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The genus *Murispora*

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Abstract – We are studying dothideomycetes with muriform ascospores and in this paper provide an account of those species in Amniculicolaceae. In this family muriform ascospores are only known in the genus *Murispora*. In this paper we introduce the new species *M. fagicola* (on dead branches of *Fagus sylvatica*), *M. galii* (on dead twigs of *Galium* sp.), *M. cardui* (on dead twigs of *Carduus* sp.), *M. medicaginicola* (on dead twigs of *Medicago* sp), *M. cicognanii* (on dead branches of *Clematis* sp.) and *M. hawksworthii* (on dead twigs of an unknown woody plant), collected from Italy and the UK. Descriptions, illustrations and justifications for the novelty are provided for each taxon. Morphological character differences and analysis of combined LSU, SSU and EF1-α sequence datasets support the validity of the new species and their placement in *Murispora* in Amniculicolaceae. The asexual morph of *M. hawksworthii* was established from single ascospore isolates.

***Murispora*/ Amniculicolaceae/ new species/ muriform/ purple stain**

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INTRODUCTION

Dothideomycetes is the largest class of Ascomycota and comprises a highly diverse range of taxa characterized mainly by bitunicate asci (Hyde *et al.* 2013). They can be found worldwide on substrates in terrestrial, freshwater and marine habitats. The class contains many important pathogens, while others are saprobes and may also be endophytes or epiphytes, or fungicolous, lichenized, or lichenicolous fungi (Hyde *et al.* 2013; Phookamsak *et al.* 2014; Schoch *et al.* 2006; Zhang *et al.* 2009c).

Numerous Dothideomycetes species can affect agriculture and forestry systems as plant pathogens (Cortinas *et al.* 2006; Crous *et al.* 2007; Wikee *et al*. 2011, 2013a, b; Manamgoda *et al*. 2012; Wijayawardene *et al*. 2014) and they are important in medical (Siu and Lzumi 2004; da Cunha *et al*. 2012, 2013; Liu *et al.* 2013) or biotechnological industries (Verkley *et al*. 2004; Damm *et al*. 2008; de Wit *et al*. 2012; Ohm *et al*. 2012; Stergiopoulos *et al.* 2012; Wijayawardene *et al*. 2014). Pleosporales is the largest order of Dothideomycetes (Kirk *et al*. 2008; Schoch *et al*. 2009a; Hyde *et al*. 2013) currently with 39 families, 202 accepted genera and 48 genera placed in Pleosporales genera incertae sedis (Wijayawardene *et al*. 2014).

We are studying various members of Dothideomycetes in order to provide a natural classification based on multigene phylogeny (Nelsen *et al*. 2009, Schoch *et al*. 2009b, 2011, Boonmee *et al*. 2011, 2012, Chomnunti *et al*. 2011, 2014, Liu *et al*. 2011, 2012, 2015, Zhang *et al*. 2011, 2012, Hyde *et al*. 2013, Ariyawansa *et al.* 2014, 2015a, 2015b, Wijayawardene *et al*. 2014). In this paper, we account for taxa with muriform ascospores that group with taxa in Amniculicolaceae. This family was introduced by Zhang *et al*. (2009c) to describe various freshwater taxa from Europe and later accepted by Shearer *et al*. (2009) with a well-supported phylogeny consisting of four freshwater sexual morph species and one aquatic hyphomycete asexual species. The family is characterized by "ascomata with a rough black surface, usually staining the woody substrate purple, narrow pseudoparaphyses and short-pedicellate asci, bearing hyaline, reddish-brown or pale, 1 – to multi-septate or muriform ascospores, generally with a hyaline gelatinous sheath" (Hyde *et al*. 2013; Zhang *et al*. 2008). Currently, the family comprises three genera, *Amniculicola* (type), *Murispora* and *Pseudomassariosphaeria* Phukhamsakda *et al*., that form a well-supported clade in the Pleosporales (Hyde *et al*. 2013 ; Zhang *et al*. 2009a; Ariyawansa *et al.* 2015a).

The aim of this paper is to provide a backbone tree and natural classification for Amniculicolaceae. We introduce six new saprobic species in the genus *Murispora* from different hosts in Italy and the UK. Combined gene (LSU, SSU, and EF1-α) analyses using maximum-likelihood (ML), maximum-parsimony (MP) and MrBayes clearly show that Amniculicolaceae is a well-supported family that incorporates the new *Murispora* species with high statistical support.

MATERIALS AND METHODS

Sample collection, morphological studies and isolation

The specimens were collected from different sites in Forlì-Cesena (Monte Comero, Valgianna & Ladino), Pesaro-Urbino (San Sisto) and Trento (Vermiglio,

Passo del Tonale) provinces in Italy and South Wales in the UK. Specimens were brought to the laboratory in Zip lock plastic bags and examined under a Motic SMZ 168 stereomicroscope. Micromorphological characters were examined under a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope mounted with a Canon EOS 550D digital camera. India ink was added to water mounts to show the presence of a gelatinous sheath around the ascospores.

Single ascospore isolation was carried out following the method described in Chomnunti *et al*. (2014). Germinating ascospores were transferred aseptically to Potato dextrose agar (PDA) plates and grown at 16 °C in the daylight. Colony colour and other characters were observed and measured after a week and again after three weeks. The specimens are deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures are also deposited at the Culture Collection at Mae Fah Luang University (MFLUCC). Facesoffungi numbers (FoF) were acquired as in Jayasiri *et al.* (2015) and Index Fungorum numbers (IF) as in http://www.indexfungorum.org/names/nam-es.asp.

DNA extraction, PCR amplification, sequencing and sequence alignment

Total fungal DNA was extracted from fresh fungal mycelium grown on PDA media at 16 °C for four weeks using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer.

Phylogenetic analyses were conducted using partial sequences of four genes, the internal transcribed spacers (5.8S, ITS), small subunit rDNA (18S, SSU), large subunit (28S, LSU) and translation elongation factor 1-alpha gene (TEF 1α). Nuclear ITS was amplified using the primers ITS5 and ITS4 (White *et al*. 1990), LSU was amplified using the primers LROR and LR5 (Vilgalys and Hester 1990), SSU was amplified using the primers NS1 and NS4 (White *et al*. 1990), TEF was amplified using primers EF1-983F and EF1-2218R (Rehner 2001).

Polymerase chain reaction (PCR) was carried out using the following protocol: The final volume of the PCR reaction was 25 µL and contained 12.5 µL of 2 × Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µl Taq DNA Polymerase, 500 µm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCL pH8.3, 100 Mm KCl, 3 mM $MgCl_2$, stabilizer and enhancer), 1 µL of each primer (10 µM), 1 µL genomic DNA extract and 9.5 µL deionised water. The reaction was then allowed to run for 35 cycles. The annealing temperature was 55 ºC for ITS, LSU, TEF and 50 °C for SSU and initially 95 °C for 3 mins, denaturation at 95 °C for 30 seconds, annealing for 1 min, elongation at 72 °C for 30 seconds, and final extension at 72 °C for 10 mins. PCR amplification was confirmed on 1 % agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (BGI, Ltd Shenzhen, P.R. China). The nucleotide sequence data acquired were deposited in GenBank (Table 1).

The other sequences used in the analyses (Table 1) were obtained from GenBank. The multiple alignments were automatically done by MAFFT v. 7.036 (Katoh and Standley 2013), but manual adjustments for improvement were made by eye where necessary using BioEdit v. 7.2 (Hall, 1999) and ClustalX (Kohli and Bachhawat 2013).

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers (Fig 1).
The newly generated sequences are indicated in bold

Taxon	*Culture Accession No*	*GenBank Accession No.*			*References*
		LSU	*SSU*	*TEF1*	
Aigialus grandis	BCC 18419 [T]	GU479774	GU479738	GU479838	Suetrong *et al.* 2009
Amniculicola immersa	CBS 123083 [T]	FJ795498	GU456295	GU456273	Zhang *et al.* 2009d
Amniculicola lignicola	CBS 123094 [T]	EF493861	EF493863	GU456278	Zhang *et al.* 2008
Amniculicola parva	CBS 123092 [T]	FJ795497	GU296134	GU349065	Zhang *et al.* 2009d
Anguillospora longissima	CCM-F10304	JN673029			Raja *et al.* 2011
Anguillospora longissima	CS869	GU266240	GU266222		Raja *et al.* 2011
Anteaglonium abbreviatum	ANM 925a [T]	GQ221877		GQ221924	Mugambi and Huhndorf 2009
Anteaglonium globosum	ANM 925.2 [T]	GQ221879		GQ221925	Mugambi and Huhndorf 2009
Anteaglonium latirostrum	GKML100Nb [T]	GQ221876		GQ221938	Mugambi and Huhndorf 2009
Anteaglonium parvulum	GKM 1218 [T]	GQ221880		GQ221922	Mugambi and Huhndorf 2009
Ascochyta pisi	CBS 126.54	DQ678070	DQ678018	DQ677913	Schoch *et al.* 2006
Ascocratera manglicola	BCC 9270 [T]	GU479782	GU479747	GU479846	Suetrong *et al.* 2009
Astrosphaeriella bakeriana	MFLUCC 11-0027	JN846730	JN846740		Liu *et al.* 2011
Astrosphaeriella stellata	MFLUCC 10-0555 [T]	JN846723	JN846733		Liu *et al.* 2011
Bimuria novae-zelandiae	CBS 107.79	AY016356	AY016338	DQ471087	Lumbsch and Lindemuth 2001
Byssosphaeria jamaicana	SMH 1403 [T]	GU385152		GU327746	Mugambi and Huhndorf 2009
Byssosphaeria rhodomphala	GKM L153N [T]	GU385157		GU327747	Mugambi and Huhndorf 2009
Corynespora cassiicola	CBS 100822	GU301808	GU296144	GU349052	Schoch *et al.* 2009
Corynespora smithii	CABI 5649b	GU323201		GU349018	Schoch *et al.* 2009
Delitschia chaetomioides	SMH 3253.2 [T]	GU390656		GU327753	Mugambi and Huhndorf 2009
Delitschia winteri	CBS 225.62 [T]	DQ678077	DQ678026	DQ677922	Schoch *et al.* 2006
Halotthia posidoniae	BBH 22481	GU479786	GU479752		Suetrong *et al.* 2009
Herpotrichia diffusa	CBS 250.62 [T]	DQ678071	DQ678019	DQ677915	Schoch *et al.* 2006
Herpotrichia juniperi	CBS 200.31 [T]	DQ678080	DQ678029	DQ677925	Schoch *et al.* 2006
Kalmusia scabrispora	KT 2202	AB524594	AB524453	AB539107	Tanaka *et al.* 2009
Katumotoa bambusicola	KT 1517a [T]	AB524595	AB524454	AB539108	Tanaka *et al.* 2009
Lentithecium fluviatile	CBS 122367 [T]	GU301825	GU296158	GU349074	Schoch *et al.* 2009b

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers (Fig 1).
The newly generated sequences are indicated in bold *(continued)*

Taxon	*Culture Accession No*	*GenBank Accession No.*			*References*
		LSU	*SSU*	*TEF1*	
Lepidosphaeria nicotiae	CBS 101341	DQ678067		DQ677910	Schoch *et al.* 2006
Leptosphaeria doliolum	CBS 505.75	GU301827	GU296159	GU349069	Schoch *et al.* 2009b
Lindgomyces breviappendiculata	MAFF 239292 **T**	AB521749	AB521734		Hirayama *et al.* 2010
Lindgomyces cinctosporae	Raja R56-1 **T**	AB522431	AB522430		Hirayama *et al.* 2010
Lindgomyces ingoldianus	ATCC 200398 **T**	AB521736	AB521719		Hirayama *et al.* 2010
Lindgomyces rotundatus	HHUF 27999 **T**	AB521740	AB521723		Hirayama *et al.* 2010
Lophiostoma arundinis	CBS 621.86	DQ782384	DQ782383	DQ782387	Schoch *et al.* 2006
Lophiostoma crenatum	CBS 629.86	DQ678069	DQ678017	DQ677912	Schoch *et al.* 2006
Lophiostoma macrostomoides	CBS 123097	FJ795439	FJ795482	GU456277	Zhang *et al.* 2009d
Lophiostoma semiliberum	CBS 626.86	FJ795441	FJ795484		Zhang *et al.* 2009d
Lophiotrema brunneosporum	CBS 123095	GU301835	GU296165	GU349071	Schoch *et al.* 2009b
Lophiotrema lignicola	CBS 122364	GU301836	GU296166	GU349072	Schoch *et al.* 2009b
Lophiotrema neoarundinaria	MAFF 239461	AB524596	AB524455		Tanaka *et al.* 2009
Lophiotrema nucula	CBS 627.86	GU301837	GU296167	GU349073	Schoch *et al.* 2009b
Lophiotrema vagabundum	JCM 17674	AB619022	AB618704		Hirayama and Tanaka 2011
Massaria gigantispora	M 26 **T**	HQ599397	HQ599447	HQ599337	Voglmayr and Jaklitsch 2011
Massaria inquinans	M 19 **T**	HQ599402	HQ599444	HQ599342	Voglmayr and Jaklitsch 2011
Massarina eburnea	CBS 473.64 **T**	GU301840	GU296170	GU349040	Schoch *et al.* 2009b
Massariosphaeria phaeospora	CBS 611.86	GU301843	GU296173		Schoch *et al.* 2009b
Massariosphaeria typhicola	KT 797	AB521747	AB521730		Hirayama *et al.* 2010
Massariosphaeria typhicola	KT 667	AB521746	AB521729		Hirayama *et al.* 2010
Massariosphaeria typhicola	CBS 609.86	EF165033	EF165037		Wang *et al.* 2007
Mauritiana rhizophorae	BCC 28866	GU371824	GU371832	GU371817	Schoch *et al.* 2009b
Melanomma pulvis-pyrius	CBS 124080 **T**	GU456323	GU456302	GU456265	Zhang *et al.* 2009a
Murilentithecium clematidis	MFLUCC 14-0562 **T**	KM408759	KM408761	KM454445	Wanasinghe *et al.* 2014
Murispora rubicunda	IFRD 2017	FJ795507	GU456308	GU456289	Zhang *et al.* 2009d

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers (Fig 1).
The newly generated sequences are indicated in bold *(continued)*

Taxon	*Culture Accession No*	*GenBank Accession No.*			*References*
		LSU	*SSU*	*TEF1*	
Murispora fagicola	**MFLUCC 13-0600 T**	**KT709174**	**KT709181**	**KT709188**	This study
Murispora galii	**MFLUCC 13-0819 T**	**KT709175**	**KT709182**	**KT709189**	This study
Murispora cardui	**MFLUCC 13-0761 T**	**KT709176**	**KT709183**	**KT709190**	This study
Murispora medicaginicola	**MFLUCC 13-0762 T**	**KT709177**	**KT709184**	**KT709191**	This study
Murispora cicognanii	**IT 1693**	**KT709178**	**KT709185**		This study
Murispora cicognanii	**MFLUCC 14-0953 T**	**KT709179**	**KT709186**		This study
Murispora hawksworthii	**MFLUCC 14-0918 T**	**KT709180**	**KT709187**	**KT709192**	This study
Neomassariosphaeria grandispora	CBS 613.86	GU301842	GU296172	GU349036	Schoch *et al.* 2009b
Neomassariosphaeria typhicola	CBS 123126	FJ795504	GU296174		Zhang *et al.* 2009d
Neottiosporina paspali	CBS 331.37 T	EU754172	EU754073	GU349079	Gruyter *et al.* 2009
Boeremia exigua	CBS 431.74	EU754183	EU754084	GU349080	Gruyter *et al.* 2009
Pleomassaria siparia	CBS 279.74 T	DQ678078	DQ678027	DQ677923	Schoch *et al.* 2006
Pleospora herbarum	CBS 191.86 T	DQ247804	DQ247812	DQ471090	Schoch *et al.* 2006 & Spatafora *et al.* 2006
Preussia funiculata	CBS 659.74	GU301864	GU296187	GU349032	Schoch *et al.* 2009b
Preussia lignicola	CBS 264.69	GU301872	GU296197	GU349027	Schoch *et al.* 2009b
Preussia terricola	DAOM 230091	AY544686	AY544726	DQ471063	Spatafora *et al.* 2006
Prosthemium canba	JCM 16966	AB553760	AB553646		Tanaka *et al.* 2010
Prosthemium betulinum	CBS 127468	AB553754	AB553644		Tanaka *et al.* 2010
Pseudomassariosphaeria bromicola					Ariyawansa *et al.* 2015a
Pyrenochaeta nobilis	CBS 407.76 T	DQ678096	DQ898287	DQ677936	Schoch *et al.* 2006
Quadricrura septentrionalis	CBS 125428 T	AB524617	AB524476	AB524832	Tanaka *et al.* 2010
Repetophragma ontariense	HKUCC 10830 T	DQ408575		DQ435077	Shenoy *et al.* 2006
Rimora mangrovei	JK 5246A T	GU301868	GU296193		Schoch *et al.* 2009b
Roussoella hysterioides	CBS 125434	AB524622	AB524481	AB539115	Tanaka *et al.* 2010
Roussoella pustulans	MAFF 239637	AB524623	AB524482	AB539116	Tanaka *et al.* 2010

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers (Fig 1). The newly generated sequences are indicated in bold *(continued)*

Taxon	*Culture Accession No*	*GenBank Accession No.*			*References*
		LSU	*SSU*	*TEF1*	
Roussoellopsis tosaensis	MAFF 239638	AB524625		AB539117	Tanaka *et al.* 2010
Salsuginea ramicola	KT 2597.1	GU479800	GU479767	GU479861	Suetrong *et al.* 2009
Spirosphaera cupreorufescens	A20 **T**	AY616236			Voglmayr 2004
Sporormiella minima	CBS 524.50	DQ678056	DQ678003	DQ677897	Aveskamp *et al.* 2010
Tetraploa sasicola	KT 563 **T**	AB524631	AB524490		Tanaka *et al.* 2010
Triplosphaeria maxima	KT 870 **T**	AB524637	AB524496		Tanaka *et al.* 2010
Ulospora bilgramii	CBS 110020	DQ678076	DQ678025	DQ677921	Schoch *et al.* 2006
Verruculina enalia	BCC 18401	GU479802	GU479770	GU479863	Suetrong *et al.* 2009
Westerdykella cylindrica	CBS 454.72	AY004343	AY016355	DQ497610	Lumbsch *et al.* 2005
Westerdykella ornata	CBS 379.55	GU301880	GU296208	GU349021	Schoch *et al.* 2009b

Abbreviations : **ANM** : A.N. Miller ; **ATCC** : American Type Culture Collection, Virginia, U.S.A. ; **BBH** : BIOTEC Bangkok Herbarium, Thailand ; **BCC** : Belgian Coordinated Collections of Microorganisms : **CABI** International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K. ; **CBS** : Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands ; **CS :** Carol Shearer hyphomycetes (mitosporic fungi) ; **DAOM** : Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada ; Japan ; **GKM** G.K. Mugambi ; **HHUF** Herbarium of Hirosaki University, Japan ; **HKUCC** : University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China ; **IFRD** : IFRDCC : Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China ; **JCM** Japan Collection of Microorganisms ; **JK** : J. Kohlmeyer ; **KT** : Kazuaki Tanaka ; **MAFF** Ministry of Agriculture, Forestry, and Fisheries, Japan ; **MFLUCC** : Mae Fah Luang University Culture Collection, Chiang Rai, Thailand ; **JCM** : The Japan Collection of Microorganisms ; **R** : H.A. Raja ; **SMH** : S.M. Huhndorf ; **M19, M26, A20** : H. Voglmayr ; **T** : ex-type/ex-epitype isolates.

Table 2. Alignment properties and nucleotide substitution models per locus, and combined

	LSU	*SSU*	*TEF*	*Combined LSU, SSU and TEF*
Alignment strategy (MAFFT version 7.220)	FFT-NS-i + manually	FFT-NS-i	FFT-NS-i + manually	–
Number of characters included in analysis (including gaps)	908	1052	853	2821
Number of constant characters	583	823	507	1921
Number of parsimony informative characters (%)	239 (26 %)	122 (12 %)	275 (32 %)	636 (23 %)
Number of uninformative and variable characters	86	107	71	264
Nucleotide substitution model	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G

LSU : 28S large subunit ribosomal RNA gene ; SSU : 18S small subunit ribosomal RNA gene ; TEF : partial translation elongation factor 1-alpha gene

Phylogenetic analysis

Parsimony analysis was carried with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002), with the following parameter settings, as described in Wanasinghe *et al*. (2014): characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length was zero, maxtrees set at 1000. Alignment gaps were treated as missing characters in the analysis of the combine data set, where they occurred in relatively conserved regions. Parsimony bootstrap analyses were performed using the full heuristic search option, random stepwise addition, and 1000 replicates, with maxtrees set at 1000. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino and Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were meaningfully different. Maximum parsimony bootstrap values (MP) equal or greater than 75% are given above each node in red (Fig. 1). MODELTEST v. 3.7 (Posada and Crandall 1998) following Akaike Information Criterion was used to determine the best-fit model of evolution for each data set for Bayesian and Maximum Likelihood analyses.

Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis 2008) implemented in raxmlGUI v.0.9b2 (Silvestro and Michalak 2010), employing mixed models of evolution settings of the program and Bootstrap support obtained by running 1000 pseudo replicates. The online tool Findmodel was used to determine the best nucleotide substitution (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) model for each partition. The best scoring tree was selected with a final likelihood value of – 24652.430363. Maximum Likelihood bootstrap values (ML) equal or greater than 75% are given above each node in black (Fig. 1).

A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronqvist 2001) to valuate Posterior probabilities (PP) (Rannala and Yang 1996;

Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments:

Six simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation and 20,000 trees were obtained. The first 4,000 trees, representing the burn-in phase of the analyses and discarded. The remaining 16000 trees were used for calculating PP in the majority rule consensus tree (Cai *et al*. 2006, 2008; Ariyawansa *et al*. 2015b). Branches with Bayesian posterior probabilities greater than 0.95 are given in bold. Maximum trees were visualized with Tree View (Page 1996).

RESULTS

Phylogenetic analysis

The combined LSU, SSU, and EF1-α gene dataset comprised 90 sequences from 17 families, plus taxa of Pleosporineae and Massarineae (Pleosporales), and our new strains of *Murispora,* with *Hysterium angustatum* (CBS 123334 and CBS 236.34) as the outgroup taxon (Fig 1). Three different alignments corresponding to each individual gene and a combined alignment of the three genes were analyzed. Comparison of the alignment properties and nucleotide substitution models are provided in Table 2. All trees (ML, MP and BYPP) were similar in topology and did not differ significantly (data not shown). A best scoring RAxML tree is shown in Fig. 1, with the value of – 24652.430363. Phylogenetic trees obtained from Maximum Likelihood, Maximum parsimony and Bayesian analysis yielded trees with similar overall topology at the family relationships in agreement with previous studies based on Maximum Likelihood analysis (Schoch *et al.* 2009, Suetrong *et al.* 2009, Zhang *et al*. 2009c, 2012, Hyde *et al*. 2013, Wijayawardene *et al*. 2014).

This analysis comprised 2821 characters, of which 1921 were constant, 636 parsimony informative and 264 parsimony-uninformative. Four equally parsimonious trees were generated and the first was selected (Fig. 1). Bootstrap support (BS) values of ML and MP (equal to or above 75% based on 1000 replicates) are shown on the upper branches respectively with black and blue. Branches with Bayesian posterior probabilities (PP) greater than 0.95 from MCMC analyses are given in bold. The Kishino-Hasegawa test shows length = 4196 steps with CI = 0.310, RI = 0.600, RC = 0.186 and HI = 0.690.

Our strains of *Murispora* (MFLUCC 13-0600, 14-0918, 13-0762, 13-0761, 13-0819, 14-0953 and IT1693) grouped in Amniculicolaceae, but separated from the other genera of the family with relatively high bootstrap support (97%, Figure 1). Seven strains cluster in this clade, with the type species, *Murispora rubicunda* (strain IFRD 2017).

Taxonomy

Amniculicolaceae Y. Zhang *et al.* in Zhang *et al.* Stud. Mycol. 64: 95 (2009)

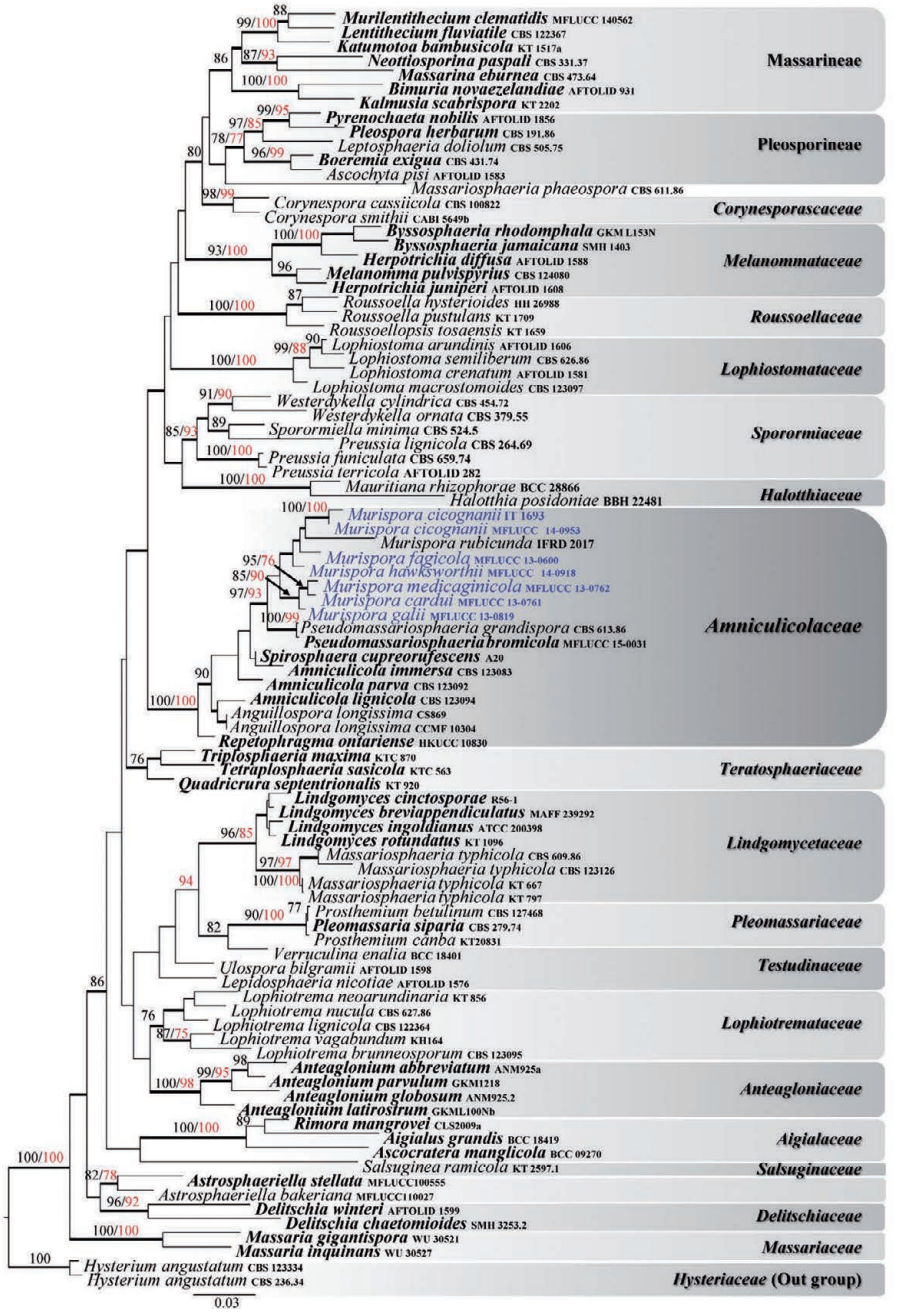

Fig. 1. RAxML tree based on a combined dataset of LSU, SSU and TEF sequence data. Bootstrap support values for maximum parsimony (MP, red) and maximum likelihood (ML, black) higher than 75 % are defined as above the nodes and branches with Bayesian posterior probabilities greater than 0.95 are given in bold. The ex-type and reference strains are in bold ; the new isolates are in blue. The tree is rooted to *Hysterium angustatum* (CBS 123334 and CBS 236.34).

Type: ***Amniculicola*** Y. Zhang & K.D. Hyde, Mycol. Res. 112 (10): 1189 (2008)

Type species: ***Amniculicola lignicola*** Y. Zhang ter & K.D. Hyde, Mycol. Res. 112 (10): 1189 (2008)

Notes: The family Amniculicolaceae is amended here to include species with 1-3 µm wide, narrow, filamentous, branched, septate pseudoparaphyses, instead of trabeculate pseudoparaphyses.

Other genera included

Pseudomassariosphaeria Phukhamsakda *et al*. in Ariyawansa *et al*. Fungal Divers. 75: 35 (2015)

Type species: ***Pseudomassariosphaeria bromicola*** Phukhamsakda *et al*. in Ariyawansa *et al*. Fungal Divers. 75: 40 (2015)

Murispora Y. Zhang bis *et al.*in Zhang *et al.* Stud. Mycol. 64 : 95 (2009)

Type species: ***Murispora rubicunda*** (Niessl) Y. Zhang ter *et al.* in Zhang *et al.* Stud. Mycol. 64: 96 (2009)

≡ *Pleospora rubicunda* Niessl, *Notiz. Pyr.*: 31 (1876)

= *Massariosphaeria rubicunda* (Niessl) Crivelli, *Ueber die Heterogene Ascomycetengattung Pleospora Rabh.; Vorschlag für eine Aufteilung (Diss. Eid genössischen Technischen Hochschule Zürich* 7318): 144 (1983)

= *Karstenula rubicunda* (Niessl) M.E. Barr, *N. Amer. Fl*., Ser. 2 (New York) 13: 52 (1990)

Murispora fagicola Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde, *sp. nov.*

Facesoffungi Number: FoF01104 ;

Index Fungorum number: IF551556 **Fig. 2**

Etymology: Name reflects the host genus *Fagus*, from which the species was collected.

Holotype: MFLU 15-2246

Saprobic on dead herbaceous branches in terrestrial habitats. **Sexual morph**: *Ascomata* 280-340 µm high 180-280 µm diam. ($\overline{x}$ = 273.2 × 246.7 µm, n = 10), globose to subglobose, solitary, dark brown to black, immersed, substrate stained purple, fused to the host tissue, ostiolate. *Ostiole* 60-80 µm high 25-50 µm diam. ($\overline{x}$= 72.7 × 34.7 µm, n = 5) short to papillate, black, smooth, opening to exterior through bark surface. *Peridium* 10-20 µm wide at the base, 15-25 µm wide in sides, comprising 3-4 layers of dark brown cells *textura angularis*, with inner 1-2 layers of cells thin-walled and hyaline. *Hamathecium* comprising numerous, 1.5-2 µm (n = 30) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 140-190 × 20–30 µm ($\overline{x}$ = 166.1 × 24.2 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 28-35 × 13-17 µm ($\overline{x}$ = 32.4 × 14.8 µm, n = 50), overlapping 1-2-seriate, hyaline when young, becoming pale reddish brown at maturity, oval to ellipsoidal, muriform, with 1-2 longitudinal septa in all cells except end cells, constricted at the septa, conical and narrowly rounded at the ends, guttulate, with rugged surface, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on MEA slow growing, light pink when young, reddish brown at maturity, flat on the surface, with mycelium composed of septate, branched hyphae producing intermediary and terminal chlamydospores.

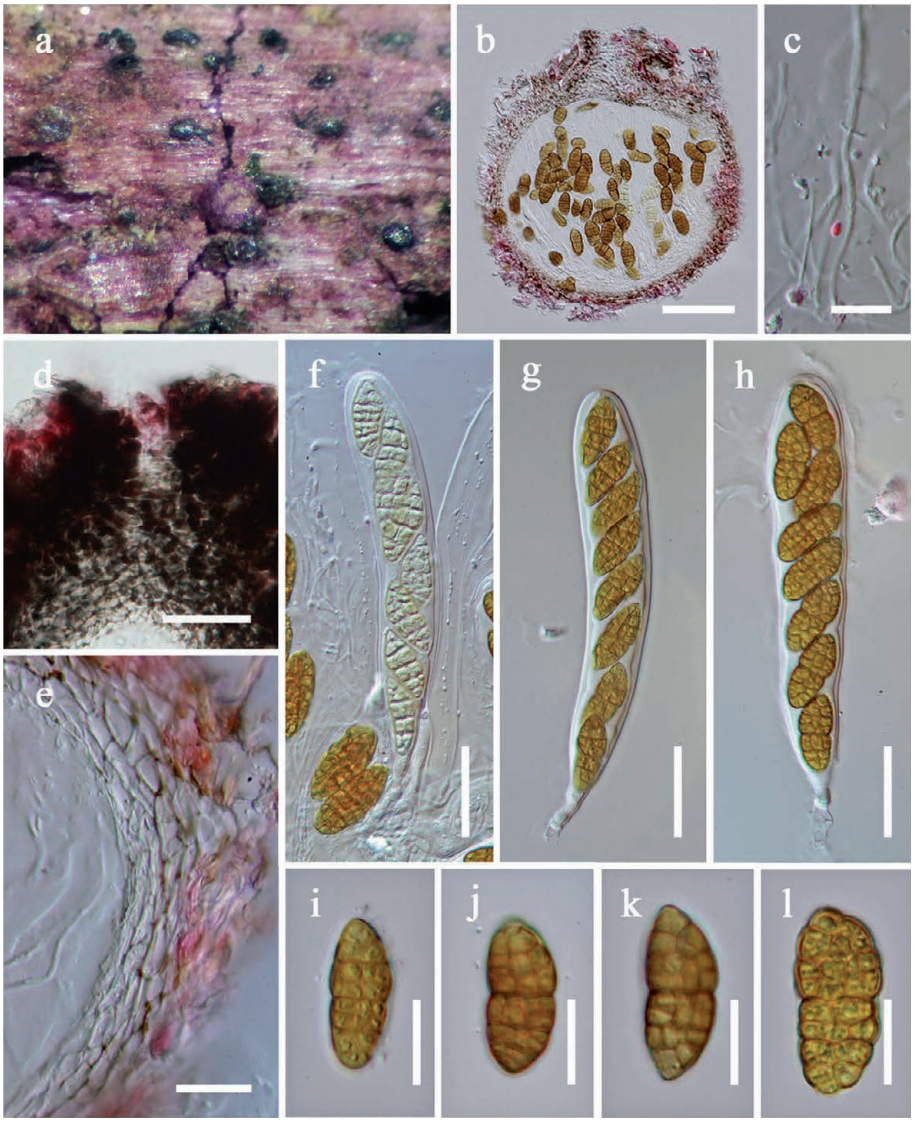

Fig 2. ***Murispora fagicola*** (**holotype**). **a.** Papilla on host substrate **b.** Section of ascoma **c.** Pseudoparaphyses **d.** Close up of ostiole **e.** Peridium **f-h.** Asci **i-l.** Ascospores. Scale bars : b = 50 µm, c,e = 10 µm, d, f-h = 20 µm, i-l = 10 µm.

Chlamydospores terminal or intercalary, thick and smooth-walled, globose, formed in abundance, after 15 d, 1.00 cm diam. at 18ºC.

Known distribution: On dead branches of *Fagus sylvatica* (Fagaceae), Italy.

Material examined: Italy, Forlì-Cesena, Bagno di Romagna, Monte Comero, dead and fallen branches of *Fagus sylvatica*, 17 Apr 2013, *E. Camporesi* (MFLU 15-2246, holotype; isotype BBH 39892; ex-type living culture = MFLUCC 13-0600).

Gene sequence data: ITS (KT736080), LSU (KT709174), SSU (KT709181), TEF (KT709188).

Murispora galii Wanasinghe, N. Camporesi, E.B.G. Jones & K.D. Hyde, *sp. nov.*

Facesoffungi Number: FoF 01105 ;

Index Fungorum number: IF551557 **Fig. 3**

Etymology: Name reflects the host genus *Galium*, from which the species was collected.

Holotype: MFLU 15-2247

Saprobic on dead herbaceous branches in terrestrial habitats. **Sexual morph**: *Ascomata* 250-350 µm high 250-310 µm diam. ($\overline{x}$ = 275.4 × 271.7 µm, n = 10), globose to subglobose, solitary, dark brown to black, erumpent to nearly superficial, substrate stained purple, ostiolate. *Ostiole* 80-130 µm high 60-90 µm diam. ($\overline{x}$=123.2 × 73.4 µm, n = 5), short to papillate, black, smooth, ostiolar canal filled with hyphae. *Peridium* 12-18 µm wide at the base, 15-25 µm wide in sides, composed of brown to dark brown or reddish brown, pseudoparenchymatous cells of *textura angularis*. *Hamathecium* comprising numerous, 1.5-2.5 µm (n = 30) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 150-200 × 20-30 µm ($\overline{x}$ = 172.1 × 23.7 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 35-40 × 10-15 µm ($\overline{x}$ = 37.5 × 13.2 µm, n = 50), overlapping 1-2-seriate, hyaline when young, becoming brown at maturity, curved-fusoid, asymmetrical with one sides flattened, muriform, with 2-3 longitudinal septa in all cells, slightly constricted at the middle septum, widest above the central septum, conical and narrowly rounded at the ends, initially guttulate, with rugged surface, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA slow growing, reaching 2 cm diam. after 3 weeks at 16ºC, light pink when young, reddish brown at maturity, flat on the surface, with mycelium composed of septate, branched hyphae.

Known distribution: On dead twigs of *Galium* sp. (Rubiaceae), Italy.

Material examined: Italy, Pesaro-Urbino [PU], San Sisto, dead and fallen twigs of *Galium* sp., 21 May 2013, *N. Camporesi* (MFLU 15-2247, holotype; isotype BBH 39893; ex-type living culture, MFLUCC 13-0819).

Gene sequence data: ITS (KT736081), LSU (KT709175), SSU (KT709182), TEF (KT709189).

Murispora cardui Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde, *sp. nov.*

Facesoffungi Number: FoF01106;

Index Fungorum number: IF551558 **Fig. 4**

Etymology: Name reflects the host genus *Carduus*, from which the species was collected.

Holotype: MFLU 15-2248

Saprobic on dead herbaceous branches in terrestrial habitats. **Sexual morph**: *Ascomata* 180-250 µm high 200-300 µm diam. ($\overline{x}$ = 201.3 × 244.7 µm, n = 10), globose to subglobose, solitary, dark brown to black, erumpent to nearly superficial, substrate stained purple, fused to the host tissue, ostiolate. *Ostiole* 60-80 µm high 45-80 µm diam. ($\overline{x}$ = 61.2 × 62.1 µm, n = 5) short to papillate, black, smooth, ostiolar canal filled with hyphae. *Peridium* 7-11 µm wide at the base, 15-25 µm wide in sides, thin-walled, comprising 3-6 layers of dark brown to black cells of *textura angularis*. *Hamathecium*

Fig 3. ***Murispora galii*** (**holotype**). **a.** Appearance of ascomata on host substrate **b.** Section of ascoma **c.** Pseudoparaphyses **d.** Close up of ostiole **e.** Peridium **f-h.** Asci **i-m.** Ascospores (Note the sheath in m). Scale bars : b = 50 µm, c,e = 10 µm, d, f-h = 20 µm, i-m = 10 µm.

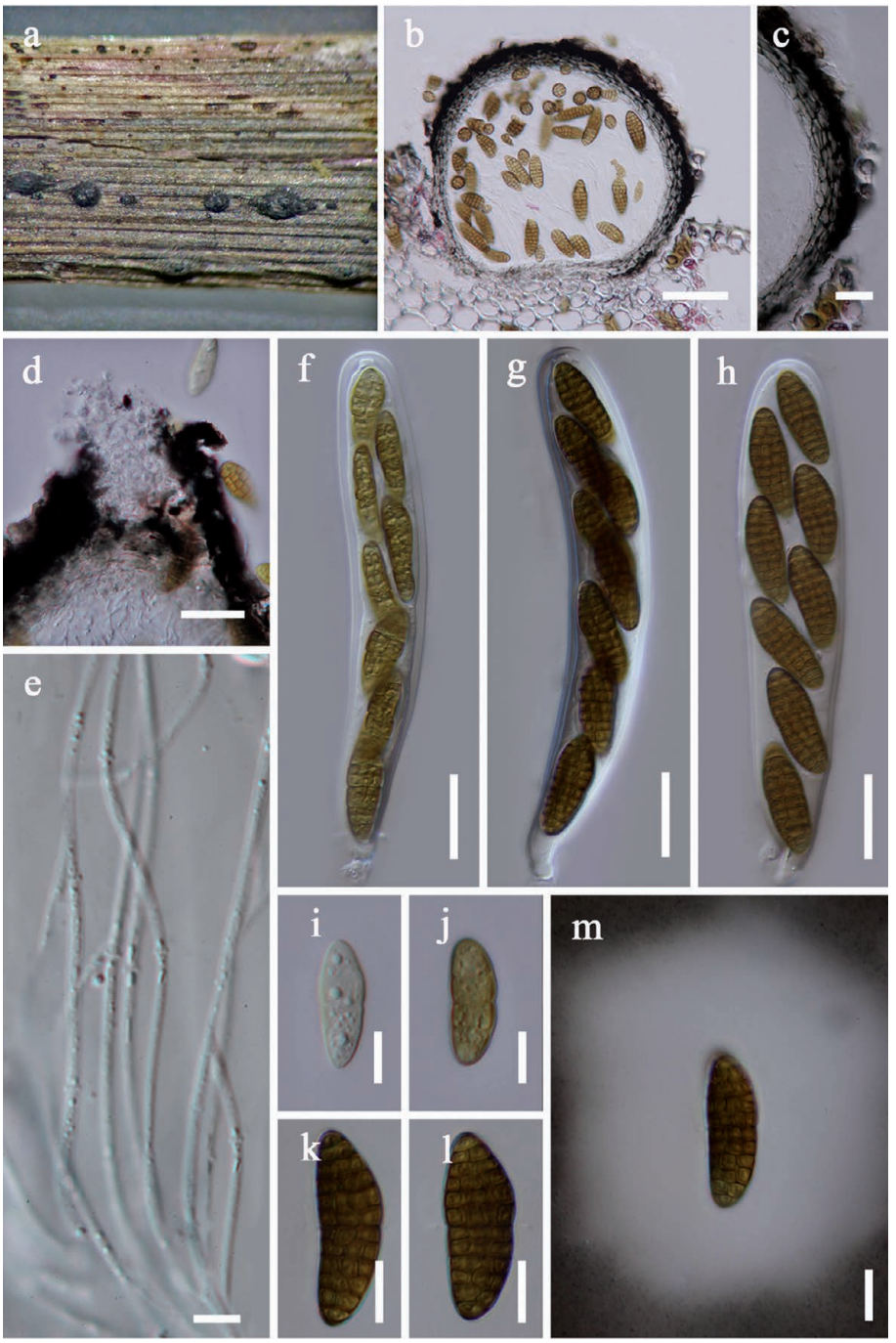

Fig 4. ***Murispora cardui*** (**holotype**). **a.** Ascomata on host substrate **b.** Section of ascoma **c.** Peridium **d.** Close up of ostiole **e.** Pseudoparaphyses **f-h.** Asci **i-m.** Ascospores (Note the sheath in m). Scale bars: b = 50 µm, c, d = 20 µm, e = 5 µm, f-h = 20 µm, i–m = 10 µm.

comprising numerous, 1-1.5 µm (n = 30) wide, narrow, filamentous, branched, septate, pseudoparaphyses. *Asci* 150-180 × 25-35 µm ($\overline{x}$ = 169.1 × 29.7 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 30-35 × 11-14 µm ($\overline{x}$ = 33.6 × 12.3 µm, n = 50), overlapping 1-2-seriate, hyaline when young, becoming dark brown at maturity, ellipsoidal to curved-fusoid, asymmetrical with one sides flattened, muriform, with 1-3 longitudinal septa in all cells and rarely in end cells, slightly constricted at the middle septum, conical and narrowly rounded at the ends, surrounded by a wide mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA slow growing, reaching 2 cm diam. after 3 weeks at 16ºC, light pink when young, reddish brown at maturity, flat on the surface, with mycelium composed of septate, branched hyphae.

Known distribution: On dead and upright stems of *Carduus* sp. (Asteraceae), Italy.

Material examined: Italy, Trento [TN], Vermiglio, dead and upright stems of *Carduus* sp., 03 Aug 2013, *E. Camporesi* (MFLU 15-2248, holotype; isotype BBH 39894; ex-type living culture, MFLUCC 13-0761.

Gene sequence data: ITS (KT736082), LSU (KT709176), SSU (KT709183), TEF (KT7091890).

Murispora medicaginicola Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde, *sp. nov.* **Fig. 5**

Facesoffungi Number: FoF01107 ;

Index Fungorum number: IF551559

Etymology: Name reflects the host genus *Medicago*, from which the species was isolated.

Holotype: 15-2249

Saprobic on dead herbaceous branches in terrestrial habitats. **Sexual morph**: *Ascomata* 220-280 µm high 150-250 µm diam. ($\overline{x}$ = 244.6 × 213.3 µm, n = 10), globose to subglobose, solitary, dark brown to black, immersed, substrate stained purple, fused to the host tissue, ostiolate. *Ostiole* 90-110 µm high 60-80 µm diam. ($\overline{x}$= 100.6 × 68.5 µm, n = 5) papillate, black, smooth, ostiolar canal filled with sparse periphyses that curve upwards. *Peridium* 10-18 µm wide at the base, 12-20 µm wide in sides, comprising 3-4 layers of brown to reddish brown cells of *textura angularis*. *Hamathecium* comprising numerous, 1.5-3 µm (n = 30) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 140-170 × 22-24 µm ($\overline{x}$ = 145.5 × 21.4 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 28-32 × 10-15 µm ($\overline{x}$ = 29.6 × 11.2 µm, n = 50), overlapping 1-2-seriate, hyaline when young, becoming dark brown at maturity, ellipsoidal to curved-fusoid, assymetrical with one sides flattened, muriform, with 2-3 longitudinal septa in all cells and rarely in end cells, slightly constricted at the middle septum, conical and narrowly rounded at the ends, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA slow growing, reaching 2 cm diam. after 3 weeks at 16ºC, light pink when young, reddish brown at maturity, flat on the surface, with mycelium composed of septate, branched hyphae.

Known distribution: On dead and upright stems of *Medicago* sp. (Fabaceae), Italy.

Material examined: Italy, Trento [TN], Val di Sole, Passo del Tonale, dead and upright stems of *Medicago* sp., 5 August 2013, *E. Camporesi* (MFLU 15-2249,

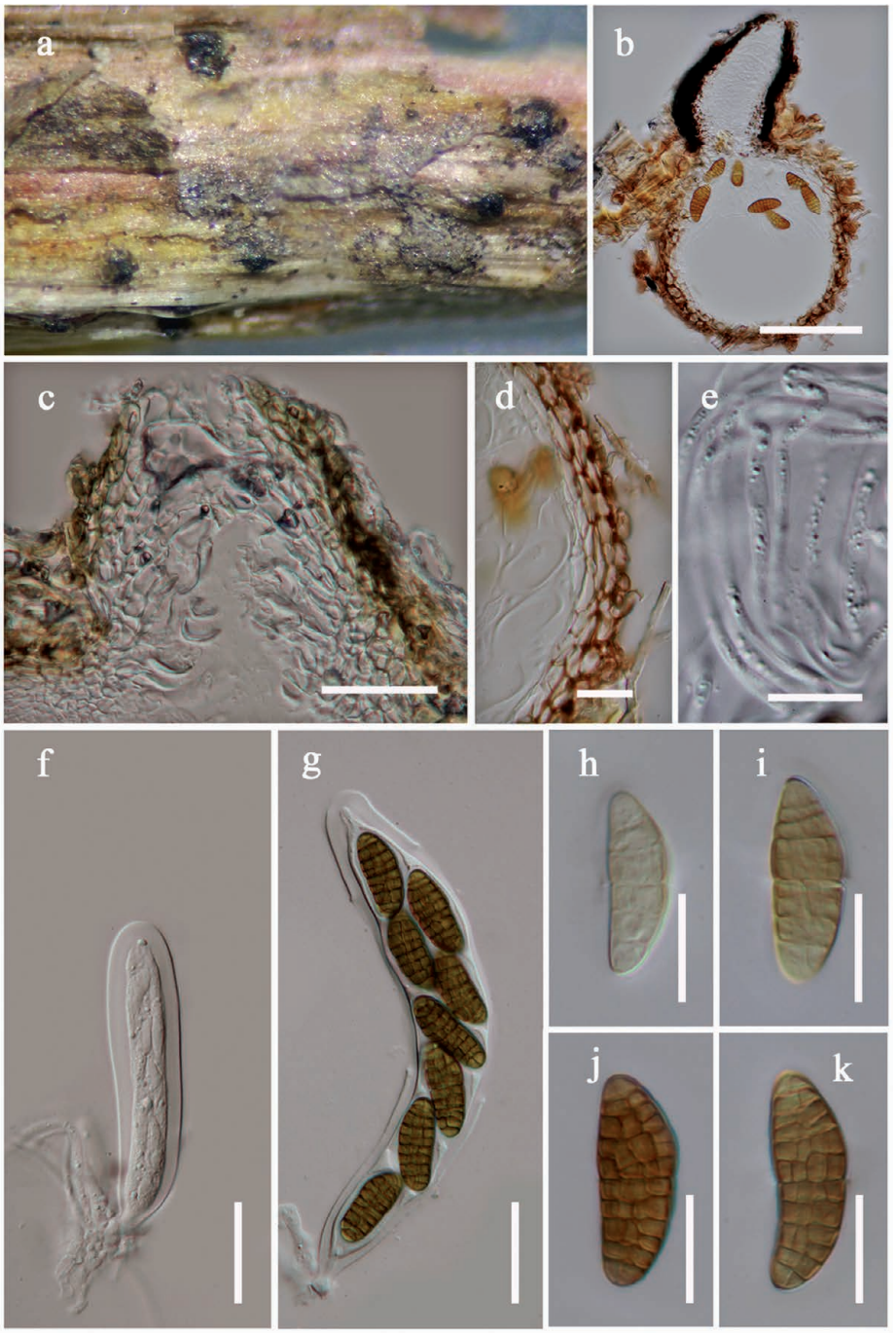

Fig 5. ***Murispora medicaginicola*** (**holotype**). **a.** Ascomata on host substrate **b.** Section of ascoma **c.** Pseudoparaphyses **d.** Close up of ostiole **e.** Peridium **f-h.** Asci **i-m.** Ascospores. Scale bars: b = 50 µm, c, e = 10 µm, d, f-h = 20 µm, i-m = 10 µm.

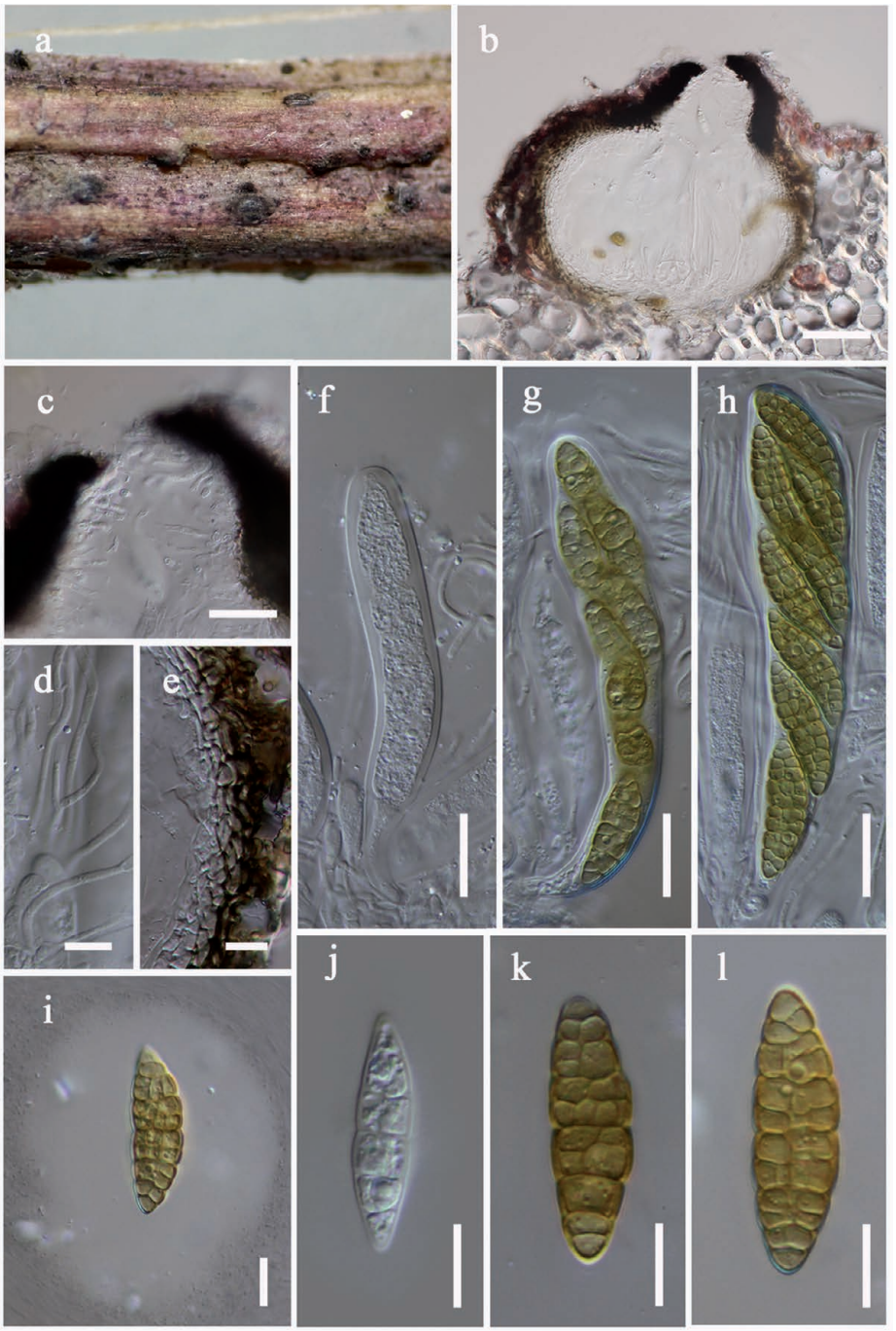

Fig 6. ***Murispora cicognanii*** (**holotype**). **a.** Ascomata on host substrate **b.** Section of ascoma **c.** Pseudoparaphyses **d.** Close up of ostiole **e.** Peridium **f-h.** Asci **i-l.** Ascospores (Note the sheath in i). Scale bars: b = 50 µm, c, e = 10 µm, d, f-h = 20 µm, i-l = 10 µm.

holotype (**isotype** in BBH, under the code of BBH 39895), ex-type living culture, MFLUCC 13-0762.

Gene sequence data : ITS (KT736083), LSU (KT709177), SSU (KT709184), TEF (KT7091891).

Murispora cicognanii Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde, *sp. nov.* **Fig. 6**

Facesoffungi Number: FoF01108 ;

Index Fungorum number: IF551560

Etymology: Named after Antonio Cicognani, a departed Italian mycologist and the founder of the A.M.B. Gruppo Micologico Forlivese.

Holotype: MFLU 15-2250

Saprobic on dead herbaceous branches in terrestrial habitats. **Sexual morph**: *Ascomata* 190-275 µm high 150-250 µm diam. ($\bar{x}$ = 232.4 × 212.7 µm, n = 10), globose to subglobose, solitary, dark brown to black, immersed, substrate stained purple, fused to the host tissue, ostiolate. *Ostiole* 50-65 µm high 30-60 µm diam. ($\bar{x}$= 57.8 × 50.2 µm, n = 5) short to papillate, black, smooth, ostiolar canal filled with periphyses-like structures. *Peridium* 9-14 µm wide at the base, 15-20 µm wide at the sides, comprising 3-4 layers of brown to reddish brown cells *textura angularis*, with inner 1-2 layers of cells thin–walled and hyaline. *Hamathecium* comprising numerous, 1-2 µm (n = 30) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 120-135 × 18-23 µm ($\bar{x}$ = 129.9 × 20.9 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 30-35 × 10-12 µm ($\bar{x}$ = 34.4 × 10.7 µm, n = 50), overlapping 1-2-seriate, golden yellow turning brown when mature, fusiform, assymetrical with one sides flattened, muriform, with 1-2 longitudinal septa in all cells and rarely in end cells, slightly constricted at the middle septum, conical and narrowly rounded at the ends, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA slow growing, reaching 2 cm diam. after 3 weeks at 16ºC, light pink when young, reddish brown at maturity, flat on the surface, with mycelium composed of septate, branched hyphae.

Known distribution: On dead branches of *Clematis* sp. (Ranunculaceae), Italy.

Material examined: Italy, Forlì-Cesena, Bagno di Romagna, Valgianna, dead and hanging branches of *Clematis vitalba*, 2 February 2014, *E. Camporesi* (MFLU 15-2250, holotype; isotype BBH 39896; ex-type living culture, MFLUCC 14-0953).

Gene sequence data: ITS (KT736084), LSU (KT709178), SSU (KT709185).

Murispora hawksworthii Wanasinghe, E.B.G. Jones & K.D. Hyde, ***sp. nov.*** **Figs 7-8**

Facesoffungi Number: FoF01109 ;

Index Fungorum number: IF551561

Etymology: In honor of David Leslie Hawksworth, to celebrate his 70th birthday and his immense contribution to mycology.

Holotype: MFLU 15-2251

Saprobic on dead herbaceous branches of terrestrial habitats. **Sexual morph**: *Ascomata* 250-310 µm high 320-380 µm diam. ($\bar{x}$ = 271.2 × 346.9 µm, n = 10), globose to subglobose, solitary, dark brown to black, superficial, substrate stained purple, fused to the host tissue, ostiolate. *Ostiole* 70-90 µm high 35-50 µm diam. ($\bar{x}$= 83.3 × 42.4 µm, n = 5), short to papillate, black, smooth, opening to

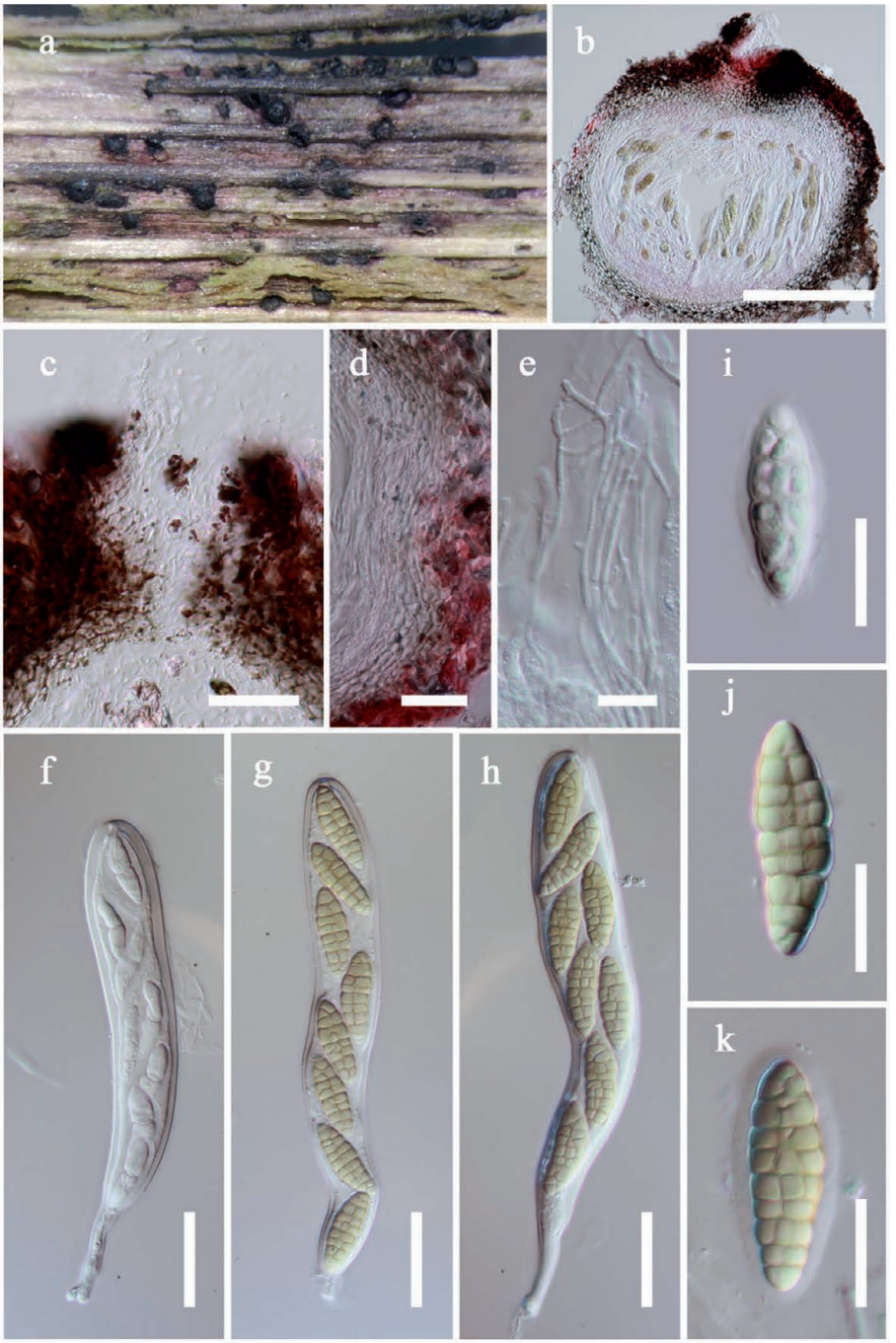

Fig 7. ***Murispora hawksworthii*** (**holotype**). **a.** Ascomata on host substrate **b.** Section of ascoma **c.** Pseudoparaphyses **d.** Close up of ostiole **e.** Peridium **f-h.** Asci **i-k.** Ascospores (Note the sheath in k). Scale bars: b = 50 µm, c, e = 10 µm, d, f-h = 20 µm, i-k = 10 µm.

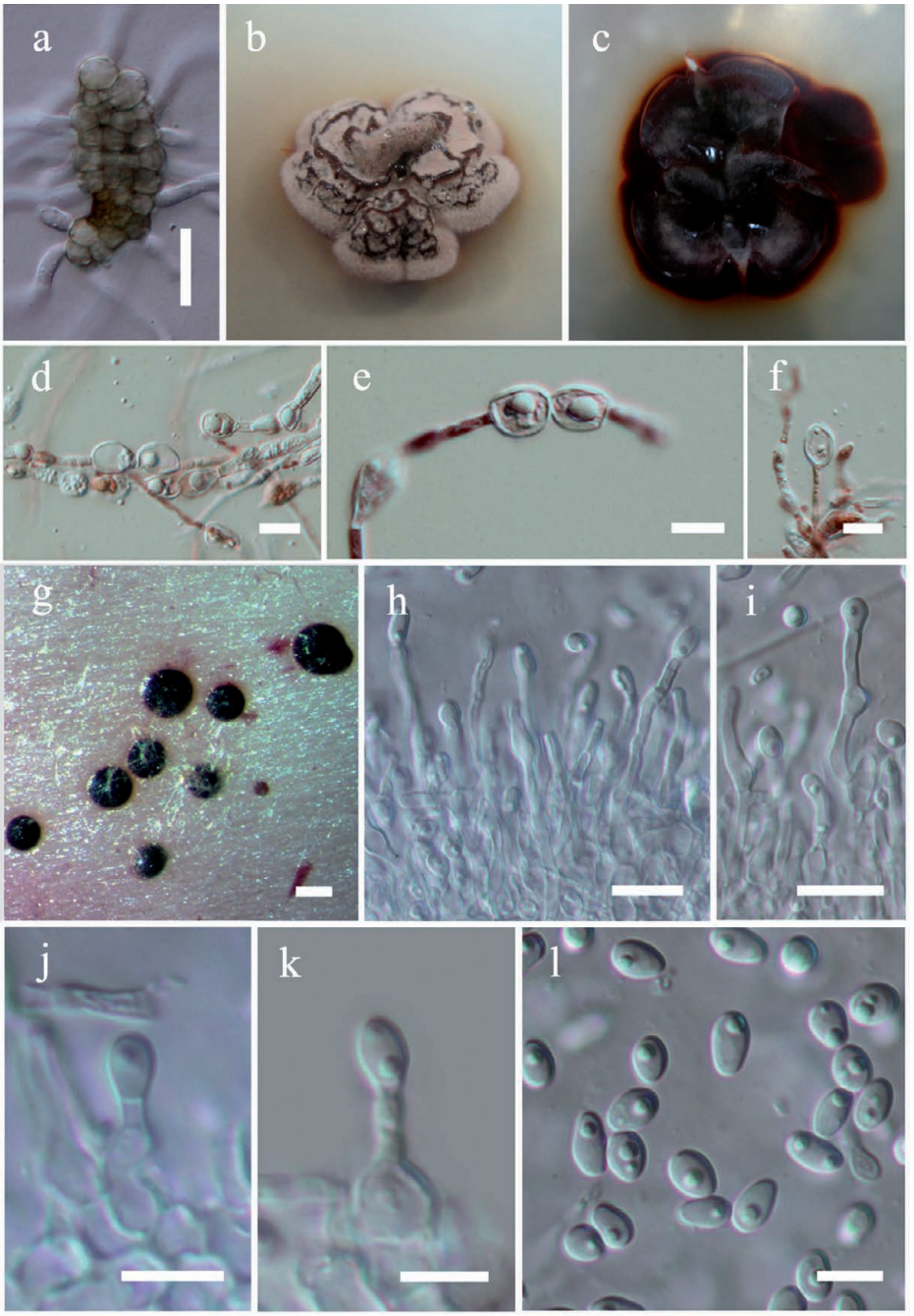

Fig 8. ***Murispora hawksworthii*** **Asexual state (holotype)**. **a.** Germinating ascospore. **b, c.** Colonies on PDA (c from below). **d.** Chlamydospores Intercalary chlamydospores. **f.** Terminal chlamydospores **g.** Close up of conidioma. **h-k.** Immature and mature conidia attached to conidiogenous cell. **l.** Conidia. Scale bars: a = 20 µm, d-f, h,i = 10 µm, g = 200 µm, j-l = 5 µm.

exterior through host surface. *Peridium* 15-25 µm wide at the base, 30-40 µm wide in sides, comprising 3-4 layers of dark brown to black cells of *textura angulari*s, with inner 1-2 layers of cells thin–walled and hyaline. *Hamathecium* comprising numerous, 1.5-2.5 µm (n = 30) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 150-200 × 20-28 µm ($\bar{x}$ = 173.1 × 22.4 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* 25-35 × 8-12 µm ($\bar{x}$ = 31.6 × 10.4 µm, n = 50), overlapping 1-2-seriate, hyaline when young, becoming pale brown at maturity, ellipsoidal to fusoid, asymmetrical with one sides flattened, muriform, with 1-2 longitudinal septa in all cells except end cells, constricted at the septa, conical and narrowly rounded at the ends, guttulate, with rugged surface, surrounded by a mucilaginous sheath. **Asexual morph**: Coelomycetous, formed in culture. *Conidiomata* 1.5-2 mm diam. pycnidial, solitary, dark brown to black, mainly immersed. Pycnidial wall reddish brown cells of *textura angularis*, with inner most layer thin, hyaline. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth, formed from the inner most layer of pycnidium wall. *Conidia* (2.5-3.5) × (1.5-2) µm ($\bar{x}$ = 3.0 × 1.7 µm, n = 50), hyaline, aseptate, guttulate, straight to curved, thin-walled, ellipsoidal.

Culture characteristics: Colonies on *PDA*: slow growing, reaching 2 cm diam. after 3 weeks at 16ºC, light pink when young, reddish brown at maturity, flat on the surface, with mycelium composed of septate, branched hyphae producing intermediary and terminal chlamydospores. *Chlamydospores* terminal or intercalary, thick and smooth-walled, globose, formed in abundance. Sporulation after 8 weeks.

Known distribution: On dead Umbeliferous stem, United Kingdom.

Material examined: UK, WALES, Dale, on dead Umbeliferous stem, 16 June 2014, *E.B.G. Jones* (MFLU 15-2251, holotype; isotype BBH 39897; ex-type living culture, MFLUCC 14-0918).

Gene sequence data: ITS (KT736086), LSU (KT709180), SSU (KT709187), TEF (KT7091892).

KEY TO SPECIES OF *MURISPORA*

1. Ascomata immersed to semi-immersed.....2
1. Ascomata superficial.....4
   2. Ascospores with less than 7 transverse septa..... ***M. fagicola***
   2. Ascospores with more than 7 transverse septa.....3
3. Asci shorter than 140 µm, ascospores with more than two longitudinal septa in all cells except end cells.....***M. rubicunda***
3. Asci longer than 140 µm, ascospores with less than two longitudinal septa in all cells except end cells.....***M. medicaginicola***
   4. Ascospores with less than 10 transverse septa.....***M. hawksworthii***
   4. Ascospores with more than 10 transverse septa.....5
5. Asci longer than 150 µm, ascospores dark brown.....6
5. Asci shorter than 150 µm, ascospores golden yellowish..... ***M. cicognanii***
   6. Pseudoparaphyses width more than 1.5 µm ..... ***M. cardui***
   6. Pseudoparaphyses width less than 1.5 µm ..... ***M. galii***

Table 3. Synopsis of *Murispora* species discussed in this study

Species	*Size*						*Septation*		*Hosts*
	Ascomata (diam)	*Asci*	*Ascospores*	*Pseudo-paraphyses*	*Peridium*				
					Apex	*Sides*	*Transverse septa*	*Longitudinal septa*	
M. fagicola	180-280 (immersed)	140-190 × 20-30	28-35 × 13-17 (symmetrical)	1.5-2	10-20	15-25	6–7	1-2 longitudinal septa in all cells except end cells	*Fagus sylvatica*
M. galii	250-310 (erumpent to nearly superficial)	150-200 × 20-30	35-40 × 10-15 (assymetrical)	1.5-2.5	12-18	15-25	10-11	2-3 longitudinal septa in all cells except end cells	*Galium* sp.
M. cardui	200-300 (erumpent to nearly superficial)	150-180 × 25-35	30-35 × 11-14 (assymetrical)	1-1.5	7-11	15-25	10-11	1-3 longitudinal septa in all cells except end cells	*Carduus* sp.
M. medicaginicola	150-250 (immersed)	140-170 × 22-24	28-32 × 10-15 (assymetrical)	1.5-3	10-18	12-20	8-11	2-3 longitudinal septa in all cells except end cells	*Medicago* sp.
M. cicognanii	150-250 (erumpent to nearly superficial)	120-135 × 18-23	30-35 × 10-12 (assymetrical)	1-2	9-14	15-20	10-11	1-2 longitudinal septa in all cells except end cells	*Clematis* sp.
M. hawksworthii	320-380 (superficial)	150-200 × 20-28	25-35 × 8-12 (assymetrical)	1.5-2.5	15-25	30-40	8-9	1-2 longitudinal septa in all cells except end cells	Unknown plant species

DISCUSSION

Murispora is based on *Pleospora rubicunda* which is characterized by immersed, erumpent or nearly superficial, globose to subglobose, elongate, weakly papillate ascomata, which stain the woody substrate purple, filamentous, narrow, branched, septate, pseudoparaphyses, 8-spored, bitunicate, cylindro-clavate asci, and oval to ellipsoidal or fusiform, pale or reddish brown, asymmetrical, muriform ascospores, with one side flattened (Zhang *et al.* 2009b, 2012, this study).

Webster (1957) provided a comprehensive account of *Pleospora rubicunda* and introduced two species (*P. straminis* Sacc. & Speg. and *P. rubelloides* (Plowr. ex Cooke) J. Webster), which were similar to *P. rubicunda*. All species produced purple staining on the host substrate and share comparable morphologies, and are distinct only in spore size and number of transverse septa. Nevertheless their spores are similar to *Pleospora*, which can also stain the wood purple. The purple staining of wood is also found in some *Leptosphaeria* species, *Ophiobolus rubellus* (Pers.) Sacc. and *Lophiotrema* species and this has resulted in some confusion in the nomenclature of these taxa (Webster 1957). Zhang *et al*. (2009c) introduced Amniculicolaceae to accommodate *Amniculicola* together with *P. rubicunda*, *Neomassariosphaeria grandispora* and *N. typhicola* whose ascomata usually stain the woody substrate purple. In view of the fact that *Pleospora rubicunda* clustered in *Amniculicolaceae*, Zhang *et al*. (2009c) introduced a new genus *Murispora* for *P. rubicunda*.

In our combined gene analyses of Pleosporales (Fig. 1), taxa from the family Amniculicolaceae formed a distinct clade with high bootstrap support (100% in ML and MP analyses) and a high PP value (1.00 in the Bayesian analysis). *Amniculicola lignicola* Y. Zhang ter & K.D. Hyde, *A. immersa* Y. Zhang ter *et al. et al.*, *A. parva* Y. Zhang ter *et al. et al.*, *Anguillospora longissima* (Sacc. & P. Syd.) Ingold, *Massariosphaeria grandispora* (Sacc.) Leuchtm., *Pseudomassariosphaeria bromicola* Phukhamsakda *et al.*, *Repetophragma ontariense* (Matsush.) W.P. Wu and *Spirosphaera cupreorufescens* Voglmayr grouped in the Amniculicolaceae clade and the type species of the family (*A. lignicola*) is included in the analyses ; thus we confirm their family placement in Amniculicolaceae. Our *Murispora* species also grouped in a separate clade (Fig. 1), with *Murispora rubicunda,* having strong support in the phylogenetic analysis (97% in ML analysis, 93 in MP analysis and 0.99 for Bayesian analysis).

The asexual morphs of Amniculicolaceae are poorly known. Phylogenies indicate that the three *Amniculicola* species cluster together with the putatively named asexual species *Anguillospora longissima*, *Spirosphaera cupreorufescens* and *Repetophragma ontariense* (Zhang *et al*. 2009b; Seifert *et al*. 2011; Hyde *et al*. 2013). Our phylogenetic analysis also support this (90% ML support). However, the paraphyletic nature of the genus *Amniculicola* is highlighted as species clustered in three different sister clades (Fig 1).

Repetophragma Subram. is characterized by macronematous conidiophores with several annellations which are produced by a few, or numerous, enteroblastic, percurrent proliferations of the conidiogenous cells, and euseptate conidia with a conicotruncate basal cell, which secedes schizolytically (Castañeda Ruiz *et al.* 2011). Shenoy *et al.* (2006) demonstrated that some *Repetophragma* species were clearly polyphyletic; as they cluster in different families and orders of Sordariomycetes and Dothideomycetes. *Spirosphaera* Beverw. (Helotiales) Leotiomycetes and *S. cupreorufescens* have features considered typical of the genus, including a spirally coiled, interwoven conidial filament, the cells of which give rise to one daughter

filament, which is also coiled and interwoven, resulting in a large, irregular, globose conidium (Hennebert 1998). The main distinctive feature of *S. cupreorufescens* is the conspicuous copper brown conidia, which are rather irregular and loose (Voglmayr 2004).

The sexual morph of *Anguillospora longissima* has been mentioned as an undescribed species of 'Massarina' (Willoughby and Archer 1973; Sivanesan 1984; Webster 1992), and agrees with the diagnostic characters of *Amniculicola* (Zhang *et al.* 2008, 2009b). The characters are typical of *Amniculicola parva,* and therefore, the sexual morph of *Anguillospora longissima* may be related to *A. parva* (Hyde *et al*. 2013).

In this study we introduce six novel species in to the genus *Murispora* and report for the first time a phoma-like (*M. hawksworthii*) asexual morph for this genus.

A combination of morphology and phylogeny has become crucial factors when describing a novel taxon. However, recent studies have made use of chemotaxonomy, and this may soon become a mandatory additional criterion for a complete description of micro fungi, especially xylariaceous taxa (Hellwig *et al* 2005; Stadler and Hellwig 2005; Bitzer *et al.* 2008, Kuhnert *et al* 2015). Chemotaxonomy may be important for Amniculicolaceae as the fruiting bodies of *Amniculicola immersa*, *A. lignicola*, *A. parva* and *Murispora rubicunda* stain the substrate purple. The phylogenetic significance of the purple staining should be further investigated.

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