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SHORT COMMUNICATION

Cantharellus pseudoformosus, a new species associated with *Cedrus deodara* from India

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Abstract The genus *Cantharellus* is known to have a global distribution and to form ectomycorrhizal associations with a diverse set of host plants. Here we describe *Cantharellus pseudoformosus* as a species new to science with a possible association with *Cedrus deodara*. ITS and LSU data demonstrated that the material from India is distinct from *Cantharellus formosus* and other closely related species.

Keywords Chanterelle · Edible mushroom · ITS · LSU · Taxonomy

Introduction

Cantharellus species are wild edible mushrooms harvested from Europe, Africa, Asia, and North America (Danell 1999; Dunham et al. 2003a,b). Species of *Cantharellus* form ectomycorrhizal associations with a wide range of economically important host trees (Redhead et al. 1997) and are known from every continent except Antarctica (Corner 1966; Watling and Abraham 1992). The economic importance and the evolutionary significance of the Cantharellaceae have resulted in considerable research on their ecology, physiology, and phylogenetics (Danell 1994; Dunham et al. 2003b). Arora and Dunham (2008) emphasized that the name of *Cantharellus cibarius* Fr. has been broadly applied regardless of its habitat, climate zone, ectomycorrhizal host, and geographic distribution. Because *Cantharellus* species exhibit limited morphological characters (Thiers 1985), the application of molecular tools plays an important role in defining species boundaries in *Cantharellus* (Arora and Dunham 2008). Phylogenetic analysis of the internal transcribed spacer (ITS) region and nuclear large subunit rRNA (LSU) in *Cantharellus* has helped to clarify the identity of different species (Redhead et al. 1997; Dunham et al. 2003a; Arora and Dunham 2008).

The forests of the northern Himalayas are diverse and contain many families of ectomycorrhizal trees such as Betulaceae, Fagaceae, and Pinaceae. In the present study, we report the occurrence of *Cantharellus pseudoformosus* as a species new to science from India. This fungus was found in mixed evergreen forests under *Cedrus deodara*, which normally supports a wide range of ectomycorrhizal fungi.

Materials and methods

Fruit bodies were collected from Khajjiyar forest (India), stands distributed over 6,400 ha, latitude 32°10′N and 33°13′N and longitude 75°45′E and 77°33′E, of Himachal Pradesh, India (Fig. 1). The searches were conducted in the months of October 2006 and September 2007. To avoid resampling, basidiocarps of all collection sites were marked, and a minimum of 5 m distance was kept between any two fruit-body collections. Ecologically, the *Cedrus deodara* stands in Indian Himalaya are in contrast to those of North America, which may be dominated by *Pseudotsuga*, *Tsuga*, *Picea*, *Abies*, *Larix*, or *Pinus* species.

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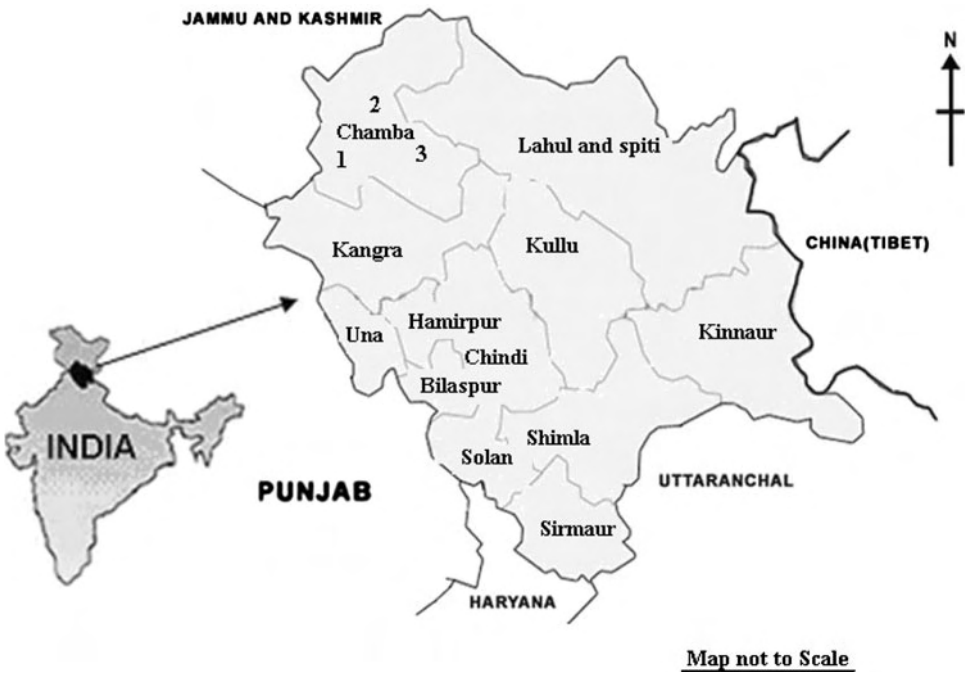

Fig. 1 A map of the Himachal Pradesh, India, showing the distribution of *Cantharellus pseudoformosus* in Chamba-Khajjiyar according to material examination. Collection sites: *1*, at Khajjiyar (near the tourist information center); *2*, Suala (25 km from the Khajjiyar guest house); *3*, Bharmour (5 km from the Bharmour bus stand) (*3*)

Macroscopic morphological details of fresh specimens, such as size, shape, color, and texture, were recorded. Microscopic features were determined from rehydrated sections of basidiomata mounted in 3% KOH and stained with 2% Congo red, 2% phloxine, and Melzer's reagent. The spores were studied from the spore deposits and from fresh material as well. Macrochemical tests were performed according to the methods of Singer (1986). The specimens have been deposited in the Punjabi University Herbarium (PUN), Botany Department, Punjabi University, Patiala, India under the voucher numbers PUN3883, PUN4126, and PUN4128.

For molecular characterization, the genomic DNA was extracted from the fruit body by the method of van Kan et al. (1991). The DNA was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific, USA). The ITS region of nrRNA was amplified by polymerase chain reaction (PCR) with ITS1 and ITS4 primers (White et al. 1990). The 5′-end of the nuclear large subunit rRNA was amplified with ITS4R (White et al. 1990) and LR5 (Vilgalys and Hester 1990) primers. The 50 µl reaction mixture for PCR amplification contained 10 ng DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, and 2.5 units of *Taq* polymerase (Fermentas, USA). Amplifications were performed in a thermal cycler (Perkin Elmer, USA) with an initial denaturation step of 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min, and a final extension of 72°C for 8 min. Before sequencing, PCR products were purified and subcloned in Ins TA clone PCR cloning kit (Fermentas) as per the manufacturer's instructions and transformed into *Escherichia coli* DH α cells. Five randomly selected clones containing the insert of ITS and LSU of nrRNA were sequenced. Among five clones of ITS, the sequence of one clone differed by about 3% with other sequences. These sequences were deposited in GenBank under the accession numbers FJ769255 and HM776721, respectively. No variations were found for the LSU sequences among the clones, and the sequence reported in this article for the LSU of nrRNA has been deposited in GenBank under the accession number GU237071. A BLAST search was performed to find the possible sister groups of the newly sequenced taxa. The sequences were edited with BioEdit 5.0.6 (Hall 1999) and aligned using MAFFT ver. 6.240 with other sequences obtained from GenBank. The final alignment included 829 bp. The sequence alignment was submitted to TreeBASE (http://www.phylo.org/treebase/) under the submission ID number 10767. Phylogenetic analysis was performed by a maximum-parsimony method with MEGA software (Tamura et al. 2007). The maximum-parsimony tree was obtained using the close-neighbor-interchange algorithm with search level 3 (Nei and Kumar 2000), in which the initial trees were obtained with random addition of sequences (10 replicates). Clade stability was assessed in a bootstrap analysis with 1,000 replicates.

Results and discussion

Taxonomy

***Cantharellus pseudoformosus* Deepika, Upadhyay & Reddy, sp. nov.** Fig. 2a–d