

# Proposal of *Mingxiaea* gen. nov. for the anamorphic basidiomycetous yeast species in the *Bulleribasidium* clade (Tremellales) based on molecular phylogenetic analysis, with six new combinations and four novel species

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The distinction and monophyletic property of the basidiomycetous yeast species in the *Bulleribasidium* clade of the order Tremellales was resolved by molecular phylogenetic analysis based on the combined sequences of the 18S rRNA gene, internal transcribed spacer (ITS) region including 5.8S rRNA gene and 26S rRNA gene D1/D2 domain. The addition to the clade of new anamorphic species identified among ballistoconidium-forming yeasts isolated from China confirmed and strengthened the separation of this clade from other clades or lineages in the order Tremellales. A new anamorphic genus, *Mingxiaea* gen. nov. (type species *Mingxiaea variabilis* comb. nov.) is therefore proposed to accommodate the anamorphic species in the *Bulleribasidium* clade. Six new combinations are proposed for the described species of this clade which were formerly assigned to the genus *Bullera*. Four novel species in the new genus were identified among 16 ballistoconidium-forming yeast strains isolated from plant leaves collected in Hainan province, southern China, by D1/D2 and ITS sequence analyses. The novel species are described as *Mingxiaea sanyaensis* sp. nov. (type strain SY-3.23<sup>T</sup> = AS 2.3623<sup>T</sup> = CBS 11408<sup>T</sup>), *Mingxiaea hainanensis* (type strain WZS-8.13<sup>T</sup> = AS 2.4161<sup>T</sup> = CBS 11409<sup>T</sup>), *Mingxiaea foliicola* (type strain WZS-8.14<sup>T</sup> = AS 2.3518<sup>T</sup> = CBS 11407<sup>T</sup>) and *Mingxiaea wuzhishanensis* (type strain WZS-29.8<sup>T</sup> = AS 2.4163<sup>T</sup> = CBS 11411<sup>T</sup>).

## INTRODUCTION

*Bullera variabilis* was described by Nakase & Suzuki (1987) to accommodate a group of yeast strains forming variously shaped ballistoconidia. Bai *et al.* (2001) redefined the concept of *Bullera variabilis* and reclassified the strains

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the 18S rRNA gene, ITS region and 26S rRNA gene D1/D2 domain sequences determined in this study are GQ438828–GQ438843 and GU327539–GU327541.

Additional phylogenetic trees are available with the online version of this paper.

previously assigned to this species based on 18S rRNA gene and internal transcribed spacer (ITS) region sequencing and DNA–DNA reassociation. The strains represented five species, four of which belong to the recently proposed genus *Derxomyces* (Bai *et al.*, 2001; Wang & Bai, 2008). Molecular phylogenetic analysis of the genus *Cryptococcus* and related tremellomycetous yeast species based on 18S rRNA gene sequencing showed that *Bullera variabilis* was not closely related to the *Bulleromyces* lineage containing the type species of the genus *Bullera*, but was located in a separate branch basal to the *Hannaella luteola* (formerly *Cryptococcus luteolus*) lineage with only moderate bootstrap support in the phylogenetic tree drawn from maximum-parsimony analysis (Takashima & Nakase, 1999). The

distinction of the *Bullera variabilis* branch was also shown in a neighbour-joining tree, but its position was not definitely resolved (Takashima & Nakase, 1999). Extensive molecular taxonomic studies on basidiomycetous yeast species based on sequence analyses of the D1/D2 variable domains of the large subunit (26S) rRNA gene and ITS region also indicated that *Bullera variabilis* was located in a separate branch that was only distantly related to the *Bulleromyces* clade which contains the type species of the genus *Bullera* (Fell *et al.*, 2000; Scorzetti *et al.*, 2002).

Boekhout *et al.* (1991) observed mating and dikaryotic hyphae with clamp connections between two *Bullera variabilis* strains; however, they did not observe basidial development. A teleomorphic species, *Bulleribasidium oberjochense*, closely related to *Bullera variabilis* was described by Sampaio *et al.* (2002). The basidium structure and morphology of *Bulleribasidium oberjochense* differ markedly from those of *Bulleromyces albus* (Boekhout *et al.*, 1991). The latter is the teleomorph of *Bullera alba*, which is the type species of the genus *Bullera*. Unlike *Bulleromyces albus*, which forms typically longitudinally to obliquely septate and globose basidia, *Bulleribasidium oberjochense* normally forms transversally septate and cylindrical basidia (Sampaio *et al.*, 2002). The differences in sexual stages support the distinction of the anamorphic species in the *Bulleribasidium* clade from the *Bullera* species in the *Bulleromyces* clade at the generic level.

The polyphyletic nature of the genus *Bullera* and related genera as defined on the basis of only phenotypical criteria (Boekhout & Nakase, 1998) has long been recognized. The requirement for revising the genus has been emphasized by the continued discovery of many new *Bullera* species in recent years, which has contributed to the increase in the polyphyletic nature of the genus. The distribution of species of the genus *Bullera* has expanded from the orders Tremellales and Filobasidiales to the order Trichosporonales (Nakase *et al.*, 2002; Fungsin *et al.*, 2006). Reclassification of the genus *Bullera* and related genera of basidiomycetous yeasts has been under way and two new genera, *Dexomyces* and *Hannaella*, have been proposed for the species of the genus *Bullera* in two distinct clades of the *Hannaella luteola* lineage in the order Tremellales (Wang & Bai, 2008).

In recent years, five novel anamorphic species closely related to *Bulleribasidium oberjochense* have been described from different countries in Asia (Bai *et al.*, 2003; Fungsin *et al.*, 2003; Nakase *et al.*, 2004), expanding the branch to a distinct clade. These studies also suggest that species of the *Bulleribasidium* clade are commonly distributed in natural sources. In a recent survey of ballistoconidium-forming yeasts associated with plant leaves in Hainan province, southern China, four novel anamorphic species represented by 16 strains were revealed to belong to the *Bulleribasidium* clade by rRNA gene sequence analysis. The result confirmed and strengthened the distinction of the *Bulleribasidium* clade. In order to avoid any further increase in the polyphyletic nature of the genus *Bullera*,

we propose a new genus, *Mingxiaea*, to accommodate the anamorphic species of the *Bulleribasidium* clade. Four novel species in the new genus, isolated from plant leaves, are described.

## METHODS

**Yeast strains and phenotypic characterization.** The strains studied are listed in Table 1. They were isolated from wilting plant leaves using the improved ballistoconidia-fall method as described by Nakase & Takashima (1993). Morphological, physiological and biochemical characteristics were examined in liquid media according to standard methods (Yarrow, 1998). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1987). Extraction, purification and identification of ubiquinones were carried out according to Yamada & Kondo (1973).

**Sequencing and molecular phylogenetic analysis.** 18S rRNA gene sequences were determined according to Wang *et al.* (2003). The ITS (including 5.8S rRNA gene) and 26S rRNA gene D1/D2 domain sequences were determined using the methods described by Bai *et al.* (2002). Sequences were aligned by using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed from the evolutionary distance data calculated from Kimura's two-parameter model (Kimura, 1980) by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analyses (Felsenstein, 1985) were performed from 1000 random resamplings. Maximum-parsimony analysis was performed using MEGA version 4 (Tamura *et al.*, 2007) and the heuristic search (close-neighbour-interchange) was used.

## RESULTS AND DISCUSSION

### Proposal of *Mingxiaea* gen. nov.

In the phylogenetic tree reconstructed from the concatenated ITS and D1/D2 sequences of almost all yeast species in the order Tremellales recognized at the time of writing, the *Bulleribasidium* clade was clearly resolved with 100 % bootstrap support (Supplementary Fig. S1, available in IJSEM Online). However, the affinity of this clade to the order Tremellales and its relationships to other tremellomycetous clades or species were not confidently shown. *Bullera miyagiana* was clustered together with *Bullera variabilis* in a strongly supported branch by D1/D2 sequence analyses (Scorzetti *et al.*, 2002; Sampaio *et al.*, 2002), whereas this affinity was not shown in the ITS tree (Scorzetti *et al.*, 2002).

In order to get a clearer phylogenetic circumscription of the *Bulleribasidium* clade, a phylogenetic tree based on the alignment of concatenated rRNA gene sequences, including 18S, ITS, 5.8S and 26S D1/D2 domain, was reconstructed using neighbour-joining analysis (Fig. 1). The sequences were from the type strains of *i*) all the described species and proposed novel species in the *Bulleribasidium* clade; *ii*) representative species of the other genera or clades in the order Tremellales; and *iii*) representative species of the other orders or lineages of tremellomycetous yeasts. The

**Table 1.** Yeast strains studied

Species	Strain	Other designation	Source
<i>Mingxiaea sanyaensis</i>	SY-3.23 <sup>T</sup>	AS 2.3623 <sup>T</sup> , CBS 11408 <sup>T</sup>	Leaf of a plant, Sanya, Hainan, China
	SY-3.39	AS 2.3450	Leaf of a plant, Sanya, Hainan, China
	JF-12.9	AS 2.3519	Leaf of a plant, Jianfa Mountain, Hainan, China
<i>Mingxiaea hainanensis</i>	WZS-8.13 <sup>T</sup>	AS 2.4161 <sup>T</sup> , CBS 11409 <sup>T</sup>	Leaf of <i>Panicum brevifolium</i> , Wuzhi Mountain, Hainan, China
	JF-4.5	AS 2.2.4026	Leaf of a plant, Jianfa Mountain, Hainan, China
<i>Mingxiaea foliicola</i>	WZS-7.24		Leaf of a plant, Wuzhi Mountain, Hainan, China
	WZS-8.14 <sup>T</sup>	AS 2.3518 <sup>T</sup> , CBS 11407 <sup>T</sup>	Leaf of <i>Panicum brevifolium</i> , Wuzhi Mountain, Hainan, China
	WZS-17.3	AS 2.4023	Leaf of <i>Melastoma candidum</i> , Wuzhi Mountain, Hainan, China
	WZS-22.20	AS 2.4019	Leaf of a Rubiaceae plant, Wuzhi Mountain, Hainan, China
	WZS-30.19	AS 2.4021	Leaf of a plant, Wuzhi Mountain, Hainan, China
	WZS-36.6	AS 2.3520	Leaf of a plant, Wuzhi Mountain, Hainan, China
	HBX-1.21	AS 2.4017	Leaf of a plant, Bangxi, Hainan, China
	HBX-2.10		Leaf of a plant, Bangxi, Hainan, China
	HBX-2.19	AS 2.4025	Leaf of a plant, Bangxi, Hainan, China
	JF-12.4	AS 2.3524	Leaf of a Orchidaceae plant, Jianfa Mountain, Hainan, China
	<i>Mingxiaea wuzhishanensis</i>	WZS-29.8 <sup>T</sup>	AS 2.4163 <sup>T</sup> , CBS 11411 <sup>T</sup>

distinction of the *Bulleribasidium* clade was confirmed in the tree with 100 % bootstrap support. The close affinity of this clade to the *Hannaella luteola* lineage was resolved with 94 % bootstrap support. The affinity of *Bullera miyagiana* to the *Bulleribasidium* clade was not supported. This species was located in another branch (Fig. 1). The *Bulleribasidium* clade was also clearly resolved by maximum-parsimony analysis from the concatenated rRNA gene sequence alignment. The bootstrap support for the close relationship of this clade to the *Hannaella luteola* lineage was 78 % (Supplementary Fig. S2).

In the *Hannaella luteola* lineage, the three clades *Derxomyces*, *Hannaella* and *Dioszegia* are closely related. Therefore, in addition to the nuclear rRNA gene sequences, mitochondrial cytochrome *b* gene sequence and morphological differences were employed to support the separation of the three clades at the generic level (Wang & Bai, 2008). However, in the case of the *Bulleribasidium* clade, its distinction from the other clades or lineages in the order Tremellales is more evident, as shown in Fig. 1 and Supplementary Figs S2 and S3, even though only nuclear rRNA genes were employed. We think it is not necessary to add more genes. Phenotypically, the species in the *Bulleribasidium* clade usually form whitish to yellowish colonies with a butyrous to matted texture, differing from those of the species of the genus *Bullera* in the *Bulleromyces* clade which usually form whitish colonies with a highly mucoid texture running to the bottom of the slant. The colonies of yeasts in the *Bulleribasidium* clade are similar to those of species of the genus *Derxomyces*, but differ from those of species of the genus *Dioszegia* which are usually orange in colour and from those of species of the genus *Hannaella* which are usually mucoid in texture (Wang & Bai, 2008).

Except for the single teleomorphic species *Bulleribasidium oberjochense*, sexual stages have not been found in the other described species of the clade. DNA–DNA reassociation

experiments indicated that *Bulleribasidium oberjochense* does not represent the sexual stage of *Bullera variabilis*, though they are closely related (Sampaio *et al.*, 2002). The occurrence of mating among strains of *Bullera variabilis* reported by Boekhout *et al.* (1991) was not observed by Sampaio *et al.* (2002). In our own mating experiments among newly isolated yeast strains belonging to described and undescribed species of the clade, we also failed to observe sexual cycles. Therefore, a new genus, *Mingxiaea* gen. nov., is proposed to accommodate the anamorphic species of the *Bulleribasidium* clade.

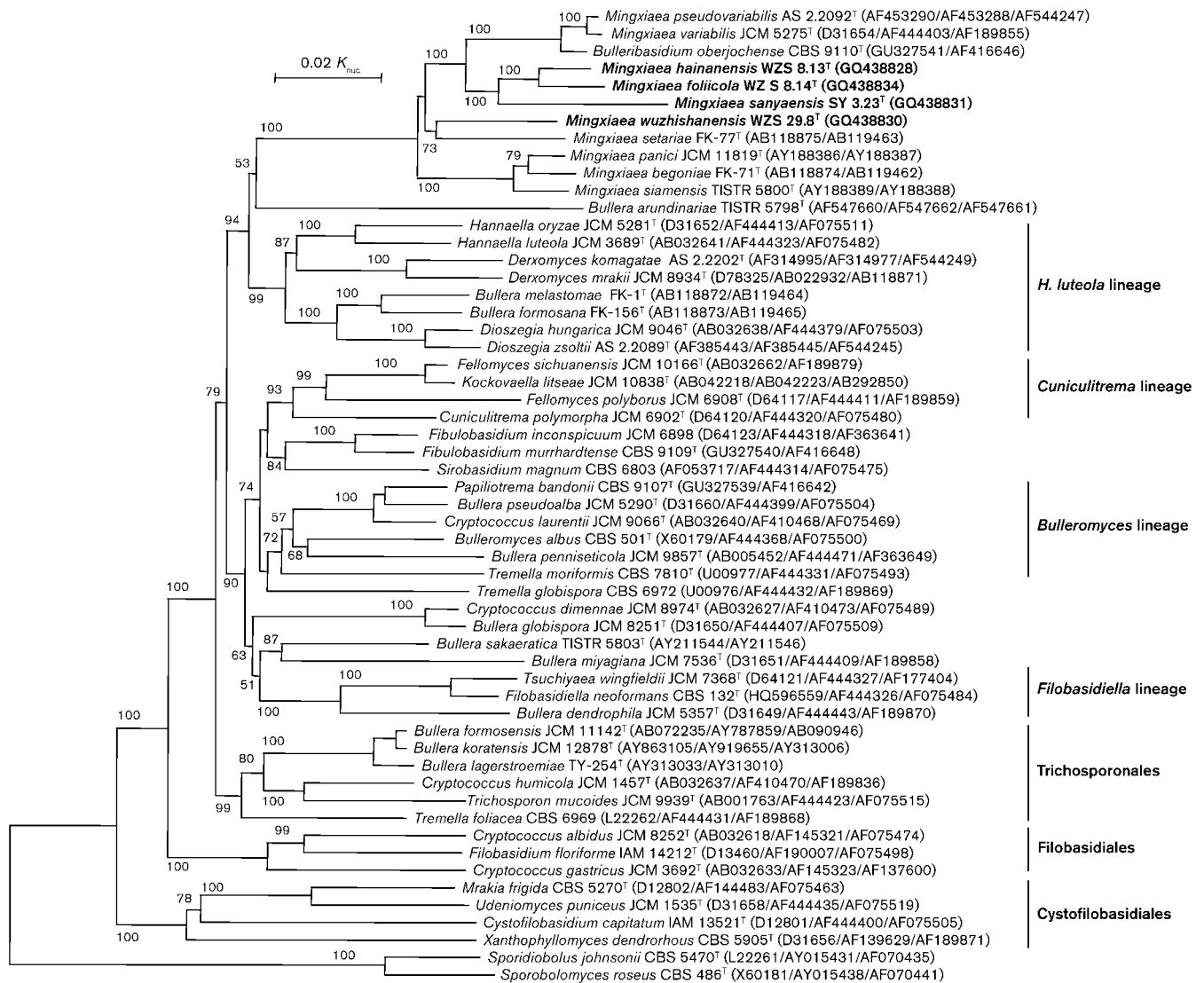
#### Latin diagnosis of *Mingxiaea* F.-Y. Bai, Q.-M. Wang, T. Boekhout & Nakase gen. nov.

*Cellulae vegetativae ovoideae, ellipsoideae, elongatae vel ampulliformes. Ballistoconidia flabelliformia, ellipsoideae, napiformia vel turbinatae. Cultura cremea aut gilvo-flavae, butyracea, non nitida vel nitida, glabra vel rugosa, margine glabra. Mycelium aut pseudomycelium formantur. Fermentatio nulla. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Species typica: *Mingxiaea variabilis* (Nakase & Suzuki) F.-Y. Bai, Q.-M. Wang & Nakase comb. nov.*

#### Description of *Mingxiaea* F.-Y. Bai, Q.-M. Wang, T. Boekhout & Nakase gen. nov.

*Mingxiaea* (Ming.xia'e.a. N.L. fem. n. *Mingxiaea* named in honour of Mingxia Li, formerly professor at the Institute of Microbiology, Chinese Academy of Sciences, for her pioneering contributions to the taxonomy of ballistoconidium-forming yeasts in China).

Budding cells are ovoid, ellipsoidal, elongate or bottle-shaped and occur singly or in pairs. Ballistoconidia are flabelliform, ellipsoidal, napiform and turbinate. Colonies are cream to pinkish-yellowish-brown, butyrous, dull or shiny, smooth or



**Fig. 1.** Phylogenetic tree reconstructed from neighbour-joining analysis of the combined sequences of the 18S rRNA gene, ITS region (including 5.8S rRNA gene) and 26S rRNA gene D1/D2 domain, depicting the relationships of the clades or lineages in the Tremellomycetes. All positions containing gaps were eliminated and there were a total of 2322 positions in the final dataset. Bootstrap percentages over 50% from 1000 bootstrap replicates are shown. Sequences are from type strains of the species compared, either determined in this study (in bold) or retrieved from GenBank under the accession numbers indicated. Bar, 0.02 substitutions per nucleotide position.

wrinkled and with an entire margin. Hyphae or pseudohyphae may be present. Clamp connections are absent. Basidia and basidiospores are not formed. Fermentation is absent. Diazonium blue B and urease reactions are positive. The major ubiquinone is Q-10. This genus is phylogenetically well separated from other genera or clades of the order Tremellales by nuclear rRNA gene sequence analysis (Fig. 1). The type species is *Mingxiaea variabilis* (Nakase & Suzuki) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase comb. nov. The following species are accepted in the genus.

*Mingxiaea begoniae* (Nakase, Tsuzuki, F. L. Lee & M. Takashima) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase

comb. nov. Basionym: *Bullera begoniae* Nakase, Tsuzuki, F. L. Lee & M. Takashima. *Mycoscience* **45**, (2004), 290.

*Mingxiaea panici* (Fungsin, Takashima & Nakase) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase comb. nov. Basionym: *Bullera panici* Fungsin, Takashima & Nakase. *Microbiol Cult Coll* **19**, (2003), 27.

*Mingxiaea setariae* (Nakase, Tsuzuki, F. L. Lee & M. Takashima) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase comb. nov. Basionym: *Bullera setariae* Nakase, Tsuzuki, F. L. Lee & M. Takashima. *Mycoscience* **45**, (2004), 292.

*Mingxiaea siamensis* (Fungsin, Takashima & Nakase) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase comb. nov. Basionym: *Bullera siamensis* Fungsin, Takashima & Nakase. *Microbiol Cult Coll* **19**, (2003), 27.

*Mingxiaea pseudovariabilis* (Bai, Takashima & Nakase) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase comb. nov. Basionym: *Bullera pseudovariabilis* Bai, Takashima & Nakase. *Antonie van Leeuwenhoek* **83**, (2003), 259.

*Mingxiaea variabilis* (Nakase & Suzuki) Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase comb. nov. Basionym: *Bullera variabilis* Nakase & Suzuki. *J Gen Appl Microbiol* **33**, (1987) 350.

### New species recognition

Among the symmetrical ballistoconidium-forming yeast strains isolated from plant leaves collected from Hainan province, southern China, 16 strains were found to belong to previously undescribed species in the genus *Mingxiaea* by D1/D2 and ITS sequence analyses. These strains were classified into four groups represented by strains SY-3.23<sup>T</sup> (three strains), WZS-8.13<sup>T</sup> (two strains), WZS-8.14<sup>T</sup> (10 strains) and WZS-29.8<sup>T</sup> (single strain), respectively (Table 1). The strains within each of the former three groups possessed identical D1/D2 sequences and identical or similar ITS sequences (0–4 mismatches), suggesting that the strains in each of these groups are conspecific.

In the tree drawn from D1/D2 sequences, groups SY-3.23<sup>T</sup> and WZS-29.8<sup>T</sup> formed two well isolated branches in the *Mingxiaea* clade (Fig. 2). Each of the two representative strains differed from the described and undescribed species in this clade by 44–51 mismatches (7–8%) in the D1/D2 domain and more than 15% mismatches in the ITS region. The data clearly indicate that the two groups represent two distinct species. Strain ST-186 from Thailand was clustered in the SY-3.23<sup>T</sup> group. This Thai strain differed from the Chinese strains by only one substitution in the D1/D2 domain, thus suggesting conspecificity with the latter.

Groups WZS-8.13<sup>T</sup> and WZS-8.14<sup>T</sup> were located in a cluster together with seven strains from Thailand. Three branches were recognized in this cluster: group WZS-8.13<sup>T</sup> formed a branch basal to the branch formed by the Thai strain TY-248 and another branch formed by the Chinese strains in group WZS-8.14<sup>T</sup> and six Thai strains (Fig. 2). The representative strains WZS-8.13<sup>T</sup>, WZS-8.14<sup>T</sup> and TY-248 of these three branches differed from each other by 14–19 mismatches in the D1/D2 domain and more than 60 mismatches in the ITS region, indicating that they represent three different species. In the branch represented by strain WZS-8.14<sup>T</sup>, the Thai strains differed from the Chinese strains by 0–4 mismatches in the D1/D2 domain. However, strains TY-154, TY-199 and TY-297, whose ITS sequences were available from GenBank, differed from the Chinese strains in the same branch by 11–12 mismatches in the ITS region, suggesting that the taxonomic status of these Thai strains remains to be confirmed. Another undescribed strain from Thailand,

ST-144, was located in a branch closely related to *Bulleribasidium oberjochense*, *M. variabilis* and *M. pseudo-variabilis*. It differed from the three described species by 42–44 mismatches in the D1/D2 domain, suggesting that strain ST-144 belongs to a separate species.

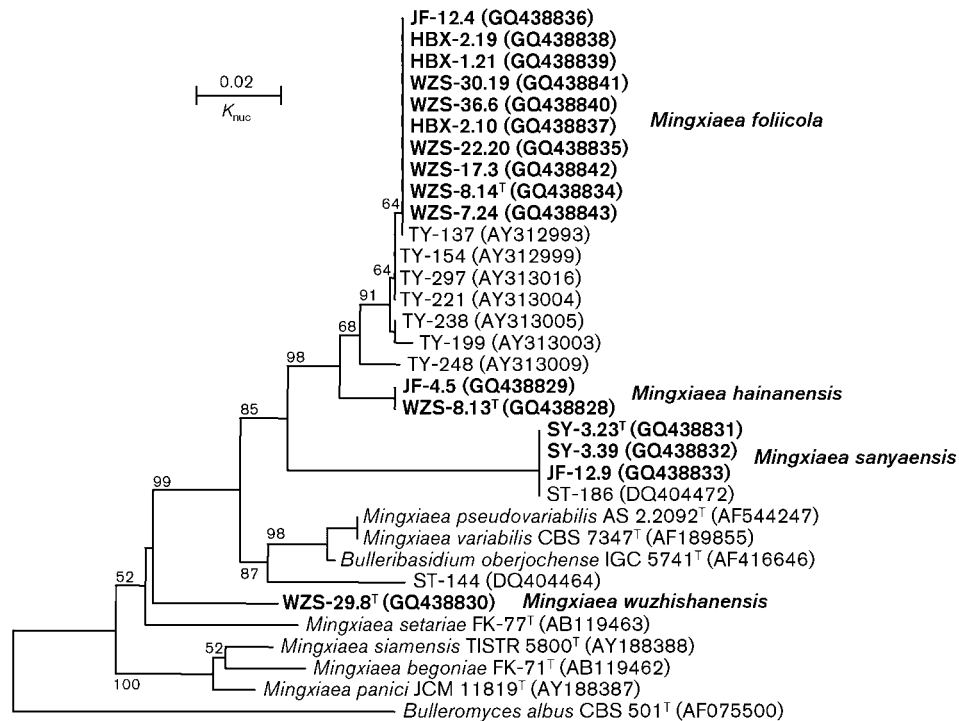
The rRNA gene sequence comparison showed that at least six novel species belonging to the *Mingxiaea* clade exist among the 16 strains isolated from Hainan, China, and the nine undescribed strains from Thailand whose rRNA gene sequences have been publicly released. Since the Thai strains have not been fully characterized phenotypically, only the four novel species represented by the Chinese strains are named and described below. Sexual cycles were not observed in the single or mixed cultures of the new strains growing on various media. Therefore, four new anamorphic species, *Mingxiaea sanyaensis* sp. nov., *Mingxiaea hainanensis* sp. nov., *Mingxiaea foliicola* sp. nov. and *Mingxiaea wuzhishanensis* sp. nov. are proposed. The physiological differences of the novel species from each other and from the described species of the genus *Mingxiaea* are summarized in Table 2.

### Latin diagnosis of *Mingxiaea sanyaensis* Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

In YM (Difco) liquido post dies 7 ad 17 °C, cellulae vegetativae ellipsoideae vel ovoideae, 2.5–4.5 × 5.0–7.2 µm. Annulus et sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura crenea aut flavae, nitida, glabra, butyracea, margine glabra. Mycelium non formantur. Ballistoconidia ellipsoideae, 3.7–5.0 × 5.5–7.5 µm. Fermentatio nulla. Glucosum, galactosum, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinose, melezitosum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum (vel lente), L-rhamnosum, D-glucosaminum (lente et exigue), galactitolum (vel lente et exigue), D-mannitolum (vel lente), glucitolum (vel lente), methyl α-D-glucosidum, salicinum (vel exigue) et inositolum assimilantur at non L-sorbosum, lactosum, inulin, methanolum, ethanolum, glycerolum, erythritolum, ribitolum (vel lente et exigue), acidum DL-lacticum, acidum succinicum, acidum citricum nec hexadecanum. Ammonium sulfatum et L-lysinum assimilantur at non kalium nitricum, natrum nitrosum, ethylaminum et cadaverinum. Ad crescentiam vitaminum non necessarium est. Maxima temperatura crescentiae: 30 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Typus: SY-3.23<sup>T</sup>, ex folio plantae incognitae, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.3623<sup>T</sup>).

### Description of *Mingxiaea sanyaensis* Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

*Mingxiaea sanyaensis* (san.ya.en'sis. N.L. fem. adj. *sanyaensis* pertaining to Sanya, China, the geographical origin of the type strain of this species).



**Fig. 2.** Phylogenetic tree reconstructed from neighbour-joining analysis of the 26S rRNA gene D1/D2 domain sequences depicting the relationships of the novel species of the genus *Mingxiaea* gen. nov. with closely related taxa. Bootstrap percentages over 50% from 1000 bootstrap replicates are shown. Sequences are from type strains of the species compared, either determined in this study (in bold) or retrieved from GenBank under the accession numbers indicated. Bar, 0.02 substitutions per nucleotide position.

In YM broth, after 7 days at 17 °C, cells are ellipsoidal or ovoid (2.5–4.5 × 5.0–7.2 µm) (Fig. 3a). Sediment is formed. On YM agar, after 1 month at 17 °C, the streak culture is cream to yellow, butyrous, shiny and smooth. The margin is entire. In Dalmau plate culture on corn meal agar, pseudohyphae and hyphae are not formed. Ballistoconidia are produced on corn meal agar and are ellipsoidal (3.7–5.0 × 5.5–7.5 µm) (Fig. 3b). Fermentation of glucose is negative. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose (or delayed), L-rhamnose, D-glucosamine (delayed and weak), galactitol (or delayed and weak), D-mannitol (or delayed), D-glucitol (or delayed), methyl α-D-glucoside, salicin (or weak) and inositol are assimilated. L-Sorbose, lactose, inulin, methanol, ethanol, glycerol, erythritol, ribitol (or delayed and weak), DL-lactic acid, succinic acid, citric acid and hexadecane are not assimilated. Ammonium sulfate and L-lysine are assimilated. Potassium nitrate, sodium nitrite, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 30 °C. Growth in vitamin-free medium is positive (weak). Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is Q-10.

The type strain, SY-3.23<sup>T</sup>, was isolated from a wilting leaf of an unidentified plant collected in Sanya, Hainan Province, south China, in December 2006. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.3623<sup>T</sup> (=CBS 11408<sup>T</sup>). The GenBank/EMBL/DBJ accession number for the sequence of the 18S rRNA gene, ITS region and 26S rRNA gene D1/D2 domain of the type strain is GQ438831.

**Latin diagnosis of *Mingxiaea hainanensis* Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.**

*In* YM (Difco) *liquido post dies 7 ad 17 °C, cellulae vegetativae ellipsoideae, ovoideae vel ampulliformes, 2.7–5.5 × 3.7–7.5 µm. Annulus et sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura flavae aut brunneo-glaucis, non nitida, glabra, butyracea, margine glabra. Mycelium formantur. Ballistoconidia napiformia aut ellipsoideae, 3.0–5.5 × 5.0–8.0 µm. Fermentatio nulla. Glucosum, galactosum, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinolum, melezitosum, amyllum solubile, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, galactitolum (vel lente), D-mannitolum (vel lente), glucitolum (variable), methyl α-D-glucosidum (lente et exigue), salicinum (exiguum) et inositolum assimilantur*

**Table 2.** Physiological and biochemical characteristics that differentiate the novel and described species of the genus *Mingxiaea* gen. nov.

Species: 1, *M. sanyaensis* sp. nov. (3 strains); 2, *M. hainanensis* sp. nov. (2 strains); 3, *M. foliicola* sp. nov. (10 strains); 4, *M. wuzhishanensis* sp. nov. WZS-29.8<sup>T</sup>; 5, *M. variabilis* (data from Boekhout & Nakase, 1998); 6, *M. pseudovariabilis* (Bai *et al.*, 2003); 7, *M. begoniae* (Nakase *et al.*, 2004); 8, *M. siamensis* (Fungsin *et al.*, 2003); 9, *M. panici* (Fungsin *et al.*, 2003); 10, *M. setariae* (Nakase *et al.*, 2004); 11, *B. oberjochense* (Sampaio *et al.*, 2002). All species were positive for assimilation of soluble starch. +, positive; -, negative; L, latent; w, weakly positive; LW, latent and weakly positive; v, variable.

Characteristics	1	2	3	4	5	6	7	8	9	10	11
Assimilation of:											
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	LW	+	+	+	+	+	+	+
D-Arabinose	+	+	+	LW	+	+	L	+	+	+	+
D-Ribose	L, +	+	+, w	-	+	L	LW	+	+	L	L
L-Rhamnose	+	+	+	-	+	+	+	+	+	+	+
D-Glucosamine	LW	-	+, w	-	+	+	+	LW	w	w	L
Ribitol	-, LW	-, LW	+, w	-	v	-	-	-, LW	-, LW	v	L
Galactitol	LW, +	+, L	+	LW	+	+	L	+	+	+	+
D-Mannitol	L, +	L, +	+	L	+	LW	v	+	+	+	L
D-Glucitol	L, +	v	-, LW	-	+	+	L, LW	-, LW	v	L, LW	+
Methyl $\alpha$ -D-glucoside	+	LW	+, w	L	+	-	+	+	+	L, LW	+
Inositol	+	+	+	-	+	L	-	+	+	-	+
Nitrite	-	-	-	-, LW	-	-	+	+	+	-	-
Ethylamine	-	-	-	-	v	-	-	+	+	-	-
L-Lysine	+	+	+	-	+	+	+	+	+	+	+
Cadaverine	-	-	-	-	-	-	LW	+	+	-	-
Growth in vitamin-free medium	w	-	+	-	-	w	-	-	-	-	-
Starch formation	-	-	-	-	+	+	+	w	w	-	+

at non L-sorbosum, lactosum, inulin, D-glucosaminum, methanolum, ethanolum, glycerolum, erythritolum, ribitolum (vel lente et exigue), acidum DL-lacticum, acidum succinicum, acidum citricum nec hexadecanum. Ammonium sulfatum et L-lysinum assimilantur at non kalium nitricum, natrum nitrosus, ethylaminum et cadaverinum. Vitaminae externae ad crescentiam necessaria sunt. Maxima temperatura crescentiae: 28–30 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Typus: WZS-8.13<sup>T</sup>, ex folio Panicum brevifolium L., depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.4161<sup>T</sup>).

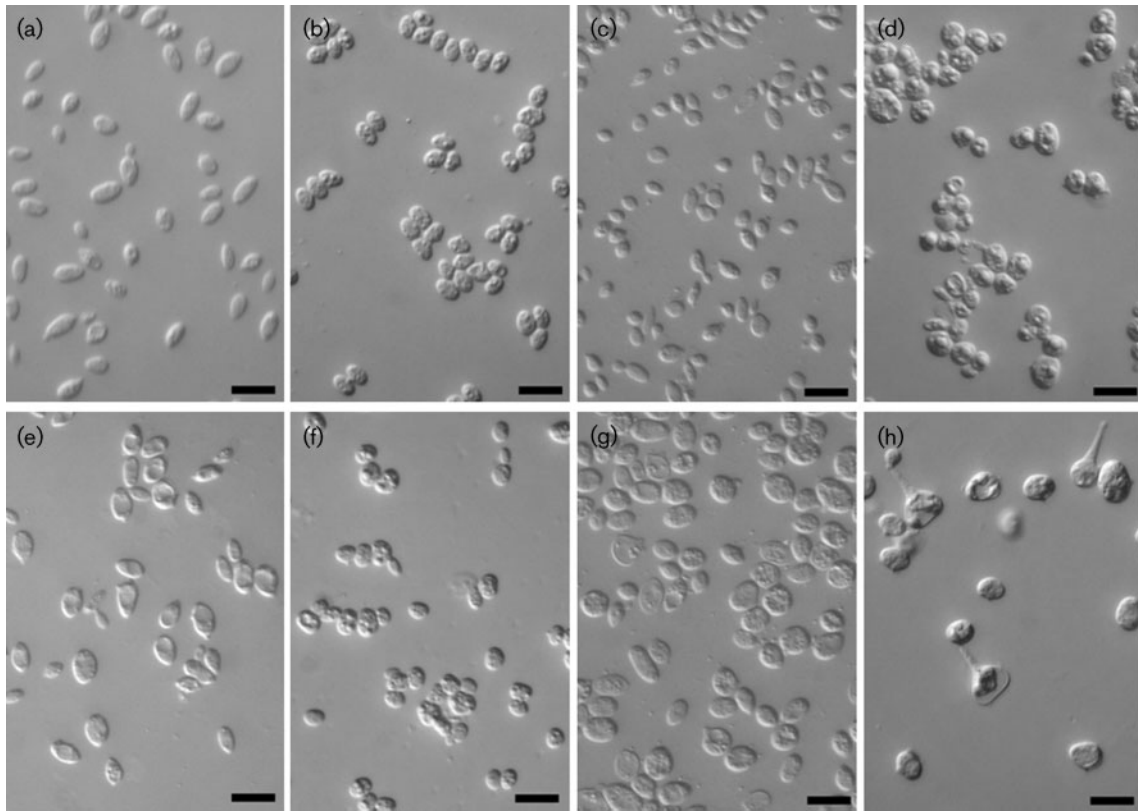
### Description of *Mingxiaea hainanensis* Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

*Mingxiaea hainanensis* (hai.nan.en'sis. N.L. fem. adj. *hainanensis* pertaining to Hainan Province, China, the geographical origin of the type strain of this species).

In YM broth, after 7 days at 17 °C, cells are ellipsoidal, ovoid or bottle-shaped (2.7–5.5 × 3.7–7.5 µm) (Fig. 3c). Sediment is formed. On YM agar, after 1 month at 17 °C, the streak culture is yellow to brownish-grey, butyrous, dull and smooth. The margin is entire. In Dalmau plate culture on corn meal agar, pseudohyphae and hyphae are not

formed. Ballistoconidia are produced on corn meal agar and are napiform or ellipsoidal (3.0–5.5 × 5.0–8.0 µm) (Fig. 3d). Fermentation of glucose is negative. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, galactitol (or delayed), D-mannitol (or delayed), D-glucitol (variable), methyl  $\alpha$ -D-glucoside (delayed and weak), salicin (weak) and inositol are assimilated. L-Sorbose, lactose, inulin, D-glucosamine, methanol, ethanol, glycerol, erythritol, ribitol (or delayed and weak), DL-lactic acid, succinic acid, citric acid and hexadecane are not assimilated. Ammonium sulfate and L-lysine are assimilated. Potassium nitrate, sodium nitrite, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 28–30 °C. Growth in vitamin-free medium is negative. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is Q-10.

The type strain, WZS-8.13<sup>T</sup>, was isolated from a wilting leaf of *Panicum brevifolium* L. collected in Wuzhi Mountain, Hainan Province, south China, in December 2006. This strain has been deposited in the CGMCC as AS 2.4161<sup>T</sup> (=CBS 11409<sup>T</sup>). The GenBank/EMBL/DDBJ accession number for the sequence of the 18S rRNA gene, ITS region and 26S rRNA gene D1/D2 domain of the type strain is GQ438828.



**Fig. 3.** Vegetative cells (a, c, e, g) grown in YM broth for 7 days at 17 °C and ballistoconidia (b, d, f, h) produced on corn meal agar after 7 days at 17 °C of novel species of the genus *Mingxiaea* gen. nov. (a, b) *Mingxiaea sanyaensis* sp. nov. SY-3.23<sup>T</sup>; (c, d) *Mingxiaea hainanensis* sp. nov. WZS-8.13<sup>T</sup>; (e, f) *Mingxiaea foliicola* sp. nov. WZS-8.14<sup>T</sup>; and (g, h) *Mingxiaea wuzhishanensis* sp. nov. WZS-29.8<sup>T</sup>. Bars, 10 µm.

#### Latin diagnosis of *Mingxiaea foliicola* Q.-M.

Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

In YM (Difco) liquido post dies 7 ad 17 °C, cellulae vegetativae ellipsoideae, ovoideae aut longi-ovoideae, 3.7–5.0 × 5.0–7.5 µm. Annulus et sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura brunneo-flavae, non nitida, glabra, butyracea, margine glabra. Mycelium non formantur. Ballistoconidia ellipsoideae, 3.0–7.5 × 7.5–10.0 µm. Fermentatio nulla. Glucosum, galactosum, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinose, melezitosum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum (vel exigue), L-rhamnosum, D-glucosaminum (vel exigue), ribitolum (vel exigue), galactitolum, D-mannitolum, methyl α-D-glucosidum (vel exigue), salicinum et inositolum assimilantur at non L-sorbosum, lactosum, inulin, methanolium, ethanolium, glycerolum, erythritolum, glucitolium (vel lente et exigue), acidum DL-lacticum, acidum succinicum, acidum citricum nec hexadecanum. Ammonium sulfatum et L-lysinum assimilantur at non kalium nitricum, natrum nitrosum, ethylaminum et cadaverinum. Ad crescentiam vitaminum non necessarium est. Maxima temperatura crescentiae: 28–30 °C. Materia amyloidea iodophila non

formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Typus: WZS-8.14<sup>T</sup>, ex folio *Panicum brevifolium* L., depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.3518<sup>T</sup>).

#### Description of *Mingxiaea foliicola* Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

*Mingxiaea foliicola* (fol.i.i'co.la. foliicola L. n. folium, a leaf; L. suff. -cola (from L. n. incola), inhabitant, dweller; N.L. n. foliicola, living in leaves, referring to the source of the type strain).

In YM broth, after 7 days at 17 °C, cells are ellipsoidal or ovoid to long-ovoid (3.7–5.0 × 5.0–7.5 µm) (Fig. 3e). Sediment is formed. On YM agar, after 1 month at 17 °C, the streak culture is brownish-yellow, butyrous, dull and smooth. The margin is entire. In Dalmau plate culture on cornmeal agar, pseudohyphae and hyphae are not formed. Ballistoconidia are produced on corn meal agar and are ellipsoidal (3.0–7.5 × 7.5–10.0 µm) (Fig. 3f). Fermentation of glucose is negative. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose,

melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose (or weak), L-rhamnose, D-glucosamine (or weak), ribitol (or weak), galactitol, D-mannitol, methyl  $\alpha$ -D-glucoside (or weak), salicin and inositol are assimilated. L-Sorbose, lactose, inulin, methanol, ethanol, glycerol, erythritol, D-glucitol (or delayed and weak), DL-lactic acid, succinic acid, citric acid and hexadecane are not assimilated. Ammonium sulfate and L-lysine are assimilated. Potassium nitrate, sodium nitrite, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 28–30 °C. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is Q-10.

The type strain, WZS-8.14<sup>T</sup>, was isolated from a wilting leaf of *Panicum brevifolium* L. collected in Wuzhi Mountain, Hainan Province, south China, in December 2006. This strain has been deposited in the CGMCC as AS 2.3518<sup>T</sup> (=CBS 11407<sup>T</sup>). The GenBank/EMBL/DDBJ accession number for the sequence of the 18S rRNA gene, ITS region and 26S rRNA gene D1/D2 domain of the type strain is GQ438834.

#### Latin diagnosis of *Mingxiaea wuzhishanensis*

Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

In YM (Difco) liquido post dies 7 ad 17 °C, cellulae vegetativae globosae, ellipsoideae vel ovoideae, 3.0–7.5 × 5.5–9.9 µm. Annulus et sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura crenea aut aurantiaca-flavae, non nitida, glabra, butyracea, margine glabra. Mycelium et pseudomycelium non formantur. Ballistoconidia ellipsoideae, 3.0–7.5 × 6.2–8.7 µm. Fermentatio nulla. Glucosum, galactosum, saccharosum, maltosum, cellobiosum (lente et exigue), trehalosum, melibiosum, raffinose, melezitosum, amyllum solubile, D-xylosum, L-arabiosum (lente et exigue), D-arabiosum (lente et exigue), galactitolum (lente et exigue), D-mannitolum (lente), methyl  $\alpha$ -D-glucosidum (lente), et salicinum (lente et exigue) assimilantur at non L-sorbosum, lactosum, inulin, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, ethanolum, glycerolum, erythritolum, ribitolum, glucitolum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum assimilantur at non kalium nitricum, natrum nitrosum, L-lysinum, ethylaminum et cadaverinum. Vitaminae externae ad crescentiam necessaria sunt. Maxima temperatura crescentiae: 28 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Typus: WZS-29.8<sup>T</sup>, ex folio plantae incognitae, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.4163<sup>T</sup>).

#### Description of *Mingxiaea wuzhishanensis* Q.-M. Wang, F.-Y. Bai, T. Boekhout & Nakase sp. nov.

*Mingxiaea wuzhishanensis* (wu.zhi.shan.en'sis. N.L. fem. adj. *wuzhishanensis* pertaining to Wuzhi Mountain, Hainan

Province, China, referring to the geographical origin of the type strain of this species).

In YM broth, after 7 days at 17 °C, cells are globose, ellipsoidal, or ovoid (3.0–7.5 × 5.5–9.9 µm) (Fig. 3g). Sediment is formed. On YM agar, after 1 month at 17 °C, the streak culture is dark-cream to orange–yellow, butyrous, dull and smooth. The margin is entire. In Dalmau plate culture on corn meal agar, mycelium and pseudohyphae are not formed. Ballistoconidia are produced on corn meal agar and are ellipsoidal (3.0–7.5 × 6.2–8.7 µm) (Fig. 3h). Fermentation of glucose is negative. Glucose, galactose, sucrose, maltose, cellobiose (delayed and weak), trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose (delayed and weak), D-arabinose (delayed and weak), galactitol (delayed and weak), D-mannitol (delayed), methyl  $\alpha$ -D-glucoside (delayed) and salicin (delayed and weak) are assimilated. L-Sorbose, lactose, inulin, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, glycerol, erythritol, ribitol, D-glucitol, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate is assimilated. Potassium nitrate, sodium nitrite, L-lysine, ethylamine hydrochloride, and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 28 °C. Growth in vitamin-free medium is negative. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is Q-10.

The type strain, WZS-29.8<sup>T</sup>, was isolated from a wilting leaf of an unidentified plant collected in Wuzhi Mountain, Hainan Province, south China, in December 2006. This strain has been deposited in the as AS 2.4163<sup>T</sup> (=CBS 11411<sup>T</sup>). The GenBank/EMBL/DDBJ accession number for the sequence of the 18S rRNA gene, ITS region and 26S rRNA gene D1/D2 domain of the type strain is GQ438830.

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