



# *Neoscytalidium dimidiatum* causes leaf blight on *Sansevieria trifasciata* in Brazil

Rodrigo Pinheiro Monteles<sup>1</sup> · Enayra Silva Sousa<sup>1</sup> · Kedma da Silva Matos<sup>2</sup> · Vinícius Soares Torquato de Brito<sup>3</sup> · Maruzanete Pereira de Melo<sup>4</sup> · José Evando Aguiar Beserra Jr<sup>1</sup>

Received: 16 March 2020 / Accepted: 16 April 2020 / Published online: 23 April 2020  
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## Abstract

*Sansevieria trifasciata* plants showing symptoms of leaf blight were observed in Teresina City, Brazil. Based on morphology and the phylogenetic analysis of DNA sequences of the EF-1 $\alpha$  and ITS regions, two isolates of *Neoscytalidium dimidiatum* were identified. Pathogenicity tests showed that the isolates caused leaf blight on *S. trifasciata*, in addition to *Sansevieria cylindrica*, and fulfilled Koch's postulates. To the best of our knowledge, this report is the first to describe *N. dimidiatum* on *Sansevieria* in Brazil.

**Keywords** Botryosphaeriaceae · Leaf blight · Saint Jorge sword · *Sansevieria cylindrica*

The species of the genus *Sansevieria* (Agavaceae) are popularly known in Brazil as Saint Jorge swords. The family includes approximately 60 species of African origin, which are extremely rustic, adapting very well to the sun or shade and heat or cold (Lorenzi and Souza 2001). Several fungi, including those from the Botryosphaeriaceae family, have been reported to cause leaf disease in *Sansevieria trifasciata* worldwide (Farr and Rossman 2019). In Brazil, there are no reports of fungal diseases in *Sansevieria* spp.

In December 2018, plants of *S. trifasciata* var. *Laurentii* (De Wild.) showing symptoms of leaf blight were observed in the gardens of the Center for Agricultural Sciences of the

UFPI, Teresina, Piauí, Brazil. Small fragments of colonized tissue were removed from the samples, disinfested in 70% ethanol for 1 min and 2% sodium hypochlorite for 2 min, then transferred to sterilized distilled water and dried on sterile paper. The isolates were cultivated in potato dextrose agar culture medium (PDA) and incubated at 26  $\pm$  2 °C for 3 to 5 days under a 12-h photoperiod.

The preliminary identification of the fungal isolates was based on morphological characteristics. A mycelial plug 6 mm in diameter taken from a 7-day-old culture was transferred to a PDA. The color and morphology of the colony were evaluated. The isolates were cultured on 2% water agar (WA) overlaid with sterilized twigs of *Pinus* to induce pycnidia formation and sporulation. Measurements of 30 selected conidia were performed. Single-spore isolates were deposited in the culture Collection of Phytopathogenic Fungi at the Phytopathology Laboratory at the UFPI (accession numbers: COUFPI 239 and COUFPI 241).

To confirm their identification, the isolates were grown on PDA for 7 days at 26 °C under a 12 h photoperiod. The aerial mycelium was scraped off the colony surface, and DNA was extracted (Moller et al. 1992). The DNA concentration was estimated visually in a 1.0% agarose electrophoresis gel stained with ethidium bromide and visualized under UV light.

The internal transcribed spacer (ITS) region was amplified with the primers ITS1 and ITS4 (White et al. 1990), and transcription elongation factor 1- $\alpha$  (EF1- $\alpha$ ) was amplified with the primers 728F (Carbone and Kohn 1999) and EF2R (O'Donnell et al. 1998). The PCR products were purified

The sequences reported in this paper have been deposited in the GenBank under accession numbers MT026926, MT026927, MT036371 and MT036372.

✉ José Evando Aguiar Beserra, Jr  
evando@ufpi.edu.br

<sup>1</sup> Departamento de Fitotecnia, Centro de Ciências Agrárias, Universidade Federal do Piauí (UFPI), Ininga, Teresina, PI 64049-550, Brazil

<sup>2</sup> Departamento de Fitotecnia, Centro de Ciências Agrárias, Universidade Federal de Roraima (UFRR), Boa Vista, RR 69310-000, Brazil

<sup>3</sup> Curso de Ciências Biológicas, Centro de Ciências da Natureza, Universidade Estadual do Piauí (UESPI), Teresina, PI 64002-150, Brazil

<sup>4</sup> Campus Floresta, Universidade Federal do Acre (UFAC), Cruzeiro do Sul, AC 698995-000, Brazil

**Table 1** Isolates and DNA sequences data used in phylogenetic analysis

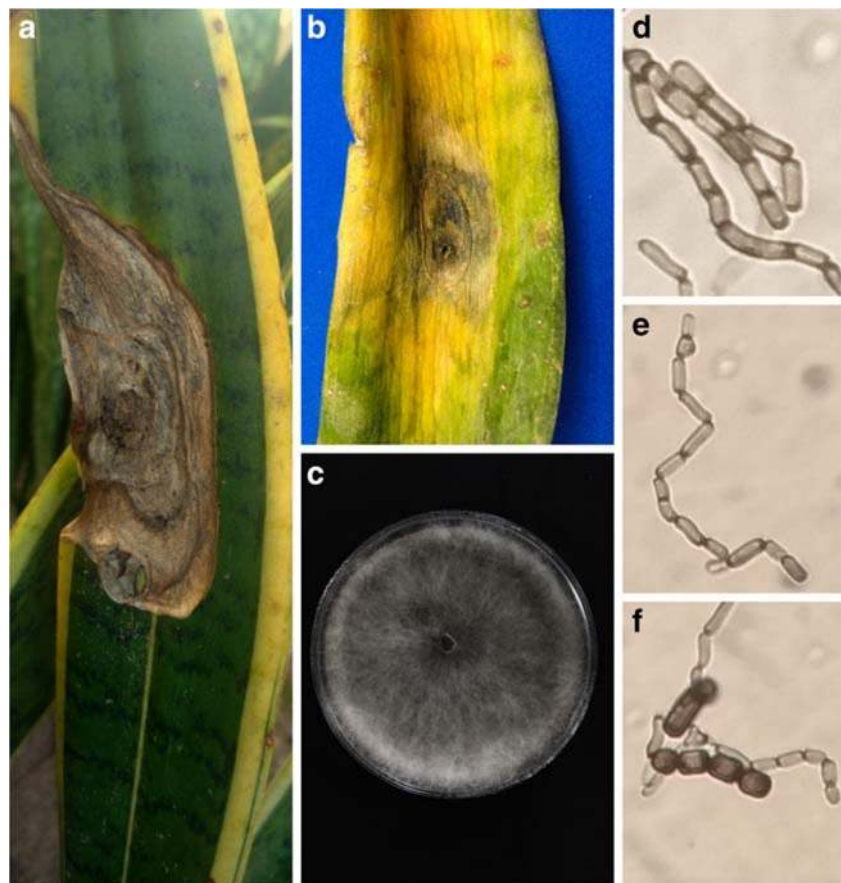
Species	Isolate	Host	Location	Reference	GenBank accession numbers	
					ITS	EF-1 $\alpha$
<i>Neofusicoccum mangiferae</i>	CMW7024	<i>Mangifera indica</i>	Australia	Slippers et al. 2005	AY615185	DQ093221
<i>Neofusicoccum vitifusiforme</i>	STE-U5252	<i>Vitis vinifera</i>	South Africa	van Niekerk et al. 2004	AY343383	AY343343
<i>Neoscytalidium dimidiatum</i>	B3	<i>Sansevieria trifasciata</i>	Malaysia	Kee et al. 2017	MF580797	–
<i>N. dimidiatum</i>	*CBS 499.66	<i>M. indica</i>	Mali	Phillips et al. 2013	KF531820	KF531798
<i>N. dimidiatum</i>	COUFPI 239	<i>S. trifasciata</i>	Teresina, Brazil	Present study	MT026926	MT036371
<i>N. dimidiatum</i>	COUFPI 241	<i>S. trifasciata</i>	Teresina, Brazil	Present study	MT026927	MT036372
<i>Neoscytalidium novaehollandiae</i>	CBS122071	<i>Crotalaria medicaginea</i>	Australia	Pavlic et al. 2008	EF585540	EF585580
<i>N. novaehollandiae</i>	CBS122072	<i>Adansonia gibbosa</i>	Australia	Pavlic et al. 2008	EF585535	EF585581
<i>Neoscytalidium orchidacearum</i>	MFLUCC 12–0533	Orchid	Thailand	Huang et al. 2016	KU179865	–

\*Epitype (B3 = Culture Collection Unit, Department of Plant Pathology, School of Biological Sciences, Universiti Sains Malaysia; CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; MFLUCC = Mae Fah Luang University Culture Collection; STE-U = Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa)

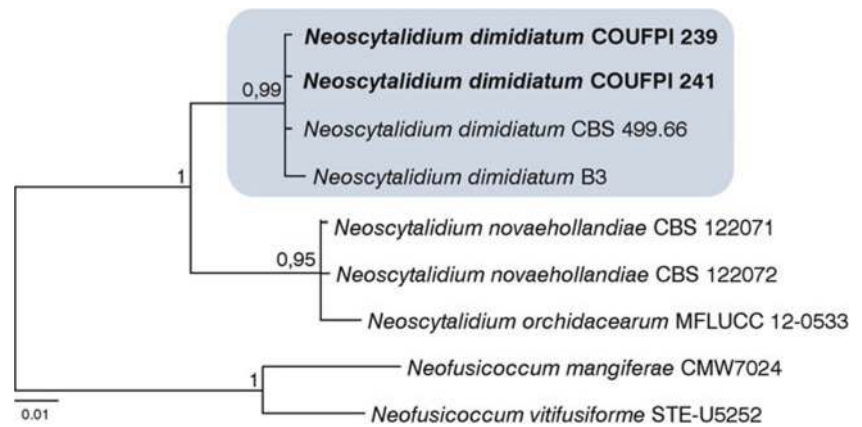
and sequenced by Macrogen Inc. (Seoul, South Korea). Additional sequences of Botryosphaeriaceae isolates were obtained from GenBank (Table 1). The obtained sequences were aligned using the multiple sequence alignment program MUSCLE® implemented in MEGA v. 8 software. The

resulting alignment was deposited in TreeBASE under accession number ID25814. Bayesian inference analyses were performed using the Monte Carlo chain method. Mr. Modeltest 2.3 (Posada and Buckley 2004) was used to determine the evolutionary model of the nucleotides that best fit the data;

**Fig. 1** *Neoscytalidium dimidiatum* pathogenic to *Sansevieria trifasciata*. (a) Necrosis symptoms observed in the field. (b) Symptomatic plant seven days after inoculation with the COUFPI 239 isolate. (c) Colony after four days of incubation in PDA medium at  $\pm 26$  °C under a 12 h photoperiod. (d–f) Different forms and pigmentation in chain arthroconidia



**Fig. 2** Bayesian phylogenetic tree of ITS-5.8S rDNA and EF-1 $\alpha$  sequences showing the phylogenetic relationships among species of *Neoscytalidium* based on the evolutionary models HKY for ITS and the HKY + I for EF-1 $\alpha$ . Posterior probability values are indicated above the nodes. The isolates used in this study are highlighted in bold. The tree is rooted with *Neofusicoccum mangiferae* and *Neofusicoccum vitifusiforme*



the models used in the phylogenetic analyses were HKY for ITS and HKY + I for EF-1 $\alpha$ .

Phylogenetic analysis was performed at the CIPRES web portal (Miller et al. 2010) using MrBayes version v. 3.2 (Ronquist et al. 2011). Markov chains were run simultaneously from random trees to 10,000,000 generations. Trees were sampled every 1000th generation for a total of 10,000 trees. The first 2500 trees were discarded as burn-in in each analysis. The sequences obtained in this study were deposited in GenBank (Table 1).

To confirm pathogenicity, isolates were cultured on PDA for 4 days at 26 °C under a 12-h photoperiod before inoculation onto 6-month-old plants of *S. trifasciata* var. *Laurentii*, *Hahnii*, *Prain*, and *Sansevieria cylindrica*. Plants were inoculated with sterile toothpicks, inserting fungal structures in the basal, middle and apical regions of the leaves. Plant inoculated with non-infected toothpicks were used as a negative control. After inoculation, the plants were maintained in a moist chamber constructed using plastic bags for 24 h. These bags were then removed, and the plants were kept in an environment with the temperature controlled at 26  $\pm$  2 °C. Disease development was observed until 30 days after inoculation. The experiment was repeated twice, and five replicates of each variety/species were tested for each isolate.

The isolates were pathogenic to *S. trifasciata* var. *Laurentii*, *Hahnii*, *Prain*, and *S. cylindrica*. Leaf blight symptoms similar to the symptoms found in the field (Fig. 1a) were observed four days after inoculation, starting with dark lesions around the inoculation point (Fig. 1b). No symptoms were observed on the control plants. The isolates were consistently recovered from the inoculated plants.

Based on multigene phylogenetic analysis, the isolates were identified as *Neoscytalidium dimidiatum*. Isolates COUFPI 239 and COUFPI 241 were grouped with the reference isolate of *N. dimidiatum* (CBS 499.66) with high support (Bayesian posterior probability = 0.99) (Fig. 2).

The isolate cultures presented a white color and cottonose mycelium, were denser at the center and border. After the third day, the colonies became light gray, and they later became

dark gray (Fig. 1c), showing the reverse pattern of the same color. The hyphae were light brown and septate. The arthroconidia were diverse in morphology and coloration; 0–1 septum was observed, and most were ovoid and light brown (Fig. 1f). Arthroconids were arranged in chains (Fig. 1d, e). The arthroconids were 10.04 to 15.94  $\times$  7.13 to 9.92  $\mu$ m. Pycnidia were observed after 10 days of incubation.

*Neoscytalidium dimidiatum* has been reported to cause leaf blight in *S. trifasciata* in Malaysia (Kee et al. 2017). In Brazil, *N. dimidiatum* has been reported to cause root rot in physic nut and cassava (Machado et al. 2012; Mello et al. 2018), black root rot in cassava (Machado et al. 2014), dieback in grapevine and mango (Correia et al. 2016; Marques et al. 2013). Our study provides the first report of leaf blight on *Sansevieria trifasciata* in Brazil caused by *Neoscytalidium dimidiatum*.

**Acknowledgements** ESS is recipient of a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) scholarship and JEABJ is recipient of PQ fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

## Compliance with ethical standards

**Disclosure of potential conflicts of interest** We declare that the authors have no conflicts of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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