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FULL PAPER

Molecular assessment of fungi in ‘‘black spots’’ that deface murals in the Takamatsuzuka and Kitora Tumuli in Japan: *Acremonium* sect. *Gliomastix* including *Acremonium tumulicola* sp. nov. and *Acremonium felinum* comb. nov.

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Abstract Unidentified black spots (or stains) appeared on the plaster walls of the Takamatsuzuka and Kitora Tumuli in the village of Asuka, Nara Prefecture, Japan. Public attention was drawn to the biodeterioration of the colorful 1,300-year-old murals. A total of 46 isolates of *Acremonium* sect. *Gliomastix* were obtained from various samples (mainly black spots) of the Takamatsuzuka Tumulus (TT) (sampling period, May 2004–December 2006) and the Kitora Tumulus (KT) (June 2004–May 2007). These isolates were assignable to four known taxa and a new species in the ‘series *Murorum*’ sensu W. Gams as inferred from the integrated analysis of phenotypic and genotypic (i.e., ITS and 28S rDNA-D1/D2 sequences) characters: these were *Acremonium masseei*, *A. murorum*, *A. felinum* comb. nov. with the neotype designation, *A. polychromum*, and *A. tumulicola* sp. nov., which have been accommodated in the validated series *Murorum* in the section *Gliomastix*. The black spots on the murals of the TT and KT were caused mainly by *A. masseei* and *A. murorum*, respectively.

Keywords Biodeterioration · Black spots on murals · Cultural properties · Dark *Acremonium* · Molecular systematics

Introduction

The anamorph-genus *Acremonium* sect. *Gliomastix* (hereafter abbreviated as dark *Acremonium*) is characterized by having “chondroid hyphae” and short, rarely sympodially proliferating phialides, which are usually darkly pigmented ameroconidia in slimy heads or in dry, more or less persistent conidial chains; it is also characterized by a lack of chlamydospores (Gams 1971; Domsch et al. 2007). These species appear commonly in soil, associated with plants, or as airborne fungi (Gams 1971; Domsch et al. 2007).

The anamorph-genus *Gliomastix* Guéguen (1905) is typified by *G. chartarum*, which was recognized by Hughes (1958) as a synonym of *G. murorum* (Corda) S. Hughes. Sixty-three years after Guéguen, the genus was monographed for the first time in 1968 (Dickinson 1968; Hughes and Dickinson 1968) and characterized by darkly pigmented phialoconidia. *Gliomastix*, the so-called dark *Acremonium*, was moved down by Gams (1971) to a section of *Acremonium*. The concept of *Gliomastix murorum* with two varieties was discussed by Hammill (1981). Recently, *Acremonium* sensu Gams (1971) with the soil-borne species has been taxonomically revised and compiled by Gams in Domsch et al. (2007). Morphology-based taxonomic treatments of dark *Acremonium* (Gams 1971)

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and *Gliomastix* (e.g., Dickinson 1968; Hughes and Dickinson 1968; Hammill 1981) have not yet been challenged. Lechat et al. (2010) demonstrated the connection of *G. fusigera* with a teleomorph in *Hydropisphaera*. This genus, similar to *Emericellopsis*, the teleomorph of *Acremonium* in the strictest sense, is a member of the Bionectriaceae, as shown by molecular evidence. Because there are too many hyaline-spored *Acremonium* species at the borderline of these genera, we do not yet follow this generic separation. On the other hand, there are a few molecular phylogenetic studies on selected *Acremonium* species (Glenn et al. 1996; Rossman et al. 2001; Seifert et al. 2003; Zuccaro et al. 2003, 2004; Sigler et al. 2004; Zare et al. 2007). To date, however, no world monographic studies based on the integrated analysis of phenotypic and genotypic characters have been made on the genus. Such actions are urgently needed because the genus contains taxa that are agriculturally, economically, and medically important. In this article, we follow Gams's concept of the anamorph-genus *Acremonium* (Gams 1971) in a very broad sense, in which *Gliomastix* was classified as a section beside the other sections *Acremonium* and *Nectrioidea*.

In Europe, fungal biodeterioration is well known to affect aspects of cultural heritage, such as murals (Dhawan et al. 1993; Guglielminetti et al. 1994; Berner et al. 1997; Karbowska-Berent 2003). The roles of fungi in the deterioration of murals, as well as their decay mechanisms, have been reviewed by Garg et al. (1995) and Caneva et al. (2008). Black stains (or spots) caused by dematiaceous anamorphic fungi (e.g., *Cladosporium*) on the mural paintings often cause problems in cultural heritage conservation (Arai et al. 1991; Ciferri 1999; Caneva et al. 2008). Fungal stains (or spots) can be caused mainly by the secretion of metabolites or the pigmentation of fungi, especially melanins (e.g., Diakumaku et al. 1995; Saiz-Jiménez 1995; Nieto-Fernández et al. 2003). Even after the fungus is dead, the pigmented cell walls remain on the surface of the substratum. These substances are particularly resistant to chemical and enzymatic degradation (Nieto-Fernández et al. 2003). Species of dark *Acremonium* or *Gliomastix* have been implicated in the biodeterioration of wall paintings by several authors; e.g., cave wall paintings in the Lascaux cave in France (Orial and Mertz 2006; Orial et al. 2009), indoor mural paintings in Europe (Nugari et al. 1993), Ajanta wall paintings in India (Dhawan et al. 1993), and Ozuka Tumulus paintings in Japan (Emoto and Emoto 1974).

In a cave with prehistoric (15,000-year-old) paintings in Lascaux, France, in 2001 white molds identified as the *Fusarium solani* (Mart.) Sacc. species complex (FSSC) initially appeared on the cave wall (Orial and Mertz 2006; Dupont et al. 2007). The next year, emergence of the black mold *Gliomastix murorum* (sic) was reported by Orial and Mertz (2006) and by Orial et al. (2009). In July 2007, novel black colonizations were observed resulting from dematiaceous molds of the anamorph-genera *Ulocladium* and *Scolecobasidium* (Bastian and Alabouvette 2009; Orial et al. 2009; Bastian et al. 2010).

Serious problems with black spots (or stains) on murals occurred in the Takamatsuzuka Tumulus (hereafter abbreviated to TT) and the Kitora Tumulus (KT), both of which are Special Historic Sites in Asuka-mura (the village of Asuka), Nara Prefecture, Japan. Both TT and KT had 1,300-year-old mural (wall) paintings, which were drawn directly onto thin plaster, in the small stone chamber interior of each tumulus. After the tumuli were excavated, molds appeared on the mural paintings at both sites (Arai 1984, 1987; Kigawa et al. 2006, 2009). In previous papers we have reviewed the history of biological issues of both tumuli (Kiyuna et al. 2008; Sugiyama et al. 2008, 2009; Kigawa et al. 2009). In February 2001, renovation work was done in the space adjacent to the stone chamber of TT. Falling soil and leakage of rainwater had occurred because the environmental preservation facility was aging (Kigawa et al. 2009). In December 2001, after remediation work in the space adjacent to the stone chamber of TT, a dark *Acremonium* was isolated for the first time near a painting named "blue dragon" (*Seiryu*) on east wall 2 and above a painting named "white tiger" (*Byakko*) on west wall 2 (Kigawa et al. 2006, 2009). In October 2002, black stains appeared near the painting of blue dragon, east wall 2, and the painting of women, east wall 3. It was too difficult to remove the stains on site. From 2004 onward, viscous gels (i.e., biofilms, which are mixtures of molds, yeasts, and bacteria) also appeared on the wall plaster (Kigawa et al. 2009). Because of the continuing deterioration of fragile supports (plaster walls and cut slabs of tuff stone) in addition to the serious contamination and blackening of the mural paintings, the Agency for Cultural Affairs decided to dismantle the stone chamber in March 2005 to save and restore the murals. In September 2005, cooling of the TT mound was started to slow down fungal growth before the stone chamber was dismantled. In February 2006, in spite of this interior cooling, black spots appeared on paintings of a group of four women, called the "Asuka beauties" (*Asuka Bijin*), on west wall 3. In May 2006, the temperature of the stone chamber interior was kept stable at about 10°C, but the black spots expanded on the plaster walls (Fig. 1) (Kigawa et al. 2007a, 2009). After the excavation of the mound, the stone walls with the mural paintings were moved to a restoration facility in the village of Asuka by the end of August 2007.

Similar black spots were also seen on the walls of the stone chamber interior of the KT in June and October 2006 (Fig. 1) (Kigawa et al. 2007b, 2008); thereafter, these spots continuously developed further on the plaster walls in the KT chamber (Kigawa et al. 2008; Sano et al. 2008).

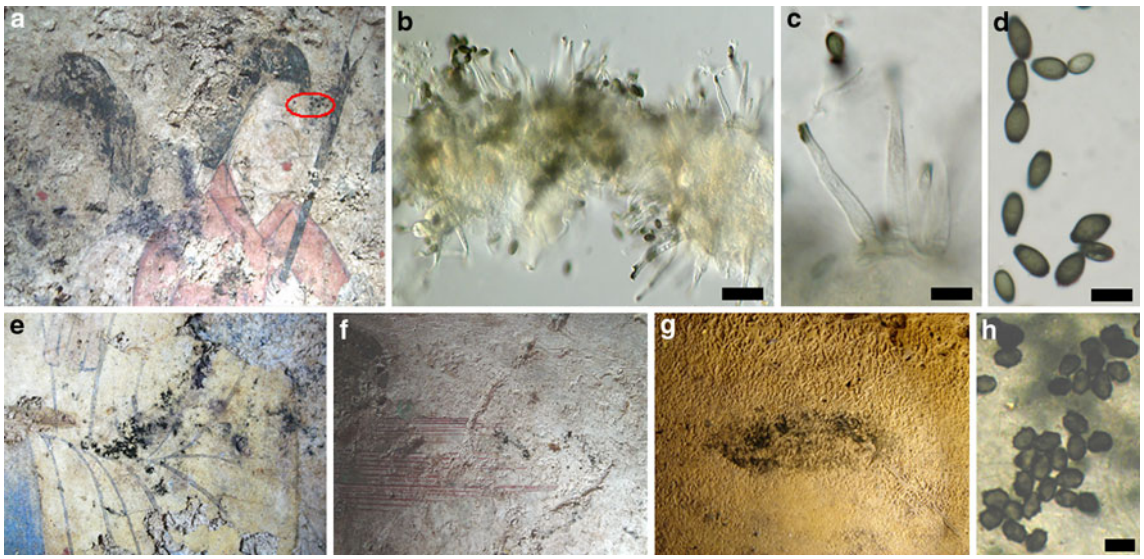

Fig. 1 Black spots on the mural paintings in the Takamatsuzuka Tumulus (**a**–**f**) and Kitora Tumulus (**g**, **h**). **a** A group of women, the ''Asuka beauties,'' on the west wall plaster [photograph taken on 17 May 2006 (sample no. T6517-1) by the Agency for Cultural Affairs, Japan]. **b–d** Conidiophores and conidia in slide preparations made directly from the part of the mural painting indicated by a *red circle* in **a**. **e** Part of a group of women on the west wall plaster (photograph taken on 13 December 2006 by the Agency for Cultural Affairs). **f** Right part of the *red line* on the west wall (photograph taken on 17 May 2006 by the Agency for Cultural Affairs). **g** Black powder-like colonies on the north wall [photograph taken on 27 October 2006 (sample no. K61027-1) by the Agency for Cultural Affairs]. **h** Conidia in a slide preparation taken directly from the part of the substrata in Fig. 1g. *Bars* **b** 20 μm; **c**, **d, h** 5 μm

Initially, the identity of these fungi at species level remained uncertain (Kigawa et al. 2007a; Sugiyama et al. 2009). To elucidate the cause of black spots of the TT and KT murals, we surveyed the mycobiota from May 2004 to December 2006 and obtained 46 isolates of dark *Acremonium*. In the course of an integrated analysis of phenotypic and genotypic characters, we attempted to identify these isolates at species level. We provide here a systematic and nomenclatural treatment of *Acremonium* sect. *Gliomastix* 'series *Murorum*' (herein validated), and propose *Acremonium tumulicola* sp. nov. based on three KT isolates and *Acremonium felinum* comb. nov. with neotypification.

Materials and methods

Sampling, isolating, and culturing

A total of 224 samples, which included moldy spots, viscous gels (biofilms), and mixtures of plaster fragments and soil, were collected from the stone chamber interior, from spaces between the stone walls, and from the stone chamber exterior of TT between May 2004 and August 2007. In addition, a total of 149 samples were collected from the stone chamber interior and exterior of KT between June 2004 and September 2007. The isolation methods used were the smear and moist chamber methods (Sugiyama et al. 2008, 2009; Kiyuna et al. 2008). The isolates have been maintained on potato dextrose agar (PDA; Nihon Pharmaceutical, Tokyo, Japan). Detailed data on the isolates identified as *Acremonium* sect. *Gliomastix* from both tumuli and accession numbers of DNA sequences in GenBank are listed in Table 1. Twenty-one selected living isolates are deposited as vouchers with the Japan Collection of Microorganisms (JCM), RIKEN BioResource Center, Wako, Saitama Prefecture, Japan, as JCM 17164–17184 (Table 1). The remaining living isolates from both tumuli are maintained at the Biology Laboratory of the National Research Institute of Cultural Properties, Tokyo, as lyophilized vouchers (Table 1).

Cultural and morphological observations

A total of 46 isolates, comprising 35 and 11 isolates from TT and KT, respectively, were used in the cultural and morphological observations. Detailed data on the isolates are shown in Table 1. All isolates were grown using the media and growth conditions mainly adopted by Gams (1971). Isolates were incubated on malt extract agar (MA; Oxoid, Cambridge, UK) at 20°C, oatmeal agar (OA; Becton-Dickinson, Baltimore, MD, USA) at 20°C, potato dextrose agar (PDA; Nihon Pharmaceutical) at 20°C, each for 20 days in darkness. In the selected isolates, growth rates were recorded using the average of three colony diameters; i.e., one conidial suspension per MA plate was

Table 1 Strain data of Takamatsuzuka and Kitora isolates, with the GenBank accession numbers for rDNA sequences determined in this study

Species	Isolate no.	JCM no.	Source[a]	Sampling date	GenBank accession no.	
					28S	ITS
Acremonium masseei	T4519-5-1	17164	White moldy colonies on the floor of the stone chamber of TT	19 May 2004	AB540433	AB540507
	T6517-1-1	17165	Black spots on the paintings of the group of women on west wall 3 in the stone chamber of TT	17 May 2006	AB540434	AB540508
	T6517-2-1		Black spots on the paintings of the group of women on west wall 3 in the stone chamber of TT	17 May 2006	AB540435	AB540509
	T6517-3-1		Black spots above the paintings of the group of women on west wall 3 in the stone chamber of TT	17 May 2006	AB540436	AB540510
	T6517-5-1		Black spots between the paintings of the moon and the white tiger on west wall 2 in the stone chamber of TT	17 May 2006	AB540437	AB540511
	T6517-6-1		Black spots below the paintings of the moon on west wall 2 in the stone chamber of TT	17 May 2006	AB540438	AB540512
	T6517-7-1	17166	Black spots above the painting of the white tiger on west wall 2 in the stone chamber of TT	17 May 2006	AB540439	AB540513
	T6517-8-1	17167	White moldy colonies on the paintings of the group of women on east wall 3 in the stone chamber of TT	17 May 2006	AB540440	AB540514
	T6517-11-1		Black spots on the paintings of the group of women on west wall 3 in the stone chamber of TT	17 May 2006	AB540441	AB540515
	T6713-1-1		Black spots on the paintings of the group of women on west wall 3 in the stone chamber of TT	13 July 2006	AB540442	AB540516
	T6713-2-1		Black moldy colonies above the paintings of the group of women on west wall 3 in the stone chamber of TT	13 July 2006	AB540443	AB540517
	T6713-4-1		Black moldy colonies below the paintings of the moon on west wall 2 in the stone chamber of TT	13 July 2006	AB540444	AB540518
	T6713-8-1		Black moldy colonies above the paintings of the group of women on east wall 3 in the stone chamber of TT	13 July 2006	AB540445	AB540519
	T6713-12-1		Black moldy colonies on the northeast area of the ceiling (stone 3) in the stone chamber of TT	13 July 2006	AB540446	AB540520
	T6713-14-1		Black moldy colonies above the group of men on west wall 1 in the stone chamber of TT	13 July 2006	AB540447	AB540521
	T61017-1-1		Black spots on the paintings of the group of women on west wall 3 in the stone chamber of TT	17 October 2006	AB540448	AB540522
	T61017-2-1		Black spots on the paintings of the group of women on west wall 3 in the stone chamber of TT	17 October 2006	AB540449	AB540523
	T61017-3-1		Black spots above the paintings of the group of women on west wall 3 in the stone chamber of TT	17 October 2006	AB540450	AB540524
	T61017-4-1		Black spots between the paintings of the moon and the white tiger on west wall 2 in the stone chamber of TT	17 October 2006	AB540451	AB540525
	T61017-5-1		Black spots below the paintings of the moon on west wall 2 in the stone chamber of TT	17 October 2006	AB540452	AB540526
	T61017-9-1		Black spots on northeast area of the ceiling (stone 3) in the stone chamber of TT	17 October 2006	AB540453	AB540527
	T61017-10-1		Black spots on northwest area of the ceiling (stone 3) in the stone chamber of TT	17 October 2006	AB540454	AB540528
	T61213-1-6	17168	Black moldy colonies and viscous gels on the paintings of the group of women on west wall 3 in the stone chamber of TT	13 December 2006	AB540455	AB540529
	T61213-2-1		Blackish viscous gels on the paintings of the group of women on west wall 3 in the stone chamber of TT	13 December 2006	AB540456	AB540530
	T61213-3-9		Black spots above the paintings of the group of women on west wall 3 in the stone chamber of TT	13 December 2006	AB540457	AB540531
	T61213-4-3		Black moldy colonies between the paintings of the moon and the white tiger on west wall 2 in the stone chamber of TT	13 December 2006	AB540458	AB540532
	T61213-5-4		Black moldy colonies below the paintings of the moon on west wall 2 in the stone chamber of TT	13 December 2006	AB540459	AB540533
	T61213-6-1	17169	Viscous gels above the painting of the white tiger on east wall 2 in the stone chamber of TT	13 December 2006	AB540460	AB540534
	T61213-9-1		Black moldy colonies on northeast area of the ceiling (stone 3) in the stone chamber of TT	13 December 2006	AB540461	AB540535
	T61213-10-1	17170	Black moldy colonies on northwest area of the ceiling (stone 3) in the stone chamber of TT	13 December 2006	AB540462	AB540536
	T61213-11-1		Black moldy colonies on the paintings of the group of women on west wall 3 in the stone chamber of TT	13 December 2006	AB540463	AB540537
	T61213-15-1		Black moldy colonies below the paintings of the group of men on west wall 1 in the stone chamber of TT	13 December 2006	AB540464	AB540538
	K61027-2-1	17171	Black powdered molds on the east wall in the stone chamber of KT	27 October 2006	AB540465	AB540539
Acremonium murorum	T6713-14-2	17172	Black moldy colonies above the group of men on west wall 1 in the stone chamber of TT	13 July 2006	AB540466	AB540540
	K6330-2	17173	Black needle-like molds on the east wall in the stone chamber of KT	30 March 2006	AB540467	AB540541
	K6630-3-1	17174	Black moldy colonies on the ceiling in the stone chamber of KT	30 June 2006	AB540468	AB540542
	K61027-1-1	17175	Black powdered molds on the north wall in the stone chamber of KT	27 October 2006	AB540469	AB540543
	K61027-3-1	17176	Black sooty molds on the south wall in the stone chamber of KT	27 October 2006	AB540470	AB540544
	K7511-1	17177	Black sooty molds in the east area of the north wall in the stone chamber of KT	11 May 2007	AB540471	AB540545

Table 1 continued

Species	Isolate no.	JCM no.	Source[a]	Sampling date	GenBank accession no.	
					28S	ITS
Acremonium felinum	K4615-9	17178	Soil from the space between the west wall and soil flowing into the stone chamber in area B of the stone chamber of KT	15 June 2004	AB540472	AB540546
Acremonium polychromum	T6713-22-1a	17179	Air in adjacent room B of TT	13 July 2006	AB540473	AB540547
	T6713-22-1b	17180	Air in adjacent room B of TT	13 July 2006	AB540474	AB540548
	K5225-12-5	17181	Air from the north area in the adjacent small room of KT	25 February 2005	AB540475	AB540549
Acremonium tumulicola	K5916-10-3	17182	Viscous substances on the stone wall in the adjacent small room of KT	16 September 2005	AB540476	AB540550
	K6303-1-7	17183	Moldy colonies on the floor in the stone chamber of KT	3 March 2006	AB540477	AB540551
	K6613-2[b]	17184	White salt-like masses on the central part of the paintings of the vermillion bird (Suzaku) on the south wall in the stone chamber of KT	13 June 2006	AB540478	AB540552

[a] TT, Takamatsuzuka Tumulus; KT, Kitora Tumulus

[b] Ex-type strain

inoculated in the center and incubated at 5°, 10°, 15°, 20°, 25°, 30°, 37°, and 40°C, each for 7 days in the dark. The colony colors of the isolates on all media were determined by using the Kornerup and Wanscher color standard (1978). Microscopic slides were prepared from portions of the colonies grown on MA plate cultures and were mounted in lactophenol mounting medium without dye (Gams et al. 1987; Bills and Foster 2004). Microscopic examinations were made using a BX51 microscope (Olympus, Tokyo, Japan) with Normarski interference contrast at up to 1,000×. All micrographs were taken with a Coolpix 5000 digital camera (Nikon, Tokyo, Japan).

Phylogenetic analyses

DNA extraction, PCR amplification, and sequencing

The isolates and reference strains from various culture collections used for the DNA sequencing are listed in Tables 1 and 2. Their genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The two gene regions sequenced were the nuclear 28S rDNA D1/D2 region (hereafter abbreviated as 28S or rDNA D1/D2) and internal transcribed spacer (ITS)–5.8S rDNA. The primers used included NL1 and NL4 (O'Donnell 1993) for 28S, ITS5, and ITS4 (White et al. 1990) for ITS. Polymerase chain reaction (PCR) was performed using puReTaq Ready-To-Go PCR beads (GE Healthcare, Buckinghamshire, UK). Thermal cycling was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). An initial denaturation at 95°C for 5 min was followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and then a final extension at 72°C for 10 min. The amplified DNA fragments were purified with a QIAquick PCR Purification Kit (Qiagen). Direct sequencing for the PCR products was performed using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems), and the tubes were incubated in a GeneAmp PCR System 9700 (Applied Biosystems). The sequencing primers ITS5, ITS2, ITS3, and ITS4 (White et al. 1990) were used for the amplification of ITS, and the primers NL1, NL2, NL3, and NL4 (O'Donnell 1993) for 28S. The completed reactions were cleaned using a DyeExTM 2.0 Spin Kit (Qiagen). Sequences were determined using electrophoresis in an ABI3130xl DNA sequencer (Applied Biosystems). The sequences determined in this study were deposited in GenBank/EMBL/DDBJ. Their accession numbers are given in Tables 1 and 2. Other known sequences downloaded for comparison for the respective molecular phylogenetic analyses from GenBank are shown in Table 3.

Table 2 Strain data and rDNA sequences determined in this study for comparison

Species[a]	Section[b]	Series[b]	Strain no.[c]	Source	Location	GenBank accession no.	
						28S	ITS
Acremonium masseei	*Gliomastix*	*Murorum*	CBS 794.69	Dung of rabbit	Italy	AB540479	AB540553
A. masseei	*Gliomastix*	*Murorum*	CBS 557.75	Stem of *Urtica dioica* together with *Leptosphaeria doliolum*	Germany, Würzburg	AB540480	AB540554
A. murorum	*Gliomastix*	*Murorum*	JCM 23082	Wine cork	–	AB540481	AB540555
A. murorum var. *murorum*	*Gliomastix*	*Murorum*	CBS 148.81	Forest soil	USA, Georgia	AB540482	AB540556
Gliomastix murorum var. *murorum*	*Gliomastix*	*Murorum*	NBRC 31241	Soil	West Germany	AB540483	AB540557
'*G. murorum* var. *murorum*'[d]	*Gliomastix*	*Murorum*	NBRC 31044	Timber block of *Fagus crenata*	–	AB540484	AB540558
G. murorum var. *murorum*	*Gliomastix*	*Murorum*	NBRC 8269	–	–	AB540485	AB540559
G. murorum var. *murorum*	*Gliomastix*	*Murorum*	NBRC 9144	–	–	AB540486	AB540560
Acremonium murorum var. *felina*	*Gliomastix*	*Murorum*	DAOM 22657	Soil	Ottawa, Ontario, Canada	AB540487	AB540561
A. murorum var. *felinum*	*Gliomastix*	*Murorum*	CBS 147.81	Forest soil	USA, Georgia	AB540488	AB540562
Gliomastix murorum var. *felina*[d]	*Gliomastix*	*Murorum*	NBRC 4871	–	–	AB540489	AB540563
'*G. murorum* var. *felina*'[d]	*Gliomastix*	*Murorum*	NBRC 8515	Soil	UK	AB540490	AB540564
'*G. murorum* var. *felina*'[d,e]	*Gliomastix*	*Murorum*	NBRC 8530	Salt-marsh soil	UK	AB540491	AB540565
G. murorum var. *polychroma*[d]	*Gliomastix*	*Murorum*	NBRC 9440	Decaying carpophore of *Coriolus* sp.	–	AB540492	AB540566
Acremonium polychromum	*Gliomastix*	*Murorum*	CBS 181.27	Bark of *Hevea brasiliensis* (Euphorbiaceae)	Indonesia, Sumatra	AB540493	AB540567
A. polychromum	*Gliomastix*	*Murorum*	JCM 23084	Stony soil along the coast	Papua New Guinea	AB540494	AB540568
A. atrogriseum	*Gliomastix*	*Murorum*	JCM 23068^{T}	Noodles	Ukraine, Russia	AB540495	AB540569
A. brachypenium	*Gliomastix*	–	CBS 866.73^{T}	Dead stem of *Cocos nucifera* (Palmae)	Sri Lanka	AB540496	AB540570
A. cereale	*Gliomastix*	*Murorum*	JCM 23071	Sand dune soil, A1 horizon	UK	AB540497	AB540571
A. dichromosporum	*Gliomastix*	–	CBS 638.73^{IT}	Rhizosphere of *Triticum aestivum* (Gramineae)	Western Australia	AB540498	AB540572
A. longisporum	*Gliomastix*	'*Luzulae*'	JCM 23080	Dead leaf sheath of Musa sapientum in greenhouse	The Netherlands	AB540499	AB540573
A. luzulae	*Gliomastix*	'*Luzulae*'	JCM 23081	Decaying *Picea* wood	Germany	AB540500	AB540574
A. persicinum	*Gliomastix*	'*Persicinum*'	JCM 23083^{T}	Coastal sand under *Ammophila arenaria*	France	AB540501	AB540575
A. glaucum	*Acremonium*	–	JCM 23076^{T}	Woolen overcoat	Solomon Islands	AB540503	AB540577
A. hansfordii	*Acremonium*	–	CBS 390.73	*Periconia cookei* on *Dendrocalamus* sp.	India, Bangalore	AB540504	AB540578
A. alcalophilum	Plectosphaerellaceae[f]	–	JCM 7366^{T}	Sludge from a compost made of pig feces	Japan	AB540505	AB540579

Table 2 continued

Species[a]	Section[b]	Series[b]	Strain no.[c]	Source	Location	GenBank accession no.	
						28S	ITS
A. rutilum	*Nectrioidea*	–	JCM 23088[NT]	Moist greenhouse wall	Germany	AB540506	AB540580
Wallrothiella subiculosa	Anam. *Pseudogliomastix*	–	JCM 23118	Old leaf of *Cocos nucifera* (Palmae)	Sri Lanka	AB540502	AB540576

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

–, Source and location are unknown

[a] Species names are noted as registered in each Culture Collection

[b] Section and series are taxonomic ranks of the genus *Acremonium*adopted by Gams (1971, 1975); series names in single quotation marks are not validly published

[c] CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM, Canadian Collection of Fungal Cultures, Ottawa, Canada; JCM, Japan Collection of Microorganisms, Wako, Japan; NBRC, NITE-Biological Resource Center, Kisarazu, Japan

[d] Strain names are doubtful in identification; details are noted in the article

[e] Strain CBS 184.30 (=NBRC 8530) was reidentified by W. Gams as *A. polychromum*

[f] Zare et al. (2007)

Table 3 List of taxa and the accession numbers of rDNA sequences retrieved from GenBank

Species[a]	GenBank accession no.	
	28S	ITS
Acremonium murorum	FJ176880	–
A. murorum var. *felina*	AY283559	–
A. strictum	AY138483	–
Ambrosiella xylebori	DQ470979	–
Gliomastix murorum	–	AM921702
G. murorum	–	EU326188
G. murorum var. *murorum*	–	EF029216
G. murorum	–	EF495243
Heleococcum japonicum	U17429	–
Hydropisphaera erubescens	AF193230	–
H. erubescens	AF193231	–
H. erubescens	AF193229	–
H. erubescens	AY545726	–
H. peziza	AY489730	–
Nalanthamala squamicola	AF373281	–
Roumegueriella rufula	DQ518776	–
R. rufula	EF469082	–

[a] Species names are noted as registered in GenBank

Molecular phylogenetic analyses

The sequences were assembled using ChromasPro 1.42 (Technelysium, Tewantin, QLD, Australia). Three subsets of the segment were also made into data sets: ITS, 28S, and ITS plus 28S. Multiple alignments were performed using CLUSTAL W version 1.83 (Thompson et al. 1994); the final alignments were manually adjusted. Ambiguous positions and alignment gaps were excluded from the analysis. The neighbor-joining (NJ) tree was constructed using the multiple alignments in MEGA ver3.1 (Kumar et al. 2004), with 1,000 bootstrap replicates (Felsenstein 1985).

The phylogenetic reconstruction approach using a Bayesian tree based on ITS plus 28S sequences (Rannala and Yang 1996) was implemented using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). The model of DNA substitution was calculated using Modeltest2.2 (Nylander 2004). The results were obtained by the SYM + I + G model. Bayesian Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) analyses (Mau et al. 1999) were performed with MrBayes for phylogenetic estimation inferred from the respective gene sequences. MrBayes was run for 2,500,000 generations. Searches were conducted with four chains (three cold, one hot) with trees sampled every 100 generations. The average standard deviation of split frequencies was 0.009 at the end of the run. The confidence levels of nodes were measured by posterior probabilities

obtained from the majority-rule consensus after deletion of the trees during burn-in.

Results and discussion

Cultural and morphological characterization of dark *Acremonium* isolates

A total of 46 representative isolates from the TT and KT stone chamber interiors and exteriors, assignable to *Acremonium* sect. *Gliomastix*, were culturally and morphologically characterized (for details, see Table 1). Using the integrated phenotypic (mentioned here) and genotypic (mentioned below) analyses, these isolates were identified as taxa of the 'series *Murorum*' (validated later in this article) in sect. *Gliomastix*: *Acremonium masseei* (Sacc.) W. Gams, *A. murorum* (Corda) W. Gams, *A. felinum* comb. nov., *A. polychromum* (J.F.H. Beyma) W. Gams, and *A. tumulicola* sp. nov. The cultural and morphological characteristics of the respective taxa are fully described later in this article (see Figs. 2, 3, 4, 5, 6).

As shown in Table 1, 33 representative strains of *A. masseei*, which were isolated from a variety of substrates such as black moldy spots and viscous gels (biofilms) on plaster walls collected in different periods, are thought to be genetically the same species. In this study, *A. masseei* was isolated only from the stone chamber interiors of the TT and KT. However, we could not detect this species from the exterior of either the TT or KT stone chamber. So far, *A. masseei* has been isolated from soil, dung, and plant substrates (Gams 1971; Matsushima 1975; CBS Fungi Database: http://www.cbs.knaw.nl/fungi/BioloMICS.aspx). This is the first case of isolation of *A. masseei* from a biodeteriorated cultural heritage such as mural paintings.

The optimum temperature for growth on MA, in the two isolates (T4519-5-1 and T6517-1-1) identified as *A. masseei*, was 20°–25°C after 7 days. However, the growth rate of isolate T6517-1-1 was somewhat higher than that of T4519-5-1 at 10°C (Fig. 7). Therefore, strain T6517-1-1, isolated after the stone chamber was cooled, is thought to be active at the low-temperature conditions of the stone chamber interior.

According to the official records (see the Agency homepage concerning the TT, http://www.bunka.go.jp/takamatsu_kitora/hekiga_hozonkanri.html), in December 2001, after remediation work in the space adjacent to the stone chamber of TT, dark *Acremonium* [mentioned as *Acremonium* (sect. *Gliomastix*) sp. in Kigawa et al. 2006, 2009] was detected on the blue dragon painting on east wall 2 and on the white tiger on west wall 2. The conidia-bearing structure appearing in the microscopic photographs in these records is very similar to that of our isolate T6713-14-2, identified as *A. murorum*. However, the isolates obtained by Kigawa and coworkers were not preserved as vouchers and are no longer available for study. Therefore, detailed comparison between the previously isolated strains and our isolates is impossible. The results suggest, however, that *A. murorum* was growing in the stone chamber interior for some time after 2001.

Molecular phylogenetic analyses of dark *Acremonium* isolates

The 28S, ITS, and combined ITS–28S dataset contained 46 isolates of *Acremonium* sect. *Gliomastix*, i.e., 35 from the TT and 11 from the KT (see Table 1), and 28 authentic

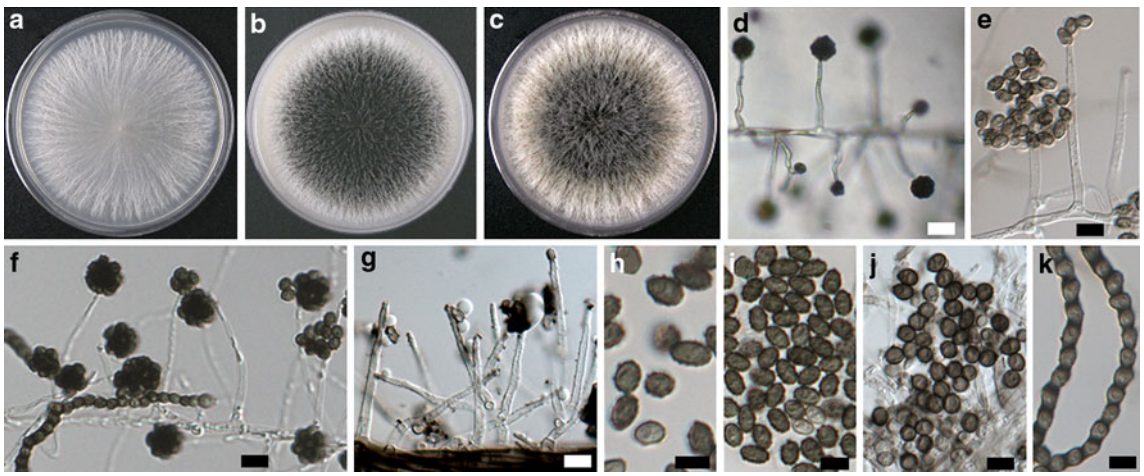

Fig. 2 *Acremonium murorum* (K7511-1). **a** Colonies on malt agar (MA) at 20°C, 20 days. **b** Colonies on oatmeal agar (OA) at 20°C, 20 days. **c** Colonies on potato dextrose agar (PDA) at 20°C, 20 days. **d**–**g** Conidiophores. **h**–**k** Conidia. *Bars* **d** 20 μm; **f** 10 μm; **e**, **g**–**k** 5 μm

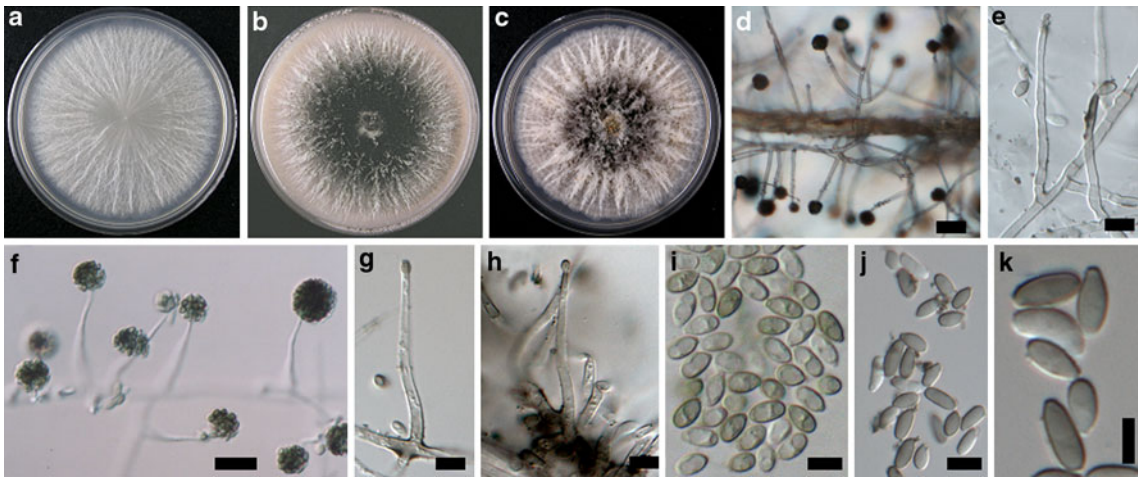

Fig. 3 *Acremonium felinum* (K4615-9). **a** Colonies on MA at 20°C, 20 days. **b** Colonies on OA at 20°C, 20 days. **c** Colonies on PDA at 20°C, 20 days. **d–h** Conidiophores. **i–k** Conidia. *Bars* **d** 20 μm; **f** 10 μm; **e, g–k** 5 μm

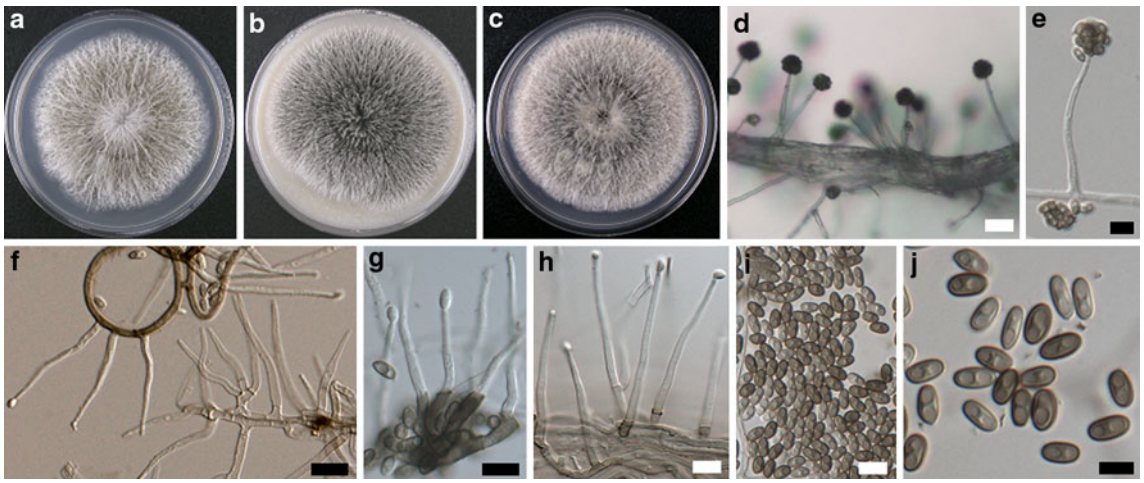

Fig. 4 *Acremonium tumulicola* (K6613-2). **a** Colonies on MA at 20°C, 20 days. **b** Colonies on OA at 20°C, 20 days. **c** Colonies on PDA at 20°C, 20 days. **d–h** Conidiophores. **i**, **j** Conidia. *Bars* **d**, **f** 10 μm; **e**, **g–j** 5 μm

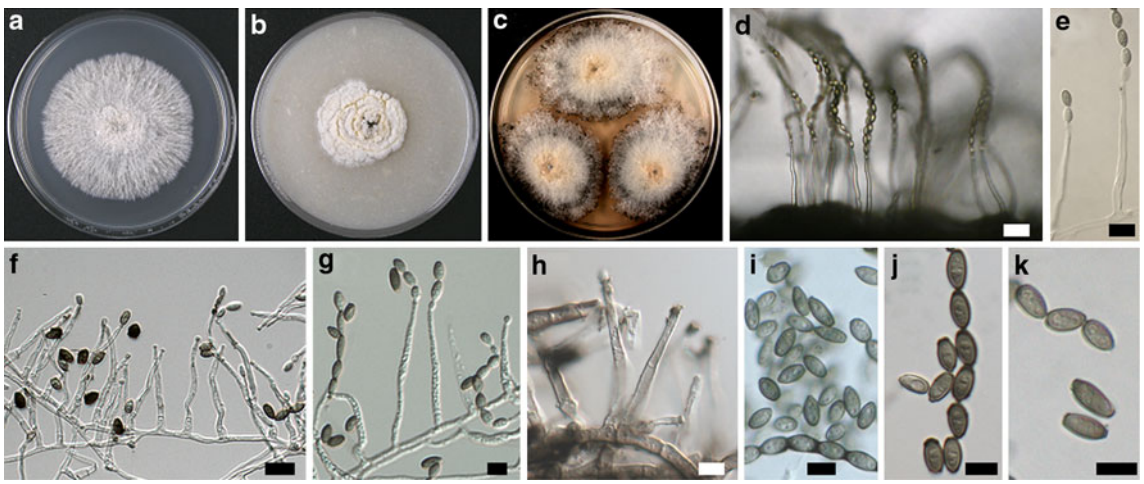

Fig. 5 *Acremonium masseei* (T6517-1-1). **a** Colonies on MA at 20°C, 20 days. **b** Colonies on OA at 20°C, 20 days. **c** Colonies on PDA at 20°C, 1 month. **d–h** Conidiophores. **i–k** Conidia. *Bars* **d** 20 μm; **f** 10 μm; **e, g–k** 5 μm

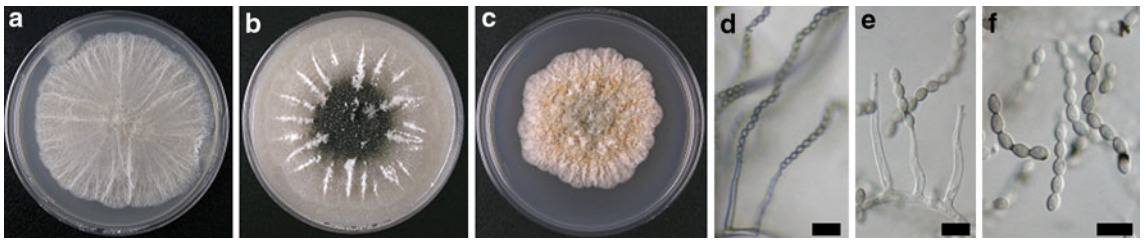

Fig. 6 *Acremonium polychromum* (K5225-12-5). **a** Colonies on MA at 20°C, 20 days. **b** Colonies on OA at 20°C, 20 days. **c** Colonies on PDA at 20°C, 20 days. **d**, **e** Conidiophores. **f** Conidia. *Bars* **d**–**f** 10 μm

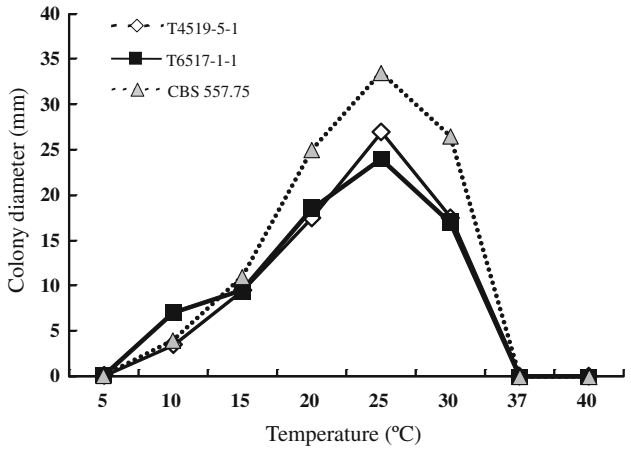

Fig. 7 Effect of temperature on colony growth in *Acremonium masseei*, Takamatsuzuka isolates (T4519-5-1 and T6517-1-1), and the reference strain (CBS 557.75)

strains obtained from the public culture collections/bioresource centers (Table 2).

Using *Acremonium alcalophilum* JCM 7366^T (which belongs to the Plectosphaerellaceae according to Zare et al. 2007) as an outgroup taxon, the NJ tree was inferred from 1,018 bp of the ITS + 28S sequences dataset. In the ITS + 28S phylogeny, all the TT and KT isolates appeared in five clades: Masseei clade, Murorum clade, Felinum clade, Polychromum clade, and Tumulicola clade (Figs. 8, 9). Each clade was supported by a high bootstrap value (99%) and high Bayesian posterior probability (1.00). Most of the isolates showed good correlation between the molecular phylogenetic placement and phenotypic characteristics.

In the Masseei clade, our 33 isolates along with *A. masseei* CBS 557.75 (ex stem of *Urtica dioica*, Germany) and 794.69 (ex rabbit dung, Italy) formed a monophyletic cluster with high bootstrap supports. In Gams's description (1971) of *A. masseei*, CBS 794.69 was cited and illustrated as fig. 46, which nicely depicts the morphological characteristics. There was no variation in the ITS–28S gene sequences from 33 isolates of *A. masseei* in the Masseei clade; 32 from TT and 1 from KT. On the other hand, there is a three-nucleotide difference between these isolates and *A. masseei* CBS 557.75 and 794.69. The genetic diversity of this Masseei clade is not great. Our molecular phylogeny (Figs. 8, 9) only suggests the existence of two haplotypes (i.e., a group of TT and KT isolates, and one of the two CBS strains) within the Masseei clade.

Three KT isolates (K5916-10-3, K6303-1-7, and K6613-2) formed an independent branch named a novel clade 1 with a high bootstrap value (99%) and high Bayesian posterior probability (1.00) as mentioned earlier, for which we introduce the new species name, *Acremonium tumulicola.*

Three TT and KT isolates (T6713-22-1a, T6713-221b, and K5225-12-5) grouped together with JCM 23084 (ex stony soil along the coast, Papua New Guinea) and CBS 181.27 of *A. polychromum* and NBRC 8530 (=CBS 184.30, reidentified by W. Gams as *A. polychromum*; ex salt-marsh soil, UK) of '*G. murorum* var. *felina*'. Among these strains, CBS 181.27 [=Herb. IMI 62332 (ex bark of *Hevea brasiliensis*, Sumatra)] is the ex-type strain of *Oospora polychroma*, which was cited and illustrated in Gams's description of *A. polychromum*. No base changes for the 28S and ITS sequences were detected among these six strains. This assemblage is here called the Polychromum clade.

One KT isolate (K4615-9) was accommodated within the Felinum clade, which included DAOM 22657 (ex soil, Ottawa, Ontario, Canada) and CBS 147.81 (ex forest soil, Georgia, USA) of '*A. murorum* var. *felinum*' (or as *Gliomastix felina*), and NBRC 31044 (ex timber block of *Fagus crenata*, locality unknown) of '*G. murorum* var. *murorum*'. DAOM 22657 was used for the molecular tree based on partial large subunit (LSU) rDNA sequences (Seifert et al. 2003), whereas CBS 147.81 was examined by Hammill (1981), who discussed the differences between *G. murorum* and *G. felina*. NBRC 31044 is listed in the NBRC online catalogue (http://www.nbrc.nite.go.jp/e/index.html).

In the Murorum clade (Fig. 9), our six isolates, CBS 148.81 and JCM 23081 of *A. murorum* var. *murorum*, three NBRC strains [NBRC 9144 = CBS 119.67, ex *Camarophyllus niveus* (now *Hygrocybe virginea*), Netherlands; NBRC 31241 = ATCC 16277, ex soil, Germany; NBRC

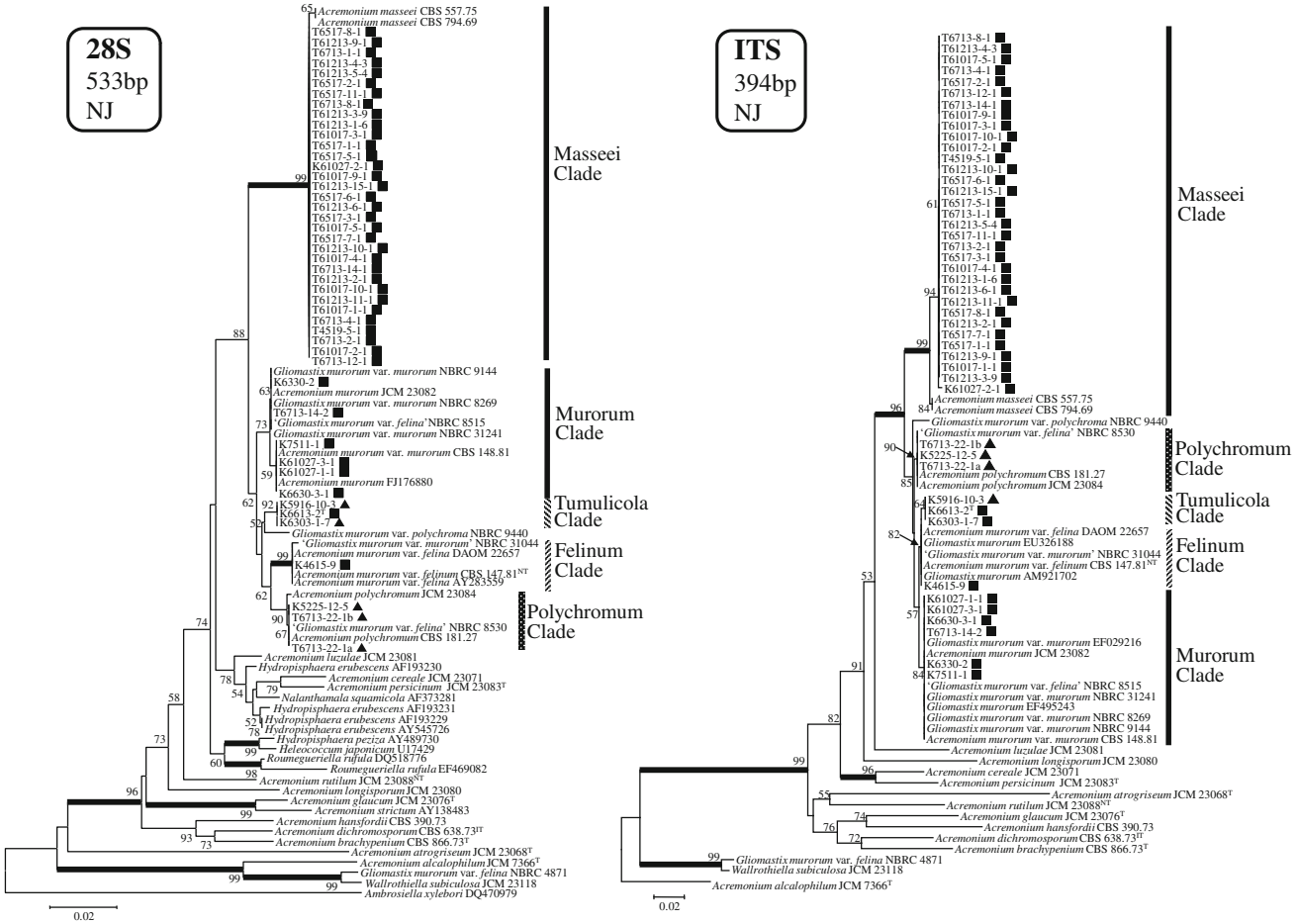

Fig. 8 Phylogenetic relationships among 46 Takamatsuzuka Tumulus (TT) and Kitora Tumulus (TK) isolates and 28 known *Acremonium* (sensu lato) species and with the accession numbers downloaded from GenBank based on neighbor-joining (*NJ*) analysis of 28S rDNA-D1/D2 and internal transcribed spacer (*ITS*)–5.8S region sequence data of 533 and 394 aligned nucleotide sites, respectively, using MEGA ver3.1. *Numbers* on the *branch nodes* represent bootstrap support values (%) based on 1,000 replications; bootstrap values greater than 50% are indicated. Branches significantly supported by bootstrap values above 95% are shown with *thick lines*. *T* and *K* indicate isolates from the TT and KT, respectively. *Filled squares* indicate the isolates from the respective stone chamber interiors, and *filled triangle* from the adjacent space or small room of both tumuli. *Right vertical bars* indicate the phylogenetic group in this study. ^{T}, ^{IT}, and ^{NT} indicate ex-type, ex-isotype, and ex-neotype strains, respectively. *Single quotes* (‘’) indicate that species names are doubtful in identification

8269, ex Herbarium IFO H-10916, K. Tubaki 103-21] of *G. murorum* var. *murorum*, and NBRC 8515 (=IMI 52460, ex soil, UK) of ‘*G. murorum* var. *felina*’ grouped together with comparatively high bootstrap value (94%) and high Bayesian posterior probability (1.00). CBS, JCM, and NBRC strains named *A. murorum* var. *murorum* or *G. murorum* var. *murorum* are authentic in having phenotypic characteristics that fit Gams’s (1971, in Domsch et al. 2007) and Hammill’s (1981) descriptions and illustrations for *A. murorum*/*G. murorum*. However, there is a slight difference between the 11 strains placed in the Murorum clade in their ITS–28S gene sequences, as shown in Fig. 9.

A first molecular phylogeny of the genus *Acremonium*, including sect. *Gliomastix*, by Glenn et al. (1996), revealed that the genus is polyphyletic. In that paper, only one species, *A. murorum*, was sampled from sect. *Gliomastix*. In our molecular phylogeny (see Fig. 9), taxa of sect. *Gliomastix* series *Murorum* (*A. masseei*, *A. murorum*, and *A. polychromum*) were accommodated in a cluster with high bootstrap support (99%) and high Bayesian posterior probability (1.00). However, the remaining branches were longer and comparatively independent and contained species of sects. *Acremonium* (*A. glaucum* and *A. hansfordii*) and *Nectrioidea* (*A. rutilum*). The identities of several strains with the names *G. murorum* var. *polychroma* NBRC 9940 (ex decaying carpophore of *Coriolus* sp.), ‘*G. murorum* var. *felina*’ NBRC 8530, and ‘*G. murorum* var. *murorum*’ NBRC 31044 and 8515, all of which have been used in this study, should be carefully reconsidered in future monographic research.

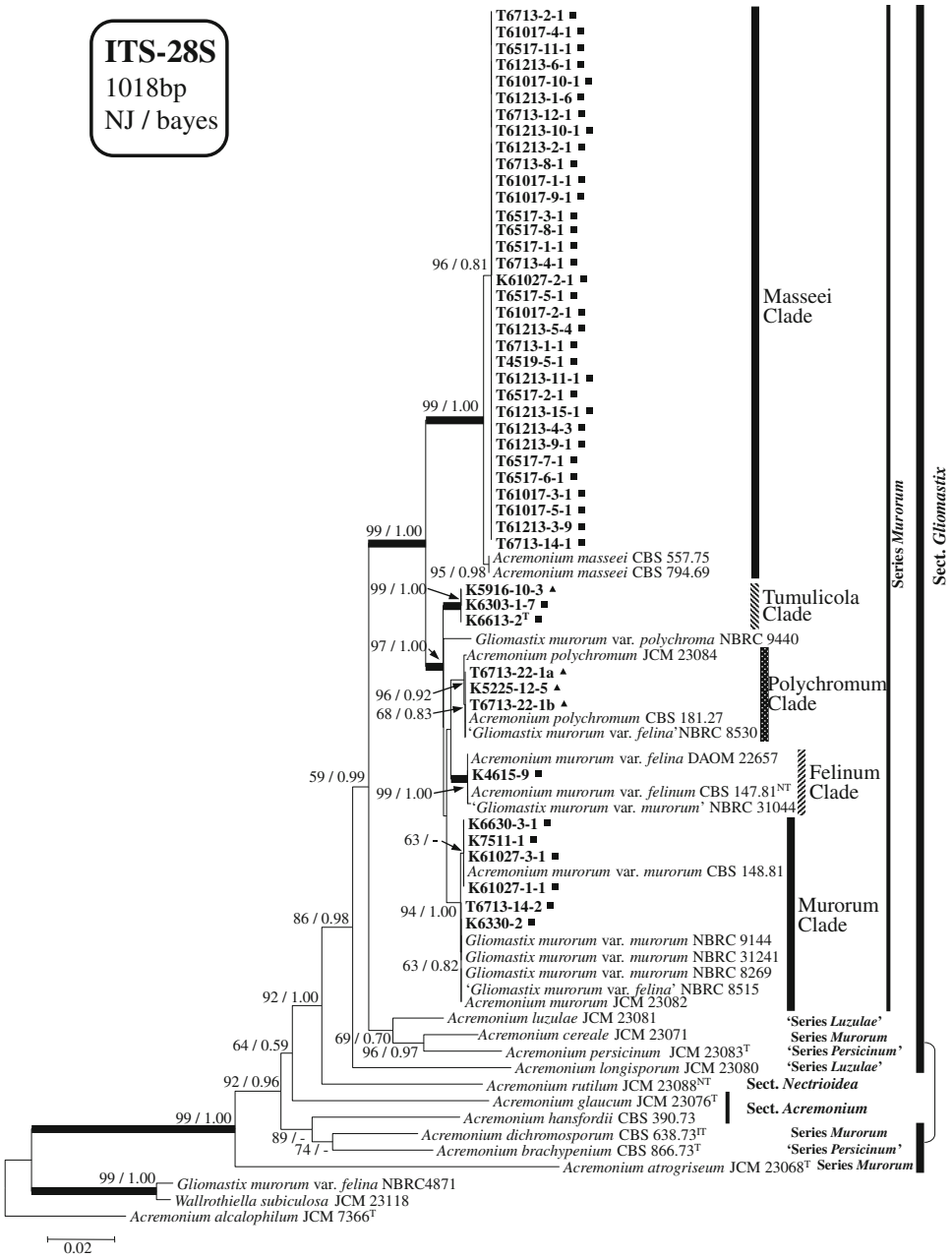

Fig. 9 Phylogenetic relationships among 46 TT and TK isolates and 28 known *Acremonium* (sensu lato) species and with the accession numbers downloaded from GenBank based on NJ and Bayesian analyses of combined ITS–5.8S and 28S rDNA-D1/D2 region sequence data of 1,018 aligned nucleotide sites using MEGA ver3.1. *Numbers* on the *branch nodes* represent Bayesian posterior probability and bootstrap support values (%) based on 1,000 replications; bootstrap values greater than 50% are indicated. Branches significantly supported by bootstrap value and Bayesian posterior probability above 99% and 1.00, respectively, are shown by *thick lines*. Further details as in Fig. 8. Section and series names according to Gams (1971, 1975)

A discussion on the roles of dark *Acremonium* spp. in the biodeterioration of mural paintings and plaster walls, and also on the invasion route to the TT and KT stone chamber interiors, will be published elsewhere.

Systematics

A total of 46 TT and KT isolates herein identified have been accommodated in the 'series *Murorum*' proposed by Gams (1971). However, his proposal lacked a Latin diagnosis/description and typification. Therefore, we validate this taxon here:

Acremonium sect. *Gliomastix* series *Murorum* W. Gams ex Kiyuna, An & Sugiy., ser. nov.

Acremonium sect. *Gliomastix* series *Murorum* W. Gams, in *Cephalosporium*-artige Schimmelpilze (Hyphomycetes), p. 81. 1971, nom. inval. Art. 36, 37.1.

Series in sectione *Gliomasticis*. Conidia pigmentata, parva, minus quam 4.5 µm lata, sursum rotundata, laevia vel incrustata, non costata.

Species typica: *Acremonium murorum* (Corda) W. Gams

The brief or full descriptions of five species of *Acremonium* sect. *Gliomatsix* series *Murorum* are provided below.

Acremonium murorum (Corda) W. Gams, *Cephalosporium*-artige Schimmelpilze (Hyphomycetes), p. 84, 1971. Fig. 2

≡*Torula murorum* Corda, Icon. Fung. 2:9, 1838 (basionym).

≡*Gliomastix murorum* (Corda) S. Hughes var. *murorum*, Can. J. Bot. 36:769, 1958.

=*Gliomastix chartarum* Guéguen, Bull. Soc. Mycol. Fr. 21:240, 1905.

Colonies on MA reaching 70–80 mm diameter in 10 days at 20°C in darkness, white (25A1), floccose, reverse concolorous. Colonies on OA reaching 70–80 mm diameter in 10 days at 20°C in darkness, blackish brown (5F8), velvety to floccose, reverse concolorous. Colonies on PDA reaching 65–70 mm diameter in 10 days at 20°C in darkness, blackish brown (5F8), floccose, reverse concolorous.

Hyphae hyaline, septate, 1.5–2 µm wide. Conidiation plectonematogenous. Phialides mostly borne singly or 2–3 on a short subtending cell, smooth walled, sometimes forming a collarette, 20–40 (–50) µm long, tapering from 2–2.5 µm near the base to 1–1.5 µm. Conidia mostly adhering in heads, rarely in chains, blackish brown (5F8), warted, ellipsoidal to subglobose, with truncate base, 4–5 × 2–3 (–4) µm. Chlamydospores absent.

Gene sequence data: The GenBank accession numbers for nuclear 28S rDNAD1/D2 and ITS regions are listed in Table 1. Isolates examined: one isolate from surfaces of murals of the stone chamber interior of the TT; T6713-14-2 (July 2006) and five isolates from the murals and walls of the stone chamber interior of the KT, from March 2006 to May 2007; K6330-2, K6630-3-1, K61027-1-1, K61027-3-1, and K7511-1. The detailed data on these isolates are shown in Table 1.

The phenotypic characteristics of the six isolates agreed well with the previous descriptions (Dickinson 1968; Gams 1971; Matsushima 1975; Hammill 1981).

Acremonium felinum (Marchal) Kiyuna, An, Kigawa & Sugiy., comb. nov. Fig. 3

MycoBank no.: MB 518430

≡*Periconia felina* Marchal, Bull. Soc. R. Bot. Belg. 34:141, 1895 (basionym).

≡*Gliomastix murorum* var. *felina* (Marchal) S. Hughes, Can. J. Bot. 36:769, 1958.

≡*Gliomastix felina* (Marchal) Hammill, Mycologia 73:231, 1981.

Neotype designated here: A dried culture of CBS 147.81 in herb. TNS F-37403: USA, Clarke County, Georgia, in forest soil, 1968, isol. et det. T. M., Hammill, No. 33 (date of accession in CBS: February 1981, received as *Gliomastix felina*).

Colonies on MA reaching 60–70 mm diameter in 10 days at 20°C in darkness, white (25A1), floccose, reverse concolorous. Colonies on OA reaching 30–40 mm diameter in 10 days at 20°C in darkness, blackish brown (5F8), velvety to floccose, zonate, reverse concolorous. Colonies on PDA reaching 30–40 mm diameter in 10 days at 20°C in darkness, blackish brown (5F8), floccose, reverse concolorous.

Hyphae hyaline, septate, 1.5–2 µm wide. Conidiation plectonematogenous. Phialides mostly single, sometimes on a short subtending cell, smooth walled, with walls darkly encrusted in the upper part, sometimes forming a collarette, 20–40 (–50) µm long, tapering from 2–2.5 µm near the base to 1–1.5 µm. Conidia mostly adhering in heads, blackish brown (5F8), some smooth walled and some rough walled, ellipsoidal, with truncate base, 4–6 × 2–2.5 µm. Chlamydospores absent.

Gene sequence data: The GenBank accession numbers for nuclear 28S rDNA D1/D2 and ITS regions are listed in Table 2. Isolates examined: one isolate (K4615-9) from soil from the space between the west wall and soil flowing into the stone chamber in area B of the stone chamber of the KT, 15 June 2004.

The phenotypic characteristics of this isolate (K4615-9) agreed with descriptions of *Gliomastix murorum* var. *felina* (Dickinson 1968) and *G. felina* (Hammill 1981). Because of the occurrence of intermediate strains, Gams (1971) included *G. murorum* var. *felina* in the synonymy *A. murorum*. Although certain isolates showed quite pronounced differences, the taxonomic situation around *A. murorum* var. *murorum* and *A. murorum* var. *felinum* has remained unsettled. *Acremonium murorum* var. *murorum* was distinguished from the variety *felinum* by the formation of more subglobose conidia in chains (Domsch et al. 2007; Crous et al. 2009). Our observations support the specific distinction of *A. felinum* from *A. murorum* as suggested by Hammill (1981). During a review process of the manuscript, W. Gams (personal communication) suggested to propose the designation of neotype because the type material of *Periconia felina* Marchal is missing. We designate a collection CBS 147.81 as neotype of *A. felinum* agreeing with the morphology-based brief descriptions by Hammill (1970, 1981). It has been characterized by DNA sequence data (rDNA D1/D2 and ITS) in this study.

Acremonium tumulicola Kiyuna, An, Kigawa & Sugiy., sp. nov. Fig. 4

MycoBank no.: MB 518348

Ab *Acremonio felino* differt conidiis ellipsoideis basi rotundata, laevibus et sequentiis regionum rDNA D1/D2 et ITS.

Holotype: Japan, Nara Prefecture, Asuka-mura, on the white salt-like masses on the central part of the painting named the vermillion bird (*Suzaku*) on the south wall in the Kitora Tumulus stone chamber interior, 13 June 2006. Herb. TNS F-37402 (dried culture); the ex-type strain has been deposited in Japan Collection of Microorganisms, RIKEN BioResource Center as JCM 17184 (originally as isolate K6613-2); it is also maintained in Centraalbureu voor Schimmelcultures (CBS), Utrecht as CBS 127532.

Etymology: The specific epithet "*tumulicola*" refers to the habitat (*tumulus*) where the sample was collected.

Colonies on MA reaching 30–40 mm diameter in 10 days at 20°C in darkness, white (25A1), floccose to funiculose, reverse concolorous. Colonies on OA reaching 30–40 mm diameter in 10 days at 20°C in darkness, blackish brown (5F8), funiculose to floccose, zonate, reverse concolorous. Colonies on PDA reaching 30–40 mm diameter in 10 days at 20°C in darkness, blackish brown (5F8), floccose, reverse concolorous.

Hyphae hyaline, septate, 1.5–2 μm wide. Conidiation mainly synnematogenous. Phialides mostly arising singly, sometimes on a short subtending cell, smooth walled, with walls darkly encrusted in the upper part, sometimes forming a collarette, 20–40 (–50) μm long, tapering from 2–2.5 μm near the base to 1–1.5 μm. Conidia adhering in heads, blackish brown (5F8), smooth walled, ellipsoidal, 4–5 × 2–3 μm. Chlamydospores absent.

Teleomorph: Unknown.

Gene sequence data: The GenBank accession numbers for nuclear 28S rDNA D1/D2 and ITS regions are listed in Table 1. Habitat: Viscous gels on the stone wall, moldy colonies on the floor, and white salt-like masses on the murals.

Isolates examined: Three strains [K5916-10-3 (September 2005), K6303-1-7 (March 2006), and K6613-2 (ex-type) (June 2006)] isolated from the stone chamber interior and adjacent small room of the KT; the full strain data are provided in Table 1.

Cultural characteristics of these three KT isolates resemble those of *A. murorum* in funiculose surface texture on the respective media (MA, OA, and PDA). In other respects, the morphological characteristics resembled *A. felinum* rather than *A. murorum*. Conidia of *A. murorum* adhere in heads or in irregular chains, whereas conidia of *A. felinum* and *A tumulicola* adhere in heads. *Acremonium felinum* differs from *A. tumulicola* by more or less roughened conidia with a truncate base whereas in *A tumulicola* they are rounded and smooth. Our molecular phylogenetic analyses of ITS–28S rDNA sequences show that these isolates should be regarded as a separate species (see Fig. 9).

Acremonium masseei (Sacc.) W. Gams, *Cephalosporium*-artige Schimmelpilze (Hyphomycetes), p. 83, 1971. Fig. 5

≡*Trichosporum masseei* Sacc., Syll. Fung. (Abellini) 22:1356, 1913 (basionym).

Based on *Trichosporium aterrimum* Masssee 1899 (nom. illegit. Art. 53).

≡*Gliomastix masseei* (Sacc.) Matsush., Icon. Microfung. Matsush. Lect. (Kobe), p. 76, 1975.

Colonies on MA reaching 30–40 mm diameter in 10 days at 20°C in darkness, white (25A1), floccose, reverse concolorous. Colonies on OA or PDA reaching 30–40 mm diameter in 10 days at 20°C in darkness, white (on OA 25A1), velvety to floccose, zonate, reverse concolorous; on PDA white (25CD6), floccose; soluble pigment produced, reddish brown (2B3); reverse yellowish white (2A2).

Hyphae hyaline to pale brown, septate, 1.5–2 μm wide. Conidiation mainly synnematogenous. Phialides mostly borne singly on a short subtending cell, smooth walled, with walls darkly encrusted in the upper part, forming a collarette, 20–40 (–50) μm long, tapering from 2.5–3.0 μm near the base to 1.5–2.0 μm. Conidia phialidic, mostly adhering in chains, rarely in heads, blackish brown (5F8), smooth walled, ellipsoidal, with truncate base, intensely black on both ends, 5–8 (–10) × 2.5–3 (–4) μm. Chlamydospores absent.

Gene sequence data: The GenBank accession numbers for nuclear 28S rDNA D1/D2 and ITS regions are listed in Table 1. Isolates examined: 32 isolates from the murals and walls of the TT stone chamber interior, from May 2004 to December 2006, and 1 isolate from the wall of the KT stone chamber interior in October 2006. The detailed data on these isolates are shown in Table 1.

Cultural and morphological characteristics of the same isolates agreed well with those of the two reference strains (CBS 557.75 and CBS 794.69) and with their published descriptions (Gams 1971; Matsushima 1975).

Acremonium polychromum (J.F.H. Beyma) W. Gams, *Cephalosporium*-artige Schimmelpilze (Hyphomycetes), p. 81, 1971. Fig. 6

≡*Oospora polychroma* J.F.H. Beyma, Verh. K. Ned. Akad. Wet., Afd. Natuurk, Sect. 2, 26 (2): 5, 1928 (basionym).

≡*Gliomastix murorum* var. *polychroma* (J.F.H. Beyma) C.H. Dickinson, Mycol. Pap. 115:11, 1968.

≡*Gliomastix polychroma* (J.F.H. Beyma) Matsush., Icon. Microfung. Matsush. Lect. (Kobe), p. 77, 1975.

Colonies on MA reaching 60–70 mm diameter in 10 days at 20°C in darkness, white (25A1), floccose, reverse concolorous. Colonies on OA reaching 50–65 mm diameter in

10 days at 20°C in darkness, blackish brown (5F8), velvety to floccose, zonate, reverse concolorous. Colonies on PDA reaching 40–50 mm diameter in 10 days at 20°C in darkness, grayish white (25CD6), floccose, reverse concolorous.

Hyphae hyaline, septate, 1.5–2 μm wide. Conidiation phalacrogenous to synnematogenous. Phialides mostly arising singly, sometimes on a short subtending cell, smooth walled, with walls darkly encrusted in the upper part, lacking a visible collarette, 20–30 (–40) μm long, tapering from 2–2.5 μm near the base to 1–1.5 (–2) μm. Conidia adhering in very long, dry chains, blackish brown (5F8), smooth walled, ellipsoidal, with truncate base, intensely black on both ends, 3–5 × 2–2.5 (–3) μm. Chlamydospores absent.

Gene sequence data: The GenBank accession numbers for nuclear 28S rDNA D1/D2 and ITS regions are listed in Table 1. Isolates examined: two isolates from the air in adjacent room B of the TT in July 2006; T6713-22-1a, T6713-22-1b, and one isolate from the air from the north area in the adjacent small room of the KT in February 2005; K5225-12-5. The detailed data on isolates are shown in Table 1.

Phenotypic characteristics of three isolates identified as *A. polychromum* agreed well with previous descriptions (Dickinson 1968; Gams 1971; Matsushima 1975). In our studies, *A. polychromum* was isolated only from air in adjacent areas of the TT and KT. We could not detect this species from the stone chamber interior or surrounding soil samples from the TT and KT.

Key to the taxa of *Acremonium* sect. *Gliomastix* series *Murorum* included in the phylogenetic analyses

1a. Conidia dry, in regular chains 2
1b. Conidia coated in black, sticky mucilage, in heads or in irregular chains 3
2a. Conidia with truncate base, almost black, mostly 3–4 μm wide *A. masseei*
2b. Conidia with almost pointed base, olivaceous-brown, mostly 2–3 μm wide *A. polychromum*
3a. Conidia almost smooth or some rough-walled, in slimy heads 4
3b. Conidia almost smooth to coarsely roughened, globose to ellipsoidal, 3–6 × 2–4.5 μm, adhering in slimy heads or in chains *A. murorum*
4a. Conidia ellipsoidal, smooth walled to some rough walled, with truncate base, 4–6 (–7) × 2–3 (–4) μm *A. felinum*
4b. Conidia almost ellipsoidal with rounded base, smooth walled, 4–5 × 2–3 μm *A. tumulicola*

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