Some keratinophilic fungi new to India

RAHUL SHARMA

J. Mycopathol, Res, 54(1) : 35-39, 2016;
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. ***54(1)*** *: 35-39, 2016;* (ISSN 0971-3719)

Some keratinophilic fungi new to India

RAHUL SHARMA
Centre for Biodiversity Exploration and Conservation (CBEC),15 Kundan Residency, 4th Mile Mandla Road, Tilhari via RFRC, Jabalpur 482021, Madhya Pradesh

Received : 02.03.2015 RMS Accepted : 22.07.2015 Published : 25.04.2016

In a previous study from central India and a recent survey of Pune city we found the presence of four keratinophilic fungi never recorded earlier from Indian soils. Three of them are Onygenalean ascomycetes viz. *Aphanoascus saturnoideus*,*Apinisia recovitzae* and *Bifidocarpus cubensis* while the fourth one *Cryptumbellata terricola* is a hyphomycete. The fungi were identified on the basis of their typical morphology revealed by scanning electron microscopy (SEM). The geographical distribution of these keratinophilic fungi is expanded, since some are very rare and have not been recorded anywhere in the world after their first description. *B. cubensis* and *C. terricola* are previously known only from their type locality Cuba and China respectively. The present study elaborates the morphology of *C. terricola* by SEM, since the original description is based on light microscopy.

Key words: Keratinophilic fungi, India, *Aphanoascus saturnoideus*, *Apinisia recovitzae*, *Bifidocarpus cubensis, Cryptumbellata terricola*, new records, SEM

INTRODUCTION

Keratinophilic fungi are specialized fungal forms that mostly live in soil surviving on keratinous substrate(s) shed by birds, reptiles and mammals. The survival of these fungi in a particular habitat largely depends on the availability of keratinous substrate like feather, hair, skin, hooves etc. The abundance and distribution of these fungi is prominent in such locations where there is regular supply of keratin like public places, burrows, poultry, barber's dump etc. True keratinophilic fungi are mostly confined to single ascomycete order, the Onygenales, having six families, Ajellomycetaceae, Arthrodermataceae, Ascosphearaceae, Gymnoascaceae, Nannizziopsiaceae and Onygenaceae.

However, members of other groups like hyphomycetes, zygomycetes and chytrids etc. are also found to degrade keratin (Karling, 1946, 1947, 1948; Malviya *et al*, 1992a; 1992b; Rajak *et al,* 1992; Udagawa and Uchiyama 1999). Several of the Onygenalean fungi are potential pathogens of humans and other animals, members of family Ajellomycetaceae are all pathogenic while other families have both pathogenic and non-pathogenic members. Recently, we described two

new keratinophilic fungi from Indian soil viz. *Auxarthronopsis bandhavgarhensis* Sharma, Graeser and Singh (Sharma *et al*, 2013) and *Gymnoascus verruculosus* Sharma and Singh (Sharma and Singh, 2013).

Soil is a reservoir of saprophytic fungi capable of degrading various natural substrates of plant and animal origin viz. cellulose, lignin, pectin etc. (plants) and chitin, keratin etc. (animals). Of the natural substrates, keratin is widespread in urban and rural settings as well as natural forests. Looking to the fact that protected forest harbours a wide variety of wild animals (birds, reptiles and mammals) which shed keratinous substrates, a study was conducted in National Parks of Central India which included Bandhavgarh, Kanha and Pench tiger reserves to look for yet unrecorded keratinophilic fungal forms. Recently a new genus *Auxarthronopsis* was described from the vicinity of Bandhavgarh National Park (Sharma *et al*, 2013). The present communication reports other keratinophilic ascomycete *Aphanoascus saturnoideus* that has previously never been recovered from Indian soils.

As part of our on-going program to study keratinophilic fungi in Indian soil, samples were collected in the city of Pune and analysed for presence of keratin degrading fungi. The present paper reports first occurrence of four keratinophilic fungi from India from our recent findings in Pune city and a previous study carried in Bandhavgarh National Park situated in central India.

MATERIALS AND METHODS

Soil was collected in sealed polythene bags using sterile spatula and brought to laboratory for processing. The soil sample was stored at room temperature. Vanbreusegham's hair baiting technique was employed for recovery of fungus from soil (Sharma and Rajak, 2003) and subsequent isolation on SDA medium by micro-dilution drop-trail method (Sharma *et al*, 2002) Mature ascomata were mounted in lacto-phenol and visualized either under Nikon E800 light research microscope. Photomicrographs were taken with either Nikon HIII camera. For scanning electron microscopy died ascomata (peridium) were mounted onto stubs with the help of double sided tape and photographs taken by JEOL EM6100 microscope at 10 KV accelerating voltage. A pure culture of the fungus if obtained was deposited in Microbial Type Culture Collection (MTCC), Chandigarh.

RESULTS AND DISCUSSION

Taxonomy

Aphanoascus saturnoideus Cano and Guarro, Mycological Research 94: 370, 1990. Soil sample B-26 collected from Uttar Godhi area of Bandhavgarh National Park, India resulted in a keratinophilic fungus *Aphanoascus saturnoideus*. The fungus was identified on the basis of its typical Saturn shaped discoid ascospores. The fungus produced numerous pale to dark brown ascomata on hair-baits as well as on SDA medium. The fungus is first time being reported from India.

Colony on SDA, initially white turning pale brownish due to formation of numerous ascomata, 9 cm after 21 days, reverse yellow to orange-brown. Ascomata numerous, pale brown initially, turning dark brown. The ascospores are subglobose to oblate with narrow equatorial ring. The ascospores appear smooth under light microscope however they are finely pitted throughout the surface when viewed under scanning electron microscope (Fig 1a-f). The ascospores of *A. saturnoideus* are largest among all *Aphanoascus* species with equatorial rim. The species was first described by Cano and Guarro from Spanish soils in 1990 (Cano and Guarro, 1990) and subsequently reported from Japanese soils (Uchiyama *et al*, 1995; Udagawa and Uchiyama, 2000). The species forms a distinct clade among the disc shaped ascospore forming *Aphanoascus* species (Cano *et al*, 2002) the phylogenetic separation from other species corresponds to its morphological distinctness i.e. unusually large ascospores.

The identity of *Aphanoascus* species largely depends on the SEM data because ascospores culturing (as revealed by SEM) is the key to species recognition apart from molecular evidence. It seems that the gap between the species of *Aphanoascus* known from India and those from around the world is due to lack of SEM in mycological studies in India. Currently there are 7 species of *Aphanoascus* known in India including *A. saturnoideus* out of total 19 species known worldwide (Cano and Guarro, 1990; Cano *et al*, 1990; Pivkin and Khudyakova, 2002; Cano *et al*, 2002) making it one of the largest genera of Onygenales. The other six species known from India are *A.*

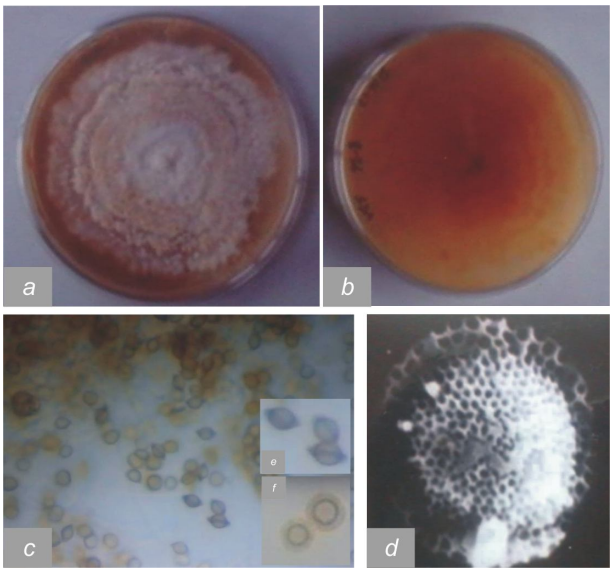

Fig. 1 : *Aphanoascus saturnoideus* Cano, and Guarro 1991; **a**-Culture front; **b**-culture reverse; **c**-smashed preparation of ascus; **d**-pitted pattern of a single ascospore (polar view); **e**-enlarged view of portion of Fig 1c showing ascospores in equatorial view; **f** –ascospores polar view.

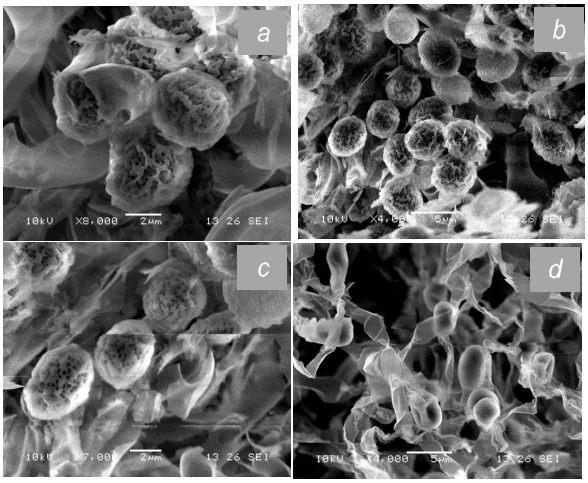

Fig. 2 : *Apinisia recovitzae* (Lagarde) Guarro, Cano and de Vroey 1991 (a-d); **a**-broken ascus with remains of ascus sheath; **b**-young and mature ascospores; **c**-ascospores showing finely punctate-reticulate walls; **d**-anamorph *Chrysosporium* stage.

Durus (Sharma *et al*, 2006; Deshmukh and Verekar 2006), *A. fulvescens* (Singh *et al*, 2009), *A. keratinophilus* (Ramesh and Hilda, 1998-1999), *A. punsolae* Deshmukh and Verekar, 2006), *A. reticulosporus* (Sharma *et al*, 2006) and *A. terreus* (Randhawa and Sandhu, 1964; Jain and Agarwal, 1977; Ghosh and Biswas; 1995; Saxena *et al,* 2004; Sharma *et al,* 2006; Sharma, 2014).

Material Examined

Soil sample B-26, leg Rahul Sharma and ND Sharma, 17.10.2006, under rock alongside road, Uttar Godhi, Bandhavgarh National Park, MP, India. Living culture MTCC 8694 (with anamorph and teleomorph stages).

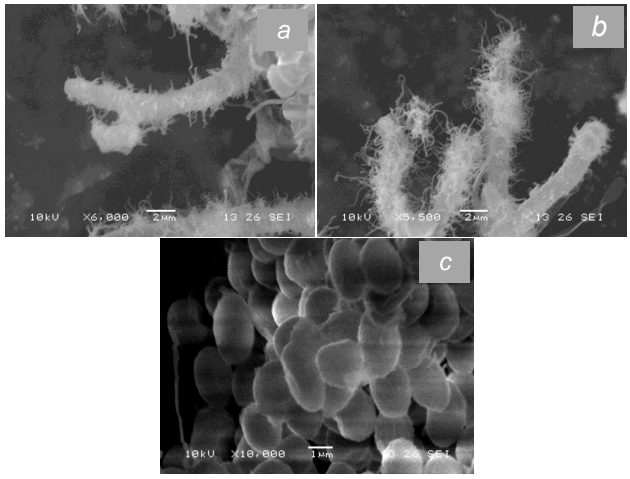

Fig. 3 : *Bifidocarpus cubensis* Cano, Guarro and R.F. Castenada 1994 (a-c)*;* **a,b**-*Bifurcating peridial* appendages; **c**-smooth walled ascospores.

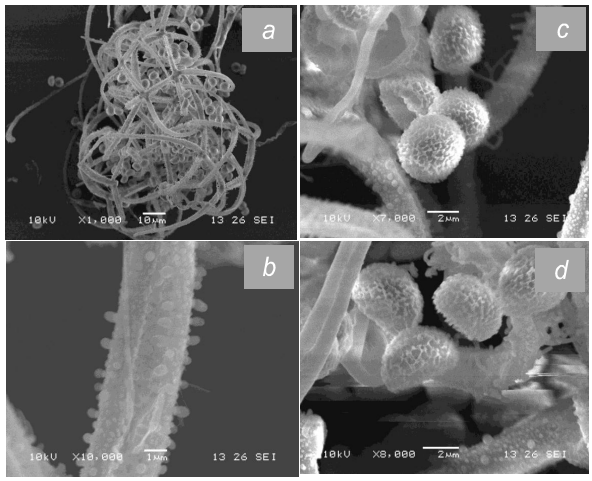

Fig. 4 : *Cryptumbellata terricola* Udagawa and Uchiyama 1999 (a-d);a- conidiomata showing perdium like structure; b-close-up of hyphal wall; c,d-conidia

Apinisia recovitzae (Lagarde) Guarro, Cano and de Vroey, Mycotaxon 42: 199, 1991. a" *Myxotrichum recovitzae* Lagarde, Arch. Zool. Exp. Gen. 53: 280, 1913. *Gymnoascus recovitzae* (Lagarde) Lagarde, Arch. Zool. Exp. Gen. 53: 281, 1913.*Kuehniella recovitzae* (Lagarde) Orr, Mycotaxon 4: 171, 1976. Fig 2a-d this fungus is first time recorded from India after its first discover in 1913 by Lagarde (Guarro *et al*, 2011). The fungus forms typical asci with sheath around ascospores, which are finely and irregularly punctate-

reticulate (see Fig 2a-c).

Material Examined

Soil sample CBEC 014, leg Rahul Sharma and Rohit Sharma, 01.V.2014, alongside road, Sangvi, MS, India. LM slide no. SLPUN006 slide from horse hair baited soil (with anamorph and teleomorph stages).

Bifidocarpus cubensis Cano, Guarro and R.F. Castenada, Mycotaxon 52: 53, 1994. (Fig. 3a-c). *Bifidocarpus cubensis* is the type species of the genus and has been recorded only once when it was first described by Guarro *et al* in 1994 (Cano *et al*, 1994; Guarro *et al*, 1991; Guarro *et al*, 2011), from forest soil in Cuba. This is the second report of the fungus from the world and was observed on goat dung in soil collected in Hadapsar, Pune city.

Material Examined

Soil sample No. Pune-67, leg Rahul and Rohit Sharma, 17.05.2014, goat sitting area under trees, industrial area, Hadapsar, Pune, India. LM slide no. SLPUN67, ascomata, asci and ascospores.

Cryptumbellata terricola Udagawa & Uchiyama, Mycotaxon 70: 186, 1999. (Fig. 4a-d). The genus *Cryptumbellata* was recorded only once when it was first described by Udagawa & Uchiyama in 19997 with *C. terricola* as its type species isolated from soil associated with beehive of *Vespula* collected in China. The fungus showed keratinophilic activity. The fungus forms appendaged sporodochia resembling a gymnothecium which covered the conidia situated centrally. This is the first reported SEM study of the fungus ever after its first discovery in 1999, revealing the spore surface pattern of the fungus more clearly (Fig. 4c,d). The present specimen developed sporodochia (Fig. 4a) on horse hair used as baits in soil collected under Banyan tree along road side, Pune city; on prolonged incubation it was overgrown by other saprophytic fungi and was lost for further isolation from same soil hair-baited plate.

Material Examined

Soil sample No. Pune-20, leg Rahul & Rohit Sharma, 08.05.2014, under Banyan tree along roadside, Ganeshkhind, Pune, India. LM slide no. SLPUN0020, sporodochia, conidia.

ACKNOWLEDGEMENTS

Author thanks Dr Rohit Sharma and Dr ND Sharma for company during soil collections in various capacities. Mr SV Shende, Pune University and Mr ML Sharma Punjab University Chandigarh for scanning electron microscopy He thanks Principal Chief Conservator of Forest (PCCF) Wildlife, Bhopal and Field Director, Bandhavgarh National Park (Mr Aseem Srivastava) for permission to collect one of the soil samples. Dr Ahilesh Kumer Pandey (RDU) and Dr San Prasad Gautam (then Head) Department of Biological Sciences, Rani Durgavati University, Jabalpur are acknowledged for laboratory facility for carrying out part of the work under National Park project (No. SR/FT/LS-36/2005) funded by Department of Science and Technology (DST), Govt. of India under Fast Track scheme for young scientists, and to CBEC where most of the study was undertaken.

REFERENCES

Cano J. and Guarro J., 1990, The genus *Aphanoascus*. *Mycological Research*, **94**: 355-377.

Cano, J., Guarro, J., and Zaror, L., 1990, Two new species of *Aphanoascus* (ASCOMYCOTINA).*Mycotaxon*, **38**: 161-166.

Cano, J., Guarro J. and Castenada R.F., 1994, Studies on keratinophilic fungi IV. *Bifidocarpus*, a new genus of the Eurotiales. *Mycotaxon* **52**: 53-57.

Cano, J., Sagues M., Barrio E., Vidal P., Castenada R. F., Gene J. and Guarro J., 2002, Molecular taxonomy of *Aphanoascus* and description of two new species from soil. *Studies in Mycology*, **47**: 153-164.

Deshmukh, S., K., and Verekar, S. A., 2006, Keratinophilic fungi from the vicinity of meteorite crater soils of Lonar (India). *Mycopathologia*, **162**: 303-306.

Ghosh, G. R., and Biswas, S. B., 1995, Keratinophilic fungi from Bharatpur bird sanctuary. *Indian Journal of Microbiology*, **35**: 153-159.

Guarro, J., Cano, J. and de Vroey, Ch., 1991, *Nannizziopsis* (Ascomycotina) and related genera. *Mycotaxon* **42**: 193-200.

Guarro, J., Gene, J., Stchigel A. M. and Figureas, M., 2011, *Atlas of Soil Ascomycetes*. CBS Biodiversity Series 10. CBS KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 45-46.

Guarro, J., Gene, J., Stchigel A. M. and Figureas, M., 2011, *Atlas of Soil Ascomycetes*. CBS Biodiversity Series 10. CBS KNAW Fungal Biodiversity Cebtre, Utrecht, The Netherlands, pp. 95-96-44.

Jain, P. C. and Agarwal, S. C., 1977. Keratinophilic fungi from soils of Mount Abu (India). *Geobios* **4:** 136-138.

Karling, J. S., 1946, Keratinophilic chytrids. I *Rhizophydium keratinophilum* n.sp., a saprophyte isolated on human hair, and its parasite *Phlyctidium mycetophagum* n.sp. *American Journal of Botany*, **33**: 751-757.

Karling, J. S., 1947. Keratinophilic chytrids. II *Phlyctorhiza variabilis* n. sp. *American Journal of Botany*, **34**: 27-32.

Karling, J. S., 1948. Keratinophilic chytrids. III *Rhizophydium nodulosum* n. sp. *Mycologia*, **40**: 328-335.

Malviya, H.K., Rajak, R.C. and Hasija, H.K., 1992a. Purification and partial characterization of two extracellular keratinases of *Scopulariopsis brevicaulis*. *Mycopathologia*, **119**: 993-995.

Malviya, H.K., Rajak, R. C. and Hasija, H.K., 1992b. *In vitro* degradation of hair keratin by *Graphium pennicilloideus*: Evidence of sulphitolysis and peptidolysis. *Cryptogamic Botany*, **3**: 197-201.

Pivkin, M. V. and Khudyakova, Y. V., 2002. A new species of *Aphanoascus* (Ascomycota) with a*Malbranchea* anamorph from marine bottom deposits. *Mycotaxon*, **81**: 7-10.

Rajak, R.C., Malviya, H.K., Despande, H. and Hasija, S. K. 1992, Keratinolysis by *Absidia cylindrospora*and *Rhizomucor pusillus*: biochemical proof. *Mycopathologia* **118**: 109-114.

Ramesh, V., M., and Hilda, A. 1998-1999. Incidence of keratinophilic fungi in the soil of primary schools and public parks of Madras city, India. *Mycopathologia*, **143**: 139-145.

Randhawa, H. S., and Sandhu, R.S. 1964. *Keratinophyton, terreum* gen. nov., sp. nov., a keratinophilic fungus from soil in India. *Sabouraudia*, **3:** 251-256.

Saxena, P., Kumar, A. and Shrivastav, J. N. 2004. Diversity of keratinophilic mycoflora in the soil of Agra (India). *Folia Microbiol.* (Praha), **49**: 430-434.

Sharma, R., Rajak, R. C. and Pandey, A. K., 2006, Keratinophilic ascomycetes of Central India.*Mycopathological Research*, **44**: 27-38.

Sharma, R., Graeser, Y. and Singh, S. K., 2013, *Auxarthronopsis*, a new genus of *Onygenales* isolated from the vicinity of Bandhavgarh National Park, India. *IMA Fungus*, **4**: 89-102.

Sharma, R., and Singh, S. K., 2013, A new species of *Gymnoascus* with verruculose ascospores. *IMA Fungus*, **4**: 177-186.

Sharma, R., 2014, Study shows some pathogenic fungi from Pune (India) soils (unpublished).

Singh, I., Mishra, A., & Kushwaha, R., 2009, Dermatophytes, related keratinophilic and opportunistic fungi in indoor dust of houses and hospitals. *Indian J. Medical Microbiology*, **27**, 242-246.

Uchiyama, S., Kamiya, S. and Udagawa, S., 1995, Five Onygenalean fungi from Japan. *Mycoscience,* **36**: 211-220.

Udagawa, S. and Uchiyama, S., 1999, *Cryptumbellata*, a new hyphomycete genus from soil. *Mycotaxon*,**70**: 185-192.

Udagawa, S. and Uchiyama, S., 2000, Materials for the fungus flora of Japan (55). *Mycoscience*, **41**: 263-267.