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First record of *Pseudofusicoccum adonsoniae* Pavlic, T.I. Burgess and M.J. Wingf. from *Ficus krishnae* (as endophyte) and new record for North India

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ABSTRACT

During our investigation of endophytic diversity of *Ficus krishnae* L. growing in Chandigarh, *Pseudofusicoccum adansoniae* is isolated as endophyte from stem tissues of *Ficus krishnae.* It is isolated and identified for the first time from *Ficus krishnae* throughout the world whereas reported only for the second time from India. So, it constitutes first record for North India.

Key words: Endophyte, *Ficus krishnae*, *Pseudofusicoccum adansoniae.*

INTRODUCTION

Endophytic fungi reside inside the host plant without causing any harmful effects to it. These have been recorded in all the plants examined till date. They belong to different taxonomic groups and are diverse in their habitat, biological activity and chemical composition (Tan and Zou 2001, Zimmerman and Vitousek 2012). The endophytic fungi play an important role in improvement of plant health and plant growth (Hallmann et al. 2007). Therefore isolation of these fungi from different plant species will provide areas for discovering diverse species and exploiting these fungi in various industrial applications (Aly et al. 2011).

Ficus krishnae is an unusal species of genus *Ficus.* It is considered as highly sacred plant species in India due to its peculiar shape of leaves (Anand et al. 2016). The plant has been used in ancient folklore medicine (Madhava et al. 2008). Stem, bark and leaves of the plant are used for diabetes (Lakshmi et al.2010).

The genus *Pseudofusicoccum* was introduced by Crous et al. (2006) who described it as closely related to *Fusicoccum* and *Neofusicoccum* morphologically but differs phylogenetically from both of these genera. *Pseudofusicoccum stromaticum* is regarded as type species of this genus whereas *Pseudofusicoccum adansoniae* was first described by Pavlic et al. 2008. It was isolated from dying branches of *Adansonia gibbosa* in Western Australia. To the best of our knowledge this species is reported only once from India (Maharastra) (Sharma et al. 2013). Apart from India it has been reported from Australia, Brazil and Thailand (Pavlic et al. 2008, Sakalidis et al. 2011, Doilom et al. 2015, Trakunyingcharoen et al. 2015a, Trakunyingcharoen et al. 2015b, Gonçalves et al. 2016)

The aim of this study is to investigate endophytic diversity of *Ficus krishnae* L. growing in Panjab University Campus, Sector 14, Chandigarh. The isolates were identified on the

basis of morphological features as well as molecular characterization.

MATERIAL AND METHODS

Ficus krishnae plant (*Ficus krishnae* L.) stem tissues were collected from Sector 14, Panjab University Campus, Chandigarh. The stem samples of plants were randomly excised and brought to the laboratory in ziplock plastic bags. Briefly, samples were washed in running tap water to remove dust and debris, dried in the air and then cut into 0.5-1cm segments. For surface sterilization, the segments were soaked in 0.01% mercuric chloride followed by washing of segments in sterilized distilled water thrice (Janardhanan et al., 1991; Ahmad, 1991; Bills, 1996; Moutia and Dookuna, 1999) and dried in a laminar air flow chamber. The sterilized segments were then placed on potato dextrose agar (PDA) supplemented with chloramphenicol (100µg/mL concentration) to inhibit bacterial growth. Developing hyphal tips of emerged colonies were collected after incubation at 25± 1℃ for 15 days and sub-cultured on PDA. Pure cultures of isolates were maintained in PDA slant tubes and 20% glycerol stock solution and deposited in the culture collection of the Panjab University Herbarium (PAN). In this study, molecular and morphological characteristic of an isolate was examined.

Identification of fungi

Microscopic examination

The Morphological characteristic of the fungus was examined from the pure cultures on PDA. The slides were prepared in 4% KOH and stained with 2% Congo-red. Then the slides were examined with Matrix VRS-2f transmission microscope. The size of conidiomata, conidia and conidiophores were measured using Promed software. Morphological characteristics of the isolate were then compared with previous descriptions.

Molecular Characterization

Pure fungal cultures were used to extract DNA. The DNA was used in PCR to amplify the ITS region using ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCG CTTATTG ATATGC) primers described in the literature (White et al. 1990). The 400 - 900 bp amplicon was gel eluted and subjected to sequencing. The sequencing results were assembled and compared with NCBI data base.The phylogenetic tree was constructed using neighbour joining methods in MEGA7.

RESULTS

Taxonomy of isolate

The colony was dull white initially turning dark olivaceous grey to olivaceous black after 7-10 days on PDA; mycelium fluffy, dense; aerial mycelium cottony intially turning olivaceous grey with age. Numerous conidiomata were observed after two weeks, along with many pycnidia covered with hyphal hairs, immersed to semi-immersed in medium. Hyphae were brown in color and much branched. Conidiogenous cells smooth, cylindrical to ellipsoidal and hyaline. Paraphyses absent. Conidia 17.6 –25.6 × 3.5–5.5 µm, ellipsoid and straight, occasionally slightly bent or irregularly shaped, apices rounded, smooth with fine granular content, hyaline, thin-walled, unicellular, covered with persistent mucous layer.

Molecular analysis

ITS region of the isolate and its related species was compared to determine the phylogenetic relationship (Fig.2). The DNA nucleotide sequence have been identified and deposited in Genbank for which accession number (MF613653.1) has been provided.

DISCUSSION

In present study, *Pseudofusicoccum adansoniae* is isolated as endophyte from stem tissues of *Ficus krishnae.* It is isolated and identified for the first time from *Ficus krishnae* throughout the world whereas reported only for the second time from India. The presence of endophytic *Pseudofusicoccum adansoniae* was recently recorded in branches of *Chrysobalanus icaco* and *Eugenia sp.* collected from Caatinga biome, Brazilian Semi-Arid Region (Gonçalves et al. 2016). Earlier, it was isolated from *Hevea brasiliensis* (as an endophyte and pathogen from leaves and petioles) (Trakunyingcharoen et al. 2015a), *Senna siamea*, *Cassia fistula* and *Dimocarpus longan* (as caulicolous fungi) (Trakunyingcharoen et al. 2015b) in Thailand. Doilom et al. 2015 found *P. adansoniae* associated with leaf spot of *Tectona grandis* growing in Chiang Rai Province of Thailand whereas it was also found to cause mango dieback and canker in Kimberley Region of Western Australia (Sakalidis et al. 2011).This species was isolated for the first time from dying branches of *Adansonia gibbosa* and asymptomatic branches *of Acacia synchronica, Eucalyptus sp. and Ficus opposite* plants growing in Western Australia (Pavlic et al. 2008). In India, It was reported only once from *Jatropha podagrica* as endophyte from Maharastra (Southern India) (Sharma et al. 2013). Here, it constitutes first record of *Pseudofusicoccum adansoniae* from North India.

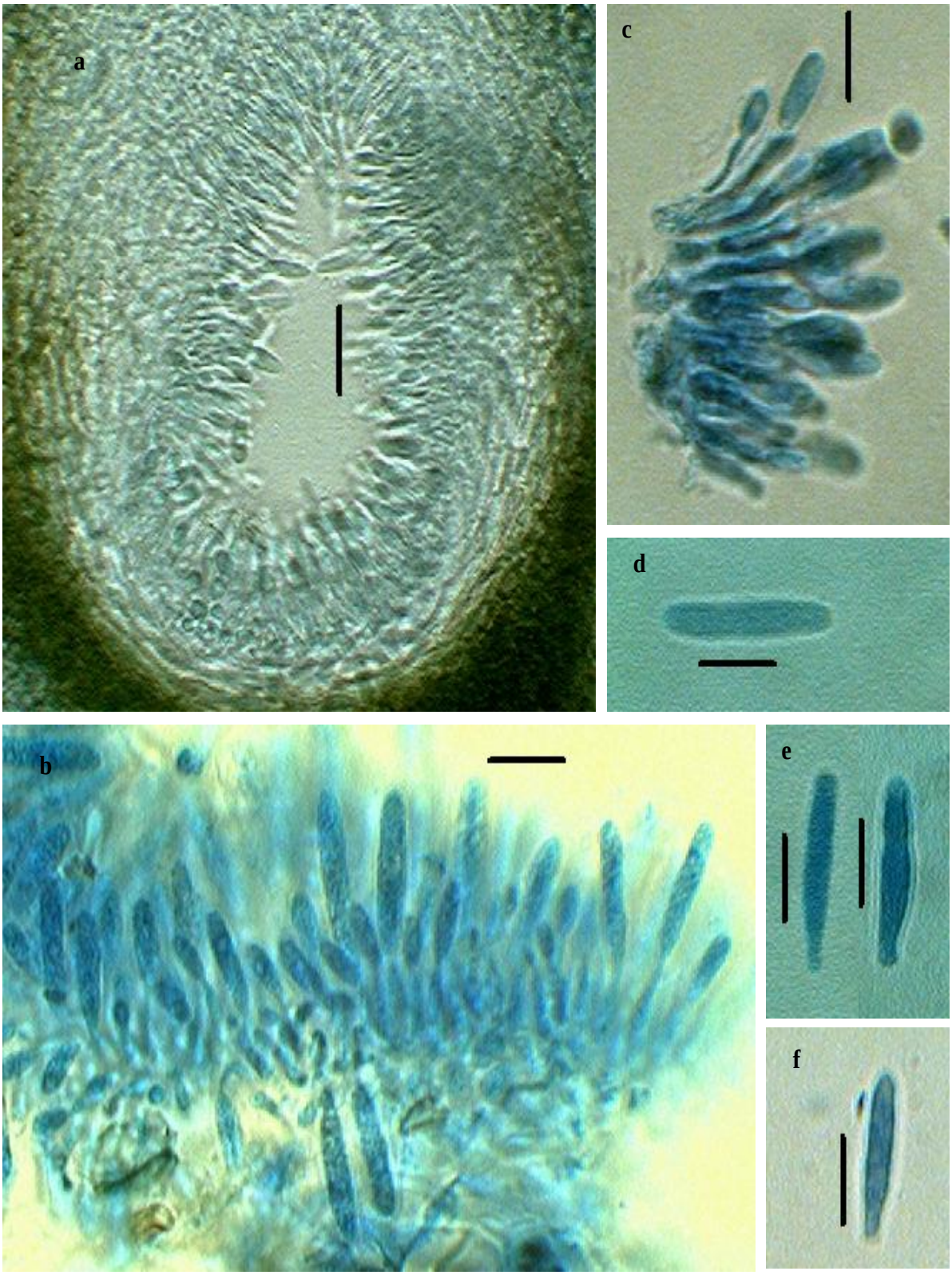

Fig. 1. ***Pseudofusicoccum adansoniae*****: a)** Cross- section of conidiomata; **b, c)** Conidiogenous cells bearing conidia; **d-f)** Hyaline aseptate conidia. **Scale Bars**- 10µm.

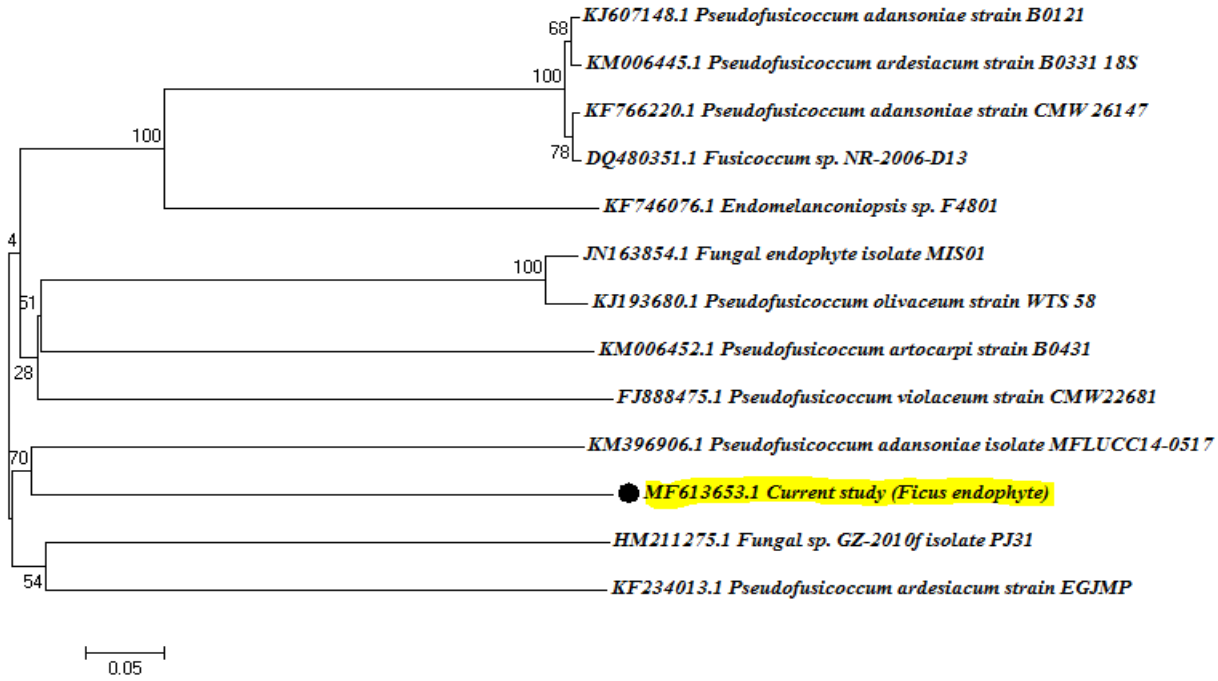

Fig. 2: The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 3.23300671 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All ambiguous positions were removed for each sequence pair. There were a total of 538 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

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