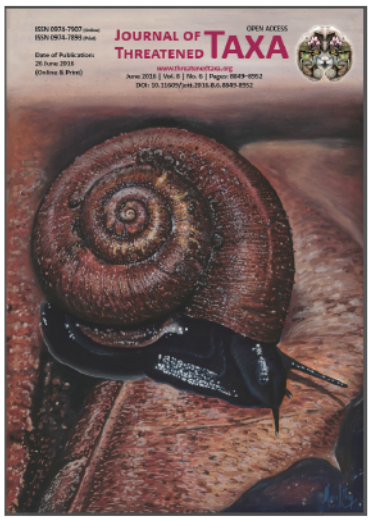

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SHORT COMMUNICATION

REDISCOVERY OF *PENICILLIUM PARADOXUM* (ASCOMYCETE: ASPERGILLACEAE) FROM MAHARASHTRA, INDIA

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Rediscovery of *Penicillium paradoxum* (Ascomycete: Aspergillaceae) from Maharashtra, India

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Abstract: *Penicillium paradoxum* has an enigmatic *Aspergillus*-like anamorphic state; earlier named as *Aspergillus paradoxus* with a teleomorph state *Hemicarpenteles paradoxus*. The present paper describes the rediscovery of this species from India after five decades and includes a phylogenetic study of this strain. This is the first record of this strain from peninsular India including the Western Ghats.

Keywords: Anamorphic ascomycete, phylogeny, taxonomy, Western Ghats

Aspergillus paradoxus Raper & Fennell was described on its type strain, NRRL 2162 [T], (J.H. Warcup, Isolate No. A-28, January 1948) on opossum dung, Wellington, New Zealand (Raper & Fennell 1955) and WB 4695 (J.N. Rai, IMI 86829, 1962), in flood inundated soil (Gomti River) from Lucknow, India. The species forms clavate vesicles, uniseriate conidiophores and abundant sclerotia and therefore Raper & Fennell (1955) placed this species in the *Aspergillus ornatus*-group. Afterwards, Sarbhoy & Elphick (1965) recollected *A. paradoxus* from dog excreta from Knole Park, Kent (IMI 117502) which formed cleistothecia, globose asci and lenticular ascospores having equatorial crusts. The teleomorph state was named as *Hemicarpenteles paradoxus* A.K. Sarbhoy & Elphick.

The exploration and ex situ conservation of fungal biological resources of the Western Ghats is the leading goal of the National Fungal Culture Collection of India (NFCCI-WDCM 932). During January 2011 a survey was conducted to study the microfungal diversity in the natural forests of Mahabaleshwar and the surrounding forests situated in the northern part of the Western Ghats, India, between 17°58'N and 73°43'E. The Mahabaleshwar forests consist of unique stunted semi evergreen patches which form suitable microhabitats for many rare and new species of fungi (Rajeshkumar et al. 2011a,b). During the survey, an unusual mycelial growth associated with carnivorous animal excrement (containing feathers and bones) was collected. The morphological characterization of the isolate showed taxonomic affinity with genus *Aspergillus*. Partial β-tubulin (BenA) gene sequences were generated as a step toward accurate identification and to study the position of the species among other closely related species.

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Conflict of Interest: The authors declare no competing interests.

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Materials & Methods

Isolates and morphology: The highly sporulated fungal mycelia formed in plenty on the animal excrement were directly isolated using a Nikon binocular stereo microscope (Model SMZ 1500 with Digi-CAM, Japan) and plated on 2% MEA and further sub-cultured on CREA, CYA, CZA, G25N, MEA and OMA media (Image 1). For morpho-taxonomic studies and photomicrographs a Carl Zeiss Image Analyzer 2 (Germany) microscope was used. Dimensions of conidiophores, phialides and conidia were measured using the software Axiovision Rel 4.8. The specimens were deposited in Ajrekar Mycological Herbarium (AMH 9426) and an axenic culture was deposited in National Fungal Culture Collection of India (NFCCI 2356), Agharkar Research Institute, Pune, India.

DNA extraction, amplification and phylogeny: Fungal colonies were grown on MEA plates, and genomic DNA was isolated following the rapid salt-extraction method (Aljanabi & Martinez 1997). A fragment of the BenA gene was amplified using primer pairs Bt_2a and Bt_2b (Glass & Donaldson 1995). The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Houbraken & Samson (2011). Analyses were carried out in MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0.) on the BenA data set using the Maximum Parsimony method with 1000 bootstrap replications. Sequence data were deposited in GenBank.

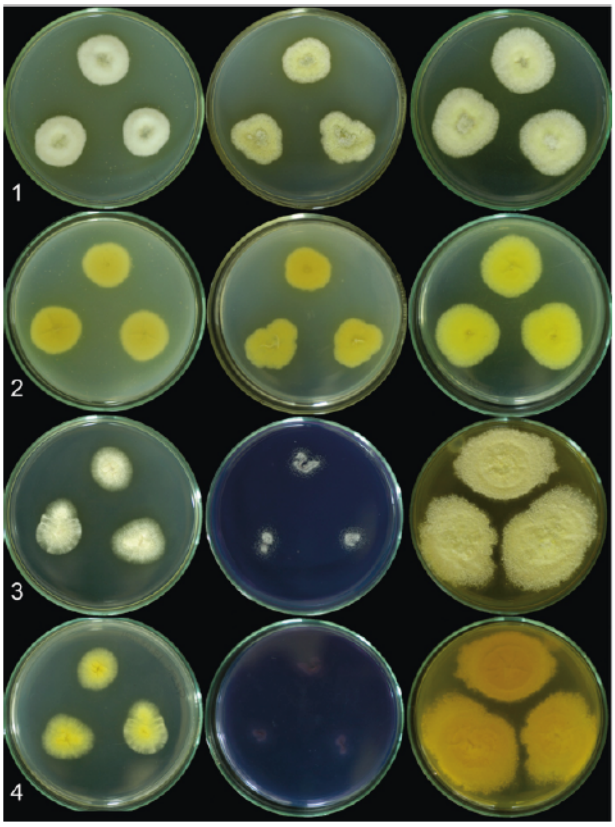

Image 1. *Penicillium paradoxum*, NFCCI 2356.
1st row, colonies obverse OMA, MEA, CYA at 25˚C after 7th day.
2nd row, colonies reverse OMA, MEA, CYA at 25˚C after 7th day.
3rd row, colonies obverse at 25˚C, G25N, CREA (7th day), CZA (10th day).
4th row, colonies reverse G25N, CREA (7th day), CZA (10th day).

Results

Penicillium paradoxum (Fennell & Raper) Samson, Houbraken, Visagie & Frisvad. MycoBank 547045.

Basionym: *Aspergillus paradoxus* Fennell & Raper, Mycologia 47: 69. 1955. MB292853

Type description: Colonies at 25^{0}C after 10 days to two weeks, 7–8 cm. fast growing, deep flocculent mass in light yellow shades near marguerite yellow to primrose yellow, bearing limited to fairly abundant conidial structures primarily from the submerged mycelium. Conidial heads loosely columnar, up to 150–200 µm, or smaller. Conidiophores 1–2 mm (up to 1cm) × 12–20 µm, thin walled, delicately roughened, several times septate, strongly phototropic; Vesicles subclavate, 15–25 µm in diameter, seldom larger. Sterigmata in a uniseriate, 10–12 × 3.5–5.5 µm, borne on the terminal portion of the vesicular areas, with tips often incurved. Conidia ovoid, delicately echinulate, 5.5–6.5 × 4.0–5.0 µm.

Material examined: AMH 9426, Mahabaleshwar, Maharashtra State, India 17^{0}58'N & 73^{0}43'E, on carnivorous animal excrement, Ajrekar Mycological Herbarium, coll. K.C. Rajeshkumar and S.V. Swami; axenic culture NFCCI 2356, 30.i.2011. GenBank: KT201347.

AMH 9426 (Image 2): Colonies persistent white to off white in nature, Conidiophores 300µm - 1.2cm. Vesicles larger, clavate, up to 50–63.5 µm wide, Conidiophores in single series, phialides ampulliform, apical pore broad, 15–21 × 4–5.5 µm. Conidia mostly ellipsoid, subglobose or variously shaped, smooth, hyaline, 5.5–9.7 × 3.5–5.7 µm.

Phylogenetic analyses: β-tubulin (KT201347): based on MegaBLAST search of NCBI GenBank nucleotide database, the closest hits using the BenA gene sequences are *Hemicarpenteles paradoxus* strain IBT 17513 (GenBank FJ530992.1, Identities = 373/373(100%), Gaps 0/373(0%), *Aspergillus paradoxus* isolate NRRL 2162 (GenBank EF669683.1, Identities = 373/373(100%), Gaps = 0/373(0%) and *Hemicarpenteles paradoxus* strain IBT 19365 (Genbank = FJ530993.1, Identities = 372/373(99%), Gaps = 0/373(0%). The analyses using *BenA* gene sequences revealed that the isolate NFCCI 2356 is closely related to *Hemicarpenteles*

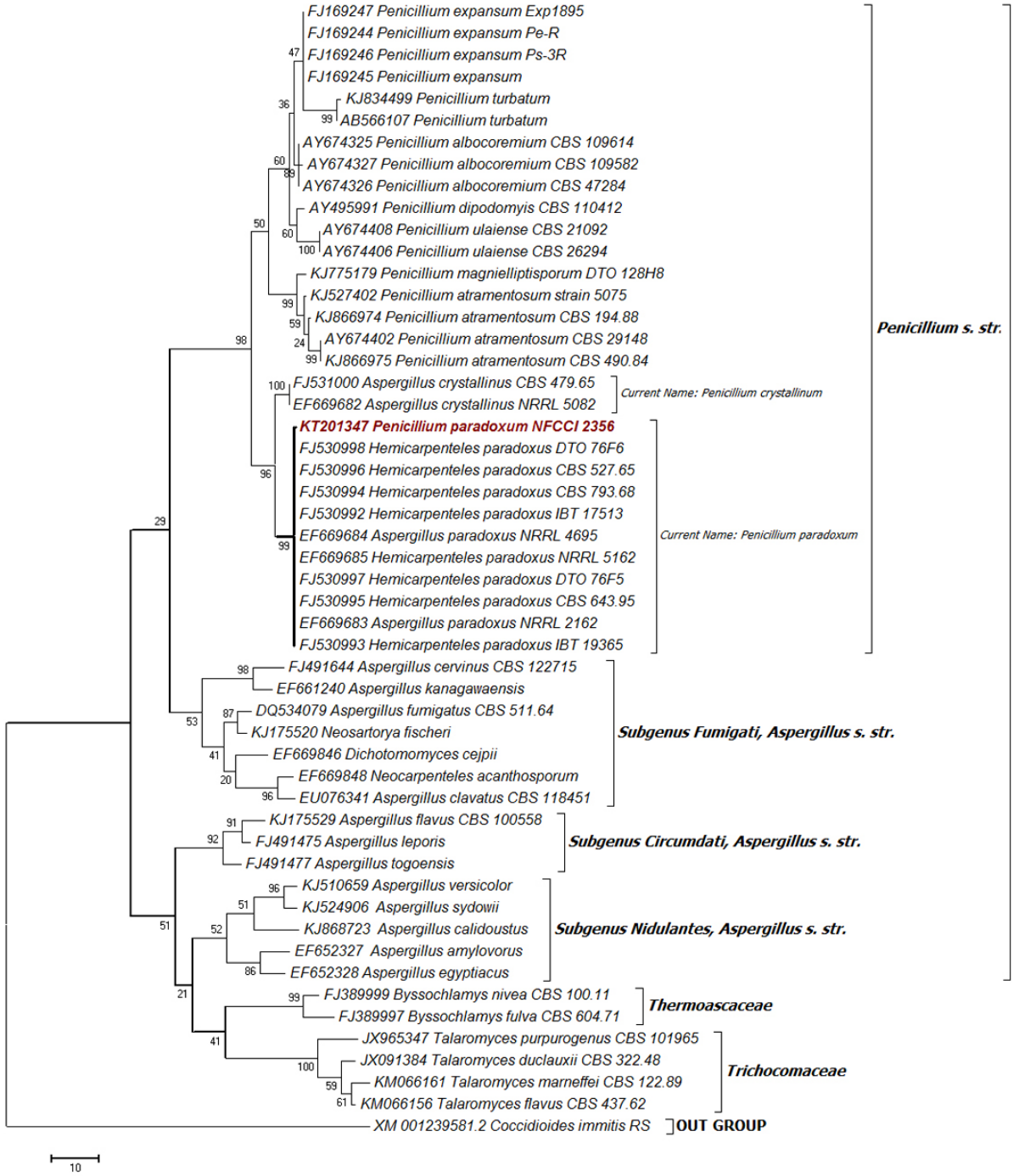

Figure 1. β-tubulin phylogenetic tree generated from Maximum Parsimony (MP) analyses of the aligned *BenA* gene sequences of *Penicillium paradoxum* (KT201347) based on the recent family concepts involving 52 sequences from the order *Eurotiales* including outgroups. Family placement of the sequences were referred to in Houbraken & Samson (2011) and Visagie et al. (2014).

paradoxus and *Aspergillus paradoxus* (current name *Penicillium paradoxum*) and phylogenetically formed a unique monophyletic lineage in *Penicillium* sensu stricto (Aspergillaceae) (Fig. 1).

Discussion

The taxonomic characterization and literature review revealed that *P. paradoxum* (as *A. paradoxus*) is only recorded from Lucknow (Rai et al. 1964) and has never been isolated and recorded so far from India. The present collection is also evident that *P. paradoxus*

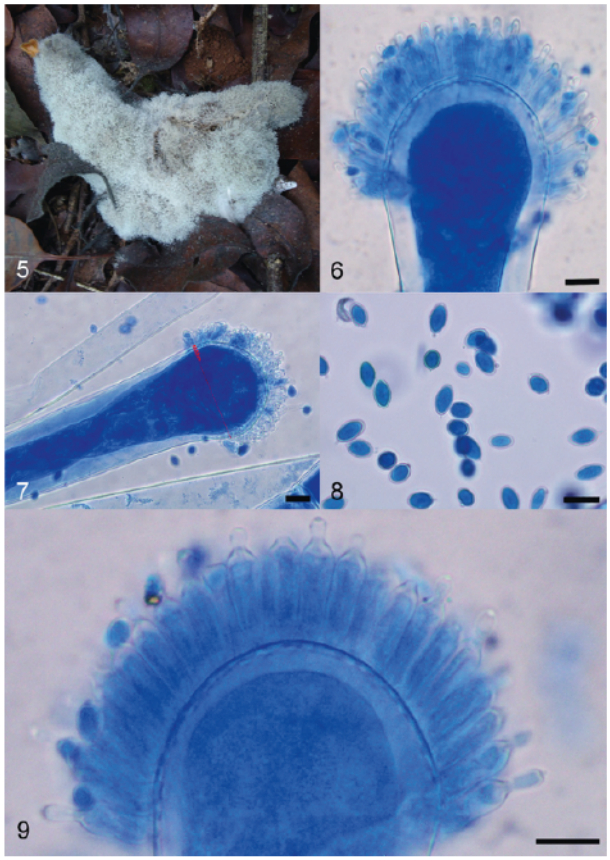

Image 2. *Penicillium paradoxum*, AMH 9426.
5 - Habitat; 6 - Vesicle; 7 - Conidiophore; 8 - Conidia; 9 - Phialides with conidia development. Scale = 10µm.

is associated with a unique microhabitat namely animal excrements as this species was found earlier in New Zealand (NRRL 2162) and England (IMI 117502). Peterson (2008) analysed the relationships of *Aspergillus* using a four gene loci (β-tubulin, calmodulin, ITS and LSU and RNA polymerase II (RPB2) sequences) stated that *H. paradoxus* is phylognetically associated with members of the *Eupenicillium* clade. Similarly, Varga et al. (2007), excluded *H. paradoxus* from the *Aspergillus* section *Clavati* and stated its affinity with *Penicillium* species. Houbraken & Samson (2011) revolutionized the concepts of *Penicillium* and *Aspergillus* taxonomy and segregated *Trichocomaceae* into three separate families Aspergillaceae, Thermoascaceae and Trichocomaceae. Their phylogenetic analysis also positioned the genus *Hemicarpenteles* under the *Penicillium* sensu stricto clade. Recently while redefining the genus concept of *Penicillium* to accommodate in the one-fungus-one-name concept (as per International Code of Nomenclature for algae, fungi and plants) Visagie et al. (2014) combined *A. paradoxus* with *Penicillium paradoxum* and positioned this species in the *Penicillium* section *Paradoxa*. The present taxonomic study and phylogenetic analysis from the Western Ghats, India also placed *P. paradoxum* under the *Penicillium* section *Paradoxa* in accordance with the current concepts of Aspergillaceae.

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