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ARTICLE

Asexual and sexual morphs of *Moesziomyces* revisited

Julia Kruse[1, 2], Gunther Doehlemann[3], Eric Kemen[4], and Marco Thines[1, 2, 5]

[1]Goethe University, Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Max-von-Laue-Str. 13, D-60486 Frankfurt am Main, Germany; corresponding author e-mail: thines@smut-fungi.net

[2]Biodiversität und Klima Forschungszentrum, Senckenberg Gesellschaft für Naturforschung, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany

[3]Botanical Institute and Center of Excellence on Plant Sciences (CEPLAS), University of Cologne, BioCenter, Zülpicher Str. 47a, D-50674, Köln, Germany

[4]Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, 50829 Köln, Germany

[5]Integrative Fungal Research Cluster (IPF), Georg-Voigt-Str. 14-16, D-60325 Frankfurt am Main, Germany

Abstract: Yeasts of the now unused asexually typified genus *Pseudozyma* belong to the smut fungi (*Ustilaginales*) and are mostly believed to be apathogenic asexual yeasts derived from smut fungi that have lost pathogenicity on plants. However, phylogenetic studies have shown that most *Pseudozyma* species are phylogenetically close to smut fungi parasitic to plants, suggesting that some of the species might represent adventitious isolations of the yeast morph of otherwise plant pathogenic smut fungi. However, there are some species, such as *Moesziomyces aphidis* (syn. *Pseudozyma aphidis*) that are isolated throughout the world and sometimes are also found in clinical samples and do not have a known plant pathogenic sexual morph. In this study, it is revealed by phylogenetic investigations that isolates of the biocontrol agent *Moesziomyces aphidis* are interspersed with *M. bullatus* sexual lineages, suggesting conspecificity. This raises doubts regarding the apathogenic nature of asexual morphs previously placed in *Pseudozyma*, but suggests that there might also be pathogenic sexual morph counterparts for those species known only from asexual morphs. The finding that several additional species currently only known from their yeast morphs are embedded within the genus *Moesziomyces*, suggests that the yeast morph might play a more dominant role in this genus as compared to other genera of *Ustilaginaceae*. In addition, phylogenetic reconstructions demonstrated that *Moesziomyces bullatus* has a narrow host range and that some previously described but not widely used species names should be applied for *Moesziomyces* on other host genera than *Echinochloa*.

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INTRODUCTION

Ustilaginales is the largest order within the smut fungi (*Ustilaginomycetes*), including species forming a blackish to brownish powdery spore mass in different organs of monocotyledonous, and exceptionally dicotyledonous plants (Vánky 2012, Begerow *et al.* 2006). The order includes nine families, encompassing 54 genera. The *Anthracoideaceae* with the occurrence on *Cyperaceae* and *Juncaceae*, and *Ustilaginaceae*, with few exceptions parasitic to the *Poaceae*, are the largest families within the order. The latter contains the three largest smut genera, *Anthracocystis*, *Sporisorium,* and *Ustilago*. The difference between these three closely related genera is the almost complete lack of a plant-derived columella within sori formed by *Ustilago* species (Vánky 2012, McTaggart *et al.* 2012). Within *Ustilago* some species are economically important pathogens, like corn smut (*Ustilago maydis*) or wheat smut (*Ustilago nuda*). *Ustilago maydis* is a species for which one of the first fungal genomes was sequenced (Kämper *et al.* 2006). Smut fungi of the *Ustilaginales* usually feature both an asexual yeast morph and a sexual morph infecting host plants in a biotrophic manner. In rare cases yeasts of the *Ustilaginales* could also be found to be affecting humans (McNeil & Palazzi 2012, Teo & Tay 2006). The earliest case of an invasive infection with an *Ustilago* species, possibly *U. maydis*, was reported in 1946 (Moore *et al.* 1946). But spores of the *Ustilaginales* potentially also cause pneumonias, allergic reactions, or asthma (Valverde *et al.* 1995).

There are several studies dealing with the phylogeny of *Ustilaginomycotina*, mostly based on the LSU or ITS locus and some of them include asexual morphs as well (e.g. Begerow *et al.* 2000, 2006, Stoll *et al.* 2005, Wang *et al.* 2006, 2015, Boekhout 2011). Wang *et al.* (2015) link many asexual yeasts to their corresponding sexual morphs, an important step within the naming of pleomorphic fungi, as dual naming of sexual and asexual morphs is now discontinued (Hawksworth *et al.* 2011).

Pseudozyma has been used for species of ustilaginomycetous yeasts belonging to *Ustilaginales* which are mostly

believed to be apathogenic (Begerow *et al.* 2000, 2006). The genus was described in 1985, by Bandoni (1985), and later refined by Boekhout (1995). After Sampaio (2004) and finally Wang *et al.* (2015) established that the type species *Pseudozyma prolifica* was a synonym of *Ustilago maydis*, the name *Pseudozyma* should no longer be used. However, the phylogenetic position of some species referred to *Pseudozyma* is still unclear. Wang *et al.* (2015) suggested using the name *Pseudozyma* now with the addition "*pro tempore*" for five *Pseudozyma* species with an unclear phylogenetic position. In the current study we give *Pseudozyma* names with reference to the new combinations recently made, where possible.

To date, about 20 *Pseudozyma* species names were validly published (www.indexfungroum.org). Fifteen are now linked to a corresponding sexually typified genus (Wang *et al.* 2015). Of these, *Pseudozyma antarctica*, *P. aphidis*, *P. parantarctica*, and *P. rugulosa* were transferred to *Moesziomyces* (Wang *et al.* 2015).

Ustilaginalean yeasts are isolated from diverse habitats, for *Ustilaginales*, but mostly from grasses (Boekhout 1995, Avis *et al.* 2001). Some were isolated from flowers, leaves or fruits of other plants, but it is also possible to isolate them from soil or even human blood or secretory fluid (Sugita *et al.* 2003, Arendrup *et al.* 2014). Apart from the few clinical cases of *Ustilago maydis* infestation, it was not known until 2003 that species referred to *Pseudozyma* could also infect humans (Sugita *et al.* 2003). However, such species can cause invasive infections, especially in immunosuppressed individuals (Arendrup *et al.* 2014). The infection risk, according to Prakash *et al.* (2014), is the same as being colonised by non-albicans *Candida* infections. Furthermore, Avis *et al.* (2001) and Gafni *et al.* (2015) reported antifungal properties of some pseudozyma-like yeast species, including *Moesziomyces aphidis,* and some strains have been reported to be natural antagonists of powdery mildews (*Erysiphales*). Colonization of leaf surfaces by these yeasts provides a natural source of protection against some plant pathogenic fungi (Gafni *et al.* 2015).

Of the ustilaginalean yeasts, especially *Moesziomyces antarcticus* and *M. aphidis*, formerly treated as *Pseudozyma* species, have been frequently isolated from various substrates. *Moesziomyces antarcticus* was isolated from plants and soil, but also from blood of humans (Boekhout 2011). *Moesziomyces aphidis* was described in 1995 and first isolated from the secretion of an aphid, but it has later been isolated from water (Boekhout 2011) and various other sources, including soil and human blood. Wang *et al.* (2015) showed that these pseudozyma-like species, together with *M. parantarcticus* and *M. rugulosus*, belong to the genus *Moesziomyces*, which before had been generally regarded as monotypic (Vánky 2005).

Moesziomyces mainly differs from other smut fungi in having remnants of ruptured sterile cells (Vánky 1977). Vánky (1977) initially included four species in the genus *Moesziomyces*. Three of them, occurring on different genera of grasses (*Leersia, Paspalum, Pennisetum*) were later considered to be conspecific and united under the oldest name, *M. bullatus* (Vánky 1986, 2005). The other species, *M. eriocauli* (Vánky 1986), was transferred to a new genus *Eriomoeszia*, because of a thin cortex of sterile cells, which surrounds the spore balls (Vánky 2005). After the transfer of four pseudozyma-like species *(M. antarcticus, M. aphidis, M. parantarcticus* and *M. rugulosus*) to this genus, it now contains five species (Wang *et al.* 2015).

Given the high host specificity observed for most *Ustilaginales* species (McTaggart *et al.* 2012, Escudero 2015, Li *et al.* 2017), it seemed doubtful that *Moesziomyces bullatus* was parasitic to seven not closely related genera, suggesting that more species might be present in the genus, some of which might be conspecific with smuts in the past named in *Pseudozyma*. It was the aim of the current study to clarify the relationships of asexual and sexual morphs in the genus *Moesziomyces*.

MATERIAL AND METHODS

Fungal material

The fungal material used in this study is listed in Tables 1 and 2. The nomenclature of the hosts is derived from the latest version of The International Plant Names Index (www.ipni.org), the nomenclature of the fungi follows Vánky (2012) and MycoBank (www.mycobank.org).

Yeast cultivation

Fresh material of *Moesziomyces bullatus* collected in 2015 (GLM-F105817) was used for yeast cultivation. A suspension of spores in 2 mL water was prepared. Three tubes with 200 µL spore suspension were exposed to three different conditions: (1) heating of the suspension on a thermomixer at 45 °C for 5 min (Shetty & Safeeulla 1979); (2) chilling the suspension overnight on ice; and (3) incubation for 5 min at room temperature (*ca.* 20 °C). From each tube 20 µL suspension was each plated on two plates of SAM (Thines lab Standard Agar Medium, consisting of 20 g agar, 20 g PDB, 10 g yeast extract, 10 g malt extract, 40 mL clarified vegetable juice, 960 mL water) with the addition of 75 mg Rifampicin/L. One set of plates was incubated at 30 °C, the other set at room temperature. After 3 d on every plate abundant yeast growth was recognized. Pure cultures were produced by picking and transferring individual single colonies to the SAM medium (isolate A1–A10). To isolate pseudozyma-morphs associated with *Albugo laibachii* on *Arabidopsis thaliana*, *A. laibachii* spores were suspended in water and treated with antibiotics to remove bacteria. Subsequently, the suspension was plated on PDA at 20 °C and colonies were singled out after 7 d.

DNA extraction, PCR and sequencing

In total 5–20 mg of infected plant tissue from herbarium specimens and yeast colonies were disrupted in a mixer mill (MM2, Retsch), using two iron beads of 3 mm and 5–8 iron beads of 1 mm diam per sample and shaking at 25 Hz for 5–10 min. Genomic DNA was extracted using the BioSprint 96 DNA Plant Kit (Qiagen, Hilden) on a KingFisher Flex robot (Thermo Scientific, Dreieich). PCR amplification of the complete ITS nrDNA was performed using the M-ITS1 forward primer (Stoll *et al.* 2003) and the ITS4 (White *et al.* 1990) or smITS-R1 reverse primer (Kruse *et al.* 2017).

Table 1. List of *Moesziomyces* specimens used in the present study.

Species	Host	Host family	Location	Year	Collector	Fungarium no.	ITS GenBank acc. no.
Moesziomyces aphidis	*Arabidopsis thaliana* infected with *Albugo laibachii*	*Brassicaceae*	UK, Norwich	2007	E. Kemen	GLM-F107578	KY930224
Moesziomyces bullatus	*Echinochloa crus-galli*	*Poaceae*	Germany, Bavaria	2013	J. Kruse	GLM-F105812	KY424439
	Echinochloa crus-galli	*Poaceae*	Germany, Bavaria	2013	J. Kruse	GLM-F105813	KY424428
	Echinochloa crus-galli	*Poaceae*	Germany, Bavaria	2013	J. Kruse	GLM-F105814	KY424429
	Echinochloa muricata	*Poaceae*	Germany, Saxony	2000	H. Jage	GLM-F047045	KY424430
	Echinochloa muricata	*Poaceae*	Germany, Saxony	2000	D. Schulz, B. Huber & F. Klenke	GLM-F047047	KY424431
	Echinochloa muricata	*Poaceae*	Germany, Saxony-Anhalt	2003	H. Jage	GLM-F065276	KY424432
	Echinochloa muricata	*Poaceae*	Germany, Saxony-Anhalt	2005	H. Jage	GLM-F076000	KY424433
	Echinochloa crus-galli	*Poaceae*	Germany, North Rhine-Westphalia	2010	J. Kruse	GLM-F105815	KY424434
	Echinochloa crus-galli	*Poaceae*	Germany, Schleswig-Holstein	2011	J. Kruse	GLM-F105777	KY424435
	Echinochloa crus-galli	*Poaceae*	Poland	1979	K. Vánky	HUV No. 283, ex TUB	KY424436
	Echinochloa crus-galli	*Poaceae*	Germany, Hesse	2015	J. Kruse	ex-GLM-F105817 culture No. A1 GLM-F107575	KY424437
	Echinochloa crus-galli	*Poaceae*	Germany, Hesse	2015	J. Kruse	ex-GLM-F105817 culture No. A3 GLM-F107576	KY424427
	Echinochloa crus-galli	*Poaceae*	Germany, Hesse	2015	J. Kruse	ex-GLM-F105817 culture No. A10 GLM-F107577	KY424438
Moesziomyces penicillariae	*Pennisetum glaucum*	*Poaceae*	Westafrica, Gambia	1973	K. Vánky	HUV No. 154, ex TUB	KY424440

Table 2. List of additional sequences used in the phylogenetic tree, downloaded from GenBank.

Species	Source	ITS GenBank acc. no.	Citation
Eriomoeszia eriocauli	*Eriocaulon cinereum*	AY740041	Stoll *et al.* (2005)
Macalpinomyces eriachnes	*Eriachne helmsii*	AY740038	Stoll *et al.* (2005)
Moesziomyces bullatus	*Paspalum distichum*	AY74015)3	Stoll *et al.* (2005)
	human preterm low birth weight infant	KF926673	Okolo *et al.* (2015)
	-	DQ831013	Matheny *et al.* (2006)
	human preterm low birth weight infant	KF926673	Okolo *et al.* (2015)
	-	DQ831013	Matheny *et al.* (2006)
Pseudozyma antarctica	-	JX094775	Gujjari *et al.* (unpublished)
	-	JN942669	An (unpublished)
	unpolished Japanese rice	AB089360	Sugita *et al.* (2003)
	Antarctica sediment	AF294698	Avis *et al.* (2001)
	Albizia julibrissin flower	AY6415)57	Wei *et al.* (2005)
	lake sediment	AB089358	Sugita *et al.* (2003)
Pseudozyma aphidis	Japanese pear fruit	AB204896	Yasuda *et al.* (2007)
	human pulmonary infection	Q743064	Parahym *et al.* (2013)
	Saccharum officinarum	AB704889	Morita *et al.* (2012)
	Leucaena glauca	HQ662536	Wei *et al.* (2011)
	human	EU105)207	Lin *et al.* (2008)
	human blood	AB089362	Sugita *et al.* (2003)
	human	HQ848933	Xie *et al.* (unpublished)
	Fallopia japonica	KC282385	Wang & Liu (unpublished)
	blood culture from hospitalized patient	KM610219	Bosco-Borgeat & Taverna (unpublished)
	Leucaena glauca	HQ647299	Wei *et al.* (2011)
	Saccharum officinarum	AB704890	Morita *et al.* (2012)
	poplar leaf	KM268868	Sun & Yan (unpublished)
	Forcipomia taiwana	KM555221	Chen (unpublished)
	seaweeds	KP269028	Wang *et al.* (unpublished)
	aphid secretion	**AF294699**	Avis *et al.* (2001)
	Neoreglia cruenta	FN424100	Garcia *et al.* (unpublished)
	Saccharum officinarum	AB704878	Morita *et al.* (2012)
	giant panda secrete	KF973199	Li *et al.* (unpublished)
	Camellia sinensis foliar lesions	HQ832804	Li *et al.* (unpublished)
	Echinochloa crus-galli	GU390690	Hamayun & Ahmad (unpublished)
	aphid secretion on *Solanum pseudocapsicum*	JN942666	An (unpublished)
	mulberry leaf	KF443199	Qiu *et al.* (unpublished)
	Citrus leaf	JQ425372	Soliman (unpublished)
	-	JN942667	An (unpublished)
Pseudozyma hubeiensis	*Magnolia denudata* wilting leaf	DQ008954	Wang *et al.* (2006)
Pseudozyma parantarctica	-	JN544036	Chen (unpublished)
	yam tuber steep water	KF619567	Babajide *et al.* (2015)
	-	KP132543	Irinyi *et al.* (2015)
	human blood	AB089356	Sugita *et al.* (2003)
	-	**NR 130693**	An (unpublished)
Pseudozyma rugulosa	mouldy *Zea mays* leaf	AB089370	Sugita *et al.* (2003)
	ex-leaf of corn	**AF294697**	Avis *et al.* (2001)
	plant leaf	HE650886	Han *et al.* (2002)
Pseudozyma sp.	*Hyoscyamus muticus*	AB500693	Abdel-Motaal & Itu (unpublished)

Table 2. (Continued).

Species	Source	ITS GenBank acc. no.	Citation
	Coffea arabica	EU002890	Vega *et al.* (unpublished)
	Hyoscyamus muticus	AB500690	Abdel-Motaal & Itu (unpublished)
	Coffea arabica	DQ778919	Vega *et al.* (2008)
	Saccharum officinarum leaves	LC05)3989	Surussawadee & Limtong (unpublished)
	shoot of tip pepper	GU975792	Sim *et al.* (unpublished)
	marine environment	DQ178645	Chang *et al.* (2008)
	Helicoverpa armigera caterpilla gut	AM160637	Molnar & Prillinger (unpublished)
	marine sediment	KC834821	Qu *et al.* (unpublished)
	-	KR047769	Wang *et al.* (unpublished)
	pharmaceutical effluent	KF922220	Selvi & Das (unpublished)
	barley kernels and leaf	HG532070	Korhola *et al*. (2014
Uncultured fungus	Ericaceae roots	HQ260042	Walker *et al.* (2011)
	cleaned rice	AB235999	Ikeda *et al.* (2007)
Uncultured fungus clone	*Axonopus compressus* soil	HQ436080	Kee & Chia (unpublished)
Uncultured *Ustilago*	tomato rhizosphere	KF493994	Johnston-Monje *et al.* (unpublished)

* type collections are highlighted in bold

The reaction was performed in a thermocycler (Eppendorf Mastercycler 96 vapo protect, Eppendorf, Hamburg) with an initial denaturation at 95 °C for 4 min, 36 PCR cycles of denaturation at 95 °C for 40 s, annealing at 56 °C for 40 s and elongation at 72° C for 60 s, followed by a final elongation at 72° C for 4 min. The resulting amplicons were sequenced at the Biodiversity and Climate Research Centre (BiK-F) laboratory using the PCR primers. Sequences were deposited in GenBank (NCBI, Table 1).

Phylogenetic reconstructions

The dataset included sequences of *Moesziomyces* species sexual and asexual morphs, both newly sequenced (Table 1) and downloaded from GenBank (Table 2). First all available sequences were extracted from NCBI on the basis of sequence similarity. Subsequently sequences were removed that were: (1) highly redundant with already-included ITS genotypes; or (2) of doubtful sequence quality, i.e. with mutations at positions highly conserved or with nucleotide changes only towards one end of the sequences.

Macalpinomyces eriachnes was selected as an outgroup, based on the phylogenetic tree in Shivas *et al.* (2013). Alignments were made using mafft v. 7 (Katoh & Standley 2013) employing the Q-INS-I algorithm and removing leading and trailing gaps. The resulting total alignment length was 576 bp. For phylogenetic analyses, Minimum Evolution (ME) analysis was done with Mega v. 6.06 (Tamura *et al.* 2013), using the Tamura-Nei substitution model, assuming partial deletion at a cut-off of 80 % and 1000 bootstrap replicates. Maximum Likelihood (ML) analysis was done using RAxML on the webserver TrEase (www.thines-lab.senckenberg.de/trease) with all parameters were set to default values. For Bayesian analysis also the webserver TrEase was used for calculating 10 M tree generations on four incrementally heated MC chains. The first 30 % of the trees obtained this way were discarded to ensure sampling of the stationary phase. All other parameters were set to default.

Morphological examination

For light microscopy, the herbarium specimens GLM-F105814 and GLM-F105812 were transferred to distilled water on a slide. Morphological examination was carried out using a Zeiss Imager M2 AX10 microscope (Carl Zeiss, Göttingen). Measurements of the spore balls and spores were performed at ×400. Measurements are reported as maxima and minima in parentheses, and the mean plus and minus the standard deviation of a number of measurements given in parenthesis; the means are given in italics (Table 3).

RESULTS

The isolated yeasts from fresh *Moesziomyces bullatus* samples from *Echinochloa crus-galli* and *E. muricatus* were fast-growing on SAM medium. The colour of the yeasts was cream to light reddish, and the shape of the colonies was regular and roundish.

A phylogenetic hypothesis for the sampled *Moesziomyces* species and the cultivated yeast asexual morphs is given in Fig. 1. The results of the Minimum Evolution, Maximum Likelihood and Bayesian Analyses were congruent. The clade comprising the type of *M. aphidis* also includes *M. bullatus s. str*. from *Echinochloa crus-galli* and *E. muricata*, as well as the sequence of the type of *M. rugulosa*, an isolate of *Moesziomyces* from *Albugo laibachii* on *Arabidopsis thaliana* and many other isolates not determined to the species level from various sources. While visual inspection of the alignments revealed that there was some sequence variation within the *Moesziomyces bullatus* clade, the relationships of the four subgroups was not resolved apart from the clustering

Table 3. Measurements from 25 spore balls and 100 teliospores for collections of *Moesziomyces bullatus* on *Echinochloa crus-galli* from two different clades.

	***Moesziomyces bullatus* ex *Echinochloa crus-galli*, GLM-F105814**						***Moesziomyces bullatus* ex *Echinochloa crus-galli*, GLM-F105812**					
	sporeballs			**spores**			**sporeballs**			**spores**		
No.	length	breadth	l/b	length	breadth	l/b	length	breadth	l/b	length	breadth	l/b
1	148,5	100	1,49	7,5	7,5	1	120,5	76,5	1,58	8,5	7	1,21
2	111	91	1,22	8	7,5	1,07	81	68,5	1,18	8	7,5	1,07
3	79	65	1,22	7	7	1	64,5	55,5	1,16	7,5	7	1,07
4	118,5	97,5	1,22	8,5	7	1,21	58,5	53	1,1	8	7	1,14
5	58,5	58,5	1	8	6	1,33	97	53,5	1,81	7,5	7,5	1
6	100,5	83,5	1,2	8	7,5	1,07	76	61,5	1,24	8	6	1,33
7	125	113	1,11	7,5	7	1,07	94	87	1,08	7,5	6,5	1,15
8	93	89,5	1,04	7,5	7	1,07	61	44	1,39	7,5	6,5	1,15
9	95,5	59	1,62	8	6	1,33	101,5	69	1,47	7,5	6,5	1,15
10	82	61	1,34	7	7	1	101	74	1,36	8,5	6	1,42
11	102	69,5	1,47	9	7	1,29	144,5	73,5	1,97	8	6,5	1,23
12	53	41,5	1,28	9	7	1,29	84	66	1,27	9	7	1,29
13	95	78	1,22	8,5	6,5	1,31	90	69,5	1,29	8	6,5	1,23
14	53	41,5	1,28	7,5	6,5	1,15	83,5	61	1,37	8,5	6,5	1,31
15	138,5	96,5	1,44	8,5	6,5	1,31	96,5	51,5	1,87	8,5	7,5	1,13
16	93	71,5	1,3	7	7	1	111,5	78,5	1,42	7,5	6,5	1,15
17	115	91	1,26	7	6,5	1,08	91,5	68,5	1,34	7	6,5	1,08
18	71	49,5	1,43	7,5	6,5	1,15	68,5	49,5	1,38	7	5,5	1,27
19	111,5	104	1,07	7,5	7	1,07	96	82,5	1,16	8,5	7	1,21
20	117,5	95,5	1,23	7,5	7,5	1	84,5	78,5	1,08	7,5	7	1,07
21	92,5	75,5	1,23	7,5	7	1,07	86,5	62	1,4	7,5	6,5	1,15
22	80,5	68	1,18	8	7	1,14	122,5	86	1,42	8,5	7,5	1,13
23	156,5	97,5	1,61	8	7	1,14	113,5	78,5	1,45	7,5	5,5	1,36
24	58,5	53,5	1,09	8,5	7	1,21	106	105	1,01	8	6	1,33
25	52,5	52	1,01	8,5	6	1,42	105,5	84,5	1,25	7	6	1,17
26				7	6,5	1,08				7	7	1
27				7,5	7,5	1				6,5	6,5	1
28				8,5	8	1,06				6,5	5,5	1,18
29				6,5	6	1,08				8,5	7,5	1,13
30				7	6	1,17				8	6,5	1,23
31				7,5	6	1,25				8	6	1,33
32				8	7,5	1,07				7,5	6,5	1,15
33				7	6,5	1,08				7	6,5	1,08
34				7	6,5	1,08				8,5	8	1,06
35				8,5	7,5	1,13				8	7,5	1,07
36				7	6	1,17				8	7,5	1,07
37				7,5	6	1,25				8,5	7	1,21
38				7	6	1,17				8	7	1,14
39				7,5	6,5	1,15				8	6,5	1,23
40				8	6,5	1,23				6,5	6	1,08
41				6,5	6	1,08				7	7	1
42				6,5	6	1,08				8,5	6	1,42
43				8,5	6,5	1,31				7	6,5	1,08
44				8,5	7,5	1,13				7	7	1
45				7,5	6,5	1,15				8	6,5	1,23
46				8,5	7	1,21				8,5	7	1,21

Table 3. (Continued).

	Moesziomyces bullatus ex *Echinochloa crus-galli*, GLM-F105814						*Moesziomyces bullatus* ex *Echinochloa crus-galli*, GLM-F105812					
	sporeballs			spores			sporeballs			spores		
No.	length	breadth	l/b	length	breadth	l/b	length	breadth	l/b	length	breadth	l/b
47				7	7	1				7,5	6,5	1,15
48				7	6,5	1,08				8	7,5	1,07
49				6,5	6,5	1				8	6	1,33
50				9	7	1,29				7,5	7	1,07
51				8	6	1,33				6	5,5	1,09
52				8	6	1,33				7,5	6	1,25
53				7,5	7	1,07				7	7	1
54				7	6,5	1,08				8,5	6,5	1,31
55				7	7	1				8	8	1
56				9	7,5	1,2				8	7	1,14
57				7,5	6,5	1,15				7	6,5	1,08
58				7,5	6,5	1,15				7	7	1
59				8	6,5	1,23				7,5	6,5	1,15
60				8	6,5	1,23				7,5	7	1,07
61				7,5	6,5	1,15				7,5	6,5	1,15
62				8,5	7	1,21				8,5	7	1,21
63				8	8	1				8,5	7,5	1,13
64				8	7,5	1,07				8	6,5	1,23
65				7,5	7,5	1				9	7	1,29
66				7,5	7,5	1				9	7,5	1,2
67				7,5	7	1,07				7,5	7	1,07
68				7	5,5	1,27				7,5	5,5	1,36
69				8,5	7	1,21				7,5	6	1,25
70				7,5	7	1,07				7,5	6,5	1,15
71				8	7,5	1,07				8,5	6,5	1,31
72				8	7	1,14				8,5	6,5	1,31
73				8	6,5	1,23				9,5	7	1,36
74				7,5	7	1,07				8,5	6,5	1,31
75				7,5	7	1,07				8,5	7	1,21
76				8	7	1,14				9,5	6,5	1,46
77				7,5	7	1,07				9	7	1,29
78				8	6,5	1,23				7,5	6,5	1,15
79				8,5	6,5	1,31				9	7	1,29
80				8	6	1,33				7,5	7	1,07
81				8	5,5	1,45				9	7	1,29
82				8	5,5	1,45				8	6,5	1,23
83				7	6,5	1,08				8,5	6,5	1,31
84				7	6,5	1,08				7,5	6,5	1,15
85				8,5	7	1,21				7,5	7	1,07
86				7,5	5,5	1,36				9	7	1,29
87				9	6,5	1,38				8	8	1
88				7,5	6,5	1,15				8,5	7	1,21
89				7	6	1,17				8,5	7	1,21
90				8	8	1				7,5	7,5	1
91				9	6,5	1,38				7,5	6	1,25
92				8	7,5	1,07				8,5	6,5	1,31

Table 3. (Continued).

	***Moesziomyces bullatus* ex *Echinochloa crus-galli*, GLM-F105814**						***Moesziomyces bullatus* ex *Echinochloa crus-galli*, GLM-F105812**					
	sporeballs			**spores**			**sporeballs**			**spores**		
No.	length	breadth	l/b	length	breadth	l/b	length	breadth	l/b	length	breadth	l/b
93				8	7,5	1,07				9	6,5	1,38
94				7,5	7,5	1				7,5	6	1,25
95				7,5	6,5	1,15				6,5	6	1,08
96				8	7,5	1,07				7,5	7	1,07
97				7,5	7	1,07				8,5	7,5	1,13
98				8,5	7	1,21				7,5	6	1,25
99				10	7	1,43				7,5	6,5	1,15
100				7,5	7,5	1				8	7	1,14

of *Pseudozyma aphidis* and the majority of *M. bullatus* isolates with the clade containing the type of *P. rugulosa*. Collections from *E. crus-galli* with smut symptoms were present in two different clades. The morphological investigation of a sexual morph from each clade (GLM-F105812 and GLM-F105814) revealed no morphological differences. *Moesziomyces bullatus* clustering within the majority of *M. aphidis* had the following spore characteristics: sporeballs variable in shape and size, 52.5–156.5 x 41.5–113 µm, spores ovoid, globose, often irregular, pale yellow-brown, (6.5–)7–*7.8*–8.5(–10) × (5.5–)6–*6.8*–7.5(–8) µm, a length/breadth ratio of 1.01–1.15–1.39 (*n* = 100) (Fig. 2). In comparison, the collection of *M. bullatus* clustering together with the sequence of the type species of *M. rugulosus* showed the following spore characteristics: sporeballs variable in shape and size, 58.5–144.5 x 44–105 µm, spores ovoid, globose, often irregular, pale yellow-brown, (6–)7–*7.9*–8.5(–9.5) × (5.5–)6–*6.7*–7.5(–8) µm, a length/breadth ratio of 1.01–1.19–1.38 (*n* = 100) (Fig. 2). The sister group to *M. bullatus* was formed by *M. antarcticus*. The four lineages of *M. bullatus* formed an isolated clade with high to maximum support in all analyses together with samples classified as *M. antarcticus*.

Apart from the groups mentioned above, four additional distinct groups were revealed. Two of these corresponded to lineages formed by sexual smuts of the genus *Moesziomyces* isolated from plants with smut disease symptoms. One of these corresponded to *M. bullatus s. lat*. on *Paspalum distichum*, and the other to *Eriomoeszia eriocauli*. The remaining two lineages formed a monophyletic clade with high support in Minimum Evolution Analysis. One lineage included sequences of yeasts classified as *M. parantarcticus,* the other a sexual morph of a plant pathogenic fungus of the genus *Moesziomyces* from *Pennisetum glaucum*, as well asexual morphs isolated from symptom-free barley and a preterm infant.

DISCUSSION

Moesziomyces is a morphologically well-defined genus in the smut fungi, mainly characterised by ruptured sterile cells in the sori around the spores. The genus was believed to be monotypic by Vánky (2012), but phylogenetic investigations of the past decade have shown that several species previously assigned to the asexually typified yeast genus *Pseudozyma,* were closely related to *Moesziomyces bullatus* (Begerow *et al.* 2000, 2006, Wang *et al.* 2006, 2015). In the latest edition of the *International Code of Nomenclature for algae, fungi and plants* (ICN) it is ruled that the dual naming for asexual and sexual morphs of fungi has been discontinued (McNeill *et al.* 2012). Consequently, Wang *et al.* (2015) attempted to resolve the names of species placed in the genus *Pseudozyma* as far as possible, and combined those related to *Moesziomyces bullatus s.lat*. into *Moesziomyces*.

The yeast asexual morphs were, for example, found to live epiphytically on different hosts (Boekhout 1995), but also to occur on a variety of other substrates. Due to their asexual reproduction with pullulating and division, it is possible for them to colonize suitable habitats in a short period of time. Of these yeasts, *Pseudozyma aphidis* is often considered as a biocontrol agent for plant pathogenic fungi (Avis *et al.* 2001, Buxdorf *et al.* 2013). Thus it is noteworthy that one isolate of this species co-occurred in *Albugo laibachii* lesions on *Arabidopsis thaliana*, indicating only no or only limited antagonism against this specialised white blister rust species (Thines *et al.* 2009). It is commonly believed that most *Pseudozyma* species have lost pathogenicity, which is seemingly supported by recent genomic analyses (Lefebvre *et al.* 2013). However, it should be noted that if a different start codon is taken for translation than the one predicted, all *Pseudozyma* yeasts included by Lefebvre *et al.* (2013) have a functional copy of PEP1, a conserved effector among smut fungi of the *Ustilaginales* (Sharma *et al.* 2014, Hemetsberger *et al.* 2015), suggesting the possibility of a misannotation of the start codon. In-depth bioinformatic analyses and functional testing will be needed to clarify this situation.

Deducing the conspecificity of *Moesziomyces bullatus* with *Pseudozyma aphidis* and *P. rugulosa* was not possible for Wang *et al.* (2015), as they did not include sequences from the type host of *M. bullatus*, *Echinochloa crus-galli*, but only from *M. verrucosus* on *Paspalum distichum*, which they erroneously assumed to be conspecific with *M. bullatus*. However, the smut sexual morphs from the type host, *E. crus-galli* from Germany, are placed in two of the four subclusters

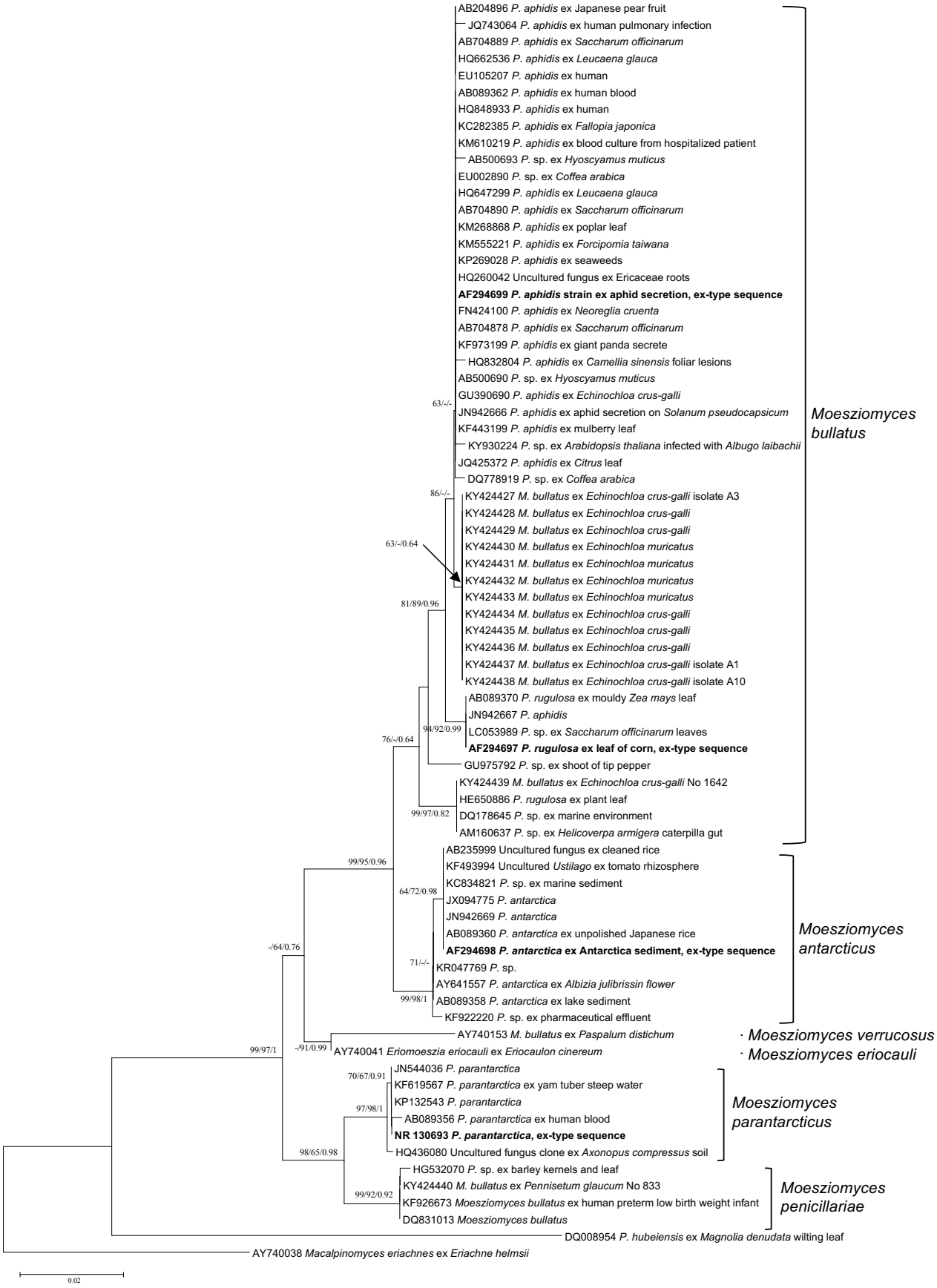

Fig. 1. Phylogenetic tree based on Minimum Evolution analyses of nrITS sequences of *Moesziomyces* spp., rooted with *Macalpinomyces eriachnes*. Numbers on branches denote bootstrap support in Minimum Evolution, Maximum Likelihood and *a posteriori* probabilities from Bayesian Analyses. Values below 55 % are not shown. The bar indicates expected substitutions per site. GenBank numbers precede taxon names, and are followed by the name of the host or isolation source of the fungus.

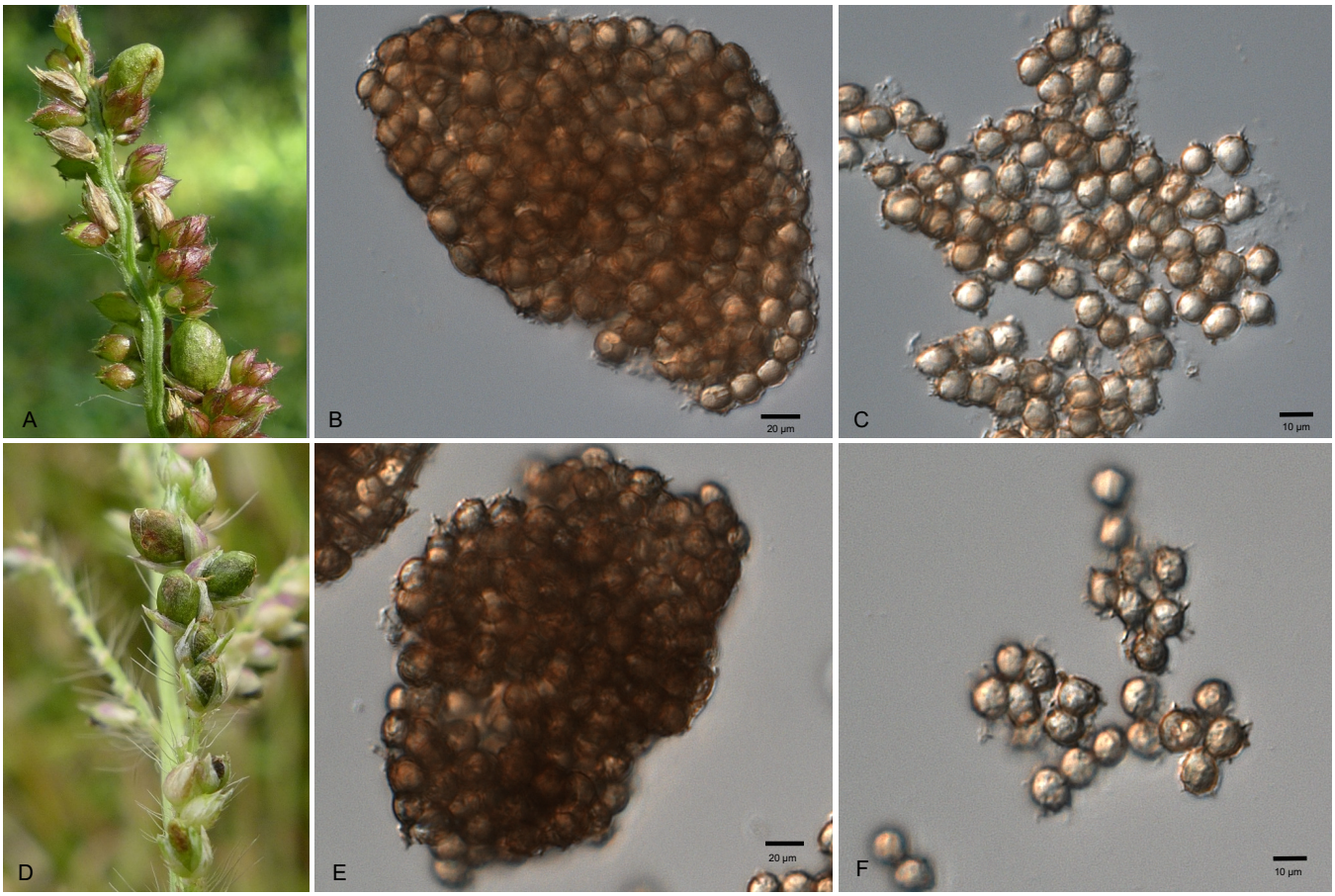

Fig. 2. Sori and spores of *Moesziomyces bullatus* on *Echinochloa crus-galli*. **A, D.** Sori. **B, E.** Teliospore balls. **C, F.** Teliospores. A–C (GLM-F105812), D–F (GLM-F105814).

of a larger cluster, which is interpreted here as representing *M. bullatus*. It is noteworthy that three of the four subclusters of *M. bullatus* contain environmental samples from various sources. In conjunction with the ease of cultivation observed for *M. bullatus* from *E. crus-galli*, it is concluded that unlike the vast majority of genera of *Ustilaginales*, the asexual yeast morph plays a major role as a proliferating life-cycle stage in *Moesziomyces* and that the plant-parasitic dikaryophase is probably mainly important for maintaining the possibility of sexual recombination. As the two subclades previously referred to as *M. aphidis* and *M. rugulosus* are interspersed with the morphologically identically lineages of *M. bullatus* from *E. crus-galli*, they are probably better included in *M. bullatus* until more sequence data become available. It seems probable that, with the inclusion of additional smut samples from *Echinochloa*, additional smut-causing members of the subclades will be discovered. Sampling in Africa seems to be promising in this respect, as the species diversity of *Echinochloa* is highest on this continent. Also, the notion that yeasts of the subclade containing the ex-type culture of *Pseudozyma aphidis* can withstand high temperatures, such as the human body temperature, is suggestive of a subtropical to tropical origin of this lineage.

Further, our investigations show that the older name *M. eriocauli* for *Eriomoeszia eriocauli*, should be taken up again, as this species was found embedded within *Moesziomyces*.

With the synonymy of the generic name *Eriomoeszia* and the reappraisal of the hardly used *Moesziomyces* names of the smut fungi of *Paspalum* and *Pennisetum*, *Moesziomyces* now includes six species. It is, however, likely that additional species will have to be added because smut samples from some *Poaceae* genera listed as host plants for the *M. bullatus* complex in Vánky (2012) could not be included in the current study, such as smuts from *Leersia, Panicum, Polytrias*, and *Uranthoecium*. Given the apparently high host specificity of *Moesziomyces* species, it seems likely that these pathogens represent species independent from *M. bullatus*.

TAXONOMY

Based on the phylogenetic data presented here, the following nomenclature and taxonomic changes are made.

Moesziomyces antarcticus (Goto *et al.*) Q.M. Wang *et al.*, *Stud. Mycol.* **81**: 81 (2015).

Basionym: *Sporobolomyces antarcticus* Goto *et al.*, *Mycologia* **61**: 759 (1969).

Synonyms: *Candida antarctica* (Goto *et al.*) Kurtzman *et al.*, *Yeasts*: 86 (1983).

Vanrija antarctica (Goto *et al.*) R.T. Moore, *Bibltheca Mycol.* **108**: 167 (1987).

Pseudozyma antarctica (Goto *et al.*) Boekhout, *J. Gen. Appl. Microbiol.* **41**: 364 (1995).

Moesziomyces bullatus (J. Schröt.) Vánky, *Bot. Notiser* **130**: 133 (1977).
Basionym: *Sorosporium bullatum* J. Schröt., *Abh. Schles. Ges. Vaterl. Cult., Abth. Naturwiss*. **72**: 6 (1869).
Synonyms: *Tolyposporium bullatum* J. Schröt, *in* Cohn., *Krypt. Fl. Schles*. **3**(1): 276 (1887).
Sterigmatomyces aphidis Henninger & Windisch, *Arch. Microbiol*. **105**: 50 (1975).
Tolypoderma bullata (J. Schröt.) Thirum. & M.J. O`Brien, *Friesia* **11**: 190 (1978) ["1977"].
Sporothrix rugulosa Traquair *et al.*, *Canad. J. Bo*t. **66**: 929 (1988).
Stephanoascus rugulosus Traquair *et al*., *Canad. J. Bot*. **66**: 929 (1988).
Pseudozyma aphidis (Henninger & Windisch) Boekhout, *J. Gen. Appl. Microbiol*. **41**: 364 (1995).
Pseudozyma rugulosa (Traquair, *et al.*) Boekhout & Traquair, *J. Gen. Appl. Microbiol*. **41**: 364 (1995).
Moesziomyces aphidis (Henninger & Windisch) Q.M. Wang *et al*., *Stud. Mycol*. **81**: 81 (2015).
Moesziomyces rugulosus (Traquair, *et al*.) Q.M. Wang *et al*., *Stud. Mycol*. **81**: 81 (2015).

Moesziomyces eriocauli (G.P. Clinton) Vánky, *Nordic J. Bot*. **6**: 71 (1986).
Basionym: *Tolyposporium eriocauli* G.P. Clinton, *Rhodora* **2**: 82 (1901).
Synonyms: *Dermatosorus eriocauli* (G.P. Clinton) M.D. Whitehead & Thirum., *Mycologia* **64**: 128 (1972).
Tolypoderma eriocauli (G.P. Clinton) Thirum., *Friesia* **11**: 191 (1978).
Eriomoeszia eriocauli (G.P. Clinton) Vánky, *Mycol. Balcanica* **2**: 106 (2005).

Moesziomyces parantarcticus (Sugita *et al*.) Q.M. Wang *et al*., *Stud. Mycol*. **81**: 81 (2015).
Basionym: *Pseudozyma parantarctica* Sugita *et al*., *Microbiol. Immun*. **47**: 186 (2003).

Moesziomyces verrucosus (J. Schröt.) J. Kruse & Thines, **comb. nov.**
MycoBank MB819410
Basionym: *Ustilago verrucosa* J. Schröt., *Hedwigia* **35**: 214 (1896).
Synonyms: *Tolyposporium evernium* Syd., *Ann. Mycol*. **37**: 443 (1939).
Tolyposporium paspali Langdon, *Univ. Queensland Dept. Biol. Pap* **2**(9): 4 (1948).
Moesziomyces evernius (Syd.) Vánky, *Bot. Notiser* **130**: 135 (1977).
Tolyposporidium evernium (Syd.) Thirum. & Neerg., *Friesia* **11**: 180 (1978) ["1977"].

Moesziomyces penicillariae (Bref.) Vánky, *Bot. Notiser* **130**: 135 (1977).
Basionym: Tolyposporium penicillariae Bref., *Unters. Gesammtgeb. Mykol.* **12**: 154 (1895).
Synonym: *Tolyposporidium penicillariae* (Bref.) Thirum. & Neerg., *Friesia* **11**: 181 (1978) ["1977"].

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