



Morphology and phylogeny of *Microdochium fisheri*, a new record from India

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Abstract

Identification, characterization and documentation of an interesting isolate exhibiting unique morphological characters on artificial nutrient media was isolated from rhizospheric soil of paddy. This isolate was characterized and identified based on morphological, and cultural characteristics. Phylogenetic analysis was conducted using internal transcribed spacer (ITS) region, and large subunit (LSU) - rDNA sequence data. The isolate was determined as *Microdochium fisheri* Hern.-Restr. & Crous, reported as an endophyte of stem of greenhouse-grown *Oryza sativa* plants in UK. This is the first report of *Microdochium fisheri* isolated as saprophyte from rhizospheric soil of paddy in India.

Key words – biodiversity – filamentous fungi – India – *Microdochiaceae* – Western Ghats

Introduction

The genus *Microdochium* was introduced with *Microdochium phragmitis* Syd. as the type species (Sydow 1924). For a long time, the morphology of this genus and its species was confused with allied genera including *Fusarium*, and its taxonomy was in a state of flux. However, the taxonomic relationships of *Microdochium* Syd., *Monographella* Petr. and *Idriella* Nelson & Wilh, were recently defined based on morphology and DNA sequence data, and a new family *Microdochiaceae* Hern.-Restr., Crous & Groenew (*Sordariomycetes*, *Xylariales*) was introduced to accommodate genera like *Microdochium*, *Idriella* and *Selenodriella* (Hernández-Restrepo et al. 2016). *Microdochium* s.str. belongs to a monophyletic clade. Most of its species are terrestrial causing diseases in economically important plants (Simpson et al. 2000, Hong et al. 2008, Matsumoto 2009, Rapacz et al. 2014, Xu & Nicholson 2009, Daamen et al. 1991, Al-Hashimi & Perry 1986, Zhang et al. 2015). Some species are non-pathogenic and may be endophytes (Ernst et al. 2011, Jaklitsch & Voglmayr 2012). A few species are antagonist to plant pathogenic *Verticillium dahliae* and inhibitory to hyphal growth of *Saprolegnia diclina*, which causes infection in fishes (Berg et al. 2005, Liu et al. 2016). Several isolates of *Microdochium* were isolated from aquatic (marine) environment from diseased as well as healthy salmon eggs, and identified as *M. lycopodium* and *M. phragmitis* (Liu et al. 2016). Recent reports on bioactive compounds from species of *Microdochium* opens new opportunity to further expand the scope of research on this

genus. An active compound, cyclosporine A, has been reported from an estuarine *M. nivale* isolate with potential to control diseases in human and animals (Bhosale et al. 2011). Cytotoxic activity against a human tumoral cell line has been reported from an extract of *M. phragmitis* isolated from Antarctic angiosperms (Santiago et al. 2012). Considering its biotechnological potential, systematic exploration of *Microdochium* and its species from natural substrates is required to explore their bioactive potential and conserve them for future research.

During an ongoing course of systematic study of exploration, identification, documentation and ex-situ conservation of *Fusarium* and its allied genera isolated from natural substrates in India, the present isolate was found. It produced characters like hyaline fusiform, obovoid, subpyriform to clavate, mostly one-septate, conidia, produced from denticulate conidiogenous cells on different culture media, which places this taxon in the genus *Microdochium*. The identity of the isolate was confirmed by sequencing and phylogenetic analysis of rDNA regions of internal transcribed spacer (ITS) and large sub unit (LSU).

Materials & Methods

Isolation, in vitro culturing and microscopic examination

About one gram of rhizospheric soil sample was collected from an experimental pot-cultivated paddy and serially diluted up to 10^{-7} . Soil suspension of about 0.5 ml was used as inoculum on two culture media, potato dextrose agar (PDA) and potato carrot agar (PCA). The plates were incubated at 25°C for 15 days. Colonies showing different morphological features were purified and transferred onto PDA slants for preservation and maintenance. Further cultural and morphological studies were undertaken on potato dextrose agar (PDA), potato carrot agar (PCA), malt extract agar (MEA), oat meal agar (OMA) and corn meal agar (CMA) at 25°C. Methuen handbook of colour was referred for recording colony colour on different media used in this study. Sporulating cultures were identified by morphology using standard literature. Microscopic details were observed using a Carl Zeiss Image Analyzer 2 (Germany) microscope with the specimens mounted in lactophenol-cotton blue and distilled water. Fungal structures were measured using the software Axiovision Rel 4.8. A pure culture is deposited in the National Fungal Culture Collection of India with accession no. NFCCI 4083 (NFCCI-WDCM 932) at MACS' Agharkar Research Institute, Pune, India.

Isolation of genomic DNA, PCR and sequencing

Genomic DNA was isolated from colonies grown on PDA plates for a week following a simple and rapid DNA extraction protocol (Aamir et al. 2015) using FastPrep®24 tissue homogenizer (MP Biomedicals GmbH, Germany). The amplification of internal transcribed spacer region 1, 5.8 ribosomal RNA gene and internal transcribed spacer region 2 was achieved using the primers ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3' and ITS 5: 5' GGA AGT AAA AGT CGT AAC AAG G 3' (White et al. 1990). Partial ribosomal nuclear large subunit (nucLSU) was amplified using primers LROR: 5' ACC CGC TGA ACT TAA GC and LR7: 5' TAC TAC CAC CAAGAT CT 3' (Vilgalys & Hester 1990) using Applied Biosystems ProFlex PCR System. PCR was performed in a 25 µl reaction using 2 µl template DNA (10–20 ng), 0.5 U Taq DNA polymerase (Genei, Bangalore, India), 2.5 µl 10X Taq DNA polymerase buffer, 0.5 µl 200 µM of each dNTP (Genei, Bangalore, India), 1 µl 10 pmol primer, sterile ultra pure water (Sigma) qsp 25 µl. The thermo-cycling conditions involved an initial denaturation at 94°C for 4 min, followed by 35 cycles of 1 min at 94°C, 30 sec at 50°C, 1 min at 72°C and final extension at 72°C for 8 min for ITS region where as in case of partial nuc LSU conditions involved 5 min denaturation step at 94°C, followed by 30 cycles of 1 min at 94°C, 50s at 52°C, and 1.2 min at 72°C with a final 7 min extension step at 72°C. The PCR products were purified with FavorPrep™ PCR Purification Kit. Purified PCR product of these marker genes was subjected to direct sequencing using BigDye® Terminator v3.1 Cycle sequencing Kit and ABI 3100 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

The sequences were analyzed using the gapped BLASTn search algorithm and aligned to the nearest neighbours. Sequences were submitted to GenBank under accession numbers KY777595 (ITS) and KY777594 (LSU) (<http://www.ncbi.nlm.nih.gov/Genbank>).

Table 1 List of *Microdochium* species reported from throughout the world

Sr. No.	Name of Taxon	Host	Place	References
1	<i>M. albescens</i>	<i>Oryza sativa</i>	Ivory Coast	Hernández Restrepo et al. 2016
2	<i>M. bolleyi</i>	<i>Gramineae</i>	North Dakota, U.S.A.	Hoog & Hermanides-Nijhof 1977
3	<i>M. caespitosum</i>	Dead leaves	Tanzania	Sutton et al. 1972
4	<i>M. citrinidiscum</i>	<i>Eichhornia crassipes</i> leaf	Peru	Hernández Restrepo et al. 2016
5	<i>M. colombiense</i>	<i>Musa sapientum</i>	Colombia	Hernández Restrepo et al. 2016
6	<i>M. consociatum</i>	-	San Jorge Prov., Ecuador	Hernández Restrepo et al. 2016
7	<i>M. cylindricum</i>	<i>Eucalyptus</i> dead leaves	Brazil	Sutton & Hodges 1976
8	<i>M. dimerum</i>	<i>Citrus medica</i>	Reggio in Calabria	Arx 1984
9	<i>M. falcatum</i>	<i>Eucalyptus tereticornis</i> dead leaves	Brazil	Sutton & Hodges 1976
10	<i>M. fisheri</i>	<i>Oryza sativa</i> stem	U.K.	Hernández Restrepo et al. 2016
11	<i>M. fusariisporum</i>	Dead straw	Kansas, U.S.A.	Hernández Restrepo et al. 2016
12	<i>M. fusarioides</i>	<i>Phytophthora syringae</i> oospores	U.K.	Harris 1985
13	<i>M. gracile</i>	Rabbit dung	Netherland	Mouchacca & Samson 1973
14	<i>M. griseum</i>	<i>Sapium ellipticum</i> dead leaves	Tanzania	Sutton et al. 1972
15	<i>M. intermedium</i>	Soil	Papua New Guinea	Hoog & Hermanides-Nijhof 1977
16	<i>M. linariae</i>	Stem	Patavino, Italy	Savulescu 1942
17	<i>M. lycopodium</i>	<i>Lycopodium annotinum</i>	Austria	Hernández Restrepo et al. 2016
18	<i>M. majus</i>			Glynn et al. 2005
19	<i>M. maydis</i>	<i>Zea mays</i> leaves	Mexico	Hernández Restrepo et al. 2016
20	<i>M. neoqueenslandicum</i>	<i>Agrostis</i> sp.	Waihi, New Zealand	Hernández Restrepo et al. 2016
21	<i>M. nivale</i>	-	-	Samuels & Hallett 1983
22	<i>M. opuntiae</i>	<i>Oputia</i> dead leaves	Louisiana, Langlois	Hernández Restrepo et al. 2016
23	<i>M. oryzae</i>	<i>Oryzae sativa</i>	Japan	Samuels & Hallett 1983
24	<i>M. palmicola</i>	<i>Roystonea regia</i> dead petiole	Cuba	Holubová-Jechová & Mercado Sierra 1982
25	<i>M. panattonianum</i>	<i>Lactuca sativa</i> leaves	Denmark	Galea et al. 1986

Table 1 continued

Sr. No.	Name of Taxon	Host	Place	References
26	<i>M. paspali</i>	<i>Paspalum vaginatum</i>	Hainan, China	Zang et al. 2005
27	<i>M. passiflorae</i>	<i>Passiflora edulis</i> dead stem	New Zealand	Samuels et al. 1987
28	<i>M. phragmitis</i>	<i>Phragmites communis</i> leaves	Lichtenrade Berlin	Sydow 1924
29	<i>M. phyllanthi</i>	<i>Phyllanthus discoideus</i> leaves	Sierra Leone, West Africa	Sutton et al. 1972
30	<i>M. punctum</i>	<i>Sisyrinchii campestris</i> stem	U.S.A.	Braun 1993
31	<i>M. queenslandicum</i>	-	Queensland	Matsushima 1989
32	<i>M. sclerotiorum</i>	Culture contaminant	Netherlands	Mouchacca & Samson 1973
33	<i>M. seminicola</i>	Barley	Canada	Hernández Restrepo et al. 2016
34	<i>M. sorghi</i>	<i>Sorghum vulgare</i> leaves	Louisiana, U.S.A.	Braun 1995
35	<i>M. stevensonii</i>	<i>Panicum hemitomom</i>	Florida, U.S.A.	Hernández Restrepo et al. 2016
36	<i>M. stoveri</i>	<i>Musa</i> sp.	Honduras, Central America	Samuels & Hallett 1983
37	<i>M. tabacinum</i>	<i>Nicotiana</i> sp. stem	Netherlands	Arx 1984
38	<i>M. tainanense</i>	<i>Saccharum officinarum</i> roots	Japan	Hoog & Hermanides- Nijhof 1977
39	<i>M. trichocladiopsis</i>	<i>Triticum aestivum</i> rhizosphere	Unknown Country	Hernández Restrepo et al. 2016
40	<i>M. tripsaci</i>	<i>Tripsacum laxum</i> leaves	Sri Lanka	Arx 1981
41	<i>M. triticicola</i>	<i>Triticum aestivum</i> roots	U.K.	Kwasna & Bateman 2007

Results

Phylogenetic analyses

The sequences of the ITS and LSU- rDNA of the isolate were subjected to BLASTn sequence homology searches and phylogenetically related species were chosen for construction of the phylogenetic trees (separately for ITS and LSU-rDNA), which included different species of *Microdochium*, *Castanediella*, *Selenodriella*, *Idriellopsis*, *Neoidriella* and *Paraidriella*. Multiple sequence alignments were performed using CLUSTAL W (<http://www.ebi.ac.uk/clustalw/>) and the phylogenetic analysis were performed by using the maximum likelihood (ML) method based on the Kimura 2-parameter model in MEGA 6.0 (Kimura 1980, Tamura et al. 2013). Optimal ML trees were found by a nearest neighbour interchanges (NNI) search, starting with a tree topology generated by the BIONJ method (Gascuel 1997) using maximum composite likelihood (MCL) distances (Tamura et al. 2004). One thousand bootstrap replicates were analyzed to obtain nodal support values.

Microdochium fisheri Hern.-Restr. & Crous, Persoonia 36: 68, 2016

Figs 1–3

On PDA colonies reached 39.2 (\pm 3.88) mm at 25°C after 5 days of incubation. Colonies were flat, margin entire, slightly raised to umbonate centre, pinkish white (7A2) with reverse greyish orange (5B4) (Fig. 1 A–D). On PCA colonies reached 41.42 (\pm 2.79) mm at 25°C after 5 days of

incubation. Colonies were flat, margin entire, floccose, and umbonate at centre, pale red (7A3) with reverse initially greyish orange (6B5), later turning orange-white (5A2) (Fig. 1 B–E).

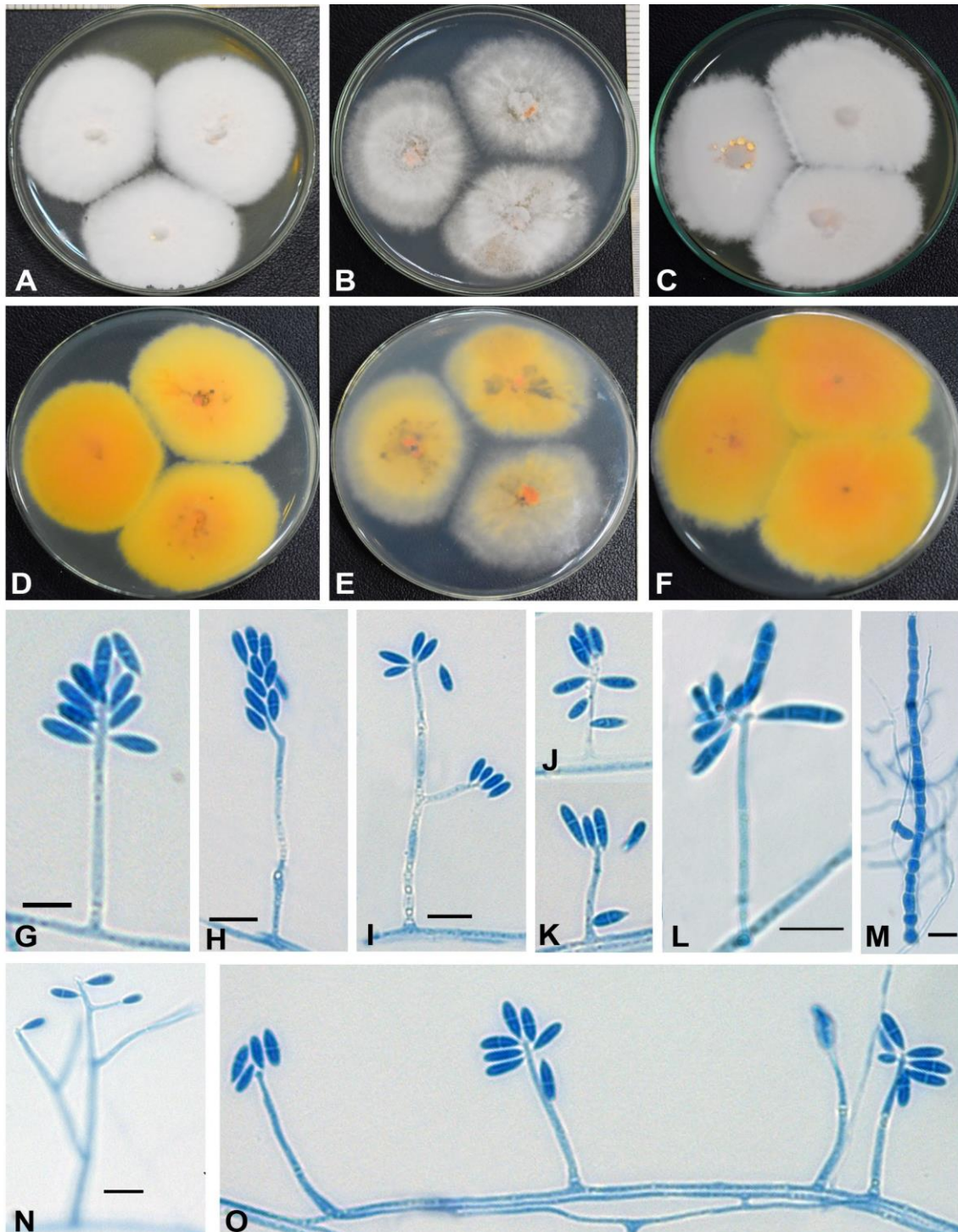


Fig 1 – Morphological characteristics of *Microdochium fisheri* (NFCCI 4083). A, D Front and reverse view of colonies on potato dextrose agar. B, E Front and reverse view of colonies on potato carrot agar. C, F Front and reverse view of colonies on malt extract agar. G, H Simple conidiophores bearing 0–1 septate hyaline conidia. I, Branched conidiophore bearing hyaline conidia. J, K Simple conidiophores bearing conidia produced from denticulate conidiogenous cells. L, Rare 2-septate conidia produced from denticulate conidiogenous cell. M, Chlamydospores in long chain. N Branched conidiophore bearing short to long denticulate conidiogenous cells. O Mycelia giving rise to simple conidiophores producing conidia attached to conidiogenous cells.

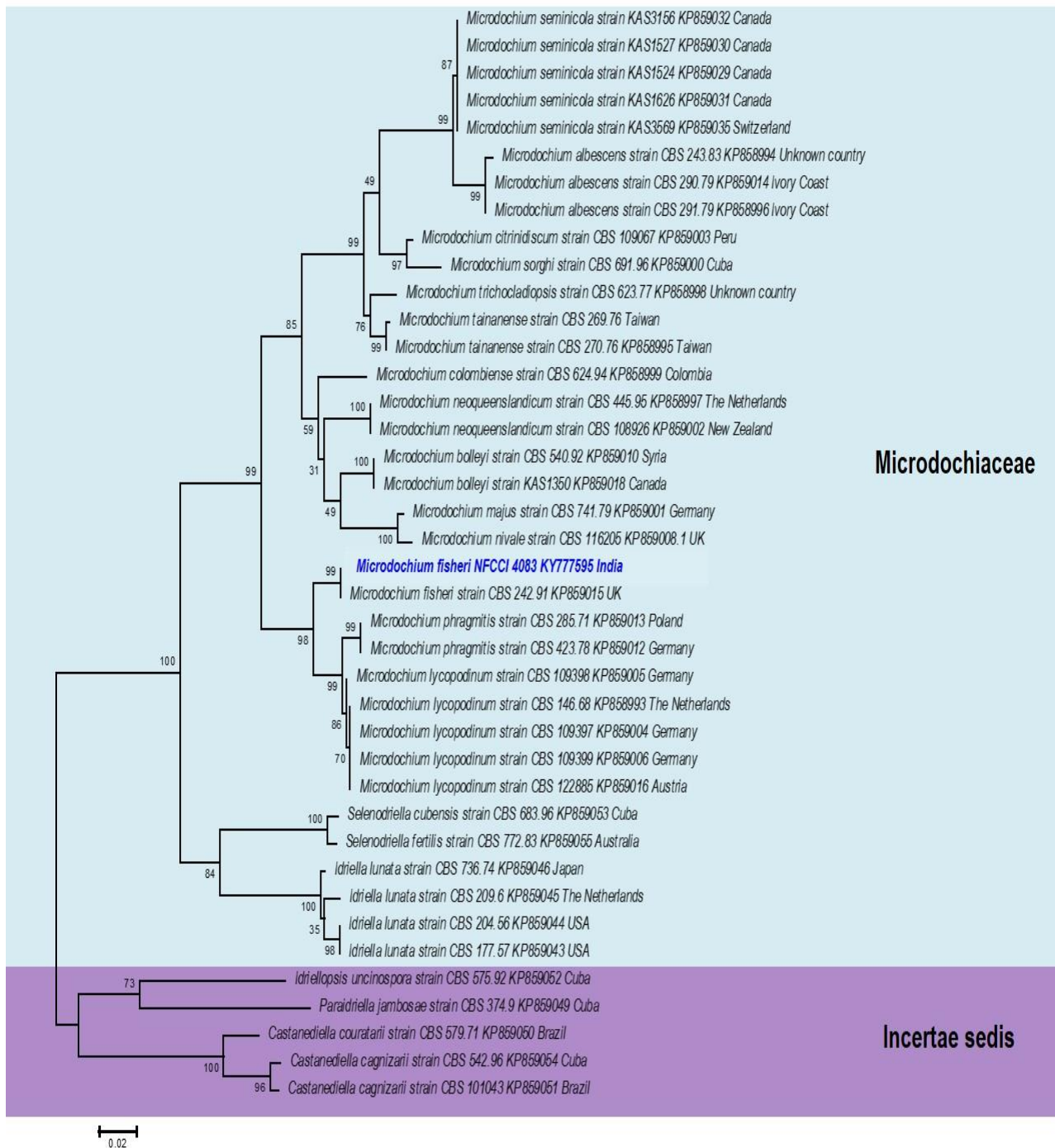


Fig 2 – Phylogram generated from ITS-rDNA sequences. The tree with the highest log likelihood (-3229.8838) is shown. *Microdochium fisheri* from India (GenBank accession number KY777595) is shown in blue. The tree was rooted to *Castanediella couratarii* strain CBS 579.71, *Castanediella cagnizarii* strain CBS 542.96 and *Castanediella cagnizarii* strain CBS 101043.

On MEA colonies reached 39.12 (\pm 3.24) mm at 25°C after 5 days of incubation. Colonies were flat, margin entire, floccose, and umbonate at centre, pale red (7A3) with reverse light orange (6A4) (Fig. 1 C–F). Mycelium was superficial and immersed. Hyphae smooth-walled, septate, branched, hyaline.

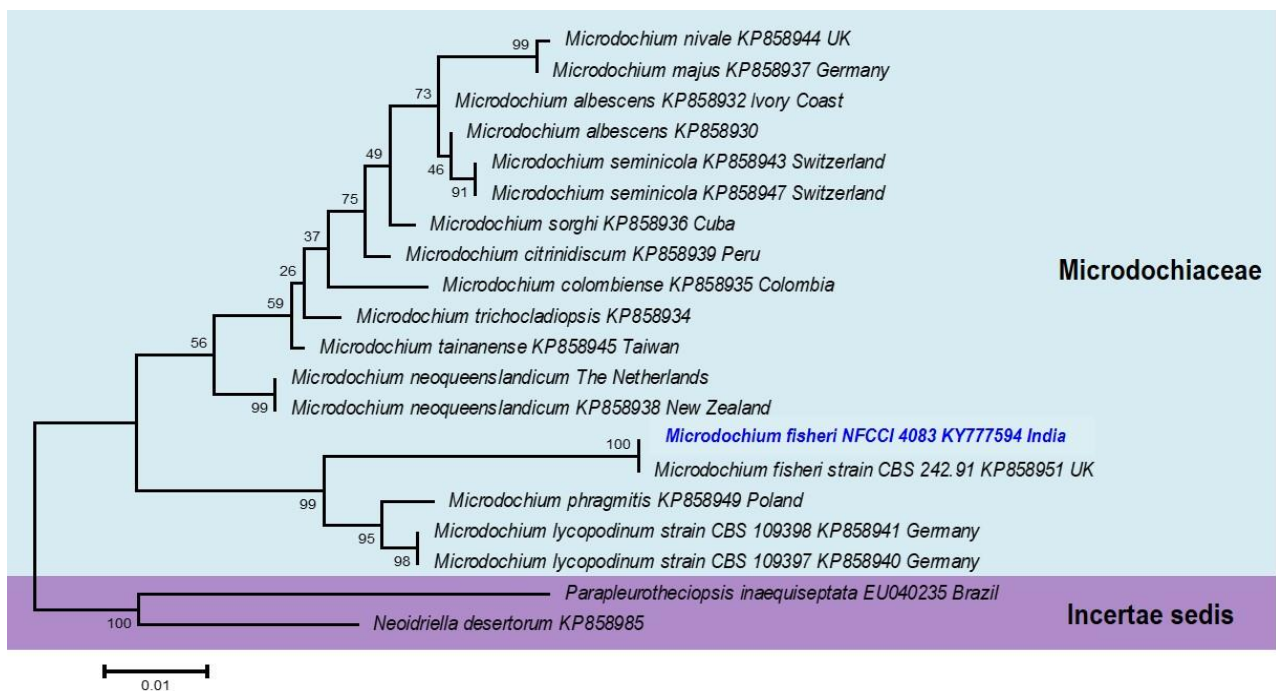


Fig 3 – Phylogram generated from LSU-rDNA sequences. The tree with the highest log likelihood (-2228.0020) is shown. *Microdochium fisheri* from India (GenBank accession number KY777594) is shown in blue. The tree was rooted with *Parapleurotheciopsis inaequiseptata* and *Neoidriella desertorum*.

Conidiophores micronematous, arising as lateral branches from superficial mycelium, smooth-walled, simple to branched, hyaline $12.5\text{--}90 \times 1.4\text{--}3 \mu\text{m}$ (mean = $36.3 \times 2.0 \mu\text{m}$, $n = 30$). Conidiogenous cells terminal to intercalary, cylindrical to denticulate, tapering towards apex, with great variation in length. Conidia solitary, simple, smooth-walled, 1-septate (rarely 2-septate), fusiform, obovoid, subpyriform to clavate, hyaline, $4.8\text{--}12 \times 1.6\text{--}3.6 \mu\text{m}$ (mean = $7.5 \times 2.6 \mu\text{m}$, $n = 30$), apex rounded, base tapering towards a subtruncate and unthickened hilum. Conidia sometimes form a floret appearance on conidiogenous cells. Chlamydospores one-celled, in chains.

Known distribution – India, UK.

Material examined – India, Maharashtra, Pune, on rhizospheric paddy soil, Nov. 2016, S.K. Singh, NFCCI 4083; in-vitro culture deposited in National Fungal Culture Collection of India (NFCCI-WDCM 932).

Notes – The isolate grew well over a wide range of artificial nutrient media (PDA, PCA, MEA, OA, CDA) with variations in colony colours and morphology. As per Index of Fungi, the genus now contains about 41 species (Table 1).

Discussion

ITS-based phylogenetic analysis conducted with 40 nucleotide sequences shows that the sequence derived from the Indian fungus (NFCCI 4083, GenBank number KY777595) clearly clustered with *Microdochium fisheri* CBS 242.91 in a strongly supported clade (99% bootstrap). Our second LSU-based phylogenetic analysis also showed the Indian isolate to clearly cluster with *M. fisheri* in a strongly statistically supported clade (100% bootstrap). The phylogenetic analysis confirms the identity of this Indian isolate as *M. fisheri*. This study also reflects the wider existence of *M. fisheri*, which is not restricted to an endophytic mode of life, but it can survive as a saprophyte/epiphyte in nature. There were only minor morphological differences on PDA between the UK *M. fisheri* (CBS 242.91), reported as an endophyte of stem of greenhouse-grown plants of *Oryza sativa* in UK, and the Indian isolate. The conidia of the Indian isolate were mostly 1, rarely 2–3 septate compared to 0–1 septate for the UK isolate. In addition, the Indian isolate formed

intercalary chlamydospores, a feature not seen in the UK isolate. Thus, based on overall morphological similarity, molecular analysis and phylogeny, the Indian isolate is treated as *Microdochium fisheri* and is reported as a saprophyte for the first time from India.

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