ORIGINAL ARTICLE



DGfM

Characterization of mating-type idiomorphs suggests that *Morchella importuna*, *Mel-20* and *M. sextelata* are heterothallic

Hongmei Chai¹ · Lijiao Chen¹ · Weimin Chen¹ · Qi Zhao¹ · Xiaolei Zhang¹ · Kaimei Su¹ · Yongchang Zhao¹

Received: 16 December 2016 / Revised: 3 May 2017 / Accepted: 10 May 2017 / Published online: 2 June 2017 © German Mycological Society and Springer-Verlag Berlin Heidelberg 2017

Abstract Morels (Morchella spp.) are highly prized for their culinary qualities and intensively collected worldwide by mycophiles. Morels are divided into three clades by phylogenetic analyses: black morels, yellow morels and the rufobrunnea clade. Morchella importuna, Mel-20 and M. sextelata are included in the black morel clade and are widely distributed in Yunnan province, China. M. importuna and M. sextelata have been artificially cultured in recent years, but their life cycles and reproductive systems are still poorly understood, which delays the progress of morel cultivation. In this study, the genomes of two ascospore isolates of M. importuna with opposite mating-type were sequenced and two idiomorphs, MAT1-1 and MAT1-2, were identified. The MAT1-2idiomorph was 6.7 kb in length containing a single MAT1-2-1 gene, and the MAT1-1 idiomorph was 10.5 kb containing a MAT1-1-1 gene and two other open reading frames (ORFs), GME3123 and GME3124. These ORFs differed greatly from the homologues of previously published mating-type genes; therefore, we speculate that they are novel mating genes found only in morels. Single-ascospore populations of M. importuna, M. sextelata and Mel-20 were analysed, and the result indicated that the ratios of MAT1-1- and MAT1-

Section Editor: Eckhard Thines

Hongmei Chai and Lijiao Chen contributed equally to this work

Electronic supplementary material The online version of this article (doi:10.1007/s11557-017-1309-x) contains supplementary material, which is available to authorized users.

☑ Yongchang Zhao yaasmushroom@aliyun.com 2-harbouring idiomorphs were not significantly different from a 1:1 ratio. The results suggest that these three black morels are heterothallic.

Keywords Morel \cdot *MAT1–1-1* \cdot *MAT1–2-1* \cdot Single-ascospore population

Introduction

As a group of edible mushrooms with excellent flavour and high medicinal value (Fu et al. 2013; Su et al. 2013; Hu et al. 2013), morels (Pezizomycetes, Ascomycota) are found in most parts of the world and are extensively traded (Pilz et al. 2007; Pildain et al. 2014). In recent years, morels have been commercially cultivated to meet the ever-growing need in China.

Phylogenetic analyses indicate that *Morchella* consists of three clades: the Elata Clade (black morels), the Esculenta Clade (yellow morels) and the rufobrunnea Clade (O'Donnell et al. 2011). *Morchella importuna, Mel-20* and *M. sextelata* are included in the Elata Clade (Du et al. 2014; Richard et al. 2015). In a previous study, some black morel species were confirmed to form secondary ectomycorrhizal symbioses with spruce (Buscot 1994), while *M. importuna* and *M. sextelata* are considered to be saprophytic fungi that can be cultivated artificially (Peng et al. 2016).

Although several species of *Morchella* have been successfully cultivated (Ower et al. 1986; Miller 2005; Masaphy 2010; Peng et al. 2016), their genetic information, life cycles and reproductive systems remain poorly understood. Based on SNP, SCAR, and AFLP markers, Pagliaccia et al. (2011) consider members of the *Morchella* sp. *Mel*-12 phylogenetic lineage to be heterothallic and to outcross in nature. Multiple nuclei are present in a single ascospore or one hyphal segment

¹ Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, 9 Xueyun Road, Wuhua, Kunming, Yunnan Province 650223, People's Republic of China

of *Morchella* (Hervey et al. 1978; Volk and Leonard 1990). However, whether the ascospores are heterokaryotic or homokaryotic remains unclear.

The sexual reproduction of Ascomycota fungi is controlled by a single mating-type locus (MAT-1) (Coppin et al. 1997) with two highly divergent nonhomologous idiomorphs (Metzenberg and Glass, 1990), designated MAT1-1 and MAT1-2 (Turgeon and Yoder 2000). The MAT1-1 idiomorph is characterized by the MAT1-1-1 gene, which encodes protein with an alpha-box domain, while the gene MAT1-2-1, which encodes a high-mobility group (HMG) domain, is generally located on the MAT1-2 idiomorph (Debuchy and Turgeon 2006). Individual isolates usually contain either the MAT1-1 or MAT1-2 idiomorph in heterothallic species. In contrast, in homothallic species, both mating types are contained in the haploid genome, usually tightly linked, although exceptions exist (Nelson 1996; Debuchy and Turgeon 2006). In Pezizomycetes, edible mushrooms that are as highly praised as morels, Tuber melanosporum and T. indicum, have been identified as heterothallic (Rubini et al. 2011; Belfoori et al. 2013).

In our previous studies, a draft genome database of the *Mes-15* strain YAAS2689 included in the Esculenta Clade was obtained (data unpublished), and the sequences of the genes *MAT1–1-1* (KP776983) and *MAT1–2-1* (KP776984) were verified. In this study, the genomes of two single ascospore isolates from *M. importuna* (YPL6) with opposite mating-type were sequenced. The aim was (1) to identify the *MAT* locus structure of *M. importuna*, (2) to clone and characterise the *MAT1–1-1* and *MAT1–2-1* mating-type genes of *M. importuna*, *Mel-20* and *M. sextelata*, and (3) to analyse whether these three black morel species are heterothallic or homothallic.

Materials and methods

Strains and isolations of ascospores

The fruiting body WXLBD7 of *Mel-20* was collected from the forests of Weixi (Yunnan province). YPL2 and YPL6 of

Table 1	Ascocarps	used	in	this	study
---------	-----------	------	----	------	-------

M. importuna and HL1 of *M. sextelata* were obtained from morel cultivation fields in Chuxiong (Yunnan province) and Huili (Sichuan province), respectively (Table 1). The corresponding strains were obtained by tissue isolation. Single-ascospore strains were isolated from the fruiting bodies after spore dilution. Every ascospore isolate was numbered with an Arabic numeral suffix corresponding to the ascocarp. For example, YPL6–3 refers to the 3rd ascospore isolate from ascocarp YPL6. All the cultures were identified by phlogenetic analyses (Table S1; Figs. S1, S2) and deposited in Mushroom Center of Yunnan Crops Genebank (in Yunnan Academy of Agricultural Sciences, YAAS), Kunming, China.

DNA extraction and PCR amplification

Each isolate was incubated on PDA at 23 °C for 7 days. Mycelia were scraped and ground in liquid nitrogen. DNA extraction was performed using an EZgeneTM Fungal gDNA Kit (BIOMIGA Inc.). All primer pairs were designed by Primer Premier v.5 and synthesized by Beijing Tsingke Biotechnology. The PCR amplifications were performed in a 25-µL mixture containing 12.5 µL of PCR Mix (TSINGKE), 1 µL of primer (10 µM/L) and 10 ng of DNA. Long-range PCR amplification was performed using LA Taq DNA polymerase (TaKaRa Biotechnology (Dalian)). The primer sequences and main PCR cycling parameters are listed in Table 2.

Genome sequencing

According to the conserved amino acid sequences of the MATI-2-1 gene HMG domain of ascomycetes, primer pair p1 was designed to amplify the corresponding gene fragments from the single-ascospore isolate group which contained 105 isolates of *M. importuna* YPL6. The isolates that contained the predicted amplification bands were considered to contain the MATI-2 idiomorph, while the isolates that lacked detectable bands were hypothesised to contain the MATI-1 idiomorph.

Ascocarp	Species	Origin	Genbank acc.Nr							
			ITS	RPB2	EF1-a	LSU				
HL1	Morchella sextelata	Huili, Sichuan	KX809732	KX809721	KX809716	_				
WXLBD7	Mel-20	Weixi, Yunnan	KX809734	KX809723	KX809718	_				
YPL2	M. importuna	Chuxiong, Yunnan	KX809735	KX809724	KX809719	_				
YPL6	M. importuna	Chuxiong, Yunnan	KX809736	KX809725	KX809720	_				
YAAS2689	Mes-15	Shanggri-La, Yunnan	KM485942	_	KM527910	KM485975				

Genomic DNA from the isolates YPL6–1 with the *MAT1–* 2 idiomorph and YPL6–3 with the *MAT1–1* idiomorph were submitted to the Beijing Genomics Institute (BGI). Genomic libraries were constructed and paired-End sequences were produced using Illumina HiSeqTM 2000. The clean reads were assembled into contigs by the SOAPdenovo assembly programme (http://soap.genomics.org.cn/soapdenovo.html), and the scaffolds were built by analysing the information of paired reads that covered different contigs. Genes were predicted using GeneMark-ES (v.2.3e)software and corresponding gene annotations were conducted by BLASTx analysis with the KEGG, KOG, SwissProt, NR and GO databases.

Identification and verification of the *MAT1–1* and *MAT1–2* idiomorphs of *M. importuna*

The MAT1-2 and MAT1-1 idiomorphs were identified by alignment analyses of two scaffolds containing MAT1-2-1 or MAT1-1-1. Ten PCR primer pairs (p2-1, p2-2, p2-3, p2-4, p2-5, p3-1, p3-2, p3-3, p3-4, and p3-5) (Table 2) were employed to verify the two idiomorphs based on their genome sequences. PCR was performed under standard conditions (Table 2). The products were recovered from agarose gels and purified using a Gel/PCR Extraction Kit (BIOMIGA), then cloned into the pGM-T vector and sequenced.

Table 2 primer sequences and PCR cycling parameters used in this study

Specified use	Code	Sequence(5'-3')	Annealing temperature (°C)	Extending time (s)
HMG domain conservation region	plf plr	CCGGAATTGGGAACAACGATG GTTTTCGGGGTGTATACTTATAC	56	30
Verify the MAT1–2 idiomorph of M. importuna	p2–1f p2–1r	GGGGTGTGTGTCGTCACTGGGGT CCGTCTGCTTTCATACTTTGGGT	62	120
	p2–2f p2–2r	ACAGAATTGTTGCCAGTAGA AGTGA CGATGAACTGATAGGGTGGT AAGAG	60	90
	p2–3f p2–3r	ACACGGACCGCACCTTGAAATACTT CGACAACAGACATTGGGCTACTTAC	60	360
	p2–4f p2–4r	TAGTAGACCATCACCGCACAGCAG ATACGCAATAATCAAGCACCCAGG	64	120
	p2–5f p2–5r	TCACTACCGACCACTCTTACCCGC CGACATTCATTGTCAACACCATGC	64	120
Verify the <i>MAT1–1</i> idiomorph of <i>M. importuna</i>	p3–1f p3–1r	GTCGTCACCACAAACCACCCACC TCTCCCAACTTCTCAACAATCCA	61	180
	p3–2f p3–2r	TCTACCAGCCATGTGAAACA AGCAA GCTCTCTTGTGCCCCTTTTGACTAT	64	120
	p3–3f p3–3r	ACTCTTGTAGTGTTCACCATAAA GGAAAAATCTCAAAAATCGTCTC	51	180
	p3–4f p3–4r	AGTCAATAGTCAATGGAGCGTTA AGGATAGAAGTAGAAGAGGGGGGG	56	100
	p3–5f p3–5r	ATGTATTATTCTCATCGTATTTT CTTTAGGTGGTAGTCTGGGTTTC	51	180
MAT1-1-1 gene conservation region	p7f p7r	CCGGTTTATCTTACTGGACTGGTTC GCTTTCCTCTTCTCCGTTGCCATA	62	40
Verify the <i>MAT1–1-1</i> gene coding region	p8f p8r	ATGTCACTCCGTCCGGTTTACCTTA TGGAATGTCTGTGATTGAGGCTGTG	65	90
Walking for the total sequence of MAT1-1-1 gene	p9-1f	TCTTTGTCTTCGTAACGCCACTTTG	65	120
	p9-2f	CCAGGAGGGTCGGTATTTCAGGTGC	65	120
	p9-3f	CCGATTGTTGATGAACCGTTGACTA	65	120
	p9-1r	CCTAGCCCTCCCAGGAATTTTGATA	65	120
	p9-2r	TAGTCAACGGTTCATCAACAATCGG	65	120
	p9-3r	GTGGGGGCACAATATATCGACAGTC	65	120
Verify the MAT1-2-1 gene coding region	p10f	GGCCAGAACAGATGCTCGAA GAAGC	64	60
	p10r	CTCCCAAAGCATGATCAAATCCCTC		

Sequence comparison and dotplot analysis were conducted to verify the two idiomorphs of *M. importuna*.

Isolation of *MAT1–2-1* and *MAT1–1-1* from *Mel-20* and *M. sextelata*

Conserved sequences could be found by analysis of the flanking regions of MAT1-2-1 gene in *M. importuna* (KY782629) and *Mes-15* (KY782632). A pair of specific primers (p2–3f and p2–3r) were designed based on the conserved regions to amplify the complete sequences of the MAT1-2-1 gene in *Mel-20* and *M. sextelata*. The PCR amplicons (approximately 5 kb) were cloned and sequenced.

Because no conserved portions could be located in the 10kb flanking regions from the upstream to the downstream region of the *MAT1–1-1* gene in *M. importuna* (KY782630) and *Mes-15* (KY782631), a primer pair (p7f and p7r) was designed to sequences within the *MAT1–1-1* gene to amplify the corresponding DNA fragments. To obtain the complete sequence of the *MAT1–1-1* gene, the DNA walking method was performed using the Genome Walking Kit (TaKaRa Biotechnology (Dalian)) with SP Primers (p9-1f, p9-2f, p9-3f, p9-1r, p9-2r, and p9-3r) based on *MAT1–1-1* fragments of *Mel-20* and *M. sextelata*.

Verification of the coding regions of *MAT1–1-1* and *MAT1–2-1* genes

Total RNA was extracted from the mycelia of YPL6, YPL6–1, YPL6–3, HL1, HL1–1, HL1–47, WXLBD7, WXLBD7–11 and WXLBD7–23 using a TaKaRa MiniBEST Plant RNA Extraction Kit. The first-strand cDNAs were synthesized using Oligo dT primer (TaKaRa PrimeScriptTM II 1st Strand cDNA Synthesis Kit). The primer pairs p8 for the *MAT1–1-1* gene and p10 for the *MAT1–2-1* gene were designed using predicted sequences adjacent to the start site and end site of the two genes. The design strategy also took advantage of conserved regions, so the primers could be used in all three morel species. Introns were verified manually by comparing the RT-PCR sequences with the corresponding DNA sequences.

Mating type ratios of ascospores from the same ascocarp

Single-ascospore populations of WXLBD7, YPL2, YPL6 and HL1 were obtained to determine the occurrence and frequency of the mating type idiomorphs. Standard PCR tests with p8 and p10 primer pairs were conducted to examine the four single-ascospore populations. A chi-square test was applied to compare the *MAT1–1/MAT1–2* ratio at the significance level P = 0.05.

Results

MAT loci structures of M. importuna

The results of genome assembly and gene annotation of isolations YPL6–1 and YPL6–3 showed that the genome sizes and components of two isolations are similar (Tables S2, S3). The *MAT1–1-1* gene was observed only on Scaffold 152 (KY782630) of the *M. importuna* isolate YPL6–3 genome, which was 80 kb long and contained 18 predicted genes. The *MAT1–2-1* gene was found on Scaffold 91 (KY782629) of the YPL6–1 genome, which was 75 kb long and contained 26 predicted genes. Sequence alignment of the two scaffold regions revealed that the *MAT1–1* and *MAT1–2* idiomorphs were 10.5 and 6.7 kb long, respectively.

The *MAT1–2* idiomorph contained only a single *MAT1–2-1* gene. However, in addition to the *MAT1–1-1* gene, there were two predicted coding sequences GME3123 and GME3124 on the *MAT1–1-1* idiomorph (Fig. 1). They were not homologues to any of the mating genes that have been published; therefore, we speculated that they are novel mating genes unique to morels. GME3123 was found to be 1688 bp containing four introns and encoding 488 amino acids, and GME 3124 was 1825 bp containing five introns and encoding 512 amino acids. BLASTx analysis revealed that GME3123 had 24% and GME3124 21% amino acids similarity to hypothetical protein (AIU38081.1) of *Tuber borchii* (Belfiori et al. 2016).

To compare this analysis with the published morel genome databases, a sequence completely identical to the MAT1-1 idiomorph was found on the Scaffold 48 of the *M. conica* genome (http://genome.jgi.doe.gov/ Morco1/Morco1.home.html) between 239.6 kb and 250. 1 kb. Three genes, 10317, 10316 and 10315, were in this area on the Scaffold 48 of the M. conica genome, and they were completely identical with the genes MAT1-1-1, GME3123 and GME3124, respectively. No sequences identical to the MAT1-2 idiomorph were founded in this genome database. Sequences completely identical to the MAT1-2 idiomorph were located on the Scaffold 20 of the M. importuna strain SCYDJ1-A1 genome (http:// genome.jgi.doe.gov/Morimp1/Morimp1.home.html) between 230.6 kb and 237.3 kb. Only an ORF known as fgenesh1 pg.20 # 79 was found in this area, and its sequence was the same as the MAT1-2-1 gene. The MAT1-1 idiomorph could not be found in this genome database.

The coding sequences flanking the two idiomorphs were highly conserved. The 5' flanking region of both idiomorphs contained APN2, which is connected to the cox13 gene, and there were four additional predicted ORFs between the cox13 gene and the MAT idiomorphs (Fig. 1). The APN2 gene displayed 66% amino acid



Fig. 1 Organization of *MAT* locus in *M. importuna*. The scale bar indicates sizes of only the *MAT* idiomorphs, the distance and sizes of other genes are not to scale. Introns in the *MAT* genes are indicated by black boxes. Thick and thin arrows indicate the orientation of genes and prime pairs respectively. Gene or superfamily names refer to a gene encoding a protein showing the highest percentage identity to the putative gene product following BLASTx analysis. *APN2* DNA-

sequence similarity to CCX30259.1 (*Pyronema* omphalodes), and the cox13 gene displayed 62% similarity to GAP92444.1 (*Rosellinia necatrix*). The end4 (also known as SLA2) gene was the second gene that located on the 3' flanking region of two idiomorphs, and it displayed 66% amino acid sequence similarity to CCX33915.1 (*Pyronema omphalodes*).

Structure of the MAT1-1-1 gene of three morel species

The lengths of the MAT1-1-1 gene sequences of *M. importuna*, *M. sextelata* and *Mel-20* were 1694, 1730, and 1651 bp, respectively. RT-PCR confirmed that the

(apurinic or apyrimidinic site) lyase 2; *cox13* cytochrome c oxidase subunit 6A, mitochondrial; *CPSF6*: cleavage and polyadenylation specificity factor subunit 6; *tfa1* transcription initiation factor IIE subunit alpha; *atp-3* ATP synthase subunit 4, mitochondrial; *SDH2* succinate dehydrogenase (ubiquinone) iron-sulfur subunit, mitochondrial; *Mba1* mitochondrial inner membrane protein; *end4* (also known as *SLA2*) endocytosis protein end4

MAT1–1-1 ORFs contained two introns and encoded proteins of 525, 537 and 511 amino acids, respectively (Table 3). Sequence alignments and dotplot analyses indicated that the *MAT1–1-1* gene in *M. importuna* displayed 92.3 and 87.2% sequence identity to those in *M. sextelata* and *Mel-20*, respectively.

The deduced amino acid sequences of these three morel species contained a conserved alpha-box motif of the MAT1-1-1 protein of ascomycetes (Fig. 2a). In addition, the three proteins shared more than 20% amino acid identities to those in *Tuber indicum* (AHE80942), *T. melanosporum* (ADU56595) and *T. borchii* (AIU38080).

 Table 3
 The variance of MAT1-1-1 and MAT-2-1 gene in three morel species

Strain Species	eccies MAT1–1-1						MATI-2-1							
		DNA	Intron (bp		Amino acids	GenBank	DNA (bp)	Intron(bp)			Amino acids (aa)	GenBank		
		(bp)	1	2	(aa)	accession number		1	2	3		accession number		
YPL6	M. importuna	1694	58	61	525	KX809728	1197	59	93	58	329	KX809731		
HL1	M. sextelata	1730	58	61	537	KX809726	1199	59	94	59	329	KX809729		
WXLBD7	Mel-20	1651	57	61	511	KX809727	1247	59	94	62	344	KX809730		

	1	TAPEK <mark>ARKA</mark> L	NAFVGF	RCYY	ITIE	PLFK)WPMKK	LSNL	IGLL	MEAD	DPNKSI	WSLM	1T <mark>KA</mark> W	STIF	DOIG	KDQA	APLDO	FFS:	LI <mark>CP</mark> H	LNL	PDPAS	YLEIH
Dothideomycetes	2	IAPEK <mark>AKKA</mark> I	NAFVGF	RCYY	ISI	POFKS	SWPMKK	(LSNL	IGLL	MEAD) PNKSI	WSLM	1AKAW	STIF	DOIG	KDKA	APLDO	FFS	LI <mark>C</mark> TH	.LK <mark>M</mark> J	PTPES	YLAVI
·	3	RTQEGKKRPL	NSFIAF	RSFY	۲ <mark>۵</mark> ۱	/IFPI	O <mark>LTQK</mark> A	AK <mark>S</mark> GI	LRFL	NQND	DP <mark>F</mark> K A F	WAII	LAK <mark>A</mark> Y	SIVF	RDDHE	SEV.	SLDQ	FLE:	ITAKE	IG <mark>L</mark>	EPAR	YLDAN
Eurotiomycetes	4	QTGEKKLRPL	NSFIAY	RSFY	r <mark>s</mark>]	CM <mark>FP</mark> E	E <mark>VTQK</mark> T	'K <mark>S</mark> GI	IKDL	NQAD)P <mark>Y</mark> K <mark>G</mark> F	WAII	LAKAY	SIIF	DDHR	TEV.	SLDT	FLE:	LTVPF	'IG <mark>L</mark>	QPED	YI GI]
	5	VRENGKLRPL	NSFIAF	RSFY	r <mark>s</mark>]	ra <mark>fp</mark> i	DLSQKI	.K <mark>S</mark> GL	LRLL	NTSD	op <mark>f</mark> kaf	WAII	LAKAY	SIIF	NDSH <mark>A</mark>	GQV.	NLES	FLE:	LNGPI	IG <mark>I</mark>	APSD	YI RVN
	6	ASCDR <mark>AKRP</mark> L	NAFMAF	RSYY	ľLP	KL <mark>FP</mark> I	D <mark>VQQK</mark> T	'A <mark>S</mark> GF	LTTL	MHKD	DP <mark>F</mark> RNF	WALI	AKVY	SFVF	RDQIG	KDKV	/SLSY	FMS:	LA <mark>C</mark> PT	MTI.	EPAA	YI NAI
Condoniomycostes	7	QAGDR <mark>AKRP</mark> I	NAFIAF	RSYY	ZVP	KL <mark>FP</mark> E	ES <mark>Q</mark> QKA	AASGF	LTTL	WNKD)P <mark>F</mark> RNF	R <mark>W</mark> AMI	AKVY	SFIF	DEMG	KKRA	APLSS	FLM	AA <mark>CP</mark> A	MD <mark>IJ</mark>	PPDE	YIQVI
Sordariomycetes	8	GQRAKRPL	N <mark>GFM</mark> AF	RTYY	MP	KL <mark>FP</mark> I	DA <mark>QQK</mark> N	JA <mark>S</mark> DF	'LTQL	MAKD) PHRNF	WALI	AKVY	SFIF	RDHV <mark>G</mark>	<mark>K</mark> AKC	CN <mark>L</mark> TA	FLS	/A <mark>CP</mark> M	MK <mark>I</mark>	EPVE	YIQTI
	9	VAGGRAKRPL	NAFMAF	RTYY	YLP	KL <mark>FP</mark> I	DT <mark>QQK</mark> T	'A <mark>S</mark> GF	'LTQL	MAKD) PHRNF	WALI	AKVY	SFIF	RDHV <mark>G</mark>	KARI	INLSA	FLS	/A <mark>CP</mark> M	MR <mark>I</mark>	[EPVD]	YLRS I
	10	GGVAR <mark>AKRP</mark> I	N <mark>AFM</mark> AF	RTY Y	ľLÞ	(M <mark>FP</mark> I	DT <mark>QQK</mark> Y	(C <mark>S</mark> GF	LTKL	WNQD	DPRRNF	WALI	AKVY	SFIF	RDHV <mark>G</mark>	KGR I	INLSA	FLG	JV <mark>CP</mark> M	MK <mark>I</mark>	[HPDD]	Y <mark>I</mark> QT <mark>I</mark>
Morchella sextelata	11	LTKRSVL <mark>KA</mark> L	NPYIAH	RTWI	I <mark>S</mark> KFI	LGGL	GFTQML	JISAL	TKGV	NQRE	KRK <mark>A</mark> M	1 <mark>W</mark> SNI	ARLY	TYHF	DQGT	LTSI	'LEDF	IKA	JIVEN	KQDJ	SPSS	FLHNC
Morchella importuna	12	LTKRSVL <mark>KA</mark> L	NPYIAH	RTW1	I <mark>S</mark> KFI	GGL	G <mark>F</mark> TQML	II <mark>S</mark> AL	TKGV	NQRE	KRK <mark>A</mark> M	1 <mark>W</mark> SNI	ARLY	TYHF	DQGT	LTSI	'LE <mark>D</mark> F	IKA	QLIEN	KQDJ	PSPSS	FLHNC
Mel-20	13	LTKHSVL <mark>KA</mark> L	NPYIAH	RTWI	I <mark>S</mark> KFI	GGL	G <mark>F</mark> TQML	ISAL	TKGV	NQRE	KRK <mark>A</mark> M	1 <mark>W</mark> SNI	ARLY	TYNF	DQGT	LTSI	'LE <mark>D</mark> F	IKA	QLVEN	KQDJ	PSPSS	FLHHC
Pezizomycetes	14	IPPSQHL <mark>RA</mark> L	N PYVAÇ	RSWI	I <mark>S</mark> KY(CNGY	G <mark>L</mark> TQAE	CISNL	TRDV	WVAE	TNKYM	1 <mark>W</mark> QNI	ASLY	TAAF	DRGD	PGLV	/LE <mark>E</mark> F	IETI	ELAKH	GHP"	TPEK.	LLREA
·	15	IPPSQHL <mark>RA</mark> L	N PYVAÇ	RSWI	I <mark>S</mark> KY(CNGY	G <mark>L</mark> TQAE	CISNL	TRDV	WVAE	TNKYM	1 <mark>W</mark> QNI	ASLY	TAAF	RDRGD	PGLV	/LE <mark>E</mark> F	IETI	ELAKH	GHP?	TPEK:	LIREA
	16	VPRSQQL <mark>RA</mark> L	NPYVAÇ	RSWI	I <mark>S</mark> KY(CNGY	G <mark>L</mark> TQAE	CISNL	TREV	WVAE	PNKYN	1 <mark>W</mark> QNI	ASLY	TAAF	RDRSE	PGLV	/LE <mark>D</mark> F	IETI	ELAKH	GHPS	STPEK:	L <mark>I</mark> RE <i>I</i>
Lecanoromycetes	17	.GKEKATRSV	NSFMMF	RCYY	(A	E I FEA	FOOKV	/ISSY	IVYL	NOSD)P <mark>F</mark> KAF	WALI	AKAY	SVIF	RDHV <mark>G</mark>	K EHA	APVDA	FLS	IVADE	VGI	DNQN	YI IAN
Lecanoromycettes	18	.RPVL <mark>PKK</mark> SI	NSWMAF	RSFY	1Q	VL <mark>FP</mark> H	ILQQKE	CA <mark>S</mark> IY	LTAL	WKRD	op <mark>f</mark> kaf	WTII	AAAY	SKIF	RNTV <mark>G</mark>	K PRA	APLDR	YLN	IV <mark>CP</mark> Ç	MG <mark>M</mark> .	GVEA	YLELI
T	10	LYNGTRRRSV	NRYILF	RAFI	KSIT						TERCE					anac	ה בדג גרחי	FT D		TGT		VT CCN
Leonomycetes	19	hindinadov	n	r		/ELQN	1 <mark>lqqk</mark> e	SASPI S	IAVL	NARL W)T <mark>L</mark> V2L	W	JAR <mark>A</mark> F	•••••	JIRD <mark>A</mark>	GVSG	JIVEA			101.	L PIDQ	1
Leonomycetes	1 19	111011440 V	n	r		/E <mark>L</mark> QI	1 <mark>LQ</mark> QKC)A <mark>S</mark> PI S	IAVI.	MARL W	DI <mark>L</mark> VOL	W	JARAF	•••	IRD <mark>A</mark>	GVSG	JIVEA			101.	LTDA	1
b	1	LINGIN <mark>a</mark> uo v	n	r		/E <mark>L</mark> QI	1 <mark>LQQK</mark> D	S	LAVL	WARL W	1 ^L V2L	W	JAKAF	•••TI	IRD <mark>A</mark>	GVSG	JIVEA	<u>. r 11</u> 1X.		101.	LTDA	1
b Eurotiomycetes	19	.PVGPLKAPKVP	n P <mark>PN</mark> AFI	r LY <mark>R</mark> Q:	HH <mark>H</mark> PI	KIKE <mark>7</mark>	JLQQKC AYP <mark>D</mark> YSI	DASPI s NN <mark>D</mark> I <mark>S</mark>	IAVL VMLG	NARL W KQ <mark>W</mark> K	(D <mark>ene</mark> e	W W IKTQI	FRNLA	TI	'IRD <mark>A</mark>	GVSG ED <mark>HP</mark> I	JT <mark>VE</mark> A D <mark>Y</mark> H <mark>Y</mark> T	PR <mark>K</mark> I	SERKI	₹ <mark>RTS</mark>	SRQFSI	
b Eurotiomycetes	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	.PVGPLKAPKVPF SVKIPARPAKVPF	n PPNAFI PPNCFI	r Ly <mark>R</mark> Q: LyRQ:	HHHP1 ANHHI	VELQN KIKE <mark>7</mark> LVKD <mark>7</mark>	JLQQKD AYPDYSI ANPG <mark>V</mark> SI	DASPI S NNDIS NNEIS	IAVL SVMLG	NARL W KQWK ARWN	DENEE INESPE	W IKTQI VREQI	FRNLA FTHLA	EELK	i RD <mark>A</mark> KKHAI K <mark>E</mark> HAI	EDH <mark>PI</mark> IKHPI	DYHYT DYQYA	PRKI PRRI	SERKI SERKI	₹RTS: ≹RTPI	SRQFSI	
b Eurotiomycetes	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	.PVGPLKAPKVFF SVKIPARPAKVFF PSLPAALKPKIFF	n PPNAFI PPNCFI PANEWI	r LyrQ LyrQ LyrA	HHHPI ANHHI DNHII	VELQN KIKE <mark>7</mark> LVKD7 PIKK7	VLQQKD AYPDYSI ANPGVSI AYPGITI	DASPI S NNDIS NNEIS NNEIS	IAVL VMLG SRILG SSIIA	NARL W KQWK ARWN G <mark>MW</mark> A	(DENEE INESPE AETPE	W IKTQI VREQI RRLKY	FRNLA FTHLA YKIRA	EELK DELK DLLK	,IRD <mark>A</mark> KKHAI K <mark>E</mark> HAI	GVSG EDHPI IKHPI KAYPI	DYHYT DYQYA IYKYA	PRKI PRRI PRKI	SERKI SERKI	₹RTS: ₹RTPI ₹RASI	SRQFSH KKTLTH	
b Eurotiomycetes Leotiomycetes	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	.PVGPLKAPKVFF SVKIPARPAKVFF PSLPAALKPKIFF LPLPVAPKPKIFF	n PPNAFI PPNCFI PANEWI PANEWI	r LYRQ LYRQ LYRA LYRA LYRA	HHHPI ANHHI DNHII DNHII	VELQN KIKE <mark>F</mark> LVKDF PIKKF	AYPDYSI ANPGVSI AYPGITI AYPGITI	DASPI S NNDIS NNEIS NNEIS	VMLG SRILG SSIIA SSIIA	NAKU W KQWK ARWN GMWA GMWA	DENEE INESPE AETPE AETPE	W IKTQI VREQI RRLKY RRLKY	FRNLA FTHLA YKIRA YKIRA	EELK DELK DLLK	IRD <mark>A</mark> KKHAI K <mark>E</mark> HAI EAHKI QAHKI	EDHPI IKHPI KAYPI RAHPI	DYHYT DYQYA IYKYA IYKYA	PRKI PRRI PRKI PRKI	SERKI SERKI SEKKI NEKKI	RTS: RTPI RASI	SRQFSH R KKTLTH KKTLTH	(N)
b Eurotiomycetes Leotiomycetes	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	PVGPLKAPKVE SVKIPARPAKVE SSLPAALKPKIE LPLPVAPKPKIE APTPVTTRGKEE	n PPNAFI PANEWI PANEWI PANEWI PPNEWI	r LyrQ LyrQ LyrA LyrA LyrA LyrT	HHHPI ANHHI DNHII DNHII DNHII	VELQN KIKE <mark>7</mark> PIKK <mark>7</mark> PIKK <mark>7</mark> PIKL <mark>7</mark>	AYPDYSI ANPGVSI AYPGITI AYPGITI AYPGITI	DASPI S NNDIS NNEIS NNEIS NNEIS	IAVL VMLG RILG SIIA SIIA SIIA	NAKL W KQWK ARWN GMWA GMWA GMWA	DENEE INESPE AETPE AETPE AESDD	W IKTQI VREQI RRLKY RRLKY RRLKY	FRNLA FTHLA YKIRA YKRA	EELK DELK DLLK LVLK	IRDA KKHAI K <mark>E</mark> HAI EAHKI QAHKI ESHAI	EDHPI IKHPI KAYPI RAHPI KAHPI	DYHYT DYQYA IYKYA IYKYA VYKYT	PRKI PRRI PRKI PRKI PRKI	SERKI SERKI SEKKI NEKKI	₹RTS: ₹RTPI ₹RASI ₹RASI	SRQFSI KKTLTI KKTLTI KKTLTI	(N (N (N (N
b Eurotiomycetes Leotiomycetes Sordariomycetes	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	PVGPLKAPKVE SVKIPARPAKVE SVLPARPAKVE DELPVAPKPKIP APTPVTTRGKIP CSU DPUVIT	n PPNAFI PANCFI PANEWI PANEWI PPNEWI PPNAYI PDNAYI	r LYRQ LYRQ LYRA LYRA LYRT LYRK	HHHPI ANHHI DNHII DNHII DNHII DHHRI	VELQN KIKE <mark>7</mark> LVKD7 PIKK7 PIKK7 QIREQ QIREQ	AYPDYSI ANPGVSI AYPGITI AYPGITI AYPGISI 2NPGLHI 2NPGLHI	S NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS	IAVL VMLG RILG SIIA SIIA SIIA VIVG	NAKL W KQWK ARWN GMWA GMWA GMWA NMWR	DENEE INESPE AETPE AETPE AESDD RDEQPH	W W VREQI RRLKY RRLKY RREKY I RDKY	FRNLA FTHLA YKIRA YKIRA YKKRA YFSMA	EELK DELK DLLK LVLK NEVK EDLK	IRDA KKHAI KEHAI EAHKI QAHKI ESHAI ARLLI	EDHPI IKHPI KAYPI RAHPI KAHPI KAHPI	DYHYT YQYA YXYA YKYA YKYA YXYT YQYA	PRKI PRKI PRKI PRKI PRKI PRKI	SERKI SERKI SEKKI NEKKI SEKKI	RTS: RTPI RASI RASI RVSI RVSI RVSI	SRQFSI SRQFSI KKTLTI KKTLTI KKRLAI PYLK.	KN K. K. K.
b Eurotiomycetes Leotiomycetes Sordariomycetes	1 1 2 2 3 3 H 4 J 5 Z 6 - 7	.PVGPLKAPKVP SVKIPARPAKVP PSLPAALKPKIP CLPPVAPKPKIP APTPVTTRGKIP KAKIP SSLPRKHIKIP	n PPNAFI PPNCFI PANEWI PANEWI PPNAYI PPNAYI PPNAYI PPNAYI	r LYRQ LYRQ LYRA LYRA LYRT LYRK LYRK LYRK	HHHPI ANHHI DNHII DNHLI DNHLI DHHR(DRHT FRHH	VELQN KIKE <mark>F</mark> LVKD PIKKF PIKLF QIREQ LVKKS	AYPDYSI AYPGVSI AYPGITI AYPGISI DNPGLHI SEPHLSI ANPCTU	DASPI S NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS	VMLG SIIA SIIA SIIA SIIA VIVG QVLG	NAK W KQWK ARWN GMWA GMWA GMWA NMWR KAWN KAWN	I FASF INESPE JAETPE JAETPE JAESDD RDEQPH IAEPPE IAESNO	W VREQI RRLKY RRLKY RREKY IRDKY VRQRY VRQRY	FRNLA FTHLA YKIRA YKIRA YKKRA YKEMS YKEMS	EELK DELK DLLK DLLK LVLK NEVK ERIK OOVK	JIRDA KKHAH KEHAJ EAHKH QAHKH ESHAH ARLLH KALLH OALLH	EDHPI IKHPI KAYPI RAHPI KAHPI KAHPI ERHP(KHPI	DYHYT DYQYA IYKYA IYKYA NYKYT DYRYN DYRYN DYQYQ	PRKI PRRI PRKI PRKI PRKI PRRI PRRI PRRI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SERKI	RTS: RTPI RASI RASI RVSI RVSI RVSI RKI	SRQFSI KKTLTI KKTLTI KKRLAI PYLK. TQDTI	KN
b Eurotiomycetes Leotiomycetes Sordariomycetes	1 1 2 2 2 3 H 4 J 5 Z 6 - 7 7 - 8	PVGPLKAPKVP SVKIPARPAKVP PSLPAALKPKIP LPLPVAPKPKIP APTPVTTRGKIP KAKIP KAKIP 	n PPNCFI PANEWI PANEWI PPNEWI PPNAYI PPNAYI PPNAYI PPNAYI PPNAYI PPNAYI PPNAYI	r LYRQ LYRQ LYRA LYRA LYRK LYRK LYRK LYRK LYRK	HHHPI ANHHI DNHII DNHII DNHLI DNHLI DHHR(DRHT) ERHHI	VELQN KIKE <mark>/</mark> PIKK/ PIKK/ DIRC UVKS YVKD <mark>/</mark> TMKOF	AYPDYSJ ANPGVSJ AYPGITJ AYPGITJ AYPGISJ NPGLHJ SEPHLSJ ANPGITJ NPGITJ NPGITJ	DASPI S NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS	VMLG RILG SIIA SIIA SIIA SVIVG QVLG QVLG QILG	NAK W KQWK ARWN GMWA GMWA GMWA KAWN KAWN KAWN	I F A SP INE SPE JAE TPE JAE TDE JAE SDD RDE QPH IAE PPE IME SND	W IKTQI VREQI RRLKY RRLKY IRDKY VRQRY VRQKY VRQKY	FRNLA FTHLA YKIRA YKIRA YKEMS YKEMS YKEMS	EELK DELK DLLK LVLK NEVK ERIK QQVK KMHK	IRDA KKHAH KEHAI EAHKI QAHKI ESHAH ARLLI KALLI QALLI FRLIM	EDHPI IKHPI KAYPI RAHPI KAHPI CAHPI ERHPI EKHPI	DYHYT DYQYA IYKYA IYKYA NYKYT DYRYN DYQYQ DYQYK	PRKI PRKI PRKI PRKI PRKI PRRI PRRI PRKI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SERKI SERKI	RTS: RTPI RASI RASI RVSI RVSI RRKI RRI RRI	SRQFSI KKTLTI KKTLTI KKRLAI PYLK. TQDTI ASPNQI	XN
b Eurotiomycetes Leotiomycetes Sordariomycetes	1 2 2 3 1 4 1 5 2 6 - 7 - 8 - 10 -	PVGPLKAPKVF SVKIPARPAKVF SSLPAALKPKIF PTPVTTRGKIF KAKIF .SSLPRKHIKIF .SPEPVCKIKVF KDKVA	n PPNAFI PANEWI PANEWI PPNAWI PPNAYI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PPNAFI	r LYRQ LYRQ LYRA LYRA LYRK LYRK LYRK LYRK LYRK	HHHPI ANHHI DNHII DNHII DNHLI DNHLI DHHR(DRHT) ERHHI DRHA'	VELQN KIKEZ LVKDZ PIKKZ PIKKZ QIREQ LVKKS YVKDZ IMKQE	AYPDYSJ ANPGVSJ AYPGITJ AYPGITJ AYPGISJ MPGLHJ SEPHLSJ MPGITJ SNSHLSJ MPDLHLSJ	S S NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS	VMLG RILG SIIA SIIA SIIA VIVG QVLG QVLG QILG ISLG	NAK W KQWK ARWN GMWA GMWA GMWA KAWN KAWN KAWN	ITASP INESPE AETPE AETPE AESDD RDEOPH IAEPPE IMESND ISESPA INEAAD	W IKTQI VREQI RRLKY RRLKY RREKY IRDKY VRQKY VRQKY VRQKY VRQKY	FRNLA FTHLA FTHLA YKIRA YKEMS YKEMS YKEMS YTELA	EELK DLLK DLLK LVLK NEVK ERIK QQVK KMHK EDIK	KKHAI KEHAI EAHKI QAHKI ESHAI ARLLI KALLI QALLI ERLLI KEHLS	EDHPI IKHPI KAYPI RAHPI KAHPI LDNPI ERHP(EKHPI MYPI SAHPI		PRKI PRKI PRKI PRKI PRKI PRKI PRRI PRKI PRK	SERKI SERKI SEKKI SEKKI SEKKI SERKI SERKI SERKI SERKI	RTS: RTPI RASI RASI RVSI RVSI RRKI RRZ IRK.	SRQFSI KKTLTI KKTLTI KKRLAI PYLK. TQDTI ASPNQI	KN KN KN KN KN ES IP
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes	1 2 3 3 1 1 5 2 6 7 - 8 9 1 1	PVGPLKAPKVE SVKIPARPAKVE SSLPAALKPKIE LPLPVAPKPKIE SSLPRKHIKIE SSLPRKHIKIE SPPVCKIKVE KDKVA KAKVA	n PPNAFI PANEWI PANEWI PPNEWI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PPNAFI	r LYRQ LYRQ LYRA LYRA LYRK LYRK LYRK LYRK LYRO	HHH PI ANHHI DNHIJ DNHIJ DNHIJ DHHR DRHT ERHHT DRHA' HHHP	VELQN KIKEZ LVKDZ PIKKZ PIKLZ QIREC UVKSZ IVKSC IVKSC	APPDYSI APPGVSI APPGUSI APPGITI APPGISI APPGISI APPGISI APPGITI SEPHLSI APPGITI SEPHLSI APPGITI SEPHLSI APPGITI SEPHLSI APPGITI	S NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNDIS	VMLG RILG SIIA SIIA SIIA VIVG QVLG QVLG QLG SIIG SIMLG	NAK W KQWK ARWN GMWA GMWA GMWA KAWN KAWN KAWN KAWN KAWN	ITASP INESPE AETPE AETPE AESDD RDEOPH IAEPPE IMESND ISESPA INEAAD	W IKTQI VREQI RRLKN RRLKN RREKN IRDKN VRQRN VRQKN VRQKN VRQKN VRQKN VRQKN VRQKN	FRNLA FTHLA FTHLA YKIRA YKEMS YKEMS YKEMS YTELA FKSMA	EELK DELK DLLK DLLK ERIK QQVK KMHK EDIK EKIK	IRDA KEHAI EAHKI QAHKI ESHAI KALLI QALLI ERLLI KEHLS KEHLS	EDHPI IKHPI KAYPI RAHPI KAHPI LDNPI ERHPC EKHPI MYPI SAHPI		PRKI PRKI PRKI PRKI PRKI PRKI PRKI PRKI	SERKI SERKI SEKI SEKI SEKI SEKI SERI SERI SERI	RTS: RTP RASI RASI RVSI RVSI RRVSI RRV RRZ IRK RRZ IRK	SRQFSI KKTLTI KKTLTI KKRLAI PYLK. TQDTI ASPNQI RKAE RKAE	KN KN K K K K K K K K K K K K K K K K K
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes	1 1 2 3 3 1 4 1 5 2 6 - 7 - 8 - 9 - 10 - 11 -	PVGPLKAPKVP SVKIPARPAKVP SSLPAALKPKIP DPLPVAPKPKIP APTPVTTRGKIP SSLPRKHIKIP SSLPRKDIKIP SPSPKDIKIP KAKVA KAKVA TKKSVAKVA	n PPNAFI PANEWI PANEWI PPNAWI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PPNAFI PANFI	r LYRQ LYRQ LYRA LYRA LYRK LYRK LYRK LYRK LYRQ LYRQ LYRQ LYRU	HHHPI ANHH DNHLJ DNHLJ DHHR(DRHT ERHH DRHA' HHHP	KIKE LVKD PIKK PIKK QIREC QIREC YVKD IMKQE IVKSC IVKSC SVSA	AYPDYS AYPGYS AYPGIT AYPGIT AYPGIT AYPGIT APGIT SEPHLS ANPGIT NSELS ONPLHI ONPLHI AYPCMH	S NNDI NNEI NNEI NNEI NNEI NNEI NNEI NNEI	VMLG SVMLG SIIA SIIA SIIA SVIVG QVLG QILG SISLG SIMLG SKIIG	KQWK ARWN GMWA GMWA GMWA KAWN KAWN KAWN KAWN KAWN KAWN KAWN KA	IT ASP INESPE AETPE AESDD RDEQPH IAE PPE IMESND ISESPA INEAAD INEAAD INEAAD INEAAD	W IKTQI VREQI RRLKN RRLKN RREKN IRDKN VRQKN VRX VRX VRX VRX VRX VRX VRX VRX VRX VRX	FRNLA FTHLA YKIRA YKIRA YKEMS YKDMS YTELA FKSMA YKORA	EELK DELK DLLK DLLK ERIK QQVK KMHK EDIK EDEK	KKHAH KEHA EAHKH QAHKH ESHAH KALLH QALLH ERLLM KEHLS KEHLS ROHA	EDHPI IKHPI KAYPI RAHPI KAHPI ERHPI ERHPI MYPI SAHPI SAHPI IAHPO	YHYT YQYA YXYY YXYY YXYY YQYQ YQYX YQYQ YQYQ	PRKI PRKI PRKI PRKI PRKI PRRI PRKI PRKI	SERKI SERKI SEKKI SEKKI SEKKI CERRI SEKRI SEKRI AEKKI	RTS: RTP RASI RASI RVSI RVSI RKI RRR RRI RRR RRT RMT RMT RMT	SRQFSI KKTLTI KKRLAI PYLK. TQDTI SPNQI SRKAE RKAEI KKRLAI	KN KN K K K K K K K K K K K K K K K K K
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes	1 2 3 3 1 4 1 4 1 5 2 6 7 - 8 9 10 - 11 11 - 12 - 12 - - 13 -	PVGPLKAPKVP SVKIPARPAKVP PSLPAALKPKIP LPLPVAPKPKIP APTPVTTRGKIP SSLPRKHIKIP SSLPRKHIKIP SPEPVCKIKVP KDKVA KDKVA KDKVA GTVAKRAP	n PPNAFI PANEWI PANEWI PPNAYI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PPNAFI PPNAFI PPNAFI PPNAFI	r LyrQ LyrQ LyrA LyrA LyrK LyrK LyrK LyrK LyrK LyrQ LyrQ LyrQ LyrQ LyrQ LyrQ	HHHPI ANHHI DNHIJ DNHIJ DHHR(DRHA ERHHI DRHA HHHP HHHP NHHAS AMHKI	KIKEZ LVKDZ PIKKZ PIKKZ PIKLZ VVKQ VVKDZ IVKQ IVKQ SVSAZ KLKS	AYPDYSI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGHH SYPSIT	NNDIS NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNDIS NNDIS VQQIS	VMLG SVMLG SIIA SIIA SIIA SVIVG QILG QILG SIMLG SIMLG SIMLG SIMLG	KQWK ARWN GMWA GMWA GMWA MMWR KAWN KAWN KAWN KAWN KAWN KAWN KAWN KAWN	DENEE INESPE AETPE AETPE IAESDD IDEOPH ISESPA INEAAD INEAAD INEAAD INEAAD INESSA	W IKTQI VREQI RRLKY RREKY IRDKY VRQKY VRQKY VRQKY VRQKY VRQKY VRQKY VRQKY VRQKY VRQKY	FRNLA FTHLA YKIRA YKIRA YKEMS YKDMS YTELA FKSMA FKSMA YKQRA WQTAA	EELK DELK DLLK LVLK NEVK ERIK QQVK KMHK EDIK EDIK EDEK KNAK	IRDA KKHAI EAHKI QAHKI ESHAI ARLLI QALLI ERLLI KEHLS REHLS RQHA EEHSI	EDHPI IKHPI KAYPI RAHPI KAHPI KAHPI ERHPI ERHPI SAHPI SAHPI SAHPI RAHPI ROHPI	YHYT YQYA IYKYA IYKYA IYKYA YXYY YQYQ YQYK YQYQ YQYQ YQYQ YQYQ YQYQ	PRKI PRRI PRKI PRKI PRKI PRRI PRRI PRRI	SERKI SERKI SEKKI SEKKI SEKKI CERRI SEKRI SEKKI SEKKI SEKKI	RTS: RTP RASI RASI RVSI RVSI RKI RRKI RRKI RRTI RMTI RMTI RMTI ROSI	SRQFSI KKTLTI KKTLTI KKRLAI PYLK. TQDTI ASPNQI RKAE: RKAE RKAEI KKLAI	KN KN KK KK KN KK KN K KN K KN K
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes Dothideomycetes	1 1 2 2 3 1 4 5 6 7 8 9 100 11 11 12 13 1	PVGPLKAPKVP SVKIPARPAKVP SLPAALKPKIP PTPVTRGKIP SSLPRKHIKIP SSLPRKHIKIP SPEPVCKIKVP KDKVA KDKVA KAKVA GTVAKRAP RAVAAGLKKAP	n PPNAFI PANEWI PANEWI PPNEWI PPNAYI PPNAYI PPNAFI PPNAFI PENAFI PENAFM PENAFM PENAFM PENAFM PENAFM	r Lyro Lyro Lyra Lyra Lyrt Lyrk Lyrk Lyrk Lyrk Lyro Lyrc Lyro Lyrd Lyrd Lyro Lyro Lyro Lyro Lyro Lyro Lyro Lyro	HHHPI ANHHI DNHIJ DNHIJ DHHR(DRHA ERHHI DRHA HHHP HHHP NHHAS AMHKI AMHKI	KIKEZ LVKDZ PIKKZ PIKKZ PIKKZ VVKDZ IVKSQ IVKSQ SVSAZ KLKSE HLRAE	AYPDYSI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGISI AYPGITI AYPGITI AYPGITI SEPHLSI AYPCHLSI AYPCHH SPDLHI AYPCHH SPDLHI CPPHLT	NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNDIS	VMLG STILG SIIA SIIA VIVG QVLG QVLG SIIA QVLG SIIA SIIA SIIA SIIA SIIA SIIA SIIA SII	KQWK ARWN GMWA GMWA GMWA GMWA KAWN KAWN KKWN QQWQ QQWQ QQWQ NMWS EIWH QIWH	IT ASP INESPE AETPE AETPE AESDD IDEOPH ISESPA INESND ISESPA INEAAD ISESQA IAFSPD IAFSPE	W IKTQI VREQI RRLKY RREKY IRDKY VRQKY VRQKY VRQKY VRQKY IKAQI VKDEY EKKVW AKRPY	FRNLA FTHLA YKIRA YKIRA YKEMS YKDMS YTELA FKSMA FKSMA YKQRA WQTAA	EELK DELK DLLK DLLK VVLK ERIK QQVK KMHK EDIK EELK KNAK QQAK	JIRDA KKHAI EAHKI QAHKI ESHAI ARLLI QALLI ERLI KEHLS EEHSI EEHLI	EDHPI IKHPI KAYPI RAHPI LDNPI ERHPC EKHPI MAYPI SAHPI IAHPC RQHPI RQHPI	DYHYT DYQYA DYRYA IYKYA IYKYA IYKYT DYRYN DYRYI DYQYQ YQYQ YQYQ YQYQ YQYQ YQYQ YQYQ Y	PRKI PRRI PRKI PRKI PRKI PRKI PRKI PRKI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI	RTS: RTPI RASI RASI RVSI RKI RKI RKI RKI RMTI RMTI RMTI RMTI ROSI RQSI	SRQFSI KKTLTI KKTLTI KKTLTI TQDTI ASSPNQI RKAEI KKAEI KKAEI KKAEI KKAEI	KN KN KK KK KK KK KK KK KK KK KK KK KK K
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes Dothideomycetes	1 1 2 3 3 1 4 1 5 2 6 7 8 9 100 11 12 13 13 1 15 5	PVGPLKAPKVF SVKIPARPAKVF SSLPAALKPKIF PLPVAPKPKIF APTPVTTRGKIF SSLPRKHIKIF SSLPRKHIKIF SPSPKDIKIF SPEPVCKIKVF KAKVA KAKVA GTVAKRAF LRAVAGLKKAP	n PPNAFI PANEWI PANEWI PPNAWI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PRAFI ERAFI EMNCWI PMNAFI PMNAFI	r LYRQ LYRQ LYRA LYRA LYRK LYRK LYRK LYRK LYRK LYRQ LYRK IFRD IFRD IFRD IFRD	HHHPI ANHHI DNHLI DNHLI DHHR(DRHT ERHHI DRHA' HHHP: HHHP: NHHA: AMHKI AMHKI	KIKEZ LVKDZ PIKKZ PIKKZ PIKKZ QIREC LVKKS VVKDZ IVKSC IVKSC SVSAZ KLKSE AVRQF	APPDYSI APPGVSI APPGITI APPGITI APPGITI SEPHLSI MPPGLHI SEPHLSI MPSLT SPDLHI APPCMHI SPDLHI APCMHI SPSLT CAPGIGI	S NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNQIS NNQIS NNQIS VQQIS NNDVS	VMLG STILG STILG STILG STILG STILG QVLG QVLG QVLG QVLG SILG STILG STILG STILG STILG STILG STILG STILG STILG	KQWK ARWN GMWA GMWA GMWA GMWA KAWN KKWN KKWN QQWQ QQWQ QNWS EIWH QIWH QIWH	KDENEE INESPE AAETPE AAETPE RDEOPH IAEPPE INESND ISESPA INEAAD INEAAD INEAAD INESPD INLSPE RELDPK	W VREQI RRLKN RRLKN RREKN IRDKN VRQKN VRX VRX VRX VRX VRX VRX VRX VRX VRX VRX	FRNLA FTHLA YKIRA YKIRA YKEMS YKEMS YTELA FKSMA FKSMA YKQRA WQTAA WQDAA	EELK DELK DLLK LVLK NEVK ERIK KMHK EDIK KNAK QQVK KNAK QSAK AKSA	JIRDA KEHAI EAHKI QAHKI ESHAI KALLI QALLI ERLIM KEHLS KEHLS RQHAI EEHSI REHHV	GUSG EDHEI IKHEI IKHEI IKAYEI CAHEI DNEI CAHEI CAHEI CAHEI COHEI ROHEI ROHEI VKHEC	DYHYT DYQYA DYQYA IYKYA IYKYA IYKYT DYRYI DYQYQ YQYQYK IYQYQ YQYQYQ IYQYQ YQYQYQ IYQYQ YQYQYQ IYYYT IYKYT	PRKI PRRI PRKI PRKI PRKI PRRI PRKI PRKI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI	RRTS: RTPI RASI RVSI RRVSI RRK. RRR. RRR. RRR. RRMTI RRMTI RMTI RMTI RRMTI RRMTI RRMTI RRMTI RRMTI	SRQFSI KKTLTI KKTLTI KKTLTI TQDTI ASPNQI RRKAE RKAEI KNKLAI KKAKQ KSKR SSAMV(KN KK SD
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes Dothideomycetes <i>Morchella importuna</i>	1 2 3 3 1 4 1 5 2 6 - 7 - 8 - 9 10 - 11 12 - 13 - 13 - 14 1 15 - 16 -	PVGPLKAPKVE SVKIPARPAKVE SSLPAALKPKIE PSLPAALKPKIE SSLPRAKIKIE SSLPRKHIKIE SSLPRKHIKIE SPPVCKIKVE KDKVA KAKVA TKKSVAKVA GTVAKRAP LRAVAAGLKKAP VE	n PPNAFI PPNCFI PANEWI PPNAWI PPNAYI PPNAYI PPNAFI PPNAFI PENAFI PENAFI PENAFI PENAFI PENAFI PENAFI PMNCWI PMNAFI	r LYRQ LYRQ LYRA LYRA LYRA LYRA LYRA LYRA LYRA LYRA	HHHPI ANHHI DNHLI DNHLI DHHLI DRHT ERHT DRHA HHPP HHHP MHHA AMHKI AMHKI TEMKI	YELQP KIKE VKIK PIKK PIKK QIR QIR QIR QIR QIR QIR QIR QIR QIR QIR	AYPDYSI ANPGVSI AYPGITI AYPGISI SEPHLSI ANPGITI AYPGISI SEPHLSI ANPGITI SEPHLSI ANPGIGI SPDLHI SPPLHI YPGMH YPGMG AYPGIGI GYPGIGI	NNDIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNEIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS NNQIS	VMLG SVMLG SIIA SIIA SIIA VIVG QVLG QVLG QVLG SISLG SI	KQWK KQWK ARWN GMWA GMWA GMWA KAWN KAWN KKWN QQWQ QQWQ QQWQ QQWQ QQWQ QQWQ QQ	KDENEE INESPE JAETPE JAETPE JAESDD IDEOPH IAESPA INEAAD INEAAD SRESOA IAFSPD INLSPE RELDPK	WITT W VREQI RRLKY RRLKY RREKY VRQKY VRQKY VRQKY VKQKY VKQKY VKQKY IRAQI IREHY IREHY	FRNLA FTHLA FTHLA YKIRA YKEMS YKEMS YTELA FKSMA FKSMA FKSMA YKQRA WQTAA WQTAA YKDLA	EELK DELK DLLK LVLK NEVK ERIK KMHK EDIK KNAK QQVK KNAK QSAK AKSA AKSA	LIRDA KKHAH KEHA: QAHKI QAHKI QALLH EESHAT KEHLS KEHLS ROHA: EEHSI REHHY REHHY	GVSG EDHEI IKHEI KAHEN ZAHEN ZDNEI ERHEZ ERHEZ ZAHEN Z	YHY YQQ YQYA YXYY YXYY YYY YQQ YQYQ YQYQ Y	PRKI PRKI PRKI PRKI PRKI PRKI PRKI PRKI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI GEKKI GEKKI SECVI	RTS: RTPI RASJ RASJ RRVSJ RRVSJ RRKI RRK. RMTI ROSJ RMTI ROSJ RRKI RRZ RKI RRKI RRKI	SRQFSI KKTLTI KKTLTI KKTLTI KKRLAI PYLK RVAEI RKAEI RKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI KKAEI	XN XX SP LL XL SD SD
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes Dothideomycetes Morchella importuna Morchella sextelata	$\begin{bmatrix} 1 & 0 \\ 2 & 0 \\ 3 & 1 \\ 4 & 1 \\ 5 & 2 \\ 6 & 0 \\ 7 & 0 \\ 8 & 9 \\ 10 & 0 \\ 11 & 0 \\ 12 & 0 \\ 13 & 0 \\ 11 & 0 $	PVGPLKAPKVP SVKIPARPAKVP PSLPAALKPKIP CPLPVAPKPKIP CPLPVAPKVF SSLPRKHIKIP SSLPRKHIKIP SSLPRKHIKIP SPEPVCKIKVP KDKVA KAKVA TKKSVAKVA GTVAKRAP LRAVAAGLKKAP VP VP	P PPNAFI PPNCFI PANEWI PANEWI PPNAYI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PMNCWI PMNCWI PMNAFI PMNAFI PMNAFI	r LYRQ LYRQ LYRA LYRA LYRA LYRK LYRK LYRK LYRK LYRQ LYRN IFRD IFRD IFRD IFRD IFRD IFRD IFRD IFRD	HHHPI ANHH DNHLI DNHLI DHHLI DHHLT DRHAT DRHAT HHHP NHAA AMHKI TEMKI TEMKI TEMKI	KIKEZ KIKEZ PIKKZ PIKKZ PIKKZ PIKKZ VKDZ IVKSC IVKSC IVKSC IVKSC IVKSC VKAC AVROP	AYPDYSI ANPGVSI ANPGUSI ANPGISI ANPGISI ANPGISI ANPGISI ANPGILH AYPGHLSI APPSLH COPSLH CYPGIG AYPGIG AYPGIG AYPGIG	NND I S NNE I NNE I NNE I NNE I NNE I NNE I NND I NND I NND I Q I Q I NND I NND V NND V NND V NND V	VMLG SRILG SIIA SIIA SIIA SIIA SIIA SIIA SIIA SII	KQWK ARWN GMWA GMWA GMWA GMWA KAWN KAWN KKWN QQWQ QQWQ QQWQ QQWQ QQWQ QQWQ QQ	ILLAS INESPE AETPE AESDD IDEOPH IAEPPE INESDD ISESPA INESPE INESPE INLSPE RELOPK RELOPK	WITTOI VREQU RRLKY RREKY VRQRY VRQRY VRQKY VRX VRX VRX VRX VRX VRX VRX VRX VRX VRX	FRNLA FTRLA FTLLA TKIRA YKIRA YKEMS YKCMS YKCMS YKCMS YKCRA YKCRA YKCLA YKDLA YKDLA YKDLA	EELK DELK DLLK DLLK NEVK ERIK QQVK KMHK EDIK EELK KNAK QQVK KMAK AKSA AKSA AKSA	IIRDA KKHAH KEHA: QAHKI ESHAH ARLLI KEHL: KEHL: KEHL: RQHA: RCHA: REHHI REHHI REHHI	CVSC EDHE KAPEI KAPEI CAPEI CDNEI CDNEI CAPE CAPEI CAP	DYHYT DYOYA IYKYA IYKYA YKYA DYRYT DYOYQ YRYT YQYQ YQYQ YQYQ YQYQ YQYQ YQYQ YQ	PRKI PRKI PRKI PRKI PRKI PRKI PRKI PRKI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI GEKKI GEKKI GEKKI SEVVI EDVVI	RTSP RASJ RASJ RASJ RRVS RRVS RRVS RRKI RRTI RRMTI ROSJ RRKI RRTI RRSI RRKI RRKI RRKI RRKI RRKI	SRQFSI KKTLTI KKRLAI PYLK. TQDTI ASPNQI RKAEI SRKAEI KKAKQI KKAKQI SSAWV(SSTV(SVTV(SVTV(KN
b Eurotiomycetes Leotiomycetes Sordariomycetes Lecanoromycetes Dothideomycetes Morchella importuna Morchella sextelata Mel-20	1 1 2 2 3 3 1 4 1 5 2 6 6 7 7 8 9 9 9 10 4 11 2 13 14 1 1 5 13 14 1 1 1 5 13 14 1 1 1 5 13 14 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	PVGPLKAPKVP SVKIPARPAKVP SSLPAALKPKIP SLPAALKPKIP SSLPAALKPKIP SSLPRKHIKIP SSLPRKHIKIP SPEPVCKIKVP KDKVA KDKVA KDKVA KAKVA GTVAKRAP RAVAACLKKAP VP VP VP VP VP VP VP VP VP VP VP VP VP	n PPNAFI PPNCFI PANEWI PPNAWI PPNAYI PPNAYI PPNAFI PPNAFI PPNAFI PMNCWI PMNCWI PMNAFI PMNAFI PANAFI PANAFI	r LYRQ LYRQ LYRQ LYRA LYRA LYRA LYRA LYRA LYRA LYRA LYRA	HHHPI ANHH DNHLI DNHLI DHHR DRHT ERHH DRHT NHHP NHHP NHHP NHHA: AMHKI AMHKI TEMKI TEMKI TEMKI	VELQN KIKEZ PIKKZ VKDZ VKDZ VKDZ VKDZ VKDZ VKDZ VKDZ V	AYPDYS AYPDYS AYPGITI AYPGITI AYPGITI AYPGIS DNPGLH SEPHLSS DNPCLH AYPGIS ONPDLH AYPCMH SPDLH AYPCMH CYPGIG CYPGIG CYPGIG YPGIG YPGIG	NND I S NNE I NNE I NND I NND I	IAVL VMLG SIIA SIIA SIIA VIVG OVLG OVLG OVLG CILG IMLG FING RIG RIG RIG RIG RIG RIG RIG RIG RIG RI	W KQWK KARWN GMWA GMWA GMWA GMWA GMWA KAWN KAWN KAWN KAWN KAWN KAWN KAWN KA	ILFASF INESPE AAETPE AAESDD DEOPH IAEPPE IMESDD INEAAD INEAAD INEAAD INESPE INLSPE KELDPK INLSPE INLSPE	IKTOI VREQI RRLKY RREKY VRQRY VRQRY VRQRY VRQCY VKAQI IKAQI VKDEY EKKVW AKRPP IRAN IRAN IRAN IRAN IRAN IRAN IRAN IRAN	ERNLA FTHLA FTHLA KIRA KIRA KIRA KIRA KIRA KIRA KIRA KIR	EELK DELK DLLK LVLK NEVK ERIK QQVK KMHK EDIK EDIK EDIK EDIK EDIK EDIK EDIK EDI	IIRDA KKHAH KEHA: QAHKI QAHKI ESHAH KEHL KEHL KEHL RQHA: RCHA: REHI REHH REHH ASHKI	GVSG EDHEI IKHBI IKHBI IKHBI DNI CRHE CRHE CRHE CRHE CRHE CRHE CRHE CRHE	DYHYII DYQYA IYKNA IYKNA IYKYA YEYN YCYQ YCYK YYKYI YKYI SYKYI SYKYI SYKYI YKYI	PRKI PRKI PRKI PRKI PRKI PRKI PRKI PRKI	SERKI SERKI SEKKI SEKKI SEKKI SEKKI SEKKI SEKKI GEKKI GEKKI GEKKI SEDVVI SEDVVI SEDVVI SEDVVI	RTS: RTS: RASJ RASJ RRSJ RRSJ RRRJ RRTJ RRTT RMTT RMTT RMTT RMTT RRTT RR	SRQFSI KKTLTI KKRLAI PYLK. TQDTI ASPNQI SRKAEI RKAEI RKAEI KKAEI KKAKQ KSKTV SAMV(SATV(SATV) SAMV(SATV)	KN SS PP LL KL DD DD JD JT DD JD JD JT DD JD D JT DD JD D JT DD D D JT DD D D

747

Fig. 2 Amino acid alignment of conserved regions of MAT proteins of Morchella spp. with those of other ascomycetes. a Amino acid alignment of the conserved alpha-box domain of the following species: (1) Stemphylium callistephi(AAR04468); (2) Alternaria brassicicola(AAK85542); (3) Aspergillus fischeri(XP_001263836); (4) Talaromyces marneffei (ABC68484); (5) Histoplasma capsulatum (ABO26868); (6) Ophiocordyceps sinensis (ALH25059); (7) Cordyceps militaris (AKM95188); (8) Fusarium poae (CAD59610.3); (9) Colletotrichum musae (CAD59611.3); (10) Fusarium avenaceum (CAD59608.4); (11) Morchella sextelata; (12) Morchella importuna; (13) Mel-20; (14) Tuber indicum (AHE80942); (15) Tuber melanosporum (ADU56595); (16) Tuber borchii (AIU38080); (17) Xanthoria polycarpa (CAI59771); (18) Pyrenopeziza brassicae

rp n

(CAA06844); (19) Sclerotinia homoeocarpa (AJW31369). **b** Amino acid alignment of the conserved HMG domain of following species: (1) Aspergillus fumigatus (XP_754989.2); (2) Penicillium chrysogenum (CAP17333); (3) Sclerotinia sclerotiorum (AGB05594); (4) Sclerotinia trifoliorum (ANN44262); (5) Sclerotinia homoeocarpa (AJW31335); (6) Sordaria fimicola (CAB63226); (7) Cordyceps militaris (AKM95197); (8) Ophiocordyceps sinensis (AEH27625); (9) Colletotrichum gloeosporioides (AKO22190); (10) Rusavskia elegans (CAI59778.2); (11) Dufourea flammea (CAI59780.2); (12) Passalora fulva (ABG49507); (13) Leptosphaeria maculans (AAO37761); (14) Bipolaris sorokiniana (AAF87724); (15) Morchella importuna; (16) Morchella sextelata; (17) Mel-20; (18) Tuber borchii (AIU38078); (19) Tuber indicum (AHE80950)

руург

Structure of the MAT1-2-1 gene of three morel species

The lengths of *MAT1–2-1* gene sequences of *M. importuna*, *M. sextelata* and *Mel-20* were 1197, 1199 and 1247 bp, respectively. The results of RT-PCR analyses confirmed that the *MAT1–2-1* ORFs contained three introns and encoded proteins of 329, 329 and 344 amino acids, respectively (Table 3). Sequence alignments and dotplot analyses indicated that the *MAT1–2-1* gene in *M. importuna* shared 94.3 and 86.3% sequence identity with the corresponding genes in *M. sextelata* and *Mel-20*, respectively. The alignment of the MAT1–2-1 protein with corresponding sequences deposited

in GenBank indicated that the HMG-box motifs of three morels were conserved (Fig. 2b).

Mating type ratios of three morel species based on single-ascospore populations

The primer pairs p8 for the *MAT1–1-1* gene and p10 for the *MAT1–2-1* gene could be used in all three morel species to examine the mating types of single-ascospore populations. Strains that contained only *MAT1–1-1* and not *MAT1–2-1* were amplified and scored as *MAT1–1*, and vice versa (Fig. 3). In the four ascospore populations (Table 4), the ratio of *MAT1–1* to

Fig. 3 MAT gene PCR products of single ascospore populations YPL2, HL1 and WXLBD7, a MAT1-1-1 gene PCR products of YPL2-1 to YPL2-24. b MAT1-2-1 gene PCR products of YPL2-1 to YPL2–24. c MAT1–1-1 gene PCR products of HL1-1 to HL1-24. d MAT1-2-1 gene PCR products of HL1-1 to HL1-24. e MAT1-1-1 gene PCR products of WXLBD7-1 to WXLBD7-24. f MAT1-2-1 gene PCR products of WXLBD7-1 to WXLBD7-24. M DNA marker 2000 (2000,1000, 750, 500, 250 and 100 bp)



MAT1–2 ranged from 0.44 to 0.96. Chi-square analysis indicated that the ratio was not significantly different from 1:1 except for the HL1 populations. No sequence difference was detected between the single-ascospore strains and the parent strains in either of the *MAT1–1-1* or *MAT1–2-1* genes.

 Table 4
 Summary of MAT type ratios of four ascospore populations

Population	п	MAT1-1	MAT1-2	Ratio ^a	Chi-Sq ^b	P-value ^c
HL1	46	14	32	0.44	7.043	0.00796
WXLBD7	53	26	27	0.96	0.019	0.89037
YPL2	66	32	34	0.94	0.061	0.80492
YPL6	105	47	58	0.81	1.152	0.28313

n number of single-ascospore populations per each ascocarp.

^a Ratio of MAT1-1-1 to MAT1-2-1.

^b Chi-square value for relative to the expected ratio of 1:1.

^c Probability of chi-square values at $P \le 0.05$ at 1 degree of freedom

Discussion

Heterothallism in M. importuna, M. sextelata and Mel-20

In ascomycetes, the mating type corresponds to two allelic forms of a single locus and, accordingly, heterothallism is bipolar (Whitehouse 1949). This property was first demonstrated in *Ascobolus magnificus* and *A. carbonarius* (Dodge 1920) and then in several *Neurospora* species (Shear and Dodge 1927). Molecular analyses of mating-type in filamentous ascomycetes began with the cloning of *A* and *a* matingtypes from *Neurospora crassa* (Glass et al. 1988). It has since been confirmed that the mating-type locus contains one of two highly divergent sequences occupying the same chromosomal locus. Metzenberg and Glass (1990) used the word idiomorph to denote that these large sequences are not obviously related by structural similarity. In heterothallic ascomycetes, ascospore populations can be divided into two groups: those harbouring the *MAT1–1* and *MAT1–2* idiomorphs. The sexual cycle occurs only between two individuals with opposite mating-type, rather than with self-fertilizing as in homothallic reproduction (Pöggeler 2001; Chilvers et al. 2014).

The genomic data of *M. importuna* YPL6 confirmed that the *MAT* locus is divided into two idiomorphs. In the YPL6–3 genome, only the *MAT1–1* idiomorph harbouring the *MAT1– 1-1* gene was found. Similarly, the *MAT1–2* idiomorph with the *MAT1–2-1* gene was only located in the YPL6–1 genome. Furthermore, the conserved flanking sequences of the two idiomorphs confirmed that they occupy the same chromosomal locus. The characterization of the mating locus is consistent with heterothallic ascomycetes. Mating-type analysis of four ascospore populations from three species, *M. importuna, M. sextelata* and *Mel-20*, indicated that two sexual groups were present, and the ratios of *MAT1–1-* and *MAT1–2-*harbouring idiomorphs were not significantly different from a 1:1 ratio. These results suggested that these morels are all heterothallic.

Because the lack of visible structures, such as the clamp connection in basidiomycetes precludes visible morphological analysis, these molecular data will facilitate crossbreeding to produce high-quality morel cultivars.

Structure of MAT idiomorphs

In heterothallic ascomycetes, the structure of the MAT idiomorph varies in different species. The mating idiomorphs of Neurospora crassa were the first to be investigated, and the MAT1-1 idiomorph contains three ORFs: MAT1-1-1, MAT1-1-2 and MAT1-1-3 (mat A-1, A-2 and A-3 in N. crassa). The MAT1-2 idiomorph contains the MAT1-2-1 and MAT1-2-2 ORFs (mat a-1 and a-2 in N. crassa) (Pöggeler and Kück 2000). Similar MAT structures have been found in many other species surveyed later (Debuchy and Turgeon 2006; Wilken et al. 2012; Hutchinson et al. 2016). In this study, M. importuna has been identified as possessing a similar MAT idiomorph structure similar to those previously described. The MAT1-1 idiomorph contains three OFRs, and the MAT1-2 idiomorph contains a single MAT1-2-1 gene. However, two ORFs,GME3123 and GME3124,in the MAT1-1 idiomorph were found to be highly divergent from the homologues of mating genes that have been published. At this point, we can only speculate that GME3123 and GME 3124 are new mating-type genes at the MAT1-1 locus of M. importuna.

APN2, *SLA2* and *APC5* are ancestral companions of the *MAT* locus in euascomycetes (Debuchy and Turgeon 2006). Similarly, homologues of *APN2* and *SLA2* were found on each side of the *MAT* locus in Sordariomycetes, and the *ATG3*, *cox13* and *CWF24* genes were also conserved in the up- and downstream regions of *MAT* (Debuchy and Turgeon 2006; Xu et al. 2016; Lu et al. 2016). For *M. importuna*, the *APN2* gene was connected to a *cox13* gene on the 5' flanking region of

both *MAT* idiomorphs. However, four predicted genes were found between the *cox13* gene and the *MAT* idiomorphs. The *SLA2* (*end4*) gene was located on the 3' flanking region of two idiomorphs. These results indicated that the *MAT* locus structure of *M. importuna* had some conserved sequences but was highly divergent from other species.

Based on the conserved flanking sequences, *MAT1–1* and *MAT1–2* idiomorphs have been cloned from many species (Martin et al. 2011; Belfiori et al. 2013). In this study, the coding sequences flanking the two idiomorphs of *M. importuna* were highly conserved, but the cloning of the whole *MAT* idiomorph of *M. sextelata* and *Mel-20* using long-distance PCR amplification was not successful (data not shown), possibly due to the variability of the sequence in the different species. Unfortunately, we were not able to identify the entire mating idiomorphs of these two morel species.

Expression of mating genes

In *Neurospora crassa* and *Tuber melanosporum*, mating genes are constitutively expressed during the vegetative as well as the sexual phases (Ferreira et al. 1996; Rubini et al. 2011). In this study, we did not analyse the expression of mating genes specially in different cultivation periods and conditions. However, we verified the coding region by successfully amplifying the *MAT1–1-1* or *MAT1–2-1* gene with cDNA from all the tested strains incubated on PDA for 10 days. For the single-ascospore isolates, *MAT1–1-1* of YPL6–3, HL1–1 and WXLBD7–11, and *MAT1–2-1* of YPL6–1, HL1–47 and WXLBD7–13 were amplified with their cDNA, respectively. In addition, the expression of the two genes was detected in the mycelia from the YPL6, WXLBD7 and HL1 tissues. These results implied that the mating genes in morels are constitutively expressed.

Mating locus and phylogenetic relationship

In ascomycetes, as throughout the eukaryotic kingdom, genes controlling sex determination are evolving more rapidly than other protein-coding genes (Whitfield et al. 1993; Ferris et al. 1997; Pöggeler 1999), which enables the study of the phylogenetic relationship of closely related fungal species. The sequences from the *gpd* (glyceraldehyde-3-phosphate dehydrogenase gene), *mat A-1* and *mat a-1* genes produced phylogenetic trees with a similar topology and strict separation of homothallic and heterothallic species within the genera *Neurospora* and *Sordaria* (Pöggeler 1999). Belfiori et al. (2013) revealed the existence of cryptic species of *Tuber indicum* by comparing the idiomorph structure and sequences of *MAT* genes.

Morchella importuna and *M. sextelata* had a closer phylogenetic relationship than did either to *Mel-20* (Fig. S1). Based on comparing the DNA sequences of the *MAT1–1-1* and *MAT1–2-1* genes, *M. importuna* showed greater identity to *M. sextelata* than to *Mel-20*, which was consistent with the molecular evolution relationship based on the analysis of ITS, *RPB1*, *RPB2* and *TEF1* (Du et al. 2012; Richard et al. 2015). Acquiring more sequence information on the mating loci will help to investigate the phylogenetic diversity of morel species, thereby producing new insights.

Acknowledgements This work was supported by the National Natural Science Foundation Program of PR China (31460014) and the China Agriculture Research System (CARS-24).

References

- Belfiori B, Riccioni C, Paolocci F, Rubini A (2013) Mating type locus of Chinese black truffles reveals heterothallism and the presence of cryptic species within the *T. indicum* species complex. PLoS ONE 8(12):e82353
- Belfiori B, Riccioni C, Paolocci F, Rubini A (2016) Characterization of the reproductive mode and life cycle of the whitish truffle *Tuber borchii*. Mycorrhiza 26:515–527
- Buscot F (1994) Ectomycorrhizal types and endobacteria associated with ectomycorrhizas of *Morchella elata* (Fr.) Boudier with *Picea abies* (L.) karst. Mycorrhiza 4:223–232
- Chilvers MI, Jones S, Meleca J, Peever TL, Pethybridge SJ, Hay FS (2014) Characterization of mating type genes supports the hypothesis that *Stagonosporopsis chrysanthemi* is homothallic and provides evidence that *Stagonosporopsis tanaceti* is heterothallic. Currr Genet 60:295–302
- Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol. Mol Biol Rev 61:411–428
- Debuchy R, Turgeon BG (2006) Mating-type structure, evolution, and function in Euascomycetes. The Mycota 1:293–323
- Dodge BO (1920) The life history of Ascobolus magnificus. Mycologia 12:115–134
- Du XH, Zhao Q, O'Donnell K, Rooney AP, Yang ZL (2012) Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China. Fungal Genet Biol 49:455–469
- Du XH, Zhao Q, Yang ZL (2014) Diversity, evolutionary history and cultivation of morels: a review. Mycosystema 33:183–197
- Ferris JP, Pavlovic C, Fabry S, Goodenough UW (1997) Rapid evolution of sex-related genes in *Chlamydomonas*. Proc Natl Acad Sci U S A 94:8634–8639
- Ferreira AVB, Saupe S, Glass NL (1996) Transcriptional analysis of the mt A idiomorph of Neurospora crassa identifies two genes in addition to mt A-1. Mol Gen Genet 250:767–774
- Fu LH, Wang YP, Wang JJ, Yang YR, Hao LM (2013) Evaluation of the antioxidant activity of extracellular polysaccharides from *Morchella* esculenta. Food Funct 4:871–879
- Glass NL, Vollmer SJ, Staben C, Grotelueschen J, Metzenberg RL, Yanofsky C (1988) DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. Science 241:570–573
- Hervey A, Bistis G, Leong I (1978) Cultural studies of single ascospore isolates of *Morchella esculenta*. Mycologia 70:1269–1274
- Hu M, Chen Y, Wang C, Cui H, Duan P, Zhai T, Yang Y, Li S (2013) Induction of apoptosis in HepG2 cells by polysaccharide MEP-II from the fermentation broth of *Morchella esculenta*. Biotechnol Lett 35:1–10
- Hutchinson MI, Powell AJ, Tsang A, O'Toole N, Berka RM, Barry K, Grigoriev IV, Natvig DO (2016) Genetics of mating in members of the Chaetomiaceae as revealed by experimental and genomic

characterization of reproduction in *Myceliophthora heterothallica*. Fungal Genet Biol 86:9–19

- Lu YZ, Xia YL, Luo FF, Dong CH, Wang CS (2016) Functional convergence and divergence of mating-type genes fulfilling in *Cordyceps militaris*. Fungal Genet Biol 88:35–43
- Masaphy S (2010) Biotechnology of morel mushrooms: successful fruiting body formation and development in a soilless system. Biotechnol Lett 32:1523–1527
- Martin SH, Wingfied BD, Wingfield MJ, Steenkamp ET (2011) Structure and evolution of *Fusarium* mating type locus: new insights from the *Gibberella fujikuroi* complex. Fungal Genet Biol 48:731–740
- Metzenberg RL, Glass NL (1990) Mating type and mating strategies in *Neurospora*. BioEssays 12:53–59
- Miller SC(2005) Cultivation of Morchella US Patent 6,907,691
- Nelson MA (1996) Mating systems in ascomycetes: aromp in the sac. Trends Genet 12:53–59
- O'Donnell K, Rooney AP, Mills GL, Kuo M, Weber NS, Rehner SA (2011) Phylogeny and historical biogeography of true morels (*Morchella*) reveals an early cretaceous origin and high continental endemism and provincialism in the Holarctic. Fungal Genet Biol 48: 252–265
- Ower R, Mills GL, Malachowski JA(1986) Cultivation of *Morchella*. US patent 4594809
- Pagliaccia D, Douhan GW, Douhan LA, Peever TL, Carris LM, Kerrigan JL (2011) Development of molecular markers and preliminary investigation of the population structure and mating system in one lineage of black morel (*Morchella elata*) in the Pacific northwestern USA. Mycologia 103:969–982
- Peng WH, Tang J, He XL, Chen Y, Tang H (2016) Status analysis of artificial cultivation of *Morchella* in Sichuan(Chinese). Edible Med Mush 24:145–150
- Pildain MB, Visnovsky SB, Barroetavena C (2014) Phylogenetic diversity of true morels (*Morchella*),the main edible non-timber product from native Patagonian forests of Argentina. Fungal Biol 118:755–763
- Pilz D, McLain R, Alexander S, Villarreal-Ruiz Berch S, Wurtz TL, Parks CG, McFarlane E, Baker B, Molina R, Smith JE (2007) Ecology and Management of Morels Harvested from the Forests of Western North America. Gen Tech Rep PNW-GTR-710. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland
- Pöggeler S (1999) Phylogenetic relationships between mating-type sequences from homothallic and heterothallic ascomycetes. Curr Genet 36:222–231
- Pöggeler S (2001) Mating-type genes for classical strain improvements of ascomycetes. Appl Microbiol Biotechnol 56:589–601
- Pöggeler S, Kück U (2000) Comparative analysis of the mating-type loci from *Neurospora crassa* and *Sordaria macrospore*: identification of novel transcribed ORFs. Mol Gen Genet 263:292–301
- Richard F, Bellanger JM, Clowez P, Hansen K, O'Donnell K, Urban A, Sauve M, Courtecuisse R, Moreau PA (2015) True morels (*Morchella*, Pezizales) of Europe and North America: evolutionary relationships inferred from multilocus data and a unified taxonomy. Mycologia 107:359–382
- Rubini A, Belfiori B, Riccioni C, Tisserant E, Arcioni S, Martin F, Paolocci F (2011) Isolation and characterization of MAT genes in the symbiotic ascomycete *Tuber melanosporum*. New Phytol 189:710–722
- Shear CL, Dodge BO (1927) Life histories and heterothallism of the red bread mould fungi of the *Monillia sitipholia* group. J Agric Res 34: 1019–1042
- Su CA, Xu XY, Liu DY, Wu M, Zeng FQ, Zeng MY, Wei W, Jiang N, Luo X (2013) Isolation and characterization of exopolysaccharide with immunomodulatory activity from fermentation broth of *Morchella conica*. Daru J Pharmaceuti Sci 21:5
- Turgeon BG, Yoder OC (2000) Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet Biol 31:1–5

- Volk TJ, Leonard TJ (1990) Cytology of the life-cycle of *Morchella*. Mycol Res 94:399–406
- Whitfield S, Lovell-Badge R, Goodfellow NP (1993) Rapid sequence evolution of the mammalian sex-determing gene SRY. Nature 364: 713–715
- Wilken PM, Steenkamp ET, Hall TA, De Beer ZW, Wingfield MJ, Wingfield BD (2012) Both mating types in the heterothallic fungus

Ophiostoma quercus contain *MAT1-1* and *MAT1-2* genes. Fungal Biol 116:427–437

- Whiterhouse HLK (1949) Heterothallism and sex in the fungi. Biol Rev 24:411-447
- Xu LS, Jardini TM, Chen WD (2016) Direct repeat-mediated DNA deletion of the mating type MAT1-2 genes results in unidirectional mating type switching in Sclerotinia trifoliorum. Sci Rep 6:27083