A new species complex including *Claviceps fusiformis* and *Claviceps hirtella*

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Isolates of *Claviceps* species with lunate to fusiform macroconidia were collected from panicoid grasses in Texas and Zimbabwe and described as new species based on anamorphs since no teleomorphs were available. Characterization was based upon morphology and partial sequences of rDNA and β-tubulin. The isolates grouped into two strongly-supported clades. The first clade contained ancestral *C. hirtella* and *C. fusiformis* from pearl millet (*Pennisetum glaucum*) in clade terminal position with Texas isolates from native cup grass (*Eriochloa sericea*) and pearl millet grouped between them. The second clade consisted of African isolates from *Urochloa* and *Eragrostis*. The isolates from Texas from pearl millet and buffel grass (*Cenchrus ciliaris*) and isolates from *E. sericea* were described as new species, *Sphacelia texensis* and *Sphacelia eriochloae*, respectively. Both species had morphology, DNA markers, and alkaloid production that was intermediate between those features exhibited in *C. fusiformis* and *C. hirtella*. The African isolates from *Urochloa* and *Eragrostis* were also described as a new species, *Sphacelia lovelessii*. In shaken cultures, *C. hirtella* readily produced a whole range of clavines with agroclavine and festuclavine predominating, but ergometrine was also detected. *Claviceps fusiformis* produced mainly agroclavine and elymoclavine, *S. eriochloae* produced mainly agroclavine, elymoclavin and festuclavine and the cultures of *S. texensis* contained small amounts of agroclavine and festuclavine. Only traces of clavines were found in cultures of *S. lovelessii* of the second clade. The alkaloid content of infected florets in the sphacelial (honeydew) developmental stage was also measured. Only *C. fusiformis* and *S. eriochloae* produced alkaloids *in planta* at this early stage.

Key words: alkaloids, β-tubulin, *Cenchrus*, *Claviceps*, clavine, conidia, *Eragrostis*, *Eriochloa*, pearl millet, *Pennisetum*, phylogeny, *Sphacelia*, rDNA, *Urochloa*

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Introduction

Since 1997, infections by *Claviceps* species with lunate to fusiform macroconidia have been found in Texas on heads of introduced pearl millet (*Pennisetum glaucum*), buffel grass (*Cenchrus ciliaris*), and native cupgrass (*Eriochloa sericea*). San Martín *et al*. (1997) and Velásquez-Valle *et al*. (1998) detected similar infections in Mexico. However, no teleomorphs of the parasites were observed, mostly due to fungal hyperinfection, precluding species description by traditional sclerotial germination studies. Ergot disease of *C. ciliaris* was observed in the late 1980's in Texas (Craig and Hignight, 1991) but the authors did not describe the causal fungus. Morphologically similar *Claviceps* anamorphs (sphacelia) with fusiform to lunate conidia of unknown species were also collected from *Urochloa* spp. and *Eragrostis* sp. in Zimbabwe during 2000-2001. In this paper we report the identity of these fungi.

Only two species of *Claviceps* with markedly fusiform to lunate conidia have been formally described, both colonizing grasses from *Paniceae*: *C. fusiformis* and the Australian endemite *C. hirtella*. In addition, there are numerous records of sphacelial forms with lunate conidia from various grasses and locations in India (Table 1). *C. fusiformis* has been described as the main ergot pathogen of pearl millet or bajra (*Pennisetum typhoideum*, now *P. glaucum*) in Africa and India (Loveless,

1967; Thakur *et al*., 1984). The oldest specimen of *C. fusiformis* came from Ghana in 1925 (Loveless, 1967). In Africa, *C. fusiformis* was the typical ergot parasite of *Pennisetum* and *Cenchrus* (Loveless, 1964a, 1967).

In India, the picture is more confusing. Pearl millet was not initially recorded as a host plant of ergot fungi with fusiform or lunate conidia, but such fungi were recorded, although not identified, on other panicoid grasses well before the first documented epiphytotics of *C. fusiformis*. The first record of an ergot with long conidia (20.7 × 6.1 µm), later identified as *C. fusiformis* (Siddiqui and Khan, 1973), refers to pearl millet in Banaskanatha district in Bombay State in 1955 (Shinde and Bhide, 1958). Shinde and Bhide (1958) also noted that the asci and conidia of the fungus from pearl millet resembled those observed by Thomas *et al*. (1945b) on *Pennisetum hohenackeri*. The following year, fully developed ergot infection at severe incidence was recorded in Bombay and Mysore states (Bhide and Hegde, 1957). Pearl millet ergot remained little known in other regions until 1966 when, with the introduction of the first generation of pearl millet hybrids (HB 1 and HB 2), epiphytotics developed in other states (Sundaram *et al*., 1969).

In addition to *Cenchrus ciliaris*, *Cenchrus setigerus* and *Pennisetum hohenackeri*, the unidentified Indian ergot anamorphs originated from *Panicum antidotale*, *Urochloa panicoides*, *Urochloa ramosa*, and *Paspalidium flavidum* (now *Setaria flavida*) (Ramakrishnan, 1937; Thomas *et al*., 1945a,b; Adyanthaya, 1946; Ramakrishnan, 1947). Cross-infection experiments have shown that some of these fungi possessed polygeneric host ranges. Ramakrishnan (1947) infected *Urochloa ramosa* with conidia collected from *Cenchrus setigerus*, whereas Thakur and Kanwar (1978) succeeded in infecting pearl millet with an ergot pathogen from *Panicum antidotale*.

The question of whether the pearl millet ergot epiphytotics in India were caused by a change in host of an indigenous population of *C. fusiformis* already present e.g. that from *Cenchrus*, or by introduction of an African *C. fusiformis* more virulent to *Pennisetum* spp. remained open. Similarities between the alkaloid composition of African and Indian isolates do not preclude an introduction (Kumar and Arya, 1978).

Langdon (1952) noted the similarity of conidial morphology and host specificity of *Claviceps* anamorphs with lunate conidia in the Indian records to those of *Claviceps hirtella* (Langdon, 1942). *C. hirtella* is the only Australian species colonizing *Urochloa* spp. Queensland Plant Pathology Herbarium harbours specimens of *C. hirtella* from species of *Urochloa*, *Paspalidium*, *Eriochloa* and *Entolasia*. Since 1976, *C. hirtella* has been repeatedly found on *Cenchrus ciliaris* (supposedly introduced in 1870-1880), presenting thus a problem for the seed industry.

In former Rhodesia (now Zimbabwe), Loveless (1964a) defined 13 groups of *Claviceps* sphacelial anamorphs on the basis of conidial morphology and host species. Six of them were assigned to teleomorphs already described (*C. paspali*, *C. digitariae*, *C. sulcata*, *C. maximensis*, *C. pusilla* and *C. cynodontis*). Since then, three new species have been identified with the corresponding morphological groups: *C. rhynchelytri*, group No. 1 (Herd and Loveless, 1965); *C. fusiformis*, group No. 7 (Loveless, 1967); and *C. africana*, group No. 10 (Loveless, 1964b; Herd and Loveless, 1965; Frederickson *et al*., 1991). Two of the original groups (No. 11 from *Hyparrhenia* spp. and No. 13 from *Loudetia* spp.) are still present in Zimbabwe (Frederickson, 1990; Pažoutová and Frederickson, 2005). No records of anamorphs with lunate conidia on *Eragrostis* in Zimbabwe, and only one for *Urochloa* and *Brachiaria* in South Africa, 1988 (conidia measuring 13 µm × 4.4 µm and identity suggested to be *Claviceps sulcata*), were mentioned in these studies.

Sclerotia of African and Indian isolates of *C. fusiformis* from pearl millet contain agroclavine and elymoclavine as the main alkaloid components; minor or trace components are chanoclavine, setoclavine, penniclavine and, occasionally, festuclavine. Both also readily produce clavines in submerged culture *in vitro* (Banks *et al*., 1974; Bhat *et al*., 1976; Singh and Husain, 1977; Kumar and Arya, 1978). However, Janardhanan *et al*. (1982) detected festuclavine as the major component, accompanied by agroclavine, and chanoclavine, in sclerotia of *Claviceps* sp. with lunate conidia

Table 1. A survey of Paleotropic ergot fungi with fusiform to falcate conidia.

	Sclerotia	Conidial shape	Conidial size (µm)	Citation
Unnamed records:				
Urochloa ramosa	unknown	falcate, pointed	14.6 < 19.8 < 29.2 × 4.4 < 5.8 < 7.3	(Ramakrishnan, 1937)
Urochloa panicoides	globose to oblong, brown, 1.5 × 0.6 mm	fusiform to lunate	12.8 < 15.4 < 19 × 3.2 < 5.1 < 6.4	(Ramakrishnan, 1947)
Urochloa distachya	subglobose to spherical	arcuate, slightly pointed	12.6-19 × 3.8-6.3	(Thirumalachar, 1945)
Setaria flavida	curved, brown 4-5 × 1 mm	lunate	12.8 < 16 < 20.8 × 4.8 < 5 < 6.4	(Ramakrishnan, 1947)
Panicum antidotale	unknown	fusiform		(Janardhanan *et al*., 1982)
Pennisetum orientale x P. purpureum	unknown	fusiform	11.4 < 21 < 28.6 × 3.9 < 5.3 < 5.8	(Sundaram *et al*., 1969)
Cenchrus setigerus	unknown	fusiform to lunate	12.8 < 17.9 < 26 × 3.2 < 4.8 < 6.4	(Ramakrishnan, 1947)
Cenchrus ciliaris	unknown	lunate	14 < 18.4 < 25.2 × 5.0 < 5.96 < 8.4	(Adyanthaya, 1946)
Described species:				
Claviceps fusiformis	obpyriform, slightly protruding	fusiform to lunate	9.5 < 15.8 < 22.5 × 3 < 3.6 < 5	(Loveless, 1967)
Claviceps fusiformis	elongated to round, brown	fusiform	12 < 15.9 < 26.4 × 2.4 < 3.9 < 6	(Thakur *et al*., 1984)
Claviceps hirtella	subglobose, yellowish brown	arcuate	11-16.5 × 4.5-6.5	(Langdon, 1942)

from *Panicum antidotale*. The same alkaloids, plus elymoclavine, were found in submerged culture isolates from this source, suggesting that the pathogen may not have been *C. fusiformis*. No information is available about alkaloids of *C. hirtella*.

Crous and Groenewald (2005) stated that taxonomic names based solely on fungal phenotype often represent species complexes or cryptic species, and not operational units. The objective of this study was to assess the relationships of the existing species *C. fusiformis* and *C. hirtella* to the anamorphs with lunate conidia to determine if they represent such a species complex. Characterization of the isolates was based on rDNA and partial β-tubulin sequences, morphological markers and alkaloid biosynthesis. An erroneous rDNA sequence, AJ133392 for *C. fusiformis*, from a previous study (Pažoutová, 2001) was also corrected.

Materials and methods

Herbarium specimens and isolates

The origin of specimens and isolates is given in Tables 2 and 3. Specimens of *Claviceps hirtella*, BRIP 16635 and 13544, and monosporic isolates of *C. hirtella* were obtained from Queensland Plant Pathology Herbarium (BRIP, courtesy of Dr Roger Shivas). Strain *C. fusiformis* 47A was isolated in 1957 from *Pennisetum* material originating from the Ivory Coast (Tyler, 1958; Pažoutová and Tudzynski, 1999). Strain *C. fusiformis* F27 was obtained from the former Farmitalia collection from Italy in 1983, but it originated from an African *Pennisetum* specimen collected in the 1960's. Pure cultures of *Claviceps* specimens from Africa and Texas were isolated by plating honeydew drops from florets of infected pearl millet onto T2 agar and subsequent transfer of agar plugs with germinating macroconidia (Pažoutová *et al*. 2002). Only *C. fusiformis* from India was purified from surface-sterilized sclerotia (Pažoutová *et al*., 2000). Herbarium Type specimens were deposited in the herbarium of the Mycological Department, National Museum in Prague (PRM). Ex-holotype and other representative strains were stored in liquid nitrogen and deposited in the Czech Collection of *Clavicipitaceae* (CCC; Institute

Table 2. Characteristics and origins of sphacelial specimens.

Species	Specimen Accession	Host	Location	Year	Collector	Alkaloids per sphacelia (µg)	Conidial dimensions (µm)			
							Length	SD	width	SD
Claviceps fusiformis	PRM 857332	*Pennisetum glaucum*	Shamva, Zimbabwe	1999	N. W. Mc Laren	6	9.5 < 18.9 < 27.5	3.7	3.9 < 4.8 < 6.3	0.4
	PRM 857331	*Pennisetum glaucum*	Matopos, Zimbabwe	2000	D.E. Frederickson	16	10.2 < 17.9 < 21.6	2.0	2.8 < 4.8 < 8.5	0.5
	PRM 857333	*Pennisetum glaucum*	Gulbarga, Karnataka, India	2006	R.H. Angadi	30	15 < 20.9 < 26.5	2.7	3.2 < 4.9 < 6.5	0.8
Sphacelia eriochloae	PRM 857335	*Eriochloa sericea*	Agua Dulce, Texas	1997	G. N. Odvody	24	6.7 < 10.6 < 14.9	1.5	2.6 < 3.6 < 4.6	0.4
	PRM 857334	*Eriochloa sericea*	Kingsville, TX	2006	G. N. Odvody	13	7.7 < 12.1 < 15.7	1.5	2.5 < 3.7 < 5.5	0.5
Sphacelia texensis	*	*Cenchrus ciliaris*	Agua Dulce, Texas	1997	G. N. Odvody	0	8.2 < 12.6 < 19.6	1.6	3.0 < 4.0 < 4.6	0.5
	PRM 857338	*Cenchrus ciliaris*	Kenedy county, Texas	1997	O. Rodriguez	0	8.8 < 12.1 < 16.8	1.4	3.1 < 3.7 < 4.6	0.4
	*	*Pennisetum glaucum*	Weslaco, Texas	1998	G. N. Odvody	0	7.1 < 11.6 < 15.1	1.5	2.8 < 3.9 < 5.5	0.4
	PRM 857336	*Pennisetum glaucum*	Corpus Christi, Texas	2003	G. N. Odvody	0	8.8 < 12.9 < 17.2	1.6	2.8 < 4.0 < 4.8	0.4
	PRM 857337	*Pennisetum glaucum*	Weslaco, Texas	2003	G. N. Odvody	0	8.1 < 13.1 < 18.2	1.6	3.5 < 4.2 < 5.3	0.4
Claviceps hirtella	BRIP13544	*Cenchrus ciliaris*	Meandarra, Queensland, Australia	1982	V. French	0	9.6 < 14.1 < 24.8	1.9	3.4 < 4.3 < 5.4	0.4
	BRIP16635	*Cenchrus ciliaris*	Blackall, Queensland, Australia	1989		0	9.1 < 12.5 < 16.6	1.5	2.2 < 3.5 < 4.5	0.4
Sphacelia lovelessii	PRM 857342	*Urochloa* sp.	Matopos, Zimbabwe	2000	D.E. Frederickson	0	15.6 < 19.9 < 25.2	2.2	2.9 < 5.2 < 5.7	0.5
	*	*Urochloa trichopus*	Matopos, Zimbabwe	2001	D.E. Frederickson	0	10.6 < 15.4 < 20.4	1.9	3.0 < 5.2 < 5.3	0.5
	PRM 857340	*Urochloa mosambicensis*	Matopos, Zimbabwe	2001	D.E. Frederickson	0	10 < 14.3 < 20.7	1.7	3.6 < 4.5 < 6.1	0.6
	PRM 857341	*Urochloa oligotricha*	Matopos, Zimbabwe	2001	D.E. Frederickson	0	12.4 < 17.6 < 23.2	2.0	4.2 < 4.7 < 6.4	0.5
	PRM 857339	*Eragrostis* sp.	Matopos, Zimbabwe	2001	D.E. Frederickson	0	12.5 < 18.1 < 24.8	2.1	4.1 < 5.6 < 6.3	0.9

* Small specimens consumed by microscopy were not deposited
SD – standard deviation

Table 3. Origin and identity of isolates used for DNA sequencing and alkaloid production.

Species	Specimen of origin	Isolate	Host	Location	Year	Collector	Accession No.	
							rDNA	β-tubulin
Claviceps fusiformis		CCC 110 (F27)	*Pennisetum* sp.	Africa	1960's		EF052275	EF473876
		CCC 106 (47A)	*Pennisetum typhoides*	French Central Africa	1957		AJ133392	AM498382
	PRM 857332	CCC 525	*Pennisetum glaucum*	Shamva, Zimbabwe	1999	N.W. Mc Laren	AJ626727	EF473867
	"	CCC 524					"	EF473866
	PRM 857333	CCC 846	*Pennisetum glaucum*	Gulbarga, Karnataka, India	2005	R.H. Angadi	EF052276	EF473877
Sphacelia eriochloae	PRM 857334	CCC 859	*Eriochloa sericea*	Kingsville, Texas	2006	G. N. Odvody	EF473864	EF473875
	"	CCC863						
	"	CCC 868						
	"	CCC 872						
Sphacelia texensis	PRM 857337	CCC 774	*Pennisetum glaucum*	Weslaco, Texas	2003	G. N. Odvody	EF052277	EF473873
	PRM 857336	CCC 776	*Pennisetum glaucum*	Corpus Christi, Texas	2003	G. N. Odvody	EF052278	EF473874
	"	CCC 778					EF052279	EF473878
	"	CCC 777						
	"	CCC 858						
Claviceps hirtella	BRIP 43959	CCC 786	*Urochloa sp*.	Goondiwindi, Queensland, Australia	2004	R.G. Shivas, T.S. Marney	EF052280	EF473872
	"	CCC 792						EF473871
	"	CCC 787						
	"	CCC 788						
	"	CCC 789						
	"	CCC 790						
	"	CCC 791						
Sphacelia lovelessii	PRM 857340	CCC 642	*Urochloa mosambicensis*	Matopos, Zimbabwe	2001	D.E. Frederickson	EF052282	EF473870
	PRM 857341	CCC 646	*Urochloa oligotricha*	Matopos, Zimbabwe	2001	D.E. Frederickson	EF052281	EF473869
	PRM 857339	CCC 647	*Eragrostis sp*.	Matopos, Zimbabwe	2001	D.E. Frederickson	AJ605996	EF473868
Claviceps viridis		CBS 125.63	*Oplismenus compositus*	India			AJ133404	EF473865

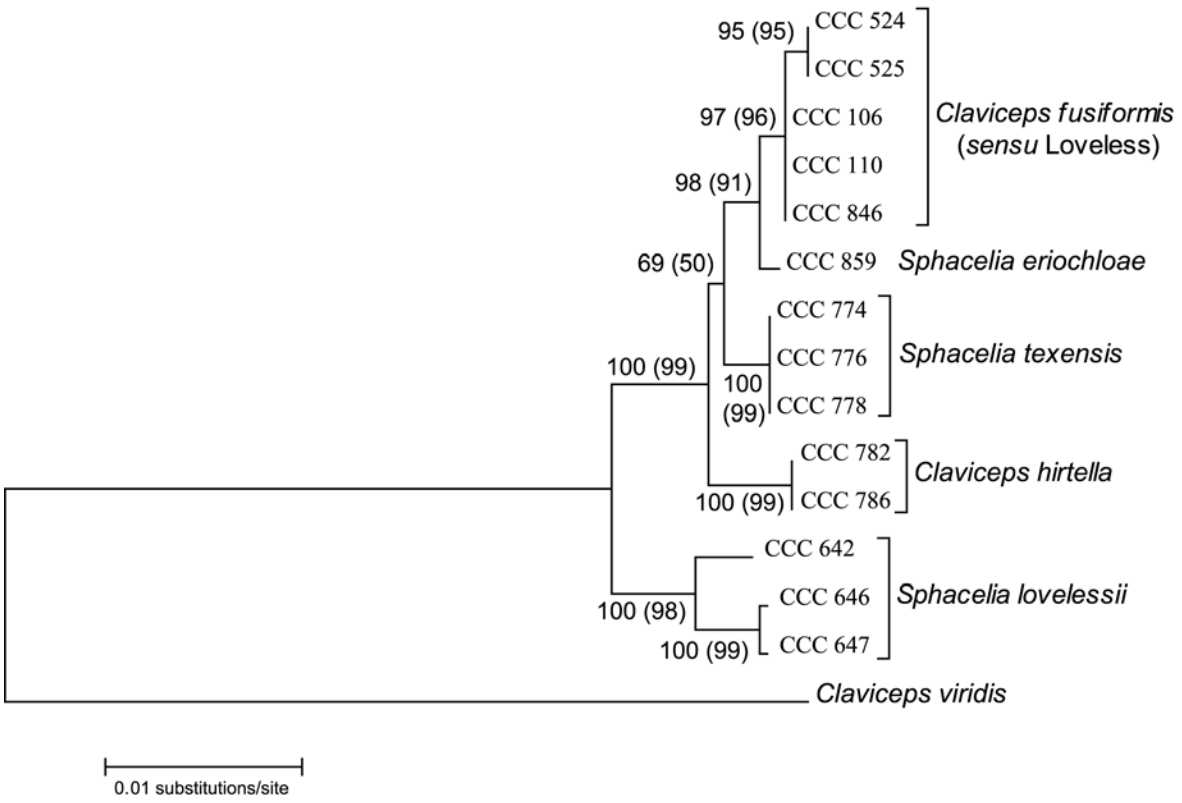

Fig. 1. Neighbor-joining tree obtained from phylogenetic analysis of a combination of rDNA and partial β-tubulin sequences with *Claviceps viridis* as an outgroup. Bootstrap confidence levels, based on 1000 replicates, are given on the appropriate branches. Bootstrap values for maximum parsimony tree (with the same topology) are given in brackets. Note that a recent *C. fusiformis* isolate from India (CCC 846) is more closely related to African isolates originating from around 1960 (CCC 106, 110) than to recent African isolates (CCC 524, 525).

of Microbiology, Academy of Sciences of the Czech Republic, Prague).

Media and cultivation

Isolates were maintained on T2 agar slants by transfer every two months. For alkaloid production, seed cultures in sucrose-asparagine medium (TI) were inoculated with 3 ml of conidial suspension from a slant culture and incubated for 10 days. Five ml of seed culture were transferred to fermentation culture (T2) and incubated for 20 - 29 days (Pažoutová *et al*., 1981) The cultivations proceeded on a rotary shaker at 24 ^{o}C in 250 ml Erlenmeyer flasks with 60 ml of the respective medium. Colony morphology was observed after 15 days of growth on T2 agar plate

Microscopy

Only honeydew specimens were used for observation and measurement of conidial size as conidia from cultures exhibit greater variability in shape and size. Conidia were stained in 1% cotton blue in 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.). At least 50 conidia from each sample were measured. Statistical analyses of spore size data were performed using Kyplot 2.0 beta 15 (Yoshioka, 2002).

Alkaloid analysis

Cultures were centrifuged and in the suitably diluted supernatant, the alkaloids were measured colorimetrically using Van Urk's reagent (Pažoutová *et al*., 1981) with elymoclavine as a standard. For qualitative alkaloid analysis, the culture was centrifuged; supernatant was alkalized with NH_4OH to pH 8-9 and twice extracted with the same volume of dichloromethane. The extracts were combined and dried using anhydrous Na_2SO_4, then

evaporated to dryness and dissolved in 200 µl of methanol. Alkaloid content was analyzed by high performance liquid chromatography (HPLC) (Pažoutová *et al*., 2000). Thin layer chromatography (TLC) of alkaloids was performed on silica gel plates (Merck) exposed briefly to NH_3 vapors, developed in chloroform:methanol (8:2), and detected using Ehrlich's reagent spray.

DNA preparation and analyses

DNA was purified from 4-7 days-old mycelium grown on T2 plates overlaid with cellophane using an UltraClean 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 D1- D2 domains of the 28S region were amplified with primers ITS5 and NL4 (White *et al*., 1990). A region of the β-tubulin gene containing part of intron 1, introns 2, 3 and 4, exons 2, 3, 4 and the first 56 base pairs of exon 5 was obtained using primers T1 and T22 (O'Donnell and Cigelnik, 1997). The reaction conditions in a Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany) were as follows: 1 cycle of 3 minutes at 95°C, 30 seconds at 55°C and 1 minute at 72°C; 30 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 1 minute at 72°C; 1 cycle 30 seconds at 95°C, 30 seconds at 55°C and 10 minutes 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 done at Macrogen Inc. (Seoul, Korea).

Phylogenetic analysis

Two sequence alignments were constructed: the datasets for rDNA and β-tubulin. Sequences were aligned using MUSCLE (Edgar, 2004) and the alignments were edited with BioEdit (Hall, 1999). Phylogenetic analyses were performed using MEGA 3.1 (Kumar *et al*., 2004). A neighbor-joining tree was constructed using the Kimura-2 parameter model with complete deletion option. A maximum parsimony (MP) tree was computed using close neighbor interchange, mini-heuristic search of the initial tree with a search factor 3 and random addition of trees with 60 repetitions. The stability of clades was evaluated by a bootstrap test with 1000 replications. The dataset and analysis were archived in TreeBase under the submission ID number SN3321.

Results

All infections sampled (Table 2) exhibitted the sphacelial stage of ergot development. Honeydew production (fresh drops or a dried crust on glumes) was the only sign of infection. Sphacelia were still hidden in the glumes and no mature sclerotia were found, except for the pearl millet specimen from India. Attempts to germinate these sclerotia into perithecial heads were unsuccessful.

Phylogenetic results

The dataset of rDNA sequences contained 1187 sites, yielding 22 variable positions with 12 singletons and only 10 sites informative for parsimony. The dataset of partial β-tubulin sequences contained 1554 sites with 60 variable positions, 36 of which were informative for parsimony. All mutations in the coding regions of the β-tubulin gene were synonymous at the third codon position. Both datasets were combined and *Claviceps viridis* was added as the outgroup. The topology of the distance tree and consensus MP tree was identical (Fig. 1). Parsimony analysis found 15 equally parsimonious trees, which differed only in the mutual positions of *C. fusiformis* isolates from Africa and India.

Two well-supported clades were found. The *fusiformis-hirtella* clade comprised four well-supported lineages with very small distances between sequences: the African and Indian *C. fusiformis* isolates from pearl millet, a Texas isolate from *Eriochloa sericea*, isolates from pearl millet from the same area and *C. hirtella* at the ancestral position. The second clade consisted of African isolates from *Urochloa* and *Eragrostis*.

Alkaloid production

Alkaloids *in planta* (Table 2) were only detected in pearl millet florets containing sphacelia and honeydew of *C. fusiformis* from

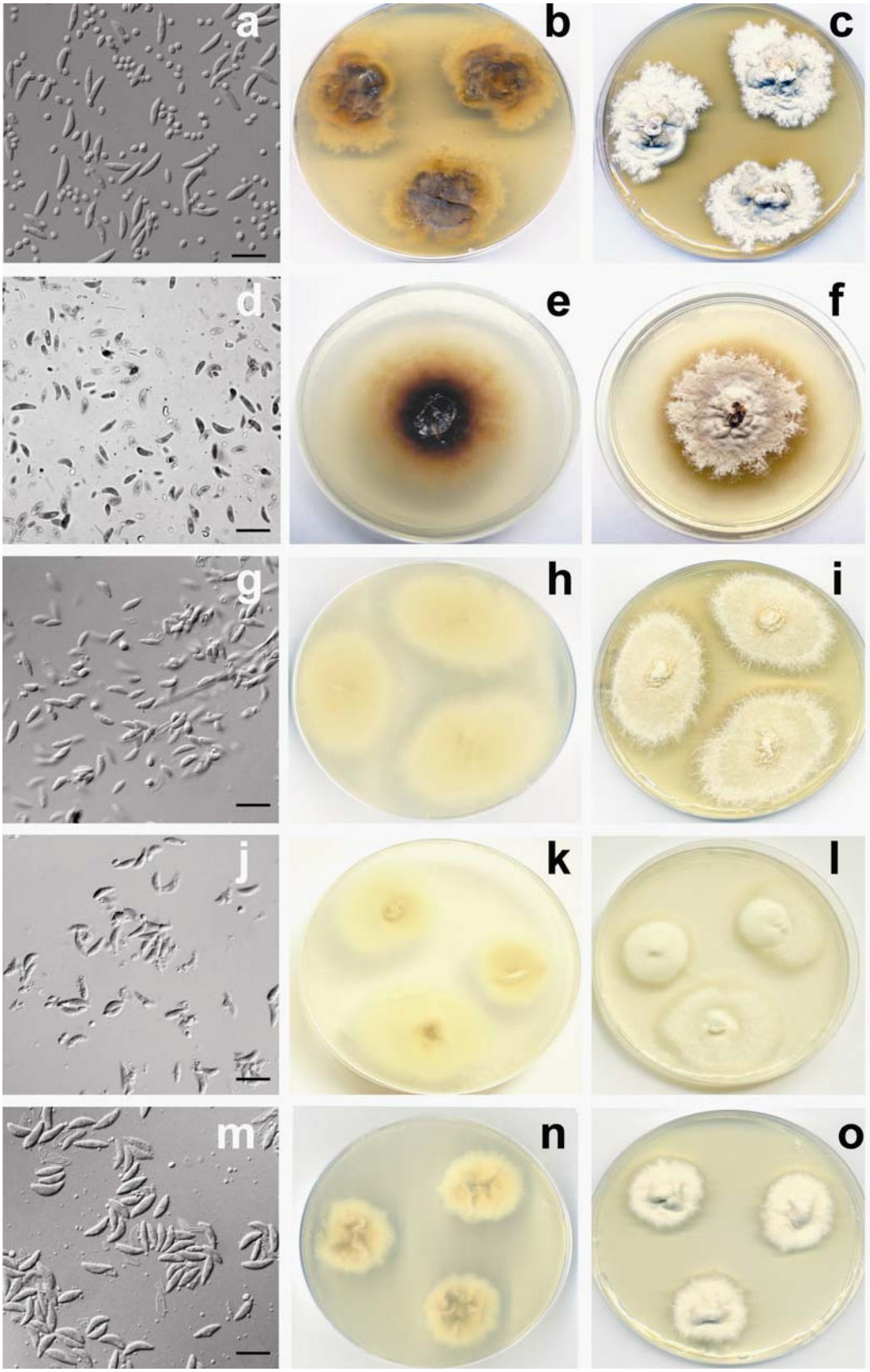

Fig. 2. Morphology of conidia from honeydew and colonies (reverse and obverse) on medium T2. **a-c.** *Claviceps fusiformis*. **d-f.** *Sphacelia eriochloae*. **g-i.** *Sphacelia texensis*. **j-l.** *Claviceps hirtella*. **m-o.** *Sphacelia lovelessii*. Bars = 20 μm.

Africa and India and in *Eriochloa* florets with *S. eriochloae* from Texas.

All lineages defined by the phylogenetical analysis were also clearly distinguishable by the specific qualitative combination of alkaloids. In shaken cultures (Table 4), *C. fusiformis* isolates from Africa and India produced agroclavine and elymoclavine as major alkaloids, accompanied by chanoclavine. *C. hirtella* readily produced a whole range of clavines with agroclavine and festuclavine predominating, but ergometrine was also detected. Of the Texas isolates, the isolate from *Eriochloa* produced mainly agroclavine, elymoclavine and festuclavine and the cultures of isolates from pearl millet contained small amounts of agroclavine and festuclavine. African isolates from *Urochloa* and *Eragrostis* produced only traces of clavines on the detection threshold limit.

Morphology

Colony morphology was observed on T2 agar, specifically suited for *Claviceps* growth and secondary metabolite production (Fig. 2). On widely-used fungal media such as malt-extract agar (MEA) and Czapek-Dox (CZD), culture growth is about half of that on T2 and the typical pigmentation and structure of the colonies is not expressed. Therefore, MEA and CZD are unsuitable for morphological characterization of ergot fungi.

Colonies of *C. fusiformis* isolates from pearl millet (Africa, India) and those from Texas from *Eriochloa* and pearl millet were similar (Fig. 2) on T2 agar. Typically, all showed rapid growth (2-4.8 cm in 14d) with a diffuse and markedly radiating margin. Colony was mostly velutinous, consisting of highly sporulating conidiophores, giving a powdery appearance; raised with cerebriform wrinkles in the centre and plane toward the margin; obverse, off-white to grayish; reverse, similar or slightly brown, typically turning reddish brown with age in some strains of *C. fusiformis* (CCC 525, CCC 846); soluble pigments yellowish to reddish brown.

African isolates from *Urochloa* and *Eragrostis* exhibited restricted growth (< 1.5 cm) and absence of sporulation and pigment production. In these isolates, conidiation ability was lost during the first year of isolation, whereas cultures of all the other isolates retained conidiation ability, sometimes for decades.

Conidia of *C. fusiformis* from Africa and India (Table 2, Fig. 2) were long (mean 18.9 µm), mostly straight and fusiform. Conidia from honeydew of Texas isolates from *Eriochloa* and pearl millet, and of *C. hirtella*, were smaller (12 µm) and more lunate. Conidia from African honeydew specimens from *Urochloa* and *Eragrostis* were the same length as *C. fusiformis* but slightly wider (5.2 µm; c.f. 4.8 µm *C. fusiformis*) and more lunate.

Taxonomy

All analyses reveal that the specimens from Africa and India, determined traditionally as *C. fusiformis* according to Loveless (1967), represent a taxonomically homogenous group with host specificity to *Cenchrus* and *Pennisetum*. Similarly, the Australian species *C. hirtella* has a distinct position. Specimens with fusiform conidia colonizing pearl millet and *C. ciliaris* in Texas were closely related to *C. fusiformis*, but showed distinct differential characters in DNA sequence, conidia size and alkaloid production; therefore this group is described here as new species. Because there was no teleomorph available (sphacelia were mostly infected in the course of development by *Epicoccum andropogonis* and did not mature) and the International Code of Botanical Nomenclature does not allow the application of a teleomorph name in the absence of the teleomorph (Greuter *et al*., 2000), the species will be described as *Sphacelia texensis*. Also a new name, *Sphacelia eriochloae*, is proposed here for the fungus from *Eriochloa* because of its unique combination of host specificity with alkaloid and DNA characteristics.

African isolates from *Urochloa* and *Eragrostis* differed from all other specimens in all aspects and the new name *S. lovelessii* is designated here for this new species.

Claviceps fusiformis Loveless, Transactions of the British Mycological Society 50: 17. (1967) (Fig. 2a,b,c)

Cultural characteristics: colonies on T2 medium, (14d, 24°C) 21-48 mm in diameter; diffuse and markedly radiating margin, colony mostly velutinous, consisting of highly sporulating conidiophores giving a powdery appearance,

Table 4 Alkaloid yield of *Claviceps*/*Sphacelia* isolates in shaken culture.

	Claviceps fusiformis				***Sphacelia eriochloae***					***Sphacelia texensis***					***Claviceps hirtella***							***Sphacelia lovelessii***		
Isolate CCC No.	524	525	106	110	846	859	863	868	872	774	776	777	778	858	786	787	788	789	790	791	792	645	646	649
Cultivation (days)*)	21	21	21	21	21	21	21	21	21	29	29	29	29	29	27	24	24	29	24	24	27	29	29	29
Total alkaloids ($mg.L^{-1}$)	1520	1050	1265	510	800	1280	7930	3780	5210	65	91	111	98	117	2472	971	1354	1433	586	326	219	37	36	31
Constituent alkaloids (%):																								
Ergometrine															6.1	2.6	3.3	3.4	3.2	8.7	7.2			
Elymoclavine	21	26.7	13.2	68	10.5	22.1	21.4	24.3	22.3						12.7	4.5	4.7	6.6	6.6	7.9	3.6			
Chanoclavine	1.3	2.9	1.4	24	0.8	2	6.5	2	6.1						1.9	1.9	1.6	3	2.5	3.3	2.6	100		ND
Chanoclavine-1-aldehyde	0.7		2.9																					
DH-setoclavine															6.7	5.7	3.1	6.7	4.9	5.9	4.2			
iso-DH-setoclavine															1	1.3	1.1	1.2	1.1	1.2	1.2			
Agroclavine	75.7	68.5	80.3	8	70.6	41.4	34.4	30.1	40.8	17.1	17.5	34.7	8.4	55.2	24	19.2	24.2	26.1	13.2	5.8	12.5		100	ND
Festuclavine						12.4	9.3	8.1	9.1	82.9	35.3	46.5	27.1	44.8	42.3	57.9	59.6	48.7	65	63.5	66.4			
Pyroclavine						1.9	1.4	10.4	1.1						4.2	5.7	1.3	3	2.4	2.5	1.2			
Lysergol, DH-lysergol					4.2										1	1.3	1.1	1.2	1.1	1.2	1.2			
unknown clavines	1.31	1.9	2.1		14	20	27	25	20	0	47.2	18.8	64.5	0										

*) Cultivations were terminated when viscosity due to glucan production rendered aeration ineffective

ND - not determined, alkaloid content too low to permit a qualitative analysis

raised with typically cerebriform wrinkles in the centre and plane toward the margin, obverse off-white to grayish, reverse similar or light brown getting typically reddish brown in age in some strains, soluble diffuse pigment yellowish to reddish brown to vinaceous.

Macroconidia: fusiform, straight, rarely lunate (10-28 × 3-9 µm, mean 19 × 5 µm).

Teleomorph: not examined, see Loveless (1967) and Thakur *et al*.(1984).

Alkaloids: production in the culture: spontaneous, high (500-2000 mg/L); main alkaloids: chanoclavine, agroclavine, elymoclavine.

Habitat: in living florets of *Pennisetum* and *Cenchrus* spp.

Known distribution: Africa, India.

Material examined: ZIMBABWE, Matopos, from floret of *Pennisetum glaucum* 2001, D.E. Frederickson (PRM 857331); Shamwa, from floret of *Pennisetum glaucum* 2001, N. McLaren, (PRM 857332) (cultures CCC524, 525); INDIA, Karnataka, Gulbarga, from floret of *Pennisetum glaucum* 2005, R.H. Angadi, (PRM 857333) (culture CCC846).

Notes: Our concept of *C. fusiformis* agrees with definition of Loveless (1967) and Thakur *et al*.(1984). The species infects typically species of *Cenchrus* and *Pennisetum*. Long fusiform conidia which are rarely lunate are also distinct.

Claviceps hirtella Langdon, Proceedings of the Royal Society of Queensland 54: 27. (1942) (Fig. 2j, k, l)

Cultural characteristics: colonies on T2 medium (14d, 24°C) from 25 mm to 38 mm in diameter, obverse off-white, reverse with shades of rose-grey. Two types of growth occurred even in colonies of the same isolate. Colonies growing more rapidly were more diffuse on margin, markedly radiating, velutinous and plane. Colonies with slower growth consisted of dense mycelial mat. Sporulation was absent in both cases.

Macroconidia: lunate to fusiform (9-25 × 2-5.5 µm, mean 13 × 4 µm).

Teleomorph: not examined, see Langdon (1942)

Alkaloids: production in the culture: spontaneous, high (200-2500 mg/L); main alkaloids: ergometrine, chanoclavine, setoclavine, elymoclavine, agroclavine, festuclavine.

Habitat: in living florets of *Urochloa*, *Cenchrus*, *Paspalidium*, *Eriochloa*, and *Entolasia*.

Known distribution: Australia.

Material examined: AUSTRALIA, Qld, Meandarra, in florets of *Cenchrus ciliaris*, 1982 (BRIP13544); AUSTRALIA, Qld, Blackall, in florets of *Cenchrus ciliaris*, 1989 (BRIP16635); AUSTRALIA, Qld, Goondiwindi, in florets of *Urochloa* sp., 2004 (BRIP 43959) (cultures CCC786-CCC792).

Notes: The typical differential character of this species is the production of ergometrine.

Sphacelia eriochloae Pažoutová & Odvody **sp. nov.** (Fig. 2d, e, f)

Etymology: Referring to the host plant name.

Hab. in ovariis et in inflorescentiis *Eriochloae sericeis*, Texas

Species similis *Claviceps fusiformis* sed differt per suam combinationem characterum; *macroconidiis* hyaliniis, fusoideis vel lunatis (6.7-15.7 × 2.5-5.5 µm, mediet. 11.6 × 3.6 µm); regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank EF473864). *Teleomorphosis* ignota.

Cultural characteristics: colonies on T2 medium (14d, 24°C) 21-26 mm in diameter, like *C. fusiformis*.

Macroconidia: lunate to fusiform (6.7-15.7 × 2.5-5.5 µm, mean. 11.6 × 3.6 µm)

Teleomorph: not observed.

Alkaloids: production in culture: spontaneous, very high (2000-8000 mg/L); main alkaloids: chanoclavine, festuclavine, elymoclavine, agroclavine.

Habitat: in living florets of *Eriochloa sericea*

Known distribution: Texas, Mexico.

Material examined: TEXAS, Agua Dulce, in florets of *Eriochloa sericea*, 1997, G.N. Odvody (PRM 857335); TEXAS, Kingsville, in florets of *Eriochloa sericea*, 2006, G.N. Odvody (**HOLOTYPE**, PRM 857334) (ex type culture CCC859), Mycobank Accession No.: MB 510710

Molecular characters: sequence of the ITS and D1D2 regions of the 28S rDNA unique (EF473864). The sequence is given in the format [three-prime 18S]ITS1*5.8S*ITS2 [five-prime 28S].

[ATCATTA]CCGAGTTTTCAACTCCCAAAC
CCACTGTGAACCTATACCAAAAACGTTGCCTCG
GCGGGACATGCGCCCCGGACCGCCCCCCCCCCT
CGCGGGGGAGGGCGCCGGATCCCACGGCCGCCC
GCCGGGGGCCCCAAACTCTGTATTCCCATAGCG
GCATGTCTGAGTGGATTTATCCAATAAATCA*AA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCGCCAGTACTCTGGCGG

GCATGCCTGTTCGAGCGTCATT*TCAACCCTCAG
GCCCCCGGGCCTGGTGTTGGGGACCGGCTCACG
GGGGGGAGGCACAGCGCCCCCCCCCCCTGCCGC
CCCCTAAATGGATCGGCGGCCACGCCGCGGCCT
CCCCTGCGCAGTAACATACCACCTCGCAGGCGG
CTGGCTCGGCGCGGCCACTGCCGTAAAACGCCC
AACTTCTCCAGAG[TTGACCTCGAATCAGGTAGG
AATACCCGCTGAACTTAAGCATATCAATAAGCG
GAGGAAAAGAAACCAACAGGGATTGCCCCAGT
AACGGCGAGTGAAGCGGCAACAGCTCAAATTTG
AAATCTGGCCCCCCGGGGCCCGAGTTGTAATTT
GCAGAGGATGCTTTTGGCGAGGCGCCTTCCGAG
TTCCCTGGAACGGGACGCCATAGAGGGTGAGAG
CCCCGTCTGGTCGGACGCCGAGCCTCTGTAAAG
CTCCTTCGACGAGTCGAGTAGTTTGGGAATGCT
GCTCTAAATGGGAGGTATATGTCTTCTAAAGCT
AAATACCGGCCAGAGACCGATAGCGCACAAGT
AGAGTGATCGAAAGATGAAAAGCACTTTGAAA
AGAGGGTTAAACAGTACGTGAAATTGTTGAAAG
GGAAGCGCCTGTGACCAGACTTGCGCCCGTCGG
ATCACCCAGCGTTCTCGCTGGTGCACTCCGGCG
GGCGCAGGCCAGCATCAGCTCGTCTCGGGGGAC
AAAGGCGGCGGGAACGTGGCTCCTCCGGGAGTG
TTATAGCCCGCCGTGCAATGCCCTGGGGCGGGC
TGAGGACCGCGCGTAAGCATGGATGCTGGCGTA
ATGGTCATCAGCGACCCGTCTTGAAACACGGAC
CAA].

Notes: The species has smaller conidia than *C. fusiformis*, *C. hirtella* or *S. lovelessii*. The only ergot fungus with similar conidia occurring in Texas is *Sphacelia texensis*, from which it differs in host, DNA sequences, and high overall production of alkaloids**.**

Sphacelia texensis Pažoutová & Odvody **sp. nov.** (Fig. 2g, h, i)

Etymology: Named after the geographical origin

Hab. in ovariis et in inflorescentiis *Penniseti glauci* et *Cenchri ciliari*, Texas.

Claviceps fusiformis var. *texensis* differt per suam combinationem characterum; *macroconidiis* hyaliniis, fusoideis vel lunatis (7-19 × 2.8-5.5 µm, mediet.12.5 × 4 µm); regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (GenBank EF052277, EF052278 et EF052279). *Teleomorphosis* ignota.

Cultural characteristics: colonies on T2 medium (14d, 24°C) from 35 mm to 53 mm in diameter, margin diffuse and markedly radiating, colony granular, velutinous, consisting of numerous conidiophores, raised with cerebriform wrinkles in the centre and plane toward the margin, obverse off-white, reverse similar or with shades of brown, soluble pigment slightly yellow.

Macroconidia: lunate to fusiform (7-19 × 2.8-5.5 µm, mean 12.5 × 4 µm).

Teleomorph: *Claviceps* sp. (not seen, based on phylogenetic inferences).

Alkaloids: production in the culture: spontaneous, low (≤ 100 mg/L); main alkaloids: agroclavine, festuclavine.

Habitat: in living florets of *Pennisetum glaucum* and *Cenchrus ciliaris*.

Known distribution: Texas.

Material examined: TEXAS, Agua Dulce, in florets of *Cenchrus ciliaris*, 1997; TEXAS, Kenedy county, in florets of *Cenchrus ciliaris*, 1997 (PRM 857338); TEXAS, Weslaco, in florets of *Pennisetum glaucum*, 1998, G.N. Odvody; TEXAS, Weslaco, in florets of *Pennisetum glaucum*, 2003, G.N. Odvody (PRM 857337); TEXAS, Corpus Christi, in florets of *Pennisetum glaucum*, 2003, G.N. Odvody (**HOLOTYPE**, PRM 857336) (culture CCC858), Mycobank Accession No.: MB 510709

Molecular characters: sequences of the ITS and D1D2 regions of the 28S rDNA unique (EF052277, EF052278 et EF052279). The consensus sequence is given in the format [three-prime 18S]ITS1*5.8S*ITS2 [five-prime 28S].

[ATCATTA]CCGAGTTTTCAACTCCCAAAC
CCACTGTGAACCTATACCAAAAACGTTGCCTCG
GCGGGACATGCGCCCCGGACCGCCCCCCCCTCG
CGGGGGAGGGCGCCGGATCCCACGGCCGCCCGC
CGGGGGCCCCAAACTCTGTATTCCCATAGCGGC
ATGTCTGAGTGGATTTATCCAATGAATCA*AAAC
TTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCGCCAGTACTCTGGCGGG
CATGCCTGTTCGAGCGTCATT*TCAACCCTCAGG
CCCCCGGGCCTGGTGTTGGGGACCGGCTCACGG
GGGGGAGGCACAGCGCCCTCCCCCCCCTGCCGCC
CCCTAAATGGATCGGCGGCCACGCCGCGGCCTC
CCCTGCGCAGTAACATACCACCTCGCAGGCGGC
TGGCTCGGCGCGGCCACTGCCGTAAAACGCCCA
ACTTCTCCAGAG[TTGACCTCGAATCAGGTAGGA
ATACCCGCTGAACTTAAGCATATCAATAAGCGG
AGGAAAAGAAACCAACAGGGATTGCCCCAGTA
ACGGCGAGTGAAGCGGCAACAGCTCAAATTTGA
AATCTGGCCCCCCGGGGCCCGAGTTGTAATTTG
CAGAGGATGCTTTTGGCGAGGCGCCTTCCGAGT
TCCCTGGAACGGGACGCCATAGAGGGTGAGAGC
CCCGTCTGGTCGGACGCCGAGCCTCTGTAAAGC
TCCTTCGACGAGTCGAGTAGTTTGGGAATGCTG
CTCTAAATGGGAGGTATATGTCTTCTAAAGCTA
AATACCGGCCAGAGACCGATAGCGCACAAGTA
GAGTGATCGAAAGATGAAAAGCACTTTGAAAA
GAGGGTTAAACAGTACGTGAAATTGTTGAAAGG
GAAGCGCCTGTGACCAGACTTGCGCCCGTCGGA
TCACCCAGCGTTCTCGCTGGTGCACTCCGGCGG
GCGCAGGCCAGCATCAGCTCGTCTCGGGGGACA
AAGGCGGCGGGAACGTGGCTCCTCCGGGAGTGT
TATAGCCCGCCGCGCAATGCCCTGGGGCGGGCT
GAGGACCGCGCGTAAGCATGGATGCTGGCGTAA
TGGTCATCAGCGACCCGTCTTGAAACACGGACC
AA]

Notes: *Sphacelia texensis* differs from *Claviceps fusiformis* and *C. hirtella* by combination of characters: smaller conidial dimensions and low alkaloid production *in vitro* and *in planta*. Sequence of the ITS, D1D2 regions of the 28S rDNA (EF052277, EF052278 and EF052279) and β–tubulin are unique and identical (EF473873, EF473874 and EF473878); next nearest known relatives being *S. eriochloae* and *C. hirtella*.

Sphacelia lovelessii Pažoutová, M. Kolařík & Freder. **sp. nov.** (Fig. 2m, n, o)

Etymology: Named after A.R. Loveless (British mycologist).

Hab. in ovariis et in inflorescentiis *Urochloae* spp. et *Eragrostidis* sp., Zimbabwe.

Sphacelia lovelessii differt ab aliis speciebus per suam combinationem characterum; *macroconidiis* hyaliniis, lunatis vel fusioideis (10-25 × 3-6.4 µm, mediet.17 × 5 µm); regio 'rDNA ITS', 'rDNA28S cum polymorphismis unicis sequentiae (EF052282, EF052281, AJ605996). Teleomorphosis ignota.

Cultural characteristics: colonies on T2 medium (14d, 24°C) from 15mm (CCC 642, 646) to 27 mm (CCC 647) in diameter, margin narrow or lobate, colony plane (CCC 647) or raised centrally and wrinkled (CCC 642, 646) consisting of dense myceliar mat, sporulation absent, obverse white, reverse off-white to slightly brown in the centre similar or with shades of brown, soluble pigment absent.

Macroconidia: lunate (10-25 × 3-6.4 µm, mean 17 × 5 µm).

Teleomorph: *Claviceps* sp. (based on phylogenetic inferences but unknown)

Alkaloids: traces of chanoclavine or agroclavine.

Habitat: in living florets of *Urochloa* spp.,and *Eragrostis* sp.

Known distribution: Zimbabwe.

Material examined: ZIMBABWE, Matopos, in florets of *Urochloa sp*., 2000, D.E. Frederickson; ZIMBABWE, Matopos (PRM 857342); in florets of *Urochloa mosambicensis*, 2001, D.E. Frederickson (PRM 857340) (culture CCC642); ZIMBABWE, Matopos, in florets of *Urochloa oligotricha*, 2001, D.E. Frederickson (culture CCC646); ZIMBABWE, Matopos, in florets of *Urochloa trichopus*, 2001, D.E. Frederickson; ZIMBABWE, Matopos, in florets of *Eragrostis* sp., 2001, D.E. Frederickson (**HOLOTYPE**, PRM 857340) (culture CCC648). Mycobank Accession No.: MB 510615

Molecular characters: sequences of the ITS and D1D2 regions of the 28S rDNA unique (EF052282, EF052281, AJ605996). The consensus sequence is given in the format [three-prime 18S]ITS1*5.8S*ITS2[five-prime 28S]. Nucleotides in bold indicate differences among the sequences: underlined – transitions/transversions; bracketed – not present in all sequences.

[ATCATTA]CCGAGTTTTCAACTCCCAAAC
CCACTGTGAACCCGTACCAAAAACGTTGCCTCG
GCGGGAGATGCGCCCCGGACCGCCCCCCCCC(**C**
C)TCGCGGGGGAGGGCGCCGGATCCCACGGCCG
CCCGCCGGGGGCCCCAAACTCTGTATTCCCATA
GCGGCATGTCTGAGTGGATTTATCCAATGAATC
A*AAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCGCCAGTACTCTG
GCGGGCATGCCTGTTCGAGCGTCATT*TCAACCC
TCAGGCCCCCGGGCCTGGTGTTGGGGACCGGCT
CACGGGGGG(**G**)**R**(**CA**)ACAGCGCCC**S**CCCTGCCG
CCCCCTAAATGGATCGGCGGCCACGCCGCGGCC
TCCC(**C**)TGCGCAGTAACATACCACCTCGCAGGC
GGCTGGCTCGGCGCGGCCACTGCCG**Y**AAAACGC
CCAACTTCTCAAGAG[TTGACCTCGAATCAGGTA
GGAATACCCGCTGAACTTAAGCATATCAATAAG
CGGAGGAAAAGAAACCAACAGGGATTGCCCCA
GTAACGGCGAGTGAAGCGGCAACAGCTCAAATT
TGAAATCTGGCCCCCCGGGGCCCGAGTTGTAAT
TTGCAGAGGATGCTTTTGGCGAGGCGCCTTCCSA
GTTCCCTGGAACGGGACGCCATAGAGGGTGAGA
GCCCCGTCTGGTCGGACGCCGAGCCTCTGTAAA
GCTCCTTCGACGAGTCGAGTAGTTTGGGAATGC
TGCTCTAAATGGGAGGTATATGTCTTCTAAAGCT
AAATACCGGCCAGAGACCGATAGCGCACAAGT
AGAGTGATCGAAAGATGAAAAGCACTTTGAAA
AGAGGGTTAAACAGTACGTGAAATTGTTGAAAG
GGAAGCGCCTGTGACCAGACTTGCGCCCGCCGG
ATCACCCAGCGTTCTCGCTGGTGCACTCCGGCG
GGCACAGGCCAGCATCAGCTCGTCTCGGGGGAC
AAAGGCGGCGGGAACGTGGCTCCTCCGGGAGTG
TTATAGCCCGCCGTGCAATGCCCTGGGGCGGGC
TGAGGACCGCGCGTACGCATGGATGCTGGCGTA
ATGGTCATCAGCGACCCGTCTTGAAACACGGAC
CAA].

Notes: *Sphacelia lovelessii* differs from *Claviceps fusiformis* by markedly lunate conidia. It differs from *C. fusiformis*, *C. hirtella*, *S. eriochloae* and *S. texensis* by absence of alkaloids *in vitro* and *in planta* and by lack of conidiation and slow and compact growth on agar medium T2.

Discussion

DNA and alkaloid analyses now confirm the hypothesis of an introduction of *C. fusiformis* to India from Africa, since the Indian isolate was very similar to African isolates, especially to those collected around 1960. Whereas *C. fusiformis*, appearing as a

clade terminal on the phylogram, is a known parasite of *Cenchrus* and *Pennisetum* spp., ancestral *C. hirtella* was collected from a broader range of native hosts. The first record of *C. hirtella* on *Cenchrus ciliaris* is from 1976 (BRIP 11355), although this grass was introduced to Australia around 1870. The occurrence of diverging populations from *Pennisetum*, *Cenchrus* and *Eriochloa* show that, in common with species complexes of other phytopathogenic fungi (Crous and Groenewald, 2005), evolution has been towards narrower host preferences.

In southern India, honeydew specimens containing lunate conidia were observed on various host grasses many years before *C. fusiformis* was introduced. At that time, neither large-scale epiphytotics of disease on pearl millet nor alkaloid toxicoses of grazing animals were recorded. Langdon (1952) suspected that the Indian records prior to 1950 might refer to *C. hirtella* or a closely related fungus. However, the descriptions of conidia on the Indian grass specimens as long (Table 1) are more consistent with *S. lovelessii*, which also does not produce any alkaloids. Unfortunately, no Indian isolates or specimens from hosts other than pearl millet are available for comparison with the other fungi of the *C. fusiformis* species complex.

Members of the *C. fusiformis* species complex also differ in their alkaloid biosynthesis gene cluster. In the ”standard” biosynthetic pathway, elymoclavine (C-17 hydroxyagroclavine) is an end product of the clavine pathway that may be further oxidized to D-lysergic acid and its amides e.g. ergometrine, containing a simple amino alcohol as the amide component (for review see Flieger *et al*., 1997). In *C. fusiformis*, Lorenz *et al*. (2007) found that the oxidation of clavines to lysergic acid is inhibited because the respective clavine oxidase gene, *cloA*, is inactive. Therefore, it seems that ergometrine-producing *C. hirtella* still possesses a functional gene that is missing in all other members of the *fusiformis-hirtella* clade. Due to only trace amounts of alkaloids being produced by *S. lovelessii* (Table 4), production of ergometrine in this species could be neither proved nor disproved.

From the whole species complex, only *C. fusiformis* and *S. eriochloae* have the potential to cause human and/or animal toxicoses as they are able to produce alkaloids *in planta* even in the sphacelial stage of development.

The origin of ergot infections on pearl millet, *Cenchrus*, and *Eriochloa* in Mexico and Texas (San Martín *et al*., 1997; Velásquez-Valle *et al*., 1998) is rather unclear. In the survey by Alderman *et al*. (2004) no records for grasses with lunate or fusiform conidial infections were mentioned. The presented results clearly preclude *C. fusiformis* from Africa or India as the causative pathogen. The close genetic similarity of *S. texensis* to Paleotropic *C. fusiformis* and *C. hirtella*, however, suggests that *S. texensis* may be yet another introduction from the Paleotropics (Africa and Southeast Asia) to the Americas, possibly with grass seed.

The presence of *S. lovelessii* in Zimbabwe represents another puzzle. Differences in conidial shape and size as well as in DNA markers among the isolates and specimens suggest considerable infraspecific variability. It is also the only *Claviceps/Sphacelia* species with markedly lunate macroconidia to be recorded in Africa – *C. fusiformis* tends to straight, fusiform conidia. The older studies of herbarium specimens and collections from southern and eastern Africa (Doidge, 1950; Langdon, 1952; Loveless, 1964a, b; Herd and Loveless, 1965; Loveless, 1965) were extensive and it is surprising that, at least until collections stopped in the late 1960’s, infections with the fusiform to lunate conidial shape occurring on a grass genus as common and widely distributed as *Urochloa* were overlooked. However, only *Claviceps sulcata* with allantoid conidia was reported (Loveless, 1964a; Loveless and Herd, 1964). Therefore, it may be that infection with *S. lovelessii* is rather rare. In contrast, the signs of ergot infection on the tiny florets of *Eragrostis*, another widely distributed grass genus, were very inconspicuous and may easily have gone unnoticed.

A polygeneric host range has been documented not only in *S. lovelessii* (although here it is even switching between host tribes), but also in *C. sorghi* (*Sorghum – Heteropogon*; Pažoutová *et al*., 2002) and *C. africana* (*Sorghum – Hyparrhenia*; Pažoutová and Frederickson, 2005) which contradicts the original

paradigm of "one *Claviceps* species – one host genus" or at least "a group of closely related host genera". From the evolutionary point of view, the less rigid host specificity may enable the parasite to colonize alternative hosts after migration to another region as a first step towards adaptive radiation.

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