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FULL PAPER

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Umbelopsis gibberispora sp. nov. from Japanese leaf litter and a clarification of *Micromucor ramannianus* var. *angulisporus*

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Abstract *Umbelopsis gibberispora* is described as a new species in the genus *Umbelopsis*, Umbelopsidaceae, Mucorales. The species differs from others in this genus by ellipsoidal sporangiospores with unilaterally thickened walls. Phylogenetic analyses based on nuclear large subunit ribosomal DNA (nLSU rDNA) partial sequences suggest that *U. gibberispora*, *U. swartii*, and *U. westeae* form a clade together with the strains of *Umbelopsis ramanniana*. The ex-type strain of *Micromucor ramannianus* var. *angulisporus* is found to be very close to *Umbelopsis vinacea*, whereas other isolates identified under the former name in the sense of Linnemann fall in the *U. ramanniana* subclade. For these isolates, a new species, *Umbelopsis angularis*, is introduced. Phylogenetic relationships among *Umbelopsis* species are discussed related to their attributes of the sporangial wall and mature spore shapes.

Key words Molecular phylogeny · Mucorales · Umbelopsidaceae · *Umbelopsis angularis* · *Umbelopsis gibberispora*

Introduction

Umbelopsis Amos & H.L. Barnett (Amos and Barnett 1966) is a genus in the Mucorales that is commonly isolated from soil or various other substrates. The members of the genus form a distinct group in the Mucorales possessing velutinous colonies and ochraceous or dull red sporangia except for *U. nana*, which has hyaline monosporous sporangia (sporangioles). However, the taxonomic position of this group was unclear for a long time mainly for two reasons: (1) small but distinct columellae are formed in *U. ramanniana*, which is one of the most frequently isolated species, while in other, closely related species the columella is reduced to a slightly convex transverse septum or completely lacking; and (2) zygospore formation has not been observed in any species of this group. Turner (1963) revised the group as a distinctive group of *Mortierella* Coemans. Linnemann (1941) and Gams (1977) also classified this group as a section or a subgenus in *Mortierella*, respectively. Because of the similar sporangial morphology, von Arx (1984) regarded the group as belonging to the Mucoraceae. He also recognized two different branching manners of sporangiophores in this group and reclassified the species in two genera, *Micromucor* Arx and *Umbelopsis*. Yip (1986b) pointed out that the umbellate branching system in *Umbelopsis* is no more than a condensed form of sympodial branching of *Micromucor*, and both branching types are observed in *U. isabellina*. Therefore, he suggested that *Umbelopsis* and *Micromucor* should be merged. Yip (1982, 1986a,b) described four new species in this group and classified all of them in *Umbelopsis,* although three of them produced sympodially branched sporangiophores.

Recently, molecular data revealed the phylogenetic position of *Umbelopsis* and also the relationships among its species. The genus was included in the Mucorales and sharply distinguished from the Mortierellaceae (Tanabe et al. 2000). Within the Mucorales, it forms the most basal branch (O'Donnell et al. 2001). Meyer and Gams (2003) analyzed the phylogenetic relationships of *Micromucor/Umbelopsis* species by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the whole rDNA internal transcribed spacer (ITS) region and phylogenetic analysis of ITS1 sequences. They reconfirmed the unrelatedness of these species with the Mortierellaceae and formally merged the two genera, classifying them in a new family, the Umbelopsidaceae in the Mucorales.

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In this article, we describe a new species in the group that resembles to *U. ramanniana* but differs in sporangiospore shape. We infer its phylogenetic position in *Umbelopsis* by using nuclear large subunit (nLSU) rDNA partial sequence data to supplement the results of Meyer and Gams (2003). The phylogenetic tree drew our attention to a discrepancy in the classification of the ex-type strain of *Micromucor ramannianus* var. *angulisporus* Naumov ex Váňová (1991) that we therefore analyzed further. As a consequence, we must regard this name as a synonym of *U. vinacea* and introduce a new species, *Umbelopsis angularis*, for isolates identified under that name in the sense of Linnemann.

Materials and methods

Strains examined

Two strains of *U. gibberispora* were isolated from leaf litter and deposited in CBS (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands) as CBS 109328 and CBS 101745. Other CBS strains of *Umbelopsis* species were taken from CBS for comparison. Species names, strain numbers, and accession numbers of the nucleotide sequence databases (DDBJ/EMBL/GenBank) used in this study are given in Table 1. Morphological observations of fungi were performed using single-spore or single-sporangium isolates grown at 25°C in darkness or at room temperature under daylight on malt extract agar (MEA; 2% Difco malt extract and 2% agar) and Miura agar medium (0.1% glucose, 0.02% $MgSO_4 \cdot 7H_2O$, 0.02% KCl, 0.2% $NaNO_3$, 0.2% Difco yeast extract, 1.3% agar, abbreviated LCA) (Miura and Kudo 1970). Capitalized color designations referred to the color charts by Rayner (1970). For descriptions and line drawings of microscopic features, aqueous mounts on slides made from LCA plates were used. Sporangiophore branch lengths were measured from aqueous in situ mounts in the sporulating area of LCA cultures. For scanning electron microscope (SEM) observation, small pieces (~5 mm^2) of culture on LCA were fixed in 2% glutaraldehyde solution at 4°C for 2 h, then dehydrated with a series of EtOH solutions; EtOH was substituted with 100% isoamylacetete. The specimens were dried in CO_2 using a critical point dryer HCP-2 (Hitachi, Tokyo, Japan) and coated with Au-Pd (approximately 100 Å thickness) using an Ion Sputter IB-3 (Eiko Engineering, Ibaraki, Japan), following the manufacturer's instructions. The specimens were observed by S-4100 (Hitachi) at 15 kV.

Sequencing

The cultivation of fungal strains and the extraction and purification of DNA from their mycelia were performed as described by Sugiyama et al. (1999). The nLSU rDNA gene including D1–D2 regions (~685 bp) was amplified by PCR protocols with NL1 and NL4 primers designed for nLSU rDNA (O'Donnell 1993).

PCR products were purified using a QIAquick PCR purification kit (QIAgen, Hilden, Germany) and sequenced directly using ABI 377 automated sequencers (Perkin-Elmer, Foster City, CA, USA) with the dye terminator method following the manufacture's instructions. The same

Table 1. Names, strain numbers, and accession numbers of species examined

Species	Strain no.[a]	Accession no.
Umbelopsis angularis W. Gams & M. Sugiyama	CBS 603.68 (T)	AB090294
Umbelopsis autotrophica (E.H. Evans) W. Gams	CBS 212.72	AB090295
Umbelopsis fusiformis Yip	CBS 385.85 (T)	AB090296
Umbelopsis gibberispora M. Sugiyama, Tokum. & W. Gams	CBS 101745	AB090297
	CBS 109328 (T)	AB090298
Umbelopsis isabellina (Oudem.) W. Gams	NRRL 1757[b]	AF157220
Umbelopsis nana (Linnemann) von Arx	NRRL 22420[b]	AF157221
Umbelopsis ramanniana (A. Möller) W. Gams	CBS 112.08[c]	AB090299
	CBS 219.47	AB090300
	CBS 243.58	AB090301
	NRRL 5844 (T)[b]	AF113463
Umbelopsis roseonana W. Gams & Gleeson	CBS 473.74 (T)	AB090302
Umbelopsis swartii Yip	CBS 868.85 (T)	AB090303
Umbelopsis vinacea (Dixon-Stewart) von Arx	CBS 222.29[d]	AB090304
	CBS 236.82	AB090305
	CBS 561.63	–
Umbelopsis westeae Yip	CBS 870.85 (T)	AB090306
Mucor hiemalis Wehmer f. *hiemalis*	NRRL 3624 (NT)[b]	AF113468
Mucor circinelloides f. *lusitanicus* (Bruderlein) Schipper	ATCC 1216b[b]	AJ271061

T, ex-type strain; NT, ex-neotype strain

[a] ATCC, American Type Culture Collection, Manassas, VA, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NRRL (ARS), Agricultural Research Service culture collection, Peoria, IL, USA

[b] Retrieved from DNA (GenBank/EMBL/DDBJ) or LSU rRNA database

[c] Referred to as *Umbelopsis* sp. in Meyer and Gams (2003)

[d] Ex-type strain of *Micromucor ramannianus* var. *angulisporus* (Váňová 1991)

primers were used for sequencing reactions and sequences were determined over both strands of each gene. Sequences determined in this study were deposited in the DNA Data Bank of Japan (DDBJ); their accession numbers are shown in Table 1.

Alignment

To obtain a reliable alignment data set, we adopted the profile alignment process described by Honda et al. (1999) and Iwamoto et al. (2002). An aligned data set of the representative nLSU rDNA sequences was downloaded from the rRNA www server at the University of Antwerp [URL: http://rrna.uia.ac.be/] (Wuyts et al. 2001). The secondary structure of nLSU rDNA was considered in their alignment; thus, we used the data set as a profile to obtain a reliable alignment for the following phylogenetic analyses. *Blastocladiella emersonii* (X90411), *Entomophaga aulicae* (U35394), *Mucor circinelloides* f. *lusitanicus* (deposited as *M. racemosus*; AJ271061 and M26190), and *Rhizomucor miehei* (AF198253 and AF205941) were selected as the aligned data set.

The sequence data of the Umbelopsidaceae and *M. hiemalis* (AF113468) were individually added to the aligned data set through a profile alignment process by Clustal X version 1.81 (Thompson et al. 1997). The gap opening and the gap extension parameters for both pairwise and multiple alignments were 16.0 and 6.66, respectively. Two variable sites in the aligned data, which corresponded to positions 281–296 and 343–370 in the complete nLSU rDNA sequence of *Saccharomyces cerevisiae* (J01355), could not be well aligned because these sites contained many gaps. Clustal X sometimes aligned differently in each profile alignment process even when the sequence data were identical. Therefore, these sites were realigned with different gap penalty parameters. We tried several combinations of the parameters to obtain a stable and appropriate alignment for these sites. The gap opening parameter for both pairwise and multiple alignments was changed to 10.00, and the gap extension parameter was changed to 0.10 for pairwise alignment and to 0.20 for multiple alignment. Finally, the aligned data were checked and optimized by eye and excess data were removed to construct an alignment data set. *Mucor circinelloides* f. *lusitanicus* and *M. hiemalis* were selected as outgroups because *Umbelopsis* was included in the Mucorales as the basal sister-group to all other mucoralean taxa (Tanabe et al. 2000; O'Donnell et al. 2001). The alignment was deposited in TreeBASE [http://www.treebase.org/treebase/] as S870. The positions with gaps were removed from the data set for subsequent phylogenetic analyses.

Phylogenetic analyses

For phylogenetic analyses, neighbor-joining (NJ) (Saitou and Nei 1987), maximum-parsimony (MP), and Bayesian analysis (BA) methods (Rannala and Yang 1996; Mau and Newton 1997; Mau et al. 1999; Murphy et al. 2001) were used. BA is a relatively new and powerful method for analyzing phylogenetic structures (Huelsenbeck et al. 2000, 2001; Hall 2001). Bayesian inference can be used to calculate the so-called posterior probability of phylogenetic trees, given a set of data and an evolutionary model. BA seeks to find the tree that maximizes this posterior probability (Larget and Simon 1999; Yang and Rannala 1997). A common approach is to sample phylogenies from the posterior probability distribution by means of a Markov chain Monte Carlo (MCMC) procedure as computed by the Metropolis–Hastings–Green (MHG) algorithm (Green 1995; Hastings 1970; Metropolis et al. 1953). The result of BA analysis is given as the best set of a large number of ($>$10 000) trees. Therefore, the statistical reliability of each clade can be estimated by the proportion of times it occurs in the obtained topology. This estimation is based on a principle different from that underlying bootstrap analysis. In addition, a variant of MCMC, called Metropolis-coupled Markov chain Monte Carlo (MCMCMC; Geyer 1991), runs several MCMCs simultaneously with random swapping of the states of each chain, thus allowing a search that avoids the arrival at suboptimal topologies.

NJ analysis was performed using PAUP* version 4.0 beta 10 (4.0b10) (Swofford 2001). Distances were estimated based on the Hasegawa, Kishino, and Yano (HKY85) model (Hasegawa et al. 1985) with or without the assumption of rate heterogeneity among sites. The topology of the tree was assessed by bootstrap analysis involving 1000 random resamplings (Felsenstein 1985).

MP analysis was performed by PAUP* (4.0b10). Heuristic searches were performed using the tree bisection and reconnection (TBR) for branch swapping. The topology of the starting tree for the heuristic search was obtained from the random stepwise addition option with 100 replicates. Bootstrap analysis was performed for 1000 random resampled data by using a heuristic search of the TBR branch swapping and the random stepwise addition option (10 replicates) for the starting topologies.

Bayesian analysis (BA) was performed by MrBayes version 2.0.1 (Huelsenbeck and Ronquist 2000). The HKY85 model with gamma-distributed among-site rate variation and empirical base frequencies was employed as the substitution model. In this model, 1 500 000 generations of MCMCMC were generated and sampled in each group of 1000 generations, from which 15 000 trees were obtained. The first 2000 trees were discarded because the likelihood values did not converge to a stable value. The remaining 13 000 trees were imported in PAUP* (4.0b10), and the best trees were selected according to their likelihood value based on the HKY85 model. The proportions of each internal clade were also calculated by computing a majority consensus tree on PAUP* (4.0b10).

The obtained tree topologies for each clade were evaluated by Kishino–Hasegawa (KH) and Shimodaira–Hasegawa (SH) tests (Kishino and Hasegawa 1989; Shimodaira and Hasegawa 1999) by PAUP* (4.0b10) with full optimization option.

Results and discussion

Taxonomy

Umbelopsis gibberispora M. Sugiyama, Tokum. & W. Gams, sp. nov. Figs. 1,2

Coloniae pallide vinaceae, parce sporulantes. Sporangiophora sympodialiter ramosa, ramis 115–885 μm longis, raro septatis, septo ultimo 28–40 μm sub columella. Sporangia globosa vel subglobosa, 19.5–35 μm diametro, rubida, tenuitunicata, sporis liberatis columellam relinquentia. Columellae globosae vel applanatae. Sporangiosporae ellipsoideae, pariete unilateraliter inspissato, 4–6 × 2–3 μm, dilute roseae. Chlamydosporae copiosae in mycelio submerso, leves, crassitunicatae, seu majores, 24.5–46 μm diametro, intercalares, seu minores, 5–17 μm diametro, terminales vel intercalares.

Holotypus: Colonia exsiccata FZ-1002 (CBM), isolata a T. Ohsono e foliis dejectis *Fagi crenatae* prope Kyoto in Japonia, in collectione fungorum Musei et Instituti Historiae Naturalis Chiba praeservatus.

Etymology: Latin *gibber* = hump, referring to the humpshaped wall thickening of the sporangiospores.

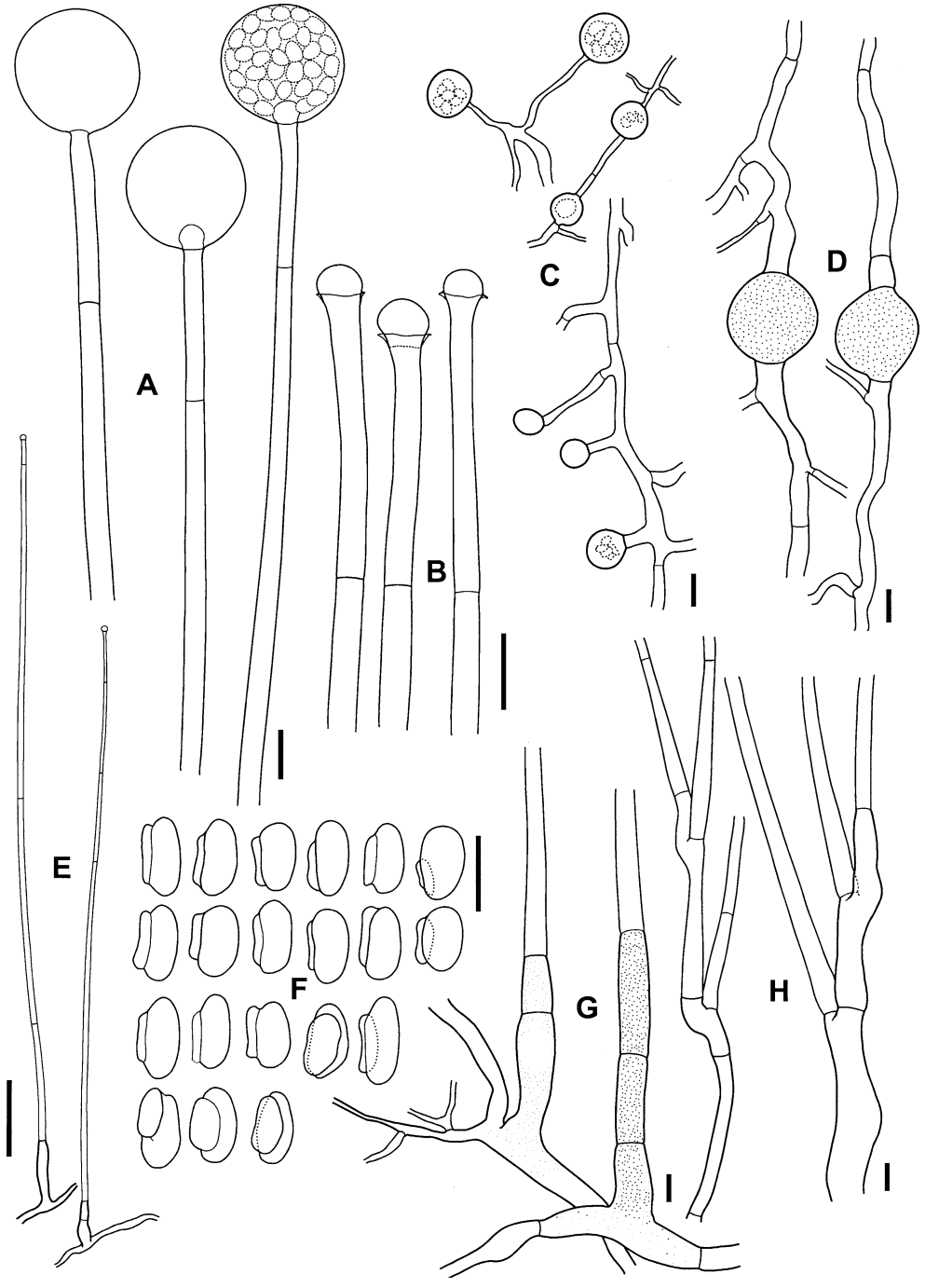

Fig. 1. *Umbelopsis gibberispora* CBS 109328. **A** Sporangia. **B** Columellae. **C** Microchlamydospores. **D** Macrochlamydospores. **E** Young sporangiophores. **F** Sporangiospores. **G** Lower portions of young sporangiophores. **H** Lower portion of sporangiophores showing branching habits. *Bars* **A–D**, **G**, **H** 10 μm; **E** 100 μm; **F** 5 μm

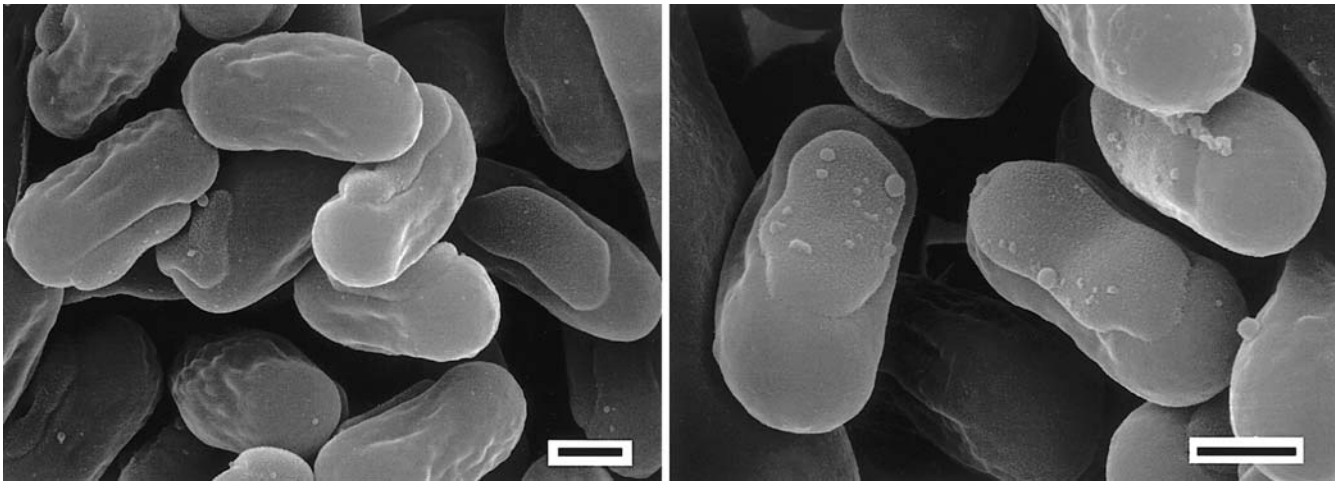

Fig. 2. Scanning electron microscopy (SEM) of sporangiospores of *U. gibberispora* (CBS 109328). *Bars* 1 μm

Colonies on MEA after 5 days reaching 39–41 mm in diameter at 25°C, velutinous with aerial growth consisting mainly of sporangiophores in the central area, at first white, later becoming Pale Vinaceous due to sporulation; sporulation scanty, restricted to the central area of the cultures; substrate mycelium dense, submerged.

Colonies on LCA after 5 days attaining 15–21 mm in diameter at 25°C, velutinous, colorless to white; sporulation more abundant in similar shade, restricted to the central area of the colony; substrate mycelium less dense than on MEA, submerged. Sporangiophores with several sympodial branches ~115–885 μm in length, often arising in succession from a widened part of the subtending stalk, 4–11 μm wide near the base and slightly tapering to 3–5.5 μm near the tip, 2–7 septate, with one septum near the base and the uppermost septum at 28–40 μm below the columella. Sporangia globose to subglobose, 19.5–35 μm in diameter, dull red; walls thin and on dehiscence leaving a collarette. Columellae subglobose to flattened, 4.5–8 μm in diameter, 1.5–5 μm high. Sporangiospores ellipsoidal, the wall thickened on one side (humpshaped), 4–6 × 2–3 μm, pale pink under the microscope, dull red in mass. Chlamydospores produced abundantly by substrate hyphae, smooth-walled, thick-walled, guttulate, of two types according to size and shape; macrochlamydospores globose to subglobose, 24.5–46 μm in diameter, intercalary, filled with lipid drops and droplets; microchlamydospores variable in shape, terminal or intercalary, 5–17 μm in diameter.

Materials examined: Holotype FZ-1002 (CBM) (dried culture) in the Natural History Museum and Institute, Chiba, Japan. Ex-type strain, CBS 109328, isolated by T. Ohsono from leaf litter of beech (*Fagus crenata* Bl.) collected at the Ashiu Experimental Forest of Kyoto University, Miyama City, Kyoto, November 18, 1999. CBS 101745, isolated by S. Tokumasu from a fallen needle of *Pinus luchuensis* Mayr, collected in Ishigaki City, Okinawa, June 4, 1984.

Umbelopsis angularis W. Gams & M. Sugiyama, sp. nov. Fig. 3A–F

Mortierella ramanniana var. *angulispora* (Naumov) Linnem., nom. inval. Art. 36, *sensu* Linnemann 1941, and all subsequent authors [non-*Mucor angulisporus* Naumov 1935]. Ab *Umbelopside ramanniana* sporis angularibus, 2.5–4 μm diametro differt, sed ceterum columellis conspicuis et chlamydosporis copiosis haec speciei similis.

Holotypus: Herbarium CBS 603.68 (dried culture) in herb. CBS, isolated by W. Gams from soil collected at Baarn, Maarschalksbos, Netherlands, 1968. Ex-type strain: CBS 603.68, preserved under same number.

Colonies on MEA after 5 days reaching 34–45 mm in diameter at 25°C, velutinous with aerial growth consisting mainly of sporangiophores, Livid Red to somewhat brownish Vinaceous due to abundant sporulation; sporulation abundant; substrate mycelium dense, submerged. Colonies on LCA after 5 days attaining 33–34 mm in diameter at 25°C, velutinous, colorless to pale vinaceous due to abundant sporulation; substrate mycelium less dense than on MEA, submerged. Sporangiophores with several cymose branches ~110–610 μm in length, often arising in succession from a widened part of the subtending stalk, 2.5–6.5 μm wide near the base and slightly tapering to 2–4 μm near the tip, 2–7 septate, one septum near the base and the uppermost septum at 20–45 μm below the columella. Sporangia globose to subglobose, 11–21 μm in diameter, reddish brown; walls thin and on dehiscence leaving a collarette. Columella subglobose, 7–8 μm in diameter. Sporangiospores polyhedral, mostly showing 5–7 angles in face view, 2.5–4 μm in diameter. Chlamydospores abundantly produced, filled with lipid droplets; macrochlamydospores 21–40 μm; microchlamydospores 5–10 μm in diameter. Very limited growth on Czapek agar suggests thiamine requirement.

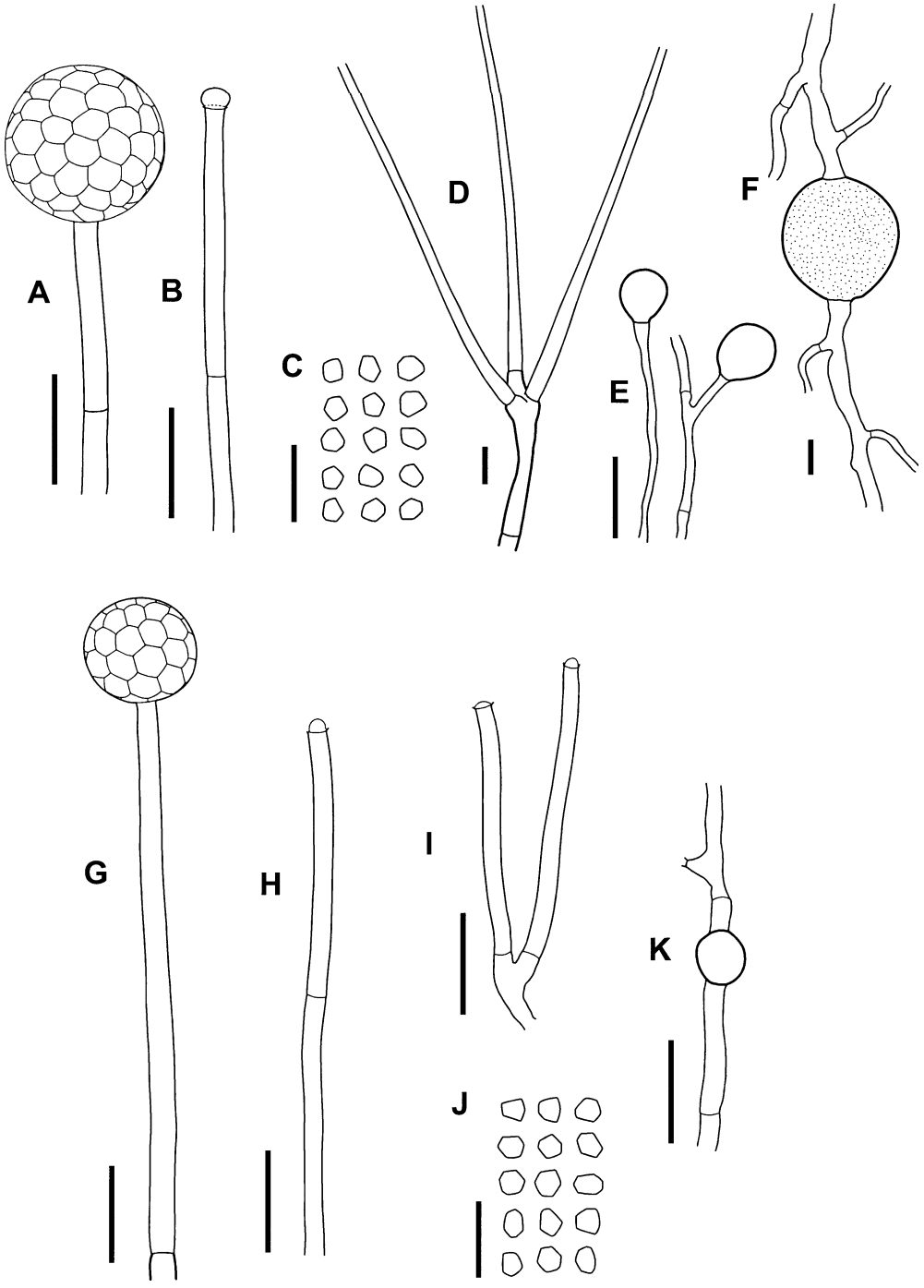

Fig. 3. Comparison of *U. angularis* (**A–F**, CBS 603.68) and *U. vinacea* (**G–K**, CBS 222.29, ex-type strain of *Micromucor ramannianus* var. *angulisporus*). **A**, **G** Sporangia. **B**, **H** Columellae. **C**, **J** Sporangiospores. **D**, **I** Sporangiophore branches. **E**, **K** Microchlamydospores. **I** Macrochlamydospore. *Bars* 10 μm

Phylogenetic relationships among *Umbelopsis* spp. based on nLSU rDNA sequence data

Figure 4 shows phylogenetic relationships among the *Umbelopsis* species. Three topologies obtained by BA showed the maximum likelihood value of −2621.95. However, the other two methods (NJ and MP) yielded almost identical topologies and the KH and SH tests showed that there was no significant difference among these topologies (data not shown). Therefore, we show one of the BA trees, adding the bootstrap values of each clade obtained by NJ and MP in Fig. 4 besides the proportion values of the BA trees. It is interesting that the proportional values of BA tend to be lower than corresponding bootstrap values of NJ and MP near the root of clade II whereas they are higher near the tip. A similar analysis applying the BA method with NJ and MP to some additional molecular data sets would be necessary to understand the statistical differences among BA and other methods.

Two major clades (I and II) are recognized in the tree, with high bootstrap values in NJ and MP, although the probabilities of clade II in BA were low. The topology of our tree is principally consistent with that of the MP tree based on ITS1 sequences by Meyer and Gams (2003), and

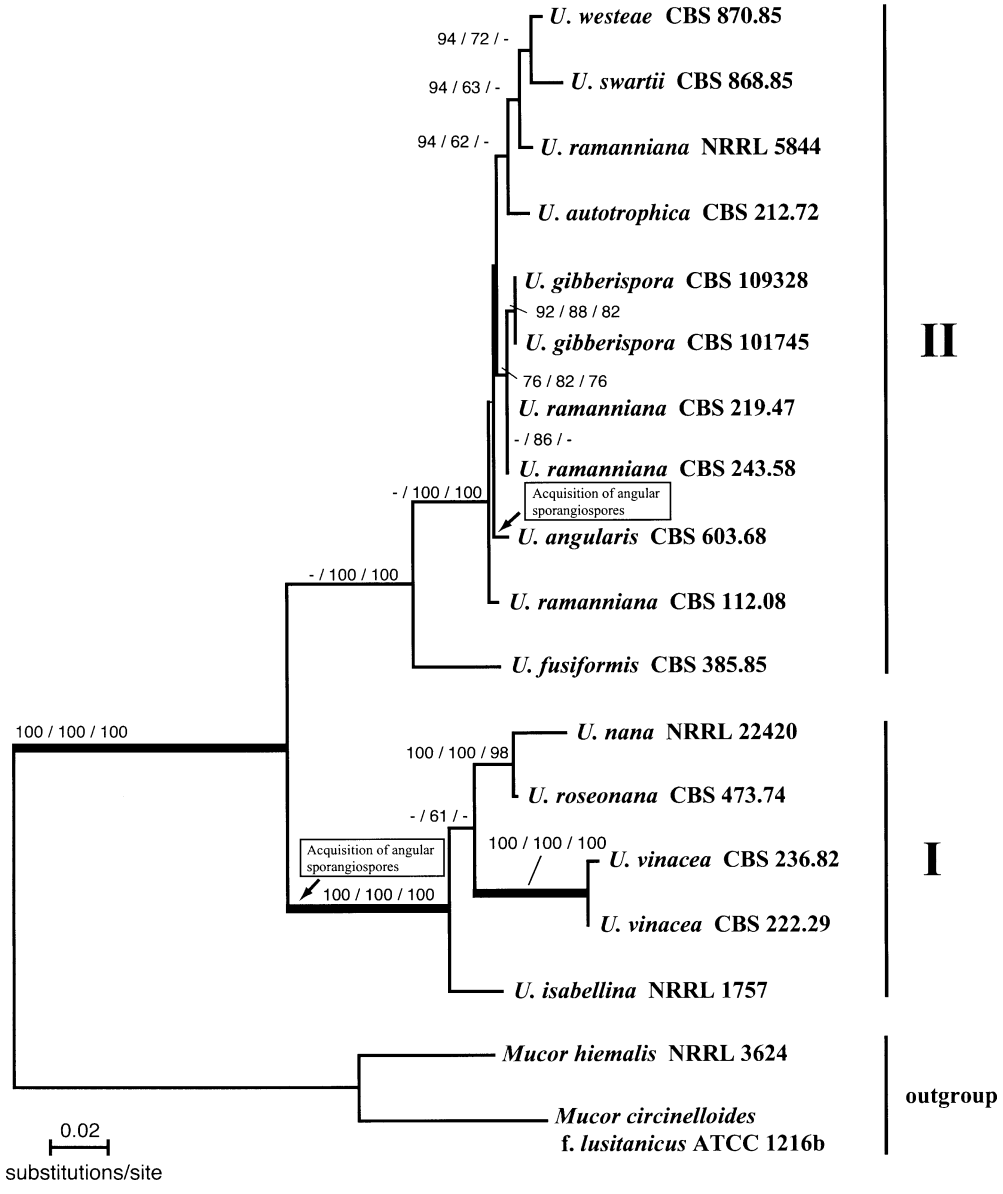

Fig. 4. One of three best Bayesian analysis (BA) trees based on nuclear large subunit (nLSU) rDNA sequence data (18 OTUs, 624 sites). Numbers above *nodes* refer to proportions among 13000 BA trees (*left*) and 1000 bootstrap samplings of values of neighbor-joining (NJ) tree (*middle*) and maximum-parsimony (MP) tree (*right*) (only values >60% shown). *Darkened branches* indicate 100% support in all methods

six different strains analyzed by us added some new findings about the phylogenetic relationships among *Umbelopsis* species.

The two strains of *Umbelopsis gibberispora* are included in clade II together with *U. angularis*, *U. autotrophica*, four *U. ramanniana* strains, *U. swartii*, and *U. westeae*. These species have several morphological characters in common, such as pinkish or reddish multi-spored sporangia, small but distinctive columellae, and a septum located at a short distance (<50μm) from the tip of the sporangiophore (subsporangial septum). *Umbelopsis fusiformis* was also included in clade II, but positioned at the root of the clade, distinct from the other three taxa in this clade. This species is characterized by fusiform sporangia lacking a columella. In the ITS analysis (Meyer and Gams 2003), *U. fusiformis* CBS 385.85 was part of a monophyletic clade together with *U. swartii* CBS 868.85 and *U. westeae* CBS 870.85 (86% bootstrap support). Further molecular data will be required to elucidate the exact phylogenetic position of *U. fusiformis.*

The six species in clade II, except for *U. fusiformis,* are morphologically close to each other. *Umbelopsis gibberispora* differs from the remaining species by its unique sporangiospore morphology (see Figs. 1, 2). Sporangiospore production of *U. gibberispora* was easily lost during cultivation and transfer on agar media. In addition, very few sporangia were formed when the fungus was grown in darkness, and only abundant macrochlamydospores were produced. Such a physiological shift from sporangium formation to multiplication by macrochlamydospores is not common in *Umbelopsis* species, but Yip (1986a) described a similar phenomenon for *U. fusiformis.* Further physiological studies are necessary to clarify the relationships between cultural conditions and morphological differentiation of *U. gibberispora*.

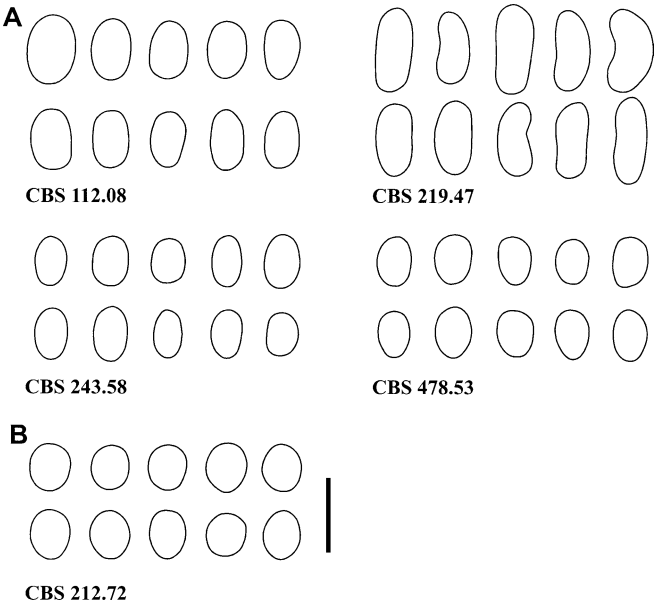

Fig. 5. Comparisons of sporangiospores of *U. ramanniana* (**A**) and *U. autotrophica* (**B**). *Bar* 10 μm

The sporangiospore shape of *U. ramanniana* is subglobose to ellipsoid without any appendage or thickening, but the length/width ratio varies among strains (Fig. 5). Evans (1971) showed that strains of *U. ramanniana* with globose sporangiospores differed from those with oval or ellipsoidal sporangiospores also in physiological characters such as growth speed or thiamine dependency. She described thiamine-independent strains with globose sporangiospores as a variety (*Mortierella ramanniana* var. *autotrophica*), which Meyer and Gams (2003) raised to species level as *Umbelopsis autotrophica*. However, an examination using 75 isolates of *U. ramanniana* showed that the spore shape varied continuously from almost globose to ellipsoidal among the isolates, and it was difficult to make a clear delimitation on morphological grounds (Sugiyama and Tokumasu, unpublished data). In Fig. 4, *U. ramanniana* isolates are included in clade II together with other *Umbelopsis* species but they do not form a clear subclade. Also, Meyer and Gams (2003) found a bipartition among isolates identified as this species, while *U. autotrophica* appeared sufficiently distinct. These results strongly suggest that the taxa included in *U. ramanniana* are still an assemblage of several species that are genetically distinct but difficult to distinguish morphologically. *Umbelopsis angularis*, *U. gibberispora*, *U. swartii*, and *U. westeae* are probably the exceptional cases in this species complex that can be recognized easily by sporangiospore morphology. Recently, phylogenetic analyses based on multiple gene sequence data have revealed the existence of cryptic species in certain pathogenic fungi (Geiser et al. 1998; Kasuga et al. 1999; Koufopanou et al. 1997, 2001). Such techniques should also be applied to analyze the phylogenetic relationships among the taxa in clade II .

CBS 222.29, designated as ex-type strain of *Micromucor ramannianus* var. *angulisporus* by Váňová (1991), originating from N.A. Naumov, is a member of clade I situated next to *U. vinacea* (CBS 236.82). Another strain previously preserved as *Mortierella ramanniana* var. *angulispora* (CBS 603.68) belongs to clade II. These taxa have in common the production of reddish-brown colonies and angular sporangiospores. Turner (1963) already recognized that the strain of *M. ramanniana* var. *angulispora* deposited to CBS by Naumov as *Mucor angulisporus* was morphologically indistinguishable from *U. vinacea.* The name of *U. vinacea* (Dixon-Stewart 1932) obviously has priority because the epithet *angulisporus* was initially invalidly introduced as *Mucor angulisporus* (Naumov 1935, 1939, without a Latin diagnosis) and validated only by Váňová (1991) at varietal level. Therefore, we identify this strain as *U. vinacea.* However, CBS 222.29 produces longer sporangiophores (up to 120 μm) and smaller sporangiospores (2.5–3.7 μm) than other *U. vinacea* isolates (Table 2), although only three base differences were observed between the nLSU rDNA sequences of CBS 222.29 and CBS 236.82, just the same magnitude of difference observed between two strains of *U. gibberispora*. Further morphological comparison using larger numbers of *U. vinacea* isolates and phylogenetic analyses based on other genes are necessary to determine whether CBS 222.29 can be regarded as conspecific with *U. vinacea*.

Linnemann (1941) applied the meager original description of *Mucor angulisporus* by Naumov (1935, 1939) to the taxon that is close to *U. ramanniana* but produces angular sporangiospores. This fungus has a worldwide distribution and differs from *U. vinacea* by sporangia with distinctive columellae, regular occurrence of a subsporangial septum, and abundant formation of large and small chlamydospores. The sporangial morphology of CBS 603.68 is consistent with Linnemann's description and clearly distinguishable from that of CBS 222.29 and other *U. vinacea* strains (see Fig. 3, Table 2). Meyer and Gams (2003) analyzed other strains deposited as *M. ramanniana* var. *angulispora* (CBS 914.85) and *U. vinacea* (CBS 236.82 and CBS 561.63) by ITS1 sequences and RFLP of the ITS1–5.8S–ITS2 region. They also found that these two taxa fell into each of the two major subclades of *Umbelopsis*, corresponding to clades I and II in this study. Therefore, we propose a new species, *U. angularis*, for the strains falling in clade II with angular sporangiospores (CBS 603.68 and CBS 914.85), while the variety *angulispora* is probably conspecific with *U. vinacea*.

Umbelopsis isabellina, U. vinacea, U. roseonana (=*U. versiformis* as shown by Meyer and Gams 2003), and *U. nana* form clade I. According to Meyer and Gams (2003), *U. ovata* Yip is also included in this clade. These species are characterized by lacking columellae or having very inconspicuous columellae, usually without a subsporangial septum. In addition, all species of clade I produce thick-walled, angular, and polygonal sporangiospores or monosporous sporangia (sporangioles) whereas most taxa in clade II form spherical or ellipsoidal sporangiospores or variations with appendages. Sporangiospores of *U. isabellina* are also slightly polygonal under SEM observation (O'Donnell 1979; M. Sugiyama, unpublished data) but show blunt angles under the light microscope. Therefore,

Table 2. Comparison of selected characters of *Umbelopsis angularis* and *U. vinacea*

	Growth on MEA[a], 25°C, 6 days	Optimum temperature (°C)	Sporangiophore[b]			Sporangia[b] diameter	Macrochlamydospores[b] diameter	Sporangiospores[b] diameter
			Length	Width (base)	Width (tip)			
U. angularis CBS 603.68	50	25	110–610 (291)	2.5–6.5 (4.8)	2.0–4.0 (2.8)	11.5–20.5 (16.3)	21.0–40.0 (31.0)	2.6–3.7 (3.1)
U. vinacea								
CBS 222.29	34	25	20–120 (55)	1.5–3.0 (2.1)	1.5–2.0 (1.6)	5.5–11.0 (8.5)	–[c]	2.6–3.7 (3.4)
CBS 236.82	31	25–30	12–50 (22)	1.5–2.0 (1.8)	1.5–2.5 (1.9)	6.0–10.0 (8.8)	–	3.2–4.7 (3.9)
CBS 561.63	36	25	15–100 (37.5)	2.5–4.0 (3.1)	2.0–4.0 (2.6)	7.0–13.5 (10.5)	–	2.9–3.9 (3.5)

MEA, malt extract agar
[a] Maximum colony diameter (mm)
[b] Range and average (in parentheses) (µm).
[c] Scant production

we assume that polygonal sporangiospores were probably acquired at the root of the clade I (see Fig. 4).

Polygonal sporangiospores are tightly packed and adhere face to face without free space in a sporangium. Tokumasu et al. (1990) suggested that this polygonal sporangiospore shape is the result of tight sporangial walls that do not allow the development of rounded spore shapes in their maturing stage. This hypothesis is consistent with sporangiospore variation observed in the species of clade I. The tight sporangial wall, a plesiomorphic feature of clade I, physically limits free expansion and further sporangiospore shape differentiation in the clade. Therefore, only a differentiation of spore size or numbers in a sporangium could evolve in this clade, whereas several kinds of appendages of sporangiospores (*U. gibberispora*, *U. swartii*, and *U. westeae*) could evolve in clade II.

The taxa of clade I produce smaller numbers of sporangiospores in a sporangium than those of clade II, or even monosporous sporangia (sporangioles). The tree topology suggested that the thick-walled and larger monosporous sporangia of *U. nana* and *U. roseonana* in the clade are probably derived from polysporous sporangia by a loss of the capacity to divide the terminal sphere into small spores (as is often observed in *Mortierella alpina*). These large propagules are expected to be more resistant to adverse conditions than are small spores from polysporous sporangia. Such a reduction of spore numbers and increase in size would cause a functional shift for the sporangiospores from the dispersal of numerous relatively vulnerable progeny (*r* strategy; MacArthur 1962; MacArthur and Wilson 1967) to the production of scantier but also more durable progeny (*k* strategy). In other words, the acquisition of a tight sporangial wall at the root of clade I would determine a different course of evolution from the taxa in clade II.

As exemplified by *U. angularis*, polygonal sporangiospores were also acquired independently in clade II (see Fig. 4). The branch length that separates *U. angularis* from the other taxa in clade II is, however, much shorter than the branches in clade I, and we have not found any additional taxon with polygonal sporangiospores in clade II. These facts suggest that relatively few mutations are required to determine a tight sporangial wall and polygonal sporangiospores, and that this phenomenon occurred at least twice in the evolution of the Umbelopsidaceae. In clade II, this was probably a recent event, and no further recognizable differentiation has occurred since then. Further physiological, ecological, and developmental studies are necessary to evaluate this hypothesis.

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