



Morphology and phylogeny of *Mycopepon*

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Abstract

Two species similar to *Mycopepon* were found on bamboo in Guizhou Province, China. These species are introduced as the new species *Mycopepon bambusae* *M. fusoidisporus*, based on their morphological characteristics and phylogenetic analyses of LSU and SSU sequences data in this paper. Descriptions and illustrations for both species are provided. *Mycopepon smithii* var. *mexicanum* is raised to specific rank as *M. mexicanus* stat. nov. *Mycopepon bambusae* differs from *M. smithii* and *M. mexicanus* by its narrower ascospores (45–61.5 × 6.5–8.5 μm). *Mycopepon fusoidisporus* has the unique features of 1-septate, dark brown, wider, ellipsoid-fusiform ascospores (45.5–57.5 × 11.5–23.5 μm). Based on a combined SSU and LSU sequence data, the phylogenetic results indicate that *Mycopepon* falls in Astrosphaeriellaceae.

Key words – 2 new species – Astrosphaeriellaceae – Dothideomycetes – Pleosporales – taxonomy

Introduction

The fungi on bamboo are a phylogenetically diverse group with many genera only known from this host (Hyde et al. 2002, Dai et al. 2016). Several of the genera are poorly known (e.g. *Linearistroma*, *Mycopepon*, *Shiraia*) and have rarely been collected (Boise 1987, Phookamsak et al. 2014, Dai et al. 2016). *Mycopepon* Boise was introduced by Boise (1987) with *M. guianensis* Boise (current name: *M. smithii* (Ellis & Everh.) Boise) as the type species and is only known from the American continent (Wijayawardene et al. 2017). The genus is characterized by superficial ascostromata with multiple ascomata with eccentric papillae that fuse into a common central papilla, giving the overall appearance of a pumpkin. *Mycopepon* has been recorded from Mexico, Nicaragua and South America in French Guiana (Boise 1987, 1994). Five epithets are listed in Index Fungorum (2018). However, only one name [*M. smithii* (Ellis & Everh.) Boise] is accepted in this genus (Index Fungorum 2018). Since *Mycopepon* lacks DNA sequence data, *Mycopepon* has been placed in Dothideomycetes genera *incertae sedis* (Boise 1987, Eriksson & Hawksworth 1987, Wijayawardene et al. 2014, 2015, 2018).

During an investigation of Xylariales in China, two mycopepon-like taxa were collected and examined. Based on morphology and molecular data, two new species, *M. bambusae* and *M. fusoidisporus*, were identified. On further study of the genus we proposed that the variety *mexicanum* of *Mycopepon smithi* should be raised to specific rank as *M. mexicanus*.

Materials & Methods

Isolates and morphology

Materials were collected from Guizhou Province in China. The fungal material were placed in plastic bags with some silica gel desiccant and brought to the laboratory. All collection details were noted and material was examined within two months. Morphological examination followed the methods from Su et al. (2016), Liu et al. (2018). Materials were mounted in water and Melzer's iodine reagent. The characteristics of ascostromata were examined by using a stereomicroscope, and were photographed by a digital camera (BX41, Olympus; Ni Nikon). Morphological characteristics of the asci and ascospores were photographed using a digital camera fitted with the compound light microscopy (Ni Nikon). All microphotographs were arranged using Adobe Photoshop CS6 and all measurements were made with Tarosoft ® image framework (v. 0.9.0.7). Single-spore isolations were carried out as described by Li et al. (2015). Cultures were grown in Petri-dishes on potato dextrose agar (PDA) at 20–25 °C. The herbarium and living cultures are deposited in Guizhou Medical University (GMB) and Kunming Institute of Botany (KUN), Chinese Academy of Sciences.

DNA extraction, PCR and sequencing

Total DNA products were extracted from fresh fungal mycelia by using a BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) (Wijayawardene et al. 2013). The amplification of the 28S large subunit (LSU) was carried out using LROR and LR5 (Vilgalys & Hester 1990) primers. Primer pairs NS1 and NS4 designed by White et al. (1990) were applied to amplify a region of the 18S small subunit rDNA. Primer pairs ITS5 and ITS4, were used to amplify ITS region (White et al. 1990). According to Su et al. (2016), the amplification conditions were carried out via PCR reactions in 25 µL volume (double distilled water 9.5 µL, PCR Master Mix 12.5 µL, 1 µL of each primer, 1 µL template DNA). Amplified PCR fragments were sequenced by Sino Geno Max, Beijing, China. The sequence data were deposited in GenBank.

Sequence alignment and phylogenetic analyses

All strains for phylogenetic analyses chosen based on published literature (Hyde et al. 2013, Phookamsak et al. 2015) were marked in RAxML tree (Fig. 1). LSU and SSU sequence data were assembled using the alignment program BioEdit (Hall 1999) and ClustalX (Kohli & Bachhawat 2003). Multiple sequence alignments were generated with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>). The alignments are uploaded in TreeBASE (www.treebase.org/treebase-web/home.html) under ID 22740 for LSU-SSU alignment. Alignment was manually improved to achieve the maximum alignment and to minimize gaps with BioEdit. The file formats were converted in CIPRES Science Gateway (<https://www.phylo.org/>). The maximum likelihood analyses were performed in RAxML 7.4.2 Black Box (<https://www.phylo.org/>, Stamatakis 2006, Stamatakis et al. 2008). The final ML search was conducted using the GTRGAMMA + I model. All free model parameters were estimated by RAxML with ML estimates of 25 per site rate categories. The MrModeltest 2.2 was used to perform the model of evolution (Nylander et al. 2008). The phylogenetic analyses were performed for Bayesian inference in MrBayes v. 3.2.1 (Ronquist et al. 2012). Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.2.2 (Ronquist et al. 2012) was used to determine the posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002). Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation. Phylogenetic trees were viewed and arranged using FigTree v1.4.0.

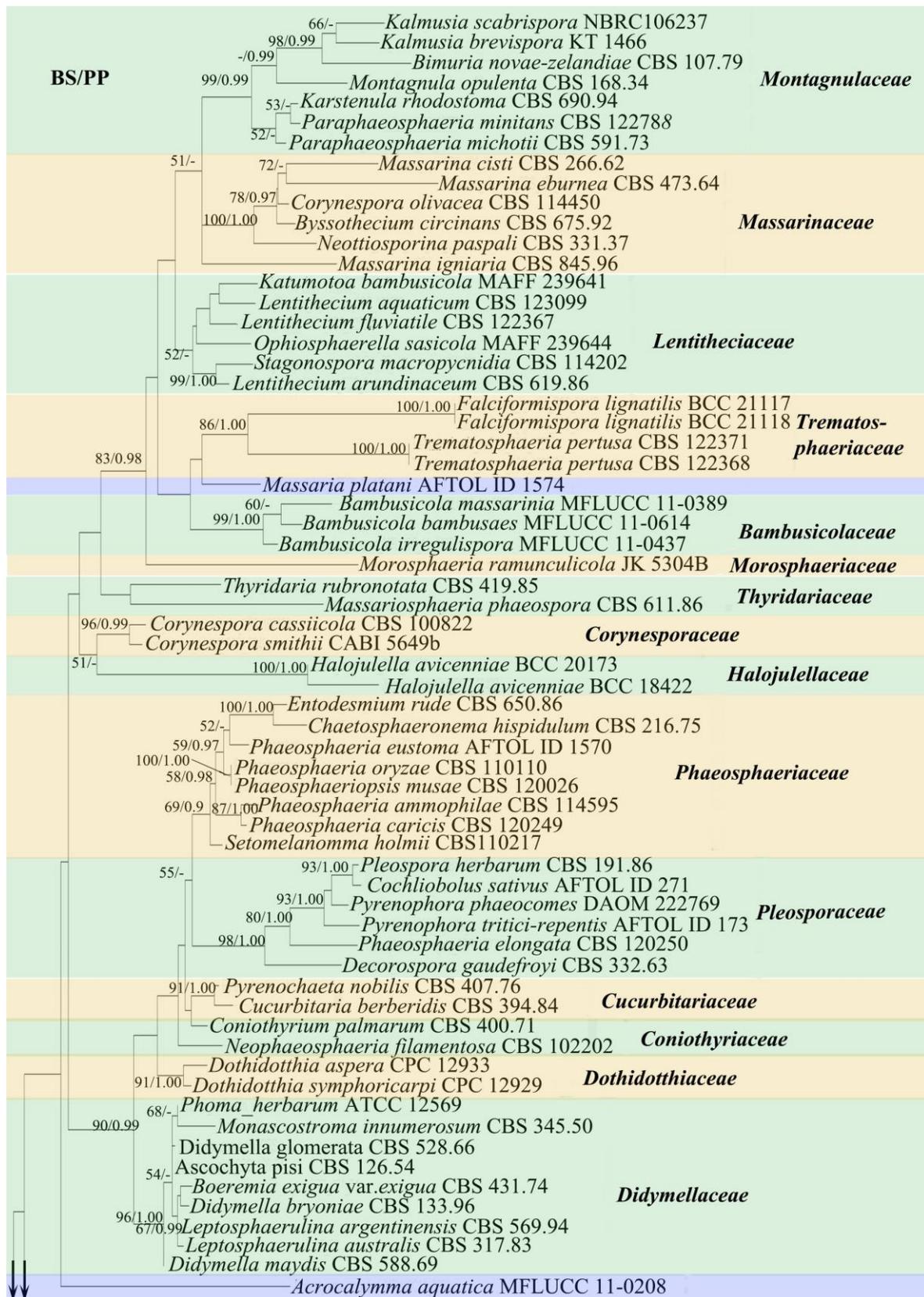


Figure 1 – RAxML tree based on analysis of a combined SSU and LSU nrDNA dataset. Bootstrap support values for maximum likelihood (ML) higher than 50% ML (left) and Bayesian posterior probabilities equal to, or higher than 0.95 PP (right) were marked above the nodes. The values below the nodes are Bayesian posterior probabilities above 0.95. Hyphen (“-”) means a value lower than 50% (BS) or 0.95 (PP). The strains numbers are noted after the species names. The tree was rooted to *Hysterium angustatum* (CBS 236.34, CBS 123334).

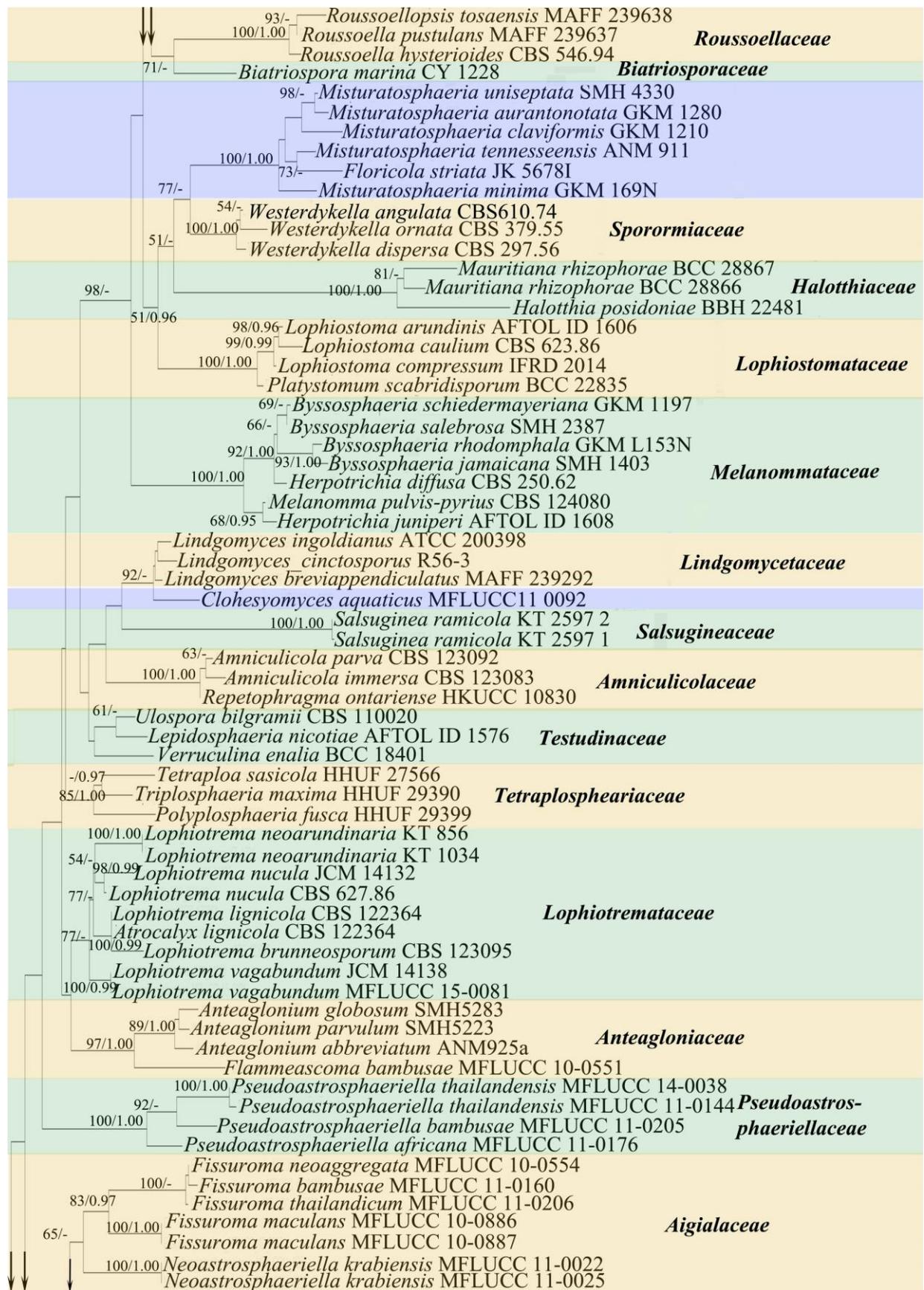


Figure 1 – Continued.

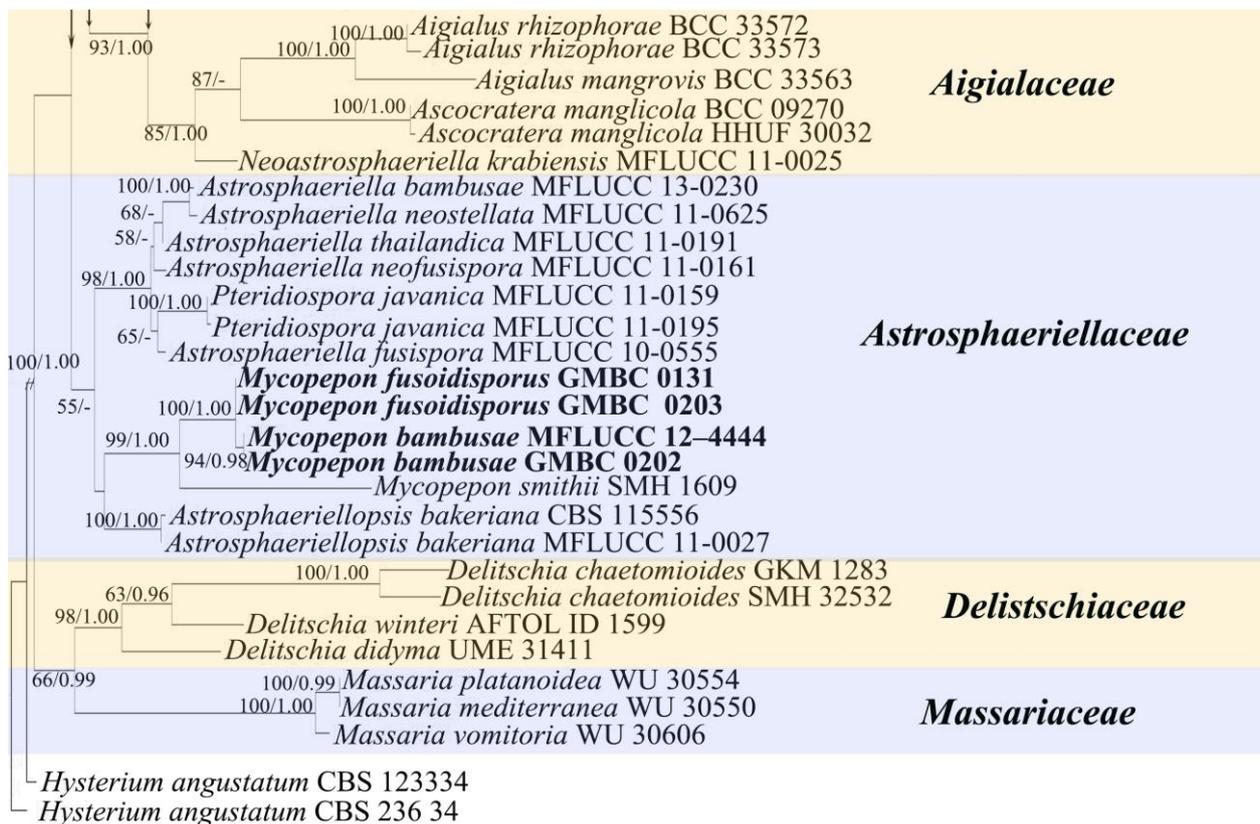


Figure 1 – Continued.

Results

ITS rDNA sequences of four strains were obtained. Initial BLAST searches suggested that our strains have high homology with *Mycopepon smithii* (SMH 1609). LSU and SSU regions were sequenced to identify these species and their classification status.

Phylogenetic analyses

A phylogenetic study using two combined loci (LSU, SSU) was conducted. All characters and gaps have equal weight. A total of 159 taxa were included in the analysis. Total length of the alignment was 1693 characters, in which 190 of those characters were variable, 1031 characters were constant, and 472 parsimony-informative characters. The final RAxML tree is shown in Figure 1, with the final ML optimization likelihood value of -19570.276304 . RAxML and Bayesian analysis of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies, and the RAxML tree is shown in Fig. 1. Our taxa gathered together with *M. smithii*. All *Mycopepon* species formed a well-supported clade (99/1.00), which is phylogenetically well supported in the family Astrosphaeriellaceae.

Taxonomy

Mycopepon mexicanus (F. San Martín) Q.R. Li & K.D. Hyde, stat. nov.

≡ *Mycopepon smithii* var. *mexicanum* F. San Martín, Acta Bot. Mexicana 35: 10 (1996)

Index Fungorum number: IF416160; Facesoffungi number: FoF 04689

Etymology – In reference to the location, Mexico.

Notes – See Gonzalez (1996) for descriptions. The variety *mexicanum* of *Mycopepon smithii* collected in Mexico was described by Gonzalez (1996). This taxon has larger ascospores ($60\text{--}84 \times 14\text{--}16 \mu\text{m}$) than those of *Mycopepon smithii* (Gonzalez 1996, Smith 1893). Because of the noticeable differences between these two taxa, we propose this variety be raised to a new species here. The holotype, San Martín 869B, was deposited at Instituto Tecnológico de Ciudad Victoria

(ITCV).

Mycopepon bambusae Q.R. Li & K.D. Hyde, sp. nov.

Fig. 2

Index Fungorum number –IF554787; Facesoffungi number –FoF 04601

Etymology – In reference to the host bamboo.

Saprobies on rotting bamboo, forming on the host surface. Sexual morph: *Ascostromata* 0.5–0.8 mm long × 0.3–0.5 mm high, scattered or clustered, superficial on host tissue, oval, with long central beak (1–1.5 mm long), black, comprising 3–6 pseudothecia. *Ascomata* 320–530 µm diam., sphaerical, black, carbonaceous, papillae eccentric, long, fusing to form a common central papilla (beak). *Hamathecium* of 1–1.5 µm wide, long, cellular, numerous, septate, branching, pseudoparaphyses Asci 161.5–219.5 × 14.5–20.5 µm (184 × 17.5 µm, n=20), 8-spored, bitunicate, cylindrical-clavate, pedicellate, apically rounded, apex not bluing in Melzer's reagent. *Ascospores* 45–61.5 × 6.5–8.5 µm (52.5 × 7.5 µm, n=30), tetra-seriate or partially overlapping, fusiform, brown to dark brown, 3-septa, slightly constricted at the central septum, rough-walled with small guttules, with broadly rounded ends, lacking sheaths or appendages. Asexual morph: Undetermined.

Culture characteristics – Colonies growing on PDA (Fig. 3 A, B, E, F), rather slow-growing, reached to 2 cm diam. in 4 weeks at 25 °C. Mycelium superficial, felty, gray at first and finally become to black, reverse of cultures black, tight, edge undulate.

Distribution – Guizhou Province, China

Material examined – CHINA, Guizhou Province, Miao and Dong Autonomous Prefecture Leishan County, Leigongshan National Nature Reserve (26° 22' 12" N, 108° 11' 47" E), on dead bamboo rhizome, 3 February 2012, Q. R. Li, LGS 20 (HKAS 87823, holotype); living cultures MFLUCC 12–4444).

Other material examined – CHINA, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture (25° 12' 52" N, 107° 59' 57" E), August 2017, Q.R. Li, LGS113 (GMB0202, HKAS100934).

GenBank accession numbers: LGS20 MH049450 (SSU), MH049442 (LSU); GMB0202 SSU MH049453, MH049445 (LSU).

Table 1 Synopsis of *Mycopepon* species.

Taxon	Ascostromata	Ascomata	Asci	Ascospores	Reference
<i>M. mexicanus</i>	0.7–1.2 mm diam., 2–6 ascomata	Globose	194–246 × 25–32 µm	60–84 × 14–16 µm, fusoid, deep brown, 3-septate, two end cells small and paler.	Gonzalez (1996)
<i>M. smithii</i>	1–1.5 mm diam., 4–8 ascomata	Ovate, 1/3 mm diam.	Not seen	52–62 × 10–14 µm, inequilateral ellipsoid, brown, 3-septate, with sharp ends.	Smith (1893)
<i>M. bambusae</i>	0.5–0.8 mm long × 0.3–0.5 mm high, 3–6 ascomata	Sphaerical, 320–530 µm diam.	161.5–219.5 × 14.5–20.5 µm	45–61.5 × 6.5–8.5 µm, fusiform, brown to dark brown, 3-septate, with broadly rounded ends.	This study
<i>M. fusoidispora</i>	0.6–0.8 mm long × 0.3–0.5 mm high, 2–8 ascomata	Sphaerical, 310–500 µm diam.	178–223 × 31.5–42 µm	45.5–57.5 × 11.5–23.5 µm, fusiform, brown to dark brown, 1-septate, occasional 3-septate, with broadly rounded end and a slightly flattened one.	This study
<i>M. guianensis</i>	0.9–1.1 mm diam. × 0.8–0.97 mm high, 3–5 ascomata	Globose to subglobose 360–660 µm high, 300–480 µm diam.,	205–240 × 28–38 µm	58–75 × 13–17 µm, fusiform, brown to dark brown, 1-septate, occasional 3-septate, ends cells paler	Doilom et al. 2018

Notes – *Mycopepon bambusae* is similar with *M. smithii* and *M. mexicanus* (Table 1). However, *M. smithii* has wider ascospores (52–62 × 10–14 µm) with small and paler ends (Smith 1893). *Mycopepon bambusae* differs from *M. mexicanus* (60–84 × 14–16 µm) by its smaller ascospores (Gonzalez 1996).

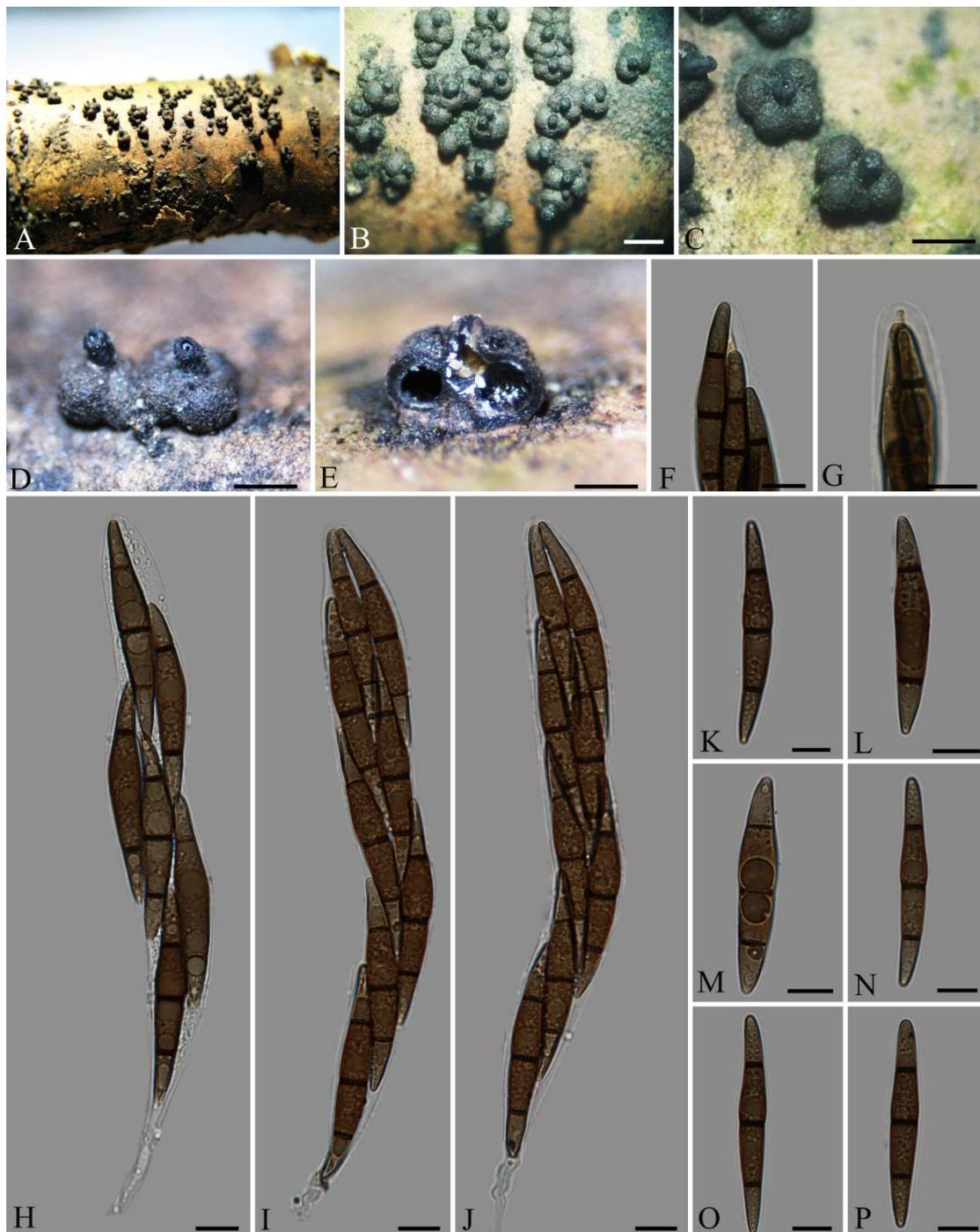


Figure 2 – *Mycopepon bambusae* (HKAS 87823, holotype) A Fresh material. B–D Stromata on the surface of host. E Section of ascostroma. F, G Ascus apex (stained in Melzer's reagent). H–J Mature asci with ascospores. K–P Ascospores. Scale bars: B–E = 200 μ m. F–P = 10 μ m.

Mycopepon fusoidisporus Q.R. Li & K.D. Hyde, sp. nov.

Fig. 4

Index Fungorum number: IF554788; Facesoffungi number: FoF 04602

Etymology – In reference to the fusoid ascospores.

Saprobic on initial rotting bamboo rhizome, forming on the host surface. Sexual morph: *Ascostromata* 0.6–0.8 mm long \times 0.3–0.5 mm high, superficial, scattered or clustered, superficial on host tissue, oval, with long central beak (0.3–0.5 mm long mm long), black, comprising 2–8 pseudothecia. *Ascomata* 310–500 μ m diam., spherical, black, carbonaceous, papillae eccentric, long, fusing to form a common central papilla (beak). *Hamathecium* comprising 1–1.5 μ m wide, long cellular, numerous, septate, branching, pseudoparaphyses. *Asci* 178–223 \times 31.5–42 μ m (203.5

× 35.5 μm, n=30), 8-spored, bitunicate, cylindric-clavate, pedicellate, apically rounded. *Ascus apex* well-developed ocular chamber, not blueing in Melzer's reagent. *Ascospores* 45.5–57.5 × 11.5–23.5 μm (51 × 17 μm, n=30), tetra-seriate or partially overlapping, brown to dark brown, 1-septate, occasional 3-septate, fusiform, smooth-walled, slightly thicker at the center, with broadly rounded end and a slightly flattened one, lacking a sheath and appendages. Asexual morph: Undetermined.

Culture characteristics – Colonies growing on PDA (Fig. 3 C, D, G, H), rather slow-growing, reached to 2.5 cm diam. in 4 weeks at 25 °C. Mycelium superficial, felty, gray at first and finally become to black, reverse of cultures black, tight, edge undulate.

Distribution – Known to inhabit dead bamboo, Guizhou Province, China.

Material examined – CHINA, Guizhou Province, Qiandongnan Miao and Dong Autonomous Prefecture, Maolan National Nature Reserve (25° 18' 08" N, 108° 04' 25" E), saprobic on the rhizome of bamboo, August 2016, Q.R. Li, ML056 (GMB0131, holotype; HKAS100936, isotype; living cultures GMBC0131)

Other material examined – CHINA, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, August 2017, Q.R. Li, LGS114 (HKAS100935, GMB0203, paratypes).

GenBank accession numbers: GMB0131 MH049451 (SSU), MH049443 (LSU); GMB0203, MH049452 (SSU), MH049444 (LSU).

Notes – *Mycopepon fusoidisporus* differs from *M. smithii* and *M. bambusae* by its wider and 1-septate ascospores. *Mycopepon smithii* has long spindle-shaped, 3-septate ascospores (30–65 × 6–16 μm) (Smith 1893, Boise 1987). Moreover, *M. fusoidisporus* has short beaks (versus long central papilla) than that of *M. bambusae*.

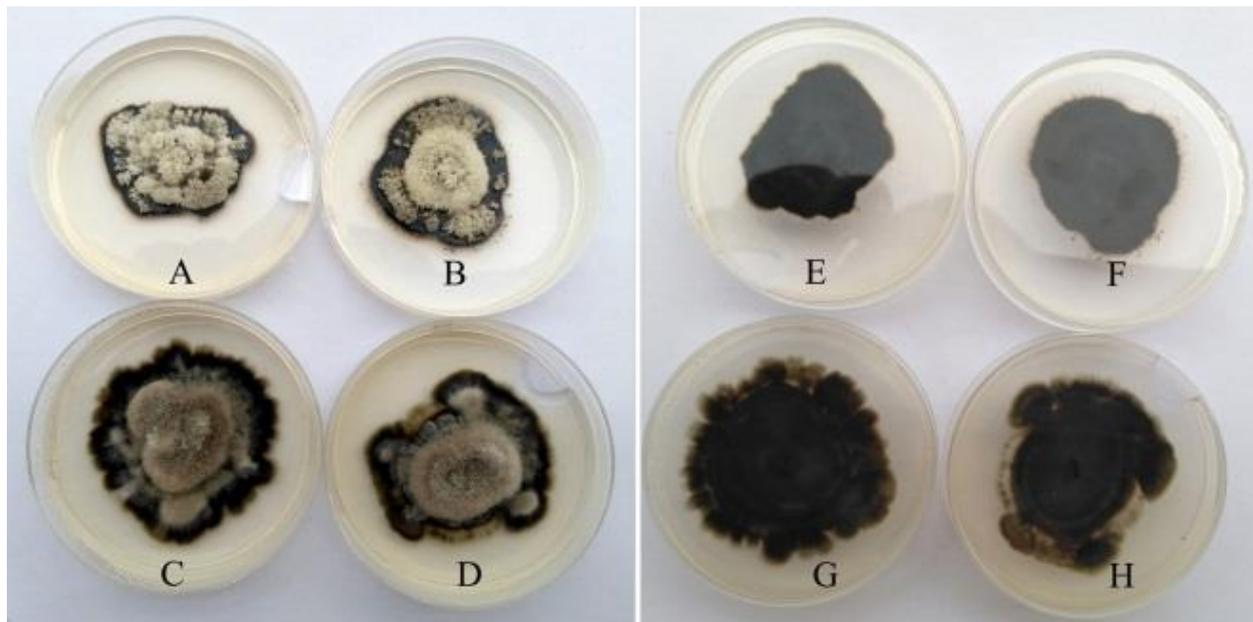


Figure 3 – Colonies on PDA after 5 week. (A GMB0202. B LGS20. C GMB0203. D GMB0131. E GMB0202. F LGS20. G GMB0203. H GMB0131.)

Discussion

Mycopepon guianensis was introduced as the type species of *Mycopepon* (Boise 1987). Boise (1994) reexamined the specimen of *Pseudovalsa smithii* Ellis & Everh. and found that these two names are synonymous: hence, *Mycopepon smithii* (Ellis & Everh.) Boise (synonym: *P. smithii* Ellis & Everh., *M. guianensis* Boise) was proposed. The variety *mexicanum* of *Mycopepon smithii* was found and identified by Gonzalez (1996). Currently, *M. smithii*, *M. guianensis* and *M. smithii* var. *mexicanum* were considered to be synonyms of *M. smithii* (Index Fungorum 2018). However, *M. mexicanus* (\equiv *M. smithii* var. *mexicanum*) has larger ascospores than those of *M. smithii* (Table 1). Two new species collected in China, were identified and are illustrated in this paper. The

hosts of *Mycopezon* have a wide diversity. *Mycopezon guianensis* (current name: *M. smithii*) was found on decorticated wood, *Mycopezon mexicanus* (\equiv *M. smithii* var. *mexicanum*) was discovered on wood lacking bark in a high perennifolia forest, while the two species described herein were from bamboo. *Mycopezon smithii* and *M. guianensis* do not have the same characters (see Table 1), particularly the dimensions of the ascospores and may not be the same taxa. Fresh collections are needed.

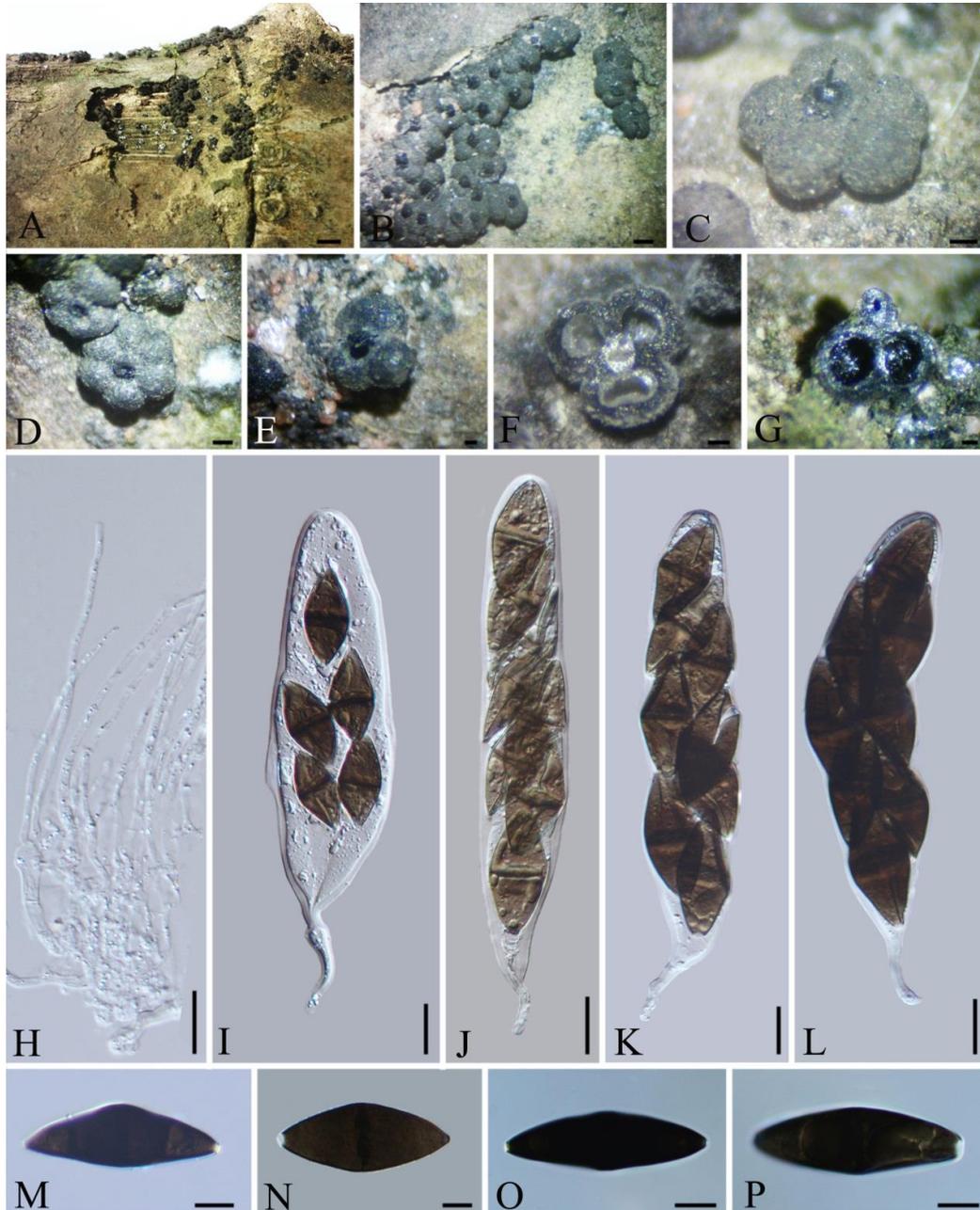


Figure 4 – *Mycopezon fusoidisporus* (GMB0131, holotype) A Fresh material. B–E Ascostromata on surface of host. F, G Section of ascostroma. H Paraphyses. I–L Asci with ascospores. M–P Ascospores. Scale bars: A = 2 mm. B = 0.5 mm. C–G = 100 μ m. H–L = 20 μ m. M–P = 10 μ m.

Mycopezon was introduced and could not be assigned to any family in Pleosporales (Boise 1987). The habit of *Mycopezon* is similar with those of Diaporthales, Diatrypaceae and members of Massariaceae *sensu* Barr (Eriksson & Hawksworth 1986). Eriksson & Hawksworth (1987) considered the genus should be placed in Melanommataceae, especially since the pseudoparaphyses were trabeculae (Liew et al. 2000). LSU and SSU sequence data for *M. smithii* (SMH 1609)

marked as a species of Dothideomycetes were used to study the placement in Sordariales (Huhndorf et al. 2004). *Mycopezon smithii* (SMH 1609) showed a close relationship with *Berkleasmiium crunisia* Pinnoi and *B. typhae* Somrith. & E.B.G. Jones, and was placed in Pleosporales genera *incertae sedis* (Pinnoi et al. 2007). In this paper, our strains cluster with *M. smithii* (SMH 1609). *Mycopezon* species were close to *Astrosphaeriellopsis bakeriana* within the Pleosporales. *Astrosphaeriellopsis bakeriana* (\equiv *Astrosphaeriella bakeriana*) formed a single clade was excluded from the genus *Astrosphaeriella*, and was accommodated in a new genus *Astrosphaeriellopsis* in the Pleosporales genera *incertae sedis* (Phookamsak et al. 2015). *Astrosphaeriellaceae* was introduced to accommodate *Astrosphaeriella* and *Pteridiospora* in Pleosporales by Phookamsak et al. (2015). Wanasinghe et al. (2018) suggested that *Astrosphaeriellopsis* and *Astrosphaeriella* should be assigned to *Astrosphaeriellaceae*. *Mycopezon* is similar to *Astrosphaeriellopsis* and *Astrosphaeriella* in its superficial ascostromata and trabeculate pseudoparaphyses (Liew et al. 2000), but differs from by its ascostromata comprising 2-8 pseudothecia. However, all of *Mycopezon*, *Astrosphaeriellopsis* and *Astrosphaeriella* have superficial carbonaceous ascostromata, trabeculate pseudoparaphyses and fusoid ascospores with septa. Therefore, *Mycopezon* falls in *Astrosphaeriellaceae* of Pleosporales in this paper.

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