Fungal Diversity (2011) 49:145–165
DOI 10.1007/s13225-011-0102-4

New *Claviceps* species from warm-season grasses

Sylvie Pažoutová · Gary N. Odvody · Debra E. Frederickson · Milada Chudíčková · Jana Olšovská · Miroslav Kolařík

Received: 18 February 2011 / Accepted: 17 April 2011 / Published online: 24 May 2011

Abstract Eight undescribed species of *Claviceps* were recognized from the combinations of molecular and morphological characters. The teleomorph was observed only for *Claviceps setariicola*. Phylogenetic affinities of the new species inside the genus were revealed by a 5.8S-ITS-28S nrDNA analysis. *Claviceps chloridicola*, *C. tenuispora*, *C. setariicola* and *C. setariiphila* are related to *C. maximensis*; *C. truncatispora* is a sister species to *C. pusilla*. *Claviceps clavispora* and *C. langdonii* cluster with species colonizing maize and sorghum. The position of *C. loudetiae* is unclear. Comparisons with herbarium specimens showed *C. setariicola* as a well-established species on *Setaria* spp. in the southern USA. *C. tenuispora* was recorded on *Cenchrus* and *Pennisetum* in Brazil, USA, and Zimbabwe. *C. setariiphila* was found on *S. geniculata* in Brazil. *C. chloridicola, C. loudetiae* and *C. truncatispora* occurred in African savannas on *Chloris, Loudetia*, and *Hyparrhenia* spp., respectively. *C. clavispora* was found on *Paspalum* sp. and *Urochloa* sp. in Mexico and *C. langdonii* colonized *Dichanthium* spp. in the southern USA and probably in Mexico. The occurrence of *C. pusilla* on pearl millet in the USA (Texas) is reported and the record of *C. sulcata* on *Urochloa brizantha* in Brazil is confirmed by nrDNA sequence comparison with an African herbarium specimen. No alkaloids were detected in sclerotia and/or sphacelia of the new species.

Keywords Ascomycota · Taxonomy · Phylogeny · *Clavicipitales* · Ergot

Electronic supplementary material The online version of this article (doi:10.1007/s13225-011-0102-4) contains supplementary material, which is available to authorized users.

S. Pažoutová (✉) · M. Chudíčková · J. Olšovská · M. Kolařík
Institute of Microbiology of the ASCR, v.v.i.,
Vídeňská 1083,
142 20 Prague 4, Czech Republic
e-mail: pazouto@biomed.cas.cz

G. N. Odvody
Texas AgriLife Research and Extension Center,
10345 State Hwy 44,
Corpus Christi, TX 78406–1412, USA

D. E. Frederickson
Formerly INTSORMIL, c/o ICRISAT Bulawayo Centre,
Bulawayo, Zimbabwe

Introduction

In the tropics worldwide, native forest and savanna are converted to pastures using warm-season C-4 grasses introduced from Africa (reviewed in Williams and Baruch 2000). Among the most ubiquitous introductions are the panicoid grasses: *Urochloa* (*Brachiaria*) *brizantha* (palisade grass), *Urochloa maxima* (guinea grass), *Panicum coloratum* (Klein grass), *Melinis* (*Rhynchelytrum*) spp., *Setaria* spp. and *Cenchrus ciliaris* (Buffel grass); the andropogonoid grasses: *Hyparrhenia rufa* (thatching grass) and *Dichanthium* spp. (bluestem) and the chloridoid grasses: *Cynodon dactylon* (Bermuda grass) and *Chloris gayana* (Rhodes grass). These grasses are hosts to many species of ergot (Loveless 1964a, b; Loveless and Herd 1964; Loveless 1967a; Loveless 1985; Frederickson et al. 1991; Pažoutová et al. 2008) and can also present a problem as invasive species.

With the exception of *C. purpurea* that, historically, has been responsible for serious human and animal toxicoses, ergot epiphytotics have remained outside the interest of both phytopathology and medicine until relatively recently. The picture began to change with the large scale introduc-

tions of African grasses and cereals to other semi-arid tropical areas worldwide and the production of F1 hybrid seed. As a consequence of transfers with their hosts, African species of *Claviceps* were increasingly recorded from other regions. The spread of *C. fusiformis* to India started in 1955 and was enhanced by the introduction of the first generation of pearl millet hybrids (Bhide and Hegde 1957; Sundaram et al. 1969). Sorghum ergot *C. africana* was introduced to India (Bogo and Mantle 1999; Pažoutová et al. 2000) and made a well-documented, dramatic sweep over the Americas and Australia starting in 1995–1996 (Reis et al. 1996; Ryley et al. 1996; Bandyopadhyay et al. 1998). Most recently, *C. cynodontis* was recorded on Bermuda grass in the USA and Mexico (Pažoutová et al. 2005; Marek et al. 2006). An outbreak of sphacelial epiphytotics on *Urochloa brizantha* occurred in Brazil, probably caused by *Claviceps sulcata* (Fernandes et al. 1995), a known pathogen of *U. brizantha* in Africa (Langdon 1954; Loveless and Herd 1964). Since then, this ergot disease has caused economic losses to Brazil, a world leader in the production of *U. brizantha* seed (Verzignassi and Fernandes 2001). Even in Africa, native *Claviceps* species described as endemic many decades previously may cause sudden epiphytotics. An outbreak of *Claviceps cyperi* (Loveless 1967b) on yellow nutsedge, followed by toxicoses in grazing cattle, was recently observed in South Africa (Naudè et al. 2005).

Numerous anamorphs and sclerotia related to *Claviceps* have been found and deposited in herbaria, but few were formally described as the essential teleomorph was lacking. Induction of formation of perithecial heads is quite difficult in most *Claviceps* species, as they require various dormancy treatments. Loveless (1964b) pointed out that some *Claviceps* species may be identified using their characteristic conidial shape and size. However, conidial dimensions vary considerably in different collections of the same species (Muthusubramanian et al. 2005; Pažoutová and Frederickson 2005). Despite this variability, conidial shape and length/width ratio are often the only useful markers for the identification of recently found anamorphs by comparison with herbarium specimens of described *Claviceps* species.

Crous and Groenewald (2005) stated that taxonomic names based solely on fungal phenotype often represent complexes of cryptic species, and not single operational units and this is especially remarkable in the phytopathogenic fungi. In the genus *Claviceps*, three closely related groups of species have been found to cluster around *C. purpurea*, *C. fusiformis*, and *C. maximensis*, each suggesting past adaptive radiations from a common ancestor (Pažoutová 2001; Pažoutová et al. 2004). The speciation process continues inside extant species: in *C. purpurea*, *C. africana* and *C. fusiformis* specialized populations/cryptic species have been found (Pažoutová et al. 2002a; Pažoutová and Frederickson 2005; Pažoutová et al. 2008).

During collections over the last decade in Mexico, Brazil, Texas, and Zimbabwe, various *Claviceps* spp. and their anamorphs were found. Some of the specimens corresponded to species recorded previously in Africa (Loveless and Herd 1964) and introduced to other locations (Pažoutová and Frederickson 2005; Pažoutová et al. 2005). In addition, parasites of *Cenchrus*, *Pennisetum*, *Setaria, Urochloa*, *Paspalum*, and *Dichanthium*, differing from any of the known anamorphs and teleomorphs, were collected. The present study aims to describe these new species and, where appropriate, to clarify descriptions of known *Claviceps* species through fresh collections and cultures, thus facilitating their routine recognition and assignment to species and lineages inside the genus.

Material and methods

Herbarium specimens

Specimens of ergotized grasses were obtained from the U. S. National Fungus Collections Specimen Database (BPI), Kew Herbarium (IMI) and Herbarium Hamburgense (HBG) (Tab. 1). Specimens of the new species were deposited in the herbarium of the National Museum in Prague (PRM).

Isolates and their cultivation

Pure cultures of *Claviceps* spp. were isolated by plating honeydew drops found on florets of infected grasses on sucrose-asparagine medium, T2, agar plates and by subsequent transfer of agar pieces containing germinating macroconidia (Pažoutová et al. 2002b). Isolates were maintained on T2 agar slants and transferred each two months. For alkaloid production, seed cultures in sucrose-asparagine medium, TI, were inoculated with 3 ml of conidial or mycelial suspension from a slant culture and incubated 10 days. Five ml of the seed culture were transferred to fermentation cultures T2 and incubated for 20 days (Pažoutová et al. 1981). Cultivations proceeded on a rotary shaker at 24°C in 250 ml Erlenmeyer flasks with 60 ml of the respective medium.

Alkaloid analysis

Alkaloid content of *in planta* sphacelia was assayed colorimetrically; three to five ergotized florets were squashed in 2 ml of distilled water and left to macerate until the honeydew dissolved. The suspension was centrifuged and 2 ml of Van Urk's reagent was added to the supernatant (Pažoutová et al. 1981). The blue color

intensity was read at wavelength 540 nm with elymoclavine (30 μg.mL^{-1}) solution in 2% tartaric acid as a standard. From shaken cultures, samples of cultivation liquid were taken in 3–day intervals and colorimetry performed as above. Alkaloids from powdered sclerotia were extracted with aqueous methanol (80%) containing 0.1% of NH_4OH (24% aqueous solution) for 2 h at laboratory temperature. Alkaloid content of sclerotia was assayed using HPLC (Shelby et al. 1997).

Morphology

Honeydew from plant specimens was used for observation and measurement of conidial size. Spores were mounted in 1% cotton blue/lactic acid and photographed and measured using an Olympus BX51 microscope equipped with a digital camera, CAMEDIA, and image-processing software (QuickPHOTO Camera 2.2.) The statistical treatment of spore size data was done using Kyplot 2.0 beta 15 (Yoshioka 2002) available at http://www.woundedmoon.org/win32/kyplot.html. In the descriptions of specimens and cultures, Anonymous (1966) color standards were followed.

DNA preparation

DNA was purified from 4–7 days-old mycelium grown on T2 plates overlaid with cellophane using an Ultra-Clean Microbial DNA Isolation Kit (Mo-Bio Laboratories, Solana Beach, California) according to the manufacturer's manual. Nuclear rDNA containing internal transcribed spacers (ITS1 and ITS2), 5.8S and the 5′ end of the 28S region was amplified with primer pairs ITS5/NL4 (ca 1.2 kbp) or ITS5/ITS4s (ca 0.56 kbp) (White et al. 1990; Kretzer et al. 1996). The reaction conditions in a Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany) were as follows: 1 cycle of 3 min at 95°C, 30 s at 55°C and 1 min at 72°C; 30 cycles of 30 s at 95°C, 30 s at 55°C and 1 min at 72°C; 1 cycle 30 s at 95°C, 30 s at 55°C and 10 min at 72°C. The reaction mixture consisted of PCR buffer, 1U of DynaZyme (both Finnzymes, Oy, Finland), 0.2 mM deoxynucleotides, 2 pmol of each primer, and 5–50 ng of DNA in 25 μl of total volume. Custom sequencing of DNA was performed at Macrogen Inc. (Seoul, Korea).

Phylogenetic analysis

The phylogenetic position of new species in the genus *Claviceps* was established using the data set consisting of ITS1, ITS2, 5.8S nrDNA and 5′ part of the 28S nrDNA containing D1 and D2 regions. Alignment of 41 sequences consisted of 1393 positions (including gaps); 228 of them were parsimony-informative. Sequences were aligned using MAFFT (Katoh and Toh 2008) under the Q-INS-i option considering secondary structure of RNA and were manually corrected in BioEdit (Hall 1999). Where the sequences of different isolates of the same species were identical, only one of them was used in the dataset. Two methods were used for phylogeny construction, one based on sequence alignment and the other using unaligned sequences. Sequences were first analyzed by maximum-likelihood (ML) method. A substitution model, General Time Reversible, with proportion of invariable sites (I) and gamma distribution (G) (Tamura and Nei 1993) with four rate categories was selected as the best-fitting one by the hierarchical likelihood ratio test using MrModeltest 2.3 (Nylander 2004). The parameters included: base frequencies A=0.23504, C=0.28531, G=0.28835, T=0.19131; rate matrix [A–C]=2.39665, [A–G]=2.19162, [A–T]=1.10453, [C–G]=1.71887, [C–T]=6.07018; [G–T]=1; proportion of invariable sites=0.5053; gamma distribution shape parameter=0.6273. The maximum likelihood tree was calculated using PhyML v2.4.4 interface at the Los Alamos HIV databases website (http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html). Bootstrap values were calculated for 1,000 replicates.

Claviceps species differ substantially in the central loop sequences of their ITS1 and ITS2 spacers (Pažoutová 2001) which cannot be unequivocally aligned. On the other hand, excluding these regions from the alignment entirely leads to a loss resolution for the relationships inside the closely related groups. Therefore, the second analysis was performed using the relative complexity measure method (RCM) (Otu and Sayood 2003; Bastola et al. 2004) that provides a computational approach to sequence comparisons not requiring their alignment. RCM compares the organization of the sequences using five distance measures leading to five matrices. However, it does not yield data for statistical support of branches. The matrices were processed by the Neighbor algorithm of the phylogeny inference package, Phylip 3.68 (Felsenstein 2004) and the consensus of the resulting five trees is presented.

Results

Taxonomy

We have chosen to describe new species from the sphacelial stage of development in the otherwise teleomorphic genus *Claviceps*. Descriptions are based on the appearance of macroconidia and sclerotia (where available), and a sequence of the nrDNA region. Later, should a teleomorph be described, the specific name can be epitypified by the teleomorph, thereby securing it as a holomorphic name, as

allowed by the Vienna Code (Art. 59. 7) (McNeill et al. 2007).

Claviceps chloridicola S. Pažoutová, *sp. nov.*
[MB519360]
Fig. 1

Diagnosis Macroconidia hyalina, oblonga vel allantoidea (11)14–18(21)×(3.7)4–5.5(5.8) μm, mediet. 16.4×4.8 μm. Microconidia globosa, 5–7 μm. Sclerotia pallido-brunnea vel brunnea, protrudentes, 2–6 mm longa. Regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank EF057429). Teleomorphosis ignota.

Typus: **South Africa**: *KwaZulu-Natal*, Cedara, on *Chloris gayana*, May 2005, *N.W. McLaren* (PRM 915382, holotype, culture CCC813)

Etymology from *Chloris*, referring to the name of the host grass

Geographic distribution/host range South Africa, Uganda, *Chloris* spp.

Macroconidia: oblong to slightly allantoid, (11)14–18 (21)×(3.7)4–5.5(5.8) μm, mean 16.4×4.8 μm; *Microconidia* globose, 5–7 μm. Sclerotia light brown to brown, protruding, 2–6 mm long. *Molecular characters*: sequence of the ITS1, ITS2 and D1D2 regions of the 28S rDNA unique (GenBank EF057429). *Teleomorph* not seen. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 35–45 mm in diameter with lobate margin, in the center irregularly raised and slightly wrinkled, abundantly conidiating areas of wet appearance, sometimes with drops of conidial mass, aerial mycelium off-white, conidiating areas reddish buff (5YR5/4), reverse dark reddish buff (5YR1/2) in the centre fading to 5YR3/6 towards the margin; colonies on MEA 45–50 mm in diameter, raised and slightly wrinkled in the center, velutinous, with diffuse margin, conidiation present, obverse cream, reverse pale reddish buff (5YR7/8). Soluble pigments absent.

Additional material examined: **Uganda**: *Kawanda*, on *Chloris pycnothrix,* 1940, C.G. Hansford (IMI 14239); *Kawanda*, on *Chloris gayana,* 1940, C.G. Hansford (IMI 14240);

Notes: Uganda specimen IMI 14239 contained conidia shaped like those of *C. chloridicola* (Fig. 1) most probably belonging to the same species. In the specimen IMI 14240, only three elongated conidia were found (17.8–18.9×3.9–4.1 μm) so that no definite conclusion about its identity could be made.

C. chloridicola is phylogenetically close to *C. cynodontis* and *C. rhynchelytri*. However, the reniform conidial shape, typical of the latter two species, is reflected by the only slightly allantoid form of the spores of *C. chloridicola*. Also, the mean length of *C. cynodontis* conidia is 13.5± 1.6 μm (Pažoutová et al. 2005), whereas those of *C. chloridicola* are longer (mean 16.4 μm).

Claviceps clavispora S. Pažoutová, G.N. Odvody *sp. nov.*
[MB 519362]
Fig. 2

Diagnosis Macroconidia hyalina, cylindrica vel clavata (10)12–19(21)×(3.5)4–5.5(6.7) μm, mediet.14.3×4.9 μm), regio 'rDNA ITS', 'rDNA28S' cum polymorphismis unicis sequentiae (GenBank AJ605995).

Typus **Mexico**: *Guanajuato state*, municipality Santa Cruz de Juventino Rosas, Centro de Desarrollo Tecnológico "Villadiego", sphacelia and honeydew on *Paspalum* sp., 2000, *S. Pažoutová* (PRM 915366, holotypus; culture CCC610).

Etymology '*clavispora*' related to the shape of conidia

Geographic distribution/host range Mexico, *Paspalum* sp. and *Urochloa* sp.

Macroconidia hyaline, cylindrical to clavate (10)12–19 (21)×(3.5)4–5.5(6.7) μm, mean 14.3×4.9 μm). *Microconidia* oval to globose, 5–6 μm in diameter. *Molecular characters*: sequences of the ITS1, ITS2 and D1D2 regions of the 28S rDNA unique (GenBank AJ605995). *Teleomorph* not seen. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 30–35 mm in diameter with lobate margin, raised centrally, slightly wrinkled and velutinous, consisting of dense myceliar mat, obverse white, reverse light reddish buff (5YR4/6); colonies on MEA 15–20 mm in diameter, raised centrally, velutinous, with slightly lobate defined margin, obverse white, reverse pale orange buff (2.5YR5/8). Sporulation absent. Soluble pigments absent.

Additional material examined: **Mexico**: *Guanajuato state*, Pueblo Nuevo, on *Urochloa* sp., 2000, *G.N. Odvody* (PRM 921841, culture CCC606)

Notes: The conidial shape was narrowly cylindric to clavate, sometimes slightly constricted in the middle (Fig. 2). There was ca 3 μm difference in the mean conidial size between *Paspalum* and *Urochloa* specimens (Tab. 1), but the cultures from these specimens have identical nrDNA sequences (AJ605995, FR732000). Conidia of *C. paspali* are smaller 8–16×3–7 μm (Langdon 1952; Tanda 1992). Although there were some superficial similarities in the elongated conidial shape of this species and that of *C. sulcata* (also occurring on *Urochloa* spp.), the sequence comparison proved that C. *clavispora* is phylogenetically unrelated.

Claviceps langdonii S. Pažoutová, G.N. Odvody *sp. nov.*
[MB 519363]
Fig. 3

Diagnosis Macroconidia hyalina, late cylindracea, (8.5) 10.5–14(17)×(4.4)5–6.5(7.7) μm, mediet.12.0×5.8 μm. Microconidia globosa, 5–7 μm. Regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank AJ605995). Teleomorphosis ignota.

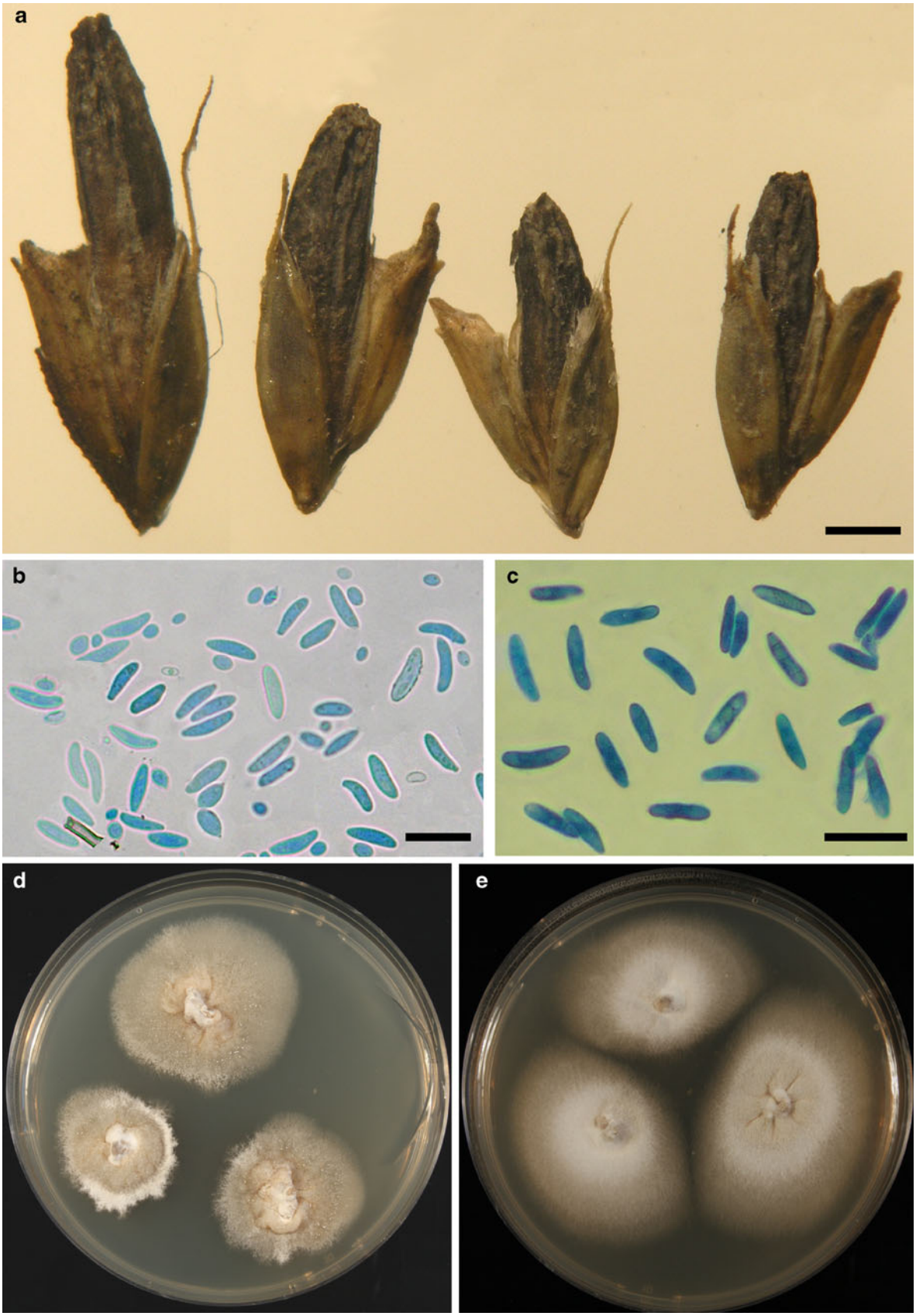

Fig. 1 *Claviceps chloridicola* (PRM 915382, culture CCC813). **a** Sclerotium (bar 500 μm). **b** Macroconidia from specimen PRM 915382 (bar 20 μm). **c** Macroconidia from specimen IMI 14239 (bar 20 μm). **d** Culture on medium T2. **e** Culture on MEA (both cultures 21 days old)

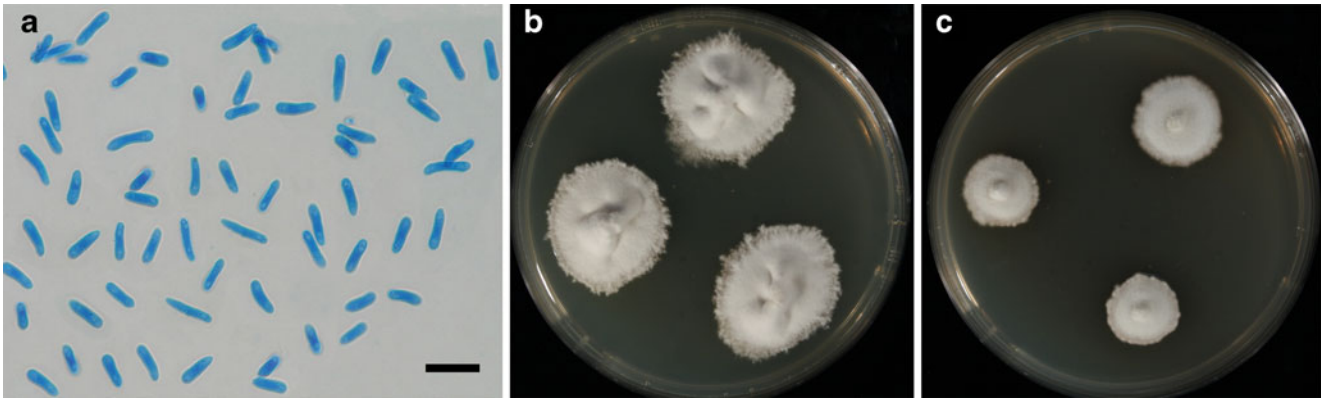

Fig. 2 *Claviceps clavispora* (PRM 915366, culture CCC610). **a** Macroconidia from the specimen (bar 20 μm). **b** Culture on medium T2. **c** Culture on MEA (both cultures 21 days old)

Typus **USA**: *Texas*, Corpus Christi, Petronilla, on *Dichanthium annulatum*, 2004, *G.N. Odvody* (PRM 915383, holotype; culture CCC820).

Etymology named after mycologist R.F.N. Langdon (Australia)

Geographic distribution/host range Southern USA and possibly Mexico, *Dichanthium* spp.

Macroconidia hyaline, broadly cylindric, (8.5)10.5–14 (17)×(4.4)5–6.5(7.7), mean 12.0×5.8 μm. *Microconidia* globose, 5–7 μm. *Molecular characters*: sequences of the ITS1, ITS2 and D1D2 regions unique (GenBank AJ605995). *Teleomorph* not seen. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 30–40 mm in diameter with diffuse margin, raised centrally, smoothly velutinous, consisting of dense myceliar mat, sporulation absent, obverse white, reverse reddish yellow (7.5YR6/6) in the center; colonies on MEA 30–40 mm in diameter with diffuse margin, raised centrally, with tufts of floccose mycelium, obverse white, reverse off-white. Sporulation absent. Soluble pigments absent.

Additional material examined: **USA**: Texas, Beeville, on *D. annulatum*, 2005, *G.N. Odvody* (PRM 915385); Corpus Christi, on *D. annulatum* 2006, *G.N. Odvody* (PRM 915386); Kingsville, on *D. annulatum*, 2005, *G.N. Odvody* (PRM 915384, PRM 921842).

Notes: The broadly cylindric conidial shape was almost identical to that of *Claviceps africana* although slightly smaller. Similarity was also found to the conidia of *C. loudetiae* from Africa (see below). However, sequence comparisons proved that C. *langdonii* is unique and only distantly related to either of the two species. San Martín et al. (1997) recorded *C. africana* on *Dichanthium aristatum* in Mexico (detection based on conidial shape only), but in the light of the present observation it may well have been *C. langdonii*.

Claviceps loudetiae S. Pažoutová, D.E. Frederickson*, sp. nov.*

[MB 519366]

Fig. 4

Diagnosis Macroconidia hyalina, cylindrica, (8.5)10–12 (13.5)×(3.5)4–5 μm, mediet. 10.9×4.2 μm. Microconidia globosa, 3–4 μm. Sclerotia anguste cylindrica vel fusiformea, brunnea vel nigra, 5–8 mm longa. Regio ‘rDNA ITS’,

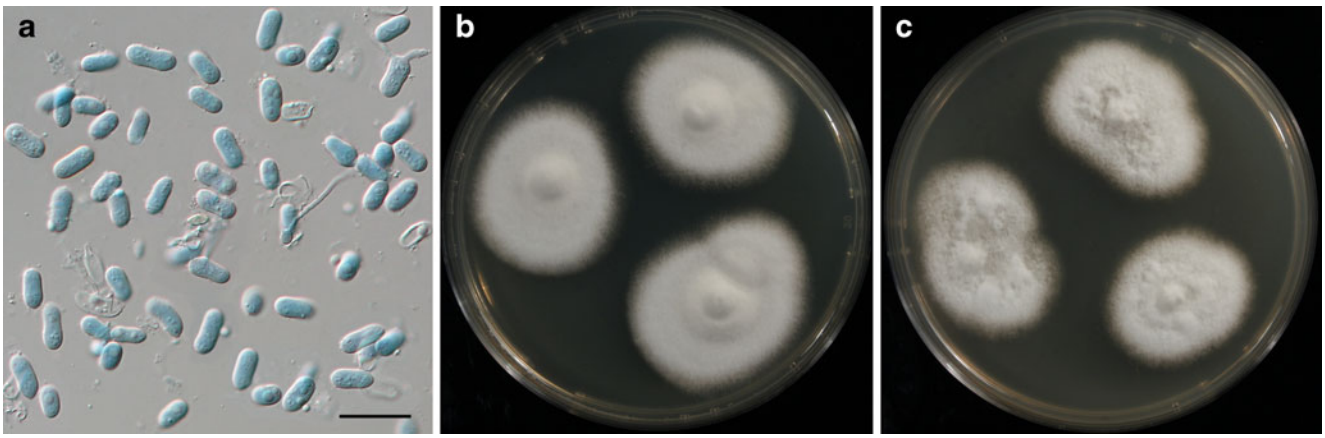

Fig. 3 *Claviceps langdonii* (PRM 915383, culture CCC820). **a** Macroconidia from the specimen (bar 20 μm). **b** Culture on medium T2. **c** Culture on MEA (both cultures 21 days old)

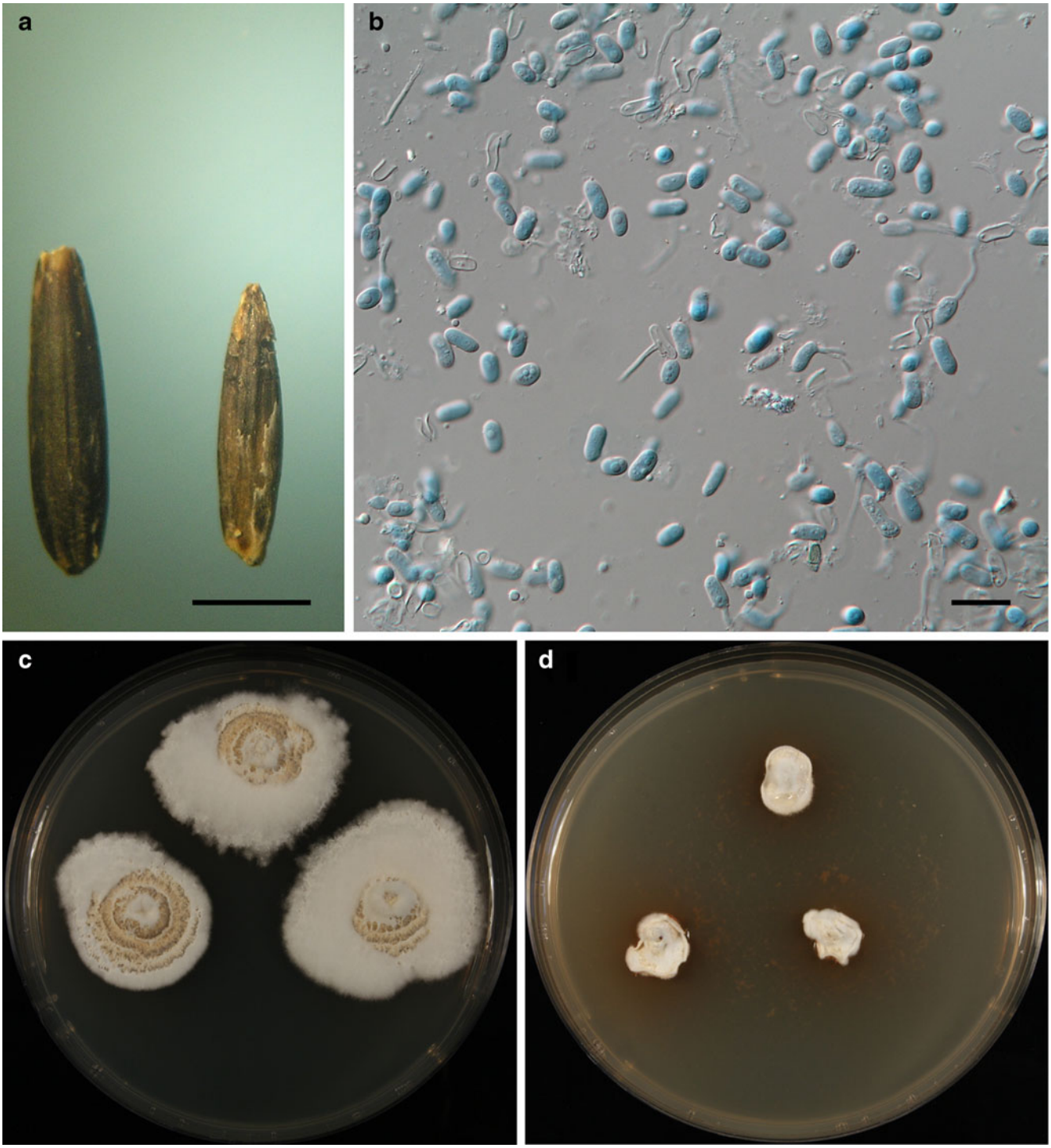

Fig. 4 *Claviceps loudetiae* (PRM 921843, culture CCC656). **a** Sclerotium (bar 500 μm). **b** Macroconidia from the specimen (bar 20 μm). **c** Culture on medium T2. **d** Culture on MEA (both cultures 21 days old)

'rDNA28S cum polymorphismis unicis sequentiae (GenBank AJ605997). Teleomorphosis ignota.

Typus **Zimbabwe**: Matopos, Lucydale Farm, dried culture ex conidia from a sphacelial stage on *Loudetia flavida*, 2000, *D.E. Frederickson* (PRM 921843, holotype; culture CCC656)

Etymology referring to the name of the host grass

Geographic distribution/host range Zimbabwe, on *Loudetia* spp.

Macroconidia: hyaline, cylindric, (8.5)10–12(13.5)× (3.5)4–5 μm, mediet. 10.9×4.2 μm. *Microconidia* globose, 3–4 μm. Sclerotia narrowly cylindric, tapering to the end,

brown to black, 5–8 mm long. *Molecular characters*: sequence of the ITS1, ITS2 and D1D2 regions of the 28S rDNA unique (GenBank AJ605997). *Teleomorph* not seen. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 30–40 mm in diameter with slightly lobate margin, raised centrally, felty, consisting of dense myceliar mat, with light brown exudate drops in concentric circles, obverse white, reverse dark reddish buff (7.5YR1/2); colonies on MEA 15–20 mm in diameter, raised centrally, felty, with defined margin, obverse white, reverse orange buff (2.5YR3/8 to 2.5YR2/8). Sporulation retained in some isolates on T2 medium, but absent on MEA. Soluble pigments produced in both media.

Notes: Presence of a *Claviceps* anamorph on *Loudetia simplex* was reported by Loveless (1964b) in Southern Rhodesia, now Zimbabwe. Conidia were cylindrical, 12–15×3.5–5.5 μm, their length to width ratio was 2.5. The conidia of several recent *Loudetia* collections from the Matopos location were always smaller, but their shape was almost identical to that found by Loveless (length to width ratio 2.6). Unfortunately, the specimens from his study were not deposited so that direct comparison with recent records was not possible. Conidia shape and size resemble phylogenetically unrelated *C. langdonii* and *C. africana* (see above for differential characters).

Claviceps setariicola S. Pažoutová & G.N. Odvody, *sp. nov.* [MB 519367]

Fig. 5

Diagnosis Ascomata stipitata, stipite 0.5 mm diam., primo viridulo-luteolo deinde luteo. Capitula juventute luteola, maturitate flava 1–2 mm diam., deinde luteo–brunnea (0.9–1.1×0.5–0.7 mm diam). Perithecia elliptica vel ovata (101)118-140(151)×(222)250–284(292) μm, (mediet. 129×267 μm). Asci cylindrici (95)105–132 (170)×2.6–3.8 μm, mediet. 119×3.3 μm. Ascosporae filiformes, (90)113–143(162)×0.9–1.2 μm (mediet. 95×1 μm). Anamorph: *Sphacelia setariicola*, macroconidia phialidica, oblonga vel navicularia, (10)14–19.5(23)×(3)4–5(6) μm (mediet. 16.7×4.6 μm), microconidia globosa vel ovata, 4–7 μm. Sclerotia pallido-brunnea vel brunnea, curvata, protrudentes, 2–5 mm longa.

Typus: **USA**: *Texas*, Kingsville, on *Setaria vulpiseta*, 2004, *G.N. Odvody* (PRM 915375, holotype)

Etymology: dwelling on *Setaria*

Geographic distribution/host range North and South America on *Setaria* spp.

Ascomata: stipitate, stipes up to 0.5 mm diam., brilliant green yellow (2.5GY9/8) when young, changing to light yellow (10Y9/10) when mature, up to 5 mm long. *Perithecial heads* 1–2 mm diam., pale yellow (10Y9/8) when young, in maturity changing to yellow (10Y9/12), 0.9–1.1×0.5–0.7 mm. Ageing heads (after ejecting ascospores) resembled raspberry and gradually darkened to brown. *Perithecia* elliptic to oval, (101)118–140(151)×(222)250–284(292) μm (mean 129×267 μm). *Asci* (95)105–132(170)×2.6–3.8 μm (mean 119×3.5 μm). *Ascospores* filiform, (90)113–143(162)×0.9–1.2 μm (mean 95×1 μm). *Sclerotia* light brown to brown, protruding, often curved, 2–5 mm long. *Anamorph*: macroconidia oblong to navicular, (10)14–19.5(23)×(3)4–5(6) μm mean 16.7×4.6 μm, globose to oval microconidia 5–7 μm in diameter. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 60–70 mm in diameter with diffuse margin, raised centrally, wrinkled and velutinous, consisting of dense myceliar mat, abundant formation of macroconidia, obverse white to cream, reverse buff (10YR6/4) in the center; colonies on MEA 30–40 mm in diameter, wrinkled in the center, velutinous, with diffuse margin, predominant formation of microconidia, obverse white, reverse yellowish buff (10YR6/10). Soluble pigments absent.

Additional material examined: **Brazil**: *Rio Grande do Sul*, Guaiba, on *S. geniculata*, 1962, J.P. da Costa Neto (BPI634247); **Unknown**: on *S. macrostachya*, 1939, H. Cull (BPI 634248); **USA**: *Arizona*, Elfrida, Cochise, on *S. viridis*, 1964, L.M. Blank (BPI 634249); Prima, on *S. macrostachya*, 1938, M. Pladeck (BPI 633262). Tucson, on *S. macrostachya*, 1940, M. Mauldin (BPI 633261); Tucson, Soil Conservation Nursery, University Station, on *S. macrostachya*, 1941, L.P. Hamilton (BPI 633259);: *New Mexico*, Dona Ana, on *S. macrostachya*, 1927, R.F. Crawford (BPI 634250), *Texas*, Beeville, on *S. vulpiseta*, 2005, *G.N. Odvody* (PRM 915377); Kingsville, on *S. vulpiseta*, 2002, *J. L. Reilly* (PRM 915374; culture CCC793); Kingsville, on *S. vulpiseta*, 2005, *G.N. Odvody* (PRM 915376); Kingsville, on *S. vulpiseta*, 2006, *G.N. Odvody* (PRM 921848; cultures CCC876 – 878); Kingsville, on *S. vulpiseta* (teleomorph), 2009, *G.N. Odvody* (PRM 921849); Presidio County, on *S. macrostachya*, 1944, V.L. Cory (BPI 633260).

Notes: Ergot infection at high enough severity to warrant fungicide treatment was recently observed in a South Texas commercial seed production of native Plains Bristlegrass (*Setaria vulpiseta*). Mature sclerotia, collected in November 2002 and placed on moist sterile sand in July 2004, were able to start germination after 2 weeks and produced perithecial heads after 40–45 days.

There are four references of ergot fungi on *Setaria*: (a) *Claviceps ranunculoides* in the Americas (Möller 1901); (b) *C. setariiphila* on *S. geniculata* (Brazil) described as a new species below (Fig. 7 in this paper); (c) "*Claviceps setariae*" from *Setaria* spp. from Uruguay, revised below (Online Resource 1) (d) *Claviceps* sp. with oblong conidia (10–13×3–4 μm) found in Southeastern Africa on various *Setaria* species (group 4 in Loveless 1964b). None of these fungi can be identified with *C. setariicola*. Sclerotia of *C.*

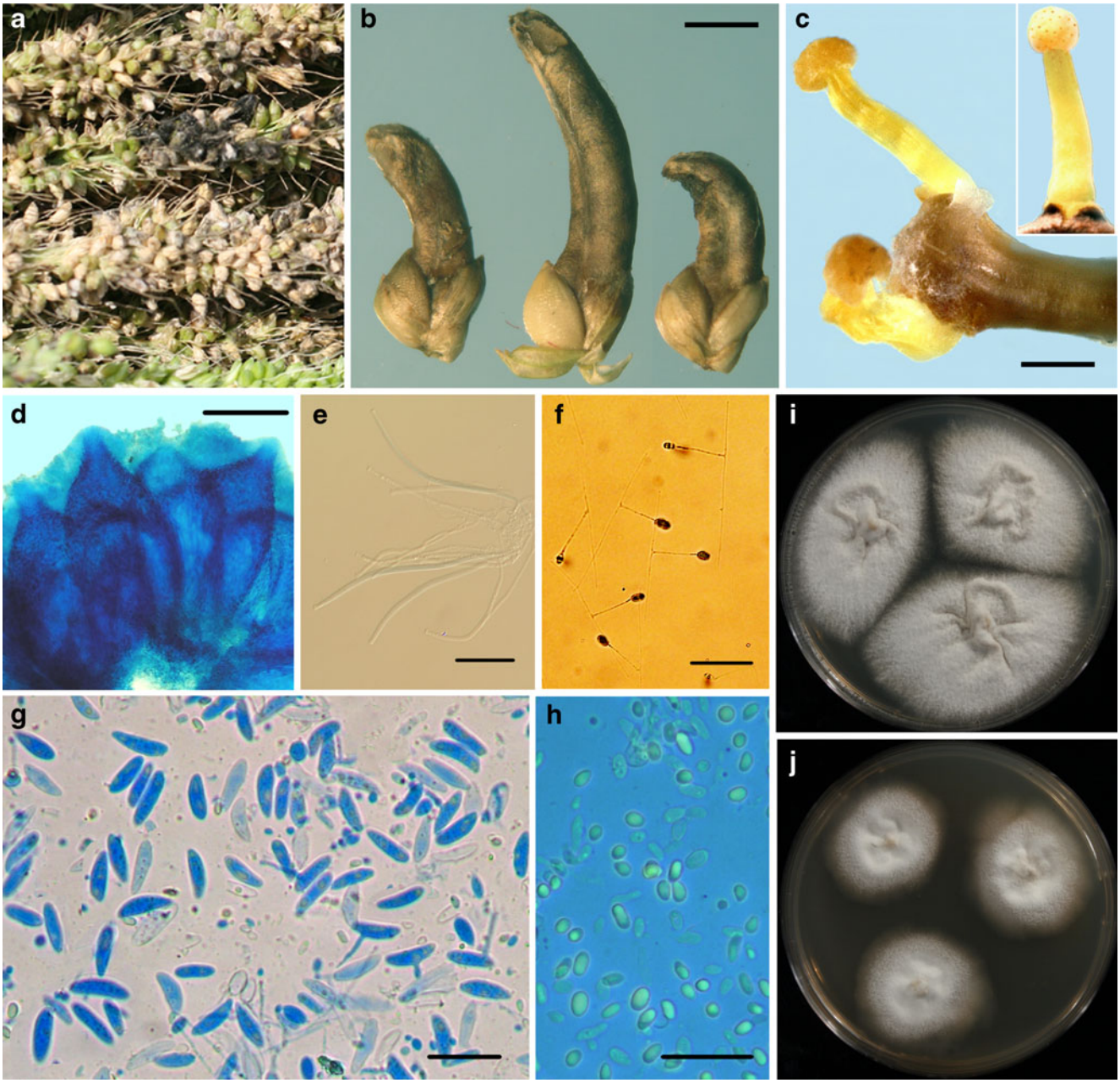

Fig. 5 *Claviceps setariicola*. **a** Flower head of *Setaria vulpiseta* with *C. setariicola* sclerotia. **b** Mature sclerotia (bar 1 mm). **c** Ascomata of different age (bar 1 mm). **d** Mature perithecia (bar 50 μm). **e** Asci (bar 50 μm). **f** Germinating filamentous ascospores producing holoblastic conidia (bar 50 μm). **g** Macroconidia (PRM 915376) (bar 20 μm). **h** Microconidia from the same specimen (bar 20 μm). **i** Culture CCC794 on the medium T2. **j** Culture CCC794 on MEA (both cultures 21 days old)

ranunculoides are bluish-black, not light brown and conidia are small (7–8×3 μm), not large (Online resource 1). The sphacelial stage of *C. setariiphila* could be mistaken for that of *C. setariicola* because its conidial dimensions fall within the range of the latter, although they are somewhat slimmer (Figs. 6 and 7). However, *C. setariiphila* sclerotia do not protrude and this species is not closely phylogenetically related (see below). Sclerotia of "*C. setariae*" are globose, do not protrude beyond the glumes and macroconidia are smaller (10–13×3–4 μm), similar to those reported by Loveless (1964b) on African *Setaria* spp. In addition, all other *Claviceps* species with yellow stromata were compared to *C. setariicola* and the identification was rejected, either based on differences in DNA, or discrepancy in morphological markers (size of conidia, ascospores and perithecia, shape and color of sclerotia).

Nine herbarium specimens of ergotized *Setaria* spp. inflorescences were examined and the shape of sclerotia

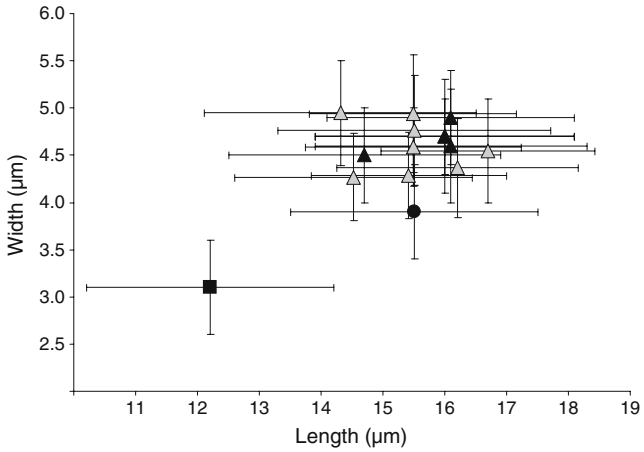

Fig. 6 Variation of conidial dimensions in *Claviceps* specimens collected from *Setaria* spp. in the Americas. *filled square* – "*C. setariae*" (HBG 3272); *filled circle* – *C. setariiphila*; *filled triangle* – *C. setariicola* (our collections); *gray triangle* – BPI collections from *Setaria* (633258, 634247, 634248, 634249, 634250, 633259, 633260, 633261, 633262). Points - mean value, bars - standard deviation

and conidial dimensions were recorded (Online Resource 1). Brazilian specimen BPI 633258 contained pointy black sclerotia and small conidia (9.7±2.3×2.6±0.3 µm), corresponding to the description of *C. ranunculoides* by Möller (1901). On the other hand, eight specimens of *Setaria* ergot from Brazil, Texas, Arizona, and New Mexico were very similar to the recent Texas collections of *C. setariicola* in both the sclerotial shape and color and conidial dimensions. Four of the herbarium specimens (BPI 634247, BPI 634248, BPI 634249, BPI 634250) had been misidentified as *C. purpurea*, although the brown to light-brown color of sclerotia and much longer conidia were in clear disagreement with this species' description. The remaining four specimens had been incorrectly determined as *C. ranunculoides*. The nrDNA sequence that was obtained from one specimen (BPI 633249, Arizona, 1964) differed by a single T-C transition from that of *C. setariicola*. Therefore, it can be concluded that all these specimens should be correctly identified as *C. setariicola* since neither *C. purpurea* nor *C. ranunculoides* descriptions correspond to their observed appearance.

Claviceps setariiphila S. Pažoutová, *sp. nov.*

[MB 519368]

Fig. 7

Diagnosis Macroconidia hyalina, anguste oblonga vel allantoidea (10)14–17.5(22)×(2.8)3.5–4.5(5.5) µm, mediet. 15.6×4.0 µm) Microconidia globosa, 5–7 µm. Sclerotia globosa, pallido-brunnea, non protrudentes, (1–2 mm). Regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank AJ557074). Teleomorphosis ignota.

Typus: **Brazil**: *Passo Fundo*, on *Setaria geniculata*, 1997, *E.M. Reis* (PRM 915379, holotype; culture CCC405)

Etymology referring to the host name, *Setaria*

Geographic distribution/host range Brazil on *Setaria geniculata*

Macroconidia hyaline, narrowly oblong to allantoid, (10)14–17.5(22)×(2.8)3.5–4.5(5.5) µm, mean 15.6×4.0 µm. *Microconidia* globose, 5–6 µm. *Sclerotia* globose, light brown, hidden in widely open glumes, not protruding, 1–2 mm in diameter. *Molecular characters*: sequences of the ITS1, ITS2 and D1D2 regions of the 28S rDNA unique (GenBank AJ557074). Teleomorph unknown. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 40–50 mm in diameter with diffuse to lobate margin, raised centrally, wrinkled and velutinous, consisting of dense myceliar mat, obverse white to cream, reverse off-white; colonies on MEA 30–40 mm in diameter, center raised and wrinkled, velutinous, with diffuse margin, obverse white, reverse reddish yellow (7.5YR6/8). Sporulation absent. Soluble pigments absent.

Notes: C. *setariiphila* resembles *C. setariicola* and "*C. setariae*" and their delimitation characters are given above.

Claviceps tenuispora S. Pažoutová, G.N. Odvody, D.E. Frederickson, *sp. nov.*

[MB 519365]

Fig. 8

Macroconidia hyalina, anguste allantoidea (10)16–21 (25)×(2)3–4(4.5) µm, mediet. 18.2×3.4 µm. Microconidia globosa, 5–7 µm. Regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank EF057430). Teleomorphosis ignota.

Typus **USA**: Texas, Corpus Christi, on *Pennisetum glaucum*, 2006, *G.N. Odvody* (PRM 915367, holotype; culture CCC853)

Etymology related to slim shape of conidia

Geographic distribution/host range Zimbabwe, Brazil, Texas, and probably South Africa, on *Pennisetum* and *Cenchrus* spp.

Macroconidia hyaline, narrowly allantoid (10)16–21 (25)×(2)3–4(4.5) µm, mean 18.2×3.4 µm; *Microconidia* globose, 5–7 µm. *Molecular characters*: sequences of the ITS1, ITS2 and D1D2 regions of the 28S rDNA unique (GenBank EF057430). *Teleomorph* not seen. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 50–60 mm in diameter, flat, diffuse and "arachnoid", abundant production of macroconidia in honeydew-like drops on the diffuse mycelium, obverse white in the center and light brownish gray (10YR6/4) in the diffuse part, reverse light brownish gray (10YR6/4); colonies on MEA 30 mm in diameter with diffuse margin, velutinous, wrinkled in the center, conidiation absent, obverse white, reverse unpigmented. Soluble pigments absent.

Additional material examined: **Brazil**: *Rio Grande do Sul*, Passo Fundo, on *Cenchrus* sp., 1997, *E. M. Reis*, (PRM 915369); **USA**: *Texas*, Weslaco, on *Pennisetum glaucum*,

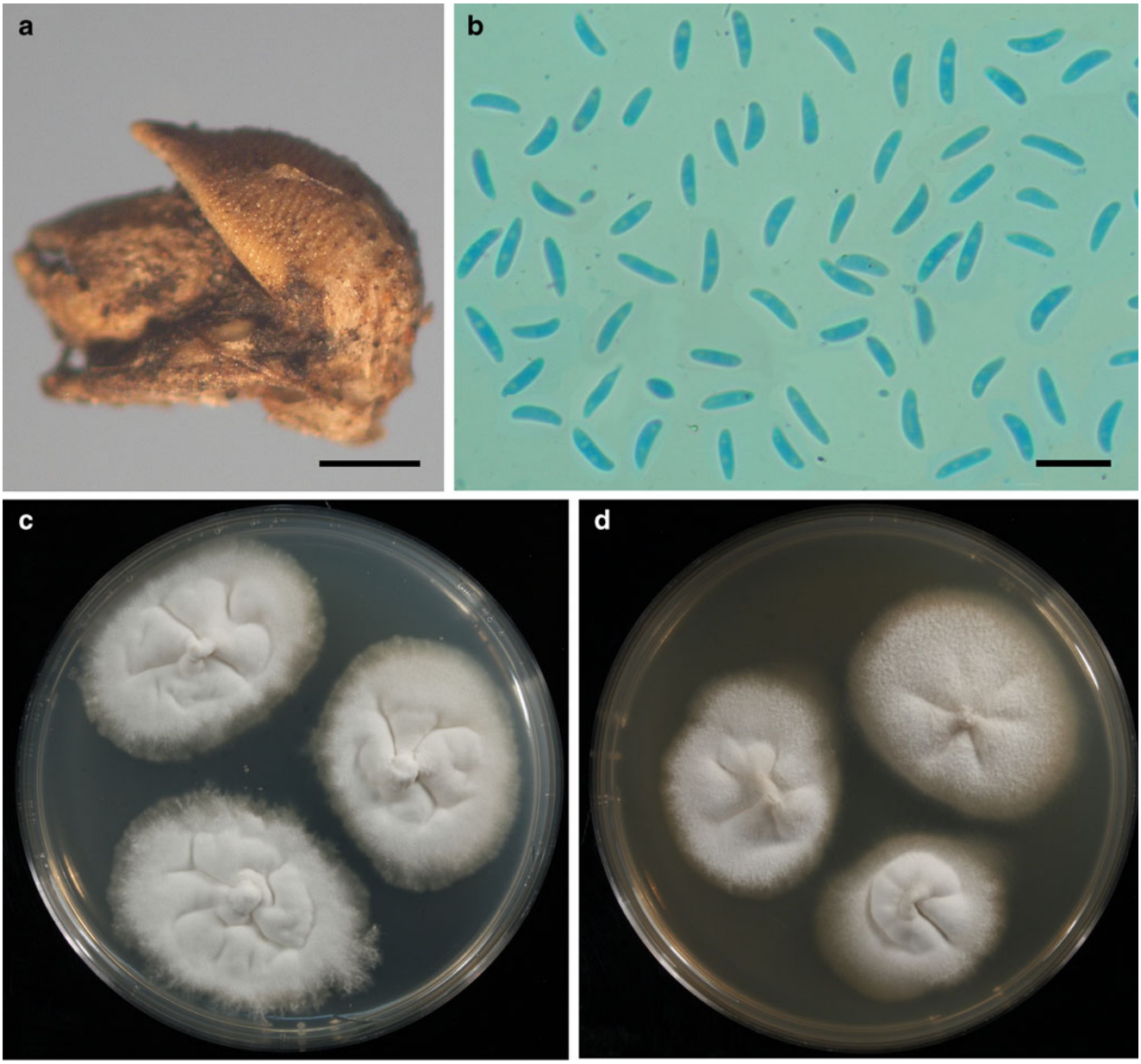

Fig. 7 *Claviceps setariiphila* (PRM 915379, culture CCC405). **a** Sclerotium (bar 500 μm). Macroconidia (bar 20 μm). **c** Culture on medium T2. **d** Culture on MEA (both cultures 21 days old)

1998, *G.N. Odvody* (PRM 915371); Corpus Christi, on *Pennisetum glaucum*, 2002, *G.N. Odvody* (PRM 915372); Corpus Christi, on *Pennisetum glaucum*, 2003, *G.N. Odvody* (PRM 915373); **Zimbabwe**: *Matabeleland*, Matopos, on *Cenchrus* sp., 2001, *D.E. Frederickson*, (PRM 915370);

Notes: Specimens of ergotised *Cenchrus* spp. from Brazil and Zimbabwe contained long and very slim allantoid conidia that did not correspond to any of the known descriptions of *Claviceps* anamorphs (Langdon 1952; Loveless 1964b; Loveless 1967a). The only other described observation of such conidia is in Langdon (1952) (p. 100), in a South African specimen PREM 33401 (=SA 33401) (Doidge 1950) with slightly arcuate narrow conidia (10–20×2.5–5 μm), that was collected from *Cenchrus ciliaris*.

Claviceps truncatispora S. Pažoutová, D.E. Frederickson, *sp. nov.*

[MB 519369]

Fig. 9

Diagnosis Macroconidia hyalina, late cylindrica, oblique truncata (11)12.5–18(20)×(6)7.5–9(10) μm, mediet. 16.5×8 μm. Microconidia globosa, 5–7 μm. Sclerotia brunnea vel nigra, fusiformea (2.5–5.0×1.0–1.5 mm). Regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank AJ537576). Teleomorphosis ignota.

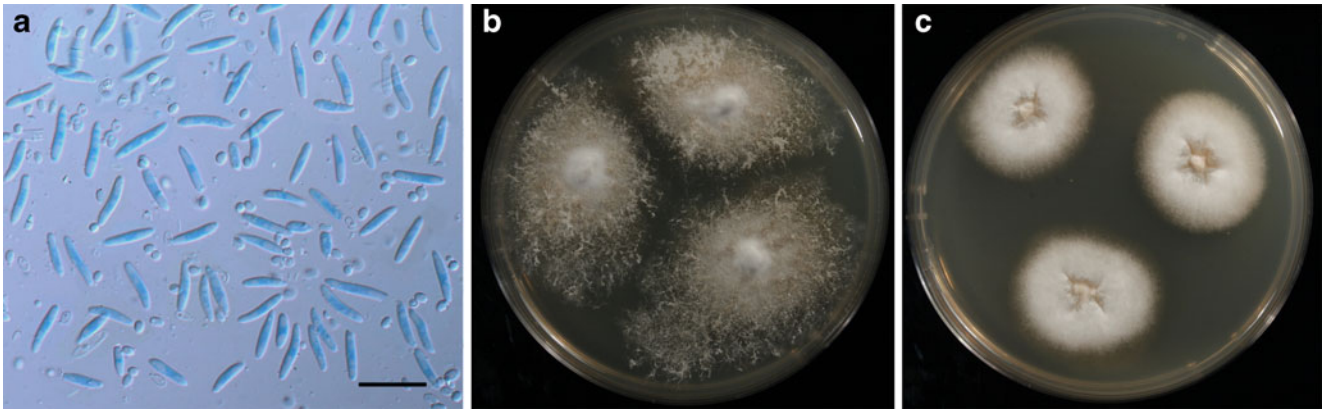

Fig. 8 *Claviceps tenuispora* (PRM 915367, culture CCC781). **a** Macroconidia from the specimen (bar 20 μm). **b** Culture on medium T2. **c** Culture on MEA (both cultures 21 days old)

Typus: **Zimbabwe**: Matopos, Lucydale Farm, on *Hyparrhenia* sp., 2000, *D.E. Frederickson* (PRM 915380, holotype; culture CCC578)

Etymology referring to the truncated shape of macroconidia

Geographic distribution/host range Southern and south-eastern Africa on *Hyparrhenia* spp.

Macroconidia: hyaline, broadly cylindric, mostly obliquely truncated, (11)12.5–18(20)×(6)7.5–9(10) μm, $_{mean}$ 16.5×8 μm. *Microconidia* globose, 5–7 μm. *Sclerotia* brown to black, fusiform (2.5–5.0×1.0–1.5 mm). *Molecular characters*: sequence of the ITS1, ITS2 and D1D2 regions of the 28S rDNA unique (GenBank AJ537576). *Teleomorph* not seen. *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 40–50 mm in diameter with diffuse margin, flat, slightly wrinkled, velutinous, sporulation absent, obverse white to cream, reverse white to light buff (7.5YR7/4); colonies on MEA 30–40 mm in diameter, with radial wrinkles, velutinous, with narrow diffuse margin, obverse white, reverse orange-buff (7.5YR7/6). Sporulation absent. Soluble pigments absent.

Additional material examined: **South Africa**: Potchefstroom, on *Hyparrhenia* sp., 2001, N.W. McLaren (PRM 915381)

Notes: *C. truncatispora* was observed on various *Hyparrhenia* species in Zimbabwe, Mozambique, Swaziland and South Africa (Loveless 1964b, 1985) and the appearance of its sclerotia and macroconidia were clearly depicted by Loveless (1985). However, due to problems with obtaining a teleomorph, the species was never formally described. *C. truncatispora* is still present at the original locations and its host grasses are now worldwide pasture and invasive species, making its appearance on another continent very probable. The species is closely related to *Claviceps pusilla* (Fig. 9d), whose triangular conidial shape might be interpreted as "twice-truncated".

Claviceps pusilla Ces., Linnaea 21: 21 (1848) – new record

Specimens examined: **USA**: *Texas*, Agua Dulce, on *Andropogon* sp., 1997, R. Velasquez (PRM 915389); Corpus Christi, TAES, on *Pennisetum glaucum*, 1998, G. N. Odvody; Corpus Christi, TAES, on *Pennisetum glaucum*, 2006, G. N. Odvody (PRM 915388); Corpus Christi, TAES, on *Dichanthium annulatum*, 2004, G. N. Odvody (not deposited).

Notes: The presence of *C. pusilla* in the Americas had been suspected during the last 10 years, but not confirmed. The diagnostic feature of this species is the uniquely triangular macroconidia (Fig. 9b). In Texas, such conidia have been observed not only on andropogonoid grasses, but also on *Pennisetum glaucum*. The conidial morphology of all specimens was identical to that of African and Australian specimens of *C pusilla*.

Conidia of Texas specimens of *C. pusilla* repeatedly failed to germinate when plated, even those from freshly collected honeydew. Therefore, DNA was isolated from a young uncontaminated sphacelium from *Pennisetum glaucum* florets artificially inoculated by spores from the same host. The nrDNA sequence (FJ685997) was identical to that of *C. pusilla* isolated in 2005 from a South African specimen (AM408174) pointing, thus, to the possible origin of the introduced population.

"*Claviceps setariae*" Herter

Material examined: **Uruguay**: Montevideo, on *Setaria caespitosa*, 1930, W.G.F. Herter (HBG 3272; No. 1422, Plantae Uruguayenses Exsiccatae).

The specimen contained spikes with fully mature sclerotia, therefore the amount of macroconidia was limited and microconidia prevailed. *Macroconidia*: oblong, 12.2×3.1 μm (mean). *Microconidia*: globose, 3–5 μm. *Sclerotia*: light brown, globose, not protruding beyond the glumes.

Notes: HBG 3272 was labeled by Herter as *Claviceps setariae*, which name was never validly published. In Africa, Loveless (1964b) found an anamorph of *Claviceps*

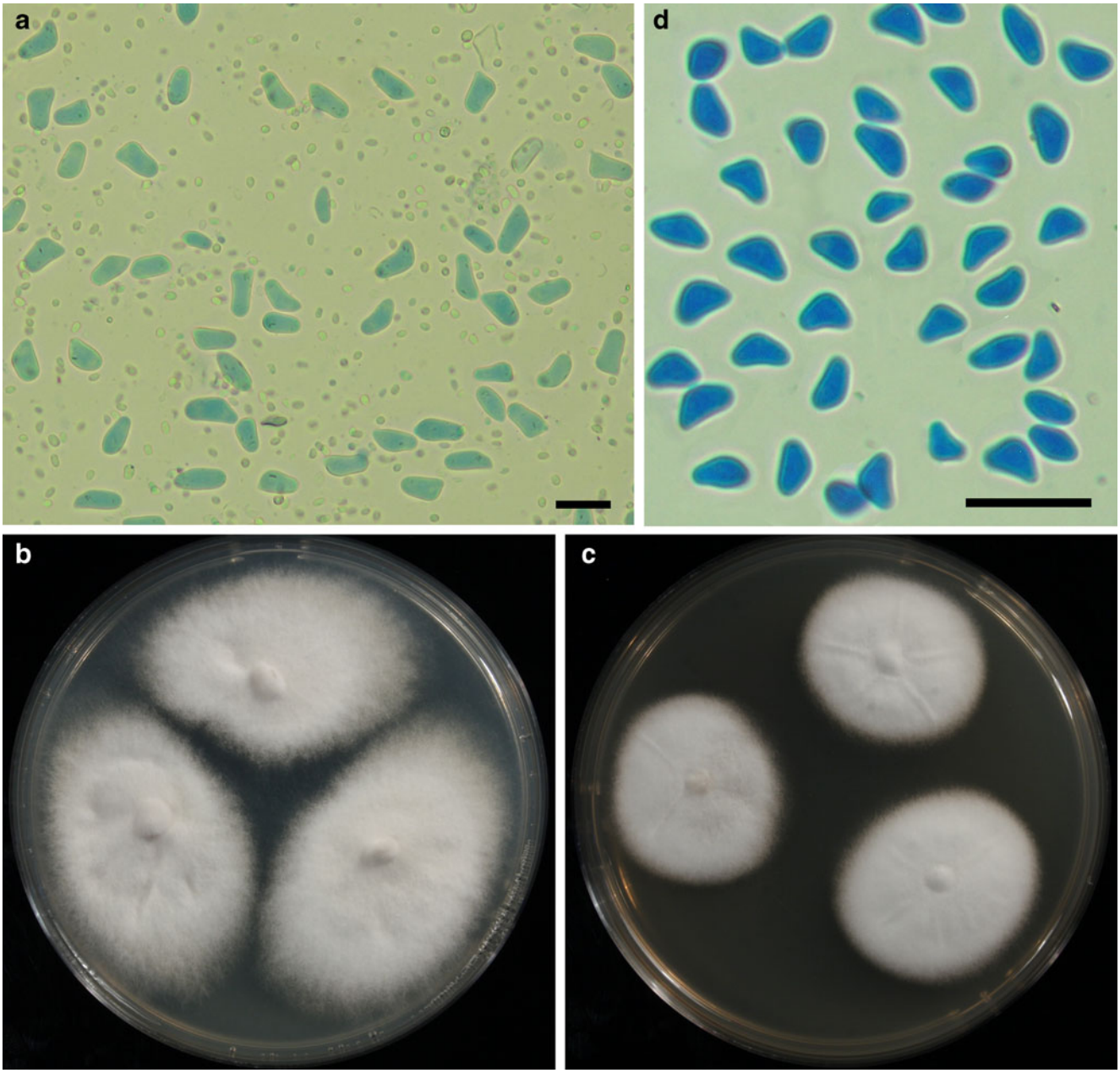

Fig. 9 *Claviceps truncatispora* (PRM 915380, culture CCC578). **a** Macroconidia (bar 20 μm). **b** Culture on medium T2. **d** Culture on MEA (both cultures 21 days old). **d** Macroconidia of the related species *C. pusilla* (PRM 915388) (bar 20 μm)

with oblong slim conidia (mean 11.8×3.3 μm,depicted as group 4 in Loveless' Fig. 1) on three *Setaria* species. These conidia resembled closely spores found in Herter's specimen (Online Resource 1); therefore it may be well possible, that they are conspecific with HBG 3272. Unfortunately, our repeated attempts at nrDNA amplification from Herter's specimen failed and the question of relatedness to *C. setariiphila* remains open. The conidial shape and dimensions differ from those of *C. setariiphila* and *C. setariicola* (Fig. 6) and the specimen most probably represents a distinct species.

Claviceps sulcata Langdon, University of Queensland Papers (Botany) **3**: 39 (1954) – confirmation of a record

[MB294990]

Fig. 10

Molecular characters: sequences of the ITS1, ITS2 and D1D2 regions of the 28S rDNA (GenBank FJ686001, FJ686002, FJ686003, FJ686004, FJ686006). *Cultural characteristics*: colonies (21 d, 24°C) on T2 medium 50–60 mm in diameter with diffuse margin, flat, consisting of thin myceliar mat, velutinous when young, then of wet appearance due to abundant production of macroconidia in

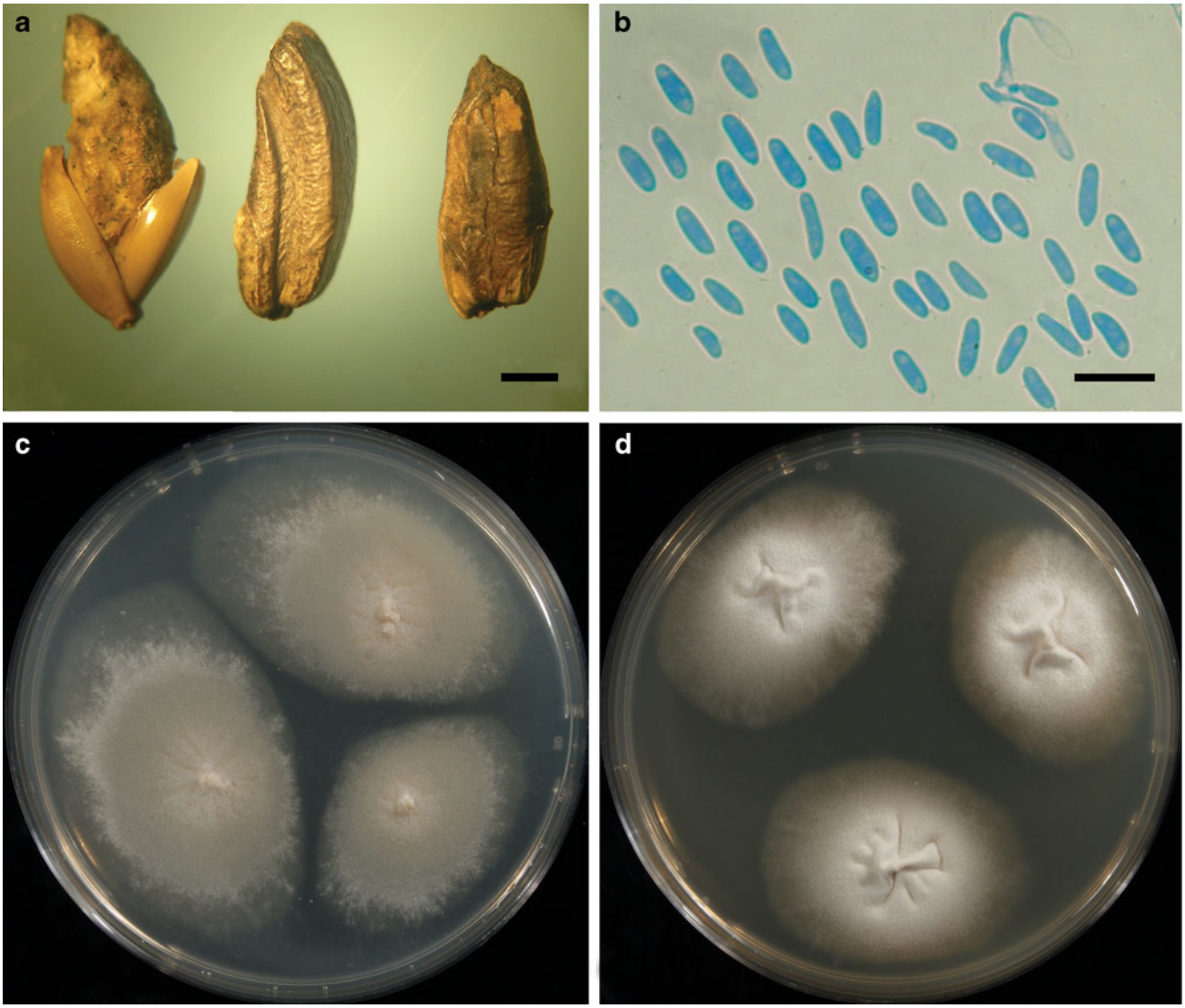

Fig. 10 *Claviceps sulcata* (PRM 915390, culture CCC400). **a** Sclerotium (bar 1 mm). **b** Macroconidia from the specimen (bar 20 μm). **c** Culture on medium T2. **d** Culture on MEA (both cultures 21 days old)

a honeydew-like substance, obverse white (aerial mycelium) to buff (7.5YR7/4), where sporulation occurs, reverse pale buff (10YR7/6); colonies on MEA 30–40 mm in diameter, wrinkled in the raised center, velutinous, with wide diffuse margin, predominant formation of microconidia, obverse white to cream, reverse apricot-buff (7.5YR7/8). Soluble pigments absent.

Specimens examined: **Brazil**: *Passo Fundo*, on *Urochloa brizantha*, 1997, E.M. Reis, (PRM 915390); **Ethiopia**: *Kaffa*, Jimma, on *B. brizantha*, 1954, R.B. Stewart (BPI 633729); **Ghana**: *Gold Coast*, Alburi Hill, on *U. maxima*, 1925, R.H. Bunting (IMI 14244); **Kenya**: Kitale, on *Brachiaria soluta*, 1973, J.W. Lightfield (BPI 632925); Kitale, Mt. Ergon, on *B. decumbens*, 1984, G.M. Lenné (IMI 289413); **Malawi**: *Lilongwe District*, on *U. maxima*, 1950, S.J. Hughes (IMI 40984); **South Africa**: *Transvaal*, Barberton, on *B. brizantha*, V.A. Wager (BPI633274); **Tanzania**: Mpwapwa, on *B. brizantha*, 1953, O.K. Courtney (BPI 633728, reported as *C. purpurea*); **Uganda**: Kawanda, on *Brachiaria* sp., 1940, C.G. Hansford (IMI 14241); **Zambia**: Mt. Makulu Research Station, on *B. brizantha*, 1957, A. Angus (IMI 70708).

Notes: Specimens of ergotized *Brachiaria*/*Urochloa* plants from various African regions were obtained from herbaria and compared to our collections of *C. sulcata* and *C. maximensis* (Figs. 10 and 11, Online Resource 2). Specimens BPI 633274 (as SA 26149, now PREM) and IMI 14241 were studied and identified as *C. sulcata* by Langdon (1954). IMI 70708 appears in the *C. sulcata* redescription by Loveless and Herd (1964).

The size ranges of *C. sulcata* and *C. maximensis* macroconidia partially overlap (Fig. 11), although conidia

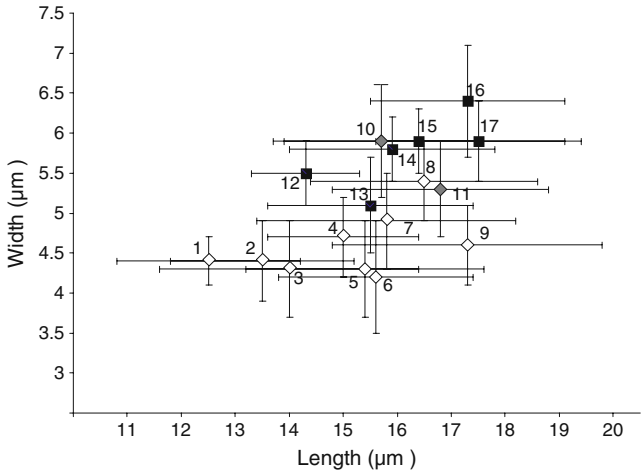

Fig. 11 Variation of *C. sulcata* and *C. maximensis* conidial dimensions. *C. sulcata* (*white diamond*): 1 – IMI 14241; 2 –PRM 921850; 3 – BPI 633274; 4 – BPI 633728; 5 – IMI 70708; 6 – BPI 632925; 7 – PRM 915390; 8 - BPI 633729; 9 – IMI 289413. Specimens from *Urochloa maxima*, denoted as *C. sulcata* (*gray diamond*): 10 – IMI 14244; 11 – IMI 40984. *C. maximensis* (*filled square*): 12 – PRM 921844; 13 – PRM 921847; 14 – PRM 921845; 15 – Chaco, 1997; 16 – PRM 915387; 17 – PRM 921846. Points – mean value, bars – standard deviation

of *C. maximensis* are somewhat bigger on average. Two specimens from *U. maxima*, IMI 14244 and IMI 40984, were denoted as *C. sulcata*, although the host plant is typical of *C. maximensis*. Apparently this is a misidentification because conidia of both these collections grouped with those of *C. maximensis* in Fig. 11.

Brazilian isolates from *U. brizantha* (Table 1) shared identical nrDNA sequences with the Tanzanian specimen, BPI 633728, confirming thus the African origin of the Brazilian epiphytotics.

Phylogeny

The PhyML and RCM trees (Fig. 12) were rooted on outgroup taxa *Cepsiclava phalaridis* (Walker 2004) and *Corallocytostroma ornicopreoides* (Shivas et al. 1997). The general topology of both trees was similar featuring four main clades.

Of the new species, *C. setariicola*, *C. setariiphila*, *C. tenuispora*, and *C. chloridicola* were associated with Clade 1, consisting of species of African origin. *C. sulcata* appeared as a sister species to *C. maximensis* in the RCM tree. *C. tenuispora* and *C. setariicola* were grouped as sister species by both methods. *C. setariiphila* appeared in the intermediary position between *C. maximensis* and *C. pusilla* groups. *C. truncatispora* clustered with *C. pusilla*. From the species in Clade 1, *C. pusilla* and *C. truncatispora* have ITS1 spacer of standard length, whereas the remaining species share a deletion of 38 bp in the central part of the ITS1 region.

C. langdonii and *C. clavispora* grouped in both trees together with species colonizing predominantly andropogonoid grasses (Clade 2); however, the clade was not well supported. *C. loudetiae* was associated with *C. viridis* in the RCM tree in all five distance matrices, whereas in the PhyML tree the position of these species was unsupported, somewhere between Clades 1 and 2.

Clade 3 was formed by species related to *C. fusiformis* that were described earlier (Pažoutová et al. 2008) and Clade 4 consisted of temperate North Hemisphere species closely related to *C. purpurea* and grouping with more ancestral *C. citrina* and *C. paspali,* (of Mesoamerican and South American origin, respectively).

Alkaloids

Mature sclerotia were available only for *C. setariicola* and *C. sulcata* (Passo Fundo, Brazil). The samples were extracted and the extracts analysed for alkaloid content. No measurable alkaloids were found. Colorimetric analysis was also performed on infected florets containing sphacelia. Again, no blue coloration was detected, suggesting the absence of alkaloid production (Tab. 1). Also, no alkaloid production was found in the shaken cultures of the above described species.

Discussion

The co-identification of dated *Claviceps* herbarium specimens with recent collections, for which cultures and DNA sequences are available, is crucial for establishing past and recent distributions and migrations of species. The characteristics of macroconidia in herbarium specimens can be valuable and in many cases form discriminating taxonomic markers (Loveless 1964b). However, although some species retain the conidiation ability *in vitro* for many years, especially on medium T2, it has been our repeated observation that the size and shape of conidia *in vitro* diverge from those produced *in planta*, and are not recommended for taxonomical comparisons. Medium MEA did not generally support conidiation and in the two cases where spores were formed (*C. setariicola* and *C. sulcata*) only microconidia were found.

With growing evidence that *Claviceps* species are less host-specialized than previously thought, and that the association of a species with a particular host genus is not necessarily the same in all parts of the world, the host plant identity offers only a preliminary clue to the parasitic species present. Unfortunately old herbarium specimens, often sweepingly assigned to *Claviceps purpurea,* are rarely suitable for obtaining DNA sequences. Most of the specimens are sclerotia and/or sphaceliae and unequivocal

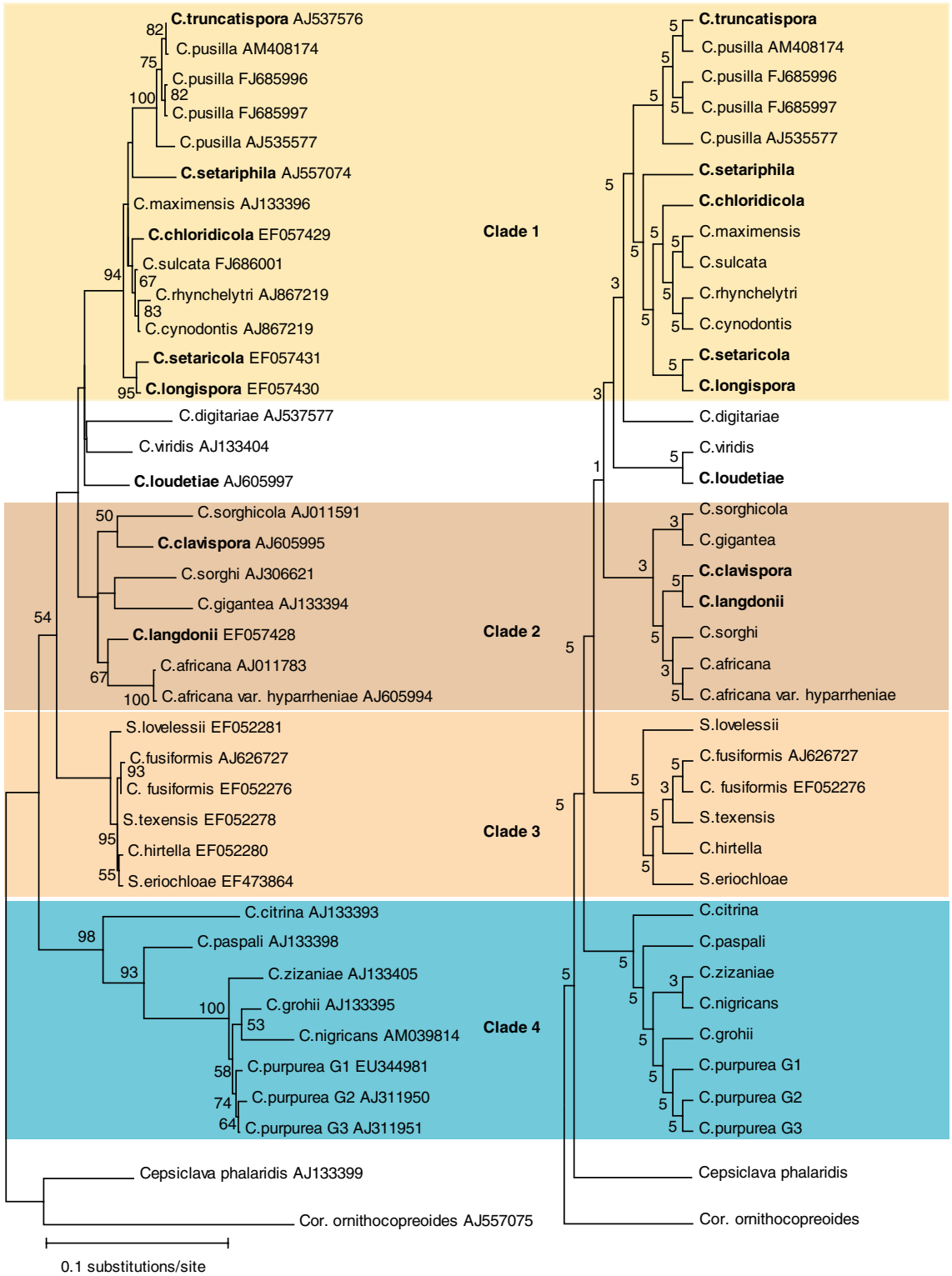

Fig. 12 Phylogenetic relationships among *Claviceps* spp. as inferred from ITS nrDNA sequence data using PhyML (**a**) and RCM (**b**). Taxon names are followed by the GenBank Accession No. of the sequence used. New species are labeled in bold. Bootstrap support values, from 1000 PhyML replicates, are assigned to the tree topology of the most likely tree found, whereas the number of distance matrices supporting the clade (0–5) is given on branches of the consensus RCM tree

taxonomic placement is not possible based on the limitations of the morphological markers available from such sources.

The poor value of these markers is exemplified by the herbarium specimens from Arizona, New Mexico, and

Table 1 Isolates of Claviceps species used for DNA analysis and morphological studies

New species	Specimen	Culture No.	Host	Location	Year	Collector	Conidia		nrDNA (ITS/LSU)	Alkaloids
							Length	Width		
Claviceps chloridicola	PRM 915382	CCC813	*Chloris gayana*	South Africa, KwaZulu-Natal, Cedara	2005	N.W. McLaren	16.3±2.2	4.8±0.5	EF057429	n.d.
Claviceps clavispora	PRM 915366	CCC610	*Paspalum* sp.	Mexico, Guanajuato, Juventino Rosas, Villadiego Exptl Station	2001	S. Pažoutová	15.4±1.9	5.2±0.5	AJ605995	0
	PRM 921841	CCC606	*Urochloa* sp.	Mexico, Guanajuato, Pueblo Nuevo	2001	G.N. Odvody	12.6±1.6	4.5±0.5	FR732000	n.d.
Claviceps langdonii	PRM 915383	CCC820	*Dichanthium annulatum*	USA, TX, Corpus Christi, Petronilla	2004	G.N. Odvody	13.1±1.6	5.7±0.5	EF057428	n.d.
	PRM 915385	CCC850	*Dichanthium annulatum*	USA, TX, Beeville	2005	G.N. Odvody	11.2±1.0	5.8±0.6	n.d.	n.d.
	PRM 915386	CCC879-886	*Dichanthium annulatum*	USA, TX, Kingsville, PMC	2006	G.N. Odvody	12.1±1.2	5.9±0.4	n.d.	0
	PRM 915384	n.a.	*Dichanthium annulatum*	USA, TX, Kingsville, roadside	2005	G.N. Odvody	11.6±0.9	6.1±0.4	n.d.	n.d.
	PMC 921842	CCC849	*Dichanthium annulatum*	USA, TX, Kingsville, PMC	2005	G.N. Odvody	12.0±1.5	5.7±0.6		n.d.
Claviceps tenuispora	PRM 915370	n.a.	*Cenchrus ciliaris*	Zimbabwe, Matopos	2001	D.E. Frederickson	18.5±2.5	3.5±0.4	n.d.	0
	PRM 915369	n.a.	*Cenchrus sp.*	Brazil, Passo Fundo	1997	E.M. Reis	19.3±2.3	3.3±0.4	n.d.	0
	PRM 915371	n.a.	*Pennisetum glaucum*	USA, TX, Weslaco	1998	G.N. Odvody	17.4±2.1	3.2±0.4	n.d.	n.d.
		n.a.	*Pennisetum glaucum*	USA, TX, Weslaco	2003	G.N. Odvody	18.4±2.2	3.2±0.4	n.d.	n.d.
	PRM 915372	n.a.	*Pennisetum glaucum*	USA, TX, Corpus Christi	2002	G.N. Odvody	16.2±1.8	3.0±0.3	n.d.	n.d.
	PRM 915373	CCC782	*Pennisetum glaucum*	USA, TX, Corpus Christi	2003	G.N. Odvody	16.8±2.6	3.6±0.3	FR732001	0
	PRM 915367	CCC853	*Pennisetum glaucum*	USA, TX, Corpus Christi	2006	G.N. Odvody	16.5±2.2	3.2±0.3	EF057430	n.d.
Claviceps loudetiae	PRM 921843	CCC656	*Loudetia flavida*	Zimbabwe, Matopos, Lucydale Farm	2001	D.E. Frederickson	10.9±1.0	4.2±0.3	AJ605997	n.d.
Claviceps setariicola	PRM 915374	CCC793-4	*Setaria vulpiseta*	USA, TX, Kingsville	2002	J. L. Reilly	16.1±2.5	4.6±1.0	EF057431	n.d.
	PRM 915375	n.a.	*Setaria vulpiseta*	USA, TX, Kingsville	2004	G.N. Odvody	16.0±2.1	4.7±0.4	n.d.	0
	PRM 915376	n.a.	*Setaria vulpiseta*	USA, TX, Kingsville	2005	G.N. Odvody	16.1±2.0	4.9±0.5	n.d.	n.d.
	PRM 915377	n.a.	*Setaria vulpiseta*	USA, TX, Beeville	2005	G.N. Odvody	14.6±2.2	4.5±0.5	n.d.	n.d.
	PRM 921848	CCC876-878	*Setaria vulpiseta*	USA, TX, Kingsville	2006	G.N. Odvody	16.0±2.1	4.7±0.6	n.d.	n.d.
	PRM921849	n.a.	*Setaria vulpiseta*	USA, TX, Kingsville	2009	G.N. Odvody	teleomorph		n.d.	n.d.
"Claviceps purpurea"	BPI 634249		*Setaria viridis*	USA, AR, Elfrida, Cochise	1964	L. M.Blank	15.4±1.6	4.3±0.5	FJ686007	n.d.
Claviceps setariiphila	PRM 915379	CCC405	*Setaria geniculata*	Brazil, Rio Grande do Sul, Passo Fundo	1997	E.M. Reis	15.5±2.0	3.9±0.5	AJ557074	n.d.
Claviceps truncatispora	PRM 915380	CCC578	*Hyparrhenia rufa*	Zimbabwe, Matopos, Lucydale Farm	2000	D.E. Frederickson	16.5±1.6	8.3±0.8	FJ686005	n.d.
	PRM 915381	n.a.	*Hyparrhenia* sp.	South Africa, Northwest, Potchefstroom, Deel Kraal	2001	N.W. McLaren	16.0±1.4	7.6±0.8	n.d.	n.d.

Table 1 (continued)

New species	Specimen	Culture No.	Host	Location	Year	Collector	Conidia		nrDNA (ITS/LSU)	Alkaloids
							Length	Width		
										n.d.
New and revised records										
Claviceps maximensis	PRM 921844	CCC641	*Urochloa maxima*	Zimbabwe, Matopos, MRS	2001	D.E. Frederickson	13.5±1.8	4.9±0.6	FJ685998	n.d.
		CCC398		Paraguay, Chaco	1997	A. Glatzle	16.4±2.7	5.9±0.4	AJ133396	n.d.
	PRM 921847	n.a.		Paraguay, Chaco	2005	A. Glatzle	15.5±1.9	5.1±0.6	n.d.	n.d.
	PRM921845	n.a.		Brazil, Rio Grande do Sul, Passo Fundo	1997	E.M. Reis	14.1±1.7	5.3±0.5	n.d.	0
		CCC698	*Urochloa maxima* var. *trichoglume*	Australia, Queensland, Grantham	2001	M. Ryley	n.d.		FJ685999	n.d.
	PRM 915387	n.a.	*Panicum coloratum*	USA, TX, Corpus Christi	2004	G.N. Odvody	17.3±1.8	6.2±0.7	n.d.	n.d.
	PRM 921846	CCC816		USA, TX, Kingsville	2005	G.N. Odvody	17.5±1.9	5.9±0.5	FJ686000	n.d.
Claviceps pusilla	PRM 915389	n.a.	*Andropogon sp.*	USA, TX, Agua Dulce	1997	R. Velasquez	9.4±1.0	5.1±0.5	n.d.	n.d.
		n.a.	*Pennisetum glaucum*	USA, TX, Corpus Christi, TAES	1998	G.N. Odvody	9.7±1.1	5.7±0.6	n.d.	n.d.
	PRM 915388	n.a.	*Pennisetum glaucum*	USA, TX, Corpus Christi, TAES	2006	G.N. Odvody	9.1±1.0	5.1±0.5	FJ685997	n.d.
Claviceps sulcata	PRM 915390	CCC400	*Urochloa brizantha*	Brazil, Rio Grande do Sul, Passo Fundo	1997	E.M. Reis	15.8±2.4	4.9±0.6	FJ686001	0
	PRM 921850	CCC401		Brazil, Rio Grande do Sul, Passo Fundo	1997	E.M. Reis	13.5±1.6	4.4±.05	FJ686002	0
		CCC323		Brazil, Mato Grosso do Sul, Campo Grande	1997	C. Fernandes	n.d.		FJ686003	n.d.
		CCC328		Brazil, Minas Gerais, Sete Lagoas	1997	S. Pažoutová	14.0±1.6	4.1±0.3	FJ686004	n.d.
Claviceps purpurea	BPI 633728			Tanzania (Tanganyika), Mpwapwa	1953	O.K. Courtney	15.0±1.4	4.7±0.5	FJ686006	n.d.

Texas. Originally wrongly denoted either *C. purpurea* or *C. ranunculoides*, they have now been correctly assigned to the newly described *C. setariicola.* The earlier classifications had already been questioned by Alderman et al. (2004), who reviewed many specimens (including the BPI ones used in this study) and found cream to buff-brown furrowed sclerotia and oblong to allantoid conidia in the range corresponding to *C. setariicola*. According to the dating of BPI specimens, *C. setariicola* had been well established in Arizona, New Mexico, and Texas since the beginning of the 20th century and a handwritten note with specimen BPI 634250 (New Mexico, 1927) informed: "Extremely common in alfalfa fields in Mezilla Valley". The BPI specimen, identified as *C. setariicola* by ITS-rDNA sequencing, was collected in Arizona in 1964. Specimen BPI 634247 confirms that the species was present also in Brazil in 1962.

C. tenuispora from Brazil, Texas and Zimbabwe is described here as a parasite of *Pennisetum* and *Cenchrus*. It is quite surprising, that its typical, conspicuously narrow, allantoid conidia were not observed during the survey of honeydews collected in South Rhodesia (now Zimbabwe) (Loveless 1964b), where only falcate conidia of *Claviceps fusiformis* were reported. However, Langdon (1952) observed a specimen PREM 33401 (Doidge 1950) collected on *Cenchrus ciliaris* from the Transvaal where slightly arcuate long and narrow conidia (10–20×2.5–5 μm), corresponding well to those of *C. tenuispora*, were found.

C. pusilla and *C. truncatispora* are closely related sister species. Whereas *C. pusilla* is widespread in the Paleotropics and subtropics, *C. truncatispora* has not been recorded outside southeastern Africa. *C pusilla* has an extended host range among the Andropogoneae and even Paniceae, whereas *C. truncatispora* has not been observed outside the genus *Hyparrhenia*. It is therefore possible that *C. truncatispora* might have originated as a specialized species from the more generalist *C. pusilla*, similarly to the *Hyparrhenia* variety of *C. africana* (Pažoutová and Frederickson 2005).

C. langdonii has so far only been found in Texas and probably also in Mexico (San Martín et al. 1997). Its host, *Dichanthium annulatum*, is an introduced Paleotropical grass. In previous studies on African ergot fungi, no such ergot on *Dichanthium* spp. was recorded (Loveless 1964b). Langdon (1952) found only *C. pusilla* on Paleotropic *Dichanthium* specimens. With regard to other andropogonoid grasses as putative hosts of *C. langdonii* in the USA, there are records of *Claviceps* sp. on several *Andropogon* species from Iowa, formerly considered to be *C. pusilla* (Gilman 1949). However, conidia are not triangular but oblong and are narrower than spores of *C. langdonii* (Alderman et al. 2004). Langdon (1952) noted that in the ergotized specimens of *A. saccharoides* and *A. furcatus* from the USA, oblong to cuneate conidia (8.5–12×3.5–5 μm) were present. These dimensions and length/width ratios correspond to those measured by Alderman et al. (2004), and not to the broader conidia of *C. langdonii* and must represent another species, different from both *C. pusilla* and *C. langdonii*.

On *Paspalum* spp., only one species of *Claviceps* has been described previously, i.e. *C. paspali*. The mention of *C. lutea* in the database originates from the persisting taxonomical error of Möller (1901), already spotted by Langdon (1952). Möller gave the host identity as *Paspalum*, although the accompanying picture clearly shows a *Panicum* plant and all other records of *C. lutea* were for *Panicum* species. Therefore the current record of a novel species, *C. clavispora*, on *Paspalum* is surprising, especially since the same fungus is found on an alternate, and unrelated, host, *Urochloa* sp.

The epithet of *C. sulcata* is derived from the latitudinal grooves on sclerotia. However, very similar furrows are found on the sclerotia of *C. setariicola* (Online Resources 1, 2). Lateral furrows on specimens from *Setaria* from Texas and New Mexico, resembling those of *C. sulcata,* were also noted by Alderman et al. (2004). The macromorphology of the florets of *Urochloa* and *Setaria* is similar, so the shape of the sclerotium seems to be partially determined by glumes.

Some problems may be encountered while establishing the identity of *Urochloa*/*Brachiaria* ergot based on conidial shape. In the species descriptions the conidia of *C. maximensis* are described as elliptic (Loveless 1964a), whereas in *C. sulcata* the conidia are defined as "predominantly allantoid, some cylindrical or elliptic" (Loveless and Herd 1964). However, as seen in Fig. 2 in Loveless (1964a) and on drawings in Loveless (1964b), somewhat allantoid, oblong, or elliptical conidia occur in both species. Our observations show that the shape of conidia is not a reliable marker for discriminating between *C. sulcata* and *C. maximensis* (Fig. 11, Online Resource 2).

Loveless (1964a) identified African *Urochloa maxima* ergot as *C. maximensis* and supposed that, despite the cosmopolitan distribution of *C. maximensis*, the species was originally indigenous to Africa. The first record of ergot on guinea grass in Australia dates from 1980 (Ryley 1981). Interestingly, *C. maximensis* was also recently collected from *Panicum coloratum* (Tab. 1), which according to recent phylogenetical studies (Aliscioni et al. 2003), is not closely related to *U. maxima*.

Phylogenetic relationships among *Claviceps* spp., based on analyses PhyML and RCM of ITS nrDNA sequence data, were revealed as four clades by both phylogenetic trees generated (Fig. 12). Clade 1 contained a group of closely related species parasitizing the most common African savanna grasses of diverse tribes. Clade 2, present in both

trees, but without statistical support, contained predominantly parasites of andropogonoid grasses with which the new species *C. clavispora* and *C. langdonii* were associated. This grouping does not permit a conclusion on geographical origin as it contains Mesoamerican endemites (*C. gigantea*) as well as Asian (*C. sorghi, C. sorghicola)* and African species (*C. africana*). The longer distances inside the clade (as compared with those in Clade 1) may suggest that the species relationships were influenced by early radiations of their hosts from the tribe Andropogoneae.

The present findings lend further support to the hypothesis (Pažoutová 2003) that *Claviceps* species did not show narrow co-evolution with their hosts but, rather, maintained flexibility and were stimulated to further evolution by migrations and subsequent colonization of the grass species newly available.

Acknowledgements The investigation reported here was partly supported by the Institutional Research Concept AV0Z50200510, by the grant 206/97/0611 of the Czech Grant Agency and partially by Sorghum, Millet and Other Grains CRSP (USAID). Thanks are due to A. Glatzle, N. W. McLaren, J. L. Reilly, E. M. Reis, M. Ryley, and R. Velasquez for sending us the specimens of ergotized grasses and reference strains and to the curators of CABI Bioscience Herbarium (Egham, UK), Herbarium Hamburgense (Hamburg, Germany), and US National Fungus Collection (Beltsville, MD) for specimen loans.

References

Alderman SC, Halse RR, White JF (2004) A reevaluation of the host range and geographical distribution of *Claviceps* species in the United States. Plant Dis 88:63–81

Aliscioni SS, Giussani LM, Zuloaga FO, Kellogg EA (2003) A molecular phylogeny of Panicum (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. Am J Bot 90:796–821

Anonymous (1966) Munsell book of color. Munsell Color Company, Baltimore

Bandyopadhyay R, Frederickson DE, McLaren NW, Odvody GN, Ryley MJ (1998) Ergot: a new disease threat to sorghum in the Americas and Australia. Plant Dis 82:356–367

Bastola DR, Otu HH, Doukas SE, Sayood K, Hinrichs SH, Iwen PC (2004) Utilization of the relative complexity measure to construct a phylogenetic tree for fungi. Mycol Res 108:117–125

Bhide VP, Hegde RK (1957) Ergot on bajri [*Pennisetum typhoides* (Burm.) Stapf and Hubbard] in Bombay state. Curr Sci 26:116

Bogo A, Mantle PG (1999) *Claviceps africana* discovered in India. Plant Dis 83:79

Crous PW, Groenewald JZ (2005) Hosts, species and genotypes: opinions versus data. Australas Plant Pathol 34:463–470

Doidge EM (1950) The South African fungi and lichens to the end of 1945. Bothalia 5:1–1094

Felsenstein J (2004) PHYLIP (Phylogeny Inference Package) version 3.63. Department of Genome Sciences, University of Washington, Seattle, USA

Fernandes CL, Fernandes ATF, Bezerra JL (1995) 'Mela': Uma nova doença em sementes de *Brachiaria* spp. no Brasil. Fitopatol Bras 20:501–503

Frederickson DE, Mantle PG, De Milliano WAJ (1991) *Claviceps africana* sp. nov.; the distinctive ergot pathogen of sorghum in Africa. Mycol Res 95:1101–1107

Gilman JC (1949) Second supplementary list of parasitic fungi from Iowa. Iowa State Coll J Sci 23:261–272

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Katoh K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinform 9:212. doi:10.1186/1471-2105-9-212

Kretzer A, Li Y, Szaro TM, Bruns TD (1996) Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: Phylogenetic and taxonomic implications. Mycologia 88:776–785

Langdon RFN (1952) Studies on ergot. Ph.D. Thesis, University of Queensland, Brisbane, Australia

Langdon RFN (1954) New species of *Claviceps*. University of Queensland, Department of Botany Papers 3:37–40

Loveless AR (1964a) Studies of Rhodesian ergots. 1. *Claviceps maximensis* Theis and *Claviceps pusilla* Cesati. Kirkia 4:35–40

Loveless AR (1964b) Use of the honeydew state in the identification of ergot species. Trans Br Mycol Soc 47:205–213

Loveless A (1967a) *Claviceps fusiformis* sp. nov., the causal agent of agalactia of sows. Trans Br Mycol Soc 50:13–18

Loveless AR (1967b) A new species of *Claviceps* on Cyperaceae. Trans Br Mycol Soc 50:19–22

Loveless AR (1985) The occurrence of ergot fungi (Clavicipitales) on the genus *Hyparrhenia* in southern Africa. Bot J Linn Soc 91:491–501

Loveless AR, Herd GW (1964) Studies of Rhodesian ergots. 2. A redescription of *Claviceps sulcata* Langdon. Kirkia 4:41–43

Marek SM, Muller RA, Walker NR (2006) First report of ergot of bermudagrass caused by *Claviceps cynodontis* in Oklahoma. Plant Dis 90:376–376

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ (2007) International Code of Botanical Nomenclature (Vienna Code), vol 146. Regnum Vegetabile 146. A.R.G. Gantner Verlag KG, Ruggell, Liechtenstein

Möller A (1901) Phycomyceten und Ascomyceten. In: Schimper AFW (ed) Botanische Mittheilungen aus den Tropen, vol 9. Jena, pp 1–319

Muthusubramanian V, Bandyopadhyay R, Tooley PW, Rajaram Reddy D (2005) Inoculated host range and effect of host on morphology and size of macroconidia produced by *Claviceps africana* and *Claviceps sorghi*. J Phytopathol 153:1–4

Naudè TW, Botha CJ, Vorster JH, Roux C, Van der Linde EJ, Van der Walt SI, Rottinghaus GE, Lv J, Lawrence AN (2005) *Claviceps cyperi*, a new cause of severe ergotism in dairy cattle consuming maize silage and teff hay contaminated with ergotised *Cyperus esculentus* (nut sedge) on the Highveld of South Africa. Onderstepoort J Vet Res 72:23–37

Nylander JAA (2004) MrModeltest 2.2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University

Otu HH, Sayood K (2003) A new sequence distance measure for phylogenetic tree construction. Bioinformatics 19:2122–2130

Pažoutová S (2001) The phylogeny and evolution of the genus *Claviceps*. Mycol Res 105:275–283

Pažoutová S (2003) Evolutionary strategy of *Claviceps*. In: White J, Bacon C, Hywel-Jones N, Spatafora J (eds) Clavicipitalean fungi: evolutionary biology, chemistry, biocontrol and cultural impacts. Marcel Dekker, New York, Basel, pp 329–354

Pažoutová S, Frederickson DE (2005) Genetic diversity of *Claviceps africana* on sorghum and *Hyparrhenia*. Plant Pathol 54:749–763

Pažoutová S, Taisinger J, Flieger M, Sajdl P, Bass A, Řeháček Z (1981) The relationship between intensity of oxidative metabolism and predominance of agroclavine or elymoclavine in submerged *Claviceps purpurea* cultures. J Nat Prod 44:225–235

Pažoutová S, Bandyopadhyay R, Frederickson DE, Mantle PG, Frederiksen RA (2000) Relations among sorghum ergot isolates from the Americas, Africa, India, and Australia. Plant Dis 84:437–442

Pažoutová S, Cagaš B, Kolínská R, Honzátko A (2002a) Host specialization of different populations of ergot fungus (*Claviceps purpurea*). Czech J Genet Plant Breed 38:75–81

Pažoutová S, Johnson N, Kolařík M, Rajasab AH (2002b) *Heteropogon triticeus* - a new host for *Claviceps sorghi* in India. J Phytopathol 150:1–4

Pažoutová S, Kolařík M, Kolínská R (2004) Pleomorphic conidiation in *Claviceps*. Mycol Res 108:126–135

Pažoutová S, Odvody GN, Frederickson DE (2005) Ergot fungus *Claviceps cynodontis* found on Bermuda grass (*Cynodon dactylon*) in the Americas. Can J Plant Pathol 27:541–546

Pažoutová S, Kolařík M, Odvody GN, Frederickson DE, Olšovská J, Man P (2008) A new species complex including *Claviceps fusiformis* and *Claviceps hirtella*. Fungal Div 31:95–110

Reis EM, Mantle PG, Hassan HAG (1996) First report in the Americas of sorghum ergot disease, caused by a pathogen diagnosed as *Claviceps africana*. Plant Dis 80:463

Ryley MJ (1981) Ergot of *Panicum maximum* in Queensland. Australas Plant Pathol 10:37–38

Ryley MJ, Alcorn JL, Kochman JK, Kong GA, Thompson SM (1996) Ergot on *Sorghum* spp. in Australia. Australas Plant Pathol 25:214

San Martín F, Lavín P, García A, García G (1997) Estados anamórficos de *Claviceps africana* y *Claviceps fusiformis* (Ascomycetes, Clavicipitaceae) asociados a diferentes pastos en Tamaulipás, México. Rev Mex Micol 13:52–57

Shelby RA, Olšovská J, Havlíček V, Flieger M (1997) Analysis of ergot alkaloids in endophyte-infected tall fescue by liquid chromatography electrospray ionization mass spectrometry. J Agric Food Chem 45:4674–4679

Shivas RG, Mitchell AA, Jubb TF (1997) *Corallocytostroma ornithocopreoides* sp. nov., an unusual toxic fungus on *Astrebla* and *Dichanthium* in north-western Australia. Mycol Res 101:849–852

Sundaram NV, Bhowmik TP, Khan ID (1969) A new host for *Claviceps microcephala* (Wallr.) Tul. Indian J Agr Sci 39:703–705

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Tanda S (1992) Mycological studies on the ergot in Japan (29). Two ergot fungi parasitic on Paspalum grasses. J Agr Sci (Tokyo) 36:292–312

Verzignassi JR, Fernandes CD (2001) Doenças em forrageiras. Campo Grande: Embrapa, Gado de Corte divulga 50

Walker J (2004) *Claviceps phalaridis* in Australia: biology, pathology and taxonomy with a description of the new genus *Cepsiclava* (*Hypocreales, Clavicipitaceae*). Australas Plant Pathol 33:211–239

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

Williams DG, Baruch Z (2000) African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. Biol Invasions 2:123–140

Yoshioka K (2002) KyPlot - a user-oriented tool for statistical data analysis and visualization. CompStat 17:425–437