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°N, 76.197°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°N, 76.076°E) and lake (34.352°N 76.076°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 ºC 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 deep.
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 °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 °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 NS5 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.
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, *Penicillium*-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).

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...
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. lagunculariae*, 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 exrolites, 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: 211–222.

Houbraken, J., P.E. Verweij, A.J.M.M. Rijss, 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.

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.

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.
The Journal of Threatened Taxa (JoTT) is dedicated to building evidence for conservation globally by publishing peer-reviewed articles online every month at a reasonably rapid rate at www.threatenedtaxa.org. All articles published in JoTT are registered under Creative Commons Attribution 4.0 International License unless otherwise mentioned. JoTT allows uses unrestricted use, reproduction, and distribution of articles in any medium by providing adequate credit to the author(s) and the source of publication.

**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**

---

**Article**

An inventory of geometrid moths (Lepidoptera: Geometroidea: Geometridae) of Kalakad-Mundanthurai Tiger Reserve, India

– Geetha Iyer, Dieter Stüning & Sanjay Sondhi, Pp. 19887–19920

---

**Communications**

Roadkills of Lowland Tapir Tapirus terrestris (Mammalia: Perissodactyla: Tapiridae) in one of its last refuges in the Atlantic Forest

– Aureo Banhos, Andressa Gatti, Marcelo Renan de Deus Santos, Leonardo Merçon, Ika Westermeyer, Natália Carneiro Ardenite, Luiz Francisco Oliveira Pereira Gonzaga, Lucas Mendes Barreto, Lucas Damásio, Tomas Lima Rocha, Vítor Roberto Schettino, Renata Valls, Helena Godoy Bergallo, Marcos Vincius Freitas Silva, Athelson Stefanon Bittencourt, Danielle de Oliveira Moreira & Ana Carolina Srbek-Araujo, Pp. 19921–19929

Scientific contributions and learning experiences of citizen volunteers with a small cat project in Sanjay Gandhi National Park, Mumbai, India

– Shomita Mukherjee, R. Nandini, P. V. Karunakaran & Nayan Khanolkar, Pp. 19930–19936

Seasonal food preferences and group activity pattern of Blackbuck Antilope cervicapra (L., 1758) (Mammalia: Cetartiodactyla: Bovidae) in a semi-arid region of western Haryana, India

– Vikram Delu, Dharambir Singh, Sumit Dookia, Priya & Kiran, Pp. 19937–19947

Recovery of vulture population in roosting and scavenging areas of Bastar and Bijapur, Chhattisgarh, India

– Sushil Kumar Dutta, Muntaz Khan, P.R.S. Nagi, Santosh Durgam & Surabhi Dutta, Pp. 19956–19963

A geographical assessment of Charringa and Arpara Beel (wetlands) of Nadia, West Bengal as a habitat of wetland birds

– Mehedri Hasan Mandal, Arindam Roy & Giasuddin Siddique, Pp. 19964–19975

Phenotypic plasticity in Cryptalaus ohira, 1967 (Coleoptera: Elateridae: Agrypninae) from two geographically distinct river basins of Indian Himalaya

– M. Pandian, Pp. 19948–19955

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

– Skarma Nonzom & Geeta Sumbali, Pp. 20118–20123

A rare photographic record of Eurasian Otter Lutra lutra with a note on its habitat from the Bhagirathi Basin, western Himalaya, India

– Ranjana Pal, Aashna Sharma, Vineet Kumar Dubey, Taapaji Bhattacharya, Jayaraj Antony Johnson, Kuppasamy Sivakumar & Sambandam Sathyakumar, Pp. 20072–20077

The first record of Medog Gliding Frog Rhacophorus translineatus Wu, 1977 (Anura: Rhacophoridae) from Chhukha District, Bhutan

– Sonam Unendup & Bal Krishna Koirala, Pp. 20078–20083

Butterflies of Amrabad Tiger Reserve, Telangana, India

– Deepa Jaiswal, B. Bharath, M. Karuthapandi, Shrikant Jadhav, S. Prabakaran & S. Rehanuma Sulthana, Pp. 20090–20097

An enumeration of the flowering plants of Kanyakosla Alpine Sanctuary in eastern Sikkim, India

– Sudhansu Sekhar Dash, Subhajit Lahiri & Ashishh Aosshii Mao, Pp. 20098–20107

New distribution data on the genus Maripanthus Maddison, 2020 (Araneae: Salticidae) and on the IUCN status of Ailurus fulgens (Mammalia: Carnivora: Ailuridae) in Arunachal Pradesh, India

– Jahan Ahmed, Sorang Tadap, Millo Tasser, Koj Rinya, Nekibuddin Ahmed & Sunil Kyrong, Pp. 20066–20071

Ailurus fulgens

– A. Asima, John T.D. Caleb, Dhruv A. Prajapati & G. Prasad, Pp. 20130–20132

Notes

New distribution data on the genus Maripanthus Maddison, 2020 (Araneae: Salticidae) and on the IUCN status of Ailurus fulgens (Mammalia: Carnivora: Ailuridae) in Arunachal Pradesh, India

– Skarma Nonzom & Geeta Sumbali, Pp. 20118–20123

Study on incidence and pathology of gastrointestinal parasitic infections in Nilgai Boselaphus tragocamelus in Hisar, Haryana, India

– Maneesh Sharma, B.L. Jangir, D. Lather, G.A. Chandratre, V. Nehra, K.K. Jakhar & G. Narang, Pp. 20124–20127

An unusual vocalization of Brown Hawk-Owl Ninox scutulata (Raffles, 1822) (Aves: Strigiformes: Strigidae) recorded from Kerala, India

– Riju P. Nair & Shine Raj Tholiludiyil, Pp. 20128–20129

New distribution data on the genus Maripanthus Maddison, 2020 (Araneae: Salticidae) and on the IUCN status of Ailurus fulgens (Mammalia: Carnivora: Ailuridae) in Arunachal Pradesh, India

– A. Asima, John T.D. Caleb, Dhruv A. Prajapati & G. Prasad, Pp. 20130–20132

On the IUCN status of Boesenbergia abolutesa and B. rubulotesa (Zingiberaceae) and typification of B. rubulotesa

– K. Aishwarya & M. Sabu, Pp. 20133–20135

New records of mass seeding Cephalostachyum latifolium Munro (Poaceae) along the mid-elevation broadleaved forest of Sarpang district, Bhutan

– Jigme Tenzin, Sangay Nidup & Dago Dorji, Pp. 20136–20139

Response

If habitat heterogeneity is effective for conservation of butterflies in urban landscapes of Delhi, India? Unethical publication based on data manipulation

– Sanjay Keshari Das & Rita Singh, Pp. 20140–20142

---

**Short Communications**

Successful rescue, medical management, rehabilitation, and translocation of a Red Panda Ailurus fulgens (Mammalia: Carnivora: Ailuridae) in Arunachal Pradesh, India

– Jahan Ahmed, Sorang Tadap, Millo Tasser, Koj Rinya, Nekibuddin Ahmed & Sunil Kyrong, Pp. 20066–20071

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

– Skarma Nonzom & Geeta Sumbali, Pp. 20118–20123

Notes

Study on incidence and pathology of gastrointestinal parasitic infections in Nilgai Boselaphus tragocamelus in Hisar, Haryana, India

– Maneesh Sharma, B.L. Jangir, D. Lather, G.A. Chandratre, V. Nehra, K.K. Jakhar & G. Narang, Pp. 20124–20127

An unusual vocalization of Brown Hawk-Owl Ninox scutulata (Raffles, 1822) (Aves: Strigiformes: Strigidae) recorded from Kerala, India

– Riju P. Nair & Shine Raj Tholiludiyil, Pp. 20128–20129

New distribution data on the genus Maripanthus Maddison, 2020 (Araneae: Salticidae) and on the IUCN status of Ailurus fulgens (Mammalia: Carnivora: Ailuridae) in Arunachal Pradesh, India

– A. Asima, John T.D. Caleb, Dhruv A. Prajapati & G. Prasad, Pp. 20130–20132

On the IUCN status of Boesenbergia abolutesa and B. rubulotesa (Zingiberaceae) and typification of B. rubulotesa

– K. Aishwarya & M. Sabu, Pp. 20133–20135

New records of mass seeding Cephalostachyum latifolium Munro (Poaceae) along the mid-elevation broadleaved forest of Sarpang district, Bhutan

– Jigme Tenzin, Sangay Nidup & Dago Dorji, Pp. 20136–20139

Response

If habitat heterogeneity is effective for conservation of butterflies in urban landscapes of Delhi, India? Unethical publication based on data manipulation

– Sanjay Keshari Das & Rita Singh, Pp. 20140–20142