New record of *Geosmithia rufescens* from a high altitude pass in the trans-Himalayan region

SKARMA NONZOM
GEETA SUMBALI
Department of Botany
University of Jammu
B. R. Ambedkar Road
Jammu-180006, India
E-mail: skarmanunzom@yahoo.com

Accepted 14. August 2018. © Austrian Mycological Society, published online 15. January 2019

NONZOM, S., SUMBALI, G., 2018: New record of *Geosmithia rufescens* from a high altitude pass in the trans-Himalayan region. – Österr. Z. Pilzk. 27: 1–4.

Key words: *Geosmithia rufescens,* new record. – Mycota of India.

Abstract: The mitosporic filamentous fungus *Geosmithia rufescens* was recovered from a high altitude pass at 4000 m s. m. of Ladakh, located in the trans-Himalayan region (northernmost area in India) and identified using morphological characters and DNA sequences. It is described and illustrated in detail The very rare fungus has characteristic lilac, cylindroidal conidia in long chains. It comes from an extreme habitat and is thus a new representative worldwide for the desert Funga. Previously it was known from Costa Rica, its find is a new contribution to the Indian Funga.

Zusammenfassung: Der mitospore filamentöse Pilz *Geosmithia rufescens* wurde aus 4000 m Seehöhe von einem Pass in Ladakh in der trans-Himalaya-Region (nördlichstes Gebiet in Indien) isoliert und anhand von morphologischen Merkmalen und DNA-Sequenzen identifiziert. Er wird detailliert beschrieben und illustriert. Der sehr seltene Pilz hat charakteristische lila, zylindroide Konidien in langen Ketten. Er stammt aus einem extremen Lebensraum und ist damit ein neuer Vertreter weltweit für die Wüstenfunga. Bisher war er aus Costa Rica bekannt, ist sein Fund ein neuer Beitrag zur indischen Funga.

Sapi La is a high altitude pass (around 4 000 m s. m.) located in Ladakh region (30˚ 45' to 35˚ 50' N latitude and 75˚ 45' to 80˚ 31' E longitude), which falls in the trans-Himalayan region. This high altitude pass is characterized by strong wind currents round the year and remains covered by thick layers of snow for about six months during the freezing cold winters as the temperature goes below 0 ºC (–35 ºC). During these six months the whole Ladakh region generally remains land locked and inaccessible from the rest of the country owing to the frequent snowfall, snowstorms and high velocity dust storms.

The genus *Geosmithia* established in 1979 by PITT is characterized by *Penicillium*-like conidiophores with roughened walls, cylindrical phialides and smooth ellipsoidal to cylindrical conidia forming long persistent chains. This genus was erected to accommodate species, previously placed in *Penicillium*, with the combination of characters such as colonies with conidia in colours other than blue-grey or grey-green, penicilli with all elements roughened, and with both phialides and conidia cylindroidal. *Geosmithia* belongs to the *Hypocreales* (*Sordariomycetes*) and currently comprises 21 taxa (www.indexfungorum.org). Most of its species (including *G. rufescens*) have been reported to be

consistently associated with subcorticolous insects (KOLARIK & al. 2004, 2005, 2015; KOLARIK & KIRKENDALL 2010; LIN & al. 2016).

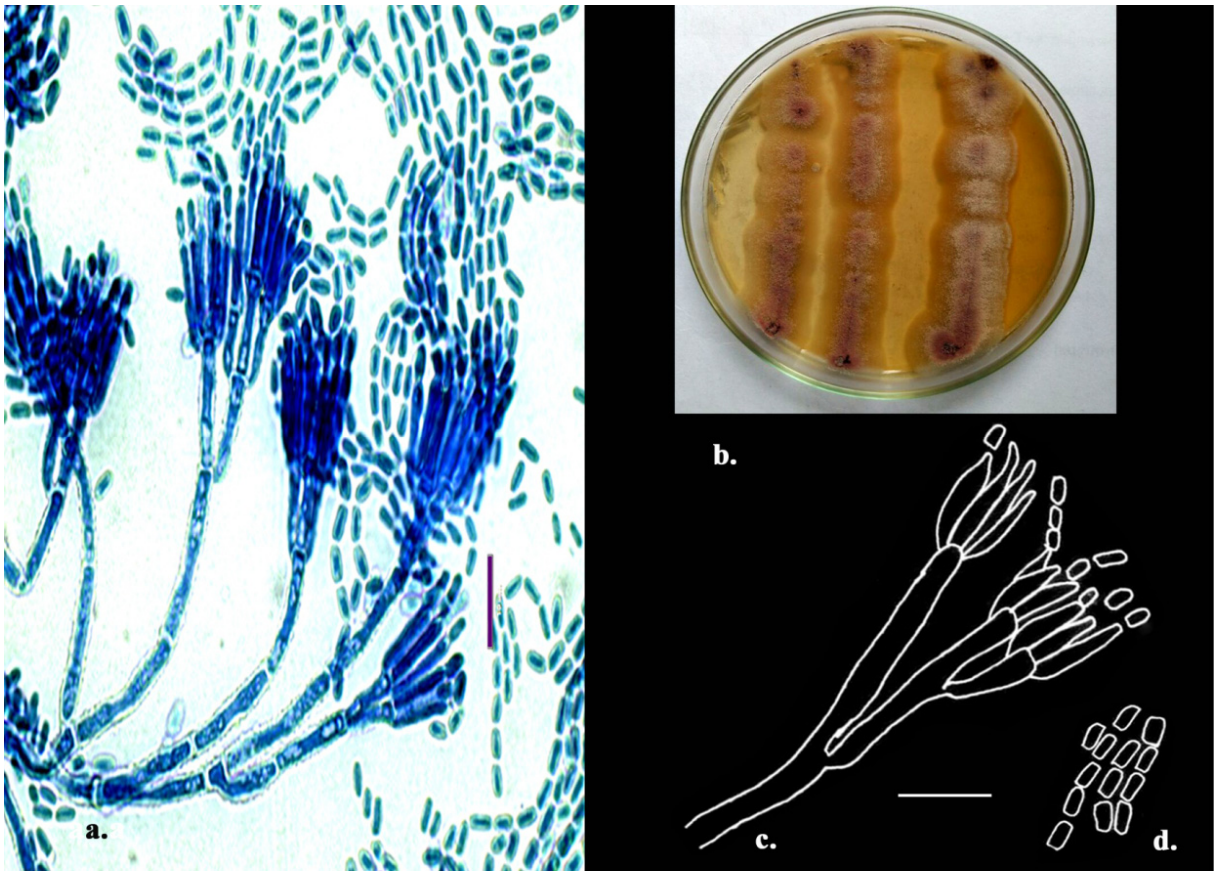

Fig. 1. *Geosmithia rufescens. a* Conidiophore bearing chains of conidia, *b* colonies on MEA, 28±2 ºC, 7 d. c, d. Conidiophore and conidia. Bars *a* 10 µm; *c, d* 14 µm.

Materials and methods

Soil samples were collected aseptically from Sapi La and brought to the laboratory in sterilized polythene bags. For the isolation of soil microfungi the dilution plate method using modified Czapek Dox agar supplemented with Rose Bengal (0.1 mg/100 ml) and streptomycin sulphate (50 mg/1000 ml) was followed. The fungal isolate obtained 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. At the facility, genomic DNA was isolated by the standard phenol/chloroform extraction method of SAMBROOK & al. (1989), followed by PCR amplification of the ITS regions using the primers ITS1 and ITS4. The PCR product was purified by PEG-NaCl precipitation and directly bidirectionally sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per manufacturer's instructions. Assembly was carried out using Lasergene package followed by NCBI BLAST against sequences from type material for tentative identification (BORATYN & al. 2013).

Microscopic drawings were made with the aid of camera lucida (Erma, Japan) at 400× and 1000× magnification. Conidiophores, metulae, phialides and conidia were measured using an ocular micrometre. Microphotography was done using Sony N50 camera attached to an Olympus CH 20i binocular microscope.

Results and discussion

Geosmithia rufescens KOLARIK, Fungal Biology 114: 685 (2010) (Fig. 1)

Description of the Indian isolate:

C u l t u r e s : growing rapidly on PDA and MEA at 28±2 ºC, margins narrow, submerged, colony plane, raised and wrinkled centrally, surface texture velutinous or granular with a crust of conidia; mycelium initially white, sporulation heavy, lilac to mauve; exudates absent; reverse rusty to dark chestnut (Fig. 1b).

C o n i d i o p h o r e s : penicillate, closely packed in the palisade; stipe 2–3 µm wide, verrucose; bi-, ter- or quaterverticillate, rarely more branched, rami symmetric or asymmetric (1-branched); metulae 8.0–10 × 1.4–2.0 µm; phialides mainly 7–12 × 1.4 µm, 3–6 per cluster.

C o n i d i a : cylindrical to subcylindrical, hyaline, aseptate, 3.5–4.3 × 1.4–1.6 µm, in highly persistent chains with narrowly truncate base (Fig. 1 a, c).

Comments:

The psychrotolerant *Geosmithia* isolate recovered during the present investigation exhibits a novel ecological strategy among the *Geosmithia* species. The ITS sequences had the closest BLAST similarity (98 %, 700bp) with the type of *G. rufescens* (GenBank Acc. no. NR_137536) and the identity was equally confirmed on the basis of morphological and cultural studies. However, the present fungal isolate has comparatively narrower branches, phialides and conidia than the original isolate. This may be probably attributed to the incessant unfavourable conditions prevailing in the study area throughout the year in the form of strong wind currents, lack of available nutrients, extremely low soil temperature for most of the time owing to the heavy snowfall in the region. Another hypothesis is that there might be more species, which could eventually be unravelled by a multigene phylogeny, because already KOLAŘÍK & KIRKENDALL (2010) wrote that the ITS rDNA sequences sometimes were not diagnostic, suggesting the use of additional markers, e. g. EF-1α, IGS rDNA or β-tubulin, together with morphology and ecology for species delimitation.

Tab. 1. Comparison of two *Geosmithia* spp. inhabiting high altitudes in Sapi La (India, 4000 m s. m.) and Qinghai-Tibet plateau (China, 4100 m s. m.).

Species	Mycelium, sporulation	Branching, metulae	Phialides	Conidia
G. rufescens	Initially white, branched, septate, smooth, hyaline, sporulation heavy, lilac to mauve	Bi-, ter- or quaterverticillate, conidiophores hyaline, verrucose, 8–10 × 1.4–2 µm	Terminal, smooth to slightly verrucose, cylindrical, 7–12 × 1.4 µm	Cylindrical to subcylindrical, hyaline, narrowly truncate base, 3.5–4.3 × 1.4–1.6 µm
G. tibetens	Yellow brown, branched, septate, smooth, subhyaline to brown, pale brown	Mono-, biverticillate, conidiophores light brown, verrucose, 6–15 × 3–5 µm	Terminal, verrucose, cylindrical, clavate or ob-clavate 8–20 × 3–5 µm	Smooth, ellipsoidal to oblong, pale brown, pointed apex, subtruncate base, 4.5–6 × 3–4.2 µm

Geosmithia rufescens was described by KOLAŘÍK (KOLAŘÍK & KIRKENDALL 2010) as a new species which inhabited the gallery of ambrosia beetles in Costa Rica. Since then, there are no reports on the occurrence and distribution of this fungus from similar or other habitats. In fact, extensive work carried out earlier on bark beetle galleries by KOLAŘÍK & al. (2004, 2005, 2007, 2015) and KOLAŘÍK & KIRKENDALL (2010) have led to the addition of diverse *Geosmithia* species. Later, LIN & al. (2016) studied fungi inhabiting beetle galleries in Taiwan and reported two new records of *Geosmithia* species indicating their common association with beetles and beetle-associated substrates. However, the role of the *Geosmithia*-beetle association is still unclear.

In addition to beetle-associated *Geosmithia* spp., few species have been reported to be geophilic, such as *G. viridis*, *G. malachitea*, *G. eburnea* and *G. tibetensis* (PITT & HOCKING 1985; YAGUCHI & al. 1993; 1994; WU & al. 2013). Among these, *G. tibetensis* has been reported from a similar high altitude (4100 m s. m.) grassland soil from Qinghai-Tibet plateau, China (WU & al. 2013). However, the two species are quite different and compared in Tab. 1.

From India, this is the first report of *Geosmithia rufescens,* isolated from a high altitude cold arid region (Sapi La) of Kargil district, Ladakh (J&K) reflecting the ability of this species to thrive in strikingly contrasting habitats.

The first author is grateful to University Grants Commission (UGC), New Delhi for the financial assistance in the form of Rajiv Gandhi National Fellowship (RGNF), which facilitated the study.

References

BORATYN, G. M., CAMACHO, C., COOPER, P. S., COULOURIS, G., FONG, A., MA, N., MADDEN, T. L., MATTEN, W. T., MCGINNIS, S. D., MEREZHUK, Y., RAYTSELIS, Y., SAYERS, E. W., TAO, T., YE, J., ZARETSKAYA, I., 2013: BLAST: a more efficient report with usability improvements. – Nucl. Acids Res. **41**: 29–33.

KOLARIK, M., KIRKENDALL, L. R., 2010: Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* PITT (*Ascomycota: Hypocreales*). – Fungal Biology **114**: 676–689.

KOLAŘÍK, M., HULCR, J., KIRKENDALL, L. R., 2015: New species of *Geosmithia* and *Graphium* associated with ambrosia beetles in Costa Rica. – Czech Mycol. **67**: 29–35.

KOLARIK, M., KUBTOVA, A., PARUTKA, P., 2004: Morphological and molecular characterisation of *Geosmithia putterillii*, *G. pallida* comb. nov. and *G. flava* sp. nov., associated with subcorticolous insects. – Mycol. Res. **108**: 1053–1069.

KOLARIK, M., KUBTOVA, A., CEPICKA, I., PARUTKA, P., 2005: A complex of three new white-spored, sympatric, and host range limited *Geosmithia* species. – Mycol. Res. **109**: 1323–1336.

KOLARIK, M., KUBTOVA, A., HULCR, J., PAZOUTOVA, S., 2007: *Geosmithia* fungi are highly diverse and consistent bark beetle associates: evidences from their community structure in temperate Europe. – Microbial Ecol. **55**: 65–80.

LIN, Y.T., SHIH, H. H., HUANG, Y. T., LIN, C. S., CHen, C. Y., 2016: Two species of beetle-associated *Geosmithia* in Taiwan. – Fung. Sci. **31**: 29–36.

PITT, J. I., 1979: *Geosmithia* gen. nov. for *Penicillium lavendulum* and related species. – Canad. J. Bot. **57**: 2021–2030.

PITT, J. I., HOCKING, A. D., 1985: Interfaces among genera related to *Aspergillus* and *Penicillium*. – Mycologia **77**: 810–824.

SAMBROOK, J, FRITSCH, E. F., MANIATIS, T., 1989: Molecular cloning a laboratory manual. – New York: Cold Spring Harbor Laboratory Press.

WU, Y.-M., XU, J.-J., WANG, H.-F., ZHANG, T.-Y., 2013: *Geosmithia tibetensis* sp. nov. and new *Gibellulopsis* and *Scopulariopsis* records from Qinghai-Tibet. – Mycotaxon **125**: 59–64.

YAGUCHI, T., MIYADOH, S., UDAGAWA, S., 1993: Two new species of *Talaromyces* from soil. – Transact. Mycol. Soc. Japan **34**: 245–254.

YAGUCHI, T., SOMEYA, A., UDAGAWA, S., 1994: Two new species of *Talaromyces* from Taiwan and Japan. – Mycosci. **35**: 249–255.