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Ergot species of the *Claviceps purpurea* group from South Africa

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ABSTRACT

Results of a survey and study of the *Claviceps purpurea* group of species in South Africa are being presented and five new species are described. Morphological descriptions are based on the anamorphs and four nuclear genetic loci. *Claviceps fimbristylidis* sp. nov. on *Fimbristylis complanata* was discovered wide-spread across five provinces of the country associated with water and represents the fourth *Claviceps* species recorded from the Cyperaceae. *Claviceps monticola* sp. nov. is described from *Brachypodium flexum* growing in mountain forests in Mpumalanga Province, as well as the northern Drakensberg southwards into the Eastern Cape Province. *Claviceps pazoutovae* sp. nov. is recorded from *Stipa dregeana* var. *dregeana* and *Ehrharta erecta* var. *erecta*, also associated with these mountain ranges. *Claviceps macroura* sp. nov. is recorded from *Cenchrus macrourus* from the Eastern Cape and *Claviceps capensis* sp. nov. from *Ehrharta villosa* var. *villosa* is recorded from the Western Cape Province. *Claviceps cyperi*, only recorded from South Africa is included in the study. Ergot alkaloid profiles of all species are provided and showed similarity to *C. purpurea*. Only *C. cyperi* and in lesser degree *C. capensis*, *C. macroura*, and *C. pazoutovae* produced ergot alkaloids in clinically significant amounts. Several reported species infect invasive grass species, native to South Africa, and thus represent potentially invasive species.

Introduction

Africa is home to numerous invasive grasses which destroyed natural grass ecosystems over the world (Williams & Baruch 2000; Foxcroft *et al.* 2010). It was also shown that as a result, their specific ergot species expanded their host range e.g. *Claviceps africana*, *Claviceps cynodontis*, *Claviceps fusiformis*, and *Claviceps sulcata* and are now spread all over the world (*viz.* Pažoutová *et al.* 2011). A crucial step in the monitoring of ergot host ranges, geographical distribution, and possible invasion is the characterisation of their host spectrum on native African grasses. Although some studies on Zimbabwean species (previously known as Rhodesia) were performed by authors such as Loveless (1964a, 1967), Loveless & Herd (1964), and

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Pažoutová et al. (2008a, 2011), no single comprehensive study of the South African species has been done; existing research papers on southern African species mostly describe single or groups of species, most of which were introduced due to the introduction of their hosts, usually for forage purposes.

The *Claviceps purpurea* species group is a phylogenetically well-defined lineage of the closely related species *Claviceps arundinis*, *Claviceps grohi*, *Claviceps humidiphila*, *Claviceps nigricans*, *C. purpurea*, *Claviceps spartinae*, and *Claviceps zizaniae* (Lorenz et al. 2009; Pažoutová et al. 2011, 2015). Among all ergot species, the most potent producers of ergot alkaloids, especially pharmaceutically and ecotoxicologically important ergopeptines, form part of this species group (Lorenz et al. 2009). Only two species from this group have been identified in South Africa to species level namely *C. purpurea* (with a wide host range including mostly introduced hosts such as *Lolium*), as well as *Claviceps cyperi* (on *Cyperus esculentus*). The conidia seemed to be the only tool for identification of these old herbarium specimens. Although the conidia of *Claviceps* species vary considerably in shape and size, for some species these features are often quite distinctive; in addition, the range of size as well as length/width (L/W) ratio is a useful character as an accessory to shape in the delimiting of species (Langdon 1954). Loveless (1964b) also demonstrated the use and value of the morphology of the conidia when examining herbarium material and concluded that in many cases they form discriminating taxonomic markers and that these characteristics have to be relied upon especially when examining old herbarium specimens.

According to Pažoutová et al. (2011), numerous *Claviceps* anamorphs are deposited in herbaria, but few were formally described as the essential teleomorph was lacking. They also stated that others have found that these conidial dimensions vary considerably in different collections of the same species (*viz.* Muthusubramanian et al. 2005; Pažoutová & Frederickson 2005) and that care should be taken to use these characters in combination with other morphological – and in recent times, also molecular characters. Pažoutová et al. (2011) concluded that the coidentification of dated *Claviceps* herbarium specimens for which cultures and DNA sequences are available is crucial for establishing past and recent distributions and migrations of species. However, 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 (Pažoutová et al. 2011).

In a comprehensive study, we screened ergotised grasses in South Africa which included invasive grass species from the genus *Ehrharta* and *Cenchrus*. In this paper we are describing five new species from the *C. purpurea* species complex; members of this species group were overlooked in South Africa so far.

Materials and methods

Herbarium specimens

Apart from the collections done over the past 8 y, other relevant unidentified specimens of ergotised grasses in the dried herbarium collection of the South African National Collections of Fungi (PREM) have been examined and are included in this study. Cultures generated have also been deposited in its live culture collection (PPRI) as well as in the Culture Collection of Clavicipitaceae at Institute of Microbiology, Academy of Sciences of the Czech Republic (CCC). Isotypes of some specimens have been deposited in the National Museum in Prague (PRM).

Isolates and their cultivation. Sclerotia were surface sterilized for 3 min with 1 % sodium hypochlorite, rinsed three times in sterilized distilled water, and plated onto Potato Dextrose Agar (PDA) containing antibiotics. Honeydew drops found on florets of infected grasses were plated onto Water Agar (WA) agar plates containing antibiotics and agar pieces containing germinating macroconidia were subsequently transferred onto PDA (Pažoutová et al. 2015). Isolates are being maintained in the PPRI and CCC collection on PDA agar punches covered with sterile water, PDA slants covered with sterile liquid paraffin, by ultra-low freezing as well as freeze dried cultures (Table 1).

Alkaloid analysis

Sample preparation and alkaloid extraction. Pulverized sclerotia (10 mg) were mixed with extraction mixture consisting of toluene–ethyl alcohol mixture (4:1, v:v, 0.5 mL) and gently stirred for 1 h. Supernatant was separated by centrifugation and kept in the freeze until use (M.F., unpubl.).

High-performance liquid chromatography (HPLC) analyses. The HPLC system consisted of a pump equipped with a 600E system controller, auto-sampler 717, and dual UV detector 2487 (Waters, Milford, MA, USA). The data were processed using Empower 2 software. Water containing mobile phases were filtered through a 0.22 mM GS filter (Millipore, Billerica, MA, USA) and degassed in an ultrasonic bath for 10 min before use.

A Luna 5 m C18(2) column (250 × 4.6 mm, Phenomenex) with a guard column was used for the analysis. The mobile phase consisted of: A.) 10 % acetonitrile in water with KH_2PO_4 (9.18 g 1 L^{-1}; pH adjusted at 3.12) and B.) acetonitrile. Gradient elution: 10 % B (0 min), increasing linearly to 35 % B (40 min), increasing linearly to 65 % B (10 min). Each analysis was followed by a column washing (65 % B, 10 min) and equilibration step (10 % B, 10 min). Flow rate, 1.0 mL min^{-1}. UV detection was performed at 288 and 315 nm, respectively.

Calibration. A standard solution of ergotamine was prepared in methanol at final concentrations of 62.5, 125, 250, 500, and 1000 mg mL^{-1}. The calibration graphs were constructed by plotting the integrated peak areas of individual compounds *versus* concentration. The linear regression equation ($y = 5946.4x$) and correlation coefficient ($r = 0.997$), were obtained.

Taxonomy. Following Pažoutová et al. (2011), we have chosen to describe new species from the asexual stage of development in the otherwise teleomorphic genus *Claviceps*. Descriptions are based on the appearance of macroconidia and sclerotia, and a sequence of the nuclear ribosomal DNA region (*nrDNA*), minichromosome maintenance complex component 7 (*MCM7*) gene, elongation factor-1α gene (*EF1-α*), and beta-tubulin gene (*Tub2*).