

Available online at www.sciencedirect.com**MYCOSCIENCE**

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc**Short communication****Misturatosphaeria mariae sp. nov. from France, a first record of Misturatosphaeria in Europe**Ying Zhang^{a,b,*}, Jacques Fournier^c, Ali H. Bahkali^d, Kevin D. Hyde^{b,e}^a Institute of Microbiology, P.O. Box 61, Beijing Forestry University, Beijing 100083, PR China^b Institute for Excellence in Fungal Research and School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand^c Las Muros, Rimont, Ariège F 09420, France^d Botany and Microbiology Department, College of Science, King Saud University, Riyadh 11442, Saudi Arabia^e International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, Yunnan 650034, PR China

ARTICLE INFO

Article history:

Received 7 November 2011

Received in revised form

29 April 2012

Accepted 4 June 2012

Available online 20 December 2012

Keywords:

Dictyosporous

Lignicolous

Phylogeny

Pleosporales

ABSTRACT

Misturatosphaeria mariae is described as new from a *Robinia pseudoacacia* post in southern France. Relationships with other species of *Misturatosphaeria* are discussed based on morphology and 28S nrDNA sequence comparisons. The geographic distribution of *Misturatosphaeria* species is also given and discussed and a key to species is provided.

© 2012 The Mycological Society of Japan. Published by Elsevier B.V. All rights reserved.

Misturatosphaeria Mugambi & Huhndorf is a monophyletic genus, which is characterized by its erumpent to superficial ascomata which are solitary or aggregated in clusters, with or without a raised papilla and have a light coloured ostiolar area. Pseudoparaphyses are septate, branched and anastomose between and above the asci. Asci are numerous, 8-spored, cylindrical or clavate, with short pedicels. Ascospores are brown or hyaline, phragmosporous or dictyosporous with external roughened or smooth wall, and with or without a gelatinous sheath (Mugambi and Huhndorf 2009). Eight species of *Misturatosphaeria* have been reported (Mugambi and Huhndorf 2009). Despite their monophyletic status and similarity in morphology, species of

Misturatosphaeria have been reported from various geographic regions, i.e. Costa Rica, Ecuador, French Guiana, Kenya, and USA (Illinois, North Carolina, Tennessee). During our study of the lignicolous fungi in southern France, several new genera and species have been identified and described (Zhang et al. 2011, 2012). In this paper we report on a new *Misturatosphaeria* species with dictyosporous ascospores from the same region.

A fresh specimen was collected in December 2006 in southern France (Haute Garonne) by M. Delpont, which was processed and examined following the method described in Zhang et al. (2009). Observations and photographs were prepared from material mounted in water, and measurements followed the protocols

* Corresponding author. Tel.: +66 8 41518136; fax: +66 8 53916029.

E-mail address: yinghku@gmail.com (Y. Zhang).

1340-3540/\$ – see front matter © 2012 The Mycological Society of Japan. Published by Elsevier B.V. All rights reserved.

<http://dx.doi.org/10.1016/j.myc.2012.09.003>

previously outlined in Zhang et al. (2011, 2012) and Liu et al. (2011).

The ex-type culture of *M. mariae* (CBS 124079) was grown on potato-dextrose agar (PDA) and malt extract agar (MEA) and total genomic DNA was extracted from mycelia following the protocols as outlined by Zhang et al. (2009). nrDNA sequences were used to study the phylogenetic relationships among species of *Misturatosphaeria*. Because the phylogenetic relationships among *Misturatosphaeria* and other pleosporalean genera/families had been well documented based on molecular and morphological data by Mugambi and Huhndorf (2009), only *Misturatosphaeria* species and out group taxa, *Westerdykella cylindrica* and *W. ornata*, were included in our phylogenetic study (Table 1).

DNA amplification and sequencing follow the protocol used by Zhang et al. (2009). Sequences generated from LROR and LR5 were analyzed with other sequences obtained from the GenBank. Multiple alignments were conducted in Mega 5 (Tamura et al. 2011) and analyses were performed in Mega 5 and MrBayes 3.1.2. Out-group taxa were *W. cylindrical* and *W. ornata*. Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. The evolutionary history was inferred using the Maximum Parsimony (MP) method. Only percentage of replicate trees over 50 in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown above the branches (Felsenstein 1985). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar 2000) with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). Eighteen nucleotide sequences were analyzed. All positions containing gaps and missing data were eliminated. Bayesian phylogenetic analysis was performed in MrBayes 3.1.2 using a uniform GRT+I+G model, as selected by hLRT in MrModeltest 2.3. The Metropolis-coupled Markov Chain Monte Carlo (MCMC) approach was used to calculate

posterior probabilities. Chains were analyzed with random starting trees for 1,000,000 generations. Trees collected before the stable likelihood value points were discarded as “burn-in”. Values of the Bayesian posterior probabilities (PP) equal to or above 70% from MCMC analysis were shown under branches. Trees were viewed in Treeview (Page 1996).

There were a total of 791 positions of DNA base pairs in the final dataset. For Maximum Parsimony analysis, the consistency index was 0.574803, the retention index was 0.693182, and the composite index was 0.474282 (0.398443) for all sites and parsimony-informative sites (in parentheses). All *Misturatosphaeria* species form a well supported clade, and *M. mariae* nested within. MrBayes analysis supports *M. mariae* as being closely related to *M. cruciformis* and *M. tennesseensis*, but this is not supported by MP analysis (Fig. 2).

Misturatosphaeria mariae Y. Zhang, J. Fourn. & K.D. Hyde, sp. nov. Fig. 1

Mycobank no.: MB 563452

Ascomata medium-sized, solitary, scattered, or in small groups, semi-immersed to nearly superficial, globose to

Table 1 – Taxa used in the phylogenetic analysis and their corresponding GenBank accession numbers (the sequence generated in this study is in bold).

Species name	Strain number	GenBank accession
<i>Misturatosphaeria aurantonotata</i>	GKM 1238	GU385173
<i>M. aurantonotata</i>	GKM 1280	GU385174
<i>M. mariae</i>	CBS 124079	JN851819
<i>M. claviformis</i>	GKM 1210	GU385212
<i>M. cruciformis</i>	SMH 5151	GU385211
<i>M. kenyensis</i>	GKM 234N	GU385188
<i>M. kenyensis</i>	GKM L100Na	GU385189
<i>M. kenyensis</i>	GKM 1195	GU385194
<i>M. minima</i>	GKM 169N	GU385165
<i>M. minima</i>	ANM 60	GU385182
<i>M. minima</i>	ANM 933	GU385195
<i>M. minima</i>	SMH 2448	GU385166
<i>Misturatosphaeria</i> sp.	SMH 3747	GU385196
<i>M. tennesseensis</i>	ANM 911	GU385207
<i>M. uniseptata</i>	SMH 4330	GU385167
<i>M. uniseriata</i>	ANM 909	GU385206
<i>Westerdykella cylindrica</i>	CBS 454.72	AY004343
<i>W. ornata</i>	CBS 379.55	GU301880

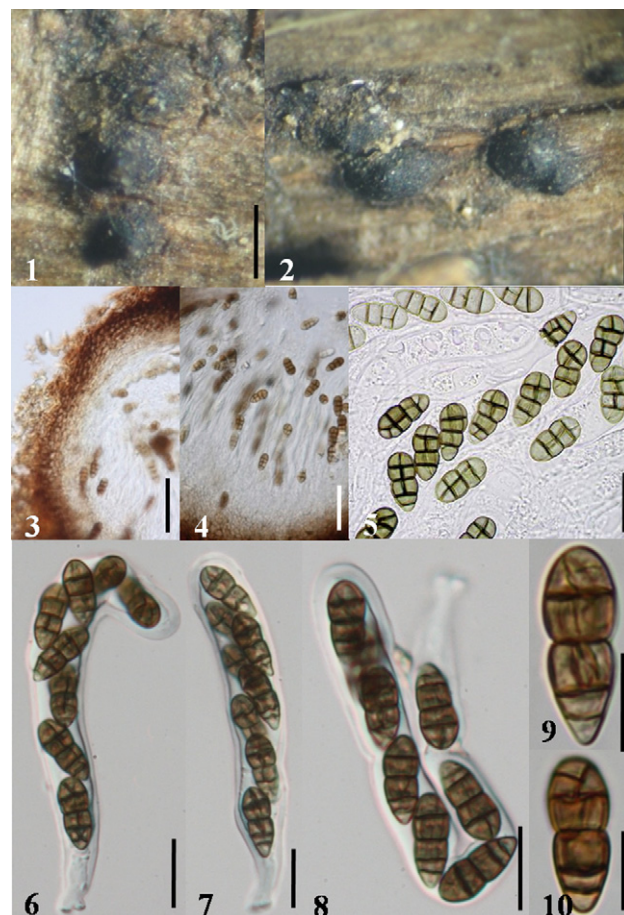


Fig. 1 – *Misturatosphaeria mariae* (holotype). (1, 2) Semi-immersed ascostroma with or without protruding papilla on the host surface; (3) section of a partial peridium comprising two types of cells; (4, 5) center of the ascomata. Note the numerous pseudoparaphyses; (6–8), cylindrical to cylindro-clavate asci with short furcate pedicel; (9, 10) released muriform ascospores. Bars: (1, 2) 0.5 mm; (3, 4) 50 μ m; (5–8) 20 μ m; (9, 10) 10 μ m.

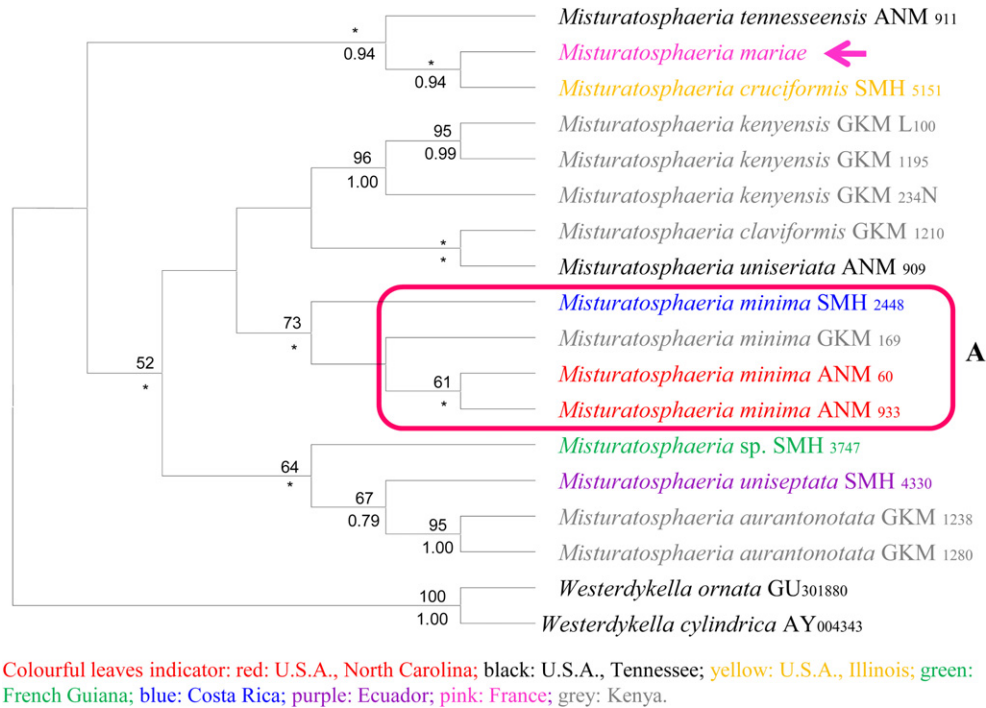


Fig. 2 – Phylogram of the Maximum Parsimony (MP) analyses generated from 28S nrDNA sequences. Bootstrap support values based on MP analysis $\geq 50\%$ are shown above the branches, and MrBayes analysis $\geq 70\%$ are shown below the branches. Indications of colourful leaves are listed at the bottom of the figure.

subglobose; apex sometimes with a protruding papilla (top area sometimes light coloured). *Peridium* 30–40 μm thick, comprising two cell types. *Hamathecium* of numerous pseudoparaphyses. Asci 8-spored, bitunicate, fissionate, cylindrical to cylindro-clavate. Ascospores broadly clavate, dictyosporous, asymmetrical, deeply constricted at the median septum.

Typus: FRANCE, Haute Garonne, Avignonet Lauragais, Canal du Midi, on submerged *Robinia pseudoacacia* post, 09 December 2006, leg M. Delpont, det J. Fournier (Holotypus, IFRD8990; ex-type culture, CBS 124079).

rDNA sequence ex holotype: JN851819.

Etymology: named after the gentle lady Mrs. Marie Fournier, the wife of Mr. Jacques Fournier.

Anamorph: Unknown.

Ascomata 360–500 μm diam., solitary, scattered, or in small groups, semi-immersed to nearly superficial, globose to subglobose, wall black; apex usually slightly conical, sometimes with a protruding papilla (top area sometimes light coloured), 60–130 μm high and 60–100 μm wide. *Peridium* 30–40 μm thick at sides, 70–100 μm thick at the apex, thinner at the base, comprising two cell types, outer layer composed of small heavily pigmented thick-walled compressed cells, cells 4 \times 8 μm diam., cell wall to 1.5 μm thick, inner layer thin, composed of smaller hyaline cells. *Hamathecium* of numerous pseudoparaphyses, septate, branching and anastomosing between and above the asci. Asci 95–120 \times 12.5–15 μm (\bar{x} = 102.5 \times 13 μm , n = 10), 8-spored, bitunicate, fissionate, cylindrical to cylindro-clavate, with a short furcate pedicel, 12–23 μm long. Ascospores 15–20 \times 7.5–8.8 μm (\bar{x} = 17.8 \times 7.6 μm , n = 10), obliquely uniseriate and partially overlapping, broadly clavate with hemispherical upper part

and somewhat tapering lower end, dark brown, dictyosporous with (3–) 4 transverse septa when mature, one trans-septum in the upper part and mostly two trans-septa in the lower part, the upper hemisphere shorter with an obtuse end, the lower part longer with narrowly rounded end, constricted at the primary median septum, 1 longitudinal septum in the two central cells, 1 oblique septum in upper end cell, external wall smooth.

Note: Although the post was in water, the absence of other aquatic ascomycetes and the external appearance of the wood strongly suggest it had not been submerged for a long period in water and thus should be considered a terrestrial species.

Misturatosphaeria is a cosmopolitan genus and has been reported in Africa, Europe, and South and North America. In the dendrogram (Fig. 2) *M. mariae* clustered with *M. cruciformis* Mugambi & Huhndorf and *M. tennesseensis* Mugambi, A.N. Mill. & Huhndorf with weak support using MrBayes analysis and with no support by MP analysis. Despite the wide ranging distribution of *Misturatosphaeria* members, *M. mariae* shares numerous morphological characters with *M. cruciformis*, such as erumpent to superficial ascomata with a protruding papilla, cylindro-clavate, 8-spored asci with short pedicels, and dictyosporous, brown ascospores (Mugambi and Huhndorf 2009). Ascospores in *M. mariae* are asymmetrical and deeply constricted at the median septum, and can be readily distinguished from those of *M. cruciformis* (Mugambi and Huhndorf 2009). Thus based on both morphology and molecular phylogenetic analysis, *M. mariae* is a well defined species of *Misturatosphaeria*. In particular, *Robinia pseudoacacia*, the host of *M. mariae*, is native to the southeastern United States, which probably indicates that *M. mariae* is not native to France either.

The Clade of *Misturatosphaeria minima* Mugambi, A.N. Mill. & Huhndorf (Clade A) received weak support from MP analysis, but no support by MrBayes analysis. Our results corroborate the study by Mugambi and Huhndorf (2009), in which they obtained MrBayes support of over 95%, while Maximum Likelihood support is 70. Strains of *M. minima* used in this study are from various locations, i.e. from Kenya, USA (North Carolina) and Costa Rica.

Morphologically, *Misturatosphaeria mariae* is comparable to *Teichospora quercina* (Ellis & Everh.) M.E. Barr, but the asymmetrical (with 3–4 transverse septa) ascospores of *M. mariae* can be readily distinguished from those of *T. quercina*. It is highly possible that some reported genera, such as *Teichospora* Fuckel or *Strickeria* Körb., may presently accommodate some species of *Misturatosphaeria*.

Key to the species of *Misturatosphaeria* (with information of related species referred to Mugambi and Huhndorf 2009).

- | | |
|---|-------------------------|
| 1a. Dictyosporous | 2 |
| 1b. Phragmosporous | 3 |
| 2a. Ascospore with 3(-4)-transverse septa, asymmetrical, median septum deeply constricted | <i>M. mariae</i> |
| 2b. Ascospore with 3(-4)-transverse septa, symmetrical, median septum not or slightly constricted | <i>M. claviformis</i> |
| 2c. Ascospore with 5-7-transverse septa, symmetrical, median septum slightly constricted | <i>M. cruciformis</i> |
| 3a. 1-septate, without sheath, brown | <i>M. uniseptata</i> |
| 3b. >1 septate (incl. 1-3-septate species), with or without sheath, brown or hyaline | 4 |
| 4a. Ascospores with 3-transverse septa, pale brown or brown, with or without sheath | 5 |
| 4b. Ascospores 1-3-transverse septa, hyaline, with sheath or terminal appendages | 6 |
| 5a. Ascospores without sheath | <i>M. tennesseensis</i> |
| 5b. Ascospores with sheath | <i>M. aurantonotata</i> |
| 6a. Ascomata solitary or in small groups of usually less than 5 individuals, ascospores 3–4 µm wide | <i>M. minima</i> |

- | | |
|---|---------------------|
| 6b. Ascomata aggregated in large clusters, ascospores 4–6 µm wide | <i>M. kenyensis</i> |
|---|---------------------|

Acknowledgements

M. Delpont is thanked for having collected and communicated the type material of *Misturatosphaeria mariae*.

REFERENCES

- Felsenstein J, 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Liu JK, Phookamsak R, Jones EBG, Zhang Y, Ko-Ko TW, Hu HL, Boonmee S, Bahkali AH, Chukeatirote E, Doilom M, Wang Y, Hyde KD, 2011. *Astrosphaeriella* is polyphyletic with species in *Fissuroma* gen. nov., and *Verrucospora* gen. nov. *Fungal Diversity* 51: 135–154.
- Mugambi GK, Huhndorf SM, 2009. Molecular phylogenetics of Pleosporales: Melanommataceae and Lophiostomataceae re-circumscribed (Pleosporomycetidae, Dothideomycetes, Ascomycota). *Studies in Mycology* 64: 103–121.
- Nei M, Kumar S, 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
- Zhang Y, Wang HK, Fournier J, Crous PW, Jeewon R, Pointing SB, Hyde KD, 2009. Towards a phylogenetic clarification of *Lophiostoma*/*Massarina* and morphologically similar genera in the Pleosporales. *Fungal Diversity* 38: 225–251.
- Zhang Y, Crous PW, Schoch CL, Bahkali HA, Guo LD, Hyde KD, 2011. A molecular, morphological and ecological re-appraisal of Venturiales—a new order of Dothideomycetes. *Fungal Diversity* 51: 249–277.
- Zhang Y, Crous PW, Schoch CL, Hyde KD, 2012. Pleosporales. *Fungal Diversity* 53: 1–221.