomycetes, is the oldest suitable name available; *Micromucor* is of later date. This name is predated by *Micromucor* (Malchevskaya 1939: 25), which, however, was not validly published (Art. 36). The name *M. nau-movii* applies to a species isolated from peat and described as growing optimally at 30–46 °; it might therefore represent a species of *Rhizomucor*, although the spores, said to measure 2 × 1.7 µm, are too small. Species of *Umbelopsis* are known to be heat-resistant (Bollen & van der Pol-Luiten 1975) but they do not grow at such high temperatures.

A bipartition of the genus is evident from our phylogenetic analysis. This grouping was also seen in the analysis by Sugiyama *et al.* (2003) using 28S sequences. This subdivision is not correlated with a particular combination of morphological features and also does not coincide with the distinction of *Micromucor* and *Umbelopsis* as suggested by von Arx (1984). Two unispored species occur in one of the subclades together with multispored species. Pink and ochraceous pigmentation of the sporangia occur in both subclades. Species with angular spores occur in both subclades. Species with pronounced verticillately branched sporangiophores are not sharply differentiated from those with more irregular cymose branching. Therefore we chose to include all taxa within one genus, *Umbelopsis*.

The former varieties of *Mortierella ramanniana* are more distantly related and must therefore be raised to specific rank. It would have been interesting to correlate this refined classification of the *U. ramanniana* complex with the variation in esterase patterns observed by Peberdy & Turner (1968) if their isolates had been preserved. The bipartition of isolates identified thus far as *Mortierella ramanniana* var. *ramanniana* is a new finding and this problem will require further studies based on a more voluminous material. Similarly, the uniformity of *U. vinacea* has not been investigated sufficiently. The justification of distinguishing varieties or species in the *U. isabellina* complex also requires further studies.

Two pairs of species, *U. roseonana*/*U. nana*, and *U. westeae*/*U. swartii* are particularly closely related so that a distinction at varietal rank might be envisaged. Still their separation is supported by small but consistent morphological and molecular differences.

NOMENCLATURE

Umbelopsidaceae W. Gams & W. Meyer, **fam. nov.**

Zygomycetes, *Mucorales*. Coloniae fere lente crescentes, ex hyphis constanter tenuibus compositae. Sporangio-phora dense

cymosa vel verticillata, ramulis vulgo ex intumescencia stipitis oriundis, stratum velutinum formantia, saepe iuxta ramificationem et sub sporangio septata. Sporangia globosa vel elongata, saepe rubida vel ochracea, multisporea vel unisporea, sporis liberatis saepe columellam fere inconspicuam praebentia. Sporae globosae vel ellipsoideae, rotundatae vel angulares, nonnumquam appendicibus praeditae, pigmentatae sicut sporangia. Chlamydosporae lipoideis repletatae vulgo copiosae. Zygosporae ignotae.

Typus: *Umbelopsis* Amos & H. L. Barnett 1966.

A family of *Zygomycetes*, *Mucorales*. Colonies rather slow-growing, consisting of initially non-septate, slender hyphae. *Sporangiophores* densely cymosely or verticillately branched, with branches commonly arising in succession from an inflation of the stipe, forming a velvety layer, often with septa near the branching and at some distance below the sporangium. *Sporangia* globose or elongate, often reddish or ochraceous, multispored or single-spored, often with a more or less conspicuous columella. *Spores* globose or ellipsoidal, rounded or angular, in some species bearing appendages, pigmented like the sporangia. *Chlamydospores* filled with lipid material often abundantly formed. *Zygosporae* unknown.

***Umbelopsis* Amos & H. L. Barnett, *Mycologia* 58: 807 (1966).**

Type: *Umbelopsis versiformis* Amos & H. L. Barnett.

Syn.: *Mortierella* subgen. *Micromucor* W. Gams, *Persoonia* 9: 381 (1977).

Micromucor (W. Gams) Arx, *Sydowia* 35: 19 (1984) ['1982']; non Malchevskaya 1939, nom. inval.

Same diagnosis as for the family.

***Umbelopsis versiformis* Amos & H. L. Barnett, *Mycologia* 58: 807 (1966).**

Syn.: *U. roseonana* (W. Gams & Gleeson) Arx, *Sydowia* 35: 19 (1984) ['1982'].

Mortierella roseonana W. Gams & Gleeson, *Persoonia* 9: 112 (1976).

Umbelopsis isabellina* (Oudem.) W. Gams, *comb. nov.

Basionym: *Mortierella isabellina* Oudem., *Archs. néerl. Sci.*, sér. 2 7: 176 (1902).

Syn.: *Micromucor isabellinus* (Oudem.) Arx, *Sydowia* 35: 19 (1984) ['1982'].

Umbelopsis ramanniana* (A. Möller) W. Gams, *comb. nov.

Basionym: *Mucor ramannianus* A. Möller, *Z. Forst- u. Jagdw.* 35: 330 (1903).

Syn.: *Micromucor ramannianus* (A. Möller) Arx, *Sydowia* 35: 19 (1984) ['1982'].

Umbelopsis autotrophica* (E. H. Evans) W. Gams, *comb. nov.

Basionym: *Mortierella ramanniana* var. *autotrophica* E. H. Evans, *Trans. Br. mycol. Soc.* 56: 214 (1971).

***Umbelopsis angularis* W. Gams & M. Sugiy., in Sugiyama *et al.*, *Mycoscience*: in press (2003).**

Syn.: *Mortierella ramanniana* var. *angulispora* (Naumov) *sensu* Linnem., *Mucorineen-Gatt. Mortierella*: 30 (1941), and all subsequent authors [non Naumov 1935].

***Umbelopsis vinacea* (Dixon-Stew.) Arx, *Sydowia* 35: 20 (1984) ['1882'].**

Syn.: *Mucor angulisporus* Naumov, *Opredelitel' Mukorovykh (Mucorales)* edn 2: 30 (1935); nom. inval. (Art. 36).

Micromucor ramannianus var. *angulisporus* Naumov ex Váňová, *Česká Mykol.* 45: 6 (1991).

Umbelopsis multisporea T. Watanabe, *Trans. mycol. Soc. Japan* 18: 244 (1977).

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