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RESEARCH ARTICLE / *ARTIGO*

Two new records of smut fungi for Panama and new combinations into the genus *Tolyposporium*

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ABSTRACT

Two species of smut fungi (Basidiomycota) are reported here for the first time for Panama. *Narasimhania alismatis* (Doassansiales) was found on *Sagittaria guayanensis* and *Tolyposporium kuwanoanum* (Ustilaginales) on *Cyperus tenuis*, a new species of host plant. The name of the latter fungus is a new combination based on the currently accepted name *Ustanciosporium kuwanoanum*. This species belongs to the genus *Tolyposporium* because of sori with peridia, teliospores in balls, teliospores without hyaline appendages, and LSU rDNA sequence data. *Ustanciosporium cyperi* is morphologically rather similar to *T. kuwanoanum*, so it is also placed into the genus *Tolyposporium*. The concept of the genus *Tolyposporium* is discussed.
Key words: *Cintractia* group, Doassansiales, *Narasimhania alismatis*, *Tolyposporium cyperi*, *Tolyposporium kuwanoanum*, Ustilaginales.

INTRODUCTION

According to Piepenbring (2003, 2006), 30 species of smut fungi are known for Panama. This country harbours a high diversity of plants including 417 species of Poaceae and 186 species of Cyperaceae (Correa et al., 2004), the most important families of host plants for smut fungi. Therefore, many more species of smut fungi are expected to exist in Panama.

The new combinations presented here concern the smut genera *Tolyposporium* and *Ustanciosporium*. The morphological concept of the genus *Tolyposporium* is based on the type species *T. junci* (J. Schröt.) Woronin ex J. Schröt. which is characterized by dark teliospores in balls which develop directly on the surface of host tissue in sori without peridia ("naked") according to Vánky (2002, 2012). Piepenbring (2000), however, observed young sori surrounded by peridia and teliospores which develop in pockets of a sterile stroma. Based on morphological and molecular data, further species have been included in the genus *Tolyposporium*, namely *T. isolepidis* (Vánky) Vánky & M. Lutz, *T. neillii* (G. Cunn.) Vánky & McKenzie, and *T. solidum* (Berk.) Vánky (Vánky 2012), with *T. isolepidis* and *T. neillii* lacking peridia and sterile stromata. Therefore, these characteristics apparently are not as obligatory for the genus as supposed by Piepenbring et al. (1999) and Piepenbring (2000) (Table 1). Approximately 20 known species of *Ustanciosporium* are characterized by dark sori around rudimentary host tissue of species of Cyperaceae, by lacking peridia, and by mostly single teliospores which often carry hyaline appendages and present a mostly foveolate ornamentation (Piepenbring, 2000; Vánky, 2012). *Ustanciosporium cubense* (M. Piepenbr.) M. Piepenbr. & Begerow, *U. cyperi* (G.P. Clinton) M. Piepenbr., *U. kuwanoanum* (Togashi & Y. Maki) Vánky, *U. rhynchosporae* Vánky, and *U. virginianum* Vánky develop teliospores in more or less permanent balls.

TABLE 1 - Concepts of selected genera of the *Cintractia* group based on data published by Piepenbring (2000), Vánky (2012), and within the present contribution

Genus	Infection	Morphology of sori	Telio-spores	Teliospore appendages	Teliospore ornamentation	Family of host plants
Cintractia s.str.	local	with peridia and sterile stromata	single	-	mostly finely warty	Cyperaceae
Tolyposporium	local or systemic	sometimes with peridia and/or sterile stromata	in balls	-	mostly irregularly warty or foveolate-reticulate	Cyperaceae, Juncaceae
Ustanciosporium	local or systemic	naked, without sterile stromata	single, rarely in balls	mostly present	mostly foveolate	Cyperaceae

According to morphological and molecular data (Piepenbring et al., 1999; Piepenbring, 2000; Begerow et al., 2006), *Heterotolyposporium piluliforme* (Berk.) Vánky should also be included in the genus *Tolyposporium*, as *T. piluliforme* (Berk.) M. Piepenbr. & Begerow. The type species of the genus *Heterotolyposporium*, *H. lepidospermatidis* Vánky however is not included in *Tolyposporium* because molecular data do not support this conclusion (Begerow et al., 2006).

Evidently, the delimitation of the genus *Tolyposporium* is not clear. Further species and more morphological as well as molecular data are needed in order to establish a generic concept in accordance with phylogenetic relationships.

MATERIAL AND METHODS

Sori of fresh specimens of smut fungi were sectioned with razor blades, mounted in lactophenol, and observed by light microscopy. For details of sori, about 15 µm thick sections were sliced with a freezing microtome (Leica CM 1510). In order to observe the germination of teliospores, fresh spores were spread onto 1.5 % water agar. For scanning electron microscopy (SEM), teliospores were placed onto double-sided adhesive tape, mounted onto a specimen stub, and sputtered with gold. The spores were observed with a scanning electron microscope Hitachi S 4500.

For isolation and sequencing of nuclear DNA, the Panamanian specimen of *Tolyposporium kuwanoanum* was processed as described by Weisenborn et al. (2010). Amplified DNA fragments were purified using a GeneJet™ Gel extraction kit (Fermentas) according to the suppliers protocol. Sequencing of DNA was carried out by Scientific Research & Development GmbH (Oberursel, Germany). Sequences were edited with CodonCode Aligner version 3.7.1 (2002-2010, CodonCode Corporation). The obtained consensus sequence of the LSU region was submitted to GenBank as JQ595299 and compared with sequences from GenBank by BLAST search (Altschul et al., 1990). For phylogenetic analyses, sequences of the gene coding for the nuclear large subunit rRNA (LSU rDNA) of species belonging to the *Cintractia* group were downloaded from GenBank and combined with own sequence data. Sequences of species of *Eriomoeszia*, *Moesziomyces* and *Ustilago* are used as outgroup. The data set was created with MAFFT v. 6.81b using automatic settings (Katoh et al., 2002). From the aligned DNA data, a subset of 537 conserved positions was selected using GBlocks v. 0.91b (Castresana, 2000) with the default options for DNA alignments, except the value for minimal block length which was set to 5, according to the specifications of the rDNA sequence homology. No additional manual editing was done within the alignment. MEGA v. 5.05 (Tamura et al., 2011) was used to perform a Neighbour-Joining (NJ) analysis using the Maximum Composite Likelihood substitution model with all positions containing gaps and missing data eliminated from the dataset (Tamura et al., 2004), followed by a bootstrap analysis with 1000 replicates.

For the molecular phylogenetic analysis, the following sequences were used: *Tolyposporium kuwanoanum* (SH 16) JQ595299 (obtained during the present investigation) and from GenBank *Cintractia amazonica* Syd. & P. Syd. AJ236142 (Piepenbring et al., 1999), *Cintractia axicola* (Berk.) Cornu DQ631906 (Matheny et al., 2006), *Cintractia* cf. *limitata* G.P. Clinton AJ236147 (Piepenbring et al., 1999), *Cintractia michellii* Vánky AJ236149 (Piepenbring et al., 1999), *Dermatosorus cyperi* Vánky AJ236157 (Piepenbring et al., 1999), *Eriomoeszia eriocauli* (G.P. Clinton) Vánky AY740094 (as *Moesziomyces eriocauli* (G.P. Clinton) Vánky, Stoll et al., 2005), *Farysia chardoniana* Zundel AF009859 (Begerow et al., 1997), *Leucocintractia leucodermoides* M. Piepenbr. & Begerow AJ236145 (Piepenbring et al., 1999), *Leucocintractia scleriae* (DC.) M. Piepenbr., Begerow & Oberw. AJ236154 (Piepenbring et al., 1999), *Moesziomyces bullatus* (J. Schröt.) Vánky DQ831011 (Matheny et al., 2006), *Pilocintractia fimbristylidicola* (Pavgi & Mundk.) Vánky AJ236143 (as *Cintractia fimbristylicola* Pavgi & Mundk., Piepenbring et al., 1999), *Stegocintractia spadicea* (Liro) M. Piepenbr. & Begerow AJ236155 (Piepenbring et al., 1999), *Tolyposporium isolepidis* EU246949 (Vánky & Lutz, 2008), *Tolyposporium junci* AF009876 (Begerow et al., 1997), *Tolyposporium neillii* EU246952 (Vánky & Lutz, 2008), *Tolyposporium piluliforme* AF009871 (as *Heterotolyposporium piluliforme*, Begerow et al., 1997), *Ustanciosporium gigantosporum* (Liro) M. Piepenbr. JN367325 (Kellner et al., 2011), *Ustanciosporium standleyanum* (Zundel) M. Piepenbr. JN367326 (Kellner et al., 2011), *Ustilago affinis* Ellis & Everh. AF133581 (Begerow et al., 2000), *Ustilago echinata* J. Schröt. AY740144 (Stoll et al., 2005), and *Ustilago maydis* (DC.) Corda AF453938 (Piepenbring et al., 2002).

RESULTS

Narasimhania alismatis **Pavgi & Thirum.**, in Thirumalachar & Pavgi, Sydowia 6:390. 1952.

Type: On *Sagittaria guayanensis* Kunth (Alismataceae, det. K. Vánky; originally cited as *Alisma* sp.). India, Uttar Pradesh, Banaras, 12 September 1951, M.S. Pavgi. Holotype HCIO 20131 (n.v.), isotypes BPI 178876, IMI 52807, H.U.V. 8637 (n.v.). For descriptions and illustrations see Soares et al. (2009) and Vánky (2012).

Specimen examined: On *Sagittaria guayanensis* (Alismataceae; det. M. Piepenbring). Panama, Chiriquí Province, Corr. Dolega, Los Algarrobos, close to the Casa de la Alemana, 8° 29´ 46,2´´N, 82° 26´ 1,8´´W, ca. 110 m a.s.l., 21 August 2007, M. Piepenbring, J. Bonilla, I. Martínez & R. Rincón 3933 (M 0140904, PMA, U.CH. = Herbarium of the Universidad Autónoma de Chiriquí, Panama). The infected plants were found in

a swamp which is used as pasture for cattle during the dry season.

Narasimhania alismatis is known from India and Mali on *Sagittaria guayanensis* Kunth (as *Lophotocarpus guayanensis* (Kunth) Durieu & Schinz; Vánky, 2012) and from Brazil on *Sagittaria montevidensis* Cham. & Schltdl. (Soares et al., 2009). It is reported here for the first time for Panama.

***Tolyposporium kuwanoanum* (Togashi & Y. Maki) M. Piepenbr., E. Yilmaz & Weisenb., comb. nov.**

Figures 1A-E, 2, 4. Basionym: *Sorosporium kuwanoanum* Togashi & Y. Maki, Ann. Phytopathol. Soc. Japan 10: 139, 1940. For further synonyms see Vánky (2012). Type: on *Bulbostylis barbata*, Japan, Chikuzen Prov., Fukuoka, 5 October 1938, K. Kuwano (isotype H.U.V. 11716, n.v.).

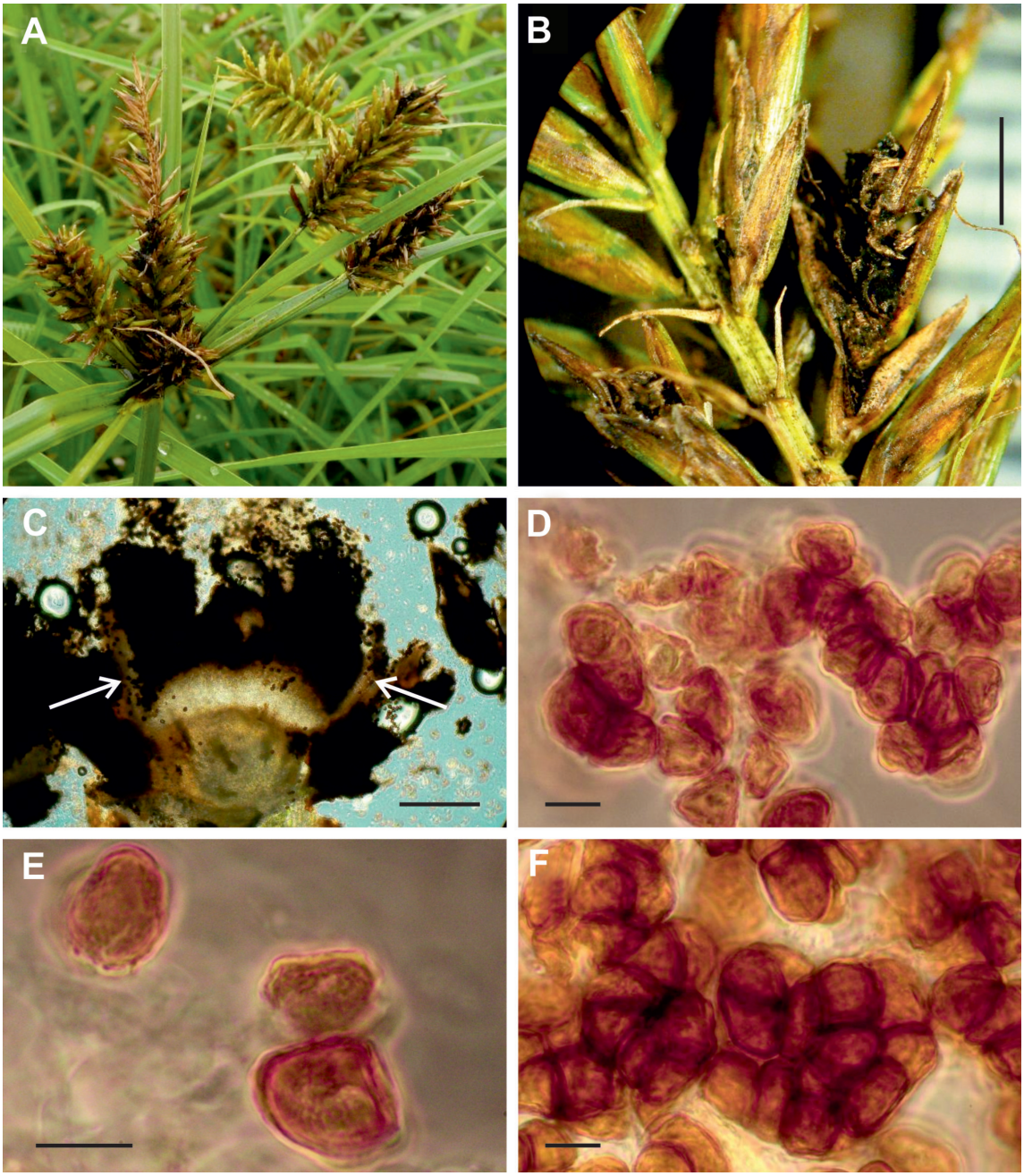

FIGURE 1 - *Tolyposporium kuwanoanum* on *Cyperus tenuis* (SH 16). **A.** Inflorescence of the host plant with infected spikelets. **B.** Two infected spikelets among healthy spikelets. Scale bar = 2 mm. **C.** Longitudinal section through a sorus as seen by light microscopy. From below upwards: host tissue, hyaline matrix with young teliospores, mature teliospores. Arrows indicate the peridium. Scale bar = 200 µm. **D, E.** Teliospores as seen by light microscopy. Scale bars = 10 µm. **F.** Teliospores of *Tolyposporium cyperi* as seen by light microscopy (type specimen, BPI 171487). Scale bar = 10 µm.

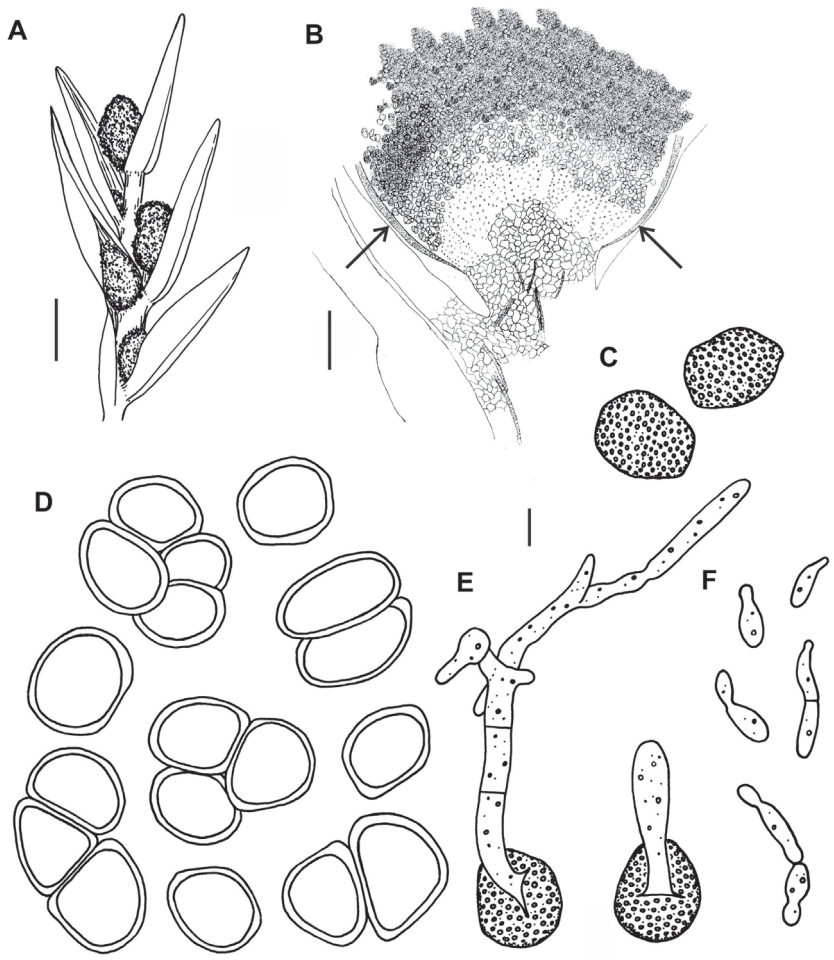

FIGURE 2 - *Tolyposporium kuwanoanum* on *Cyperus tenuis* as seen by light microscopy except Figure 2A (SH 16). **A.** A spikelet with five sori. Scale bar = 1 mm. **B.** Longitudinal section through a sorus at the tip of a spikelet. Cellular details are drawn for host tissue with vascular strands in the axis of the spikelet and at the basis of one glume, the hyaline matrix with sporogenous fungal cells, young (light colored) teliospores, and mature (dark colored) teliospores. Note the peridium (arrows) at the base of the sorus. Scale bar = 100 µm. **C.** Single teliospores drawn in surface view. **D.** Teliospores, single or in balls, drawn in optical section. **E.** Teliospores germinating with phragmobasidia. **F.** Basidiospores initiating a yeast stage. **C-F.** Scale bar = 5 µm.

Sori in groups of spikelets or in individual spikelets in otherwise healthy inflorescences (infection local), mostly hidden between the glumes of the spikelets, spherical to ovoid, about 0.5-1.5 mm diam., composed of a black mass of teliospores which is agglutinated when young and powdery when mature, located around host tissue corresponding to the basis of flowers, up to five sori in a single spikelet, which does not present any floral organ. Young sorus surrounded by a *peridium*, about 20-30 µm thick, hyaline to reddish brown, composed of gelatinized, septate hyphae. In mature sori, only basal remnants of the peridium are evident in sections seen by light microscopy. Teliospores develop in a hyaline matrix on the surface of host tissue (no sterile stroma).

Teliospore balls early disintegrating into single cells or somewhat persistant, easily crumbling during preparation for light microscopy, composed of (2-)6-14(-18) teliospores (n= 30) with large balls evident mainly by SEM, globose to ovoid or irregular, (15-)21-31(-37) x (12-)18-27(-33) µm (n = 40, measured in light microscopical preparations).

Single *teliospores* mostly subpolyangular, sometimes globose to ovoid, (10-)12-15(-17) x (8-)10-12(-14) µm (n = 80), light to medium brown, *teliospore wall* inside the balls mostly ca. 0.5 µm thick, on the surface of the balls mostly ca. 1 µm thick, in some angles up to 2 µm thick, granular foveolate as seen by light microscopy, irregularly reticulate with fine warts as seen by SEM, ornamentation more prominent at the surface of the teliospore balls than inside the balls, less developed at the edges of the teliospores.

Germination of teliospores on water agar with irregular phragmobasidia, basidial cells growing with hyphae or forming basidiospores, basidiospores initiating a yeast stage.

Specimen examined: On *Cyperus tenuis* Sw. (Cyperaceae; det. M. Piepenbring). Panama, Chiriquí Province, Cerro Punta, Finca of Roberto Rubio close to the village, on cultivated land, border of a field, 8º 50´ 56´´N, 82º 34´ 34´´W, ca. 2000 m a.s.l., 24 August 2009, M. Piepenbring, C. Williams, and participants of the workshop on phytopathology, SH 16 (M 0141289, PMA, U.CH.).

Based on Vánky (2012), the Panamanian collection is identified as *Ustanciosporium kuwanoanum*, although the sizes of teliospore balls and teliospores slightly differ from measurements published in this monograph [balls 15-70

µm long, teliospores (9-)10.5-20(-22) x 7-13 µm]. Vánky (2012) does not mention peridia, but these might have been overlooked in mature sori. *U. kuwanoanum* is known from Africa, South and East Asia, as well as Australia (Vánky, 2012). The present record from Panama is the first time this smut fungus is recorded from the Americas. Known host plant species belong to the genera *Bulbostylis*, *Cyperus*, *Fimbristylis*, *Rikliella*, and *Scirpus* (Vánky, 2012). *Cyperus tenuis* is recorded here for the first time as a host plant species.

Molecular sequence data of LSU rDNA suggest a basal position of *T. kuwanoanum* in a monophylum formed by *Tolyposporium* spp. including the type species of the genus, *T. junci* (Figure 3). The new combination of *U. kuwanoanum* into the genus *Tolyposporium* is also supported by characteristics like the presence of peridia, teliospores in balls, and absence of appendages (Table 1). The ornamentation of the teliospores of *T. kuwanoanum* differs from the ornamentation of the type species of *Tolyposporium*, *T. junci*, by being relatively fine and foveolate as seen by light microscopy. As seen by SEM, however, the ornamentation is foveolate-reticulate with fine warts on irregular ridges.

A species of smut fungi morphologically very similar to *T. kuwanoanum* is *Ustanciosporium cyperi* (G.P. Clinton)

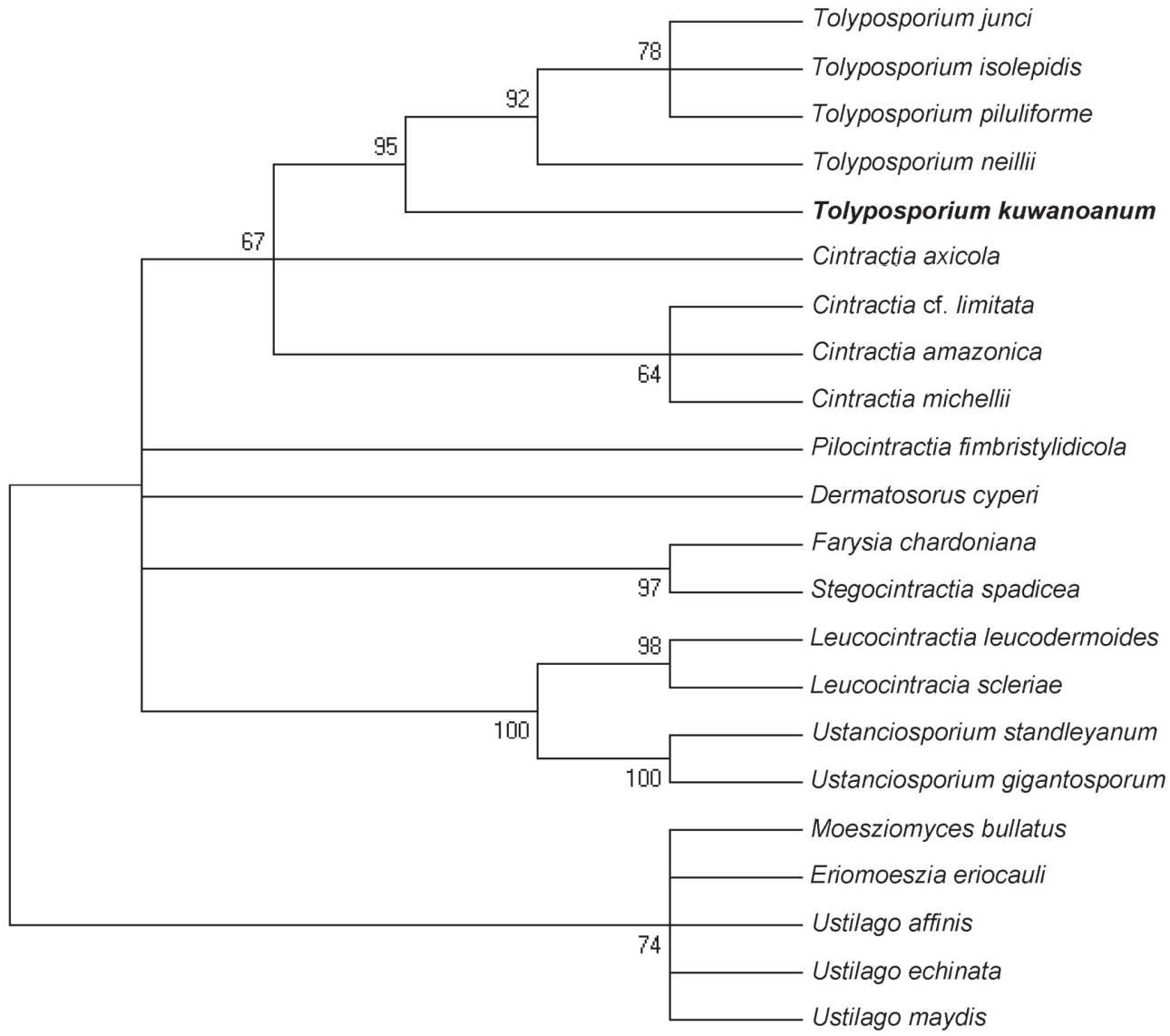

FIGURE 3 - Phylogenetic hypothesis obtained by neighbour joining analysis based on LSU rDNA data for the systematic position of *Tolyposporium kuwanoanum* among species of Ustilaginales, Basidiomycota. Sequence data of selected species of *Eriomoeszia*, *Moesziomyces*, and *Ustilago* (also Ustilaginales) are used as outgroup. Bootstrap values larger than 60 are indicated next to the branches.

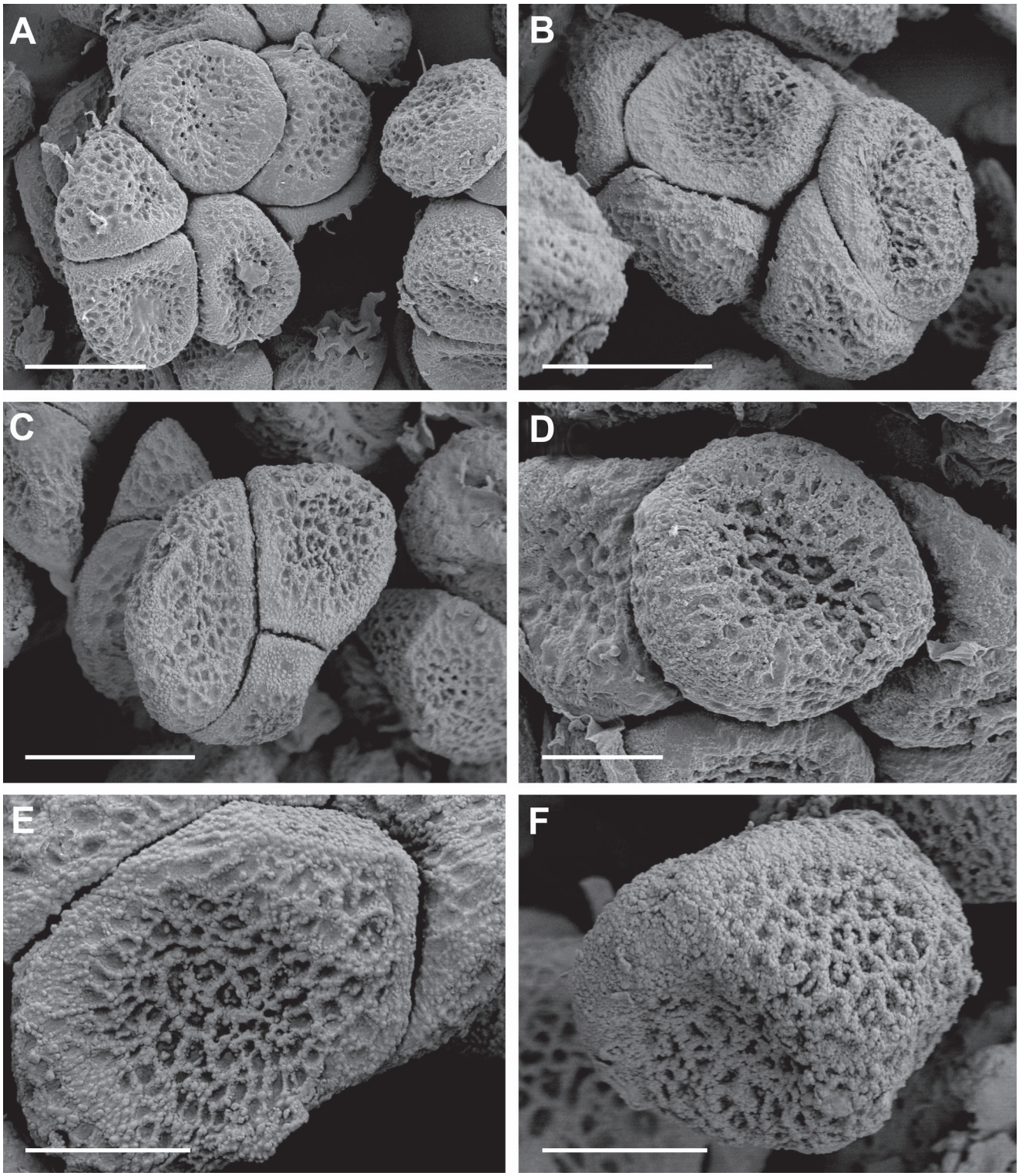

FIGURE 4 - Teliospores of *Tolyposporium kuwanoanum* as seen by scanning electron microscopy (SH 16). **A-C.** Teliospore balls. Scale bars = 10 µm. **D, E.** Teliospores surrounded by adjacent teliospores in balls. Scale bars = 5 µm. **F.** One teliospore separated from a ball. Sides which were located inside the teliospore ball are exposed. Scale bar = 5 µm.

M. Piepenbr., which also infects species of *Cyperus*. Similar characteristics are the sori around rudimentary tissue in spikelets, the absence of a sterile stroma, the presence of crumbling teliospore balls (Figure 1F), and irregular warts which appear foveolate as seen by light microscopy. As molecular data are not available for *U. cyperi*, it was placed into the genus *Ustanciosporium* because of the morphology of its sori.

U. cyperi as described by Piepenbring (2000) differs from *T. kuwanoanum* by sori in all the spikelets of an infected inflorescence (apparently systemic) and the absence of peridia which might, however, have been overlooked. The teliospore balls of *U. cyperi* (4-40 teliospores per ball) are often larger and somewhat more permanent than those of *T. kuwanoanum*, the teliospores of *U. cyperi* [(11-)14-21(-26) x (9-)11-14(-16) µm] are larger, and the ornamentation is less prominent.

Due to the morphological similarities of *T. kuwanoanum* and *U. cyperi*, they should belong to the same genus. Therefore, the following new combination is proposed:

***Tolyposporium cyperi* (G.P. Clinton) M. Piepenbr., comb. nov.**

Basionym: *Cintractia cyperi* G.P. Clinton, Proc. Boston Soc. Nat. Hist. 31:400, 1904. Further synonym: *Ustanciosporium cyperi* (G.P. Clinton) M. Piepenbr., Nova Hedwigia 70:335, 2000. Type: On *Cyperus filiculmis* Vahl, U.S.A., Connecticut, North Haven, sand plains, 26 July 1902, G.P. Clinton s.n. (holotype BPI 171484!, isotypes distributed by Seymour & Earle, Economic Fungi C 102, BPI 171485!, BPI 171487!, NY!).

Molecular data would be helpful to confirm this taxonomic conclusion but they are not yet available for this species. Teliospores in balls are also documented for *Ustanciosporium cubense*, *U. rhynchosporae*, and *U. virginianum*. Neatly delimited foveolae on the teliospores of these species, apparently lacking peridia, hyaline appendages in *U. rhynchosporae*, as well as molecular data available for *U. cubense* support the placement of these species in *Ustanciosporium*. Molecular data are not yet available for *U. rhynchosporae*, because the sequence AJ236144, deposited in genbank under the name "*Gymnocintractia rhynchosporae*" belongs to *Ustanciosporium gigantosporum* (Liro) M. Piepenbr. & Begerow and not to *U. rhynchosporae*, as erroneously assessed by González et al. (2007).

DISCUSSION

By including more species into the genus *Tolyposporium*, its species present sets of character states forming a phenetic continuum with species of other genera in the *Cintractia* group. The value of characteristics of sori (peridia, sterile stromata) and teliospores (balls, ornamentation) considered to be good characteristics by Piepenbring et al. (1999) are now not strictly essential for a species to belong to a certain genus. This situation is often observed in increasing groups of species (Piepenbring & Oberwinkler, 2003). If a genus concept becomes too fuzzy due to the inclusion of species with differing character states, however, its concept is not helpful for identification any more and might be abolished by taxonomic lumping.

Since the last publication on smut fungi from Panama (Piepenbring, 2006), we frequently looked for plant parasitic microfungi in the field in this country. In the interim only two further species of smut fungi new for Panama were found. This suggests that the diversity of smut fungi is rather well known for Panama. Many forays, however, have been performed in more or less primary forests in Panama, where no smut fungi could be detected. The two new species for Panama were found in disturbed vegetation (swampy pasture, cultivated land) confirming the hypothesis that smut fungi occur in open vegetation and typically not in tropical forests (Piepenbring et al., 2011). By focussing on vegetation dominated by species of Cyperaceae and Poaceae during the rainy season, it should still be possible to find more species of smut fungi in Panama.

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