10.11609/jott.2021.13.13.19887-20142
www.threatenedtaxa.org

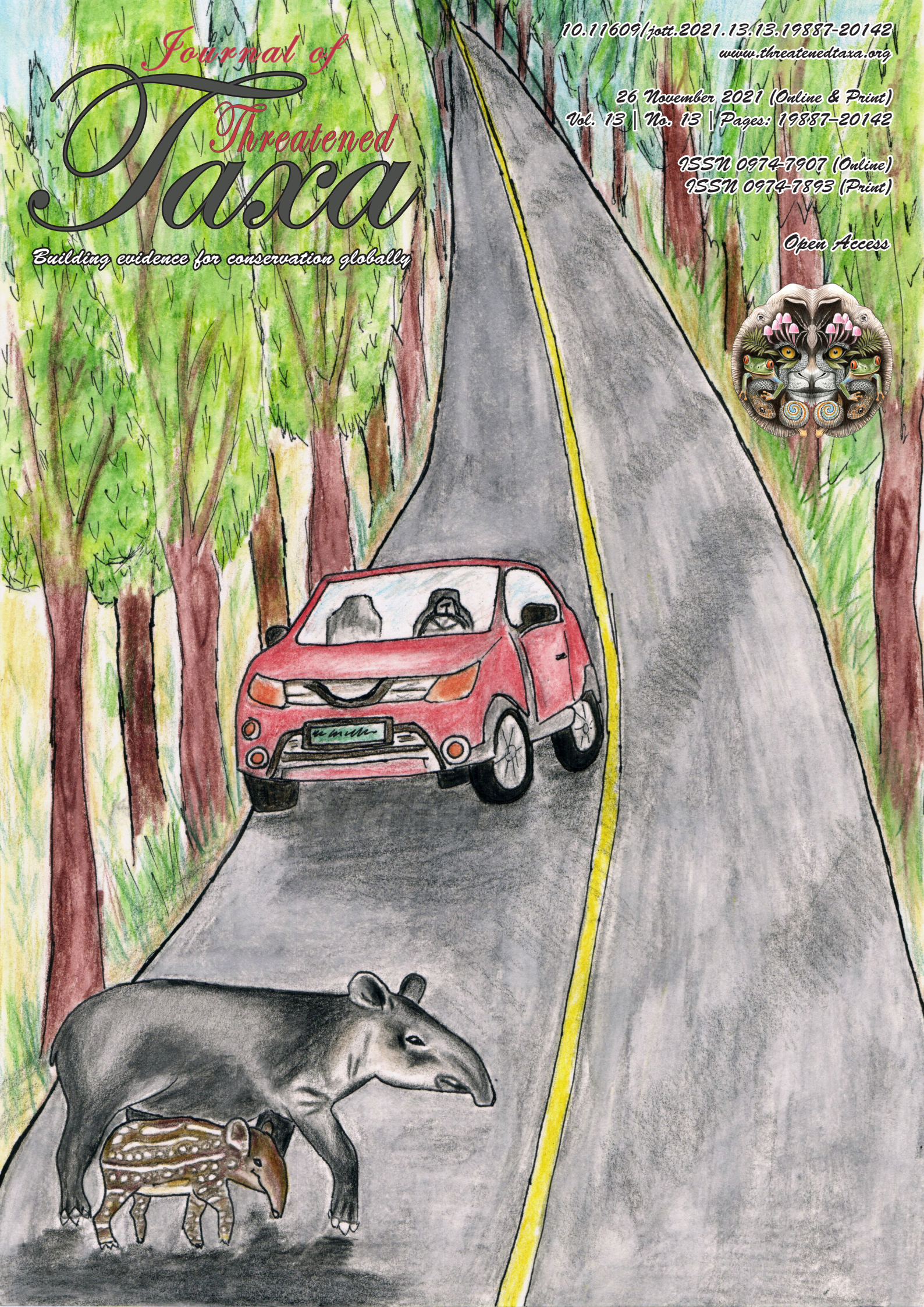
26 November 2021 (Online & Print)
Vol. 13 | No. 13 | Pages: 19887–20142

ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

Open Access

Journal of Threatened Taxa

Building evidence for conservation globally



ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road, Saravanampatti,
Coimbatore, Tamil Nadu 641035, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

For Focus, Scope, Aims, and Policies, visit https://threatenedtaxa.org/index.php/JoTT/aims_scope
For Article Submission Guidelines, visit https://threatenedtaxa.org/index.php/JoTT/about/submissions
For Policies against Scientific Misconduct, visit https://threatenedtaxa.org/index.php/JoTT/policies_various

continued on the back inside cover

Caption: Lowland Tapir *Tapirus terrestris* (Medium—watercolours on watercolour paper) © Aakanksha Komanduri.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2021 | 13(13): 20118–20123
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.6967.13.13.20118-20123
#6967 | Received 09 December 2020 | Final received 09 November 2021 | Finally accepted 12 November 2021
SHORT COMMUNICATION

A new record of psychrotrophic *Paecilomyces formosus* (Eurotiales: Ascomycota) from India: morphological and molecular characterization

Skarma Nonzom [1] & **Geeta Sumbali** [2]

[1,2] Department of Botany, University of Jammu, B.R. Ambedkar Road, Jammu, Jammu & Kashmir 180006, India.
[1] skarmanunzom@yahoo.com (corresponding author), [2] geetasumbalippl@yahoo.co.in

Abstract: A filamentous fungus *Paecilomyces formosus* (Eurotiales, Ascomycota) was detected for the first time from the region while surveying fungal diversity of a cold arid high-altitude pass (4,000 msl) located in Kargil district (Ladakh), India. The fungal isolate was characterized morphologically with camera lucida drawings and microphotographs, and identified using internal transcribed spacer (ITS) ribosomal DNA sequences. *P. formosus* has not been reported from India, or from arid/semi-arid/cold regions before, thus this represents a new record of Indian hot/cold desert mycoflora that is psychrotrophic in contrast to the more common thermophilic fungi.

Keywords: Fungal diversity, Kargil district, Indian mycoflora, internal transcribed spacer, new record, taxonomy.

The genus *Paecilomyces* (Eurotiales, Ascomycota) was first described by Bainier in 1907, and established as closely related to the genus *Penicillium*. Nevertheless, these genera differ in many aspects, such as colony and spore colour (green in *Penicillium,* white, pink, buff or other colours besides green in *Paecilomyces*), phialide shape and form of conidiophores. Later, based on morphological characters, Brown & Smith (1957) and Samson (1974) provided comprehensive monographs of *Paecilomyces* with a number of additions including the sexual stages of several species. Luangsa-ard & Hywel-Jones (2004) used molecular approaches with 18S rDNA sequencing in phylogenetic studies of *Paecilomyces* sensu lato. Similarly, Samson et al. (2009) combined data from the internal transcribed spacer (ITS) region and β-tubulin and calmodulin genes and extrolite profiles, and provided detailed taxonomy and comprehensive description of nine accepted taxa (five sexual morphs and four asexual morphs).

Sapi La (34.371^{0}N, 76.197^{0}E) is a high altitude pass (4,000 m) between two villages located in Kargil district in the trans-Himalayan region that is well known for the Sapi glacier (34.352^{0}N, 76.076^{0}E) and lake (34.352^{0}N 76.076^{0}E; Image 1 a–d). During a mycological survey of this barren pass, which experiences continual strong winds, low temperatures (below 20 ºC during summer and 0 to -35 °C in winter) and high UV radiation throughout the year, more than 30 psychrotolerant fungi were recovered. Of these, a rare microfungus belonging to the genus *Paecilomyces* (P. *formosus* syn. *P. maximus*) was detected, which is being reported for the first time from India. In this report, we describe the characteristics of this cold desert isolate.

Materials and Methods

Isolation of fungal isolates

For fungal isolation, soil samples were collected by scraping the superficial layer, not exceeding 3–5cm

Editor: Richard Mibey, Nairobi, Kenya. **Date of publication:** 26 November 2021 (online & print)

Citation: Nonzom, S. & G. Sumbali (2021). A new record of psychrotrophic *Paecilomyces formosus* (Eurotiales: Ascomycota) from India: morphological and molecular characterization. *Journal of Threatened Taxa* 13(13): 20118–20123. https://doi.org/10.11609/jott.6967.13.13.20118-20123

Funding: UGC-Rajiv Gandhi National Fellowship (RGNF) F117.1/2012-13/RGNF-2012-13-ST-JAM18891/

Competing interests: The authors declare no competing interests.

Acknowledgements: The first author is grateful to the University Grants Commission (UGC), New Delhi for the financial assistance in the form of Rajiv Gandhi National Fellowship (RGNF), which facilitated the study. The authors are also thankful to UGC SAP DRS-II for providing laboratory facilities.

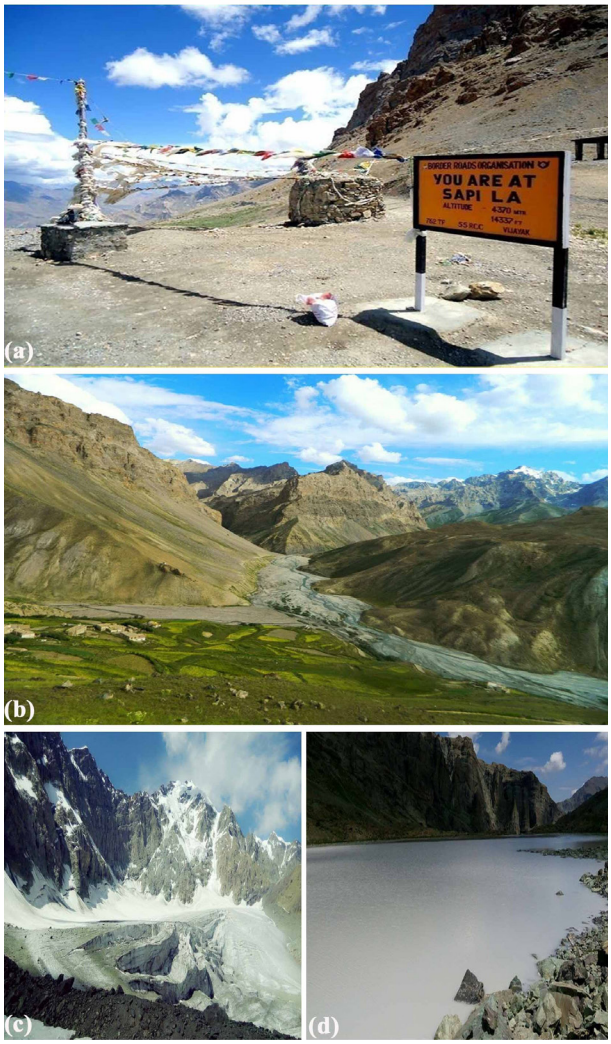


Image 1. A—Study area Sapi La (34.371^{0}N, 76.197^{0}E) | b—Sapi Valley (34.352^{0}N, 76.134^{0}E) | c—Sapi glacier (34.352^{0}N, 76.076^{0}E) | d—Sapi Lake (34.352^{0}N, 76.076^{0}E). © Skarma Nonzom.

in depth, with sterilized spatulas in pre-sterilised polythene bags, and brought to the laboratory within 18 hours. Prior to fungal isolation the samples were stored in refrigerator at -4 ^{o}C for 24–48 hours. Fungal isolation was performed using the dilution pour plate method with modified Czapek Dox Agar (CDA) supplemented with Rose Bengal (0.1mg/100ml) and streptomycin sulphate (5mg/100mL). The plates were incubated at 25 ^{o}C for 7–14 days in a BOD incubator. The morphologically different fungal isolates were further plated on potato dextrose agar (PDA) and malt extract agar (MEA) plates, and then incubated for 3–7 days at 25°C. The pure fungal isolates of DI1A (Desert Isolate 1A) were preserved and maintained on PDA slants at 4 °C for further use.

Morphological characterization

The fungal isolate described was cultured on MEA and PDA plates for three to four days at 25 °C. For microscopic observations, the fungal cultures were either teased directly by using dissecting needles and mounted on glass slides using lactophenol cotton blue/lactophenol, or by using a transparent adhesive tape. Microscopic line drawings were made with the aid of camera lucida (Erma, Japan) at 400x and 1000x magnifications. Dimensions (average of at least 20 measurements) were determined for conidiophores, phialides and conidia using an ocular micrometer. Microphotography was done using Sony N50 camera attached to an Olympus CH 20i binocular microscope.

The isolate was identified morphologically by following the description given by Samson et al. (2009).

Molecular characterization

DNA extraction and sequencing was carried out at the sequencing facility of the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune, India. Genomic DNA was isolated by the standard phenol/chloroform extraction method of Sambrook et al. (1989). This was followed by PCR amplification of the ITS regions using universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G -3'] and ITS4 [5'-TCC TCC GCT TAT TGA TAT GC-3'] (White et al. 1990). The amplified PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) sequencing was carried out from both ends so that each position was read at least twice. Assembly was carried out using Lasergene package followed by NCBI BLAST against sequences from type material for tentative identification (Boratyn et al. 2013). The confirmed sequences were submitted to Genbank, National Centre for Biotechnology Information (NCBI), Maryland, USA to obtain GenBank accession number-MK255020.

The construction of phylogenetic trees was accomplished by maximum-likelihood method implemented in the program MEGA version 6 with 500 bootstrap replicates (Figure 1). Sequences were retrieved from GenBank based on their closest related species showing maximum identity.

Colonization index of the recovered fungal species

Percentage colonization frequency (CF%), A/F ratio, abundance and cfu /g were calculated for the isolated fungal species using formulae given in Table 1.

Table 1. Formulae used.

	Reference
$CF(\%) = \frac{\text{Number of soil samples colonized by a specific fungus}}{\text{Total number of samples studied}} \times 100$ A/F ratio = Abundance/Colonization frequency where, abundance = $\frac{\text{Total number of colonies of a specific fungus}}{\text{Number of soil samples colonized by a specific fungus}}$ A/F ratios describe the distribution pattern of each fungal species into one of the following three categories: A/F ratio of <0.025 depicts that the fungal species has regular distribution. A/F ratio between 0.025 & 0.05 depicts that fungal species has random distribution. A/F ratio of >0.05 depicts that the fungal species has contagious distribution.	Raunkiaer 1934
CFU/g = a×d/s where, a = average number of colonies on the petriplate; d= dilution factor (10,000) & s= dry weight of the soil sample	Parikh & Shah 2006

Results and Discussion

Taxonomic notes

Paecilomyces formosus Sakag., May. Inoue & Tada ex Houbraken & Samson, in Samson, Houbraken, Varga & Frisvad, Persoonia 22: 21 (2009)

Ecology and distribution of the species: Tropical soil, subtropical soil, sponge, wood, air and pot plant soil in Denmark (Samson et al. 2009); current isolate examined: India, trans-Himalaya, Kargil district, Sapi La, isolated from a high altitude extreme habitat, July 2017

Characteristics of the cold desert isolate- Asexual stage; sexual stage-not observed

Morphological identification

Colony characters

Colony characters of the fungal isolate *Paecilomyces formosus* desert isolate 1 (DI1A) are depicted in Image 2a. Colonies on PDA show fast growth, initially light buff, plane, later turning golden yellow to dark yellow, becoming powdery as spores are produced, reaching a diameter of 25 to 30 mm within 3–4 days at 25 °C; reverse pale buff.

Micromorphology

Hyphae branched, hyaline, 2.8–5.6 µm in width; conidiophores simple to irregularly branched, *Penicillum*-like, arising from simple or funiculose hyphae; metulae 7.0–8.4 × 4–2.8 µm; phialides cylindrical, slightly swollen at the base with a long tapering narrow zone, sometimes tapering slightly at the extreme apex, measuring 9.8–21 × 2.5–2.8 µm (Image 2b–e); conidia variable in shape and size, ovate to fusoid, hyaline and small when young; large, yellow, mostly with pointed to rounded apex and truncate base when mature, measuring 4.9–9.1 × 2.1–4.2 µm, smooth-walled, in exceedingly long chains (Image 2b–e, shown in arrows).

Table 2. CF%, cfu/g calculated for *P. formosus* DI1A

Number of soil samples analysed= 25				
Number of samples detected positive	CF (%)	Abundance	A/F ratio	cfu/g
3	12	1.00	0.083	0.3×10^3

Molecular identification

Blast analysis of the ITS region (700 bp) showed its closest similarity to the type material *Paecilomyces formosus* Samson et al. (2009) (GenBank: NR_149329.1; E-value 0; identity: 96.36% and coverage: 100%). Phylogenetic analysis of the sequences of the current isolate DI1A (GenBank: MK255020) based on combined sequences of 15 selected isolates of closest type strains confirmed that our isolate forms a strongly supported clade (99% bootstrap value) with *P. formosus* (Figure 1). *Aspergillus* was used as outgroup.

Discussions and Recommendation

During a mycological survey of a high altitude pass located in the trans-Himalayan region, a psychrotrophic *Paecilomyces* isolate DI1A was recovered which represents a new record to Indian and desert fungi. *P. fusisporus* was detected earlier from cold desert in Himachal Pradesh by Sagar et al. (2007). Similarly, Kotwal & Sumbali (2011) reported three species of *Paecilomyces*, viz., *P. lilacinus, P. marquandii* and *P. variotii* from a similar high altitude pass (5,359 m) located in Ladakh. From Sapi La high-altitude region, Nonzom & Sumbali (2019) have also reported another microfungus, *Geosmithia rufescens*, of rare occurrence.

This identified *Paecilomyces* species, described by Samson et al. (2009) as *Paecilomyces formosus* (Sakag., May. Inoue & Tada) Houbraken & Samson, comb. nov., wherein they illustrated and revised many sexual and

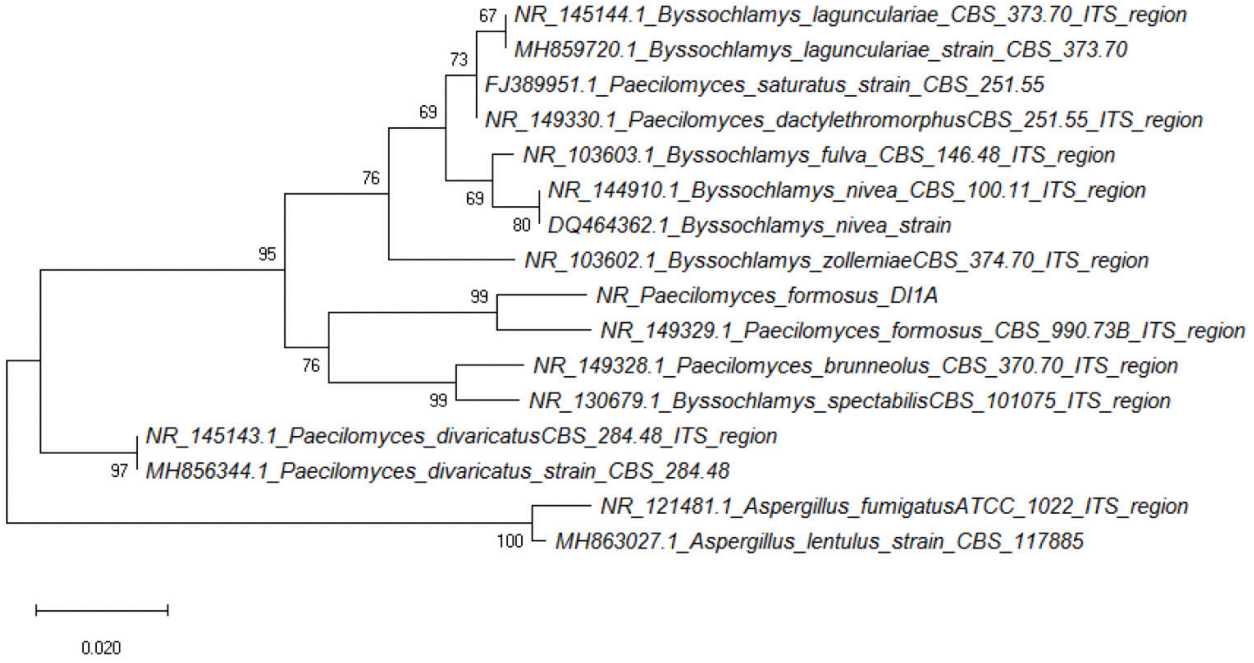


Figure 1. Phylogenetic tree based on maximum likelihood analysis of ITS data for *Paecilomyces* (DI1A) related to the isolate obtained in this study. Bootstrap values of >70% are indicated at the nodes. Scale bar represents the number of substitutions per site.

asexual morphs of *Byssochlamys* and *Paecilomyces*, respectively. Previously, the genus constituted a single species, *P. variotii* (Bainer, 1907). However, later a number of species were added to this genus with some revisions and reshufflings (Brown & Smith 1957; Samson 1974; Houbraken et al. 2006; Samson et al. 2009). As such, *P. variotii* is considered as a variable species, which has been described under diverse names from the beginning. As discussed earlier, Samson et al. (2009) presented an elaborated description of *Paecilomyces* and *Byssochlamys*, the latter comprising of five species with known sexual morphs, i.e., *B. fulva, B. nivea, B. spectabilis, B. zollerniae*, and *B. laguncullariae*, while the former included four species with only asexual morph known, i.e., *P. divaricatus*, *P. formosus*, *P. saturatus*, and *P. brunneolus*. Further, based on the ITS sequences and partial β-tubulin genes, they suggested that, *P. formosus* may constitute three distinct species, viz., *P. formosus*, *P. lecythidis*, and *P. maximus*. However, these three taxa appear morphologically similar and could not be identified on the basis of microscopic and analysis of extrolites, whereas molecular phylogeny data can prove helpful. One of the distinguishing feature observed by Samson et al. (2009) for the *P. maximus* clade and the other members of this diverse group was the rapid growth of this species at 37˚C than at 30 ˚C and based on their study they proposed *P. lecythidis* and *P. maximus* as synonyms of *P. formosus*.

As observed by Samson et al. (2009), on growth tests on PDA and MEA, colonies of the current isolate were also fast growing, reaching a diameter of 15–25 mm within 3–4 days of incubation. Morphologically, the conidia were resembling the isolate described by Samson et al. (2009) in terms of truncate shape (dominant), length, size range (3–10 µm) and variable shapes exhibited. However, the isolate from this study had larger conidia and were exhibiting slightly more diameter (up to 4.2 µm) compared to the results of Samson et al. (2009) (up to 3.5 µm) and in contrast to the formation of chlamydospores that were observed to be produced on short stalks, no such structures were observed in the current desert isolate DI1A. In other related asexual *Paecilomyces* morphs, Samson et al. (2009) observed the presence of chlamydospores (*P. brunneolus* and *P. saturatus*) but their absence in *P. divaricatus.*

Formerly, *P. maximus* was described to be associated with tropical and subtropical soils, wood and human bone marrow (Samson et al. 2009). Later, this species was found to be plant pathogenic in Iran causing dieback diseases in oak and Pistachio (Heidarian et al. 2018; Sabernasab et al. 2019). So far, there are no reports on the incidence of this isolate from arid or semi-arid regions and particularly from cold arid soils. Moreover, *Paecilomyces* species are usually considered thermophilic (Samson et al. 2009; Houbraken et al. 2010; Heidarian et al. 2018). However, in contrast,

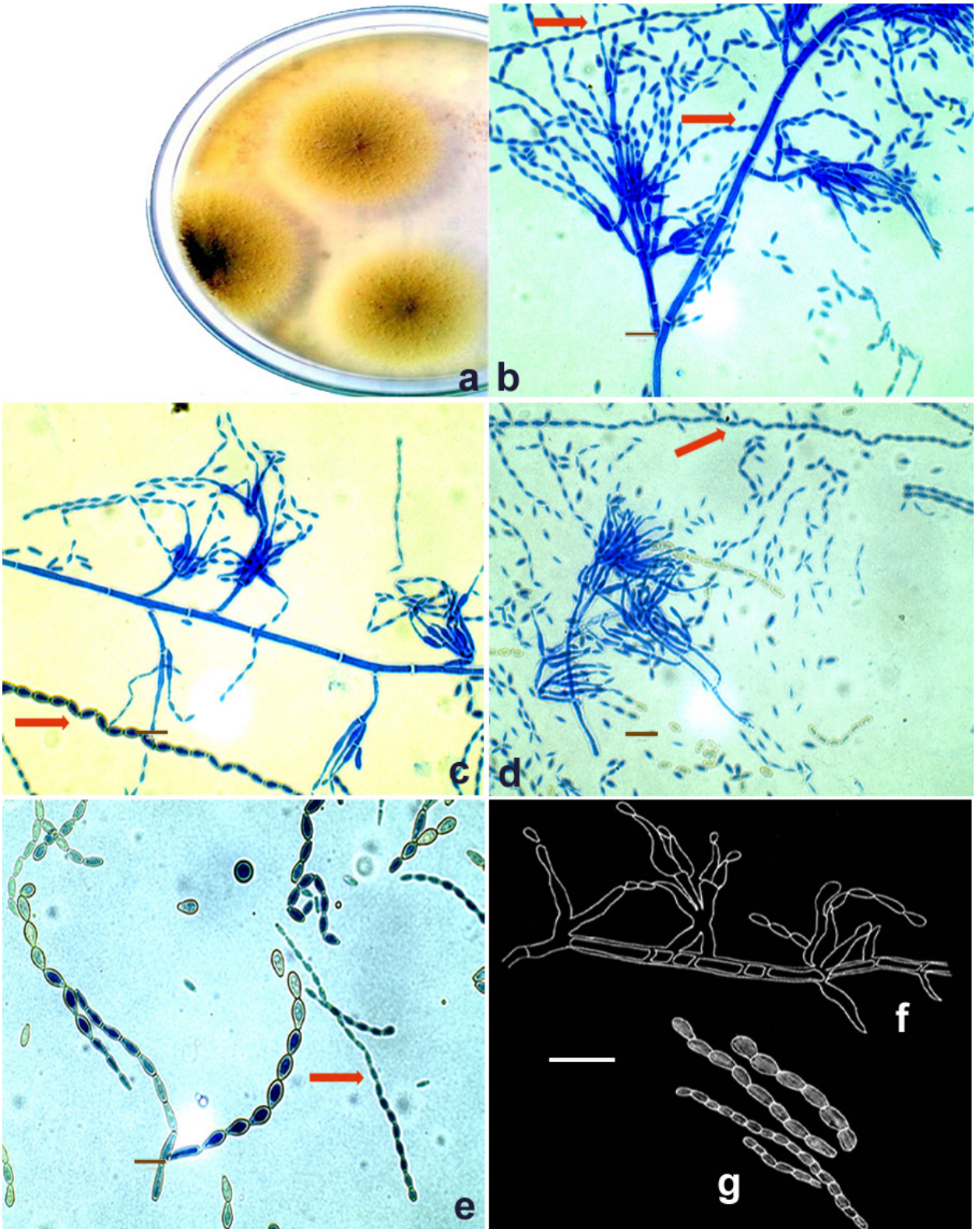


Image 2. *Paecilomyces formosus*: a—Colonies growing on PDA | b—Conidiophores with conidia | c. d, e—conidia of variable size, conidia in very long chains indicated by arrows | f,g—camera lucida drawings of conidiophores and conidia. Bars a–e= 10 µm | f–g= 14 µm. © Skarma Nonzom.

the current *Paecilomyces* isolate is a psychrotolerant or psychrotrophic with the capability to survive and surmount extremely low temperatures (up to -35°C) along with other harsh conditions such as intense UV radiation (4,000 m altitude), strong wind currents, low oxygen concentration, and oligotrophic environments. Therefore, through the present investigation we report that *Paecilomyces* can thrive and sustain their activities at temperature ranging from -35°C to 50°C. This indicates that further research in extreme habitats may unveil the diversity and distribution of described and undescribed fungal species.

References

Bainier, G. (1907). Mycotheque de lecole de pharmacie XL *Paecilomyces* genre nouveau de mucedinees. *Bulletin Trimestrielle De La Societe De Mycology Francaise* 23: 26–27.

Boratyn, G.M., C. Camacho, P.S. Cooper, G. Coulouris, A, Fong, N. Ma, T.L. Madden, W.T. Matten, S.D. McGinnis, Y. Merezhuk, Y. Raytselis, E.W. Sayers, T. Tao, J. Ye & I. Zaretskaya (2013). BLAST- a more efficient report with usability improvements. *Nucleic Acids Research* 41: 29–33.

Brown, A.H.S & G. Smith (1957). The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. Transactions of the British Mycological Society 40: 1789.

Heidarian, R., K. Fotouhifar, A.J.M. Debets & D.K. Aanen (2018). Phylogeny of *Paecilomyces*, the causal agent of pistachio and some other trees dieback disease in Iran. *Plos One* 13: 1–14.

Houbraken, J., R.A. Samson & J.C. Frisvad (2006). *Byssochlamys*: significance of heat resistance and mycotoxin production. *Advances in Experimental Medicine and Biology* 571: 211–224.

Houbraken, J., P.E. Verweij, A.J.M.M. Rijis, A.M. Borman & R.A. Samson (2010). Identification of *Paecilomyces variotii* in clinical samples and settings. *Journal of Clinical Microbiology* 48: 2754–2761.

Kotwal, S. & G. Sumbali (2011). Incidence of myco-keratinophiles in cold arid soil at high altitude Khardung village of Ladakh, India. *Journal of Mycology and Plant Pathology* 41: 72–76.

Luangsa-ard, J.J & N.L. Hywel-Jones (2004). The polyphasic nature of *Paecilomyces* sensu lato based on 18Sgenerated rDNA phylogeny. *Mycologia* 96: 773–780.

Nonzom, S. & G. Sumbali (2019). New record of *Geosmithia rufescens* from a high altitude pass in the trans-Himalayan region. *Austrian Journal of Mycology* 27: 1–4.

Sabernasab, M., S. Jamali, A. Marefat & S. Abbasi (2019). Molecular and Pathogenic Characteristics of *Paecilomyces formosus*, a new causal agent of oak tree dieback in Iran forest. *Science* 1–8.

Sagar, A., S. Raghwa, Bhallan & T.N. Lakhanpal (2007). Studies on the mycoflora of cold desert area of Himachal Pradesh. *Indian Phytopatholgy* 60: 35–41.

Sambrook, J., E.F. Fritsch & T. Maniatis (1989). *Molecular Cloning A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, 1626 pp.

Samson, R.A (1974). *Paecilomyces* and some allied Hyphomycetes. *Studies in Mycology* 6: 1–119.

Samson, R.A., J. Houbraken, J. Varga & J.C. Frisvad (2009). Polyphasic taxonomy of the heat resistant ascomycete genus *Byssochlamys* and its *Paecilomyces* anamorphs. Persoonia 22: 14–27.

Taylor, J.W., D.J. Jacobson, S. Kroke, T. Kasuga, D.M. Geiser, D.S. Hibbett & M.C. Fisher (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31: 21–32.

White,T.J., T. Bruns, S. Lee & J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–322. In: Innis, M.A., D.H. Gelfand & J.J. Sninsky (eds.). *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc, San Diego, CA. USA.

Journal of Threatened Taxa is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64

Print copies of the Journal are available at cost. Write to:
The Managing Editor, JoTT,
c/o Wildlife Information Liaison Development Society,
No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road,
Saravanampatti, Coimbatore, Tamil Nadu 641035, India
ravi@threatenedtaxa.org

Journal of Threatened Taxa

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

November 2021 | Vol. 13 | No. 13 | Pages: 19887–20142
Date of Publication: 26 November 2021 (Online & Print)
DOI: 10.11609/jott.2021.13.13.19887-20142

Publisher & Host

WILD ZOOREACH

Threatened Taxa