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Strelitziana africana newly recorded from China

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Abstract — We document the first report of *Strelitziana africana* from China. This fungus was isolated from stems of *Dioscorea cirrhosa* and *Sabia parviflora* collected from Nanning, Guangxi Province. *Strelitziana africana* can produce flyspeck signs on inoculated apple fruit and is distinguished from the other known species in the genus by morphological characters and phylogenetic analysis based on ITS sequences.

Key words — biodiversity, *Dioscoreaceae*, *Sabiaceae*, reservoir hosts, sooty blotch

Introduction

Sooty blotch and flyspeck (SBFS), a disease complex comprising more than 60 putative species of fungi, colonizes the waxy cuticle of many plants in humid production regions worldwide, inciting cosmetic damage that causes significant economic losses (Batzer et al. 2005). The common name "flyspeck" refers to species in the SBFS complex whose morphology on fruit surfaces consists of clusters of black dots lacking a mycelial matrix. Colby (1920) reported that flyspeck was caused by *Leptothyrium pomi* (Mont. & Fr.) Sacc. Von Arx (1959) synonymized 14 species, including *Leptothyrium pomi*, under *Schizothyrium pomi* (Mont. & Fr.) Arx, the currently accepted name for this taxon. Arzanlou & Crous (2006) reported *Strelitziana africana* Arzanlou & Crous from leaf speckle symptoms of *Strelitzia* in South Africa. The genus *Strelitziana* Arzanlou & Crous was named after the host genus from which it was collected and shown to be a member of the *Chaetothyriales*. We have identified two isolates that

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are described as the first record of *Strelitziana africana* from China based on morphological comparison and their phylogenetic relationships, as shown by analysis of sequence data of the internal transcribed spacer (ITS) of the rRNA repeat (Harrington & Rizzo 1999).

The species *Dioscorea cirrhosa* and *Sabia parviflora* are economically important medicinal plants in China because their stems and leaves are used as ingredients in Chinese traditional medicine. Recently, during a survey of alternate host plants for flyspeck fungi in China, these medicinal plants were found to be reservoir hosts of flyspeck fungi, including *Strelitziana africana*.

Materials and methods

Isolates. Individual sclerotium-like bodies (Batzer et al. 2005), growing in clusters on culms, were transferred to slants containing potato-dextrose agar (200 g peeled potato, 20 g dextrose, 10 g agar in 1 L water; PDA) and cultured at 22 ± 1°C in darkness (Sun et al. 2003). Colony descriptions were made after 1 month of growth on oatmeal agar (3%; 30 g oatmeal, 10 g agar in 1 L water; OA) plates at 22 ± 1°C in darkness. After 1-month-old axenic cultures were transferred to new OA plates, a sterile cover slip was partially inserted into the agar adjacent to the colony and angled away from the colony at approximately 60 degrees to the agar surface in order to enable the fungus to grow onto the cover slip. Measurements of fungal structures were conducted based on isolates growing on cover slips.

DNA sequencing. Template DNA was extracted from the fungal mycelium according to the method of Barnes et al. (2001), and primer pairs used for amplification and sequencing of the ITS region were ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Amplification was completed with the following cycling parameters: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s and a final extension of 72°C for 10 min. The PCR products were sequenced by Organism Technology Co., Ltd., Shanghai, China.

The ITS nucleotide sequences generated in this study were added to sequences downloaded from GenBank (Table 1) that had high similarity according to a BLAST search (National Center for Biotechnology Information's nucleotide blast program). Preliminary alignments were performed using CLUSTAL-X (Thompson et al. 1997). The alignments were imported into BioEdit v. 5.0.9.1 (Hall 1999) and manually adjusted. Phylogenetic analysis of aligned DNA sequences was performed with PAUP v. 4.0b10 for 32-bit Microsoft Windows (Swofford 2001). Heuristic searches were performed with 1000 random sequence additions. Clade stability was evaluated by 1000 bootstrap replications. Other measures for parsimony, including tree length, consistency index, retention index, and rescaled consistency index (CI, RI and RC, respectively), were also calculated. *Curvularia affinis* was used as the outgroup taxon.

Koch's postulates. After the two isolates in this paper were grown on OA for 1 month, a piece of the colony was picked up into an 1.5 ml Eppendorf tube and blended with 1.0 ml sterile deionized water for 1 minute. This suspension of mycelial fragments

Table 1. Sequences used in the phylogenetic analysis

Species	GenBank	Reference
GX01	GQ850385	This paper
GX02	GQ850386	This paper
Capronia acutiseta	AF050241	Untereiner & Naveau 1999
Capronia pulcherrima	AF050256	Untereiner & Naveau 1999
Cladophialophora devriesii	AB091212	Abliz et al. 2003
Cladophialophora sp.	EU137326	De Hoog et al. 2007
Curvularia affinis	EF187909	Di Maiuta & Schwarzentruber 2007
Mycosphaerella vietnamensis	DQ632675	Burgess et al. 2007
	DQ632678	Burgess et al. 2007
Pseudocercospora syzygiicola	AF309600	Crous et al. 2000
Pseudocercospora sp.	DQ303082	Crous et al. 2006
Rhinocladiella anceps	AY163559	De Hoog et al. 2003
	DQ826740	De Hoog et al. 2003
Rhinocladiella atrovirens	AY163558	De Hoog et al. 2003
Rhinocladiella basitona	AY163561	De Hoog et al. 2003
Rhinocladiella similis	AY857529	Prenafeta-Boldu et al. 2006
Strelitziana africana	DQ885895	Arzanlou & Crous 2006
Strelitziana australiensis	GQ303295	Cheewangkoon et al. 2009

and conidia was used within 2 hours. Three mature, non-wounded apples were chosen for each isolate, surface-sterilized with 75% ethanol and allowed to dry completely, then swabbed with suspension of one isolate per apple. Two control apples were surface-sterilized and swabbed with sterile deionized water only. All the inoculated apples were incubated in a moist chamber at 22 ± 1°C.

Results

DNA phylogeny

A multiple alignment of the rDNA-ITS was generated with 18 sequences obtained from GenBank plus the sequences of isolates GX01 and GX02 (GX01 = GQ850385, GX02 = GQ850386). From a MP tree with a length of 750 bp (CI = 0.7080, RI = 0.7830, RC = 0.5512), two major clades were resolved (Fig. 1). One clade, with 100% bootstrap support, contained three species in *Mycosphaerella* and *Pseudocercospora*. The other major clade had a bootstrap value of 93%. The *Strelitziana* species grouped in a well-supported subclade (100%). Our isolates and a *Strelitziana africana* isolate from *Strelitzia* that was identified by Arzanlou & Crous (2006) fell within a single clade with 100% bootstrap support, indicating that they might represent the same species.

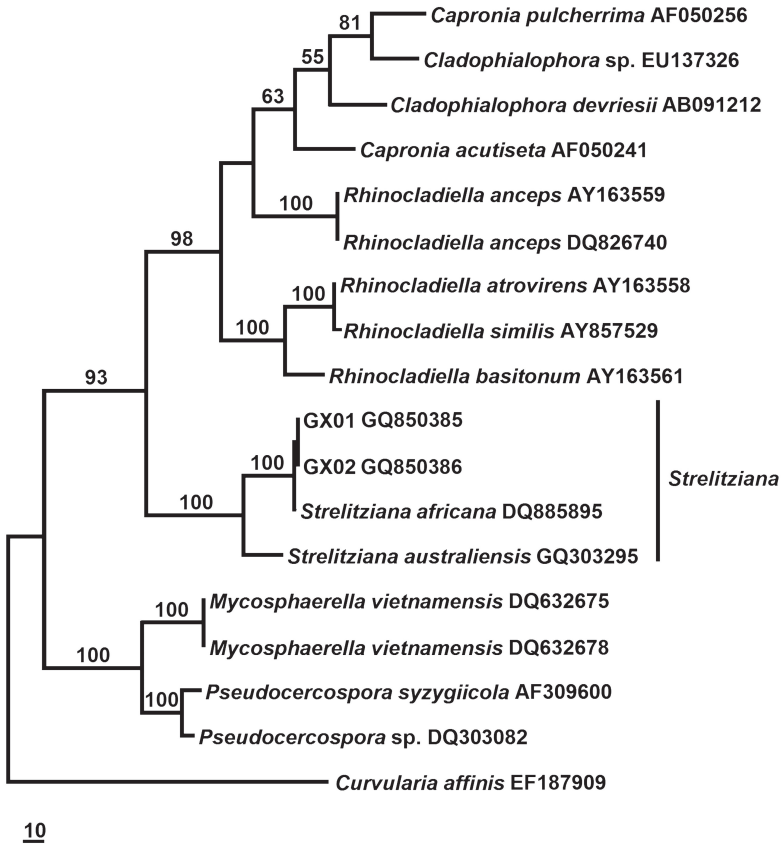

Fig. 1 The parsimony tree (TL = 750, CI = 0.7080, RI = 0.7830, RC = 0.5512) derived from a heuristic search option in PAUP v. 4.0b10 with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of ITS1, 5.8S and ITS2. Bootstrap values higher than 50% are indicated above the tree branches. The tree was rooted to *Curvularia affinis*.

Taxonomy

Description: Mycelium superficial, consisting of smooth, septate, branched hyphae, 2–3 μm wide. Conidiophores erect, solitary, arising from aerial and submerged mycelium, subcylindrical, straight to geniculous-sinuous, pale brown, concolorous with hyphae, smooth, 0–4-septate, 3–20(–50) × 1.5–4.5 μm. Conidiogenous cells terminal, integrated, rejuvenating percurrently, proliferating apically via several short, conspicuous denticles, conidiogenesis rhexolytic. Conidia pale brown, smooth, long obclavate, widest in middle of

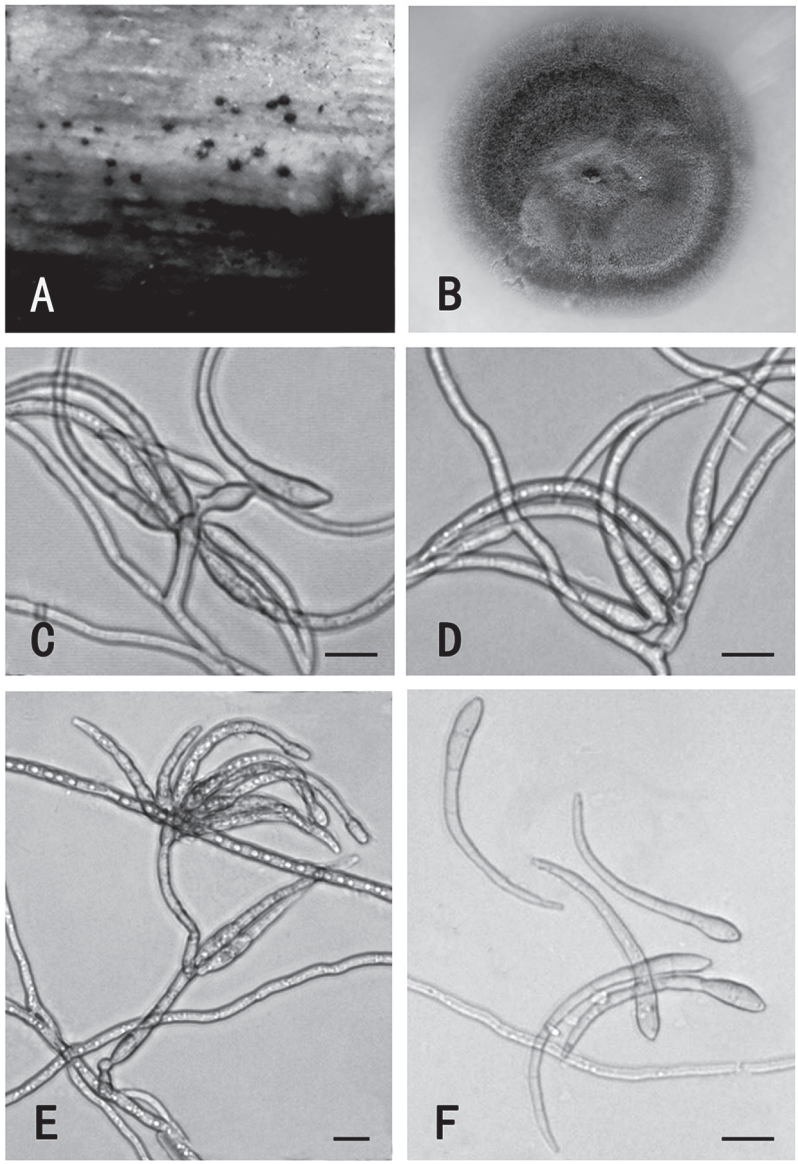

Fig. 2 *Strelitziana africana* GX01. A. Signs on *Dioscorea cirrhosa*. B. Colony on oatmeal agar after 30 days. C–D. Conidia, conidiogenous cells and hyphae. E. Conidiogenous cell giving rise to conidia, and microcyclic conidia. F. Conidia. Bars (C–F) =10 μm.

basal cell, tapering to a subobtusely rounded apex and obconically subtruncate base with minute marginal frill, 1 µm wide, (12–)35–70(–100) × 2.5–5 µm, 3–10(–21)-septate, microcyclic conidiation observed in culture (Fig. 2).

Specimens examined: On *Dioscorea cirrhosa* Lour. (*Dioscoreaceae*), China: Guangxi, Nanning, Guangxi Medicinal Botanical Garden, 22°51'N 108°19'E, alt. 72 m, 23 Sep. 2008, J.L. Zhuang & H.L. Yang, HMUABO (the Fungal Herbarium of Northwest A&F University) 822516 (with dried culture), culture GX01. On *Sabia parviflora* Wall. (*Sabiaceae*), China: Guangxi, Nanning, Guangxi Medicinal Botanical Garden, 22°51'N 108°19'E, alt. 76 m, 23 Sep. 2008, J.L. Zhuang & H.L. Yang, HMUABO 822517 (with dried culture), culture GX02.

Cultural characteristics: The colony diameter after 1 month on PDA at 22 ± 1°C reached 28 mm with even margins and smooth, felty aerial hyphae; colony centers were purplish gray and outer zones pale white. On OA the colony was flat, spreading, with sparse aerial mycelium, reaching 33 mm diam after 1 month at 22 ± 1°C, surface olivaceous, colonies fertile.

Host characteristics: On stems, the fungus produced dark, shiny, round to oval, slightly protuberant sclerotium-like bodies (Fig. 2A). The flyspeck on stems did not damage the plants, but greatly reduced their ornamental and retail value. As a result, these fungi can cause significant economic losses to producers of these medicinal plants.

Koch's postulates: The inoculated apples were examined after incubating for 1 month. All inoculated apples with the two isolates exhibited flyspeck signs similar to that on the original plant stems, although with a sparser density of the sclerotium-like bodies. Control apples did not show any flyspeck signs.

Discussion

Based on phylogenetic analysis of the ITS region and morphological characters of the anamorph, we identified the two isolates as *Strelitziana africana*. This species was described from *Strelitzia* (Arzanlou & Crous 2006) and was previously known only from that host. This study is the first report of *S. africana* from medicinal plants. Currently there are only two species in *Strelitziana*, and no potential teleomorph connection is known. *Strelitziana africana*, the type species of this genus, has rhexolytic conidiation and conidial dimensions similar to *S. australiensis* Cheewangkoon & Crous (Cheewangkoon et al. 2009). However, *S. africana* lacks an apical mucilaginous appendage and chlamydospores and has obclavate conidia, making it easy to distinguish from *S. australiensis*.

Morphologically, our two isolates are similar to *Strelitziana africana*, though our isolates produced longer conidia and conidiophores ((12–)35–70(–100) µm, 3–20(–50) µm) than *S. africana* ((18–)50–70(–95) µm, 3–15(–40) µm). Furthermore, conidiophores and conidia of the Chinese isolates produced

more septa (0–4 in conidiophores, 3–10(–21) in conidia for these isolates, vs. 0–1(–5) in conidiophores, and 3–5(–10) in conidia of ex-type strains of *S. africana*). In ITS sequence analysis, our isolates and *S. africana* isolates from *Strelitzia* identified by Arzanlou & Crous (2006) fell within a single clade with 100% bootstrap support.

The results of Koch's postulates show that: 1) the fungi from medicinal plant can produce flyspeck signs on apple fruit; 2) medicinal plants may therefore act as reservoir hosts, providing inoculum for SBFS infestations on apple. Based on the ITS sequence analysis and morphological comparison, we identified the isolates as *Strelitziana africana*, which represent a new record for China.

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