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New record of *Chalastospora gossypii* from cold arid soil of the most isolated region in trans-Himalayas

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Abstract

A psychrotolerant hyphomycete *Chalastospora gossypii* was recovered from cold arid soil of Zanskar valley (3,500 msl), which is the most isolated region located in the trans-Himalayas that presents extreme environmental challenges for biotic system including microfungi. Complete description and illustration of the fungus based on morphology and rRNA gene sequences in ITS regions are provided. The report represents a new record in Indian sub-continent and reflects the ability of the reported fungus to thrive in extreme habitats of Indian sub-continent thus representing a new addition to cold desert as well as Indian mycoflora.

Key words – *Chalastospora gossypii* – India – new record – taxonomic description

Introduction

Zanskar is a high altitude semi-arid valley in Ladakh (30˚45' to 35˚50' N Latitude and 75˚45' to 80˚31' E Longitude), Jammu and Kashmir state (India), which lies on the northern flank of Great Himalayan range. During summer months, a warm and dry climate prevails with low moisture and scanty rainfall and a temperature range of 20-27 ºC as the mountain range acts as a climatic barrier. On the other hand, winter in this valley is freezing cold as the temperature goes below 0 ºC (-30ºC) due to heavy snowfall and some parts of it are considered as the coldest inhabited places of the world. Zanskar region usually remains land locked and isolated from rest of the country during the winters (November to May) because of the constant snowfall, snow blizzards and high velocity dust storms. Therefore, the soil remains covered by thick layers of snow and ice and its temperature usually remains below zero. In view of this, investigations were carried to explore the microfungal diversity of this secluded region.

Among the various microfungi recovered, a hyphomycete with an ambiguous history, *Chalastospora gossypii* (syn *Alternaria malorum* var. *polymorpha*) was isolated and identified, which represents a new report from such type of environment/habitat and from Indian subcontinent. The genus *Chalastospora* was established by Simmons (2007) based on *Chalastospora cetera*, formerly recognized as *Alternaria cetera*, characterised by production of conidia, which are usually narrowly ovoid to ellipsoid with 1-6 transverse eusepta, lacking oblique/longitudinal septa (Crous et al. 2009). *Chalastospora gossypii* has been initially described as *Cladosporium malorum* by earlier mycologists (Heald 1930, Ruehle 1931, Matsushima 1975). Some mycologists like De Vries (1952), Ellis (1971, 1976) included *Chalastospora* under the genus *Cladosporium* on the basis of the morphological similarities they shared. *Cladosporium malorum*, which is mostly saprobic hyphomycete has been isolated from different substrates including soil, grain, fruits, and grass litter

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(Braun et al. 2003). It was reported as early as 1974 by Marasas & Bredell (1974) from South Africa, although under the epithet *Phaeoramularia* (*P. kellermaniana*). Later, Matsushima (1975) assigned it to *Cladosporuium porophorum*. However, Braun & Feiler (1995) excluded *P. kellermaniana* from *Phaeoramularia* and treated it as *Cladophialophora* on the basis of morphological similarities. Later, Braun (1998) placed it under *Pseudocladosporium*, a genus which was introduced for anamorphs of *Caproventuria*. Ho et al. (1999) treated *Cladosporium porophorum, C. malorum* and *P. kellermaniana* as conspecific. However, comprehensive morphological investigations of varied cultures of *C. malorum* raised doubts concerning the accurate placement of this species under *Cladosporium* or *Pseudocladosporium*. These studies revealed conidiogenous loci to be distinctly tretic suggesting exclusion of *C. malorum* from *Cladosporium, Cladophialophora* and *Pseudocladosporium* (Braun et al. 2003). Conidiogenesis in this species closely resembled with that of the genus *Alternaria* assigned to the order Pleosporales and based on its ITS and SSU phylogenetic assessment, *C. malorum* appeared to be best assigned to *Alternaria* (Braun et al. 2003). The taxonomic decision to place this species in *Alternaria* was further supported by data on metabolites (altersolanol and macrosporin) produced by this species (Holler et al. 2002). Further, Crous et al. (2009) on the basis of molecular studies (sequence data of the ITS and LSU regions) assigned *Alternaria malorum* to the genus *Chalastospora* under its oldest epithet *C. gossypii* and introduced two new species of *Chalastospora* (*C.ellipsoidea* and *C. obclavata*). *Chalastospora* belongs to the order Pleosporales of the Class Dothideomycetes and 4 taxa have been enlisted under this genus viz., *C. cetera, C. ellipsoidea, C. gossypii* and *C. obclavata* (ref: www.indexfungorum.org).

Materials & Methods

Isolation and morphology

Soil samples were collected by scraping the superficial layer, not exceeding 3-5cm in depth, with the help of properly sterilized spatula. These samples were kept in pre-sterilised polythene bags and brought to the laboratory for the isolation of psychrotolerant microfungi. Dilution pour plate method was adopted using modified Czapek Dox agar (CDA) supplemented with Rose Bengal (0.1mg/100 ml) and streptomycin sulphate (50mg/1000ml) was followed for the isolation of microfungi. Microscopic line drawings were made with the help of camera lucida (Erma, Japan) at 400x and 1000x magnifications. Using an ocular micrometer, dimensions were determined for hyphae, conidiophores, ramoconidia and conidia. Microphotography of the lactophenol mounted fungal cultures was done using Sony N50 camera attached to an Olympus CH 20i binocular microscope.

PCR Amplification and sequencing of ITS Region

The fungal isolate was identified on the basis of its cultural and morphological characters and further confirmed by molecular characterization, which was carried out at the sequencing facility of National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune (India). At the facility, 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']. The amplified ITS PCR products were purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per manufacturer's instructions. Essentially, 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).

Results and Discussion

Chalastospora gossypii (Jacz.) Braun & Crous, Persoonia 22: 144 (2009)

Synonymy
Alternaria malorum var. *polymorpha* Dugan, in Braun, Crous, Dugan & de Hoog, *Mycol. Progr.* 2 (1): 8 (2003)
Alternaria malorum U. Braun, Crous & Dugan, in Braun, Crous, Dugan & de Hoog, *Mycol. Progr.* 2 (1): 5 (2003)
Alternaria malorum U. Braun, Crous & Dugan, in Braun, Crous, Dugan & de Hoog, *Mycol. Progr.* 2 (1): 5 (2003) var.*malorum*
Cladophialophora kellermaniana (Marasas & I.H. Bredell) U. Braun & Feiler, *Microbiol. Res.* 150 (1): 83 (1995)
Cladosporium gossypii Jacz., *Khlopkovoe Delo* 1929 (5-6): 564 (1929)
Cladosporium malorum Ruehle, *Phytopathology* 21: 1146 (1931)
Cladosporium malorum Heald, *Bull. Wash. agric. exp. Stn* 245: 48 (1930)
Cladosporium porophorum Matsush., *Icon. microfung. Matsush. lect.* (Kobe): 36 (1975)
Phaeoramularia kellermaniana Marasas & I.H. Bredell, *Bothalia* 11(3): 217 (1974)
Pseudocladosporium kellermanianum (Marasas & I.H. Bredell) U. Braun, *Monogr.*(1974)
Cercosporella, Ramularia Allied Genera (Phytopath. Hyphom.) 2: 393 (1998)

Taxonomic description

***Chalastospora* Simmons**

Conidiophores solitary, brown, smooth, arising from surface hyphae or as short, lateral branches from ropes of aerial hyphae; short, subcylindrical to flask-shaped, 0–2-transversely euseptate, seldom once geniculate or branched. Conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, monotretic, determinate to polytretic, sympodial, conidiogenous loci visible as minute pores, without or with somewhat darkened and slightly thickened rim. Conidia in acropetal, branched chains, narrowly ellipsoid to narrowly ovoid, beakless, pale to medium brown, rarely 0-3 transversely euseptate, generally lacking longitudinal or oblique septa; conidial apex functioning as secondary conidiophore, proliferating laterally.

***Chalastospora gossypii* (Jacz.) Braun & Crous**

Colonies on PDA 30-35 mm in diameter after 7 days of incubation at 28±2ºC, effuse, floccose, velvety to woolly, olivaceous-grey to deep olivaceous-green, reverse olivaceous to blackish olive. Mycelium septate, hyphae hyaline to pale brown 2.4-5.0µm wide, conidiophores macronematous, pale brown, smooth, long, 2.4-5.4µm wide; ramoconidia 0-3 septate, polyblastic, pigmented, cylindrical with prominent scars, 4.8-25× 5-8 µm bearing conidia in long chains simple or branched; conidia elongated, fusiform or narrowly ellipsoid to ovoid, golden brown, measuring 3.2-6.4 × 2.4-3.2 µm (Fig. 1a-e).

In an attempt to explore one of the most isolated regions i.e. Zanskar valley for its microfungal diversity, *Chalastospora gossypii* (syn *Alternaria malorum* var. *polymorpha*) was recovered, which is a new report of psychrotolerant microfungal species from India. Some other mitosporic fungal diversity has been reported from a similar peculiar barren habitat i.e. Moonland Ladakh (Nonzom & Sumbali 2015). As recommended by Jeewon & Hyde (2016), nucleotide sequences were compared and the ITS-rDNA sequence analysis showed the closest BLAST match of 99% (700 bp) similarity with *Alternaria malorum* var. *polymorpha* strain STE-U 4570 (Accession No. AY251080.2) and the identity was likewise confirmed on the basis of morphological and cultural characters. It was first reported from overwintered dormant buds of *Vitis vinifera* in Washington State, USA and was assigned distinct var. *polymorpha* on the basis of various characters (Braun et al. 2003). Its unique mode of conidiogenesis (alternarioid and catenulate conidia) in addition to DNA phylogeny studies support assigning *C. malorum* to *Alternaria malorum var. polymorpha* and can be distinguished from var. *malorum* mainly by the degree of septation, wider hyphae, thicker walls, greater width, and greater pigmentation. In addition, these additional conidia (having more than one septation) could become longitudinally

septate and, in rare cases, distinctly alternarioid/ muriform (Braun et al. 2003). However, the fungal isolate described during the present investigation lacks the muriform nature, although they exhibited larger size, deeper pigmentation and septation >1 (Fig. 1b, d). These conidia were produced on the same type of conidiophores as those producing normal conidia and occurred together with the regular conidia in the same chain (Fig. 1a-d).

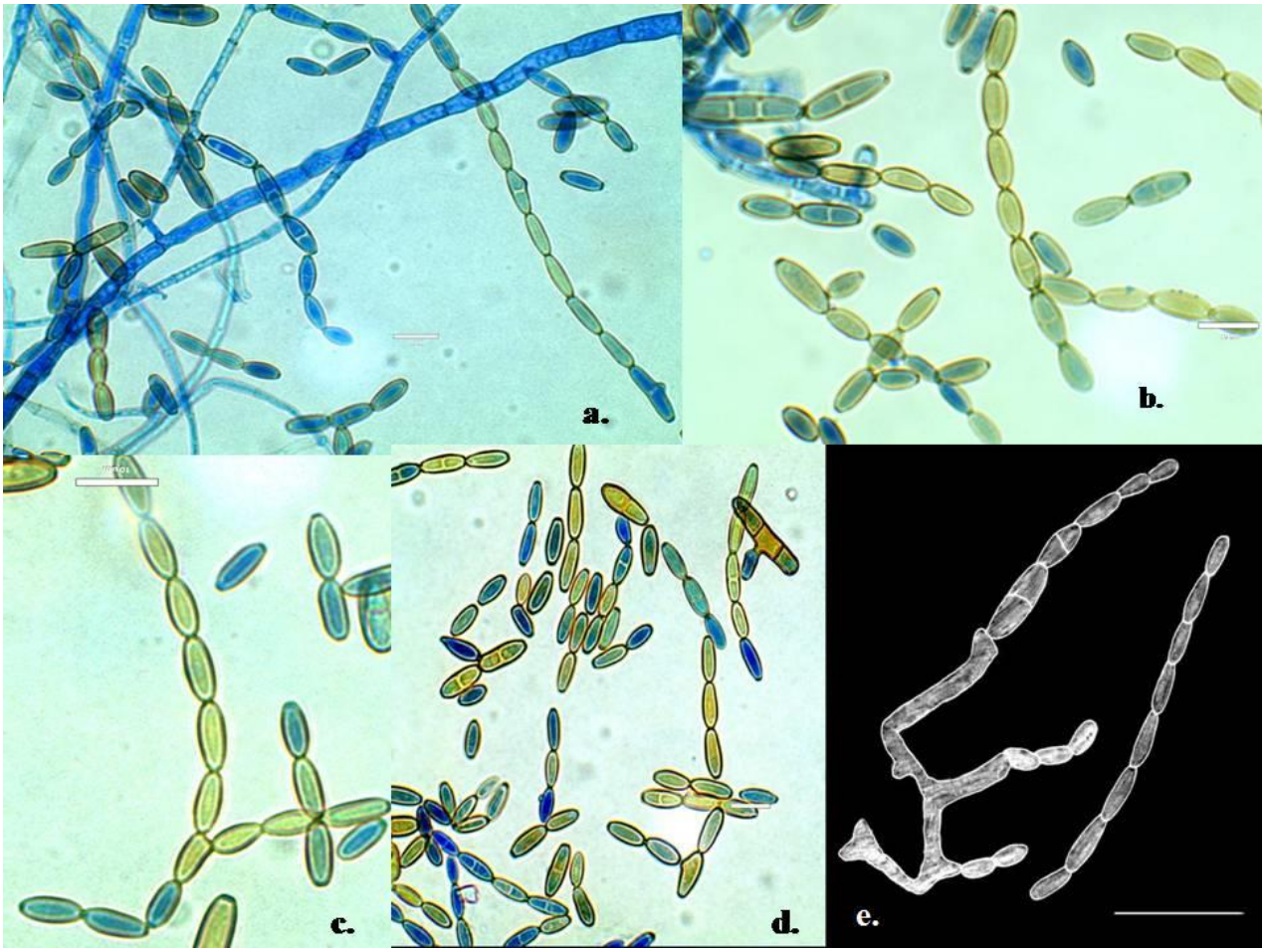

Fig. 1 – *Chalastospora gossypii* (Jacz.) Braun & Crous a-d Microphotographs. a Hyphae and conidia in chains. a, b, d Septate ramoconidia and conidia. c Conidiogenesis with two condial loci. e Camera lucida drawings of conidiophore and conidia. Scale Bars: a-d=10µm, e=14µm.

The current name assigned to *Alternaria malorum var. polymorpha* is *Chalastospora* as provided by Crous et al. (2009) on the basis of further molecular studies. It was as early as in 1929 that Jaczewski introduced the name *Cladosporium gossypii*. Type material of *C. gossypii* that was re-examined was found to be identical to *C. malorum* (Braun et al. 2003). This placement was certainly influenced by the cladosporioid habit of this fungus having pigmented, ramoconidia, conidia (0-2-septate) formed in long acropetal chains. The type of conidiogenesis (that appeared holoblastic) and the nature of the conidiogenous loci were undoubtedly misinterpreted by most of the previous mycologists who placed this fungus in *Cladosporium*, *Cladophialophora*, *Phaeoramularia* or *Pseudocladosporium*. As discussed earlier, Holler et al. (2002) recognized the ability of the said fungus to produce various metabolites known to be produced by various species of *Alternaria*, which was later confirmed by studies made by Braun et al. (2003), thereby shifting it from *Cladosporium malorum* to *Alternaria malorum*. In addition, based on the molecular and biochemical studies, Crous et al. (2009), Andersen et al. (2009) assigned *Alternaria malorum* and *Alternaria malorum var. polymorpha* to *Chalastospora*.

Some common *Alternaria* species such as *A. alternata*, *A. longipes* have been reported earlier by Nonzom & Sumbali (2015) from a similar cold arid soil (3200 msl), whereas there are no reports on the incidence of *Alternaria malorum* from arid regions, although they have been reported growing as saprophyte from soil, stored grains and so on (Nonzom & Sumbali 2015). On the other hand, *Alternaria malorum var. polymorpha* has been found to be solely associated with overwintered dormant buds, thereby indicating its ability to grow under cold conditions (Braun et al. 2003). There are no reports on the incidence or occurrence of this species thereafter. Therefore, the psychrotolerant fungal isolate recovered during the present investigation is reflecting its ability to survive not only in cold but also in oligotrophic arid conditions. Its prevalence and existence in such an extreme niche may be attributed to various morphological and physiological strategies that needs further considerations.

Conflict of interest

There is no conflict of interest among the authors for publishing this manuscript.

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