



Pattersoniomyces tillandsiae gen. et comb. nov.: linking sexual and asexual morphs of the only known smut fungus associated with Bromeliaceae

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Received: 7 June 2017 / Accepted: 20 July 2017 / Published online: 3 August 2017
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Abstract The enigmatic species *Ustilago tillandsiae* is the only known smut fungus associated with Bromeliaceae. Its generic position is evaluated by morphological, physiological, and molecular phylogenetic analyses using large subunit rDNA sequences. Phylogenetic analyses resolved *U. tillandsiae* as a member of the Ustilaginales in a sister relationship to the lineage containing *Tranzscheliella* species. However, *U. tillandsiae* differs from *Tranzscheliella* species by the development of sori in flowers, a different structure of sori and a different type of spore ornamentation. Consequently, a new genus *Pattersoniomyces* is described to accommodate *U. tillandsiae*. The new combination *Pattersoniomyces tillandsiae* is substantiated. In the sexual stage (teleomorph), this species infects bromeliads: *Tillandsia flabellata*, *Tillandsia leiboldiana*, and *Tillandsia* sp. in Central America between southern Mexico and Costa Rica. The yeast stage (anamorph) of *P. tillandsiae* was found associated with the phylloplane of *Canistrum improcerum* and

in water tanks (phytotelmata) of *Vriesea minarum*, two bromeliads occurring in northeast and southeast Brazil, respectively. The link between the teleomorph and anamorphic strains is supported by identical sequences of the D1/D2 domains of the large subunit rDNA. *Pattersoniomyces* represents the tenth endemic smut genus to the Americas, but the only one that occurs in both North and South America, being a truly neotropical genus. The host plant families of Ustilaginales are extended to the Bromeliaceae. As far as we know, *Pattersoniomyces* represents the single event of a host jump from Cyperaceae or Poaceae to Bromeliaceae, apparently without further species radiation on multiple bromeliad species and genera growing in South America.

Keywords Basidiomycota · Molecular phylogeny · Plant pathogens · Smut fungi · Ustilaginomycotina · Yeasts

Introduction

The generic classification of smut fungi (Ustilaginomycotina) has changed greatly during the recent two decades, principally in order to better reflect natural relationships and evolution within this group of Basidiomycota. The most drastic changes concerned the circumscription of *Ustilago* (Pers.) Roussel. This genus, typified by *Ustilago hordei* (Pers.) Lagerh. (Vánky 2013; McTaggart et al. 2016), was initially described as a subgenus within *Uredo* Pers. (Persoon 1801) and was soon elevated to genus rank (Roussel 1806). *Ustilago* became a catch-all for diverse more or less closely related (though sometimes distantly related) smuts, mostly characterized by having single spores and lacking sterile cells (Zundel 1953; McTaggart et al. 2012c). The heterogeneity of the genus *Ustilago* was discussed by several researchers (e.g., Lindeberg 1959);

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however, only ultrastructural and molecular phylogenetic analyses (Bauer et al. 1997, 2001; Begerow et al. 1997, 2006, 2014) made a significant step towards resolving its generic boundaries. Currently, *Ustilago* is restricted to species parasitic on Poaceae having single spores germinating with phragmobasidia and lacking both columellae and sterile cells (McTaggart et al. 2012a) and to some associated pseudozyma-like anamorphic species (Wang et al. 2015). Many species originally classified in *Ustilago* have been reallocated to other genera placed in diverse families and orders of the Ustilaginomycotina and even in a different subphylum, the Pucciniomycotina (Piepenbring et al. 1996; Vánky 1998; Ershad 2000; Vánky 2002; Bauer et al. 2007, 2008; Vánky et al. 2008; Lutz et al. 2012; McTaggart et al. 2012b; Piątek et al. 2013).

The enigmatic *Ustilago tillandsiae* F. Patt., which is the only smut fungus known on Bromeliaceae, is one of the few remaining species of *Ustilago* that does not occur on Poaceae, and for which the systematic position is not resolved. *U. tillandsiae* was described as a smut fungus parasitic on *Tillandsia* sp. in Costa Rica (type) and *Tillandsia leiboldiana* Schldl. in Mexico (in Clinton 1902, but the species name is attributed to Flora W. Patterson). In the protologue, Clinton (1902) expressed some uncertainty whether it is a smut fungus or a hyphomycete but suspected that it was rather a smut. This view was adopted by Durán (1987) and Piepenbring (1996), who included *U. tillandsiae* in the monograph of Mexican and Costa Rican smut fungi, respectively. Piepenbring (1996) concluded that without germination and ultrastructural data the systematic placement of this species could not be resolved. Subsequently, Piepenbring (2003) excluded *U. tillandsiae* from the smut fungi, indicating that it was most probably an imperfect fungus. In contrast, Zundel (1953) placed *U. tillandsiae*, without any doubt, in his world monograph of smut fungi. Vánky (2012) included it in a chapter of doubtful, excluded or invalidly published taxa, but commented that this species could be a smut fungus and probably belongs to a distinct genus.

In addition to germination and ultrastructural characters, molecular phylogenetic analyses are suitable to resolve the taxonomic position of smut fungi. In the absence of recently collected material for germination and ultrastructural analyses, a herbarium specimen of *Ustilago tillandsiae* from Honduras collected in 1976 was used to isolate DNA and to conduct molecular phylogenetic analyses based on sequences of the D1/D2 domains of the large subunit rDNA (LSU). In the meantime, four yeast strains identified as *Pseudozyma* sp. were isolated from the phylloplane of *Canistrum improcerum* Leme & J.A. Siqueira and from water tanks (phytotelmata) of *Vriesea minarum* L.B. Sm., two bromeliads occurring in Brazil. Sequences of the D1/D2 domains of the large subunit rDNA of these isolates were identical to the LSU sequence of the

specimen from Honduras, which suggested that these strains were conspecific. The aim of the present study was to resolve the phylogenetic placement of *U. tillandsiae* using LSU sequence data from the Honduras specimen and the four new Brazilian strains of this species, and consequently to clarify the generic position of this fungus. To this end, several additional herbarium collections were also analyzed applying light and scanning electron microscopic examinations.

Materials and methods

Specimen sampling and documentation

The historical teleomorphic specimens of *Ustilago tillandsiae* examined in this study are listed in Table 1. The voucher specimens are deposited in the US National Fungus Collections, USDA-ARS (herbarium BPI). Yeast strains were isolated from the phylloplane of *Canistrum improcerum* (subfamily Bromelioideae, Bromeliaceae), and from the water deposited in the phytotelmata of *Vriesea minarum* (subfamily Tillandsioideae, Bromeliaceae). Leaves of *C. improcerum* were collected in an Atlantic rain forest site of the Serra da Saudinha in the city of Maceió, Alagoas, Brazil. The leaf samples were aseptically collected in February 2015. Yeast isolation was described by Landell et al. (2015). Collections of *V. minarum* were performed from rocky outcrops in the Serra da Piedade and Serra do Cipó regions, Minas Gerais, Brazil. These areas are of rupestrian fields (“campos rupestres”) characterized by the presence of sclerophyllous shrubs. Samples of phytotelmata were collected from 30 individuals of *V. minarum* in each region. Collections were performed in April 2009 in the Serra da Piedade region and in March 2014 in the Serra do Cipó region. Water samples were collected aseptically with sterile pipettes and transferred to sterile flasks, which were transported to the laboratory on ice for processing within 24 h. Aliquots of 0.1 mL of appropriate decimal dilutions were spread on YM agar (yeast extract malt extract agar; glucose 1.0%, peptone 0.5%, yeast extract 0.3%, malt extract 0.3%, agar 2.0%) and supplemented with 0.02% chloramphenicol and 0.0033% rose Bengal (Gomes et al. 2015). The plates were incubated at 25 °C for 3 to 8 days, after which the different yeast morphotypes were counted. All strains were stored at –80 °C for further identification. The yeast strains examined in this study are listed in Table 2. They are deposited in the Collection of Microorganisms and Cells of Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil. Nomenclatural novelties were registered in MycoBank (www.MycoBank.org, Crous et al. 2004).

Table 1 List of examined specimens of the sexual stage of *Pattersoniomyces tillandsiae*, with host species, spore size range, mean spore size with standard deviation, GenBank accession number, and reference specimen

Host species	Spore size range (μm)	Mean spore size with standard deviation (μm)	GenBank acc. no. (LSU)	Reference specimen
<i>Tillandsia flabellata</i>	(5.5–)6.5–11.5(–12.5) × (5.0–)6.0–9.5(–10.5)	$8.7 \pm 1.8 \times 7.5 \pm 1.4$	N/A	Mexico, Vera Cruz (intercepted at Brownsville-007786-Texas), 21 Nov. 1977, leg. J.M. Van Valkenburgh, BPI 168187
<i>Tillandsia leiboldiana</i>	(6.0–)7.0–11.5(–16.0) × 6.0–10.5	$9.8 \pm 2.3 \times 8.1 \pm 1.1$	JQ362412	Honduras (intercepted at Miami-014910-Florida), 17 Nov. 1976, leg. F. Matthews, BPI 168184
<i>Tillandsia leiboldiana</i>	(5.5–)7.0–11.0(–12.5) × 5.0–9.5(–10.5)	$8.8 \pm 1.4 \times 7.5 \pm 1.3$	N/A	Mexico, Valle de Cordova, 23 Jan. 1865, leg. Bourgeau 64, BPI 168185 – paratype
<i>Tillandsia leiboldiana</i>	(5.0–)6.0–10.5 × 5.0–9.5	$7.6 \pm 1.4 \times 6.9 \pm 1.1$	N/A	Guatemala (intercepted at Miami-010189-Florida), 23 Jan. 1975, leg. E.B. Lee, BPI 168186

Smut specimens (sexual morph, teleomorph): morphological examination

For light microscopy (LM), dried fungal spores of the investigated specimens were mounted in lactic acid, heated to boiling point, and then examined under a Nikon Eclipse 80i light microscope at a magnification of $\times 1000$, using Nomarski optics (DIC). Spores were measured using the NIS-Elements BR 3.0 imaging software. Extreme measurements were adjusted to the nearest 0.5 μm . The spore size range, mean, and standard deviation of 50 spore measurements from each specimen (100 spore measurements from the paratype specimen) are shown in Table 1. The species description includes combined values from all measured specimens. LM micrographs were taken with a Nikon DS-Fi1 camera. The spore ornamentation in the specimen BPI 168184 from Honduras was studied using scanning electron microscopy (SEM). For SEM, dry spores were mounted on carbon tabs and fixed to an aluminum stub with double-sided transparent tape. The tabs were sputter-coated with carbon using a Cressington sputter-coater and viewed with a Hitachi S-4700 scanning electron microscope, with a

working distance of ca. 12 mm. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences, Jagiellonian University, Kraków (Poland).

Yeast strains (asexual morph, anamorph): morphological and physiological analyses

Yeast strains were characterized morphologically and by their growth responses on different carbon and nitrogen sources as recommended by Kurtzman et al. (2011). Presence of budding cells, hyphae, and pseudohyphae was investigated by cultivation on potato dextrose agar (PDA), diluted V8, and YM agar at 20 and 25 °C for up to 21 days and MM containing 0.5% of ammonium sulfate at 30 °C for up to 5 days. Possible sexual reproduction was investigated on 5% malt extract agar (*w/v*), corn meal, PDA, and diluted V8 agars at 17 and 25 °C for up to 30 days. The four yeast isolates were examined individually or mixed in pairs on these media.

Table 2 List of examined yeast strains of *Pattersoniomyces tillandsiae*, with source and locality, GenBank accession numbers, and reference strains

Source of yeast strain and locality	GenBank acc. no. (LSU/ITS)	Reference strain
Phylloplane of <i>Canistrum improcerum</i> , Serra da Saudinha, Alagoas, Brazil	KX348018/–	UFMG-CM-Y6109 (= BSS144)
Water tank of <i>Vriesea minarum</i> , Serra da Piedade, Minas Gerais, Brazil	JX280390/–	UFMG-BRO-110B
Water tank of <i>Vriesea minarum</i> , Serra do Cipó, Minas Gerais, Brazil	KT784807/KT321120	UFMG-CM-Y1455
Water tank of <i>Vriesea minarum</i> , Serra do Cipó, Minas Gerais, Brazil	KT784808/–	UFMG-CM-Y1466

DNA extraction, PCR, and sequencing

The methods of isolation of genomic DNA from the Honduras specimen (BPI 168184), amplification of the LSU, purification of PCR products, sequencing, and processing of the raw data followed Lutz et al. (2004, 2012). Processing of the Brazilian yeast strains, including the sequencing the LSU and ITS rDNA regions, was performed as described previously (White et al. 1990; O'Donnell 1993; Lachance et al. 1999; Gomes et al. 2015). DNA sequences determined for this study were deposited in GenBank, accession numbers are given in Tables 1 and 2 and Fig. 1.

Phylogenetic analyses

To elucidate the phylogenetic position of *Ustilago tillandsiae* (using the Honduras specimen BPI 168184) and the Brazilian yeast strains, their LSU sequences were analyzed within a dataset covering all genera of Ustilaginales for which sequences were available in GenBank. If present in GenBank, the sequences of the respective type species of genera were used. Additionally, LSU sequences for all available species of smut genera that were the closest relatives of *U. tillandsiae*, namely *Leucocintractia* M. Piepenbr., Begerow & Oberw., *Tranzscheliella* Lavrov, and *Ustanciosporium* Vánky, were added. GenBank accession numbers of the sequences used (Begerow et al. 1997, 2006; Piepenbring et al. 1999, 2002; Avis et al. 2001; Sugita et al. 2003; Hendrichs et al. 2005; Stoll et al. 2005; Matheny et al. 2006; Vánky et al. 2006, 2013; Bauer et al. 2007; González et al. 2007; Kellner et al. 2011; Morita et al. 2011; Lutz et al. 2012; McTaggart et al. 2012a; Li et al. 2017) are given in Fig. 1.

Sequence alignment was obtained using MAFFT 7.215 applying the L-INS-i option (Katoh and Standley 2013). To obtain reproducible results, manipulation of the alignment by hand as well as manual exclusion of ambiguous sites were avoided, as suggested by Giribet and Wheeler (1999) and Gatesy et al. (1993), respectively. Instead, highly divergent portions of the alignment were omitted using GBlocks 0.91b (Castresana 2000) with the following options: “Minimum Number of Sequences for a Conserved Position”: 28, “Minimum Number of Sequences for a Flank Position”: 28, “Maximum Number of Contiguous Non-conserved Positions”: 8, “Minimum Length of a Block”: 5, and “Allowed Gap Positions”: “With Half.” The alignment [new number of positions: 623 (29% of the original 2108 positions), number of variable sites: 273] was used for phylogenetic analyses using a maximum likelihood (ML) and a Bayesian approach (BA) following Vasighzadeh et al. (2014). Trees were rooted with *Melanotaenium endogenum* (Unger) de Bary, *Pericladium grewiae* Pass., *Restiosporium*

meneyae Vánky, and *Websdanea lyginiae* (Websdane, Sivasith., K.W. Dixon & Pate) Vánky following Begerow et al. (2006) and Lutz et al. (2012).

Results

Morphological and physiological analyses

The morphological and physiological characteristics of analyzed smut specimens and yeast strains are included in the species description and depicted in illustrations (Figs. 2, 3, 4, 5, and 6).

Phylogenetic analyses

The LSU sequences of the four Brazilian yeast isolates and the sequence of the *Ustilago tillandsiae* specimen from Honduras were identical over their full length. Blast searches (Altschul et al. 1997) for both the LSU sequences and the single ITS sequence (from the yeast culture UFMG-CM-Y1455, GenBank acc. no. KT321120) obtained from the yeast isolates and *U. tillandsiae*, respectively, revealed closest similarity to various species of Ustilaginaceae. The closest hits using the ITS sequences were *Tranzscheliella williamsii* (Griffiths) Dingley & Versluys (in GenBank cited as *Ustilago williamsii*: JN367310, max score/identity, 693/85%), *Ustilago* sp. (AM262979, 684/86%), and another *T. williamsii* sequence (in GenBank cited as *U. williamsii*: AF045869, 576/85%).

The different runs of the BA that were performed and the ML yielded consistent topologies. To illustrate the results, the consensus tree of one run of the BA is presented (Fig. 1). In all analyses, the *Ustilago tillandsiae* specimen clustered together with the four Brazilian yeast strains within the Ustilaginaceae in a sister relationship to the *Tranzscheliella* species sampled altogether forming a monophyletic group with the sister taxa *Leucocintractia* and *Ustanciosporium*.

Taxonomy

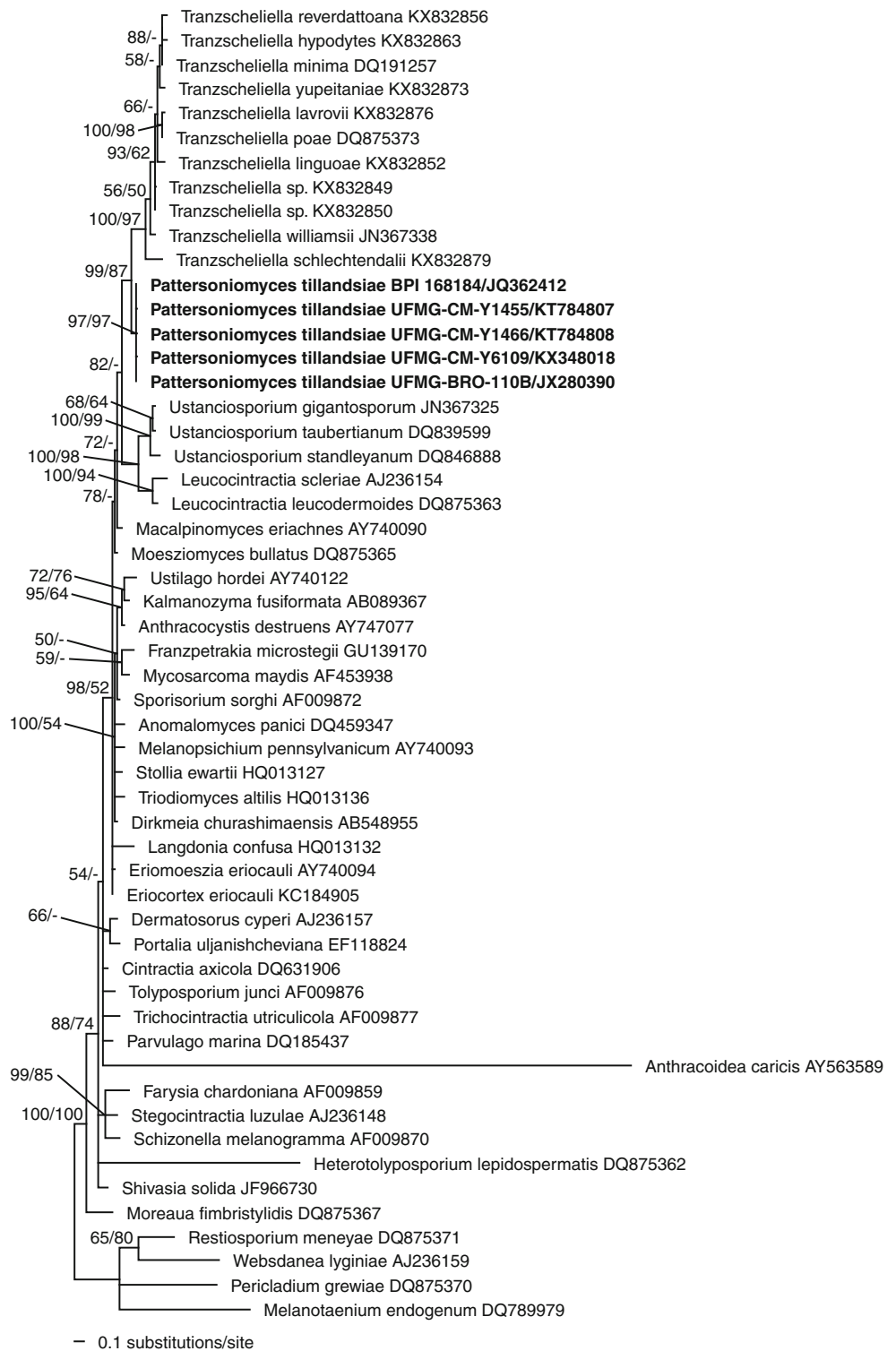
Pattersoniomyces Piątek, M. Lutz & C.A. Rosa, **gen. nov.**

Mycobank no. MB821983

Etymology: The genus is named in honor of Flora Wambaugh Patterson (1847–1928), an American mycologist and the first woman mycologist working at the US Department of Agriculture (Rossman 2002). Among others, she described *Ustilago tillandsiae*, which is the type species of the new genus.

Description: Teleomorph parasitic on living plants. Sori in the flowers of *Tillandsia* spp. (Bromeliaceae), producing naked, dusty spore masses around the rudimentary developed

Fig. 1 Bayesian inference of phylogenetic relationships within the sampled Ustilaginales: Markov chain Monte Carlo analysis of an alignment of LSU base sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees, and default starting parameters of the DNA substitution model. A 50% majority-rule consensus tree is shown computed from 75,000 trees that were sampled after the process had reached stationarity. The topology was rooted with *Melanotaenium endogenum*, *Pericladium grewiae*, *Restiosporium meneyae*, and *Websdanea lyginiae*. Numbers on branches before slashes are estimates for a posteriori probabilities; numbers on branches after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site



innermost floral organs, peridium lacking, sori protected only by perianths and bracts. Spore balls absent. Spores pigmented (brown), collapsed or hemispherically cupped, ornamented with fine ridges. Anamorph free-living, pseudozyma-like, producing pseudomycelium and true mycelium, cells budding

on short denticles, assimilating myo-inositol, showing positive diazonium blue B reaction, negative starch-like production. Anamorph linked with the teleomorph by DNA sequence analyses. Type: *Pattersoniomyces tillandsiae* (F. Patt.) Piątek, M. Lutz, M.F. Landell & C.A. Rosa.



Fig. 2 Macroscopic symptoms of the infection of *Tillandsia leiboldiana* by the sexual stage of *Pattersoniomyces tillandsiae* (all from BPI 168184): **a** sori in all flowers of the inflorescences, most are hidden by perianths and bracts; **b–d** partly exposed sori with naked, dusty spore

masses around the destroyed innermost floral organs, note remnants of the staminal filaments in the sori center indicated by *white arrows*. Scale bars = 1 cm

Pattersoniomyces tillandsiae (F. Patt.) Piątek, M. Lutz, M.F. Landell & C.A. Rosa, **comb. nov.**

Figs. 2, 3, 4, 5, and 6

Mycobank no. MB821984

Basionym: *Ustilago tillandsiae* F. Patt., J. Mycol. 8: 135 (1902)

Description of the teleomorph: Sori in all flowers of the inflorescences of *Tillandsia* spp. suggesting systemic infection, 1–2 cm long, producing naked, dusty spore masses around the rudimentary developed innermost floral organs—staminal filaments and ovary remain partly intact in the sori center, anthers not developed, peridium lacking, sori protected only by closed perianths and bracts. Spores usually single, sometimes glued together forming small groups, variable in color, shape, and size (also between collections, see Table 1), pale brown to olive-brown, globose or subglobose, more or

less collapsed or hemispherically cupped, (5.0–)6.0–11.5(–16.0) × 5.0–10.5 μm, usually with one or exceptionally two spherical bodies (lipid granules) in the cytoplasm; wall even 0.4–0.8(–1.0) μm thick, somewhat darker than the rest of spore, often somewhat lighter on one side, surface variably ornamented, smooth or with brittle episporium breaking up into thin polygonal areas, cracked or covered with fine ridges as seen by LM, almost smooth, granulose or covered with fine, circular, or irregular ridges as seen by SEM.

Specimens examined: Guatemala (intercepted at Miami-010189-Florida), on *Tillandsia leiboldiana*, 23 Jan. 1975, leg. E.B. Lee (BPI 168186); Honduras (intercepted at Miami-014910-Florida), on *Tillandsia leiboldiana*, 17 Nov. 1976, leg. F. Matthews (BPI 168184); Mexico, Valle de Cordova, on *Tillandsia leiboldiana*, 23 Jan. 1865, leg. Bourgeau 64 (BPI 168185–paratype); Mexico, Vera Cruz

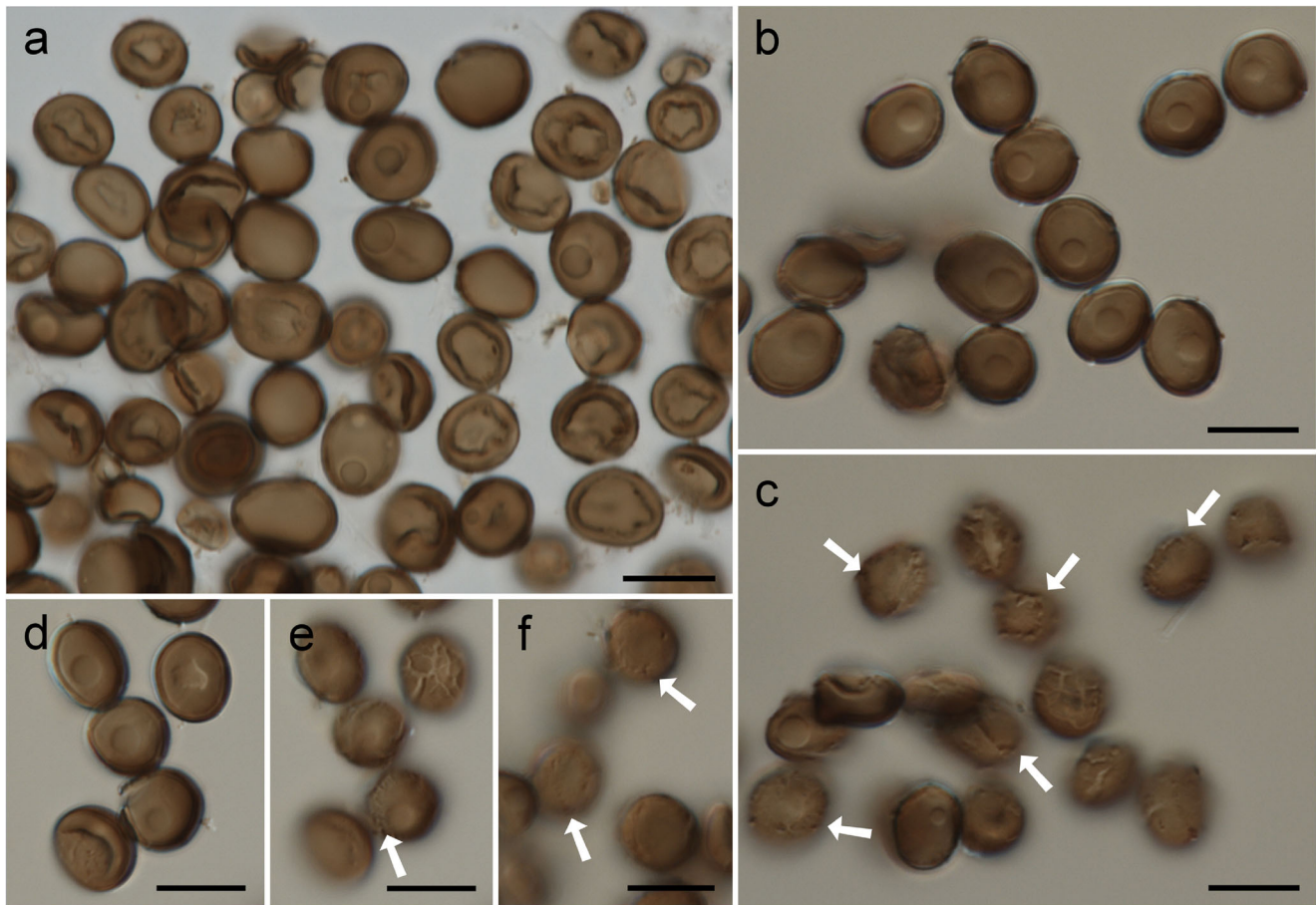


Fig. 3 Spores of the sexual stage of *Pattersoniomyces tillandsiae* (all from BPI 168184) seen by LM, median (**a**, **b**, **d**) and superficial (**c**, **e**, **f**) views, note cracked spore surface and fine ridges indicated by white arrows. Scale bars = 10 μ m

(intercepted at Brownsville-007786-Texas), on *Tillandsia flabellata*, 21 Nov. 1977, leg. J.M. Van Valkenburgh (BPI 168187).

Description of the anamorph: Growth on YM agar after 3 days at 25 °C: colonies smooth to wrinkled, butyrous, glistening, cream-colored to light salmon with an entire margin, cells ellipsoidal to fusoid and variable in size, 3–9 \times 1.5–3 μ m, budding is polar on a short denticle. Growth in Dalmau plate culture on cornmeal agar after 3 weeks: pseudomycelium and true mycelium formed. Sexual reproduction not observed. Ballistoconidia not produced. Fermentation absent. Assimilation of carbon sources: D-glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose (slow), maltose, melezitose, cellobiose (slow), salicin (slow), L-sorbose, D-xylose, L-arabinose, D-ribose (slow), glycerol, erythritol, ribitol (slow), D-mannitol, D-glucitol, myo-inositol (latent), succinic acid, citric acid, D-gluconate, D-glucosamine (latent), N-acetyl-D-glucosamine and xylitol (variable) positive. No growth was detected in L-rhamnose, D-arabinose, ethanol, methanol, galactitol, DL-lactate and hexadecane. Assimilation of nitrogen compounds: positive for nitrate, lysine and cadaverine (latent). Growth in amino-acid-free medium negative. Growth at 37 °C negative.

Growth on YM agar with 10% sodium chloride negative. Growth in 50% glucose negative. Acid production negative. Starch-like compounds not produced. In 100 μ g cycloheximide mL^{-1} growth is negative. Diazonium Blue B reaction positive.

Yeast strains were isolated from the phylloplane of *Canistrum improcerum* and water tanks of *Vriesea minarum*. The strains have been deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais (Coleção de Micro-organismos e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, as strains UFMG-CM-Y1455, UFMG-CM-Y1466, UFMG-CM-Y6109 (= BSS144), and UFMG-BRO-110B, and are permanently preserved in a metabolically inactive state.

Host plants/source and distribution: *Tillandsia flabellata* Baker, *Tillandsia leiboldiana* Schldl., *Tillandsia* L. sp. (teleomorph), *Canistrum improcerum* Leme & J.A. Siqueira, *Vriesea minarum* L.B. Sm. (anamorph) (Bromeliaceae)—the distribution of *Pattersoniomyces tillandsiae* is neotropical. The sequenced teleomorph material is from Honduras (new country report), the remaining morphologically analyzed smut specimens are from Guatemala (new country report) and

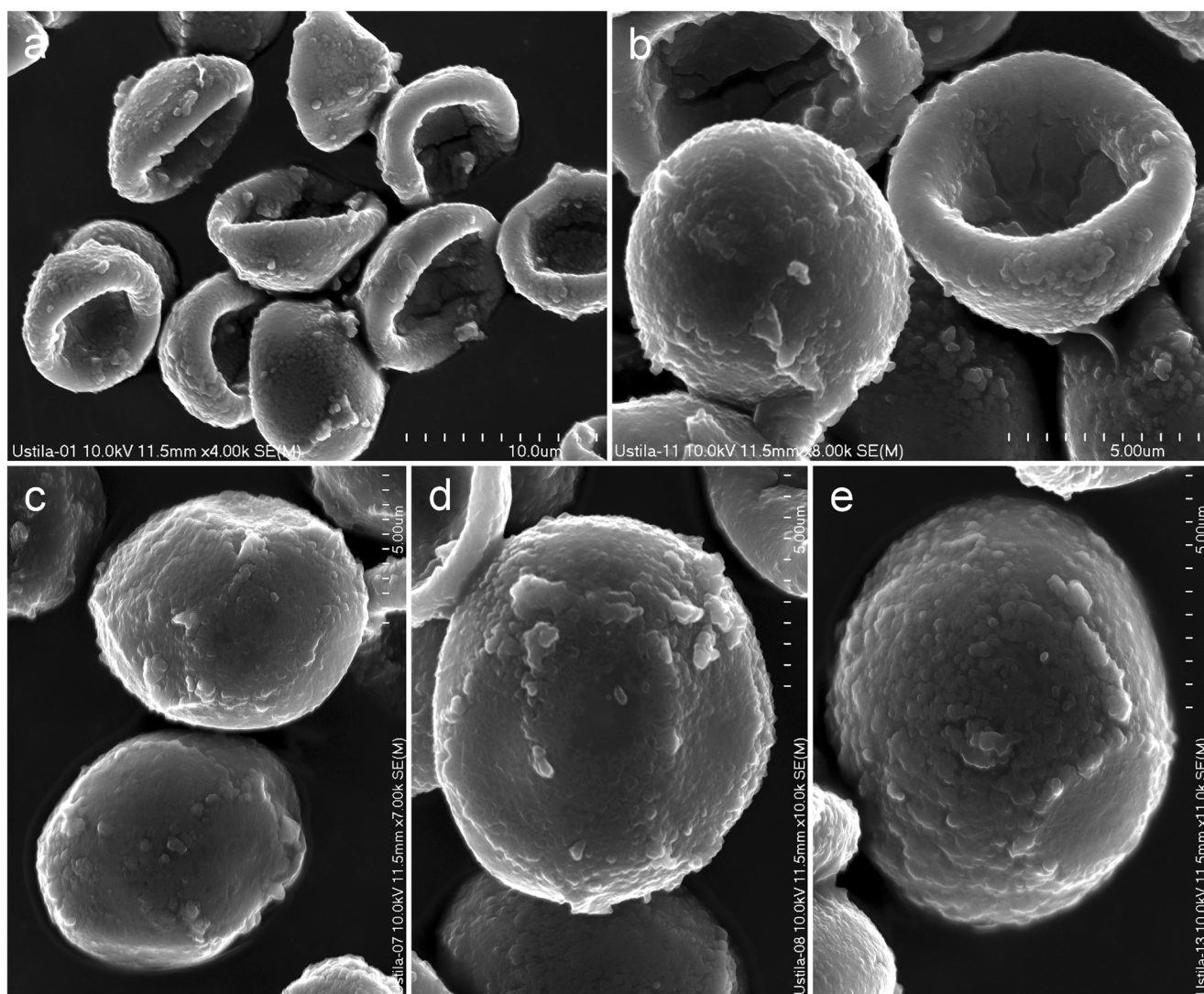


Fig. 4 Spores of the sexual stage of *Pattersoniomyces tillandsiae* (all from BPI 168184) seen by SEM. Scale bars: **a** = 10 µm, **b–e** = 5 µm

Mexico, and this smut has been additionally reported from Costa Rica and Mexico (Clinton 1902; Zundel 1953; Durán 1987; Piepenbring 1996, 2003; Vánky 2012). Yeast strains of this species are from Brazil (new country report).

Discussion

The systematic placement of *Ustilago tillandsiae*, an enigmatic fungus species parasitic on *Tillandsia* species in the neotropical North America, remained unresolved and conflicting opinions suggested that this species was either a smut fungus or a hyphomycete (Clinton 1902; Zundel 1953; Durán 1987; Piepenbring 1996, 2003; Vánky 2012). Molecular phylogenetic analyses conducted in this study unequivocally showed that *U. tillandsiae* is a smut fungus and belongs to the order Ustilaginales, family Ustilaginaceae. Likewise, morphological analyses of fully developed sori revealed that in their

overall appearance the sori of *U. tillandsiae* are similar to typical smut symptoms produced by diverse smut genera, but host relationship and phylogenetic placement indicate that this species cannot be assigned to any genus described to date. Therefore, a new genus *Pattersoniomyces* is described to accommodate this taxon formerly treated as a species of *Ustilago*.

Molecular phylogenetic analyses inferred that *Pattersoniomyces tillandsiae* occupies a sister position to the highly supported cluster of *Tranzscheliella* species with *Tranzscheliella schlechtendalii* Y.M. Li, R.G. Shivas & L. Cai being basal to the remaining sequenced species of this genus. *Pattersoniomyces* and *Tranzscheliella* share some phenotypic similarities but exhibit several phenotypic dissimilarities, especially in the soral structure and the morphology of spores. Particularly, the sori are naked in species of both genera, enclosed by perianth and bracts in *Pattersoniomyces* (this study) and enclosed, at least partly, by leaf sheath, rarely by a

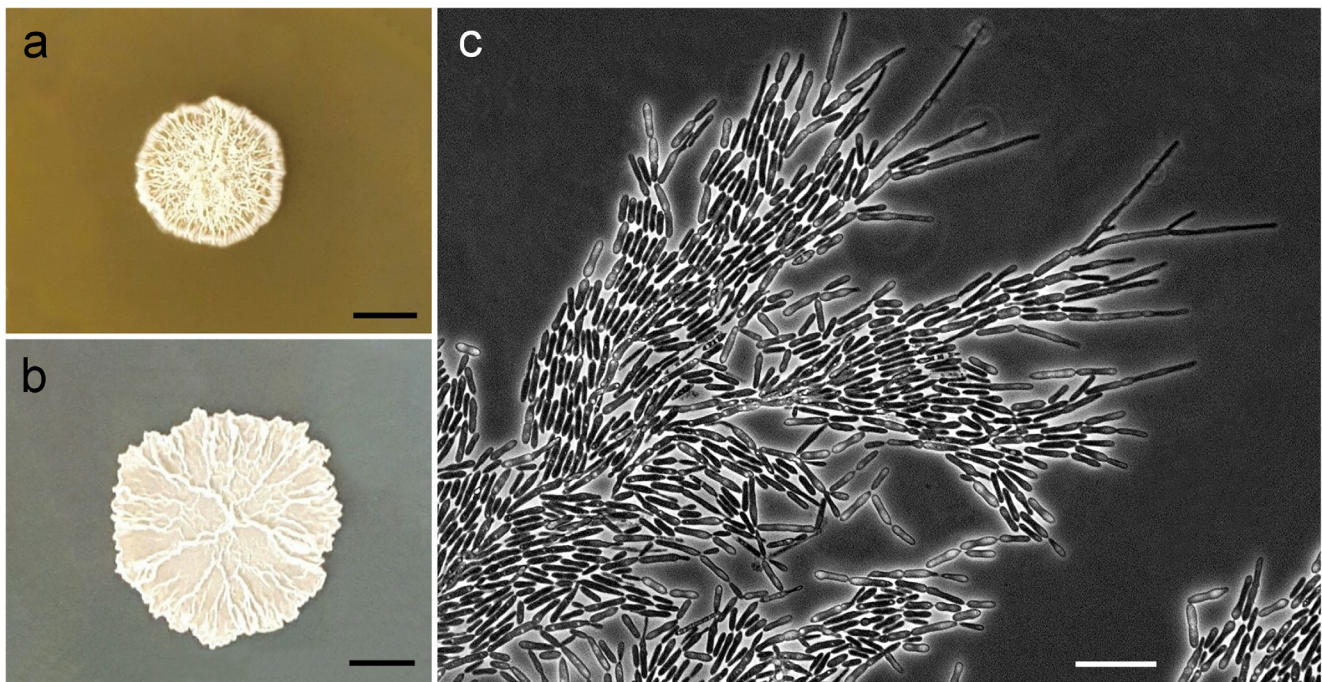


Fig. 5 Morphology of the yeast asexual stage of *Pattersoniomyces tillandsiae* (all from strain UFMG-CM-Y1455): **a**, **b** colony morphology on YM and 5% malt agars, respectively, after 5 days at

25 °C; **c** budding yeast cells and pseudohyphae on YM agar after 5 days at 25 °C. Scale bars: **a**, **b** = 1 cm, **c** = 5 µm

thin peridium, in *Tranzscheliella* (Vánky 2012, 2013; Li et al. 2017). However, sori in *Pattersoniomyces* are restricted to flowers of hosts in the Bromeliaceae and destroy the innermost floral organs—staminal filaments and ovaries remain partly intact in the sorus centre (this study), while the sori in *Tranzscheliella* are produced on stems or, rarely in all aborted inflorescence branches of hosts in Poaceae (Vánky 2012, 2013; Li et al. 2017). The soral characters appear to be an important feature to delimit smut genera, likewise demonstrated for smuts of the *Ustilago-Sporisorium-Macalpinomyces* complex (McTaggart et al. 2012a, b, c).

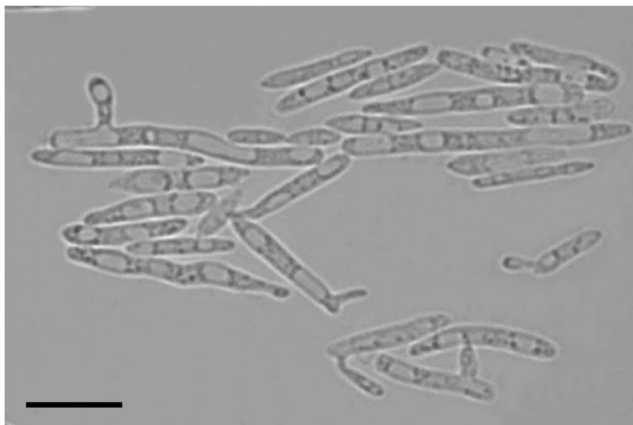


Fig. 6 Yeast cells, some budding on short denticles, of *Pattersoniomyces tillandsiae* (from strain UFMG-CM-Y1455) on YM agar after 5 days at 25 °C. Scale bar = 10 µm

The spores are lighter coloured on one side, collapsed and cracked (because of the thinner spore wall) in both *Pattersoniomyces* and some *Tranzscheliella* species (Vánky 2012; Li et al. 2017). However, the finely ridged spore surface is unique to *Pattersoniomyces tillandsiae*. Moreover, this type of spore ornamentation is unique for all smut fungi and cannot be assigned to any of the ornamentation types delineated by Vánky (1991).

Smut fungi are usually dimorphic having a free-living asexual yeast morph and a plant parasitic sexual morph in their life cycle (Begerow et al. 2014). Ustilaginomycotinous yeasts are commonly detected from very diverse habitats, using both culture dependent and culture-independent methods (e.g., Boekhout et al. 2003; Inácio et al. 2008; Sipiczki and Kajdacs 2009; Bourret et al. 2013; Nasr et al. 2014; Nasanit et al. 2015, 2016; Tantirungkij et al. 2015; Wang et al. 2015; Kijpornyongpan and Aime 2017; Kruse et al. 2017). The links between asexual and sexual morphs were demonstrated only for a few species, for example between *Pseudozyma prolifica* Bandoni and *Mycosarcoma maydis* (DC.) Bref. (Sampaio 2004; Wang et al. 2015), *Rhodotorula acheniorum* (Buhagiar & J.A. Barnett) Rodr. Mir. and *Farysia thuemenii* (A.A. Fisch. Waldh.) Nannf. (Inácio et al. 2008), *Pseudozyma tsukubaensis* (Onishi) Boekhout and *Macalpinomyces spermophorus* (Berk. & M.A. Curtis ex de Toni) Vánky (Sampaio 2004), or between *Pseudozyma aphidis* (Henninger & Windisch) Boekhout, *Pseudozyma rugulosa* (Traquair, L.A. Shaw & Jarvis) Boekhout & Traquair and *Moesziomyces bullatus* (J. Schröt.) Vánky (Kruse et al.

2017). The identical LSU sequences of *Pattersoniomyces tillandsiae* from Honduras and yeast strains from Brazil suggest that both stages represent the same species and this finding constitutes another link of asexual and sexual morphs within Ustilaginomycotina. It is interesting that both the sexual and asexual morphs of *P. tillandsiae* are associated with Bromeliaceae: with species of *Tillandsia* and *Vriesea minarum*, respectively, which belong to core lineages of Tillandsioideae: Tillandsieae and Vrieseae, respectively (Barfuss et al. 2016) as well as with the more distantly related *Canistrum improcerum* that belongs to Bromelioideae (Heller et al. 2015). The strict association of the yeast stage with bromeliads may be supported by the fact that surveys for yeasts conducted in the Americas have not recovered strains related to *P. tillandsiae* so far. This is in contrast to the majority of yeast species within Ustilaginomycotina, which are usually not strictly associated with a particular habitat or host plant family. The prominent exception is *Violaceomyces palustris* Albu, Toome & Aime, representing the distinct order Violaceomycetales, which is strictly associated, as an endophytic yeast, with *Salvinia minima* Baker and *Salvinia molesta* D.S. Mitch. (Albu et al. 2015). However, several other ascomycetous or basidiomycetous yeasts were found to be consistently associated with bromeliads (Landell et al. 2010, 2015; Safar et al. 2013; Gomes et al. 2015, 2016) but it is currently unknown which factors are responsible for such a habitat preferences of these yeast species.

The geographical range of *Pattersoniomyces* is neotropical, and the teleomorphic stage is restricted to a relatively small area in Central America between southern Mexico and Costa Rica, as far as we know. The teleomorphic stage of *Pattersoniomyces tillandsiae* follows the occurrence of the host plants *Tillandsia flabellata* and *Tillandsia leiboldiana*, both restricted to Central America (www.gbif.org/species/2694789; www.gbif.org/species/2694764). The anamorphic stage of this fungus is reported in an area of Atlantic rain forest of northeast Brazil associated with the endemic species *Canistrum improcerum* (Martinelli et al. 2008), and in a small area in southeast Brazil associated with the endemic and endangered species *Vriesea minarum* (Versieux 2011; Gomes et al. 2015). Considering the usually high level of host specificity of smut fungi in the teleomorphic stage (e.g., Hendrichs et al. 2005; Piątek et al. 2011, 2013, 2016; Li et al. 2017), it is not likely to find the teleomorph of *P. tillandsiae* outside its current area of host distribution. Future studies should question whether the anamorphic stage is indeed restricted to *C. improcerum* and *V. minarum* or if it may be also associated with other bromeliads or other habitats.

The description of *Pattersoniomyces* increases the number of endemic smut (and false smut) genera in the Americas to ten. Interestingly, only *Pattersoniomyces* shares its occurrence between North and South America, being a truly neotropical genus. *Clintamra* Cordas & Durán, *Exoteliospora* R. Bauer,

Oberw. & Vánky, *Microbotryozyma* S.O. Suh et al., *Planetella* Savile, *Salmacisia* D.R. Huff & A. Chandra, and *Violaceomyces* Albu, Toome & Aime are endemic to North America, while *Kuntzeomyces* Henn. ex Sacc. & P. Syd., *Oberwinkleria* Vánky & R. Bauer, and *Uleiella* J. Schröt. are endemic to South America (Lutz et al. 2012; Suh et al. 2012; Albu et al. 2015). The low endemism of smut genera in South America is noteworthy as it contrasts with the very high host plant diversity, but one reason may be that a vast area of South America is covered with rainforest that is not a suitable ecosystem for the occurrence of smut fungi (Piepenbring et al. 2011). On the other hand, putative suitable habitats for smut fungi such as the Brazilian Cerrado are still relatively weakly surveyed for the occurrence of smut fungi.

The order Ustilaginales currently includes 49 genera (Begerow et al. 2014; Piątek et al. 2015; Wang et al. 2015; McTaggart et al. 2016; Kruse et al. 2017). With the exception of teleomorphic genera, namely *Clintamra*, *Exoteliospora*, *Geminago* Vánky & R. Bauer, *Melanopsichium* Beck, *Melanotaenium* de Bary, *Pericladium* Pass., and *Yelsemia* J. Walker, and the anamorphic genera *Dirkmeia* F.Y. Bai et al. and *Kalmanozyma* Q.M. Wang et al., all include species that are parasitic on host plants from the order Poales. The genera infecting non-Poalean hosts are mostly placed in the basal lineages of Ustilaginales: in Melanotaeniaceae (*Exoteliospora*, *Melanotaenium*, *Yelsemia*) or Pericladiaceae (*Pericladium*). The phylogenetic placements of *Clintamra* and *Geminago* are not resolved. The core lineage of Ustilaginales, containing the families Anthracoideaceae and Ustilaginaceae, includes smut species infecting Cyperaceae, Eriocaulaceae, Juncaceae, and Poaceae of the order Poales. In this lineage, only one very remote host jump to non-Poalean hosts was reported until now, namely the host jump from the monocot family Poaceae to the dicot family Polygonaceae in the genus *Melanopsichium* (Weiss et al. 2004; Sharma et al. 2014). *Pattersoniomyces* is nested within the core Ustilaginales in the cluster containing smuts infecting Cyperaceae and Poaceae and represents an extension of the host plant families of Ustilaginales to Bromeliaceae. In contrast to the great diversity of ustilaginalean smuts on Cyperaceae and Poaceae, *Pattersoniomyces* is represented by only one species. In phylogenetic reconstructions Bromeliaceae, together with Typhaceae, are early diverging, and Cyperaceae and Poaceae are more derived Poales (Bouchenak-Khelladi et al. 2014). In an evolutionary time-scale Bromeliaceae originated much later (17.4–32.3 Mya) than Cyperaceae (73.7–87.6 Mya) and Poaceae (65–74.4 Mya) (Bouchenak-Khelladi et al. 2014). This implies that *Pattersoniomyces* likely represents a single host jump event from either Cyperaceae or Poaceae to Bromeliaceae, apparently without further species radiation on multiple bromeliad species and genera currently present in South America.

Acknowledgements We thank Amy Y. Rossman—the former curator of BPI for loan of specimens and permission to take parts of the material for molecular analyses—and Anna Łatkiewicz (Kraków) for help with scanning electron microscopy. This work was funded by the statutory funds of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (awarded to MP), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process 457499/2014-1, awarded to CAR, and process 475378/2013-0, awarded to MFL), the Fundação do Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG, APQ-01525-14, awarded to CAR and FCOG) and the Financiadora de Estudos e Projetos (FINEP, process 2084/07, awarded to CAR).

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