

***Sporothrix ranii*, a new wood-inhabiting fungus associated with date palm trees in Iran**

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In the winter of 2023 and during routine examinations of date palm trees in Safiabad, Dezful (Khuzestan Province, Iran), an intense white mass of fungal molds was observed on the leaf scars of the said trees. Purified isolates were subjected to morphological and molecular examinations. Typical morphological characteristics viz. single-celled, hyaline, smooth and obovate to ellipsoidal conidia arising by sympodial growth with basal scar prominent produced holoblastically on denticulate conidiogenous cells revealed that, all fungal isolates belong to the genus *Sporothrix*. Furthermore, based on a combination of morphological and sequence data of internal transcribed spacer (ITS) of rDNA, the recovered fungal isolates were determined as *S. ranii*. To the best of author's knowledge, it is the first report of this species for the funga of Iran and its second report in the world.

**Keywords:** Fungal mold, epiphyte, ITS-rDNA, morphology, *Phoenix dactylifera****Sporothrix ranii*, گزارشی جدید از قارچ همراه با درختان نخل در ایران**

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**خلاصه**

در زمستان ۱۴۰۱، طی پایش‌های متداول نخلستان‌های مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی صفی‌آباد دزفول (استان خوزستان)، پوشش سفید و متراکمی از کپک‌های قارچی روی تنه درختان به ویژه در انتهای برگ‌های بریده شده مشاهده گردید. جدایه‌های قارچی پس از جداسازی و خالص‌سازی تحت بررسی‌های ریخت‌شناسی و مولکولی قرار گرفتند. براساس ویژگی‌های ریخت‌شناسی همچون کنیدیوم‌های تک‌سلولی، شفاف، صاف و بیضوی تا تخم‌مرغی توسعه یافته سیموپودیالی با زخم پایه‌ای برجسته تولید شده به صورت هولوبلاستیک روی سلول‌های کنیدیوم‌زای دنداندار، مشخص گردید که تمامی جدایه‌های قارچی متعلق به جنس *Sporothrix* می‌باشند. در ادامه و براساس تلفیقی از داده‌های ریخت‌شناسی و اطلاعات ناحیه ژنومی ITS-rDNA، مشخص شد که جدایه‌های قارچی به گونه *S. ranii* تعلق دارند. براساس اطلاعات موجود، این نخستین گزارش از این گونه برای قارچ‌های ایران و دومین گزارش آن در دنیا است.

واژه‌های کلیدی: اپیفیت، ریخت‌شناسی، کپک قارچی، ناحیه فاصله‌انداز داخلی-دی‌ان‌ای ریبوزومی، *Phoenix dactylifera*

## Introduction

*Sporothrix* is a ubiquitous fungal genus comprising different species occupying a wide range of ecological niches (de Beer & Wingfield 2013). This fungus is a dimorphic ascomycete that was first established by Hektoen & Perkins (1900) based on the morphological description of *Sporothrix schenckii*, a mammalian pathogen causing sporotrichosis (Ostafińska *et al.* 2021). Members of the *Sporothrix* are mostly found in soil, however a wide variety of environments, including hardwood, plant debris, peat moss, bark beetle, mites and on the fruiting bodies of some Basidiomycota can be their habitat (de Beer *et al.* 2016). Single-celled, hyaline or occasionally pigmented conidia sympodially arranged on clusters of denticles at the ends of conidiophores are of main characterizations of *Sporothrix* members which is now considered as one of the nine clearly defined genera in the *Ophiostomataceae* (Rodrigues *et al.* 2016, Ostafińska *et al.* 2021). Because of limited morphological characteristics and their overlap with other closely related genera, the identification of *Sporothrix* spp. using only morphological data is difficult (Rodrigues *et al.* 2016). In recent years, sequence data of internal transcribed spacer (ITS) of ribosomal DNA has been considerably helpful as a universal DNA barcode marker for the recognizing of fungal species including *Sporothrix* spp. (Schoch *et al.* 2012, Zhou *et al.* 2014). During our surveys of date palm trees in Khuzestan Province (Iran), a remarkable mass of whitish fungal structures was noticed on the leaf scars. Pure cultures were made using single spore technique and morphological and molecular studies were further performed to determine the fungal isolates.

## Materials and Methods

In a survey of date palm trees at Safiabad Agricultural Research and Education and Natural Resources Center in Khuzestan Province (Iran) in 2023, white mycelial masses were observed on the leaf scars of date palms near the main trunk (Figs 1A & B). Sampling was conducted from the leaf scars and the samples were

examined under a stereo microscope (SMZ 645, Nikon, Japan). The masses of white mycelia and spores on the surface of the leaf scars were then transferred to water-agar medium using a sterile needle and the isolates were purified by the single spore technique. Morphological characteristics were studied and photographs were captured using a high-resolution Leica camera system mounted on the Olympus BX41 light microscope.

Genomic DNA was extracted from the pure culture of fungal isolates using the CTAB method as described by Möller *et al.* (1992). In polymerase chain reactions, the primer pairs of ITS1 and ITS4 (White *et al.* 1990) were used to amplify partial regions of ribosomal DNA. Each PCR was adjusted to a final volume of 25  $\mu$ l. The reaction mixture consisted of 50 ng genomic DNA, 1X, PCR Mastermix (Taq 2x master mix Red Amplicon, Denmark) and 0.2 M of each primer. The PCR mixture was subjected to initial denaturation at 95 °C for 5 min and followed by 35 cycles, 1 min at 94 °C, 30 s at 52 °C and 1 min at 72 °C, with a final extension of 7 min at 72 °C. PCR products were separated on 1% agarose gel stained with ethidium bromide, and their length was estimated in comparison with the Gene Ruler molecular marker (GeneRuler™, #SM0241). PCR products were sent to Microsynth (Switzerland) company for sequencing.

Consensus sequences were made from raw trace files of forward and reverse sequences using Staden Package Program Ver. 2.0.0b9 (Staden 1996). After a preliminary blast search, sequences with the highest similarity together with sequences of the type strains of the close species were obtained from NCBI's GenBank database (<http://ncbi.nlm.nih.gov/genbank>) and added to the alignment as reference strains. The sequences were aligned with the Muscle software (Edgar 2004) implemented in MEGA7 (Tamura *et al.* 2013), followed by checking the alignment manually when necessary. In phylogenetic analysis, all gaps were considered as missing data. For each alignment, the best evolutionary model was calculated using the software MrModelTest Ver. 2.3 (Nylander 2004). All analyses were performed using

MrBayes Ver. 3.2.1 (Ronquist & Huelsenbeck 2003), with heating parameter set at 0.15, and four MCMC running up to 1000000 generations and sampling trees every 1,000 generations. The first 25% of saved trees were discarded as the burn-in phase of the analysis. Consensus tree and posterior probability (PP) were determined from the remaining trees. The generated phylogenetic trees were inspected and printed using FigTree Ver. 1.3.1 (Rambaut 2009). The sequence derived from this study was deposited at NCBI's GenBank nucleotide database with the accession number: OR159502 (<http://www.ncbi.nlm.nih.gov>).

## Results

### - Taxonomy

*Sporothrix ranii* Moustafa, Persoonia 11(3): 392–394 (1981)

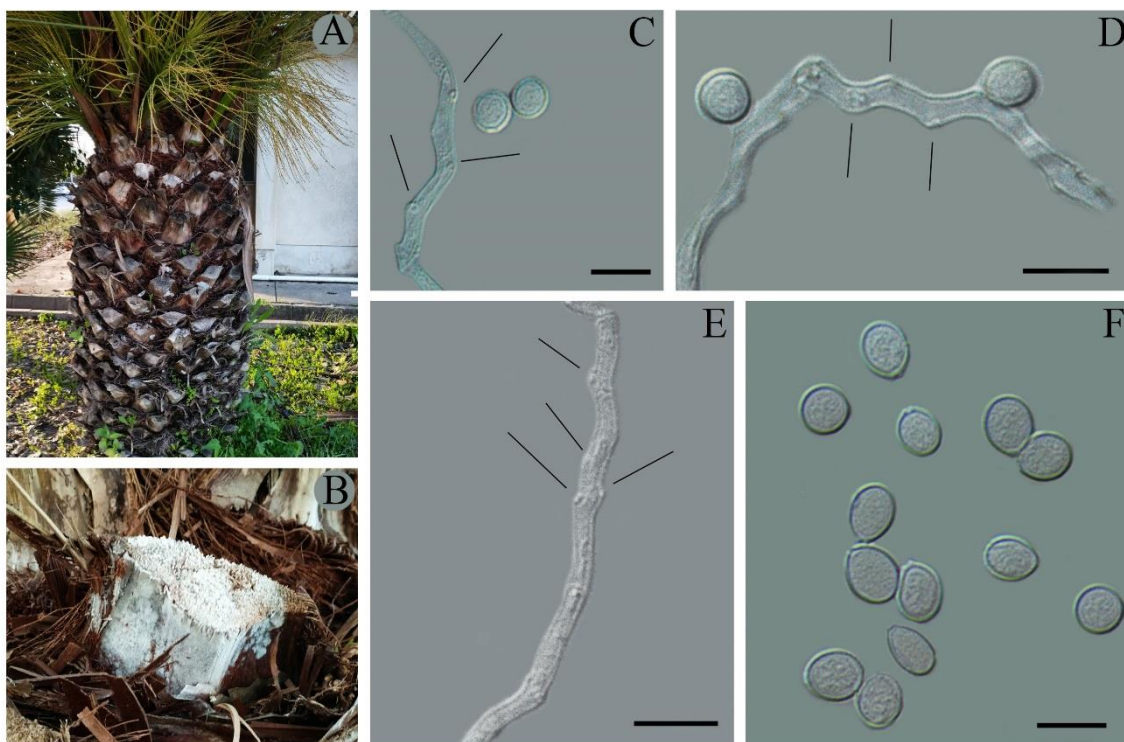
Holoblastic conidiogenous cells straight or slightly curved, cylindrical, arising terminally and laterally from suberect fascicles, with usually orthotropic branching. Conidiiferous apices often proliferate to form irregularly nodose or geniculate rachids and 33–65  $\mu\text{m}$  long.

Single-celled conidia arising by sympodial growth, rarely produced from intercalary clusters of denticles, hyaline, smooth, obovate to ellipsoidal, (7–)7.5–8.2(–9)  $\times$  (5.5–)6.2–6.5(–7)  $\mu\text{m}$ ; arising by sympodial growth, basal scar prominent, about 1  $\mu\text{m}$  wide (Fig. 1). Secondary conidia absent. Sexual morph not observed.

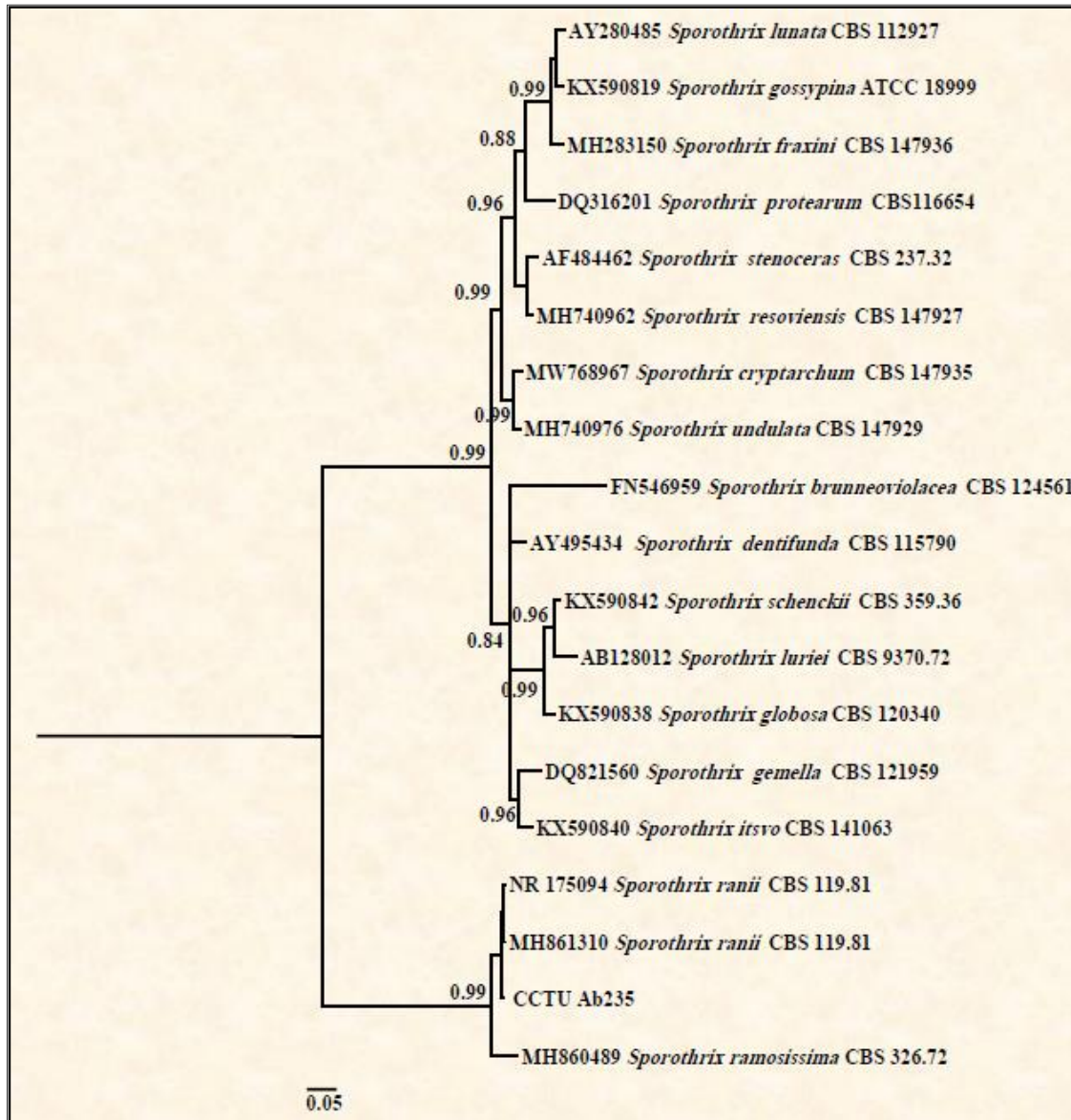
Specimen examined: IRAN: Khuzestan Province, Dezful, N 32° 26' 25", E 48° 42' 98", 86 m, on the leaf scars of *Phoenix dactylifera* (*Arecaceae*), Feb. 2023, K. Karimi, Culture Collection of Tabriz University, Tabriz, Iran (CCTU Ab235).

### - Molecular phylogeny

The final aligned ITS dataset contained 19 taxa with 573 characteristics containing 213 unique site patterns. MrModeltest Ver. 2.3 found GTR+G+I to be the most fitting replacement model for ITS dataset. Bayesian inference placed all taxa in two main clades and the sequence generated in this study (CCTU) resided in the *S. ranii* clade as sister clade of *S. ramosissima* with a high posterior probability (Fig. 2).



**Fig. 1.** Symptoms and morphological features: A–B. White mycelial mass on the leaf scars of date palm tree near the main trunk, C–E. Conidiogenous cells with sympodial growth, F. Conidia with basal scar (Bars = 10  $\mu\text{m}$ ).



**Fig. 2.** ITS phylogenetic tree inferred using Bayesian analysis based on GTR+G+I recommended by Mrmodeltest Ver. 2.3. Posterior Probability Values are indicated above the branches. The isolate generated in this study is marked as CCTU Ab235 (Posterior Probabilities <0.7 are not shown).

## Discussion

*Sporothrix* spp. have been reported as either pathogen on animals and plants or nonpathogenic often isolated from wood, soil and peat moss (de Meyer *et al.* 2008). Many species of this genus have been previously found in association with hardwood trees (Ostafińska *et al.* 2012). In this study, *S. ranii* was also isolated from the leaf scars of date palm and it is here reported for the first time in Iran. All *S. ranii* isolates of the present study were morphologically similar to those previously described by Moustafa (1982) (Fig. 1). Moreover,

sequence data of ITS-rDNA resided our fungal strain in *S. ranii* clade as sister group of *S. ramosissima*, further confirming the identity of fungal isolates obtained in this study (Fig. 2). In addition, Moustafa (*l.c.*) noticed that, *S. ranii* is morphologically similar to *S. ramosissima* in terms of shape and size of conidia and conidium-bearing denticles, however, their conidigenous cells, fertile hyphae and conidiiferous apices show obvious differences.

It is ecologically well known that, *Sporothrix* spp. need fresh wounds for colonization (Jankowiak *et al.*

2019). They occupy the plant tissues in and under the bark on the twig, branch or stem, and kill the area around wounds caused by animals, wind, frost, silvicultural practices (pruning and harvesting) and various arthropods (Jankowiak *et al. l.c.*). In the present study, all fungal isolates were similarly obtained from the leaf scars of date palm trees and no fungal structures of *S. ranii* and disease symptoms were seen on living plant organs (Figs 1A & 1B). It has also been postulated that, the epiphytic activity of wood-inhabiting members of *Sporothrix* may show their involvement in the weakening or healing process of

the tree (Jankowiak *et al. l.c.*). It appears that, still more study is needed to reveal unknown aspects of the ecological behaviors of wood-inhabiting members of the genus *Sporothrix*.

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