



## Aquatic Mycobiota of Sanjay Gandhi National Park, Maharashtra, India: Taxonomy & Ecology

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**Abstract:** The present paper is concerned with description of taxonomic diversity and study of aspects of ecology of water-borne microfungi biota of Sanjay Gandhi National Park (SGNP). The study area is home to freshwater streams, lakes and a saline creek. The study resulted in 28 isolates of water fungi obtained from 20 water samples. After morpho-molecular analysis, a total of 22 species were documented under 5 genera. *Aspergillus* was the dominant genus with 12 species, whereas *Aspergillus flavus* and *A. terreus* were dominant species, each represented by 3 isolates. Gini-Simpson's index was 0.9439, Shannon's index was 2.9978. Pielou's evenness index was 0.9698, causing observed species richness (22) to be greater than true diversity, calculated as the effective number of species (20).

**Key words:** *Ascomycota*, Diversity indices, Jaccard's dissimilarity index, Maharashtra – water-borne fungi, *Mucoromycota*, true diversity.

### INTRODUCTION

Sanjay Gandhi National Park (SGNP), located in Mumbai Metropolitan Region, is a respite in the fast-paced financial capital city of India. It covers an area of 103.09 km<sup>2</sup> spread over 3 districts viz., Mumbai Suburbs (to the west and south), Palghar (to the north) and Thane (to the east). Sanjay Gandhi National Park consists of three forest ranges: Tulsi, Krishangiri, and Yeoor. Vasai (Bassein) creek flows through the Yeoor Range, dividing SGNP into two unequal halves. The chief sources of freshwater in the national park consist of lakes (rain-fed) of Vihar and Tulsi, Dahisar river, rivulets, and springs on hill slopes. The first two (lakes of Vihar and Tulsi) are two of the sources of water

supply to Mumbai. Dahisar river has its origin in Tulsi Lake, flows through Magathana, joins Manori Creek before draining into the Arabian Sea. Rewat and Laxmi rivers are the other two rivers of the national park. Many streams of the Yeoor range culminate into Chena Lake. In abandoned quarries of peripheral areas, as well as some places in forests north of Bassein Creek, many water reservoirs have developed over the years. A varied ecosystem exists in the National Park which motivated the scientists of BSI to undertake research on the microfungi diversity of the National Park.

In India, 362 species of freshwater mitosporic fungi (Patil & Borse 2015) and 207 species of marine fungi have been reported (Borse et al. 2013). From Maharashtra, 78 freshwater mitosporic fungi (Nemade et al. 2016) and 93 species of marine fungi (Borse et al. 2013) are reported. Gosavi & Borse (2017 & 2018) reported ten species of marine fungi from the coast of the Thane District of Maharashtra. Borse et al. (2016) present the current state of knowledge about freshwater aquatic fungi from different states of India.

Some of the notable studies which include ecological aspects of aquatic fungi include works on the diversity of aquatic fungi from Kali River of state of Karnataka, India (Sridhar & Sudheep 2011, Sudheep & Sridhar 2012, 2013a, b). Over the years publications have appeared on different aspects of the microfungi biota of SGNP, viz., keratinophilic fungi from selected soils of SGNP were documented by Deshmukh & Verekar (2014); diversity of fungi of the areas with anthropogenic activities in some of the zones of greenery in Mumbai Metropolitan Region, which included some areas of SGNP, was studied by Sharda et al. (2015).

Distribution and diversity of Arbuscular Mycorrhizal Fungi (AMF) of five medicinal trees from Thane region of SGNP were reported by Chahar & Belose (2018); Dubey & Pandey (2017 & 2019) detailed foliicolous fungi of SGNP in their works foliicolous fungi of Maharashtra.

Therefore, there was a need for a detailed study of the water-borne microfungi biota of such a diverse region, for which field surveys were undertaken from 2016 to 2020 for four years, during which 3 forest ranges (Krishangiri, Tulsi and Yeoor) and 10% surrounding area outside of the National Park were intensively explored.

## MATERIAL AND METHODS

In the period 2016–2020, six collection tours were carried out to SGNP and 10% of surrounding areas. All major areas of SGNP, including buffer and core zones, were visited in different seasons: monsoon, post-monsoon, winter, and summer, to study the biodiversity of water-borne microfungi biota of the National Park. Coordinates of collection locations were also recorded. QGIS 2.8 Wien version was used for plotting GPS data to prepare a survey map showing collection sites visited during the field tours. The survey map, alongside the range map of SGNP (source: forest authorities, Government of Maharashtra), is shown in Fig. 1. Fig. 2 provides a glimpse of the study area. The field tours resulted in a collection of a total of 20 water samples from the study area. Water samples were collected in sterilized bottles and brought to the laboratory for further processing. The isolation of water-borne fungi was done by serial dilution method (Warcup 1950) using Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Potato Carrot Agar (PCA). The slides of fungi were prepared under aseptic conditions in lactophenol-Cotton blue solution.

The slides were observed under Olympus compound microscope model CX-41 attached DP22 and DP27 camera, the fungal structures were measured and finally, photographs were captured. The fungal isolates were identified based on morphological characteristics, for which Barlocher (1992), Coker (1923), Goh et al. (2003), Ingold (1975), Khuble (2001), Nizamydeen (2014) were consulted. HiPurA Fungal DNA Purification Kit (HiMedia, India) was used for extraction of Genomic DNA from the growing mycelia as per the manufacturer's instructions. PCR amplification was conducted (using SimpliAmp Thermal cycler, Applied Biosystems, USA) with Primer pair ITS4 and ITS5 to amplify the 5.8S rRNA gene and flanking internal transcribed spacer regions (ITS) (White et al. 1990).

The amplified PCR products were purified with HiPurA PCR Product Purification Kit (HiMedia, India) as per manufacturer's instructions. The purified PCR products were rechecked using agarose gel electrophoresis and were submitted for sequencing to Avanira Biotech Pvt. Limited, Pune, India. Based on a

MegaBLAST search on the NCBI GenBank nucleotide database, the Phylogenetic trees were prepared using Mega7 (Kumar et al. 2016). All the cultures are deposited at the herbarium of BSI, WRC, Pune (BSI). The isolates were assigned to respective genera and species using the aforementioned approaches based on morphology, SEM, and molecular phylogeny.

## Statistical Methodology

To analyse aspects of microfungi ecology, we employed the analytical framework consistent with, for instance, Dubey & Pandey (2022), where it is explained in much greater detail.

We calculate the following two diversity indices. In both, higher values signify higher diversity.

$$\begin{aligned} \text{Gini - Simpson's index} &= 1 - D = 1 - \sum_{i=1}^S p_i^2 \\ &= 1 - \sum_{i=1}^S (n_i / N)^2 \\ \text{Shannon's index (H)} &= \sum_{i=1}^S p_i \ln(1 / p_i) \end{aligned}$$

Where  $p_i$  is the proportion of  $i^{\text{th}}$  species,  $n_i$  = number of isolates of  $i^{\text{th}}$  species,  $N$  = total number of isolates of all species,  $\ln$  = natural logarithm,  $S$  = number of distinct species.

Pielou's evenness index  $J$  (Pielou 1995), which is essentially a normalized Shannon's index, measures the equitability of species distribution. It is given by:

$$\text{Pielou's evenness index (J)} = \frac{H}{\ln(S)}$$

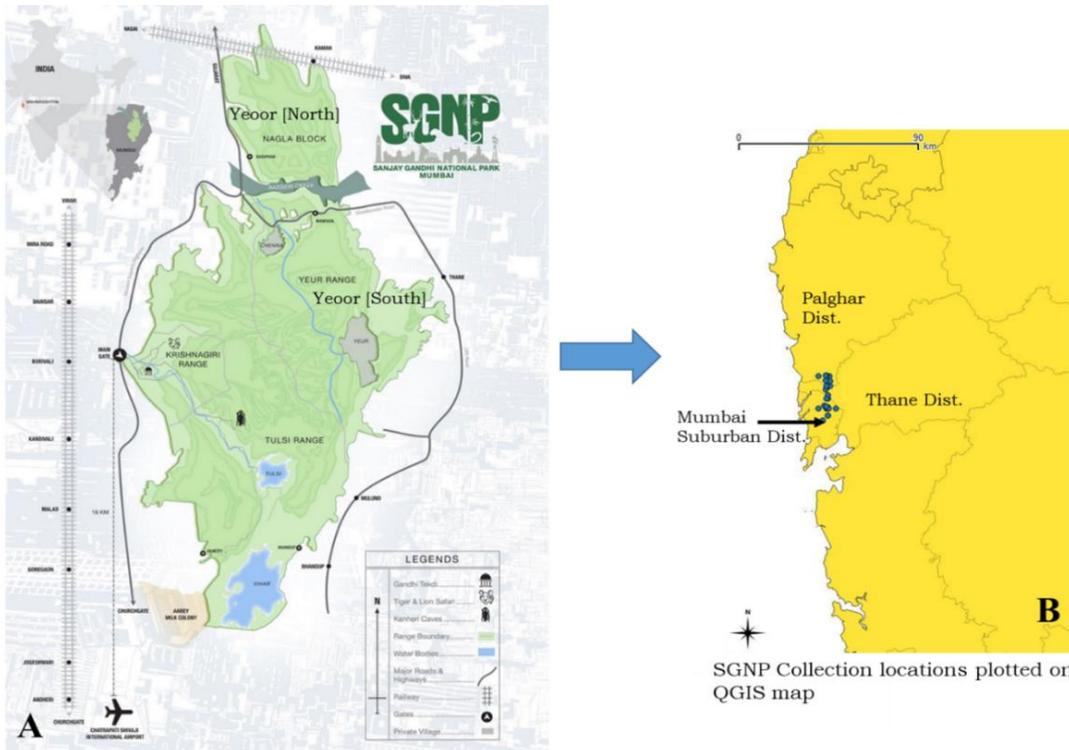
Next, we examine true diversity by calculating effective number of species obtained by transforming Shannon's index as follows (Jost 2006):

$$\text{ENS}_H = e^H$$

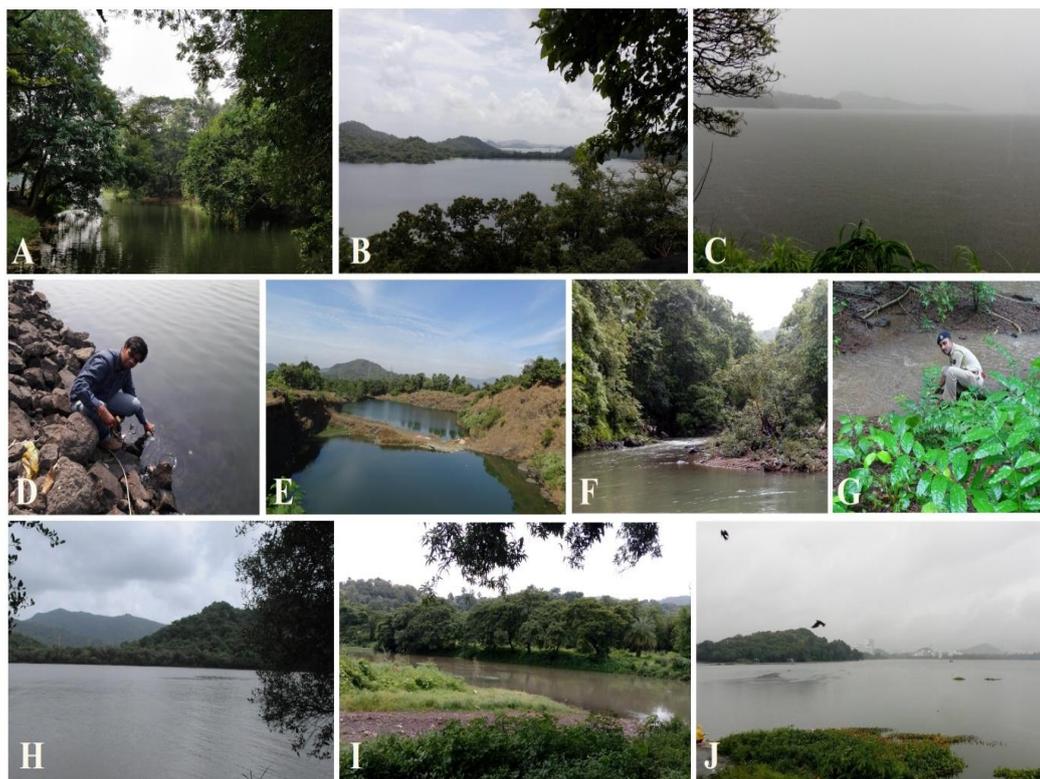
The above transformation has two-fold advantages. First, when comparing two communities, non-linearity of diversity indices blurs the difference between the two (Jost 2006). As an illustrative hypothetical example from Dubey & Pandey (2022) shows, a 25% difference in Shannon's Index between two locations ( $H_1 = 4$ ,  $H_2 = 5$ ) leads to 174.07% difference in true diversity, calculated as above. Secondly, the above transformation deflates species richness by incorporating abundance.

The choice of Shannon’s index for the calculation of effective number of species is due to the fact that being the first-order Hill Number it weighs both common and rare species equally, unlike Simpson’s effective number of species (second-order Hill Number, based on Simpson’s index) and species

richness (zeroth order Hill Number), which overweigh common species and rare species, respectively (Gotelli & Ellison 2004).



**Fig. 1.** Map of Sanjay Gandhi National Park (SGNP) showing collection locations. A. Map of SGNP ranges provided by forest authorities, Government of Maharashtra; B. Survey map prepared by plotting GPS of collection locations using QGIS 2.8 Wien version.



**Fig. 2.** Overview of Sanjay Gandhi National Park. A. Water body in Krishnagiri Range; B. Tulsi Lake, Tulsi Range; C. Vihar Lake, Tulsi Range, D. Water collection at Vihar Lake; E. Sarjamori, North of Vasai Creek, Yeoor Range; F-G. Water streams in Yeoor Range, South of Vasai Creek; H. Vasai Creek; I. Chena Lake; J. Powai Lake.

## RESULTS

A total of 22 species (28 isolates), under 5 genera, of water-borne fungi, were identified from 20 water samples, as detailed in Table 1, along with associated taxonomic and collection details. Morphological characters of the recorded water-borne fungal species are given below:

**1. *Aspergillus brasiliensis*** Varga, Frisvad & Samson, *International Journal of Systematic and Evolutionary Microbiology* 57 (2007) Fig 3A

Colonies on PCA (Potato Carrot Agar) initially white after then dark brown to black at  $28 \pm 1^\circ\text{C}$ . Exudates absent, cream coloured to light brown in reverse. Conidial heads globose initially and later radiate developing into several conidial columns; stipes  $720\text{--}1700 \times 9\text{--}13 \mu\text{m}$ , walls thick, pale brown smooth, vesicles  $32\text{--}45 \mu\text{m}$  wide, globose, biseriate, metulae covering virtually the entire surface of the vesicle, measuring  $21\text{--}30 \times 4\text{--}6 \mu\text{m}$ ; flask-shaped

phialides,  $7\text{--}9 \times 3\text{--}4 \mu\text{m}$ ; conidia subglobose,  $3.6\text{--}5.0 \mu\text{m}$  diam, echinulate.

Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, India, date 20 August 2017, RD, 205507 BSI (WC), Accession no. BSI-F876.

**2. *Aspergillus flavus*** Link, *Magazin der Gesellschaft Naturforschenden Freunde Berlin* 3(1): 16 (1809) Fig. 3B

Colonies fast growing on PDA, heavily sporulating, conidial head becoming brownish in age, reverse uncoloured to pink; Conidiophores generally less than  $1 \mu\text{m}$  in length,  $11\text{--}20 \mu\text{m}$  diam., colourless heavy walled, coarsely roughened; Vesicle globose to subglobose,  $25\text{--}43 \mu\text{m}$  in diam. sterigmata uni and biseriate. Conidia globose to subglobose; Sclerotia white at first becoming red brown or black.

Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, India, date 08

September 2016, RD, 205284 BSI (WC), Accession no. BSI-F877; Isolated from water of Vasai Creek, Peripheral to SGNP, Thane, Maharashtra, India, date 21 August 2017, RD, 205515 BSI (WC), Accession no. BSI-F839; Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, India, date 26 January 2017, RD, 205519 BSI (WC), Accession no. BSI-F878.

**3. *Aspergillus fumigatus*** Fresen., *Beiträge zur Mykologie* 3: 81 (1863) Fig. 3C

Colonies fast growing on PDA at 28± 1°C., velvety - floccose, mycelium white, conidial heads long compact columns, reverse yellow - purple, castor gray; Conidiophores short, smooth. Vesicles flask shaped or hemispherical, 21 - 29 µm in diam., fertile over upper half. Sterigmata uniseriate, 6-7 × 3-3 µm; Conidia subglobose, 2.4 -3.0 µm diam.

Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, India, date 08 September 2016, RD, 205384A BSI (WC), Accession no. BSI-F888.

**4. *Aspergillus insuetus*** (Bainier) Thom & Church, *Manual of the Aspergilli* (Maryland): 153 (1929)

Colonies on PCA slow growing at 28± 1°C., orange yellow, slightly floccose or velvety, exudates clear to wine red, reverse yellow purple red. Conidial heads radiate, hemispherical; Conidiophores upto 500 (-700) × 4.7 µm, widening near vesicle; vesicle 12 - 16 µm, fertile areas hemispherical; sterigmata biseriate, primaries 5.2-8.0 × 3.0 µm; secondaries 5.5 -7 × 2.1-2.5 µm, Conidia globose, delicately echinulate, 2.3.3 µm, in radiating chains.

Based on ITS phylogenetic identification, the isolate SGNP SM21 was identified as *A. insuetus*

Isolated from water of lake in Krishnagiri Range, SGNP, Mumbai, Maharashtra, India, date 23 December 2017, RD, 205389A BSI (WC), Accession no. BSI-F879, culture no. SGNP SM21, NCBI Accession no- ON668309.1.

**5. *Aspergillus nidulans*** (Eidam) G. Winter, *Rabenhorst's Kryptogamen-Flora, Pilze Ascomycetes* 1(2): 62 (1884) Fig. 3D

Colonies honey yellow or dark green on PDA at 28± 1°C., growing moderately, exudate lacking. Conidial head are short columnar. Conidiophore brown 65-129 × 2.4 - 3.0 µm; vesicle hemispherical, 8-9 µm in diameter; sterigmata biseriate, primaries 5.1-6 × 2-2.5 µm; conidia, globose, 3-3.5 µm, ascospores purple red smooth walled, lenticular with two equatorial crests; Cleistothecia purple, 125-150 µm, surrounded by a mantle of hulle cells up to 24.5 µm diam.

Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, 08 September 2016, RD, 205284A BSI (WC).

**6. *Aspergillus niger*** Teigh, *Annales des Sciences Naturelles Botanique* 8: 240 (1867) Fig. 3<sup>E</sup>

Colonies on PCA, PDA fast growing at 28± 1°C, mycelium white to yellow, heavily sporulating in deep brownish black colour. Conidial head, globose to radiate, in well-defined columns. Conidiophores walls thick, smooth, colourless to brownish; vesicle globose, 45-73 µm diam. or smaller, fertile all over; sterigmata biseriate, primaries some time septate; Conidia globose, 4.0-4.9 µm, walls heavy, brown, irregularly roughened, ridged or echinulate.

Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, 08 September 2016, RD, 205284B, BSI (WC); Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, 08 September 2016, RD, 205281 BSI (WC).

**7. *Aspergillus ochraceus*** G. Wilh., *Inaugural Dissertation* (Strassburg): 66 (1877) Fig. 3F

Colonies on PCA fast growing, zonate, conidial heads abundant, ochraceous buff, globose, conidia adhering into 2-3 divergent compact columns, brownish to purplish; Conidiophores light brown with coarsely roughened walls; Vesicle globose, 35-50 µm diam.; sterigmata biseriate; primaries 16-20 × 5-6 µm; secondaries 8-11 × 2-3 µm; conidia globose to subglobose, 2.7-3.5 µm diam., smooth to delicately roughened. sclerotia globose, white to pale pink when young to vinaceous purple at maturity.

Based on ITS phylogenetic identification, the isolate SM 30 was identified as *Aspergillus ochraceus*.

Isolated from water of Tulsi Lake, Tulsi Range, SGNP, Mumbai Maharashtra, 19 October 2018, RD, 205536 B BSI (WC), SM 30.

**8. *Aspergillus parasiticus*** Speare, *Report Exp. Stat. Hawaiian Sugar Planters' Assoc., Path. & Phys. Bull* 12: 38 (1912) Fig. 3G

Colonies sparse moderately growing on PCA at 28± 1°C, compact basal felt bearing conidial heads, bright yellow to dull green, loosely radiate, reverse cream; Conidiophores walls smooth or roughened; Vesicle subglobose to flask shaped, 20-35 µm in diam; Sterigmata uniseriate, 7-9 × 3-4 µm, closely packed over entire surface; Conidia globose, coarsely echinulate, 3.0-5.5 µm diam., bright yellow green. No sclerotia or cleistothecia reported.

Isolated from water of Powai Lake, peripheral to Sanjay Gandhi National Park, Mumbai, Maharashtra, 20 August 2017, RD, 205522C BSI (WC).

**9. *Aspergillus pseudoelegans*** Frisvad & Samson, in Frisvad, Frank, Houbraken, Kuijpers & Samson, *Stud. Mycol.* 50(1): 35 (2004)

Colonies growing on PDA at 28± 1°C, honey yellow or dark green, growing moderately. Vesicle spherical, 35 µm in diam. Conidiophores long, wall smooth,

thick, colourless,  $520\text{--}700 \times 5.4\text{--}7.6 \mu\text{m}$ . Conidia brown to purple, globose,  $2.4\text{--}2.7 \mu\text{m}$ .

Based on ITS phylogenetic identification, the isolate SM22 SGNP was identified as *Aspergillus pseudoelegans*. NCBI Acession no: ON668321.1

Isolated from Water of Vasai Creek, Peripheral to SGNP, Thane, Maharashtra, 21 August 2017, RD, 205515B BSI (WC).

**10. *Aspergillus sydowii*** (Bainier & Sartory) Thom & Church, *The Aspergilli* (1926)

Colonies blue–green with reddish–brown shades on PDA and PCA at  $28 \pm 1^\circ\text{C}$ . The conidial heads are spread out, while the stalks of the conidiophores are hyaline, smooth and measure up to  $500 \mu\text{m}$ ; Vesicle are spherical; Conidiogenous cells are biseriolate; conidia are echinulate, roughly spherical and measure  $2.4\text{--}4.0 \mu\text{m}$  in diam.

Isolated from water of Ghodbandar, Thane, Maharashtra, 10 September 2016, RD, 205516 BSI (WC).

**11. *Aspergillus terreus* Thom**, *American Journal of Botany* 5 (2): 85 (1918) Fig. 3H

Colonies moderately growing, reverse dull yellow to brown on PCA at  $28 \pm 1^\circ\text{C}$ . Conidial heads brown and in long compact columns, Conidiophores  $100\text{--}250 \mu\text{m}$  in length. sterigmata biseriolate, primaries parallel, crowded,  $5\text{--}7 \mu\text{m}$ , secondaries closely packed,  $5.5\text{--}7 \mu\text{m}$ . Conidia slightly elliptical to globose, smooth,  $1.5\text{--}2.5 \mu\text{m}$  diameter.

Isolated from water of Chena Lake, Yeoor Range [South], Outside SGNP, Thane, Maharashtra, 21 August 2017, RD, 205514, BSI (WC); Isolated from water of Vihar Lake, Tulsi Range, SGNP, Mumbai, Maharashtra, 20 August 2017, RD, 205517, BSI (WC); Isolated from Water of Vihar Lake (Stagnant water), Tulsi Range, SGNP, Maharashtra, 26 January 2017, RD, 205520 BSI (WC).

**12. *Aspergillus versicolor*** (Vuill.) Tirab., *Annali Bot.*: 9 (1908)

Colonies slow growing white on PCA at  $28 \pm 1^\circ\text{C}$ , slightly floccose or velvety, exudates clear to wine red, reverse yellow, red, purple red; Conidial heads radiate, hemispherical; Conidiophores upto  $500\text{--}700 \times 4.7 \mu\text{m}$ , widening near vesicle; vesicle  $12.4\text{--}16 \mu\text{m}$ , fertile area hemispherical; Sterigmata bisertate, primaries  $5\text{--}8.0 \times 3.0 \mu\text{m}$  or less; secondaries  $5.5\text{--}7 \times 2.1\text{--}2.4 \mu\text{m}$ , may be pigmented; conidia delicately echinulate globose,  $2.3\text{--}3 \mu\text{m}$ , in radiating chains; sclerotia and cleistothecia not found.

Isolated from water of Mori no 63, Tulsi Road, Tulsi range, SGNP Mumbai, Maharashtra, 20–08–17, RD, 205506 BSI (WC).

**13. *Fusarium solani*** (Mart.) Sacc., *Michelia* 2(no. 7): 296 (1881) Fig. 3I

Colonies on MEA fast growing at  $28 \pm 1^\circ\text{C}$ , off-white. Mycelium thin, hyaline, smooth, branched,  $2\text{--}3 \mu\text{m}$  wide. Setae and hyphopodia absent; Conidiophores macronematous, mononematous, straight to flexuous, profusely branched, septate, smooth, hyaline; Conidiogenous cells terminal, monophialidic, integrated,  $10\text{--}15 \times 2\text{--}4 \mu\text{m}$ ; macroconidia solitary, endogenous, fusiform, simple, hyaline, smooth, pointed at both the ends, septate,  $20\text{--}25 \times 3\text{--}5 \mu\text{m}$ ; microconidia ellipsoidal, smooth, hyaline, aseptate,  $7\text{--}15 \times 2\text{--}4 \mu\text{m}$ .

Based on ITS phylogenetic identification, the isolate SM 42 SGNP was identified as *Fusarium solani*

Isolated from water of Karnal Pada, Sarjamori, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist., Maharashtra, 20 December 2017, RD, 205496 BSI (WC), culture no. SM 42 SGNP

**14. *Penicillium citrinum*** Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 61 (1910) Fig. 3J

Colonies  $25\text{--}30 \mu\text{m}$  in length on MEA at  $28 \pm 1^\circ\text{C}$ . conidiophores arising mostly from substratum,  $50\text{--}200 \mu\text{m}$  long. Conidiophores usually unbranched but occasionally bearing one or more branched,  $25\text{--}35 \mu\text{m}$  long. Metulae  $12\text{--}20 \mu\text{m}$ , each supporting a cluster of  $6\text{--}10$ , more or less crowded and parallel phialides. Phialides  $8\text{--}11 \mu\text{m}$ , bearing in parallel chains to produce well–defined columns up to  $100\text{--}150 \mu\text{m}$  long. Conidia globose to subglobose, mostly  $2.2\text{--}3.2 \mu\text{m}$  in diameter.

**15. *Penicillium glabrum*** (Wehmer) Westling, *Ark. Bot.* 11(no. 1): 131 (1911) Fig. 3K

Colonies reaching to  $40\text{--}50 \mu\text{m}$ , on PDA, MEA at  $28 \pm 1^\circ\text{C}$ , velutinous, radially sulcate, rich green–brown in age, exudate produced yellow or brown with similar soluble pigment, reverse colourless to variously coloured. Conidiophores smooth, vesiculate, bearing monovericillate penicili (occasionally bimetulate); phialides ampuliform,  $8\text{--}12 \times 2.5\text{--}3.5 \mu\text{m}$ ; conidia globose,  $3\text{--}3.5 \mu\text{m}$  diam., forming well defined columns, smooth to roughened.

Isolated from water of Sasunavghar at foothills, Nagla Block, North of Vasai Creek, Yeoor range, SGNP,

Palghar Dist., Maharashtra, 21-12-17, RD, 205498 BSI (WC)

**16. *Penicillium oxalicum*** Currie & Thom, *Journal of Biological Chemistry* 22(2): 289 (1915)

Colonies 35–60 µm on PDA at 28± 1°C, plane to radially sulcate, velutinous to floccose; Conidiophores long, penicili biverticillate with 2–3 metulae closely appressed, or monovertilillate; metulae 15–25 × 3.5–7 µm, with smooth walls, borne in long columns.

Isolated from water of Sasunavghar at foothills, Nagla Block, North of Vasai Creek, Yeoor range, SGNP, Palghar Dist., Maharashtra, 21-12-17, RD, 205498 BSI (WC)

**17. *Rhizopus stolonifer*** (Ehrenb.) Vuill., *Revue mycol.*, Toulouse 24: 54 (1902) **Fig. 3 (L)**

Colonies on PCA at 28± 1°C, pale brown to brown; sporangiophores usually straight, wall slightly rough or smooth, 1500–2000 µm in length, 15–30 µm in diameter; sporangia subglobose, black in colour; 90–200 µm in diam. Columellae globose or oval, pale brown in colour, 60–160 µm in diameter; Sporangiospores: globose, oval, striated polygonal, 4–12 µm in diam.; rhizoids abundant, stolons slightly rough; almost colourless; 13–20 µm in diameter.

Isolated from water of Sasunavghar, Nagla Block, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist, Maharashtra, 21 December 2017, RD, 205499 BSI (WC).

**18. *Rhizopus* sp.**

Colonies on PCA at 28± 1°C, sporangiophores mostly formed on stolons opposite rhizoids, either single or in clusters, unbranched, bearing multispored terminal sporangia; sporangia globose, distinctly columellate, apophysate, greyish to brownish at maturity 100–130 µm in diameter.; sporangiospores subglobose to ellipsoidal and angular 4–10 µm in length; zygosporangia with spines or warts, formed in aerial mycelium between non-ornamented, isogamous, opposite suspensors.

Isolated from water of Vasai Creek, Peripheral, Mumbai Maharashtra, 21 August 2017, RD, 205515D BSI (WC).

**19 *Trichoderma austrokonigii*** Samuels & Druzhin., in Samuels, Dodd, Lu, Petrini, Schroers & Druzhinina, *Stud. Mycol.* 56: 92 (2006)

Colonies on MEA, PCA at 28± 1°C, white to olivaceous green; Mycelium septate, hyaline, profusely branched, smooth and 2.5–3 µm wide; conidiophores macronematous, mononematous, branched at right angles to the main axis forming primary and secondary branches, hyaline, smooth, straight to flexuous, up to 100 µm long, 2–2.5 µm wide; conidiogenous cells monophialidic, hyaline, discrete, verticillate, in groups of 2–3, terminal,

lageniform, 12–20 × 2–2.5 µm. conidia in green slimy heads, simple, spherical smooth, aseptate, sub-hyaline, 3.5–5 µm in diam.

On the basis of ITS phylogenetic identification, the isolate SM 41 was identified as *T. austrokonigii* Isolated from water of lake in Krishanagiri Range, SGNP, Mumbai Maharashtra, 26 January 2017, RD, 205521 BSI (WC), culture no. SM 41, NCBI accession no.– ON738559.1.

**20 *Trichoderma erinaceum*** Bissett, C.P. Kubicek & Szakács, in Bissett, Szakacs, Nolan, Druzhinina, Gradinger & Kubicek, *Can. J. Bot.* 81(6): 583 (2003)

Colonies on MEA, PCA at 28± 1°C, white to olivaceous green; Stromata scattered or aggregated in small numbers; pulvinate, 0.2 mm high × 0.5–1 mm wide, margin free, flat or convex, appearing grey–brown at maturity, often covered by white powdery spore mass; Peridium 12.5 µm wide with few layers of textura angularis cells, yellow;

Asci 8–spored or 16–part–spored, unitunicate, cylindrical; Ascospores 1.2–1.9 × 1.0–1.4 µm, obliquely biserial, cells dimorphic, globose, oblong with 1–3 guttules, hyaline.

Isolated from water of Tulsi lake, Tulsi range, SGNP Mumbai, Maharashtra, 23-12-17, RD, 205510, BSI (WC), NCBI accession no.- ON803453.1

**21. *Trichoderma harzianum*** Rifai, *Mycol. Pap.* 116: 38 (1969)

Colonies growing rapidly on PDA at 28± 1°C, white green to bright green to dull green Mycelium: septate, colourless, smooth, 1.5–2.5 µm. Chlamydospores: mostly globose, smooth, 6–12 µm in diam. Conidiophores: loose tuft, main branch produced numerous side branches especially lower portion. Phialides: arise false verticillate upto five, short, slitte shaped, narrow at the base, attenuate abruptly, sharp pointed neck, –75×3–4 µm, Phialospores: accumulated at the tip of phialides, subglobose, short obovoid, often broad truncate base, smooth, pale green, much darker in mass, 2.8–3.2 × 2.5–2.8 µm.

Isolated on PDA from water of Karnal Pada, Sarjamori, North of Vasai Creek, Yeoor range, SGNP, Palghar Dist, Maharashtra, 20-12-17, RD, 205496B, BSI (WC).

**22. *Trichoderma viride*** Pers., *Neues Mag. Bot.* 1: 92 (1794)

Colonies growing rapidly on PDA at 28± 1°C, surface smooth, become hairy, dark green typical coconut odour is emitted in old cultures; Mycelium: Hyaline, smooth, septate much branched; Chlamydospores: Intercalary, globes, rarely ellipsoidal, 10–15 µm in diam. Conidiophores: Arise in compact or loose tuft, main branches produced several side branches in groups of 2–3, all branches stand at wide angles; Phialides: In false whorls beneath

each terminal phialides, usually more than 2–3 phialides, 8–15 x 2–3 µm in size. Curved, pin shaped, narrower at the base, widening above the middle, attenuate into long neck. Phialospores: Globose or short obovoid broadly ellipsoidal, at distal apiculus like base, minute roughing of their wall, 3.5–4.5 µm, accumulated at the tip of each phialides, pale green, smooth.

**Material examined:**

Isolated from water of Karnal Pada, Sarjamori, North of Vasai Creek, Yeoor range, SGNP, Palghar Dist, Maharashtra, 20-12-17, RD, 205497, BSI (WC).

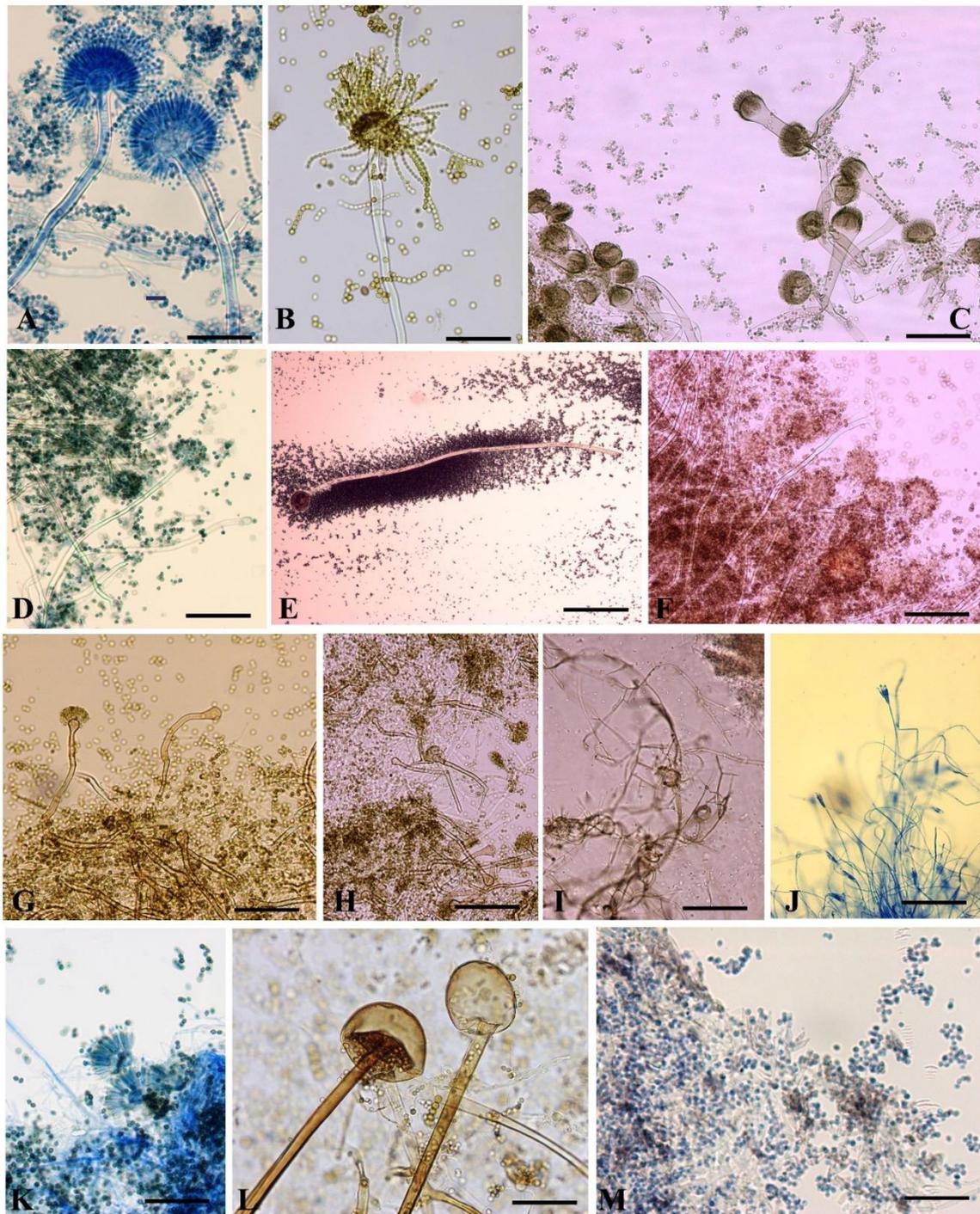
## DISCUSSION

Aquatic fungi remain one of the under-explored groups, even within fungi, with respect to their taxonomy and ecology (Grossart et al. 2019). In India, few works are available on the taxonomic characterisation of aquatic fungi, with fewer works exploring their ecological aspects. Checklists of freshwater fungi are available at the national level (Patil & Borse 2015) and at state level for Maharashtra (Nemade et al. 2016). Checklist of marine fungi is available at national level (Borse et al. 2013). These works report much higher number of fungi as they cover much larger geographical area. Taxonomic and ecological dimensions were explored for aquatic fungal diversity of Kali River in Western Ghats region of neighbouring state of Karnataka (Sridhar & Sudheep 2011; Sudheep & Sridhar 2012, 2013a, 2013b). No checklist of aquatic fungi is available for Sanjay Gandhi National Park or even at district-level in which the national park falls. The present study is unique in molecular identification of selected species for which morphological identification was inadequate. The present study is also unique in examining ecological aspects of water-borne fungi by calculating diversity measures. In the present paper, we studied diversity of water-borne fungi of SGNP from taxonomic and ecological perspectives and have provided the checklist and description of 22 species of

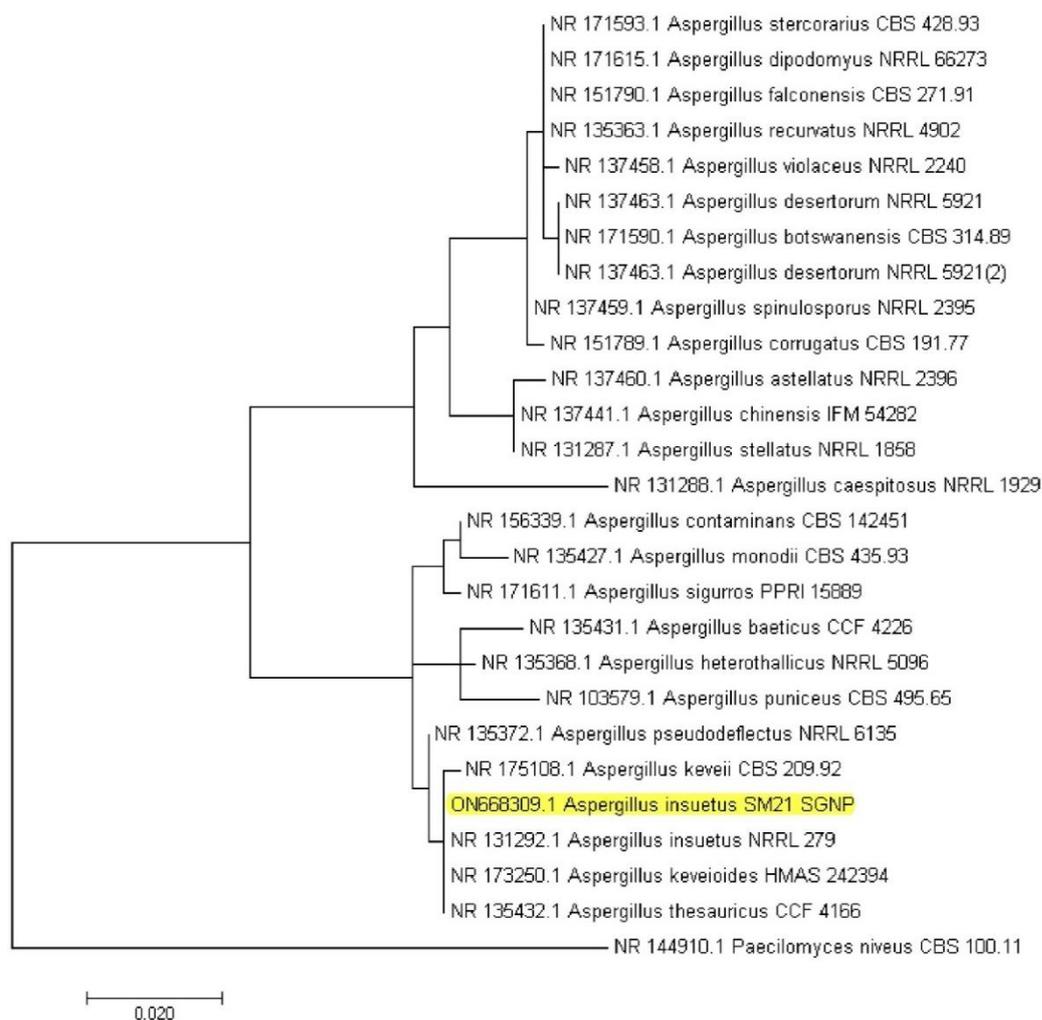
water-borne fungi documented along with their taxonomic and collection details. The molecular phylogeny was conducted for 06 waterborne fungi using the ITS gene regions. *Aspergillus* was the dominant genus with 12 species. *Aspergillus* alone accounted for about 55% of identified species. As detailed in Table 1, out of 28 fungi, 7 were documented from 10% peripheral areas outside the national park, while rest were found within the forest areas of the national park. Among those 7 fungi, 5 were from saline waters of Vasai Creek characterised by mangrove vegetation. The study area is diverse, as evidenced by values of Gini-Simpson's Index (=0.9439) and Shannon's index (=2.9978). The high value of Pielou's evenness index ( $J = 0.9698$ ) shows high equitability in species distribution. To correct observed species richness for observed evenness, we examined true diversity, a measure of which is Effective Number of Species, calculated from Shannon's index. True diversity or the effective number of species for the study area (20) was less than species richness or the observed number of species (22) due to the absence of a perfectly equitable distribution of species ( $J \neq 1$ ). Thus, the present work offers important insights into taxonomy, distribution and ecology of water-borne fungi of SGNP.

## ACKNOWLEDGMENTS

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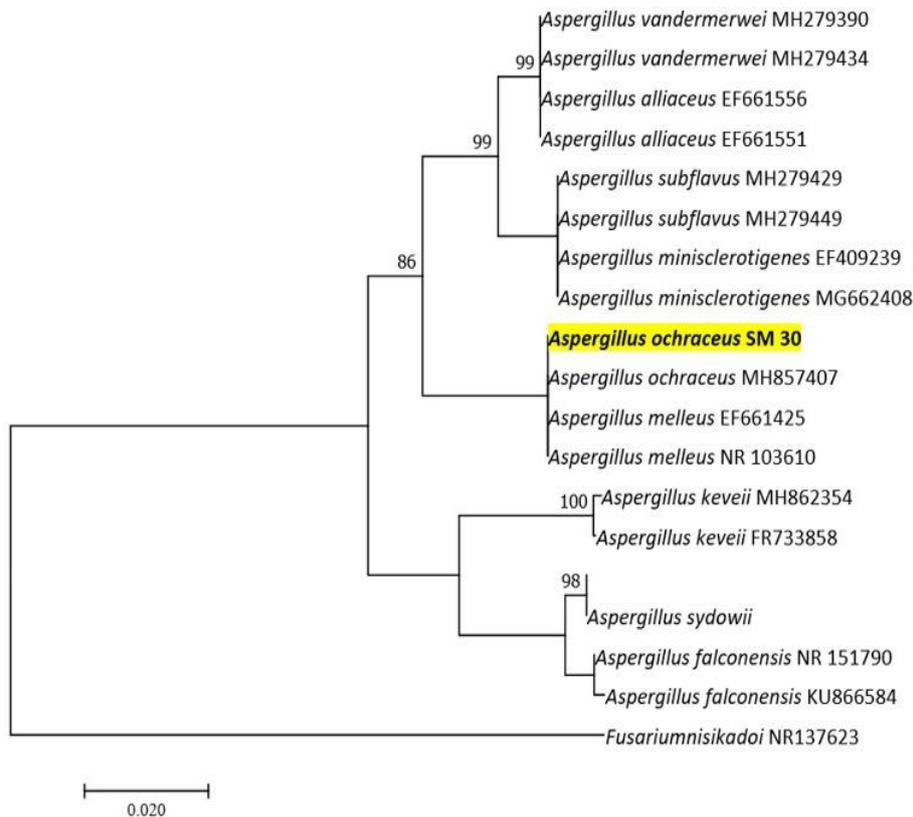


**Fig. 3.** Some of the water-borne fungi isolated from the waters of Sanjay Gandhi National Park. A. *Aspergillus brasiliensis*; B. *A. flavus*; C. *A. fumigatus*; D. *A. nidulans*; E. *A. niger*; F. *A. ochraceus*; G. *A. parasiticus*; H. *A. terreus*; I. *Fusarium solani*; J. *Penicillium citrinum*; K. *P. glabrum*; L. *Rhizopus stolonifer*; M. *Trichoderma viride* (Scale Bar–A,B,C,D,E,F,I,K,L=100  $\mu$ m; G,H,J,M=200  $\mu$ m)



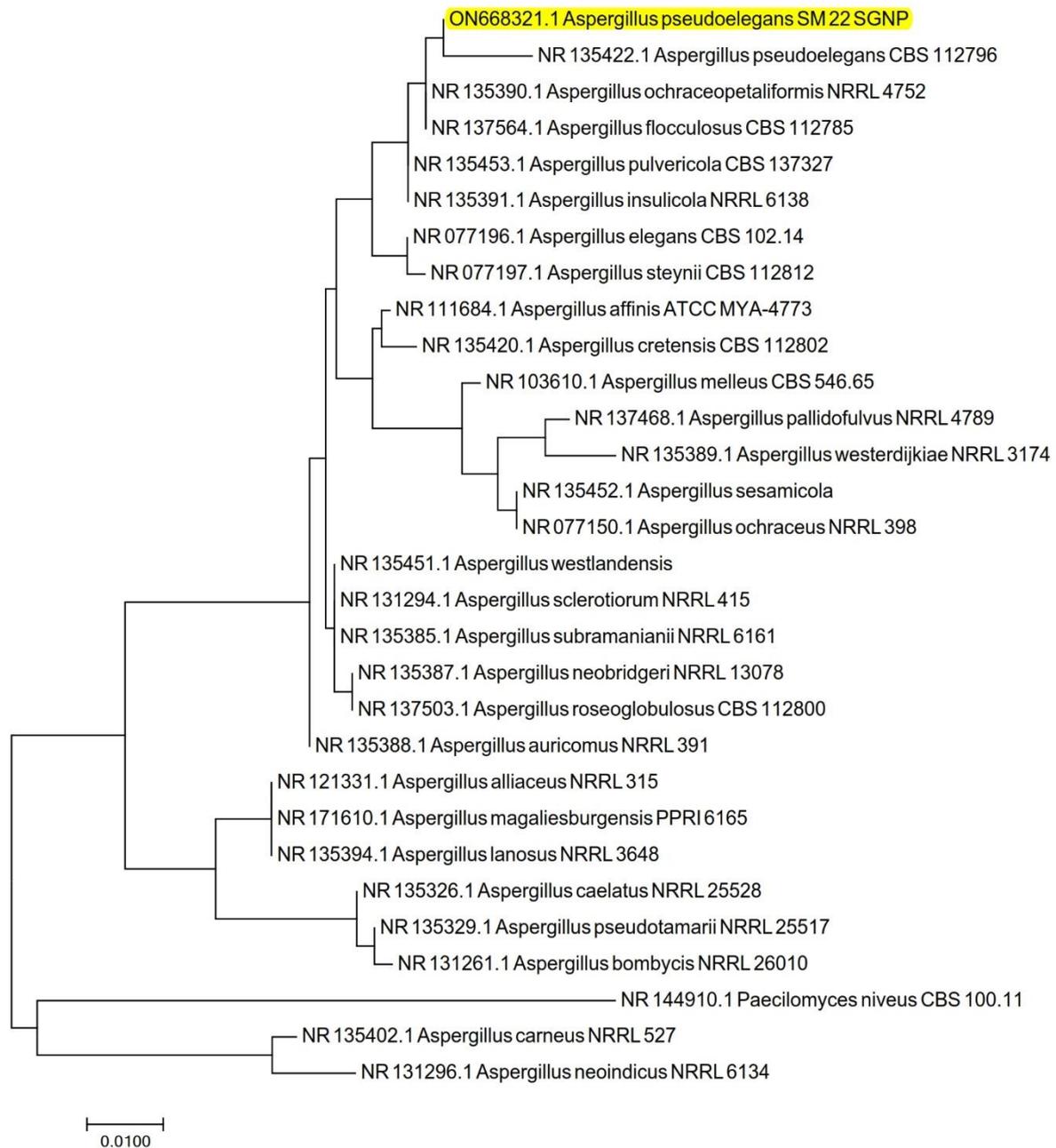
**Fig. 4.** Molecular Phylogenetic analysis of *Aspergillus insuetus* SM21 SGNP (NCBI-ON668309.1)

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.19278484 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 [2]. 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 Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. The analysis involved 27 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 443 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. *Paecilomyces niveus* was used as an outgroup.



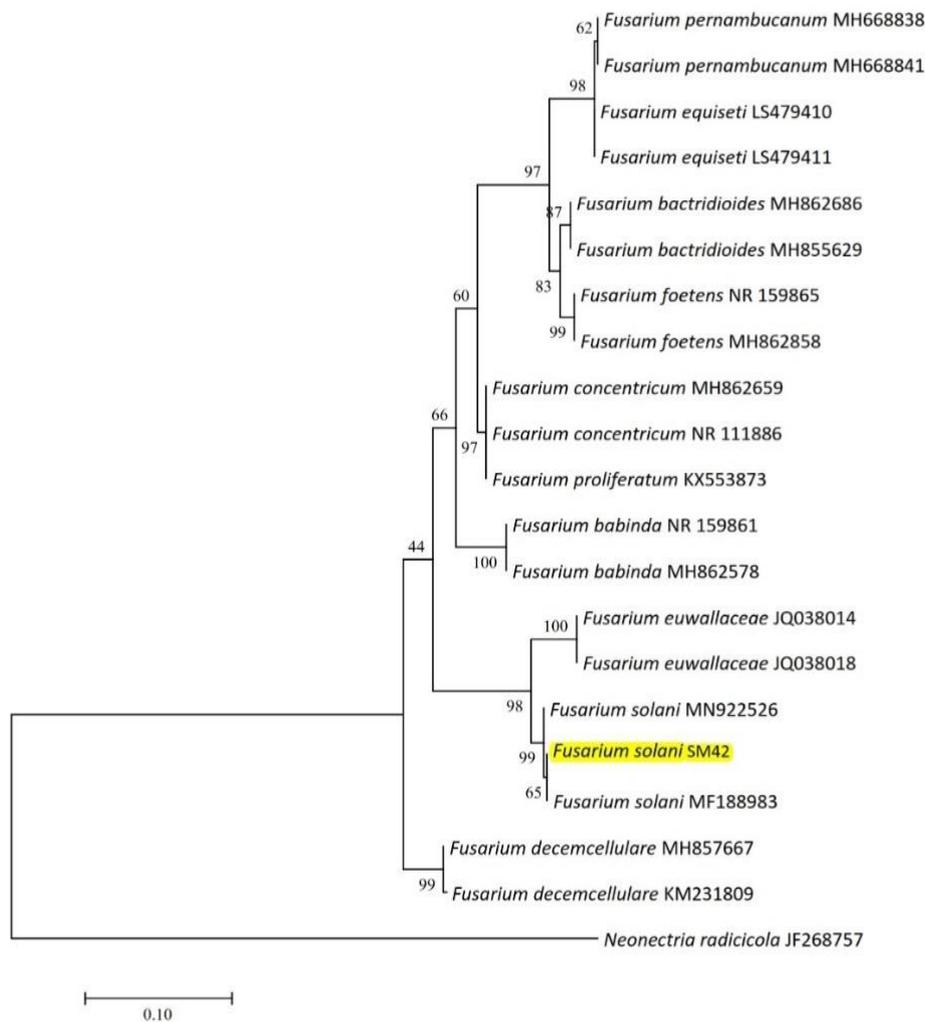
**Fig. 5.** Molecular Phylogenetic analysis of *Aspergillus ochraceus* by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree with the highest log likelihood (-1104.72) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1217)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 411 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. *Fusarium nisikadoi* was used as an outgroup.



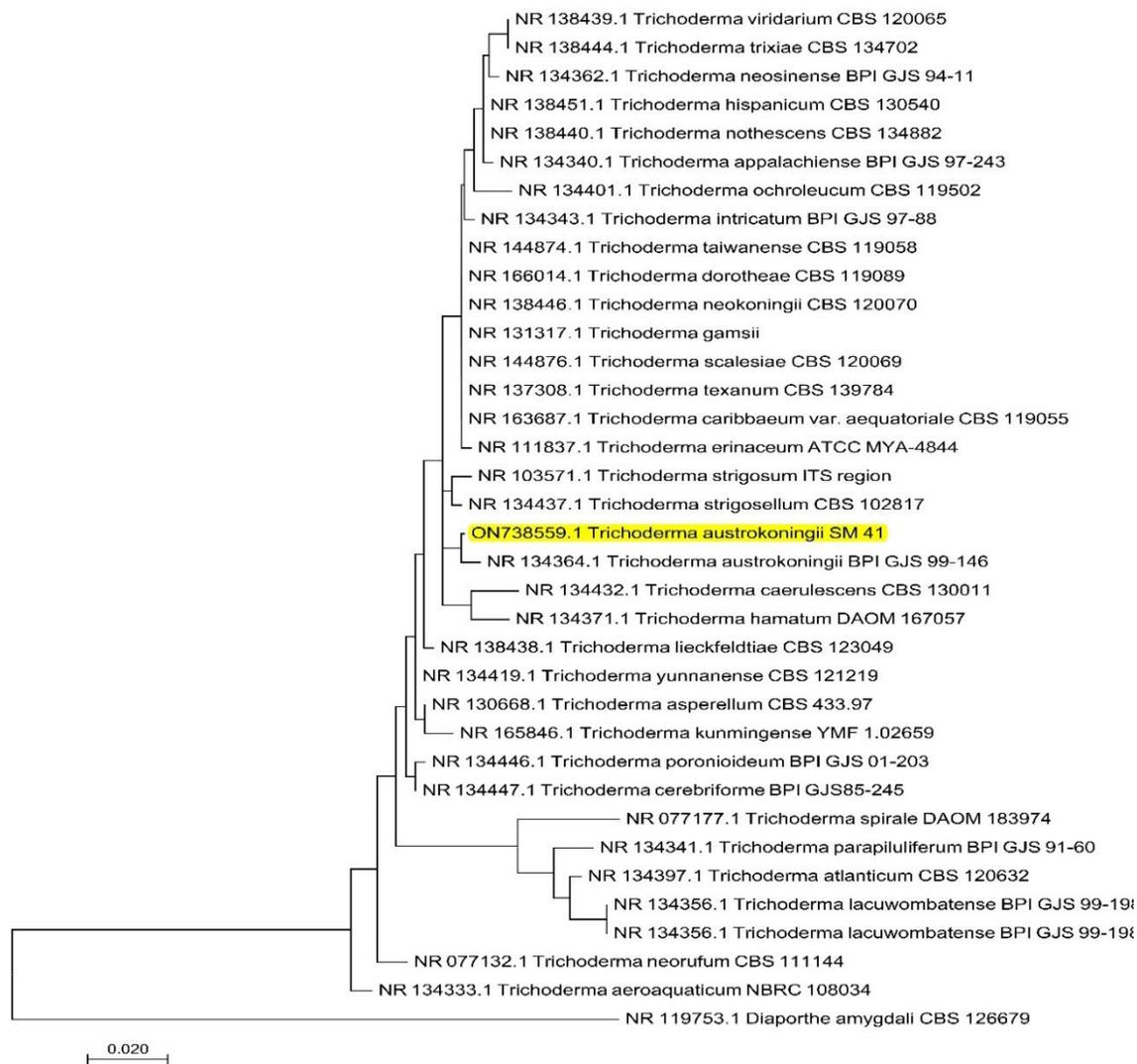
**Fig. 6.** Molecular Phylogenetic analysis of *Aspergillus pseudoelegans* by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura–Nei model [1]. The tree with the highest log likelihood (−1328.85) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor–Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 433 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al 2016). *Paecilomyces niveus* was used as an outgroup.



**Fig. 7.** Molecular Phylogenetic analysis of *Fusarium solani* by Maximum Likelihood method .

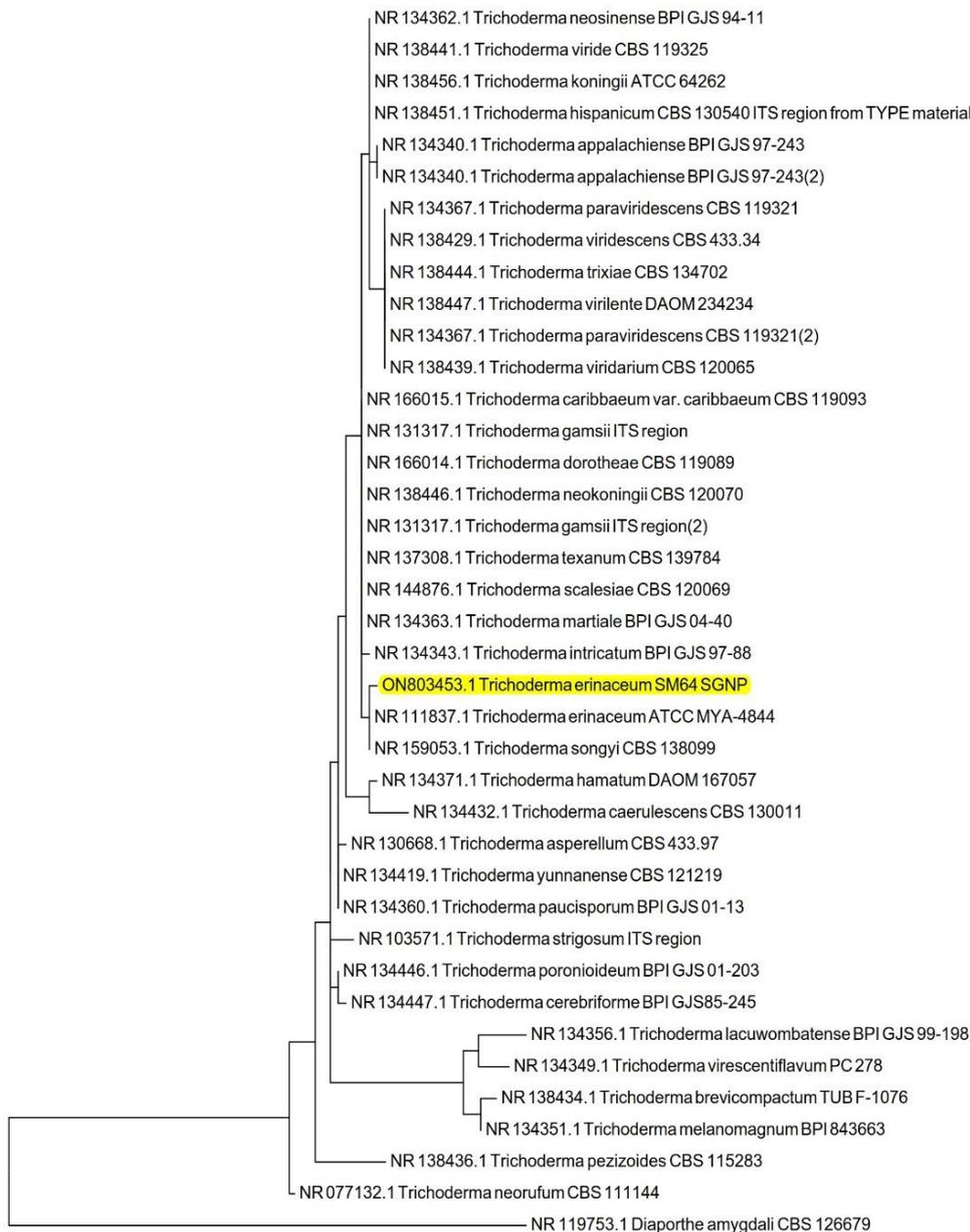
The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-1870.9515) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 21 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 439 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. *Neonectria radicola* was used as an outgroup.



**Fig.8.** Molecular Phylogenetic analysis of *Trichoderma austrokonigii* by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura–Nei model [Tamura and Nei 1993]. The tree with the highest log likelihood (–1511.08) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor–Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 36 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 428 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

*Diaporthe amygdali* was used as an outgroup.



0.020

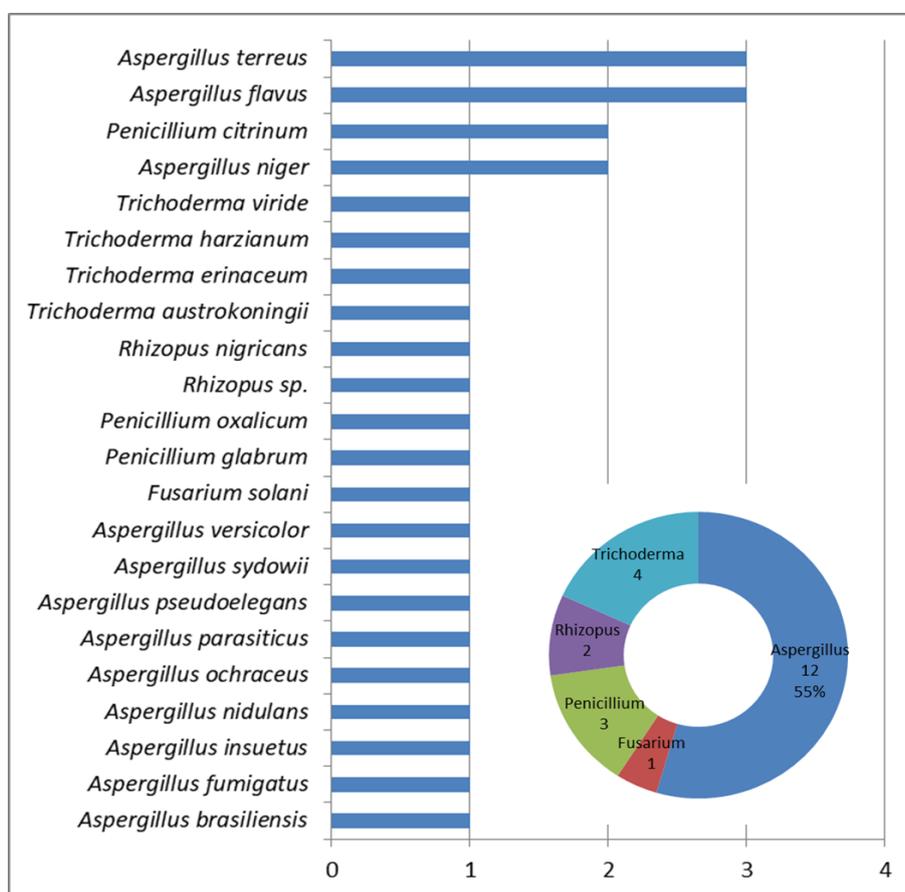
**Fig. 9.** Molecular Phylogenetic analysis by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree with the highest log likelihood (-1367.47) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 39 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 418 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016) *Diaporthe amygdali* was used as an outgroup.

Fig. 3 provides the microphotographs and Figs. 4–9 show the molecular phylogeny of six of the fungi isolated from the waters of SGNP, viz., *Aspergillus insuetus*, *Aspergillus ochraceus*, *Aspergillus pseudoelegans*, *Fusarium solani*, *Trichoderma austrokonigii* and *Trichoderma erinaceum*. As shown *A. flavus* and *A. terreus* were the dominant species, each represented by 3 isolates. Water samples collected from areas of Yeoor Range lying to the south of Vasai Creek showed only bacterial growth but no fungal growth; hence were discarded. Diversity measures (Table 2) were calculated by combining

in Fig. 10, *Aspergillus* was reported as a dominant genus with 12 species. *Aspergillus* alone accounted for about 55% of identified species.

species richness with evenness (Table 2). Gini–Simpson’s index was 0.9439, and Shannon’s index was 2.9978. Pielou’s evenness index was 0.9698, causing true diversity to be less than observed species richness. The calculated effective number of species (20) was less than the observed number of species (22).



**Fig. 10.** Distribution of water-borne fungal species among five genera recovered from Sanjay Gandhi National Park

**Table 1** Checklist of water-borne fungi of Sanjay Gandhi National Park (SGNP)

Acc. No.	Species	Family	Date	Locality
BSI-F876	<i>Aspergillus brasiliensis</i>	Aspergillaceae	20/08/2017	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSI-F877	<i>Aspergillus flavus</i>	Aspergillaceae	08/09/2016	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSI-F839	<i>A. flavus</i>	Aspergillaceae	21/08/2017	Vasai Creek, Peripheral to SGNP, Thane
BSI-F878	<i>A. flavus</i>	Aspergillaceae	26/01/2017	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSI-F888	<i>A. fumigatus</i>	Aspergillaceae	08/09/2016	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSI-F879	<i>A. insuetus</i>	Aspergillaceae	23/12/2017	Lake of Krishnagiri Range, SGNP, Mumbai
BSIF-1190	<i>A. nidulans</i>	Aspergillaceae	08/09/2016	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSI-F880	<i>A. niger</i>	Aspergillaceae	08/09/2016	Tulsi lake, Tulsi Range, SGNP, Mumbai
BSI-F881	<i>A. niger</i>	Aspergillaceae	08/09/2016	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSIF-1191	<i>A. ochraceus</i>	Aspergillaceae	19/10/2018	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSIF-1192	<i>A. parasiticus</i>	Aspergillaceae	20/08/2017	Powai lake, Peripheral to SGNP, Mumbai
BSI-F895	<i>A. pseudoalegans</i>	Aspergillaceae	21/08/2017	Vasai Creek, Peripheral to SGNP, Thane
BSI-F883	<i>A. sydowii</i>	Aspergillaceae	10/09/2016	Ghodbandar, Peripheral to SGNP, Thane
BSI-F885	<i>A. terreus</i>	Aspergillaceae	21/08/2017	Chena Lake, Yeoor Range [South], Outside SGNP, Thane
BSI-F886	<i>A. terreus</i>	Aspergillaceae	20/08/2017	Vihar Lake, Tulsi Range, SGNP, Mumbai
BSI-F884	<i>A. terreus</i>	Aspergillaceae	26/01/2017	Near Vihar Lake (stagnant water), Tulsi Range, SGNP, Mumbai
BSI-F887	<i>A. versicolor</i>	Aspergillaceae	20/08/2017	Mori no 63, Tulsi Road, Tulsi Range, SGNP, Mumbai
BSIF-1193	<i>Fusarium solani</i> (	Nectriaceae	20/12/2017	Karnal Pada, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist.
BSI-F889	<i>Penicillium citrinum</i>	Aspergillaceae	21/08/2017	Vasai Creek, Peripheral to SGNP, Thane
BSI-F890	<i>Penicillium citrinum</i>	Aspergillaceae	08/09/2016	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSI-F891	<i>Penicillium glabrum</i>	Aspergillaceae	21/12/2017	Foothills at Sasunavghar, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist.
BSI-F892	<i>Penicillium oxalicum</i>	Aspergillaceae	20/12/2017	Karnal Pada, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist.
BSI-F893	<i>Rhizopus</i> sp.	Rhizopodaceae	21/08/2017	Vasai Creek, Peripheral to SGNP, Thane
BSI-F894	<i>Rhizopus nigricans</i>	Rhizopodaceae	21/12/2017	Sasunavghar, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist.
BSI-F897	<i>Trichoderma austrokingii</i>	Hypocreaceae	26/01/2017	Lake in Krishanagiri Range, SGNP, Mumbai
BSI-F896	<i>Trichoderma erinaceum</i>	Hypocreaceae	23/12/2017	Tulsi Lake, Tulsi Range, SGNP, Mumbai
BSIF-1194	<i>Trichoderma harzianum</i>	Hypocreaceae	20/12/2017	Karnal Pada, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist.
BSI-F898	<i>Trichoderma viride</i>	Hypocreaceae	20/12/2017	Karnal Pada, North of Vasai Creek, Yeoor Range [North], SGNP, Palghar Dist.

**Table 2.** Diversity Measures of Water-borne Fungi of Sanjay Gandhi National Park

<b>Species Richness = Observed number of species</b>	<b>22</b>
Simpson's Index ( <i>D</i> )	0.0561
Gini-Simpson's Index ( <i>1-D</i> )	0.9439
Shannon's Index ( <i>H</i> )	2.9978
Pielou's evenness index ( <i>J</i> )	0.9698
True Diversity =	20
Effective number of Species = $e^H$	

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## قارچهای آبی پارک ملی سانجای گاندی مهاراشترای هندوستان: تاکسونومی و اکولوژی

راشمی دوبی<sup>✉</sup> و آمیت دیواکار پاندی

مرکز منطقه ای بررسی های تخصصی گیاهشناسی هندوستان، پونا، ایالت مهاراشترا، هندوستان

**چکیده:** مقاله حاضر به بررسی تنوع تاکسونومیک و مطالعات جنبه های اکولوژیکی قارچهای آبی پارک ملی سانجای گاندی هندوستان می پردازد. منطقه مورد بررسی شامل آبراه های شیرین، دریاچه ها و خورهای شورااست. در این مطالعه ۲۸ جدایه قارچ آبی از ۲۰ نمونه آب بررسی شده، بدست آمد و پس از تجزیه و تحلیل های موفولوژیکی و مولکولی، مجموعاً ۲۲ گونه متعلق به پنج جنس تایید شد. جنس غالب *Aspergillus* spp. با ۱۲ گونه می باشد که در این بین دو گونه *A. terreus* و *A. flavus* هر کدام با سه جدایه غالب بودند. ضریب جینی-سیمپسون ۰/۹۴۳۹ و ضریب شانون ۲/۹۹۷۸ بود. ضریب پیلو ۰/۹۶۹۸ که باعث بیشتر شدن غنای گونه های مشاهده شده از تنوع واقعی می شود، به عنوان تعداد موثر گونه ها محاسبه شد.

**کلمات کلیدی:** آسکومیکوتا، ضرایب تنوع، ضریب تشابه جاکارد، قارچهای آبی مهاراشترا، تنوع واقعی موکورومیکوتا

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