Diversity of Moesziomyces (Ustilaginales, Ustilaginomycotina) on Echinochloa and Leersia (Poaceae)

Ying-Ming Li1,3, Roger G. Shivas2, Bao-Ju Li1, Lei Cai3

1 Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China
2 Centre for Crop Health, University of Southern Queensland, Toowoomba 4350, Australia
3 State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Corresponding author: Bao-Ju Li (libaoju@caas.cn)

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Abstract
A combined ecological, morphological, and molecular approach was used to examine 26 herbarium specimens and eight strains of Moesziomyces. The phylogenetic analysis resolved eight well-supported clades, of which three contained type specimens of known species of Moesziomyces. One clade contained two specimens that produced a teleomorph in the flowers of Echinochloa kimberleyensis in Australia. The name Moesziomyces kimberleyensis is proposed for this smut fungus. Another clade contained specimens that produced sori in the flowers of Leersia hexandra. The name Thecaphora globuligera (now Moesziomyces globuligerus) is available for this species, which is lectotypified. The teleomorph of Moesziomyces antarcticus, previously known only from Japan, is found for the first time in China, on Echinochloa crus-galli.

Keywords
Ecology, plant pathogens, phylogeny, Ustilaginaceae, Ustilaginomycotina

Introduction
The genus Moesziomyces (Ustilaginales, Ustilaginaceae) was established by Vánky (1977) for smut fungi that produce sori in the ovaries of grasses, lack a columella, and have spores with irregular meshes and wings on the surface, bound in firmly
agglutinated spore balls. Vánky (1977) recognized four species, *M. bullatus*, *M. evernius*, *M. globuligerus*, and *M. penicillariae*. Vánky (1986, 2012, 2013) later synonymised these names with the oldest available name, *M. bullatus*, and considered *Moesziomyces* as monotypic. Species of *Moesziomyces* are known to produce both free-living saprobic anamorphs (yeast-like) and plant pathogenic teleomorphs (smuts) (Wang et al. 2015; Kruse et al. 2017). The anamorphs of *Moesziomyces* are readily culturable on artificial media and have been isolated from a range of substrates, while the teleomorphs are formed in ovaries of seven genera of grasses (Poaceae). Wang et al. (2015) recombined four species known only by their anamorphs (*Pseudozyma antarctica, P. aphidis, P. parantarctica, and P. rugulosa*) into *Moesziomyces*, based on a molecular phylogenetic analysis. Subsequently, Tanaka et al. (2019) showed that one of these species, *M. antarcticus*, produced a teleomorph on *Echinochloa crus-galli* in Japan. A further five species, *M. bullatus, M. eriocauli, M. evernius, M. penicillariae*, and *M. verrucosus*, have been characterized from teleomorphs (Vánky 2012; Wang et al. 2015; Kruse et al. 2017). Kruse et al. (2017) recognized six species of *Moesziomyces* based on phylogenetic analysis, and treated *M. aphidis* and *M. rugulosus* as synonyms of *M. bullatus*.

The teleomorphs of Ustilaginaceae are mostly host specific (Stoll et al. 2003, 2005; Skibbe et al. 2010; McTaggart et al. 2012; Li et al. 2017a, 2017b). Given that species of *Moesziomyces* have been reported from seven different genera of grasses (*Echinochloa, Leersia, Panicum, Paspalum, Pennisetum, Polytrias*, and *Uranthoecium*), it is likely that additional species remain to be discovered. The aim of this study was to build on the work of Kruse et al. (2017) by examining specimens of *Moesziomyces* held in herbaria BRIP (Queensland Plant Pathology Herbarium), HMAS (Herbarium Mycologicum Academiae Sinicae), and HUV (Herbarium Ustilaginales Vánky, now deposited in BRIP), as well as eight yeast strains deposited in LC Culture Collection (personal culture collection held in the laboratory of Dr Lei Cai).

**Materials and methods**

**Specimen examination**

Specimens borrowed from several herbaria were examined by light microscopy (Table 1) by mounting the spores in lactic acid (100% v/v). Teliospore measurements were expressed as ranges (min–) mean-standard deviation-mean + standard deviation (–max) (*n* = 50). Images were captured by using a Nikon DS-Fi1 camera attached to a Nikon Eclipse 80i microscope with Nomarski differential interference contrast. Helicon Focus ver. 4.46.1 (Helicon Soft Ltd) was used to combine images to increase depth of field. Nomenclatural novelties and descriptions were registered in MycoBank (http://www.MycoBank.org).
DNA extraction, PCR amplification and sequencing

Sori were carefully removed from herbarium specimens, up to 149 years old, with a fine needle, sterilized by dipping in 75% ethanol for 30 s, air-dried on sterilized filter paper, and deposited in cell lysis solution (CTAB). Pure yeast colonies grown on yeast extract peptone dextrose (YPD) plates were transferred to cell lysis solution directly. Genomic DNA was extracted following the protocol of Cubero et al. (1999). Fragments of internal transcribed spacer rDNA were amplified by PCR with primers M-ITS1/ITS4 (White et al. 1990; Stoll et al. 2003).

PCR amplifications were carried out in 25 μl reactions containing 1 μl of genomic DNA template, 9.5 μl distilled water, 12.5 μl of 2 X Taq Plus Master Mix (Nanjing Vazyme Biotech Co. Ltd, Nanjing, China) and 1 μl of each primer (10 μM). Amplification reactions were run as follows: initial denaturation of 95 °C for 5 min followed by 35 cycles at 95 °C for 30 s, 45 s at 58 °C (annealing temperature) and 1 min at 72 °C with a final extension of 10 min at 72 °C. PCR products were sent to Tianyihuiyuan (Beijing, China) for sequencing with the forward and reverse primers indicated above. AB1 sequence traces were assembled with Sequencher version 5 (Genecodes, Ann Arbor, USA).

Phylogenetic analyses

The sequences included in this study (Tables 1, 2) were aligned online with MAFFT (https://mafft.cbrc.jp/alignment/server/index.html) using auto strategy, and observed in MEGA 5 (Katoh and Toh 2008). Phylogenetic analyses were based on both maximum likelihood (ML) and Bayesian Inference (BI). RAxML (Stamatakis 2006) and PhyML 3.0 (Guindon et al. 2010) were used for ML analyses. GTRGAMMA was specified as the model of evolution in both programs. The RAxML analyses were run with a rapid Bootstrap analysis (command -f a) using a random starting tree and 1 000 ML bootstrap replicates. The PhyML analyses were implemented using the ATGC bioinformatics platform (available at: http://www.atgcmonpellier.fr/phylml/), with six substitution type and SPR tree improvement, and support obtained from an approximate likelihood ratio test (Anisimova et al. 2011).

For BI, MrBayes was used with a Markov Chain Monte Carlo algorithm incorporating four runs, each consisting of four chains, until the standard deviation of split frequencies was reached. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 50 generations and trees were saved every 5000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander et al. 2008) (available at: http://ceb.csit.fsu.edu/awty/). A user-defined tree obtained from the PhyML analyses was used as a starting point for all the Bayesian analyses, which helped to improve convergence of the four runs.
Results

The ITS dataset comprised the newly sequenced *Moesziomyces* specimens and strains (Table 1) together with the reference sequences of *Moesziomyces* from Kruse et al. (2017) and Tanaka et al. (2019) (Table 2) and *Triodiomyces altilis* and *Ustilago echinata* as the outgroup based on the phylogenetic analyses of Wang et al. (2015). The topology of the ML and BI analyses (Fig. 1) were congruent. The phylogenetic

Table 1. Collection details for *Moesziomyces* specimens newly sequenced in this study.

| Species                  | Specimen/strain no. | Host              | Source         | Location       | Year of collection | ITS GenBank accession number |
|--------------------------|---------------------|-------------------|----------------|-----------------|--------------------|-----------------------------|
| *Moesziomyces antarcticus* | HMAS 248025         | *Echinochloa crus-galli* | Sorus         | China           | 2017               | MK027038                   |
| *M. antarcticus*         | HMAS 248026         | *E. crus-galli*   | Sorus         | China           | 2017               | MK027039                   |
| *M. antarcticus*         | HMAS 60130          | *E. crus-galli*   | Sorus         | China           | 1989               | MK027043                   |
| *M. bullatus*            | HMAS 50052          | *E. crus-galli*   | Sorus         | China           | 1985               | MK027041                   |
| *M. bullatus*            | LC-CLS58-3-2        | *Setaria faberi*  | Leaf surface  | China           | 2017               | MK024201                   |
| *M. bullatus*            | LC-CLS58-3-21       | *S. faberi*       | Leaf surface  | China           | 2017               | MK024202                   |
| *M. bullatus*            | LC-CLS58-3-22       | *S. faberi*       | Leaf surface  | China           | 2017               | MK024203                   |
| *M. bullatus*            | LC-CLS60-2-22       | *Pennisetum sp.*  | Leaf surface  | China           | 2017               | MK024204                   |
| *M. bullatus*            | LC-CLS60-2-4        | *Pennisetum sp.*  | Leaf surface  | China           | 2017               | MK024205                   |
| *M. bullatus*            | LC-SY1-2-11         | *Digitaria sp.*   | Leaf surface  | China           | 2017               | MK024206                   |
| *M. bullatus*            | LC-SY1-2-21         | *D. sp.*          | Leaf surface  | China           | 2017               | MK024207                   |
| *M. bullatus*            | LC-SY1-2-22         | *D. sp.*          | Leaf surface  | China           | 2017               | MK024208                   |
| *M. bullatus*            | HMAS 50454          | *E. crus-galli*   | Sorus         | Japan           | 1985               | MK027042                   |
| *M. bullatus*            | HMAS 70876          | *E. crus-galli*   | Sorus         | China           | 1991               | MK027045                   |
| *M. bullatus*            | HMAS 73871          | *E. crus-galli*   | Sorus         | China           | 1996               | MK027046                   |
| *M. bullatus*            | HUV 2442            | *E. crus-galli*   | Sorus         | Poland          | 1869               | MK027047                   |
| *M. bullatus*            | HUV 305             | *E. crus-galli*   | Sorus         | Germany         | 1905               | MK027050                   |
| *M. globuligerus*        | BRIP 27384          | *Leersia hexandra* | Sorus         | Australia       | 1998               | MK027025                   |
| *M. globuligerus*        | BRIP 44301          | *L. hexandra*     | Sorus         | Australia       | 2004               | MK027029                   |
| *M. globuligerus*        | BRIP 44569          | *L. hexandra*     | Sorus         | Australia       | 2004               | MK027030                   |
| *M. globuligerus*        | BRIP 47767          | *L. hexandra*     | Sorus         | Thailand        | 2005               | MK027031                   |
| *M. globuligerus*        | BRIP 47768          | *L. hexandra*     | Sorus         | Thailand        | 2005               | MK027032                   |
| *M. globuligerus*        | BRIP 51872          | *L. hexandra*     | Sorus         | Australia       | 2008               | MK027035                   |
| *M. globuligerus*        | HMAS 248027         | *L. hexandra*     | Sorus         | China           | 2017               | MK027037                   |
| *M. kimbrelleyensis*     | BRIP 51843*         | *E. kimbrelleyensis* | Sorus         | Australia       | 2008               | MK027034                   |
| *M. kimbrelleyensis*     | BRIP 52498          | *E. kimbrelleyensis* | Sorus         | Australia       | 2009               | MK027036                   |
| *M. penicillarii*        | HUV 2487            | *Pe. glaucum*     | Sorus         | Gambia          | 1973               | MK027048                   |
| *M. penicillarii*        | HUV 2488            | *P. glaucum*      | Sorus         | India           | 1912               | MK027049                   |
| *M. verrucosus*          | BRIP 39886          | *Pupatorium distichum* | Sorus     | Australia       | 2003               | MK027026                   |
| *M. verrucosus*          | BRIP 43727          | *P. distichum*    | Sorus         | Australia       | 2004               | MK027027                   |
| *M. verrucosus*          | BRIP 43755          | *P. distichum*    | Sorus         | Australia       | 2004               | MK027028                   |
| *M. verrucosus*          | BRIP 51772          | *P. distichum*    | Sorus         | India           | 1992               | MK027033                   |
| *M. verrucosus*          | HUV 66447*          | *P. distichum*    | Sorus         | India           | 1992               | MK027044                   |

1BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; HMAS: Herbarium Mycologicum Academiae Sinicae; HUV: Herbarium Ustilaginales Vánky (located at BRIP). 2GenBank accessions derived from this study are shown in bold. * Type specimens.

Figure 1. Phylogram obtained from a ML analysis based on the ITS sequence alignment. Values above the branches represent ML bootstrap values (> 70%) from RaxML and PhyML analysis respectively. Thickened branches represent Bayesian posterior probabilities (> 0.95). The scale bar indicates 0.03 expected substitutions per site. * indicates type specimens or type strains.
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M. buffius

M. antarcticus

M. parantarcticus
**Table 2.** List of *Moesziomyces*, *Triodiomyces*, and *Ustilago* sequences taken from GenBank and used in the phylogenetic analysis.

| Species                        | Source                                    | ITS GenBank accession number | Reference                        |
|--------------------------------|--------------------------------------------|------------------------------|----------------------------------|
| *Moesziomyces antarcticus*     | —                                          | JX094775                     | Gujjar et al. (unpubl.)          |
|                                | —                                          | JN942669                     | An (unpubl.)                     |
| unpolished Japanese rice       | AB089360                                   |                              | Sugita et al. 2003               |
| Antarctica sediment            | AF294698                                   |                              | Avis et al. 2001                 |
| *Albizia julibrissin* flower   | AY641557                                   |                              | Wei et al. 2005                  |
| lake sediment                  | AB089358                                   |                              | Sugita et al. 2003               |
| tomato rhizosphere             | KF493994                                   |                              | Johnston-Monje et al. (unpubl.)  |
| *Echinochloa crus-galli*       | LC368624                                   |                              | Tanaka et al. 2019               |
| *Echinochloa crus-galli* sorus | LC368624                                   |                              | Tanaka et al. 2019               |
| *Echinochloa crus-galli* sorus | LC368624                                   |                              | Tanaka et al. 2019               |
| *Echinochloa crus-galli* sorus | LC368624                                   |                              | Tanaka et al. 2019               |
| *Echinochloa crus-galli* sorus | LC368624                                   |                              | Tanaka et al. 2019               |
| *Echinochloa crus-galli* sorus | LC368625                                   |                              | Tanaka et al. 2019               |
| *Moesziomyces bullatus*        | human preterm low birth weight infant      | KF926673                     | Okolo et al. 2015                |
|                                | —                                          | DQ831013                     | Matheny et al. 2006              |
| Japanese pear fruit            | AB204896                                   |                              | Yasuda et al. 2007               |
| *Saccharum officinarum*        | AB704889                                   |                              | Morita et al. 2012               |
| *Leucaena glauca* human        | HQ662536                                   |                              | Wei et al. 2011                  |
| human blood                    | AB089362                                   |                              | Sugita et al. 2003               |
| human                         | HQ848933                                   |                              | Xie et al. unpubl.               |
| *Fallopia japonica* human      | KC282385                                   |                              | Wang & Liu (unpubl.)             |
| *Leucaena glauca* human        | KM610219                                   |                              | Bosco-Borgeat & Taverna (unpubl.)|
| *Leucaena glauca* human        | HQ647299                                   |                              | Wei et al. 2011                  |
| *Saccharum officinarum* poplar leaf | AB704890                               | Morita et al. 2012           |
| *Forecypomia taiwana* seaweed  | KM555221                                   |                              | Chen (unpubl.)                   |
| *aphid secretion*              | KP269028                                   |                              | Wang et al. (unpubl.)            |
| *Neoreglia cruenta*            | AF294699                                   |                              | Avis et al. 2001                 |
| *Saccharum officinarum* giant panda secretion | AB704878 | Morita et al. 2012 |                                |
| *Camellia sinensis* leaf lesions | HQ832804                               |                              | Li et al. (unpubl.)              |
| *Echinochloa crus-galli* aphid secretion on Solanum pseudocapsicum | GU390690 | Hamayun & Ahmad (unpubl.) |                                |
| *Citrus* leaf                  | JQ425372                                   |                              | Soliman (unpubl.)                |
|                                | JN942667                                   |                              | An (unpubl.)                     |
| *mouldy Zea mays* leaf         | AB089370                                   |                              | Sugita et al. 2003               |
| *plant leaf*                   | HE650886                                   |                              | Han et al. 2012                  |
| *ex-leaf of corn*              | AF294697                                   |                              | Avis et al. 2001                 |
| *Hyoscyamus nucifera*          | AB500693                                   |                              | Abdel-Motaal & Isu (unpubl.)     |
| *Coffea arabica*               | EU002890                                   |                              | Vega et al. (unpubl.)            |
| *Coffea arabica*               | DQ778919                                   |                              | Vega et al. 2008                 |
| *Saccharum officinarum* leaf   | LC053989                                   |                              | Surussawadee & Limtong (unpubl.) |
| marine environment             | DQ178645                                   |                              | Chang et al. 2008                |
| *Helicoverpa armigera* larva gut | AM160637                               |                              | Molnar & Prillinger (unpubl.)    |
analyses revealed eight distinct groups with high support values, including six clades consistent with those recovered by Kruse et al. (2017). The largest clade included specimens of *M. bullatus* on *Echinochloa crus-galli* (the host for the type specimen of *M. bullatus*) and *E. muricata* from Europe, related yeast strains as well as strains formerly assigned to the synonymous species names *Pseudozyma aphidis* and *P. rugulosa* (Kruse et al. 2017). Four well-supported clades comprised teleomorphic specimens on *Echinochloa kimberleyensis*, *Leersia hexandra*, *Paspalum distichum*, and *Pennisetum glaucum* (the latter with related yeast strains). One well-supported clade comprised yeast strains assigned to *M. parantarcticus*. One moderately supported clade comprised teleomorphic specimens on *E. crus-galli* from China and Japan and related yeast strains, assigned to *M. antarcticus*. The remaining single-sequence lineage was formed by *Moesziomyces eriocauli* on *Eriocaulon cinereum* (Eriocaulaceae).
Taxonomy

Based on the phylogenetic analysis and the hosts of the teleomorphs, a new species of *Moesziomyces* is described and another species resurrected. Additionally, the teleomorph of *M. antarcticus* is reported for the first time from China.

*Moesziomyces antarcticus* (Goto, Sugiyama & Iizuka) Q.M. Wang, Begerow, F.Y. Bai & Boekhout, Stud. Mycol. 81: 81 (2015)

Figure 2h–k

*Sporobolomyces antarcticus* Goto, Sugiyama & Iizuka, Mycologia 61: 759 (1969).

[Basionym]
*Candida antarctica* (Goto, Sugiyama & Iizuka) Kurtzman et al. Yeasts: 86 (1983).

*Vanrija antarctica* (Goto, Sugiyama & Iizuka) R.T. Moore, Bibliotheca Mycol. 108: 167 (1987).

*Pseudozyma antarctica* (Goto, Sugiyama & Iizuka) Boekhout, J. Gen. Appl. Microbiol. 41: 364 (1995).

*Trichosporon oryzae* H. Ito, Iizuka & T. Sato, Agric. Biol. Chem. 38: 1599 (1974). (synonymy by Q.M. Wang, Begerow, F.Y. Bai and Boekhout).

Description. Sori in scattered ovaries, sometimes deciduous, globose to ovoid, 2–3 mm in length, covered by a smooth green membrane of host tissue origin that becomes brown and ruptures irregularly to expose a granular, black to dark brown mass of spore balls; columella absent. Spore balls variable in shape and size, globose, subglobose, ovoid, elongate to irregular, 130–200 μm in diameter, dark brown, composed of up to several hundred spores, united firmly by fungal sterile cells and spore meshes and wings. Spore globose, ovoid to irregular, slightly polyhedral, (8–) 8.5–9.5 (–10) × (6–) 7–9 (–10) μm, usually with well-developed meshes and wings, subhyaline to pale yellowish-brown; wall 0.5 μm thick, smooth. Some of the sterile cells empty at maturity, thin-walled, with irregular meshes or wings on the spore surface when the spores separates; other sterile cells, globose, ovoid to irregular, slightly polyhedral, (8–) 8.5–9.5 (–10) × (6–) 7–9 (–10) μm, subhyaline to pale yellowish-brown; wall 1–1.5 μm thick, smooth.

Specimens examined. CHINA, Sichuan, Chengdu, on *Echinochloa crus-galli*, 15 Sept. 1989, L. Guo leg., HMAS 60130; Guangxi, on *E. crus-galli*, Oct. 2017, R.G. Shivas, M.D.E. Shivas & Y.-M. Li leg., HMAS 208025; Guangxi, on *E. crus-galli*, Oct. 2017, R.G. Shivas, M.D.E. Shivas & Y.-M. Li leg., HMAS 208026.

Notes. The teleomorph of *Moesziomyces antarcticus* was previously reported from Japan, on *Echinochloa crus-galli* (Tanaka et al. 2019). The current report from China, also on *E. crus-galli*, suggests that this smut fungus may be common in the teleomorphic stage, at least in East Asia.
**Moesziomyces globuligerus** (Berk. & Broome) Vánky, Bot. Not. 130: 135 (1977)

Figure 2e–g

*Thecaphora globuligera* Berk. & Broome, Trans. Linn. Soc. London, Bot., Ser. 2, 1: 407 (1880). — Type: AUSTRALIA, Queensland, Brisbane, on *Leersia hexandra*, F.M. Bailey, No. 86 (K(M) 252436, **lectotype designated here**, MBT 385180, not seen; K(M) 252437, **syntype**). [Basionym]

*Tolyposporium globuligerum* (Berk. & Broome) Ricker, J. Mycol. 11:112 (1905).

*Testicularia leersiae* Cornu, Ann. Sci. Nat. Bot., Sér. 6, 15: 275 (1883).

**Description.** Sori in some of the ovaries, often deciduous, ellipsoidal to oval, 2.5–4 × 1.5–3 mm, green at first, later brown, smooth, ruptures irregularly to reveal a granular, dark brown mass of spore balls; columella absent. Spore balls subglobose, ellipsoidal or irregular, 75–150 μm in diameter, yellowish brown, composed of up to several hundred spores that separate by moderate pressure. Spores subglobose, ovoid to irregularly polyhedral, (8–) 8.5–11 (–13) × (6–) 7–9 (–10) μm (x̄ = 9.6 ± 1.2 × 7.9 ± 0.9 μm, n = 50), subhyaline to pale yellowish brown, attached together by multiple narrow cylindrical protuberances about 2 μm wide and 1–2 μm long; wall with irregular meshes and wings, less than 0.5 μm thick, smooth. (Based on specimen BRIP 27384).

**Specimens examined.** AUSTRALIA, Queensland, Willowbank, on *Leersia hexandra*, 9 Mar. 1998, C. Vánky & K. Vánky leg., BRIP 27384; Queensland, Mareeba, on *L. hexandra*, 1 May 2004, M.D.E. Shivas & R.G. Shivas leg., BRIP 44301; Queensland, Mt Garnet, on *L. hexandra*, 5 May 2005, T.S. Marney & R.G. Shivas leg., BRIP 44569; Northern Territory, Darwin, on *L. hexandra*, 15 Apr. 2008, J. Ray, A.A. Mitchell, A.R. McTaggart & R.G. Shivas leg., BRIP 51872. CHINA, Guangxi province, on *L. hexandra*, Oct. 2017, R.G. Shivas, M.D.E. Shivas, Y.-M. Li, P. Zhao & X.-H. Qi leg., HMAS 248027. THAILAND, Kanchanaburi, on *L. hexandra*, 16 Dec. 2005, R.G. Shivas & M.D.E. Shivas leg., BRIP 47767; Chiang Mai, on *L. hexandra*, 26 Dec. 2005, R.G. Shivas & M.D.E. Shivas leg., BRIP 47768.

**Notes.** Vánky (1986) considered that *M. globuligerus* was a synonym of *M. bullatus* based on their similar morphologies. Phylogenetic analyses support *M. globuligerus* as a distinct species (Fig. 1), with a teleomorph specific to the pantropical grass *Leersia hexandra* (Berkeley and Broome 1880). The name *Testicularia leersiae* (Cornu 1883), described from infected *Leersia hexandra* in Algeria, is likely a heterotypic synonym of *M. globuligerus*, but this has not been checked by molecular phylogenetic analysis. The type material of *Thecaphora globuligera* was collected circa 1878 from near the Brisbane River, Queensland, Australia by the botanist F. M. Bailey (Berkeley and Broome 1880). Original material of this specimen (F.M. Bailey, No. 86) could not be found in the Australian herbaria BRI and BRIP, where most of F.M. Bailey’s specimens are held. Two syntypes were located in K(M), of which K(M) 252436 ex C.E. Broome herbarium (BM) was selected as lectotype of *T. globuligera* (now *M. globuligerus*). The material in the second specimen, K(M) 252437 from the Berkeley herbarium, was scant (Dr Begoña Aguirre-Hudson pers. comm).
Moesziomyces kimberleyensis Y.M. Li, L. Cai & R.G. Shivas, sp. nov.
MycoBank: MB827986
Figure 2a–d

Type. AUSTRALIA, Western Australia, Kununurra, Mulligan’s Lagoon Road, on Echinochloa kimberleyensis, 9 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. Shivas & R.G. Shivas leg. (holotype: BRIP 51843).
**Etymology.** Named after the Kimberley region of northern Western Australia from where it was collected.

**Description.** Sori in some of the ovaries, often deciduous, globose to ovoid, 3–6 × 2–4 mm, green at first, later brown, smooth, ruptures irregularly to reveal a granular, dark brown mass of spore balls; columella absent. Spore balls subglobose, ovoid, elongate or irregular, 275–100 μm diam, dark brown, composed of up to several hundred spores, separated by moderate pressure. Spore globose, ovoid to irregular, slightly polyhedral, (9–) 9.5–12 (–14.5) × (8–) 8.5–9.5 (–10) μm (x̄ = 10.5 ± 1.2 × 8.9 ± 0.7 μm, n = 50), subhyaline to yellowish brown, attached together by multiple narrow cylindrical protuberances about 2 μm wide and 1–2 μm long; wall with irregular meshes and wings, 0.5 μm thick, smooth.

**Additional specimen examined.** AUSTRALIA, Western Australia, Kununurra, Mulligan’s Lagoon Road, on *E. kimberleyensis*, 7 May 2009, A.R. McTaggart, M.J. Ryley, M.D.E. Shivas & R.G. Shivas leg. (BRIP 52498).

**Notes.** *Moesziomyces kimberleyensis* was shown in the phylogenetic analysis to reside in a well-supported clade sister to *M. bullatus*. *Moesziomyces kimberleyensis* is only known from the teleomorph, which forms sori in flowers of *E. kimberleyensis*, and thereby differs from *M. bullatus* by host association. *Moesziomyces kimberleyensis* is only known from one location in Western Australia on *E. kimberleyensis*, which is an endemic grass in the tropical and subtropical woodlands of northern Australia.

**Discussion**

The phylogenetic analyses in this study supported the host specificity of the teleomorphic stage of six species of *Moesziomyces*, specifically, *M. antarcticus* on *Echinochloa crus-galli*, *M. bullatus* on *E. crus-galli* and *E. muricata*, *M. globuligerus* on *Leersia hexandra*, *M. kimberleyensis* on *E. kimberleyensis*, *M. penicillariae* on *Pennisetum glaucum*, and *M. verrucosus* on *Paspalum distichum*. The teleomorph of *M. eriocauli* may be specific to *Eriocaulon* spp., although this cannot be ascertained from the sequence data of one specimen. Specimens that have been assigned to *M. bullatus* were not well resolved and formed a number of smaller clades with varying degrees of support (Fig. 1). The *M. bullatus* clade contained several anamorphic yeasts isolated from diverse habitats (Wang et al. 2015; Kruse et al. 2017), including leaves of *Digitaria* sp., *Pennisetum* sp., and *Setaria faberii*. This shows that the anamorphs of *Moesziomyces* are widespread in the environment as saprobes.

The anamorphs of *Moesziomyces*, together with most members of the Ustilaginales, have a dimorphic lifecycle comprised of a parasitic dikaryotic phase characterized by teliospores, together with a saprobic yeast-like haploid phase (Brefeld 1883; de Bary 1884; Sampson 1939; Begerow et al. 2014). The teliospores are generally thick-walled and darkened, which protects against desiccation and UV radiation, thereby facilitating survival and long-distance dispersal (Piepenbring et al. 1998). The basidiospores
are usually thin-walled, hyaline, and survive as free-living saprobic yeasts that may occur on a vast diversity of substrates (Wang et al. 2015; Kruse et al. 2017; Tanaka et al. 2019). There is genomic evidence that some saprobic ustilaginalean yeasts, e.g. *M. antarcticus*, *Kalmanozyma brasilienisi* (= *P. brasilienisi*), *Pseudozyma hubeiensis*, and the yeast stage of *M. bullatus* (= *P. aphidis*), have retained the capacity to produce effector proteins, which hints at the possibility that undiscovered plant pathogenic stages may exist for these fungi (Sharma et al. 2018). Indeed, a teleomorph for *M. antarcticus* (= *P. antarctica*) was recently reported for the first time on *Echinochloa crus-galli* (Tanaka et al. 2019). Further collections are needed to resolve the ecological relationships and elucidate the life cycles of the ustilaginalean fungi and their hosts.

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