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Strelitziana mali, a new species causing sooty blotch on apple fruit

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Abstract — *Strelitziana mali*, a new species isolated from the cuticle of apple fruit (*Malus* ×*domestica*), is described and illustrated. Samples were collected from two orchards in Shaanxi and Henan Provinces, China. The fungus, pathogenic to apple fruits, is distinguished from the other known species in the genus both by morphological characters visible using optical and scanning electron microscopes and by phylogenetic analysis based on ITS sequences.

Key words — *Chaetothyriales*, internal transcribed spacer, scanning electron microscopy

Introduction

The genus *Strelitziana* Arzanlou & Crous (2006) was named after the host genus, *Strelitzia*, from which the type species was collected as a member of *Chaetothyriales*. Features include conidiophores that are erect, solitary, subcylindrical, straight to geniculous-sinuous, pale brown and arise from aerial and submerged mycelia; conidiogenous cells that are terminal, integrated, rejuvenate percurrently, and proliferate apically via several short, conspicuous denticles; rhexolytic conidiogenesis; conidia that are pale brown, smooth, long obclavate, and multi-euseptate; and microcyclic conidiation in culture.

The genus was established to accommodate *Strelitziana africana* Arzanlou & Crous (Arzanlou et al. 2006) collected from leaves of *Strelitzia* sp. in Africa. Recently, during investigations of sooty blotch and flyspeck of apple in China, four isolates were found that appeared to be closely related to *Strelitziana*.

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They are described as a new species of *Strelitziana* based on morphological comparison, observation with scanning electron microscope and by ITS sequence analysis.

Materials and methods

Isolates. Apples were collected from Yangling and Qianxian in Shaanxi Province, and Zhengzhou in Henan Province. Thalli were transferred from colonies on the apple surface to a potato dextrose agar (PDA) slant and cultured at 25°C in darkness (Sun et al. 2003). One–month–old cultures on PDA were described and photographed. Then 1–month–old pure cultures were transferred to fresh PDA plates, a sterile cover slip was partially inserted into the agar adjacent to the colony, 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.

Scanning electron microscopy. For scanning electron microscopy (SEM), cover slips with attached hyphae were fixed in 3% glutaraldehyde and 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 6.8. After dehydration in a series of ethanol rinses, the hyphae were dehydrated in a critical point drier, sputter-coated with gold, and examined under a scanning electron microscope (Joel JSM 6360LV) at accelerating voltages of 15 and 25 KV.

DNA sequencing. Template DNA was extracted from 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 et al. 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 25 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 10 min. The PCR products were sequenced by Organism Technology Co., Ltd., Shanghai, China.

One sequence was compared to the GenBank NR database, and target sequences with high similarity were downloaded. Preliminary alignments with downloaded sequences and those obtained in this study were performed using CLUSTAL-X (Thompson et al. 1997). The alignments were imported into BioEdit 5.0.9.1(Hall 1999) and manually adjusted. Phylogenetic analysis of aligned DNA sequences was performed with PAUP version 4.0b10 for 32-bit Microsoft Windows (Swofford 2001). Heuristic searches were performed with 1000 random sequence additions. Clade stability was evaluated by bootstrap analysis using 1000 replications. Other measures calculated for parsimony analyses included tree length, consistency index, retention index, and rescaled consistency index (CI, RI and RC, respectively). *Pseudocercospora syzygiicola* was used as the outgroup taxon.

Results

Four isolates (YL12, YL06, QX01, ZZ21) were obtained from the apple fruit cuticle. The sequences were deposited in GenBank (QX01 = FJ917556, YL06 = FJ917557, YL12 = FJ917558, ZZ21 = FJ917559). The ribosomal DNA ITS region (ITS1, 5.8S rDNA gene, ITS2) was sequenced for each isolate, and

Table 1. Sequences used in the phylogenetic analysis

Species	GenBank	Reference
Capronia acutiseta	AF050241	Untereiner et al. 1999
C. fungicola	AF050246	Untereiner et al. 1999
C. nigerrima	AF050251	Untereiner et al. 1999
C. pulcherrima	AF050256	Untereiner et al. 1999
Cladophialophora devriesii	AB091212	Abliz et al. 2003
Cladophialophora sp.	EU137326	de Hoog et al. 2007
Coniosporium sp.	AM279681	Sert et al. 2007
Cyphellophora hylomeconis	EU035415	Crous et al. 2007
Exophiala attenuata	EF025392	Zeng et al. 2007
Heteroconium kleinziense	EF110616	Crous et al. 2007
H. triticicola	AJ748260	Kwasna et al. 2007
Melanchlenus eumetabolus	AY163554	De Hoog et al. 2002
M. oligospermus	AY163555	De Hoog et al. 2002
Metulocladosporiella musae	DQ008138	Crous et al. 2006
M. musicola	DQ008136	Avila et al. 2006
Phaeococcomyces catenatus	AF050277	Untereiner et al. 1999
P. nigricans	AF050278	Untereiner et al. 1999
Phialocephala fluminis	AF486124	Gruenig et al. 2002
Pseudocercospora syzygiicola	AF309600	Crous et al. 2000
Rhinocladiella anceps	EU041805	Arzanlou et al. 2007
Sarcinomyces phaeomuriformis	AJ244259	Hoog et al. 1999
Strelitziana africana	DQ885895	Arzanlou et al. 2006
Strelitziana mali (QX01)	FJ917556	This paper
S. mali (YL06)	FJ917557	This paper
S. mali (YL12)	FJ917558	This paper
S. mali (ZZ21)	FJ917559	This paper
Thysanorea papuana	EU041814	Arzanlou et al. 2007
Veronaea compacta	EU041819	Arzanlou et al. 2007
V. japonica	EU041818	Arzanlou et al. 2007
Zasmidium cellare	EU041821	Arzanlou et al. 2007

related sequence data from GenBank was used to construct a strict consensus tree with tree length = 1689, consistency index (CI) = 0.5281, retention index (RI) = 0.5981, and rescaled consistency index (RC) = 0.3159 (Fig. 1). One major clade had 100% bootstrap support, and another clade included three species

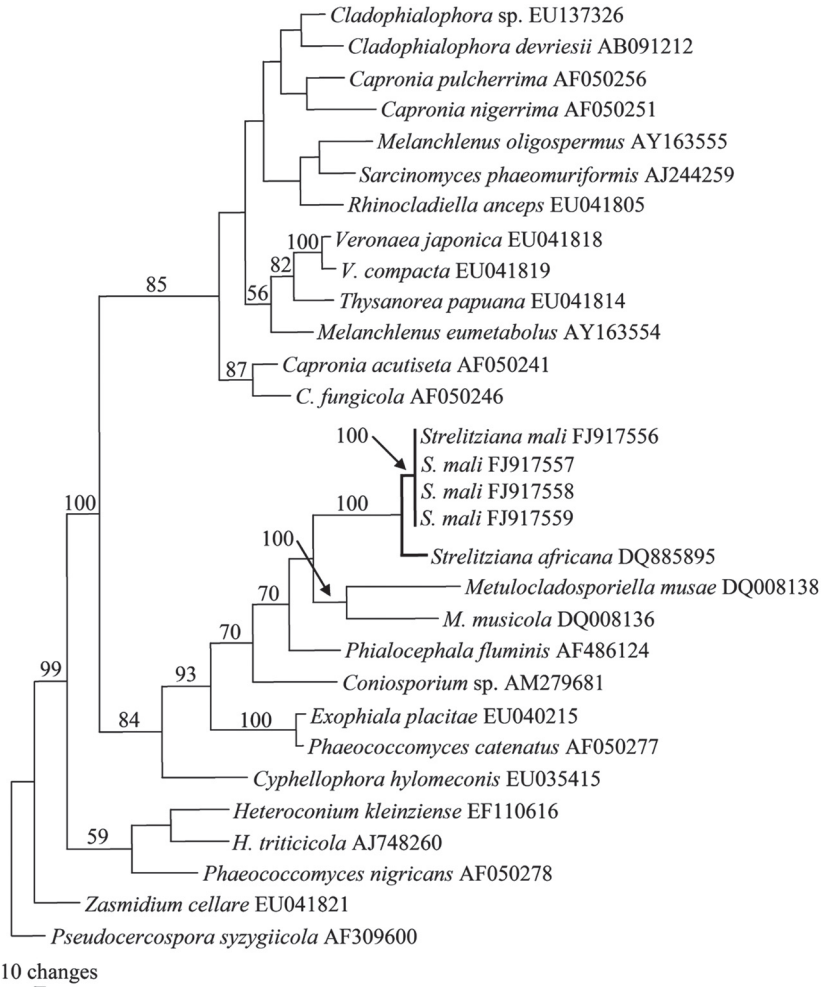

Fig. 1 The majority consensus tree (length = 1689, CI = 0.5281, RI = 0.5981, RC = 0.3159) derived from a heuristic search option in PAUP version 4.0b10 for 32-bit Microsoft Windows with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set ITS1, 5.8S and ITS2. Bootstrap values higher than 50% are indicated above the tree branches.

with a lower (59%) bootstrap support. In our tree, our four strains — YL12, YL06, QX01, ZZ21 — clustered together with *Strelitziana africana* with a 100% bootstrap value, indicating that they might represent a new species. Based on

morphological characteristics and molecular phylogenetic analysis, we propose that the four isolates represent a new species of *Strelitziana*.

Taxonomic description

Strelitziana mali Rong Zhang & G.Y. Sun, **sp. nov.** Figs. 2–3

MycoBank MB 515170; GenBank FJ917556

Coloniae in PDA post 30 dies temperature ambiente ad 18 mm diam., purpureo-brunnea, coactae, in medio 3 mm altae; Hyphae hyalinae aut pallide brunneae, septatae, ramosa, aeriae 2–3 µm crassae, submersae saepe ad 3–5 µm inflatae; Conidiophora unicellularia ex hyphis indistinctis vegetativis oriunda, constanter brunnea, cellulae conidiogenae terminal. Conidia hyaline, fusiformia, longa obclavate, (2–)5–10-septata, (12–)35–60(–100) × 7 (–35) µm, conidiogenesis microcyclica visa in vitro.

Holotypus: ex cuticulae fructi Malus ×domestica *Borkh., Liquan, Shaanxi, China, HMUABO (Herbarium Mycologicum Universitatis Agriculturalis Boreali-Occientalis) 822502; cultus QX01.*

Isolate QX01 was obtained in China from apple (*Malus ×domestica*), where it forms mycelial mats with sclerotium-like bodies on the fruit surface. The colony diameter after 1 month on PDA at 25°C reached 24 mm with even margins and smooth, felty aerial hyphae; colony centers are purplish gray and outer zones pale white. Hyphae are hyaline to brown, ramose, septate with aerial hyphae 2–3 µm diam. and submerged hyphae inflated, 3–5µm diam. Conidiogenous cells terminal, pigmented, thinner than the conidia. Conidia hyaline, thin walled, solitary, fusiform to long obclavate, (12–)35–60(–100) × 7(–35) µm, (2–)5–10–septate. Microcyclic conidiation is present in culture (Fig. 2).

Scanning electron microscope studies showed that conidiogenous cells were erect, solitary, arising from aerial and submerged mycelium, subcylindrical, straight to geniculous-sinuous, terminal, frequently constricted, abscising irregularly or regularly, and produced by a hypha that was thicker than the conidiogenous cell. Conidiogenesis is rhexolytic with remnants of the separating cell clearly visible on both the conidiogenous cell and conidia. Conidia produce secondary spores; hypha and/or conidia are often anastomose (Fig. 3).

Discussion

Previously there was only one species in the genus, *Strelitziana africana*, for which the anamorph–teleomorph connection is unknown. *Strelitziana africana* resembles species of *Pseudocercospora* Speg., which are morphologically variable (Crous et al. 2000). *Pseudocercospora syzygiicola* hyphae give rise to stromata or single conidiophores. The conidiophores are fasciculate and can also arise from a stroma. The conidiogenous cells have unthickened conidial scars, and the conidia have unthickened hilum (Sutton et al. 1997). *Strelitziana africana* lacks the non-thickened conidial scars and stroma.

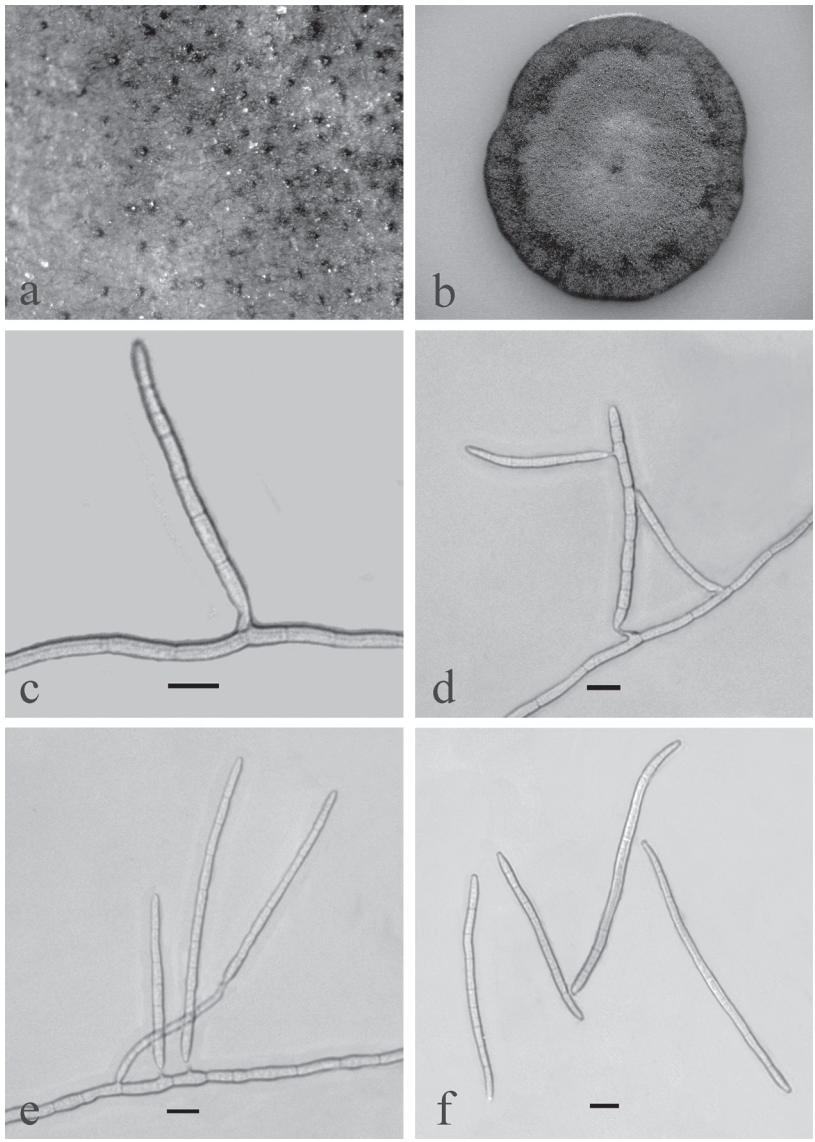

Fig. 2 *Strelitziana mali* QX01

a. Signs on apple peel; b. Colony on PDA; c. Conidia and conidiogenous cell; d. Secondary spores; hypha and conidia often anastomose; e. Single spore fallen off hypha, secondary spores; f. Conidia. Bars (c–f) =10 μm.

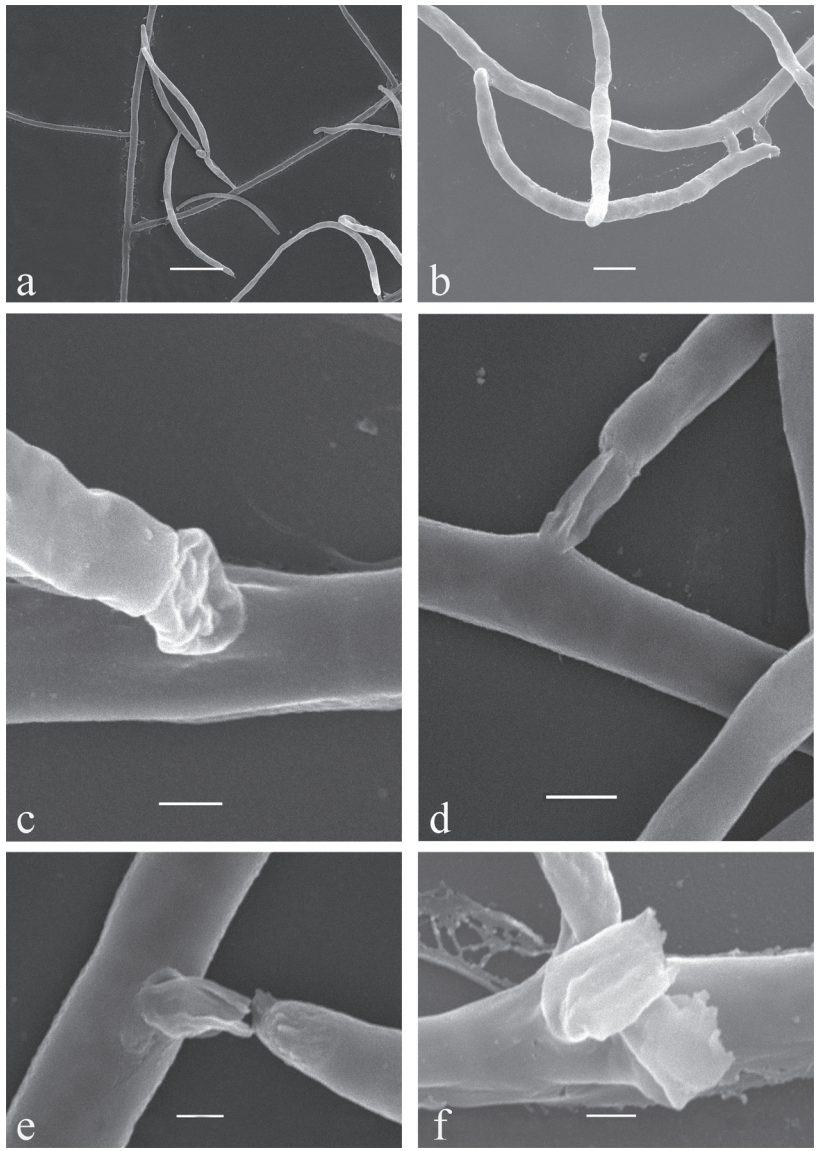

Fig. 3 *Strelitziana mali* QX01 under scanning electron microscopy

a. Secondary spores; b. Anastomosing conidia; c–d. Fasciculation of conidiogenous cell; e. The remnants of separating cell clearly visible on conidia and conidiogenous cell; f. Remnants on conidiogenous cells. Bars: a = 20 μm; b = 5 μm; c–e = 1 μm; f = 2 μm.

The longer [(12–)35–60(–100) µm] conidia easily distinguish *Strelitziana mali* from *S. africana*. Furthermore, the conidiogenous cell of *S. mali* is very tiny, not easily observed under a light microscope, and produced directly from the hypha in contrast to the *S. africana* conidiogenous cell, which is produced by a conidiophore. The ITS sequence analysis and morphological comparison clearly support describing the isolates from *Malus ×domestica* as a new species of *Strelitziana*.

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