Studies on the diversity and nutritional value of Cantharellaceae of Western Himalayas, India

A Thesis

Submitted in fulfillment of the requirements

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By

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CERTIFICATE

Certified that the thesis **"Studies on the diversity and nutritional value of Cantharellaceae of Western Himalayas, India"** which is submitted by Miss. Deepika Kumari, in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology and Environmental Sciences (DBTES), Thapar University, Patiala, is a record of the candidate's own independent and original research work carried out by her under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.

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DECLARATION

I hereby declare that the work which is being presented in this thesis "**Studies on the diversity and nutritional value of Cantharellaceae of Western Himalayas, India**" submitted by me for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology, Thapar University, Patiala, is true and original record of my own independent and original research work carried out under the supervision of Dr. M. Sudhakara Reddy, Professor, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, and Dr. R. C. Upadhyay, Principal Scientist, Directorate of Mushroom Research, Solan, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

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Abstract

The Western Himalayas, India is known to be one of the word's hotspots of biodiversity but still the diversity of some economically important fungi like Cantharellaceae has not been explained properly from this region. In the present research work several fruiting bodies of Cantharellaceae were collected from different parts of Western Himalayan region and characterized. Macromorphological and microscopical characters followed by molecular characterization (both the ITS and LSU dataset) i.e., morpho-genetic studies confirmed the identity of these fungi. The present research work described 13 species of *Cantharellus*, among these, 8 were new taxa which includes *C. applanatus* sp. nov., *C. elongatipes* sp. nov., *C. fibrillosus* sp. nov; *C. himalayensis* sp. nov., *C. indicus* sp. nov., *C. natarajanii* sp. nov., *C. pseudoformosus* sp. nov., and *C. umbonatus* sp. nov. as new to the world while *C. miniatescens* Heinem was new record from the Indian subcontinent. Three species of *Craterellus* (*Craterellus cornucopioides* var. *mediosporus* Corner, *Cr. dubius* Peck and *Cr. cinerius* Fries) were reported as new records from the Indian subcontinent. *Craterellus indicus* sp. nov. was new report from the world. All these Cantharellaceae fruit bodies were also analyzed for nutritional properties. Nutritional profile of such Cantharellaceae species revealed that, these mushrooms provide key nutrients such as protein and carbohydrates, beside antioxidants, amino acids and vitamins. The bacteria associated with the fruit bodies of Cantharellaceae were studied. Bacteria were isolated from internal tissue of sporocarps and differentiated on the basis of REP- and BOX-PCR fingerprinting. Biochemical and 16S rRNA profile revealed that the isolates belonging to nine different genera which includes *Hafnia* sp., *Enterobacter* sp., *Ewingella* sp., *Rahnella* sp., Gamma proteobacterium, *Pseudomonas* sp., *Stenotrophomonas* sp., *Alcaligenes* sp. and *Bacillus* sp. So, the present study was undertaken to explore and assess the diversity and distribution of Cantharellaceae from Western Himalayas for first time along with their nutritional properties and host association between fruit bodies of Cantharellaceae with endophytic bacteria.

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Abbreviations

Abbreviation	Word (s)
%	Percent
°C	Degree centigrade
AIC	Akaike information criterion
ANOVA	Analysis of Variance
bp	Base pair
BS	bootstrap values
C	Carbon
C	*Cantharellus*
cm	Centimeter
Cr	*Craterellus*
Da	Dalton
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
dNTP	2'-deoxynucleoside-5'-triphosphate
ECM	Ectomycorrhiza
EDTA	Ethylenediamine-tetra acetic aci
ERIC	Enterobacterial Repetitive Intergenic Consensus
g	Centrifugal force
g	Gram
HPLC	High Performance Liquid Chromatography
hr	Hour(s)
IGS	Intergenic spacer
IPTG	Isopropyl-β-thiogalactoside
kb	Kilo base
KOH	Potassium hydroxide
LB	Luria-Bertani
LSU	Large Subunit
m	Metre(s)
M	Molar
mg	Milligram
Min	Minutes
ml	Millilitre(s)
ML	Maximum likelihood
mm	Millimetre(s)
mM	Millimolar(s)
MP	Maximim Parsimony
N	Nitrogen
NA	Nutrient agar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram(s)
NTYSYS	Numerical taxonomy and multivariate analysis system

p.I	Isoeletric point
PAUP	Phylogenetic Analysis Using Parsimony
PCR	Polymerase chain reaction
ppm	Parts per million
REP	Repetitive extragenic palindromic
RFLP	Restriction fragment length polymorphism
RT	Reverse transcription
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SE	Standard error
TBR	tree bisection-reconnection
Tris	Tris-(hydroxymethyl-) aminomethane
U.V	Ultraviolet
v/v	Volume by volume
w/v	Weight by volume
X-Gal	5-Bromo-4-chloro-3-indolyl-β-D-galactoside
µg	Microgram
µl	Microlitre
µM	Micromolar

Chapter 1

Introduction

1.1 General Introduction

Biodiversity as defined by the "International Union for Conservation of Nature and Natural Resources" encompasses all life forms, ecosystems and ecological processes and acknowledges the hierarchy at genetic, taxon and ecosystem levels. Fungi are among the most diverse groups of living organisms on earth, though still inadequately studied worldwide. Most fungi are largely invisible to the naked eye, living for the most part in soil, organic dead matter and as symbionts of plants, animals, or other fungi. Some fungi become noticeable when fruiting, either as mushrooms or molds. Mushrooms have long been valued as nutritional foods by many scientists throughout the world. They are rich source of proteins, vitamins and minerals; low in fat with high proportion of unsaturated fatty acids and contains no cholesterol. Nutritionally they rank between low grade vegetables and high grade milk and proteins (Garcha *et al*. 1993) and therefore can contribute significantly in overcoming proteins deficiency in developing nation where people are fed on grains, tuber and small quantities of pulses.

In ancient times, mushrooms have been treated as special kind of nutritious food. The term "mushroom" and its variations may have been derived from the French word *mousseron* in reference to moss (*mousse*). The Greeks regarded mushrooms as providing strength for warriors in battle and the Romans regarded mushrooms as the "Food of God". In the earlier times, mushrooms were collected from their natural growing habitats, but with the passage of time, several attempts have been made to domesticate mushroom under control conditions. So far, more than 2,000 edible fungal species are widely accepted for human consumption, but only a few of them are commercially cultivated worldwide and in India, only 5 mushroom species

namely *Agaricus bisporus, Pleurotus* spp., *Volvariella volvacea, Calocybe indica* and *Lentinula edodes* are popularly cultivated in different parts. There are several mushroom species which are not well documented from India. Cantharellaceae is one of such unexplored families of Indian region.

Family Cantharellaceae belong to one of the important group of ectomycorrhizal fungi, generally found at high altitude and popularly known as *chanterelles*. *Cantharellus* Fr. and *Craterellus* Pers. are two genera of Cantharellaceae (Feibelman *et al*. 1996). Various members of Cantharellaceae such as *Cantharellus cibarius*, *C. formosus*, *C. lateritius* and *Craterellus* (*Cr*.) *cornucopioides* are considered the most popular in different countries including India, and are consumed as a food due to high nutraceutical values in their fruit bodies. Because of nutritive value of such mushrooms, they are widely attracted by mushroom marketing industry. However, there are several members of Cantharellaceae whose nutritional properties have not been elucidated.

The Himalayan biodiversity in India is known to be very rich but still the diversity of Cantharellaceae has not been explained properly from this region. Although work on the diversity of Cantharellaceae was carried out in other parts of the world, very little work has been done in India. Despite an extensive body of literature and intense interest, the taxonomy of Cantharellaceae is still poorly known. Classical fungal taxonomy relies heavily on using the shape, size and colour of the fruit bodies, spore morphologies, spore release mechanisms and habitat to define taxa. The microscopic features of the taxon are quite homogeneous and basidiocarp morphology is highly variable and mostly dependent on ecological conditions, so classical taxonomy is insufficient to identify them to solve the taxonomic riddles and to establish evolutionary relationship. In 1980, development of molecular biological tools and techniques helped in improvement of molecular evolution analysis to facilitate studies on relatedness phylogeny and evolution of organism including fungi. To unravel the taxonomic complexity and to establish the evolutionary relationship in Cantharellaceae, best approach was taken by Feibelman *et al* (1996), Dunham (2003)

and Moncalvo *et al* (2006). So additional criteria, rather than relying only on morphological features are required to study the molecular taxonomy. Nucleotide sequences from certain genes reflect phylogeny at various taxonomic levels. Molecular studies based on conserved genes/DNA segments such as the internal transcribe spacer (ITS), large subunit ribosomal DNA (LSU), small subunit ribosomal DNA (SSU), mitochondrial small subunit (mtSSU) and β-tubulin genes, have been used in taxonomy and phylogeny of Cantharellaceae.

Despite being one of the highly nutritive sources, members of Cantharellaceae have been neglected for detailed nutritional analyses. Elevated levels of proteins, lipids, minerals, vitamins, and neutraceuticals in Cantharellaceae fruit bodies are of significant value (Pilz *et al*. 2003). They have been prized since antiquity for their esculent nutritive qualities, and are currently an economically significant factor in the lucrative wild edible mushroom marketing industry. However, the major challenge to the scientific community is domestication of Cantharellaceae and not only the fructification even raising pure culture of Cantharellaceae is still a difficult task. The bacteria associated with fruit bodies of Cantharellaceae might play an important role as 'mycorrhiza helper bacteria' in the fructification as well as cultivation of such species. So beside classical, molecular taxonomy and nutritional analysis, detailed bacterial diversity and its characterization would be required for comprehensive analyses of Cantharellaceae.

1.2 Definition of chanterelles

The term "chanterelle" is used for a variety of edible and highly prized mushrooms with ridges (instead of gills) on the underside of the cap. The word "chanterelle" is derived from the Greek "kantharos" meaning "cup," "goblet," or "drinking vessel," a reference to their funnel-like shapes (Persson and Mossberg 1997). As the species name for the golden chanterelle, "*cibarius*" is derived from the Latin word for "food," the combined species name, *Cantharellus cibarius*, quite appropriately translates as "cup of food." Indeed most chanterelles are highly prized for their

flavor and can be safely collected and consumed because they are easily identified (Moser and Jülich 2000). Mushrooms are the reproductive structures (fruit body or sporocarp) of certain fungi, and in the case of chanterelles, the fungus lives in the soil and derive its carbohydrate nutrition from a symbiotic mycorrhizal association with fine tree roots (Smith and Read 1997).

Two genera, *Cantharellus* and *Craterellus* are commonly referred to as "chanterelles" because their spore-bearing surfaces appear similar without magnification. The fertile or spore-bearing surface of mushrooms is called the hymenium. The chanterelle hymenium can be smooth, wrinkled, veined, or ridged, but never forms bladelike gills (as in mushrooms like *Agaricus*) or tubes (as in *Boletus*). Most chanterelles have spore-bearing ridges that typically extend from the edge of the cap (pileus) well down the tapered stems (stipes). Chanterelles can be brittle, fleshy or leathery, but they are never woody in texture.

1.3 Systematic position of Cantharellaceae

The family Cantharellaceae is placed with the Clavariaceae in the Aphyllophorales (Donk 1964; Corner 1966; Petersen 1971). Morphological characters such as the overall vase-like or infundibuliform shape, stichic basidia and smooth spores support the monophyly of the Cantharellaceae (Singer 1986). *Cantharellus* is a homobasidiomycete and characterized by the production of small, medium to moderately large fleshy, funnel shaped basidiocarps with folded or smooth hymenium.

Noteworthy, taxonomic works on Cantharellales in 19th and early 20th centuries include those of Fries (1821-1832), Persoon (1825), Smith and Morse (1947), Corner (1966, 1969), Bigelow (1978), Petersen (1979), Feibelman *et al* (1996) and Dahlman *et al* (2000) which have made it possible to deal quite effectively with cantharelloid mycoflora. The two genera of Cantharellaceae, *Cantharellus* and

Craterellus have been distinguished based on morphological characters such as clamp connections which are present in *Cantharellus* and absent in *Craterellus*; and the configuration of the hymenophore which is folded to almost gill like in *Cantharellus* and smooth to rugulose in *Craterellus* (Kuhner and Romagnesi 1953). Corner (1966) placed *Cantharellus* Fr. and *Craterellus* Pers. and *Pseudocraterellus* in Cantharellaceae, however, later *Pseudocraterellus* was proved as *Craterellus* (Feibelman *et al*. 1996). Hawksworth *et al* (1995) placed species of *Cantharellus* in the family Cantharellaceae and species of *Craterellus* in a separate family Craterellaceae; both in order Cantharellales, but Kirk *et al* (2008) has assembled both *Craterellus* and *Cantharellus* in the family Cantharellaceae. Distinction between these genera are currently being revised, however, several species have been recently been moved from the genus *Cantharellus* to *Craterellus.*

Scientific Classification (Kirk *et al*. 2008):

Kingdom: Fungi

Division: Basidiomycota

Class: Agaricomycetes

Order: Cantharellales

Family: Cantharellaceae

Genus: *Cantharellus/ Craterellus*

1.4 Problem and gap in studies

India is known for its varied ecological conditions ranging from tropical, subtropical to temperate climatic conditions, which are very conducive for the growth of different wild mushrooms. From all over the world, more than 90 species have been reported in Cantharellaceae while India contributes only 7 species of *Cantharellus* and 5 species of *Craterellus.* Large numbers of species of Cantharellaceae are considered as highly appreciated edible mushroom and many efforts have been made

to cultivate these fungi. In India, research on Cantharellacea*e* systematic is scarce. Identification of members of Cantharellaceae is also a major problem, as identification was done previously by only classical taxonomic methods, which does not give any idea at generic level; so many species were found misidentified. Considering the biotechnological applications and diversity of Cantharellaceae in India, it is important to report identification of various species using classical taxonomy combined with molecular taxonomy. Characterization with molecular techniques offers a possible aid to identification when morphological, anatomical, ecological data are unavailable or cannot alone resolve a taxonomic problem (Cappelli 1984). In addition, the nutritive value or edibility of Cantharellaceae has not been reported extensively. Despite being one of the promising groups of nutritive values, such nutritional properties of many species of Cantharellaceae have not been studied.

Studies related to understanding of the ecology of Cantharellaceae have also not been explored and a reproducible method for isolating and maintaining cultures of Cantharellaceae is still not known. The bacterial interaction with fruit bodies might play an important role in fructification, however, the dynamics of the bacterial community that inhabits Cantharellaceae fruit bodies has been poorly studied.

1.5 Aim and Objectives

The diversity of climatic conditions prevalent in Western Himalaya, India makes the region a natural habitat of a large number of mushroom species. The aim of present work was to explore the diversity of Cantharellaceae from such region and to analyze nutritional properties along with the diversity of bacteria associated with fruit bodies of Cantharellaceae.

This work contributed with collections of the members of Cantharellaceae from Western Himalayan region and to interact with local people of such locality to know about the edibility and habitat of these various species. However, this precious

knowledge is limited to the old aged villagers only and common people still do not know about the edibility of various species of Cantharellaceae. Different species were identified based on classical and molecular taxonomy. The edible nature and nutritional value of these mushrooms were also studied. The other goal of this study was to isolate and characterize bacteria, intimately associated with fruit bodies with potential to promote the growth and yield of members of Cantharellaceae.

The objectives of the present investigations are:

1. To study the diversity of Cantharellaceae of western Himalayas
2. To assess the nutritional value of mushrooms of Cantharellaceae
3. To document the diversity of bacteria associated with these fungi

Chapter 2

Literature review

If we ask a mushroom picker to define chanterelles, he or she may answer, "Yellow mushroom". But how do chanterelles differ from other mushroom? Let us first look at the historic background of chanterelles.

The genus *Cantharellus* Fries belongs to the family Cantharellaceae of order Cantharellales. Earlier Swedish naturalist Linnaeus (1747) noted that "chantarelles" were common edible mushrooms, but used the scientific name *Agaricus chantarellus* for the golden chanterelle (Linnaeus 1755). The Swedish scientist Elias Fries, now regarded as the "father of mycology" for his pioneering work on fungal taxonomy, coined the current scientific name for the golden chanterelle as *Cantharellus cibarius*. Most of the members of this family are edible. About 97 species in genera *Cantharellus* and *Craterellus* have been described worldwide (source: Mycobank). The total number differs according to the authors and how they define the species (Corner 1966; Danell 1994a; Feibelman *et al*. 1997; Pegler *et al*. 1997; Persson and Mossberg 1997; Watling and Turnbull 1998; Dahlman *et al*. 2000; Eyssartier and Buyck 2001). Over 70 species of true chanterelles have been described thus far, and many more are yet to be named. They are found on almost every continent that has forests with ectomycorrhizal host trees.

2.1 Chanterelle evolution

Chanterelles belong to a group of fungi called basidiomycetes, members of the phylum basidiomycota (Alexopoulos *et al*. 1996), a taxonomic category of fungi that also includes gilled fungi and boletes (among others). The protein analysis suggests that basidiomycetes branched off from other fungi about 1.2 billion years ago during the Precambrian era (Heckman *et al*. 2001), but the first undisputed fossils of land plants and fungi do not appear until the Ordovician period 480 to 460 million years

ago. Although genetic analyses suggest mycorrhizal fungi diverged 130 million years ago (Berbee and Taylor 1993), the oldest actual fossil of an ectomycorrhizal root tip is 50 million years old (Selosse and Le Tacon 1998), and the oldest gilled mushroom (preserved in amber) is about 90 to 94 million years old (Hibbett *et al*. 1995). Pegler *et al*. (1997) speculate that chanterelles are more primitive than gilled fungi (also subject to revision), but regardless of their actual antiquity, chanterelles have had ample time to colonize every continent except Antarctica and to differentiate into the several genera and numerous species now found worldwide.

Pilz *et al* (2003) reported that the chanterelle is composed of a network of microscopic hyphae (one-cell-wide fungal filaments). Collectively, a network of hyphae is called a mycelium, and chanterelle may be referred to as a mycelial colony. Fruit bodies of basidiomycete fungi develop into a variety of forms, such as truffles or conks in case of chanterelles. Chanterelle fruit bodies begin as dense clots of mycelium and form primordia (miniature mushrooms) that have the potential to grow to full size under favorable conditions. Fruit bodies have a layer of fertile tissue called the hymenium (in chanterelles, the ridges found under a cap and down the stem) that in turn generates microscopic reproductive structures (basidia in this case) where spores are produced and released.

Chanterelles form a type of mycorrhizae called ectomycorrhizae, the prefix *ecto-* referring to a fungal sheath or mantle that forms around the root tips of a host tree (Smith and Read 1997). The species of chanterelles symbiotically associated with host trees, colonizing the fine roots of trees and forming structures called mycorrhizae and form long-lived mycelial colonies (Jahn and Jahn 1986) if their tree partners continue to provide nutrition. In Oregon, a long-term study conducted by Oregon Mycological Society members found a significant correlation between warm summers and chanterelle abundance (Norvell 1995; Norvell and Roger 1998). Chanterelle ectomycorrhizae are not distinctive under field conditions; thus they were not well described until they were created under sterile laboratory and greenhouse conditions (Danell 1994a, 1994b; Danell and Camacho 1997). Although

all chanterelles are thought to be mycorrhizal, but this has not been experimentally confirmed with all the species of chanterelles.

Recently Moncalvo *et al*. (2006), Hibbett *et al.* (2007) and Lawrey *et al*. (2007) worked on the phylogenetic evolution of Cantharelloid. Moncalvo *et al*. (2006) reassessed the circumscription of the cantharelloid clade and identified monophyletic groups based on nLSU, nSSU, mtSSU and RPB2 sequence data and the results placed the taxon of *Tulasnella* and Cantharelloid in the same clade, so, relationship between *Tulasnella* and members of the Cantharelloid clade will require further scrutiny, although there is cumulative evidence that they are probably sister groups.

Hibbett *et al* (2007) established the phylogeny of the entire fungal kingdom. The phylogenetic classification of the fungi of major groups within the Agaricomycotina remains unsettled. It has been divided into Heterobasidiomycetes (jelly fungi) and Homobasidiomycetes (mushroom-forming fungi) based on the structure of the septal pore apparatus and the spindle pole body. Homobasidiomycetes, a preliminary phylogenetic outline with 8 major clades was revealed using nSSU (nuclear small subunit) and mt-ssu (mitochondrial single subunit) rDNA sequences. These major clades coincided with Polyporoid, Euagarics, Bolete, Russuloid, Thelephoroid, Hymenochaetoid, Cantharelloid and Gomphoid-phalloid clades.Lawrey *et al* (2007) reassessed the fungi in the cantharelloid exhibit a mixture of homobadiomycete and heterobasidiomycete morphological and anatomical characters. The members of this clade vary widely in mode of nutrition, with plant pathogens, saprobes and mutualists.

2.1.1 World distribution

Pilz *et al*. (2003) reported that the impressive chanterelle mycotas exist in southeastern and eastern Asia, Japan, Africa, Australia, and Central and South America. Chanterelles are especially appreciated in Europe and North America. Corner (1966) mentioned 17 species of Cantharellaceae of which 14 are found in Europe.

2.1.1.1 *Europe:* In Europe, the golden Chanterelle (*C. cibarius*) is the primary commercial species. Other species that occur throughout seasons are *Cantharellus melanoxeros*, *Craterellus cornucopioides*, *Cr. cinereus*, *Cr. tubaeformis*, and *Cr. lutescens*. The *C. friesii* and *C. cibarius* var. *amethysteus* have a more southern distribution. The European pale chanterelle (*C. pallens*), the wavy capped chanterelle (*Cr. undulatus*) are primarily ectomycorrhizal associates of hazels and oaks. Eyssartier and Buyck (1999a) also described two other species, *C. pseudominimus* and *C. romagnesianus* from Europe.

2.1.1.2 *Africa:* In Africa, few species including *C. congolensis*, *C. longisporus*, *C. pseudocibarius and C. platyphyllus* were described decades ago (Corner 1966). The taxonomy of such fungal species was explored by various researchers (Harkonen *et al*. 1995; Buyck *et al*. 1996, 2000; Buyck and Eyssartier 1999b; Eyssartier and Buyck 2001).

2.1.1.3 *Australia:* Eyssartier and Buyck (2001) reviewed 17 possible Australian chanterelle species and concluded that only three, namely *C. ochraceoravus* Grgurinovic, *C. concinnus* Berk (*C. cibarius* var. *australiensis*), and *C. viscosus* Berk) were true chanterelles in Australia.

2.1.1.4 *Central and South America:* Several *Cantharellus* species, including *C. cibarius* and *C. cinnabarinus* have been reported from Central and South America, and the West Indies (Pilz *et al*. 2003). In 2000, Lorelei Norvell collected *C. lateritius* and *C. cibarius* in oak forests of the Talamanca Mountains in Costa Rica. They also found a new chanterelle species previously collected by Halling (Halling and Mueller 2000) that has been given the provisional name *C. atrolilacinus* Halling & Mueller nom. prov. Spegazzini described three chanterelles from Argentina in c1909 (Farr 1973). *Craterellus* species are poorly known in Central and South America, but *Cr. tubaeformis*, *Cr. cornucopioides* (discussed as *Cr. fallax*), *Cr. ignicolour*, and *Cr. undulatus*, have been reported along with *Cr. boyacensis* and *Cr. costaricensis* (Wu and Mueller 1995; Halling and Mueller 2000). *C. cascadensis* was described from

American Pacific Northwest (Dunham *et al*. 2003). Henkel *et al*. (2006) described a new species *C. pleurotoides* from Guyana. Recently Arora and Dunham (2008) reported a new species *C. californicus*, associated with Live Oak in California, USA.

2.1.1.5 ***Asia:*** C*antharellus cibarius*, and the close relative *C. subcibarius*, are reported from Pakistan, India, China, Thailand, Malaysia, Japan, and Philippines (Pilz *et al*. 2003). *C. lateritius* is reported from Thailand, and *C. ianthinus*, *C. pudorinus*, and *Cr. odoratus* from Malaysia, Singapore, (Jones *et al*. 1994). Recently Eyssartier *et al*. (2009) reported five *Cantharellus* species among them, *C. cerinoalbus* and *C. subamethysteus* are described as new species

2.1.2 India

India has a luxuriant forest flora with different topographical area and it is possible that it would have some unexplored species in the family Cantharellaceae. However, to date very few species of *Cantharellus* such as *C. cibarius* Fries, *C. minor* Peck and *C. cinnabarinus* Schw, are reported from Kashmir and Western-Ghat region by Abraham *et al*. (1995). Dhancholia *et al* (1991) reported *C. appalachiensis* Peterson and *C. lateritius* (Berk) Singer, Lilloa from Uttarakhand, while two species *C. friesii* Quel and *C. luteocomus* Biglow are reported by Bhatt and Lakhanpal (1988) from Western Himalayas, India. Watling and Abraham (1992) reported *C. cibarius* from Kashmir where it was widespread and grew under Himalayan spruce, although Reddy et al. (2002) was also reported to found under spruce, oak, and pine trees. Thind and Rattan (1972) has reported four species of *Craterellus* namely *Cr. cornucopioides* Fr., *Cr. mussooriensis* Reid, Thind & Adlakha, *Cr. odoratus* var. *solidostipite* Schw. ex. Fr. and *Cr. sinuosus* Fr. and among these one species *C. mussooriensis* is new to the world from Uttarakhand. The considerable intra-specific morphological variations within the Cantharellaceae raise doubts about the delimitation and integrity of this family. No phylogenetic studies of Cantharellaceae using molecular tools have been undertaken in India till date.

2.2 Taxonomy: a classical approach

Classification of fungi, traditionally, has been based on morphology. Classical fungal taxonomy relies on the size and shape of fruiting structures, spore morphology, release mechanism, colouration and habitat to define taxa, while the adequate description of grouping does not always helps to explain its origin.

The Dutch herbalist Lobelius (1581) was the first to mention chanterelles in the European literature. The Belgian botanist Clusius (1601), who traveled extensively and wrote the first scientific monograph on fungi, cited German "Reheling" and Hungarian "Niwl Gomba" as local common names for the golden chanterelle. The existence of these old vernacular names suggests that Europeans started eating chanterelles in medieval times. French language and traditions influenced much of medieval Europe, so the name "chanterelle" and the practice of eating chanterelles likely spread from France to other parts of Europe.

The genus *Cantharellus* Fries was described in 1821 in Systema Mycologicum (Fries, 1821-1832). The major drawback found in Fries's important work that it lacks any illustration. Smith and Morse (1947) and Smith (1968) worked on micro-morphological characters that made recognition easier and distinguished between two *Craterellus* species of western and eastern North America, *Cr. tubaeformis* and *Cr. infundibuliformis*. Redhead (1979) noted that Smith (1968) used inconsistent features to distinguish the pairs of species in eastern versus western North America, and also that the name *Cr. infundibuliformis* was unavailable, because it was considered to be synonymous with *Cr. tubaeformis*.

Some individual mycologists (Donk, 1964; Corner, 1966; Petersen, 1971) placed family *Cantharellaceae* with the *Clavariaceae* in the Aphyllophorales. Corner (1966) attempted to combine macroscopic and microscopic characters of Cantharellaceae and divided this family into three genera *viz*. *Cantharellus*, *Craterellus* and a new genus *Pseudocraterellus*. Corner's monograph included 7 new species; *Cantharellus cuticulatus*, *C. subcibarius*, *C. formosus*, *C. ianthinus*, *C. pudorinus*, *Craterellus sordidus* and *Cr. reniformis*.

The new described genus *Pseudocraterellus* (Corner, 1966) of Cantharellaceae was emphasized by fruit body development and secondary septation of tramal hyphae as distinguishing characters, separating the genus from *Cantharellus* (similar developmental pattern and clampless, but not secondarily septate hyphae). However, no new combinations in *Pseudocraterellus* were made. Therefore, even though the type species of the genus was plainly stated as *Cantharellus sinuosus* Fr., the species was not nomenclaturally transferred to the new genus. Heinemann (1958) perpetuated the oversight by stating no basionym for the combination *Pseudocraterellus sinuosus*. Reid (1962) was forced validly to publish the combination, and correctly ascribed it to himself as *P. sinuosus* (Fr.) D. Reid. Later Corner (1966} insisted on retaining authorship by stating the combination as *P. sinuosus* (Fr.) Corner ex Heinemann.

Petersen (1969) worked on "Notes on cantharelloid Fungi-II" and described new species of *Craterellus*, *Cr. carolinensis.* Descriptions were also given for the type specimens of *Thelephora subundulata* and *Stereum calyculus* and for representative specimens of *Cr. sinuosus*, *Cr. crispus*, and *Cantharellus lutescens* and also accept the relative taxonomic relevance of *Pseudocraterellus* at generic rank.

Later on, Petersen (1976) worked on the type specimen of cantharelloid taxa proposed by Peck (1873) and redescribed and judgment was made on their modern taxonomic placement. Studies concluded that virtually every medium–sized to large, yellow–orange *Cantharellus* has at one time or another passed under the name *C. cibarius* or been mistaken for that species regardless of its habitat, climate zone, and ectomycorrhizal host and even within Europe there may be more than one species lumped under that name. Petersen (1979) published species circumscriptions and nomenclatural notes on *Cantharellus confluens, C. lateritius* and *Craterellus odoratus* along with description of the type specimen of *Craterellus aureus* was also provided. The data described several diagnostic characters that differentiate *C. lateritius* and *Cr. odoratus*.

Bigelow (1978) reported cantharelloid fungi of New England and adjacent area and reported 20 taxa of Cantharellaceae, including *Gomphus* within Cantharellaceae,

among these one species *Cantharellus luteocomus* was new report. Singer (1986) demonstrates the morphological characters such as the overall vase-like or infundibuliform shape, stichic basidia and smooth spores support the monophyly of the Cantharellaceae.

Härkönen *et al*. (1995, 2003) reported six species, and Buyck *et al.* (2000) reported 11 species and introduced two new taxa: *Cantharellus tomentosus* Eyssart. & Buyck and *C. isabellinus* var. *parvisporus* Eyssart & Buyck from Tanzania. Recently Tibuhwa *et al.* (2008) also reported a new species *Cantharellus fistulosus* from Tanzania is characterized by having a hollow, smooth stipe and a pink hymenium that contrasts with the yellowish brown stipe and cap surface.

Hankel *et al*. (2005) reported a new species *Cantharellus pleurotoides* from the Pakaraima Mountains of Guyana, occurring in rainforests dominated by ectomycorrhizal Dicymbe spp. (Caesalpiniaceae). This fungus is singular among *Cantharellus* species described worldwide in possessing a pleurotoid basidioma.and other macromorphological, micromorphological, and habitat data are provided for the new species.

Olariaga and Salced (2008) reported *Cantharellus ilicis from Quercus* forest of Mediterranean Basin, differs from other taxa by a unique combination of features. Because of its fleshy basidiomata, whose pileipellis hyphae are thick-walled, together with the presence of clamps, *C. ilicis* belongs to the section *Cantharellus*

Arora and Dunham (2008) reported a new species, *Cantharellus californicus,* associated with Live Oak in California, USA. The prominent golden chanterelle of California's oak woodlands is characterized as a new species using molecular and morphological data. The observations indicated that it is a largest *Cantharellus* species in the world, with individual sporocraps commonly weighing 1/2 kilogram (kg) (or 1 pound) or more when mature.

Eyssartier *et al*. (2009) described and illustrated five *Cantharellus* species collected in Peninsular Malaysia, associated with *Dipterocarpaceae*. Among them, *Cantharellus cerinoalbus* and *C. subamethysteus* are described as new species. Important characters of *C. subamethysteus* is its *Hygrophorus*-like habit, absence of

clamp-connections and more brightly coloured, fits in genus *Cantharellus*. *C. subamethysteus* having purplish lilac squamules on the cap and distinctly accrescent hymenophore indicated as a new species.

Fries and Mueller (1984) studied based on data from sexual incompatibility tests and other experimental approaches to characterize more completely the systematic of particular taxa suggested that species circumscriptions cannot be based solely on morphological criteria. It has been suggested that morphological classification is not precise enough to accurately describe fungal communities (Mehmann *et al*. 1995) and also time consuming. While the use of such descriptions has enabled the classification of many taxa, similarities between some characteristics has led to misidentification of some species and failure to identify cryptic species within complex taxa. In these situations it is very difficult to define the limits of a species or know for sure whether one morphological type is same as another. The absence of remarkable morphological differences between the species has probably discouraged some mycologists from describing new species. Thus, species with similar morphology were historically lumped under the binomen *C. cibarius* (Eyssartier & Buyck.., 2000; Dunham *et al*. 2003 and Guevara et al., 2004). Twentieth century research, supported by molecular data, demonstrated that slight morphological differences can mask different species of *Cantharellus* (Feibelman e*t al*., 1996; Dunham *et al*., 2003).

2.3 Taxonomy: a molecular approach

A reliable taxonomy is crucial for the assessment of biodiversity and for the categorization of habitats based on their species composition (Göker *et al*. 2011). The emergence of phylogenetic mycology as a paradigm for fungal biology studies has been greatly accelerated by numerous advancement in phylogenetic methods, especially in the area of molecular systematic. The use of polymerase chain reaction (PCR) has dramatically increased our ability to document the diversity of the fungi. The PCR method have advantages when applied to any stage in the life cycle of a fungus including fruit bodies, mycorrhizas, extra radical mycelia and isolated

mycelia growing *in vitro*. Molecular phylogenetic approaches to fungal evolution have proved valuable information towards the goal of understanding relationship among specific fungal groups (Mitchell *et al*. 1995). Molecular studies on different genera of mushrooms helped to illuminate natural classification (Hibbett and Vilgalys 1993; Bunyard *et al*. 1994; Drehmel *et al*. 1999; Liu *et al*. 1999). There are two important advances that stimulated the use of molecular techniques. Firstly, the advent of fungal cells or even single spores, dried herbarium materials or extinct organism. Second, the selection of universal oligonucleotide primers specific to fungi, have provided easy access to nucleotide sequences (Guarro *et al*. 1999). Molecular techniques are becoming increasingly important as means for obtaining characters for studying taxonomic and phylogenetic relationships among fungi (Zambino and Szabo 1993). The limitation of identification of mushroom strains based on a few morphological characters can be overcome by the use of DNA based techniques like RAPD, AFLP, RFLP or DNA sequence analyses (ITS, nLSU, nSSU, mtSSU and RPB2). Any of the molecular methods could be combined with morphological methods to make identification of fungal species reliable (Khush *et al*. 1992; Calonje *et al*. 1997).

2.3.1 Internal transcribed spacers (ITS)

The most popular locus for DNA-based mycological studies at the subgeneric level for species identification is the internal transcribed spacer (ITS) region of the nuclear ribosomal repeat unit (Horton and Bruns 2001; Bridge *et al*. 2005). Ribosomal DNA (rDNA) in eukaryotes is arranged in tandemly repeated units containing the coding regions for 18S, 5.8S, and 28S ribosomal RNA separated by spacers (Fig. 2.1).

The variation in the spacers has proven useful for distinguishing among a wide diversity of difficult-to-identify taxa. The ITS region is now perhaps the most widely sequenced DNA region in fungi. It has typically been most useful for molecular systematics at the species level, and even within species (e.g. to identify geographic

races). Gardes and Bruns (1993) designed two taxon selective primers, ITS1-F and ITS4-B, intended to be specific for fungi and basidiomycetes, respectively.

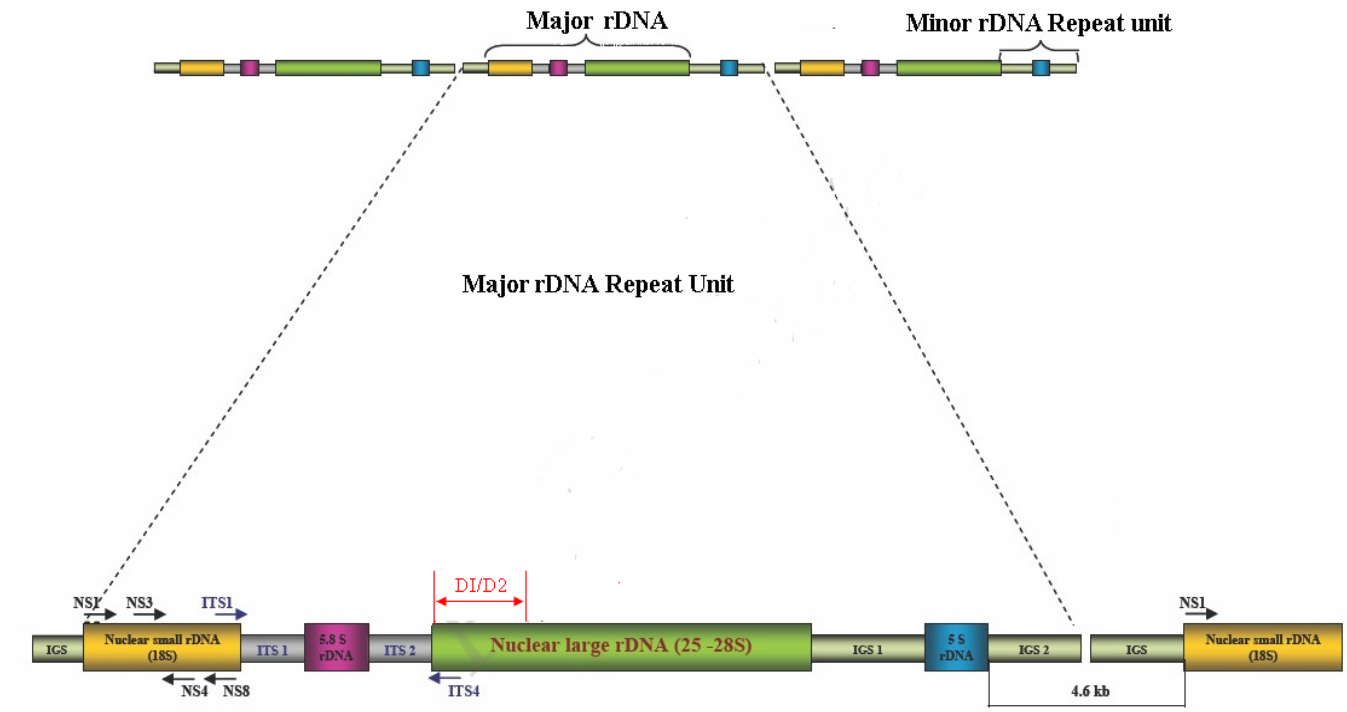

Fig. 2.1 Systematic representation of fungal rRNA gene. The primer used to amplify the various rDNA region used in this study are represented as one direction (5' to 3') arrow. The D1/D2 region of the 25-28S rDNA is represented by a double sided arrow (http// plantbio edu/burn/picts/result/map.pdf; Sugita & Nishikawa, 2003).

Little information is available on the use of ITS in the taxonomy of Cantharellaceae isolates. Feibelman *et al* (1994) studied molecular taxonomy of *Cantharellus* species by amplifying the internal transcribed spacer (ITS) between the 18S and the 28S genes of the nuclear ribosomal DNA. Except for *Cantharellus tubaeformis* and *C. infundibuliformis*, the ITS region was longer in the Cantharellaceae than in the other fungi and highly variable in length. The ITS-1 region between the 18S and 5.8S genes was the site of most variation. The *Cantharellus cibarius* group had the longest ITS-1, ranging from 820 to 1100 base pairs (bp), compared with 240 to 350

bp in other basidiomycetes. They concluded that North American chanterelles with *C. cibarius*-like morphology exhibit significant length variability in the nuclear ribosomal internal transcribed spacer (nrDNA ITS), suggesting that this general morphology might mask a species complex.

Danell (1994) amplified ITS region using ITS1 and ITS4 primers to confirm the identity between fruit body and obtained mycelium from a mushroom. The result identified both as *C. cibarius*. The size of the characteristic ITS from rDNA of vegetative mycelium corresponded with that of the *C. cibarius* fruit body ITS was same (1.4 kb).

Feiblman *et al* (1996) described a new species *C. tabernensis* from mixed pine and hardwood forests of southern Mississippi. A yellowish brown pileus with a dark brown disk and a bright orange hymenophore and stipe morphologically separate this chanterelle from other species. The new species is shown to have a long internal transcribed spacer (1400bp) region of the nuclear ribosomal DNA, consistent with other *Cantharellus* species.

Dunham *et al* (2003) worked on phylogenetic analyses of the ITS regions of yellow chanterelle from the central Cascade Mountains of Oregon. They indicated that the ITS, especially ITS-1, and the 5` end of the 28S nuclear rDNA are potentially informative regions for systematic studies within the Cantharellaceae. The result of phylogenetic analyses proved a new species *C. cascadensis* and data also showed that two species *C. cibarius* var. *roseocanus* and *C. cibarius* (European species) are more closely related to white chanterelles (*C. subalbidus*).

Ying *et al* (2011) worked on 12 samples of *C. tubaeformis* from North America and Europe. The data were analyzed by internal transcribed spacer (ITS) sequences to reveal the correlation between ITS genotypes and geographic locations and to provide molecular evidence for the identification of *C. tubaeformis* from different habitats in North America and Europe. The analyses identified abundant sequence variations within *C. tubaeformis.* The length of the ITS region varied from 571 to 640 bp. Phylogenetic analysis showed some correlations between the ITS genotypes

and geographic locations of *C. tubaeformis*; however, some discrepancies between geographical location and affinity were also found.

Redman (2006) sampled 96 basidiomes from Olympic Peninsula of the Pacific Northwest for genetic analysis based on amplification of the ribosomal ITS regions and identified specimens as *Cantharellus formosus* populations.

2.3.2 Nuclear Large Subunit Ribosomal RNA (LSU)

The large subunit ribosomal DNA (LSU rDNA) has been widely used as a potential marker for fungal species identification in recent years. The longer chain length of LSU rDNA (>3000 bp) has retarded the acquisition of sequences and larger differences in length and variability of divergent (D) domains (Hassouna *et al*. 1984). The LSU is a part of the rDNA gene sequence of the nuclear genome, which is arranged in ribosome clustered at tandem repeat manner (Long and Dawid 1980; Sonnenberg *et al* 2007). Ribosomal genes are highly conserved in different forms of living organisms (Woese *et al*. 1990), but are actually composed of a mixture of conserved and divergent regions.

Phylogenetic analysis of the 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). In order to resolve the internal or external evolutionary relationship of Cantharellaceae, it is clear that more sets of comparative molecular data, such as complete LSU rDNA must be examined.

Feibelman *et al* (1997) studied on the phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of nuclear large subunit rDNA. Approximately 325 bases near the 5' end of nuclear 28S ribosomal gene were sequenced, and the sequences were compared. Sequence analyses demonstrated that *Cantharellus* and *Craterellus* should be treated as distinct genera. The phylogeny generated using parsimony suggested that *Cantharellus tubaeformis* and *Pseudocraterellus sinuosus* should be considered species of *Craterellus*.

Dahlman *et al*. (2003) amplified, large subunit rDNA sequenced and cladistically analysed approximately 650 bp of the 5' end of the nuclear large subunit ribosomal DNA gene. The results provide molecular evidence for the classification of *Cantharellus* species (*C. ignicolour* and *C. lutescens*) within *Craterellus*. Furthermore, the data also predict that all *Leptocantharellus*-like species currently classified within *Cantharellus* are more accurately classified within *Craterellus*. The species complexes of *Cr*. *tubaeformis* and *Cr*. *cornucopioides* were also investigated.

Dunham *et al* (2003) amplified LSU and demonstrated that *C. formosus, C. subalbidus,* and *C. cascadensis* are biological species with boundaries congruent with those delineated by nLSU rDNA phylogenetic clades. Moncalvo *et al* (2006) reassessed the circumscription of the cantharelloid clade and identified monophyletic groups by using nLSU, nSSU, mtSSU and RPB2 sequence data. The data supported a sister-group relationship between these two genera; *Cantharellus* and *Craterellus*. *Craterellus* species complexes can be recognized among northern temperate taxa: the *Cr. cornucopioides* complex (including *Cr. fallax* and *Cr. konradii*), the *Cr. tubaeformis* complex (*including Cr. infundibuliformis*), *Cr. odoratus*, *Cr. lutescens*, and *Cr. ignicolour*. The result also revealed two smaller, slender ''yellow chanterelles'', *C. appalachiensis* and *C. minor* are more closely related to the red species of the *C. cinnabarinus* group than they are to the core group of yellow chanterelles.

2.3.3 Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. This technique is frequently used in area of fugal diversity to differentiate at species level. Vilgalys and Gonzales (1990) used RFLP analysis of ribosomal RNA subunit for the determination of genotypic classes of fungal species. Horton and Bruns (2001) reviewed the molecular techniques applied to studies of

fungal communities and suggested that the RFLP typing using ITS sequences is an effective tool for identification purposes.

Feiblman *et al*. (1996) described a new species *Cantharellus tabernensis* by using Restriction fragment length polymorphism analysis of the 5' end of the nuclear large ribosomal subunit gene and described *C. tabernensis* as a distinct species. The 5' end of the nuclear large subunit gene was amplified with ITS4R and LR5 and amplified products were cut with restriction enzymes, including *Hae III*, *Hha I*, *Hinf I*, *Rsa I*, and *Taq I*, Digestion with the enzyme *Taq I* produced the most informative restriction fragment length polymorphisms (RFLPs). Although the RFLPs generated with *Taq I* of *C. cibarius*, *C. minor*, and *C. cinnabarinus* are the same, the RFLP pattern of *C. tabernensis* is different from those of other species of *Cantharellus*.

Inter- and intra- specific variation among 180 collections of *Cantharellus* was evaluated by analysis of the internal transcribed spacer (ITS) of the rDNA region using restriction fragment length polymorphism (Dunham *et al*. 2003). The ITS region was first amplified by PCR with specific primers (ITS1-F and ITS4) and then cleaved with different restriction enzymes. Amplified products, which ranged between 1490 and 1690 base pairs, were obtained for all the isolates analyzed. Cleavages of amplified fragment with the restriction enzymes *Alu*I, *Hinf*I, *Dpn*II, and *Hae*III, revealed extensive polymorphism. Information pooled from individual restriction fragment profiles was used to characterize six unique RFLP types assigned across the 180 collections analyzed. Species identifiable by specific pattern belonged to *C. formosus*, *C. subalbidus*, *C. cibarius* var. *cibarius*, *C. cibarius* var. *roseocanus* and *C. cascadensis*, while the one collection exhibiting different RFLP pattern to *C. formosus*, which was taken from under *Quercus* in southern California, possibly represents an undescribed relative of *C. formosus*.

Arora and Dunham (2008) also studied the molecular characterization of *Cantharellus* species by rDNA PCR-RFLP. Variation within rDNA genes of 50 isolates of *Cantharellus* from different geographical region was examined by PCR using primers ITS1-F and ITS4, coupled with RFLP analysis. The ITS amplification

products (1490, 1600 or 1690 bp) were digested with three different restriction endonucleases *i.e*., *Alu*I, *Hinf*I and *Hae*III. Cluster analysis based on restriction fragments grouped the isolates into 4 distinct groups, which coincide with their geographical origin and differentiate the species; *C. californicus*, *C. formosus*, *C. cibarius* var. *roseocanus* and *C. subalbidus*. Among these *C. californicus* was new report from California. These studies confirmed the potential of ITS region PCR-RFLP for the molecular characterization of Cantharellaceae and their identification. These results clearly show that the genus *Cantharellus* consists of many species and the molecular tools play an important role in identifying these species.

In summary, the molecular approaches to identify the fungal diversity include DNA extraction, amplification of ITS region by PCR using ITS1 and ITS4 primers, amplification of LSU region, and RFLP analysis. The PCR fragments are subjected to sequencing and then are compared with the existing databases of either EMBL or NCBI for sequence homology. A phylogenetic analysis is employed to identify different species of *Cantharellus* at species level. All heritable information is potentially accessible using DNA sequencing is expected to provide the solution to the problem associated with the taxonomy and phylogeny of Cantharellaceae species. Recent the analysis of DNA sequences of the LSU, SSU, β-tubulin and 5.8 region of ribosomal gene cluster of Cantharellaceae suggested several distinct biological species exist within group and proposed it as an excellent example of molecular approaches in systematic studies of morphologically heterogeneous taxa of Cantharellaceae (Moncalvo 2006; Hibbett *et al*. 2007). So this is an important technique in assessing diversity, which can identify different fungi to species, and in some cases, even strain level without the need for the comparison of morphological characteristics (Albee *et al*. 1996; Bruns *et al*. 1998). This technique has been used successfully in the past to clarify the taxonomic status of fungal genera and provide useful molecular data to compare with original morphological descriptions of species. Further, revisions of current fungal taxa using this technique along with the investigation of more ecosystems that may not have been well studied in the past is likely to increase our understanding of the taxonomic diversity of Cantharellaceae.

2.4 Nutritional value of mushrooms

Mushrooms have long been valued as nutritional foods by many scientists throughout the world (Chang and Miles 2004). They have received an incredible interest in recent decades with the realization that these are good sources of delicious food with pleasing flavor, aroma, exotic tasteful appeal and high nutritional traits because they contain good quality proteins, unsaturated fatty acids, minerals and vitamins (Wahid *et al*. 1988). They can be recommended for the countries suffering from the insufficient nutrition, especially developing or third world countries. In recent years, it has been well proven and documented in the world literature that mushrooms do provide definite nutrition and health benefits for human. Wild edible mushrooms are traditionally used by many Asian countries as food and medicine (Manzi *et al*. 1999; Sanmee *et al*. 2003) and are becoming more and more important in our diet for their nutritional characteristics. Some edible mushroom species are sources of physiological agents for medicinal applications, possessing antitumour, cardiovascular, antiviral, antibacterial and other activities (Chang 1996; Halpern and Miller 2002; Ferreira *et al*. 2009; Ferreira *et al*. 2010). Still there are several varieties of wild mushrooms whose nutritive profiles have not been described well. The members of *Cantharellus* species are one of such edible mushrooms that needed to study. The world consumption of chanterelles has been estimated at 150,000-200,000 metric ton a year (Walting 1997).

In India, mushrooms have long been popular food in such villages where climate is wet and having large forests. Local people collect various types of mushrooms for their own food and for sale. They recognize the benefit of the additional foods, added flavor and the income from local sales and from export to other states or countries. However, the nutritional values of many of these mushrooms have not been known. The last 20 or 30 years have seen an increasing movement of chanterelles, morels and *Boletus edulis* from the northern to the southern hemisphere. Several studies have confirmed that chanterelles are nutritious and rich sources of amino acids, fat, carbohydrates, fibers and energy (Caglarirmak *et al*. 2002; Colak *et al*. 2007).

2.4.1 Carbohydrates

Carbohydrates appear in fungi as amino polysaccharide cell wall constituents in the form of matrix glycoproteins, free polysaccharides, some oligosaccharides, monosaccharides and sugar alcohols as well. The main cell wall component, chitin is a linear molecule constituted entirely of β -1, 4 linked N-acetyl glucosamine residues. Mushrooms also contain fiber fraction, the main component is probably chitin, which is an important structural polysaccharide in the mushroom cell wall (Kreger, 1954; Michalenko *et al*., 1976).

The total amount of carbohydrates in fungi ranges from as low as 8% in *Cantharellus* sp. to 76% in *Armillariella mellea* (Leonowic 2002). Caglarirmak *et al*. (2002) studied a comparison of chemical and nutritional contents of three different edible mushrooms, *C. cibarius*, *L. piperatus* and *B. edulis* and determined 8.86% carbohydrates in *C. cibarius*.

Barros *et al*. (2008) reported the most popular oligosaccharides *i.e*., trehalose, which appears in fruit bodies of *C. cibarius* and *Cr. cornucopioides* as 6.12 g/100 g and 0.11g/100g respectively and disaccharide like, mannitol, maltose and melezitose were also identified. Although the contents of maltose and melezitose were negligible in both species, but the highest amount of mannitol (10.67g/100g) was estimated in *Cr. cornucopioides*.

2.4.2 Protein and Amino acids

Mushrooms have good nutritional value particularly as a source of protein that can enrich human diets especially in some developing countries where animal protein may not be available and are expensive. The nutritional value of proteins is usually very high in majority of fungi (Ilievska and Petrovska 2000) and fungal proteins are considered to be of equal quality to those of animal origin (Longvah and Deosthale 1998).

Several members of Cantharellaceae have been reported as good protein source so far. Danell (1994) estimated the protein content in *C. cibarius* and revealed that it contained approximately 10 percent protein by dry weight and emphasized that the

protein content depending on the genotype and the age of the fruit bodies. Caglarirmak *et al* (2002) reported that the value of protein contents of 3 edible mushrooms *viz*, *C. cibarius*, *L. piperatus* and *B. edulis* and determined highest (3.1 mg/g) protein content in *C. cibarius*.

Colak *et al* (2009) reported detailed nutrition analysis in 8 different mushrooms (*Craterellus cornucopioides*, *Armillaria mellea*, *Sarcodon imbricatus*, *Lycoperdon perlatum*, *Lactarius volemus*, *Ramaria flava*, *Cantharellus cibarius* and *Hydnum repandum*). They found major compounds of mushrooms as proteins varying from 21-50%, with highest protein content for *Craterellus cornucopioides* (50.10%) followed by *C. cibarius* (34.17%).

Pandey and Budhathoki (2007) studied on protein contents of 35 different species of mushrooms including some species of Cantharellaceae and reported that the highest amount of protein *i.e.*, 1.58 mg/g in C*antharellus subcibarius* while moderate amount of protein found in *Cantharellus cibarius* (1.06 mg/g) and documented that the amount of protein varies from species to species in the same genus and depends on photographical condition. Colak *et al* (2009) reported the nutritional composition of the dry wild mushrooms and it comprised the protein contents of *Cr. cornucopioides* and *C. cibarius* as 50.10% and 34.17%, respectively.

Beside protein contents in *C. cibarius*, Danell (1994) also reported this species as rich source of amino acids, which are essential for human body. *Cantharellus cibarius* contained highest content of glutamic acid (15.6 µg/mg) and lowest content of methionine (1.2 µg/mg). Degreef *et al* (1997) reported protein and amino acid composition of 9 different wild edible mushrooms including 4 species of Cantharellaceae (*C. cibarius var. defibulatus*, *C. congolensis*, *C. miniatescens* and *C. symoensii*) and showed that the highest amount of protein and essential amino acids was found in *C. miniatescens*.

Mdachi *et al* (2004) worked on Tanzanian wild edible mushrooms including *C. cibarius* and reported 16 known essential amino acids except isoleucine in it. The results revealed that the highest amount of threonine, tryptophan, alanine and glutamic acid were found in *C. cibarius* compared to other edible mushrooms.

2.4.3 Fatty acid

Mushrooms are very low in fat contents. On dry weight basis, different species of mushrooms contain fat contents ranging from 1.1 to 8.3% and from 0.1 to 0.3% on fresh weight basis. In general, the crude fat of mushrooms has representative of all classes of lipid compounds including free fatty acids, monoglycerides, triglycerides, sterols, sterol esters and phospholipids.

Several studies have been carried out on the fatty acid composition and nutritional qualities of wild edible mushrooms including different species of Cantharellaceae (Mattila *et al*. 2002; Barros *et al*. 2007; Colak *et al*. 2007) and demonstrated that the presence of low fat content and high protein values in different species of Cantharellaceae can be concluded as good alternative food for that persons those with heart or weight problems

Kavishree *et al* (2008) studies 23 species of naturally grown edible mushrooms, including *C. cibarius* from four geographic regions of India. Detailed analysis of fat and fatty acids of different species was performed and provide a useful database for taxonomic/nutritional/nutraceutical evaluation. *Cantharellus cibarius* contained linoleic and oleic acids in the ratio of 0.48%, and saturated fatty acids accounted for 39%. They concluded that presence of unsaturated fatty acids, particularly linoleic acid provides the flavor compounds in different species of Cantharellaceae.

2.4.4 Antioxidant activity and phenolic compounds

Mushrooms contain various polyphenolic compounds recognized as an excellent antioxidant due to their ability to scavenge free radicals by single-electron transfer (Hirano *et al*., 2001). Some common edible mushrooms, which are widely consumed in Asian culture, have currently been found to possess antioxidant activity, which is well correlated with their total phenolic content (Yen and Hung, 2000; Cheung and Cheung, 2005). In the last few years, an increasing interest in the consumption of mushrooms has arisen, due to their elevated polyphenol concentration, which correlates with an elevated antioxidant activity.

A significant antioxidant activity has been reported in several species of *Cantharellus*. Kasuga *et al* (1993) studied antioxidant activity of ethanol extract of 150 mushrooms species and found higher antioxidant activity in *Cantharellus* sp. compared to many other mushrooms such as *Amitake* and *Oagitake* spp. Valentao *et al* (2005) showed that *C. cibarius* is characterized by the presence of six phenolic compounds (3-, 4-, and 5-O-caffeoylquinic acid, caffeic acid, p-coumaric acid, and rutin).

The pink-red *C. cinnabarinus* and the orange *C. friesii* are composed almost entirely of canthaxanthin (Fig. 2.3), a pigment also found in salmon and flamingo feathers (Gill and Steglich 1987). Canthaxanthin (chemically described as β, β-carotene-4, 4-dione) was first isolated from the edible mushroom, *Cantharellus cinnabarinus* (Haxo 1950). It has been reported to protect human tissues from oxidative damage (Chen and Tappel 1996) and is sold as an antioxidant.

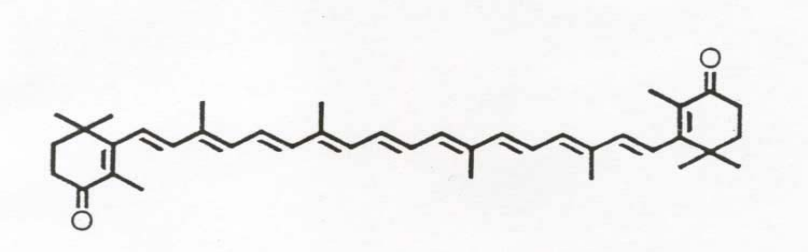

Fig. 2.3 Chemical structure of Canthaxanthin ($C_{40}H_{52}O_2$)

Puttaraju *et al* (2006) analyzed the total antioxidative status, employing multimechanistic antioxidative assays such as inhibition of lipid peroxidation, determination of reducing power, and free radical scavenging ability, in addition to determination of total phenolics in two mushrooms collected from Indian forests, namely *C. cibarius* and *C. clavatus*. The phenolic contents present in the fruit bodies of *C. cibarius* and *C. clavatus* were 2.8 mg/g and 13.5 mg/g, respectively. *Cantharellus clavatus* expressed very high activity scavenging activity offering 0.98 mg of BHA (Butylated HydroxyAnisole) equiv/g of sample, while *C. cibarius* offered low activity of 0.23 mg of BHA equiv/g of sample. Reducing power abilities

of *C. cibarius* and *C. clavatus*, expressed as milligrams of GAE (Gallic Acid Equivalent) per gram of sample were recorded as 3.4 mg of GAE/g and 9.1 mg of GAE/g, respectively.

Recently, Palacious *et al* (2011) reported that C. *cibarius* and *Cr. cornucopioides* contained between 1 and 6 mg of phenolics per gram of dried mushroom, while the flavonoid concentrations ranged between 0.9 and 3.0 mg per gram of dried matter. Later they evaluated the antioxidant properties of the methanolic extracts from these mushrooms by monitoring the linoleic acid autoxidation, and found *C. cibarius* being the most effective against lipid oxidation (74% of inhibition).

2.4.5 Carotenoids

The carotenoids constitute the largest class of naturally occurring pigments and are synthesized in plants from acetyl-coenzyme A, by a series of well-defined condensation reactions. Carotenoids are found almost everywhere in nature, but particularly among organisms that bask in the sun. Those interesting compounds, most of which show a yellow to red colour, have attracted the attention of chemists and biologists.

Bicyclic carotenoids are the compounds responsible for the yellow colour of many chanterelles (Arpin and Fiasson 1971; Gill and Steglich 1987; Mui *et al*. 1998). Common in green plants, where they act as antioxidants, ultraviolet protectors, and pigments, these chemicals are rare in mushrooms (Gill and Steglich 1987). The golden chanterelle, *C. cinnabarinus* and *C. minor* contained beta-carotene and small amounts of other carotenoids (Gill and Steglich 1987). Vitamin A, synthesized from beta-carotene, (Jensen and Salisbury 1984) is essential for good night vision (Stryer 1988), a fact that might explain the use of chanterelles by Chinese herbalists to treat night blindness.

2.4.6 Vitamins

The higher fungi are a relatively good source of some important vitamins. Scientific explorations of chanterelles have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine (Solomko and Eliseeva, 1988). Chanterelles are also rich source of vitamins A and D (Pilz *et al*. 2003). Hawksworth et al. (1983) reported that the basidiomes of *C. cibarius* is an edible fungus with high source of vitamin A.

Mushrooms rank very high with regard to vitamins B1 and B2 supply and provide up to 40% of daily requirements (Kelley 1997) and they contain thiamine up to 0.1 and ascorbic acid (vitamin C) 3 mg per 100 g fresh mushrooms (Priestly 1984). It has been established for example that 100 g of fresh mushroom may provide 20% of human day demand of riboflavin (B2) and 25% of niacin (Szuecs 1958).

Caglarirmak *et al* (2002) studied on the nutritional values of *C. cibarius*, *L. piperatus* and *B. edulis* and reported that vitamins B1 and B2 contents of *C. cibarius* is 0.3 mg/100 g and 0.12 mg/100 g, respectively were highest among these three species and contained approximate value of ascorbic acid (vitamin C), pantothenic acid and niacin.

Chanterelles are one of the most concentrated natural dietary sources of vitamin D, and are certainly an excellent choice for vegetarians. This vitamin is synthesized when the mushroom tissues are illuminated with sunlight. Mattila *et al* (1994) have shown that *C. cibarius* and *C. tubaeformis* contain high amounts of ergocalciferol, 128 and 298.2 mg/kg of fresh weight respectively, possibly because in the genus *Cantharellus*, the pileus and gills are often more effectively exposed to light than they are in other species of fungi.

Rangel-Castro *et al* (2002) reported that ergocalciferol (vitamin D2) content in the fruit bodies of *C. cibarius* varied between 0.12 and 6.30 μg/g of dry weight. Next to cod liver oil, chanterelles are one of the most concentrated sources of vitamin D. High vitamin D concentrations might play a role in chanterelle ultraviolet protection

and resistance to insect predation (Rangel-Castro 2002). The influence of illumination conditions in determining ergocalciferol content is a factor that should be taken into account in case of cultivated fungi.

Outila *et al* (1999) have shown that human subjects can easily absorb ergocalciferol from lyophilized and homogenized *C. tubaeformis*. Thus, for some groups, for example vegetarians or individuals allergic to fish, fungi can be an important dietary source of vitamin D. Eating such fungus frequently can help in preventing one from abnormal sight ophthalmia, night blindness, dryness of skin and mucous membrane from losing its power of secretion. It resists certain infectious disease of the respiratory tract and also used for the treatment of abscesses and wounds.

2.5 Bacteria associated with fungi

Although microorganisms are perhaps the most diverse (Torsvik *et al*. 2002; Venter *et al*. 2004) and abundant (Whitman *et al*. 1998) type of organism on Earth, the distribution of microbial diversity at continental scales is poorly understood. Ecologists describing microbial biogeography typically invoke Beijerinck (1913) from a century ago; ‘‘everything is everywhere, the environment selects.’’ However, few studies have attempted to verify this statement or specify which environmental factors exert the strongest influences on microbial communities in nature (Papke and Ward 2004).

Microorganisms living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence are defined as endophytes (Wilson 1993; Saikkonen *et al*. 2004). They are also reported to be associated with fungal fruit bodies. Bacteria are ubiquitous colonizers of the ectomycorrhizosphere, and occur on the mycorrhizal mantle, and at inter- and intracellular locations within the mantle and Hartig net. Extensive bacterial biofilms have also been observed covering the foraging hyphal front (Nurmiaho-Lassila *et al*. 1997). Different taxa of microbes in mycorrhizosphere have been reported to be associated either with the hyphae or

with the sporocarps of different mycorrhizal fungi (Garbaye 1991; Nurniaho-Lassila *et al*. 1997).

Despite numerous accounts of bacterial isolations from healthy ectomycorrhizal roots and sporocarps of a wide range of fungal species (Li and Castellano 1987; Danell *et al*. 1993; Varese *et al*. 1996), the functional significance of the bacterial associate has rarely been elucidated. The role of bacteria has been explored in various ECM fungi like *Laccaria bicolour*, *Amanita muscaria* and *Suillus bovinus* (Frey-Klett *et al*. 1997; Maier *et al*. 2004; Schrey *et al*. 2005), but it has not been explored with different species of *Cantharellus*. *Cantharellus* species are ectomycorrhizal fungi that grow in mutually beneficial, or symbiotic, association with green plants and their associated bacteria. They are widely used in Europe, Africa, Asia and northwestern USA, but knowledge about their biology and symbiotic association is still scarce. The ecology of *Cantharellus* spp. has not been explored and a reproducible method for isolating and maintaining such cultures is still not known.

Danell *et al*. (1993) isolated and identified aerobic bacteria from fruit bodies of *C. cibarius* and found that most of them belong to the *Pseudomonas fluorescent* group. *Bacillus* spp., *Xanthomonas* spp., and *Streptomyces* spp. were also found, though in significantly lower amounts. Interestingly, they also observed that the proportion of *P. fluorescent* in soil samples of fruit bodies growing sites was only 12%, while inside the fruit bodies these bacteria represented an average of 78% of the total culturable community. Danell and Camacho (1997) reported greenhouse production of *C. cibarius* from mycelia associated with one-year-old pine seedlings and these fruit bodies were also colonized by bacteria. The amino acids, organic acids, and sugar released by chanterelles serve as a likely nutrient source for the bacteria (Rangel-Castro *et al*. 2002). But they also reported that faster growth of bacteria in medium might possess inhibitory role in the cultivation of chanterelles. However, the dynamics of the bacterial community that inhabits *Cantharellus* spp. has not been

studied extensively. Thus, the study of fruit body-associated bacteria is important for understanding their ecological role as well as characteristics features.

2.6 Phenotypic and Biochemical approach for bacterial diversity analysis

Phenotypic approaches for the identification of bacteria using either conventional or commercial systems are still used in the majority of laboratories. The historic way to characterize bacteria is to describe quantitatively as many phenotypic properties as possible, such as morphology, structure, cultivation, nutrition, biochemical metabolism, pathogenicity, antigenic properties and ecology. Phenotypic similarities do not necessarily indicate phylogenetic relationships (relationship based on the ancestry of organisms) (Gillis and De Leg 1992). In contrast to animals and plants, the morphology of microorganisms is in general too simple to serve as a basis for a sound classification and to allow for reliable identification.

Generally the identification of bacteria is carried out according to Bergey's Manual on Systematic Bacteriology that includes several biochemical tests such as catalase, oxidase, nitrate reduction, starch hydrolysis and antibiotic profiling. Catalase activity was determined by detective bubble formation with 3% H_2O_2 solution. Oxidase was determined by using paper disc with tetramethyl-p-phenylenediamine. Nitrate reduction by using nitrate agar plate; DNAase test by DNAase agar medium with methyl green as an indicator. Starch hydrolysis was determined on starch hydrolyzing agar by detecting cleared zones formed around the colonies. Antibiotic profiling was also done by using ICOSA universal-1 kit (Hi-Media Laboratories, India) having twenty different antibiotics of various Concentrations (Karn *et al*. 2010).

Tripathi and Garg (2010) characterized bacterial isolate based on morphology, physiology and biochemical tests as per Bergey's manual of determinative bacteriology. Isolate was identified by performing various tests like Gram's staining, spore formation, motility, catalase, cytochrome oxidase, arginine dihydrolase, lysine and ornithine decarboxylase, nitrate reduction, starch, casein and urea hydrolysis.

Roy *et al* (2009) reported that morphological characterization of the isolate was based on the light and electron microscopy. Other than simple and gram staining, the detailed characterization was conducted through various other staining procedures for detection of endospore, capsule, flagella as per standard procedure.

Biochemical characterization included the test for presence of enzymes namely protease, DNase, lipase, lecithinase, oxidase & catalase. The specific readymade culture media (such as media from Himedia laboratories) were also used for the enzymatic assay. Thus, until very recently, microbial identification required the isolation of pure cultures (or defined co-cultures) followed by testing for multiple physiological and biochemical traits (Amann *et al*. 1995). Determining physical and chemical factors, such as temperature, pH, or geography, that correlate with differences between diverse microbial communities will reveal how easily microbes tolerate different kinds of environmental change and will increase our understanding of microbial ecology and evolution. These methodologies fit well on every type of bacteria including endophytes.

Endophytic bacteria promote the growth of fungi and plants in various ways, for example through secretion of plant growth regulators; e.g. indole-acetic acid (Lee *et al*. 2004), via phosphate solubilizing activity (Wakelin *et al*. 2004), by enhancing hyphal growth and mycorrhizal colonization (Will and Sylvia 1990), production of siderophores (Costa and Loper 1994) and by supplying biologically fixed nitrogen (James *et al*. 1994). The bacterial strains use effectively C sources which are common components of plant root exudates, e.g. glucose, sucrose, maltose and mannose as well as compounds synthesized by fungi. The fungal metabolites and of C source use of associated bacteria can successfully contribute to accelerate the selection of capable plant growth promoting combinations. These parameters can be used to characterize endophytic bacteria.

2.7 Molecular approach for bacterial diversity analysis

Early traditional characterization of the microorganism depended upon phenotype, biochemical and serological tests. But these are not fully reliable as mutation and environmental conditions can affect the physiological traits. Ribosomal RNA

(particularly 16S rRNA) is considered to be most reliable candidate molecule for identification-classification study. The analysis of 16S rRNA genes, aided by using PCR to amplify target sequences in environmental samples, has enabled microbial ecologists to identify and characterize microorganisms in natural community like activated sludge. The taxonomic position of an organism can be determined by comparing the sequence with those of other bacteria (Amann *et al*. 1995). Analysis of 16S rRNA gene is now widely used for analysis of bacterial population and has been given most attention (Woese *et al*. 1990). Ribosomal RNA is one of the best candidates and it has been used for the studies on bacterial evolution.

Since rDNA-based fingerprinting lays too much emphasis on a single locus for deciphering phylogenetic affinities, isolates are subjected to multi-locus analysis like BOX-PCR, ERIC-PCR and random amplification of polymorphic DNA (RAPD). The BOX-PCR, in contrast to ARDRA, is the multi-locus analysis and produces higher degree of resolution among the isolates. The repetitive sequences in the form of BOX elements are randomly located within the whole genome and the BOX primers amplify genomic regions between the two BOX elements. The distribution of these repetitive sequences (BOX and ERIC) is nearly a true reflection of genomic structure and amplification of inter-REP elements often detects similarities in a given group of bacteria. It is anticipated that REP- and ERIC-like sequences are virtually ubiquitous in bacteria and facilitate a rapid molecular characterization by PCR-based fingerprinting (Selenska-Pobell *et al*. 1995).

Although 16S rRNA analysis represents a very useful technique for culture independent analysis of complex microbial communities, the clone frequencies in the clone libraries do not reflect the *in situ* quantities of the respective microorganisms. Possible reasons are differences in the numbers of rRNA operons, efficacies of cell lyses and DNA extraction or shifts due to PCR amplification (Eschenhagen *et al*. 2003). The aim of the study was to elucidate different endophytic bacteria associated with fruit bodies of *Cantharellus* spp. Bacteria were first differentiated based on REP- and BOX-finger printing. Further, the bacteria were characterized for their physiological and biochemical basis and identified by 16S rRNA gene sequence analyses.

Chapter 3

Materials and methods

3.1 Diversity, identification and characterization of Cantharellaceae of Western Himalayas

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3.1.1 Geographic features

3.1.1.1 Region: The Western Himalayas region, extended from Kashmir to Nepal, is about 800 km long and 195-400 km broad. It extends between 29-30º latitude and 74-81º longitude. In the present study, the area of Western Himalayas lying in Himachal Pradesh and Uttarakhand was explored (Fig 4.1). Himachal Pradesh is situated 30° 22'-33°12'N and 70°47'-74°04'E and Uttarakhand is between 29°37'-30°15'N and 77°53'-79°15'E.

3.1.1.2 Climate: The distribution of the rainfall is quite uneven and the mean annual precipitation varies between 600-3200 mm during the rainy season of July to September. The direction of monsoon winds and other factors like altitude, location and direction of the ranges play an important part in the distribution of the rainfall. During winter, it snows in many parts of Himachal Pradesh and Uttarakhand. Snow fall, usually takes place in the month of December to February, although unusual falls may occur early or late. The mean annual temperature varies from 15 to 30ºC, the average being about 25ºC. The temperature is lowest during winter season (minimum -10ºC). Majority of the collections has been done from the temperate regions of Western Himalayas. The trees usually had a height of 20-30 m, and the species occurring mixed, singly or in groups of varying extent (Kumar *et al*. 1990). Greater part of the forests consists of oak (*Quercus incana*), rhododendron (*Rhododendron arboretum*), other common species are *Cornus capitata*, *Pieris ovalifola*, *Rhamnus* sp., *Populus ciliate*, *Quercus dilatata*, *Quercus semicarpifolia* and *Cedrus deodara*.

3.1.2 Sample collections

During the rainy season of 2006-10, the collections were done from the forest forays of Himachal Pradesh and Uttarakhand. Most of the sites were near to Solan and Shimla, visited regularly after spell of rain. Distant sites were, however, visited during mushroom growing season every year to make mushroom forays to different localities in the vicinity. After every collection, field characteristics were taken including spore prints and chemical spot test and the sites were marked to avoid problem during re-sampling of basidiocarps. Further, the steps were described as below:

(i) The basidiocarps were picked up by digging them out carefully with the help of sharp knife. Attempts were made to collect all the developmental stages of the basidiocarps to have an idea of range of size, colour and shape. The specimens were wrapped in the wax paper and placed in the collection bags. Associated habitat and relevant ecological data like altitude, forest type, temperature and humidity were recorded in the field.

(ii) In the laboratory, collections were systematically placed and each sample was allotted a collection number.

(iii) The collections were dried in drier (hot air oven) at 40-55ºC. The dried collections were sealed. 1,4-p-dichlorobenzene and naphthalene balls were used as insect repellants.

3.1.3 Macroscopic studies

The various perishable macroscopic characters, which help in the identification of mushrooms, were recorded on the date of collection. These characteristics were carefully recorded in the field keys specifically designed for the purpose and photographed in natural conditions using digital camera. The following macroscopic characters were also notes:

(i) Pileus: Shape, size colour, consistency, dry/wet/sticky/smooth/scaly, marginal characters, veil present or absent.

(ii) Stipe: Shape, size, colour, solid, hollow, consistency, surface smooth/scaly/striate/fibrillose/dry/viscid.

(iii) Lamellae: Distinctly formed or not, attachment, crowded or distant, consistency, thickness, width, shape, size, colour and arrangement of (decurrent to subdecurrent, asantomosing) gill types.

(iv) Context: Colour consistency (colour change after cutting and brusing) and thickness.

(v) Smell and taste: It was studied manually.

(vi) Spore print: Spore prints were taken from the fresh collections on clean glass slides by placing the smaller pileus/pieces on the slide and covering them with petriplates. Spores were deposited on the slides after 5-8 hours. Colour of the spores in mass was noted and slides were kept carefully.

(vii) Colour observation: Colour standard used were as given by Kornerup and Wanscher (1978) and Mearz and Paul (1930).

(viii) Ecology: For the ecological studies, some parameters were noted in the field during collection: altitude, substratum, growth habit (solitary, scattered, gregarious and caespitose), prevalence, occurrence and edibility.

3.1.4 Microscopic studies

The microscopic characters were studied either from free hand section cutting or from the tissue maceration. For this purpose, the tissue was first treated with 5% KOH and then stained with 2% alkaline congo red or 2% phloxine. After staining the period of 8-10 minutes, the tissue was macerated by taping the slide or with direct pressure on the cover glass. The specimens were examined with a Leica DM LS2 (Glattbrugg, Switzerland) microscope with light and phase contrast optics and characters was recorded by camera lucida attached on the microscope. The following criteria were found particularly for the identification and confirmation of family Cantharellaceae.

(i) Spore morphology: The spore wall shows varieties of colour reactions with certain stains or chemicals and all these spore characters are significant in mushrooms taxonomy. The colour reactions exhibits by spore wall are as follow:

(a) ***Cyanophilic reactions*:** The spores were stained with cotton blue (0.2%). When spore wall show the retention of blue colour known as cyanophilic, and if they do not retain colour, known as non-cyanophillic.

(b) ***Amyloid reaction:*** Spores when treated with iodine solution like Melzer reagent, the wall may either show blue or red colouration or may yellow colouration or remain colourless; accordingly, they are known as amyloid, pseudoamyloid and nonamyloid respectively.

(ii) Basidia: Size and shape of basidia were studied, data were noted, single celled and thin walled, clavate or cylindrical-clavate, clubed shaped structures. The basidia bear basidiospores on sickle shaped apical extension called sterigmata. The number of spores on basidia is also a typical feature.

(iii) Hymenophoral trama: The sterile and fundamental tissue, making the main core of the gills of mushrooms, is known as gill or hymenophoral trama. The hyphae within this tissue are variously arranged and arrangement of cell in different configuration showed a diagnostic criterion in taxonomy. The four different types of tramal hyphae arrangement are namely, (a) Intermixed to irregular, (b) Subregular to regular, (c) Bilateral, (d) Inverse.

(iv) Cuticular layer and context: Cuticular and context of the pileus and stipe were studied by cutting thin section of pileus or stipe tissue or same ways as tramal hyphae.

(v) Cystidia: These are sterile elements present in month of the mushrooms on pileus, gill and stipe edge. Cystidia present on lamellae surface and lamellar edge, known as pleurocystidia and chielocystidia respectively. These structures do not occur in family Cantharellaceae.

(vi) Clamp connections: It is well known that the dikaryotic mycelium of higher basidiomycetes is provided with a special structure at the septa of the hyphae, known as clamp connection. The presence or absence of clamp connections showed a great taxonomic variation within family Cantharellaceae.

3.1.5 Molecular studies

3.1.5.1 Procedure for DNA Isolation (VanKan *et al*. 1991)

For molecular characterization, the genomic DNA was extracted from the fruit bodies of collected specimens of Cantharellaceae.

1. The dried fruit body's powder of Cantharellaceae (100 mg) was added in an extraction buffer containing Tris-HCl (200 mM) pH 7.5, NaCl (250 mM), EDTA (25 mM) and SDS (0.5%), and treated with 1µg/µl ribonuclease A. During incubation at 65°C for 20 min, the cell suspension was mixed thoroughly by inverting the Eppendorf tube several times

2. Added 0.5 ml of pure equilibrated phenol, mixed well and allowed it to stand for 15 minutes.

3. Added 0.5 ml of chloroform: isoamylalcohol mixture (24:1) mixed well and left for 15 minutes and centrifuge for 20 minutes at 12,000 rpm. Removed the upper aqueous phase carefully without taking any of the interphase material and transformed it into a fresh eppendorff tube.

4. Again 0.4 ml of chloroform: isoamylalcohol (24:1) mixture was added and mixed by inversion. Centrifuged at 12,000 rpm for 10 minutes and transferred the supernatant into a new tube.

5 Added 0.54 volumes of isopropanol to precipitate the DNA. Mixed by inversion, left for 15 minutes at room temperature and centrifuged at 10.000 rpm for 10 minutes. Supernatant was discarded.

6. DNA pellet obtained was washed with 70% ethanol to remove salts. Centrifuged for 5 minutes, removed the supernatant and dried the pellet for 10-15 minutes.

7. Resuspended the DNA pellet in 300 µl of 0.2 M ammonium acetate and was left overnight at 4°C.

8. Afterwards, DNA was precipitated by adding 600 µl of ethanol.

9. DNA pellet obtained after centrifugation was washed with 70% ethanol and dried under vacuum.

10. Resuspended the pellet in 25 µl of MQ water.

3.1.5.2 DNA Purification

The DNA was purified by elution through the Wizard DNA Clean up system (Promega, USA) according to manufacturer's instructions in order to remove contaminants, which can hamper in manipulation of DNA.

3.1.5.3 Electrophoresis of DNA on agarose gels

DNA was loaded on agarose gels (0.7% w/v) prepared in 0.5× TBE, pH 8.0 using a 6x loading dye (Appendix I). Ethidium bromide (0.5 µg/ml) was added to stain the gel prior to pouring. The DNA was then electrophoresed at 3 volts/cm for 45-60 minutes and visualized on a U.V. transilluminator.

3.1.5.4 Spectrophotometric quantification of DNA

The concentration of extracted DNA in suspension was estimated by spectrophotometric measurement at A_{260}. For double-stranded DNA suspensions, an OD of 1.0 at a wavelength of 260 nm and using a cuvette with 1 cm light path is equal to a concentration of 50 µg/ml. The quality of the DNA was evaluated by measurement of the A_{260}/A_{280} and the A_{230}/A_{260} ratios. Ideally, the A_{260}/A_{280} ratio should be 1.8-2.0 while the A_{230}/A_{260} ratio should be 0.3-0.9. Ratios (A_{260}/A_{280}) less than 1.8 indicate protein or phenol contamination, while ratios greater than 2.0 indicate the presence of RNA.

3.1.5.5 Ethidium bromide fluorescent DNA quantification

DNA was migrated electrophoretically in an agarose gel containing ethidium bromide (0.5 µg/ml). The quantity of DNA was visually determined with reference to

a known DNA concentration of lambda phage (Fermentas, USA) by comparing the intensity of fluorescence.

3.1.5.6 Amplification of internal transcribe spacer (ITS) region

The polymerase chain reaction (PCR) provides a rapid and highly sensitive method for the primer-mediated enzymatic amplification of specific target sequences resulting in the exponential increase of target DNA copies.

Internal transcribe spacer (ITS) region of the rDNA from genomic DNA was amplified by PCR using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*. 1990). The 50 µl reaction mixture for PCR amplification contained: 10 ng DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 µM of each primer and 2.5 units of Taq DNA polymerase (Fermentas, USA). Amplifications were performed in a thermal cycler (Perkin Elmer, USA) with an initial denaturation step of 94ºC for 5 min followed by 35 cycles of 94ºC for 1 min, 54ºC for 1 min and 72ºC for 1 min and a final extension of 72ºC for 10 min. Controls containing no DNA template were included for the presence of contamination of reagents and reaction buffer. Aliquots (5µl) of amplification products were electrophoresed on a 1.5% agarose gel and visualized on a UV transilluminator.

3.1.5.7 Amplification of Large subunit (LSU) region

Nuclear large subunit from genomic DNA was amplified by ITS4R (5'-GCATATCAATAAGCGGAGGA-3') and LR5 (5'-ATCCTGAGGGAAACTTC-3') (Vilgalys and Hester 1990). 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 dNTPs, 0.5 µM of each primer and 2.5 units of Taq DNA polymerase (Fermentas, USA). Amplifications were performed in a thermal cycler (Perkin Elmer, USA) with an initial denaturaton step of 94ºC for 3 min followed by 35 cycles of 94ºC for 1 min, 50ºC for 1 min and 72ºC for 1.5 min and a final extension of 72ºC for 8 min.

Successful amplifications were confirmed by agarose gel (0.8% w/v) electrophoresis and ethidium bromide staining.

3.1.5.8 RFLP analysis of ITS and LSU products

Different isolates of Cantharellaceae were subjected to ITS-RFLP analysis. ITS-PCR products were digested with 5 different restriction enzymes *viz*. *Alu I*, *Mbo I*, *Hinf I*, *Hae III* and *Taq I* (Fermentas, USA).

1. Sterile water was added in a sterile microfuge tube containing DNA solution and made up a volume of 17 µl (500 ng).
2. The appropriate 10× restriction enzyme assay buffer was added and mixed thoroughly by tapping the tube.
3. 1 µl (2-5 units) of the restriction enzyme was added, mixed by tapping the tube.
4. The mixture was incubated at the appropriate temperature for 1-2 hr.
5. To stop the reaction, 4-5 µl gel-loading buffer was added, mixed by vortexing briefly.

3.1.5.9 Purification of PCR products

Amplified ITS products and LSU region were purified by agarose gel (0.8%) electrophoresis prior to cloning. The DNA fragment was excised from the gel, using the QIAquick gel extraction kit (Qiagen Inc., USA) as per manufacturer's direction. Purified PCR products were eluted with 40 µl TE buffer (pH 8.0). In this manner, purified PCR products were directly applied for the cloning in T-vector.

3.1.5.10 Ligation in T-vectors

The ITS and LSU PCR products were cloned using the restriction independent InsTA Cloning Kit (Fermentas, USA). The respective amplicon was ligated into pTZ57R/T vector. The final reaction volume for ligation was 30 µl (Appendix I) and incubated at 22°C for 12 hrs. The reaction mixture was kept overnight and analyzed on 0.7% agarose gel.

3.1.5.11 Genetic Transformation of ITS and LSU products into *E. coli* DH5α cells

A single colony of *E. coli* DH5α from a freshly grown plate was inoculated into 25 ml of LB broth in a 250 ml flask and incubated the culture for 16-20 hrs at 37°C under shaking condition (120 rpm). Aseptically 200 µl of the above-saturated culture was transferred into 25 ml of fresh LB broth in a 250 ml flask. The culture was further incubated with vigorous shaking at 37°C for 2-3 hrs. To monitor the growth of the culture, the OD_{590} was determined at every one-hour (OD_{590} should be ~ 0.5). The above culture was transferred to sterile, disposable, ice-cold 50 ml polypropylene tubes. The culture was cooled to 0°C by storing the tubes on ice for 10 min. The cells were harvested by centrifugation at 8,000 rpm for 10 minutes at 4°C. The pellet was resuspended in 10 ml of ice-cold 0.1 M $CaCl_2$ and store on ice for 15 min. Further, the cells were recovered by centrifugation at 8,000 rpm for 10 min at 4°C. The cell pellet was resuspended in 1 ml of ice-cold 0.1 M $CaCl_2$. $CaCl_2$ treatment for 2 hours induces considerably a transient state of "competence" in the *E. coli* cells. One hundred micro liter of the suspension of competent cells were transferred to a sterile and prechilled microfuge tube (1.5 ml capacity). The plasmid DNA sample (~100 ng in a volume of 5 µl or less) was added to each tube. The content of the tubes were mixed gently and stored the tubes on ice for 30 min. The tubes were incubated in a circulating water bath that has been preheated to 42°C for exactly 2 min without shaking. The tubes were rapidly transferred to an ice bath and chilled the cells for 1-2 min. One ml of LB broth was added to each tube and incubated the cultures for 45-60 min at 37°C to allow the bacteria to recover and to express the antibiotic resistance marker encoded by the plasmid. One hundred micro liters of transformed cells were spreaded on Luria agar-Ampicillin-X-Gal-IPTG plates and incubated at 37°C. Transformed colonies appeared in 12-16 hrs.

3.1.5.12 Blue/white screening for recombinant plasmids

After transformation of the ligated product, the *E. coli* DH5 α (LacZ-) bacterial host cells were plated on Luria Agar (Appendix I) medium containing 50 μg/ml ampicillin, for selection of transformants. X-Gal and IPTG were used to screen for colonies containing a recombinant plasmid. The cloning site in the pTZ57R/T vector is located in the multiple cloning site (MCS) of the plasmid's lacZα gene; if insert was present, non-functional β-galactosidase is produced, and the transformed bacterial colony is white. White colonies were picked and grown in 2 mL LB containing ampicillin (50 μg/mL) and simultaneously patching of these cultures were done on Luria agar containing ampicillin. Plasmid was isolated (described in the proceeding section) and reamplification of the insert was done using vector's promoter specific sequences

3.1.5.13 Isolation and purification of plasmid DNA from recombinant bacteria by alkaline lysis method

The plasmid DNA was isolated based on the alkaline lysis method. A single transformed *E. coli* white colony was transferred into 2 ml of Luria broth containing appropriate antibiotic (ampicillin, used in a final concentration of 50 μg/ml) in a loosely capped 15 ml tube and incubated the culture overnight at 37°C with vigorous shaking. 1.5-2.0 ml of the above-saturated culture was poured into a microfuge tube and cells were harvested by centrifugation at 8,000 rpm for 1 min. The bacterial pellet was resuspended in 200 μl of ice-cold Solution I (Appendix I) by vigorous vortexing to ensure that the bacterial pellet is completely dispersed in this solution. Further 200 μl of freshly prepared Solution II (Appendix I) was added and the contents were mixed by gentle inversion of the tubes, five to ten times. Vortexed is avoided here. The tubes were stored on ice for 5 min. Finally 300 μl of ice-cold Solution III (Appendix I) was added and mixed by inversion to disperse Solution III (Appendix I) through the viscous bacterial lysate. The tubes were stored on ice for 10

min. The tubes were centrifuged at 12,000 rpm for 10 min in a microfuge. The upper aqueous phase was then extracted with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). To precipitate extracted plasmid DNA, 0.7 volumes isopropanol were added to the aqueous phase, followed by 10 min centrifugation at 12,000 rpm. The DNA pellets were washed with 750 µl ethyl alcohol (70%) and microfuged another 10 min. Finally; the pellets were resuspended in 40 µl TE buffer/milliQ water and stored at 4°C for further use.

3.1.5.14 Size screening for recombinant plasmids

Clones containing ITS products of approximately 750-1600 bp and LSU-PCR ~900 bp inserts were identified by PCR screening, using the rapid protocol for preparation of template DNA from single bacterial colony using M13-forward (5'-GTAAAACGACGGCCAGT-3') M13-reverse (5'-CAGGAAACAGCTATGAC-3') plasmid primers. The amplification products were checked by agarose gel (1.0% w/v) electrophoresis.

3.1.5.15 Sequencing

The ITS and LSU products were sequenced for both strands using M13 forward and reverse primers, used for pTZ57R/T vectors. The sequence was generated by chain termination method (Sanger *et al*. 1977) using an Applied Biosystems automatic sequencer (DNA Sequencing Facility, Department of Biochemistry, South Campus, Delhi University, New Delhi, India).

3.1.5.16 Sequence analysis

The ITS and LSU of nrDNA sequences of all the isolates were compared with those available in GenBank databases using BLAST program (Altschul *et al*. 1997). The sequences of ITS products and nrDNA large subunit were aligned to minimize the number of inferred gaps. The sequences were edited with BioEdit 5.0.6 (Hall 1999) and aligned using MAFFT v 6.240 with other sequences obtained from GenBank. All sequences were submitted to NCBI database (Table 4.2).

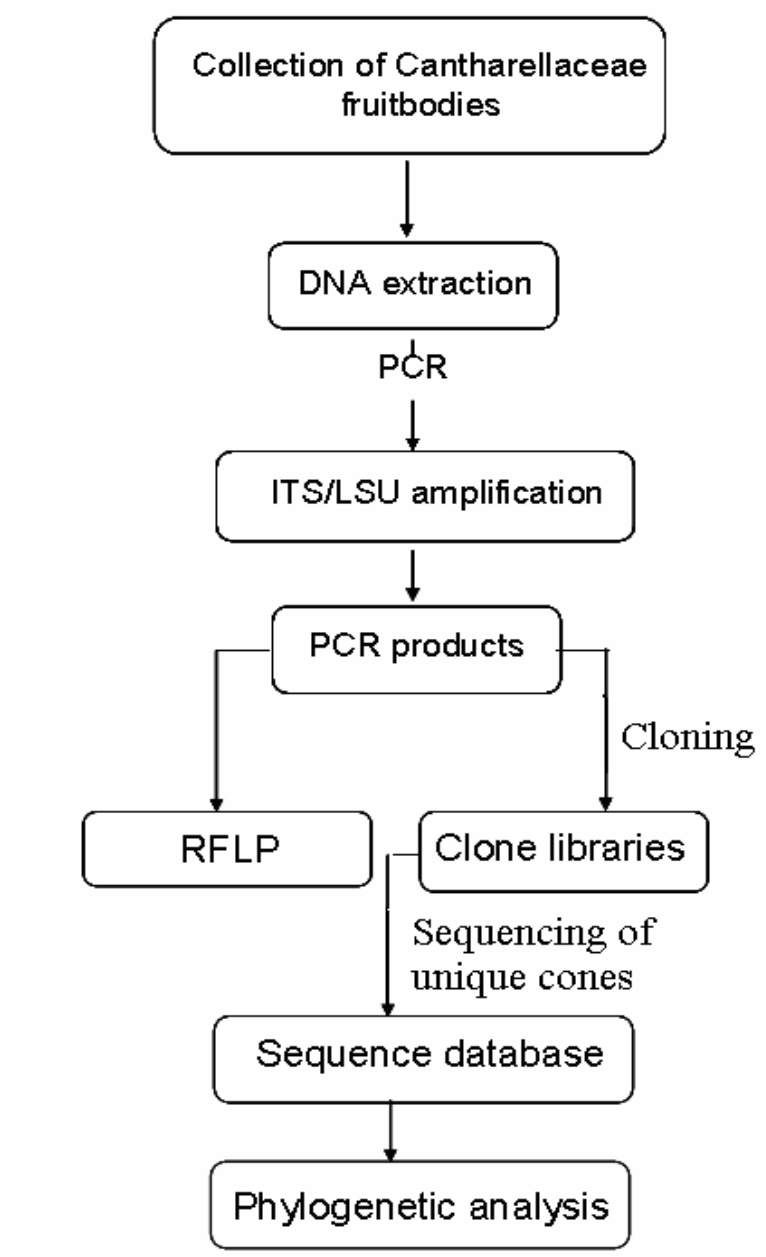


Fig. 3.1 Different steps towards molecular taxonomy of Cantharellaceae used to amplify the various rDNA region used in this study are represented.

3.2 Nutritional Properties

The collected fruit bodies were investigated for their different nutritional properties. The samples of Cantharellaceae were analyzed for chemical composition (Protein, fat, carbohydrates and ash) using AOAC procedures (1995) and other methods as described below.

3.2.1 Determination of Moisture

Five grams of mushroom samples were taken in glass Petri plates and placed in oven at 105ºC for 12 hours for drying The following formula was used to calculate the percentage of moisture (AOAC 1995).

$$\text{Moisture (\%)} = \frac{\text{Wt. of original sample (g)} - \text{Wt. of dried sample (g)}}{\text{Wt. of original sample (g)}}$$

3.2.2 Estimation of Ash

For ash estimation, one gram of dried mushroom samples was taken in a crucible and heated as oxidizing flame till smoke subsided. The crucible was transferred to muffle furnace at 250ºC for 6 hours. The sample was cooled in desiccator and weighed. The ash in the sample was calculated as:

$$\text{Ash (\%)} = \frac{\text{Wt. of Ash in sample (g)}}{\text{Wt. of original sample (g)}} \times 100$$

3.2.3 Estimation of crude Fat

For crude fat estimation one gram of dried powder of each Cantharellaceae specimen was taken in extraction tubes of Soxhlet apparatus (AOAC 1995). The temperature of the heater was adjusted so that the continuous rop of ether fall on the samples in the extraction tube. The process of extraction was carried out with petroleum ether (B.P. 40-60ºC) for 16 hours. The sample was removed and the solvent was allowed

to evaporate under the fume hood. The extract was completely dried in an air oven for 30 minutes at 105ºC. The weight of the extract was recorded after cooling in desiccators. Crude fat was calculated with the help of following formula:

$$\text{Crude fat (\%)} = \frac{\text{Wt. of fat in sample (g)}}{\text{Wt. of sample (g)}} \times 100$$

3.2.4 Estimation of crude Protein

Total nitrogen content and crude protein content was determined by microkjeldahl method. Nitrogen of sample is converted into ammonia sulphate by digestion with concentrated sulphuric acid in presence of digestion mixture **(**Kahlon and dass 1987). The clear digested solution is distilled with excess alkali in boric acid and is back titrated with hydrochloric acid and mixed indicator:

Reagents used:

a) Digestion mixture [$K_2 SO_4$: $CuSO_4$ 10:1(w/w)]
b) Concentrated H_2SO_4
c) 0.1N HCl
d) 40% NaOH
e) Boric acid
f) Mixed indicator- Bromophenol green (0.5 gm) and methyl red (0.1 gm) dissolved in 100 ml of 95% ethanol and pH adjusted to 4.5 with diluted HCl

Procedure: One gram of sample was taken in digestion flasks. To it, 5 gm of digestion mixture and 30 ml of concentrated H_2SO_4 were added. The flasks were kept on hot plate till the contents became clear. After cooling, the volume of digested sample was made up to 50 ml an aliquot of 25 ml was distilled in microkjeldahl distillation apparatus with 50 ml of 40% NaOH.

The liberated ammonia was trapped in 20 ml boric acid containing 2-3 drops of mixed indicator. About 25 ml of distillate was collected in 25 ml conical flasks and

titrated with 0.1N HCl till the end points. A change in colour from blue to light pink appeared. A blank was also run simultaneously. Nitrogen was calculated as follow:

$$\% \text{ N} = \frac{(\text{X - Y}) \times 14 \times \text{Normality of acid} \times \text{V1}}{\text{V2} \times \text{S}} \times 100$$

X	=	Volume of 0.1 N HCl used for sample titration
Y	=	Volume of 0.1 N HCl used for blank titration
V_1	=	Total volume made
V_2	=	Volume of aliquot taken
S	=	Weight of sample taken

$$\% \text{ Crude protein} = \%\text{N} \times 4.38$$

In order to overcome the error introduced by the chitin nitrogen, the factor 4.38 was adopted in case of fungi (Delmas 1989).

3.2.5 Estimation of Carbohydrates and Energy:

Total carbohydrates were calculated by difference Total carbohydrate = 100 – (g moisture + g protein + g fat + g ash) and total energy was calculated according to the equations (Barros *et al.* 2007): Energy (kJ) = 17 × (g protein + g carbohydrate) + 37 × (g lipid).

3.2.6 Estimation of total Sugar

Total sugars were estimated by phenol-sulphuric acid method of Dubois *et al* (1956) using glucose as a standard.

Sample preparation: One gm of Cantharellaceae fruit body's power was extracted with 50 ml of 80% ethanol. This suspension was shaken for 45 minutes at room temperature and filtered through Whatman No 4 filter paper. The residue was washed 5 times with additional 25 ml of 80% ethanol. The combined filtrate was then rotary evaporated at 40ºC and redissolved in deionized water to the final volume of 5 ml.

Reagents used:

(a) 95% Sulphuric acid: 95 ml of concentrated sulphuric acid was mixed with distilled water to make volume 100 ml.

(b) 5% phenol (w/v): it was prepared by dissolving 5 gm of phenol in 60 ml distilled water then volume was made to 100 ml with distilled water.

Procedure: For the estimation of total sugars in the samples, 0.5 ml of prepared sample aliquots were taken in test tubes and distilled water was added to make the volume to 1 ml. It was followed by addition of 1 ml of 5% phenol and 5 ml of sulphuric acid. Sulphuric acid was poured directly in the centre of the test tube to ensure that temperature rises to 70ºC for optimum colour development and proper mixing of the solution. The tubes were cooled to room temperature. After 20 minutes, the intensity of brown colour was measured at 490 nm against reagent blank. The concentration of total sugar was calculated from the standard curve. The standard curve was prepared by using glucose in the range of 10-100 µg.

3.2.7 Estimation of Reducing Sugar

Reducing sugars were estimated by Dinitrosalicylic acid method of Miller (1959).

Reagents used:

a) Dinitrosalicylic acid (DNS) solution: Ten gram of DNS and 0.5 gm of sodium sulfate were added in 500 ml of 2% sodium hydroxide solution. The solution was allowed to cool, 2 gm of phenol was dissolved in it and final volume was made to 1000 ml, the solution was filtered and stored in dark bottle in refrigerator.
b) Potassium sodium tartarate solution (40%): 40 gm of potassium sodium tartarate was dissolved in distilled water to make its final volume 100 ml, the solution was filtered and stored at room temperature.

Procedure: To 1 ml of prepared sample of Cantharellaceae in 80% ethanol was taken in test tubes and added 3 ml of DNS, then tubes were kept in boiling water bath for 15 minutes followed by addition of 1 ml of potassium sodium tartarate solution. The contents were cooled to room temperature. The absorbance of resulting solution was recorded at 575 nm against the reagent blank. The corresponding sugar contents were estimated from standard curve of glucose in the concentration range of 0-1000 µg/ml.

3.2.8 Quantitative estimation of Amino acids

Extraction of amino acids and their quantification was determined by the methods of Mattila *et al*. 2002.

Reagents used:

(a) Ethanol (70% v/v)

(b) Isopropanol (10% v/v)

(c) Methanol

Procedure: Dry Cantharellaceae fruit body's (10 g) were ground for 10 min with 100 ml of 70% (v/v) ethanol in water in a glass mortar. The suspension was centrifuged, and the clear supernatant was collected in a conical flask. The solid residue was re-extracted three times. After centrifugation, all the clear supernatants were pooled and evaporated to dryness on a water bath. The residue was extracted with absolute alcohol to remove salts and other interfering material was filtered and then evaporated to dryness. This residue was dissolved in 5 ml of 10% (v/v) isopropanol in water and was used for the analysis of free amino acids using High Performance Liquid Chromatography (HPLC) (Perkin-Elmer). The operation conditions of HPLC consistded a flow rate of methanol and water (80:20) at 0.5 ml/min through Lithium-carbonate column. The solvent used was methanol and water in 80:20 ratios. Amino acid identification was made by comparing the relative retention times of sample peaks with standards.

3.2.9 Antioxidant potential studies

The fruit bodies of Cantharellaceae were used for testing their radical scavenging potential. The fruit bodies used were in dried and powdered form. The radical scavenging potential of all the species of Cantharellaceae was determined by three methods as described below:

- Hydroxyl radical scavenging potential
- Antioxidant activity in linoleic acid emulsion
- Improved ABTS radical decolourization assay

All the samples were used in all the three assays for carrying out the present studies.

3.2.9.1 Extract preparation

Two types of extracts; alcoholic extract and aqueous extract were used in the present study.

(i) Alcoholic extracts preparation: Preparation of methanolic extracts of Cantharellaceae was done as described by Barros *et al* (2007) with little modifications. A fine-dried mushroom powder sample (5 g) was extracted by stirring with 10 ml of methanol at 25°C at 150 rpm for 24 h and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 10 ml portions of methanol. The methanol was evaporated by keeping the extracts open in 100 ml beakers at 37ºC for 2-3 days in an incubator. The organic solvent in the extracts was removed by a rotary evaporator. The yield was calculated after evaporation and the remaining residue from the extracts was re-suspended in methanol to a concentration of 10 mg/ml. Extracts were kept in the dark at 4°C for not more than 1 week prior to use. The yield was calculated by the following formula:

$$\text{Yield} = \frac{\text{Amount of residue left}}{\text{Amount of sample taken}} \times 100$$

(ii) Aqueous extracts: Cold water extracts was prepared for the antioxidant studies. Five gm of each sample was homogenized, soaked in 10 ml of distilled water and then left overnight at room temperature. After 12 hour, the extracts were filtered through Whatman No. 1 filter papers and were stored at 4ºC in refrigerator until use.

3.2.9.2 Hydroxyl radical scavenging potential: Hydroxyl radical scavenging potential was determined by deoxyribose degradation method of Li *et al* (2010). This method provides measurement of hydroxyl radicals scavenging activity bsed on Fenton reaction.

Reagents used:

(a) Deoxyribose (28mM): 375.6 mg of deoxyribose was dissolved in 100 ml of distilled water.

(b) Ferric chloride (1mM): 16.2 mg $FeCl_3$ was dissolved in 100 ml of distilled water.

(c) Ethylelediamine tetraacetic acid (1mM): 37.2 mg of EDTA was dissolved in 100 ml of distilled water.

(d) Ascorbic acid (1mM): 17.66 mg ascorbic acid was dissolved in 100 ml of distilled water.

(e) Hydrogen peroxide (10mM): 113 microlitres 30% of H_2O_2 was dissolved in 100 ml of distilled water.

(f) 8% Thiobarbituric acid (TBA) in 15% trichloroacetic acid (TCA): TBA (8%) - Dissolved 8 gm TBA in 100 ml of 0.05N NaOH. TCA (15%) - Dissolved 15 gm TCA in 100 ml distilled water.

(g) Phosphate buffer (20mM), pH 7.4

Procedure: The reaction mixtures consisted of 0.1 ml of FeCl3, 0.1 ml of EDTA, 0.1 ml ascorbic acid and 0.1 ml of H_2O_2. Various concentrations of the extracted samples were added in the reaction mixture. The final volume was made 1 ml with phosphate buffer. A control without addition of sample was also run. The reaction mixture was

incubated at 37ºC.for one hour in water bath followed by addition of 3 ml of TBA The percentage inhibition was determined by comparing light absorbance values of the test and control samples at 532 nm.

The percentage inhibition/reduction was calculated as:

$$\% \text{ Inhibition} = \frac{\text{OD (control)} - \text{OD (test sample)}}{\text{OD (control)}} \times 100$$

3.2.9.3 Antioxidant activity in linoleic acid emulsion: The antioxidant activity was determined using thiocyanate method (Kızıl *et al*. 2010).

Reagents used:

(a) Linoleic acid emulsion (pH 7.0): Prepared by mixing 0.28 gm of linoleic acid and 0.284 gm of tween 20 as an emulsifier.

(b) Phosphate buffer (0.2 M, pH 7.0)

(c) 75% Ethanol

(d) 30% Ammonium thiocyanate: 30 gm of ammonium thiocyanate was added in 100 ml of distilled water.

(e) Ferrous chloride (0.02M): 38. 2 gm of ferrous chloride was dissolved in 3.55 of HCl.

Procedure: Different concentration of extracts of all the samples were mixed with 2.5 ml of linoleic acid emulsion and 50 ml of phosphate buffer and the mixture was homogenized. The reaction mixture was then incubated at 37ºC. Aliquots (0.1 ml) were taken at different time intervals during incubation. The degree of incubation was measured according to the thiocyanate method by sequentially adding 4.7 ml ethanol, 0.1 ml ammonium thiocyanate, 0.1 ml sample solution and 0.1 ml ferrous

chloride. The mixture was allowed to stand for three minutes and absorbance was recorded at 500 nm. A control was run in an identical manner but without the addition of extracts. Vitamin-C was used as a positive control.

3.2.9.4 Improved ABTS radical decolourization assay: For ABTS assay, the method of Re *et al* (1999) was used.

Reagents used:

(a) ABTS [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt] solution (7mM): 3.84 g of ABTS was dissolved in 1000 ml of distilled water.

(b) Potassium persulfate solution (2.4 mM): 0.64 g of potassium persulfate was dissolved in 1000 ml of distilled water.

(c) Methanol

Procedure: The working solution was prepared by mixing ABTS solution and potassium persulfate solution in equal quantities and then allowing them to react for 12 hours at room temperature in the dark. The solution was then diluted by mixing one ml $ABTS^+$ solutions with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using a spectrophotometer. Fresh ABTS was prepared for each assay. The sample (1 ml) was allowed to react with one ml of $ABTS^+$ solution and absorbance was taken at 734 nm after 7 minutes using the spectrophotometer. The $ABTS^+$ scavenging capacity of the samples was compared with that of the standard and percentage inhibition was calculated as ABTS radical scavenging capacity:

$$\% \text{ Inhibition} = \frac{\text{OD (control)} - \text{OD (test sample)}}{\text{OD (control)}} \times 100$$

where O.D. (control) is the absorbance of $ABTS^+$ radical + methanol and

O.D. (sample) is the absorbance of $ABTS^+$ radical + sample extract/standard

3.2.10 Estimation of total phenolics: For Phenol assay, the method of Barros *et al*. (2007) was used.

Reagents used

(a) Folin-Ciocalteau reagent

(b) 20% Sodium carbonate (20 gm dissolved into 100 ml of distilled water)

(c) Gallic acid

Procedure: Phenolic compounds in the mushroom methanolic extracts were estimated by a colourimetric assay, wherein 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 minutes, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 minutes, after which the absorbance was measured spectrophotometrically at 765 nm. Gallic acid was used to calculate the standard curve (0.01-0.4 mM). The mean values of results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract.

3.2.11 Estimation of Bioactive molecules

3.2.11.1 Flavonoid contents: Flavonoid was estimated by using method of Yoo *et al* (2008).

Reagents used

(a) 5% $NaNO_2$

(b) 10% $AlCl_3.H_2O$

(c) NaOH (1mM)

(d) Chatequin

Procedure: For flavonoid contents determination, the methanol extracts sample (250 µl) was mixed with 1.25 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of a 10% $AlCl_3.H_2O$ solution was added. After 6 min, 500 µl of 1M NaOH and 275 µl of distilled water were added to the mixture. The solution was

mixed well and the intensity of pink colour was measured at 510 nm. Chatequin was used to calculate the standard curve (0.022–0.34 mM; Y = 0.9629X-0.0002; R^2 = 0.9999) and the results were expressed as mg of chatequin equivalents (CEs) per g of extract.

3.2.11.2 β-carotene estimation

Reagents used:

(a) Acetone

(b) Hexane

(c) β-carotene

Procedure: For β-carotene determination, the dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper (Barros *et al*. 2008). The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β-carotene was calculated according to the equations (Barros *et al.* 2007) of β-carotene (mg/ 100 ml) = $0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$. The results were expressed as μg of carotenoid/g of extract.

3.2.12 Estimation of Vitamins

3.2.12.1 Estimation of Vitamin A: vitamin A was determined by using method of Nield *et al* (1963)

Reagents used:

(a) 2N KOH

(b) Petroleum ether

(c) Anhydrous sodium sulphate

Procedure: One gram sample was homogenized with 1.0 ml of saponification mixture (2N/KOH in alcohol) and heated under gentle reflux for 20 min at 60ºC). 25 ml of water was added to the mixture after cooling to room temperature and the solution was transferred to a separating funnel. It was then extracted thrice using 25,

15 and 10 ml of petroleum ether (40-60ºC). The ether extracts were pooled and washed with 50-100 ml of distilled water repeatedly until the wash water was free from alkali. The petroleum ether extract was then dried by adding anhydrous sodium sulphate. Vitamin A was estimated using reverse phase gradient HPLC. The separation works with an isocratic method at 30°C and heptane: 1-propanol (99:1) solution was used as a mobile phase. The 10µL sample was injected into the system; flow rate was 1 ml/minute. Chromatograms were detected by a UV-detector at 325 nm. The separation takes 30 minutes for each run. Results were calculated by the "external standard-method" by integration of the peak areas. The vitamin A standard solution was used for recognition of the peaks.

3.2.12.2 Estimation of Vitamin B-complex: Vitamin B-complex was estimated by using method of Thomas *et al* (2008).

Reagent used:

(a) Vitamins B1, B2, B3 and B12 (Standard)
(b) Methanol
(c) Acetonitrile
(d) 1-hexane sulphonic acid sodium salt
(e) Triethylamine
(f) Glacial acetic acid.

Diluent preparation: Diluent wad made by addition of Water: acetonitrile: glacial acetic acid (94: 5: 1). Water was used since the vitamins under soluble in water, while acetonitrile was used to increase the solubility of riboflavin and glacial acetic acid was used to adjust the pH approximately near to that of the mobile phase

Preparation of vitamins B1, B2, B3 and B12 stock solution:

Accurately weighed amounts, 30 mg of thiamine (vitamin B1), 20 mg of riboflavin (vitamin B2), 36 mg of niacinamide (vitamin B3) and 25 mg of Cynocobalmine (vitamin B12), were taken into 100 mL volumetric flasks separately and 50 mL of diluent was added and sonicated to dissolve. The volume was made up to the mark

with diluent. The working standard solutions of vitamins contained 300 µg/mL of vitamin B1, 200 µg/mL of vitamin B2, 3360 µg/mL of vitamin B3 and 250 µg/mL of vitamin B12.

Standard preparation: Stock solutions, 10 mL of vitamin B1, 10 mL of vitamin B2, 5 mL of vitamin B3 and 4 mL of vitamin B12 were transferred to a 100 mL volumetric flask and the volume was made up with diluent and mixed well. The solution was then filtered through 0.2 µm glass nylon filter. This final solution contains 30, 20, 168 and 10 µg/mL of vitamin B1 vitamin B2, vitamin B3 and vitamin B12 respectively. The filtered solution was injected into the HPLC system.

Sample preparation: One gram of sample homogenized with 100 ml of 0.1 M HCl. Transfer the prepared sample into four different test-tubes and added 1 ml of glacial acetic followed by 0.5 ml 4% (w/v) $KMnO_4$. Allowed the mixture to stand for 3 minutes and then added 0.5 ml of 3% (v/v) H_2O_2. Shaking vigorously until excess O_2 is expelled. The extract sample was cleanup by specific ion-exchange resin column. Collected the eluate in 25 ml of volumetric flask and diluted the volume with KCl solution. To it, 15 mL of diluent was added and sonicated for 20-25 min with intermittent shaking. The solution was filtered through 0.2 µm glass nylon filter. The filtered solution was injected into the HPLC system.

Induced degradation of vitamins:

a) Acid base degradation: Syrup (5 mL) equivalent to 0.5 mg of vitamin B6 was transferred to 50 mL volumetric flask. To it, 35 mL of diluent was added and sonicated for 20-25 min with intermittent shaking. To it, 5 mL of 1 N HCl and 5 mL of 1 N NaOH was added separately. The sample was diluted to volume with diluent and mixed well. The solution was immediately injected into the HPLC system.

b) Hydrogen peroxide-induced degradation: The method described above (i) was followed except the 5 mL of 3% H_2O_2 was added in place of HCl/NaOH.

c) Thermal degradation: The sample solution was heated on a boiling water bath for one hour, cooled to room temperature and injected into HPLC system.

d) Photolytic degradation: The sample solution was kept in photolytic chamber at 2600 lux for 24 h, followed by analysis as per proposed method.

Chromatographic conditions: Chromatographic separation was achieved at 30 °C on a reverse phase column using mobile phase consisting of solution (A) (6.2 g of 1-haxane sulphonic acid sodium salt dissolved in 2L of water and 2mL of triethylamine was added to it and pH was adjusted to 3 ± 0.05 with glacial acetic acid) and methanol, (B) Gradient elution was performed slowly at 99: 1 of A: B (v/v) composition. After 7 min, isocratic elution, the composition was changed to 80: 20 (v/v) of A: B and elution was continued isocratically for next 28 min. Thereafter the composition was changed back to 99:1 of A: B (v/v) and elution was continued isocratically for next 30 min. The flow rate was kept at 1.5 mL/min and detection was performed at 280 nm. The injection volume was 20 µL in all HPLC runs.

3.2.12.3 Estimation of Vitamin C: Vitamin C was estimated by using method of Aletor 1995.

Reagents used:

(a) Methanol
(b) Metaphosphoric acid
(c) Dichloro Indophenol
(d) Ascorbic acid
(e) Oxalic acid

Procedure: Twenty gram of fresh sample was ground in 50 ml of metaphosphoric acid. The final volume was made to 100 ml and filtered through Whatman filter paper 1.The extract was taken in flask. Dichloro Indolphenol blue dye solution was standardized with ascorbic acid. The burette was filled with dye. The mixture present

in the flask was titrated with dye until the pink colour appeared. Pink colour should persist for 20 seconds.

$$\text{Dye factor} = \frac{0.5}{\text{x ml}}$$

where x ml is the amount of dye used for neutralizing 5 ml of ascorbic acid. Sample was also titrated in the same way by taking extract instead of ascorbic acid solution.

$$\text{Ascorbic acid (mg/g)} = \frac{\text{Dye factor} \times \text{dye used} \times \text{vol. of sample made}}{\text{Sample taken in gram} \times \text{extract taken}} \times 100$$

3.2.12.4 Estimation of Vitamin D: The sample was extracted by the AOAC procedure, with slight modifications for estimation of vitamin D.

Reagents used:

(a) Methanol

(b) Propanol

(c) Haxane

(d) Egrocalciferol (> 99% purity)

(e) Acetonitrile

Procedure: One gram of sample was homogenized with 350 µL of methanol–2-propanol (80:20 by v/v). The extraction was repeated three times with 2 ml of hexane. The phases were separated by centrifugation, and the upper organic phase was transferred to a conical tube and dried under nitrogen. The residue was dissolved in 100 µL of methanol and injected into the HPLC system. The HPLC separation works with an isocratic method at 30°C with a "normal phase" column. Chromatograms were detected by a UV-detector. The separation takes 30 minutes for each run. Results were quantified by the "external standard-method" by integration of the peak areas.

The ethanol standard solution was used for recognition of the peaks. Quantification was based on the using the internal standard method. Vitamin stock solution of crystalline vitamin D_3 (cholecalciferol) or vitamin D_2 (ergocalciferol) (Sigma Chemical Co., St. Louis, MO) was dissolved in acetonitrile and contents in mushroom samples were expressed in 1 gm per gm of dry mushroom.

3.2.12.5 Estimation of Vitamin E: The sample was analyzed by the method of Barros *et al*. 2008, with slight modifications for estimation of vitamin E.

Reagents used:

(a) Hexane

(b) Tocopherol

(c) Methanol

(d) NaCl

Procedure: The samples (500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000g) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream and redissolved in 1 mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.22 µm disposable LC filter disk, transferred into a dark injection vial and analysed by HPLC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the injection volume was 20 µL. The compounds were identified by chromatographic comparisons with standards and quantification was based on the using the internal standard method.

3.2.13 Estimation of Cholesterol: Total cholesterol was determined by slight modified method of Folch *et al* (1957).

Reagents used:

(a) HPLC-grade methanol
(b) Saturated NaCl solution
(c) Petroleum ether
(d) 0.1 HCl
(e) Diethyl ether
(f) 0.1 M Na_2CO_3
(g) Cholesterol (>99% purity)

Procedure: Five gram of each Cantharellaceae fruit body's was homogenized with 50 ml of chloroform: methanol (2: 1 v/v) mixture and allowed for 3 days. The solution was filtrated and centrifuged at 1000 g.. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating and cholesterol content was determined.

HPLC analysis was performed on Perkin Elmer (Germany) isocratic system consisting of a K-100 pump, 10 µl sample loop injector and a 250 × 4 mm Eurospher -100 C18, 5µm particle size column with integrated pre-column. The mobile phase was a mixture of acetonitrile and ethanol (60:40 v/v). The flow rate was maintained on 1.5 ml/min throughout the run and the detection carried out at 235 nm. For standard, the stock solution of cholesterol was prepared by dissolved in acetonitrile in concentration of 500 µg/ml and 1:10 dilution of the stock solution in the same diluents used as working standard. Results were calculated by the "external standard-method" by integration of the peak areas.

3.3 Diversity of endophytic bacteria associated with fruit bodies of Cantharellaceae

3.3.1 Isolation of bacterial species

Bacteria were isolated from inside fruit bodies of Cantharellaceae collected from different forest habitats (coniferous, mixed hardwood-coniferous) of Western Himalayan region, IndiaAll the sporocarps of each fungal species were washed with sterile distilled water and then surface disinfected with 96% ethyl alcohol (1 min), 5% sodium hypochloride (3 min) and again 96% ethyl alcohol (30 sec). The fruit bodies were subsequently rinsed in sterile distilled water and excess water was removed by squeezing between sterile filter papers. The internal tissue of sporocarps was ground in a sterile handheld tissue grinder with a pestle. Different dilutions of sporocarp tissue extracts were plated onto Nutrient Agar medium (HiMedia, India) and incubated at 30°C for the development of colonies.

3.3.2 Extraction of DNA

3.3.2.1 Genomic DNA extraction from bacteria isolates

A single colony of bacterial isolate was inoculated into 25 ml of nutrient broth in a 250 ml flask and incubated for 14-18 hrs at 30°C under shaking condition (120 rpm). Liquid cultures (2.0 ml) were harvested by centrifugation (Eppendorf microfuge) at 8,000 rpm for 1 min. The cell pellets were resuspended with 800 µl saline-EDTA, and approximately 10 µg crystalline lysozyme was added. During incubation at 37°C for 30 min, the cell suspension was mixed thoroughly by inverting the Eppendorf tube several times. After addition of 200 µl SDS (10%), the cell suspension was incubated again at 65°C for 15 min. The cell suspension was extracted with organic solvents to remove proteins and cell debris: first, with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) solution, and centrifuged 10 min at 12,000 rpm. The upper aqueous phase was then extracted with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). To precipitate extracted nucleic acids, 0.7 volume isopropanol was added to the aqueous phase, followed by 10 min

centrifugation at 12,000 rpm. The DNA pellets were washed with 750 µl ethanol (70%). Finally, the pellets were resuspended in 40 µl TE buffer/milliQ water and stored at 4°C.

3.3.2.2 REP-PCR based DNA fingerprinting

The primers used for REP-PCR reaction were REP1R (5'-III ICG ICG ICA TCI GGC-3') and REP2I (5'-ICG ICT TAT CIG GCC TAC-3') (Versalovic *et al*. 1994). Reaction mixture for the REP PCR contained 1X PCR buffer (Invitrogen, USA), each deoxynucleotide triphosphate at a concentration of 200 µm, 1.5 mM $MgCl_2$, each primer at a concentration of 0.1 µm and 2.5U of Taq DNA polymerase (Invitrogen, USA) in a final volume of 100 µl. DNA amplification was performed with Genamp PCR system Applied Biosystem, USA, by using the following program; initial denaturation 95°C for 5 min, 35 cycles of 92°C for 30 sec, 50°C for 80 sec and 68°C for 120 sec, final extension at 68°C for 8 min and final soak at 4°C.

3.3.2.3 BOX-PCR amplification

BOX-PCR was carried out to obtain the genomic fingerprinting of the all efficient bacteria that targets the highly conserved repetitive DNA sequences of the BOXA subunit of the BOX element.

BOXA1R: 5'CTACGGCAAGGCGACGCTGACG-3' primer (Koueth *et al*. 1995) was used to study distinctly different species. Reaction mixture for the PCR contained 1× PCR buffer (Fermentas, USA), each dNTPs at a concentration of 200 µM, 1.5 mM $MgCl_2$, each primer at a concentration of 0.1 µM and 2.5U of Taq DNA polymerase (Fermentas, USA) in a final volume of 50 µl. The reaction procedure was: an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 90°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 8 min and a final extension at 72°C for 10 min. PCR products were then examined through horizontal electrophoresis in 2% agarose gel containing ethidium bromide at 40 V in 1×TAE buffer.

3.3.2.4 Molecular Statistical Analysis

Similarities among the bacterial assemblages represented fingerprint patterns were analyzed using NTSYS v.2.0 (Exeter Software, USA) version 8.0. The banding patterns for each isolate were converted to a binary matrix, denoting presence or absence of a band. The similarity matrix was calculated using the Jaccard coefficient, and the clustering of the similarity matrix analyzed via the nearest neighbor clustering.

3.3.3 Phenotypic and biochemical characterization of bacterial isolates

To characterize all the bacterial isolates, conventional physiological and biochemical characterization tests were carried out as described in Bergey's Manual of Systematic Bacteriology (Holt *et al*. 1994).

3.3.3.1 Gram staining and temperature test

Bacterial smear from actively growing cells were spread on a glass slide and heat fixed. Smear was flooded with filtered crystal violet for 10 sec and then washed briefly in water to remove excess crystal violet. Later it was flooded with Gram's iodine for10 sec and washed briefly in water. Smear was decolourized with acetone until the moving dye front has passed the lower edge of the section and washed immediately in tap water. Counterstaining was done with safranin for 15 sec and washed with water to remove the excessive stain. Finally samples were visualized under microscope at different magnification.

Six temperatures (20, 25, 28, 30, 37 and 40°C) were chosen to test the growth pattern of bacterial isolates. Bacterial isolates were grown in liquid and solid media and incubated at different temperatures as stated above.

3.3.3.2 Catalase test

A small amount of bacterial cells was placed onto a clean microscope slide and few drops of H_2O_2 (3%) were added. A rapid evolution of O_2 as evidenced by bubbling

supports positive result. No bubbles or only a few scattered bubbles indicated this test as negative.

3.3.3.3 Oxidase test

One drop of reagent (N,N,N',N'-tetra-methyl-p-phenylenediamine dihydrochloride) was added onto the bacterial culture on an agar plate. Positive reactions turned the bacteria violet to purple immediately or within 10 to 30 seconds. Delayed reactions were ignored.

3.3.3.4 Nitrate reduction test

Nitrate broth is used to determine the ability of an organism to reduce nitrate (NO_3) to nitrite (NO_2) using the enzyme nitrate reductase. It also tests the ability of organisms to perform nitrification on nitrate and nitrite to produce molecular nitrogen. Nitrate broth contained nutrients and potassium nitrate as a source of nitrate. After incubating the nitrate broth, 2-3 drops of sulfanilic acid and α-naphthylamine were added. If the organism has reduced nitrate to nitrite, the nitrites in the medium will form nitrous acid. Sulfanilic acid was added; which reacted with the nitrous acid to produce diazotized sulfanilic acid. This reacts with the α-naphthylamine to form a red-coloured compound. Therefore, if the medium turns red after the addition of the nitrate reagents, it was considered a positive result for nitrate reduction.

3.3.3.5 Fermentation of carbon substrate by bacterial isolates

Total of 35-carbohydrate fermentation tests were performed with isolated bacterial species according to the manufacturer's direction (HiMedia, India). Inoculum was prepared by growing the cells in nutrient broth at 30°C in shaking condition until the inoculum turbidity was ≥0.5 O.D at 600nm. Citrate utilization, lysine, ornithine, TDA, nitrate reduction, acid phosphatase, urease and H_2S production tests were performed with all bacterial isolates by standard methods.

3.3.3.6 Antibiotic profiling

Antibiotic profiling was performed using ICOSA universal-1 kit (Hi-Media Laboratories, India) having twenty different antibiotics of various concentrations according to the manufacturer's instructions. The antibiotics and their concentrations were: Norfloxacin (10 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Cefuroxime (30 µg), Ciprofloxacin (5 µg), Cefaperazone (75 µg), Ceftazidime (30 µg), Roxithromycin (30 µg), Calarithromycin (15 µg), Co-Trimoxazole (25 µg), Netillin (30 µg), Cefaclor (30 µg), Cephotaxime (30 µg), Cephadroxil (30 µg), Azithromycin (15 µg), Ampicillin/Cloxacillin (10/10 µg), Penicillin (10 units), Amikacin (30 µg), Sparfloxacin (5 µg) and Ampicillin/sublactam (10/10 µg).

3.3.4 Indole-3-acetic acid (IAA) Estimation

The production of Indole-3-acetic acid by Salkowski reagent was given by Gordon and Weber (1951).

Reagents used:

(a) Salkowski reagent

(b) Indole-3-acetic acid

Procedure: Production of indole acetic acid was determined colourimetrically by mixing 1 mL of Salkowski reagent (12 gL^{-1} $FeCl_3$ in 8M H_2SO_4) (Glickmann and Dessaux 1995) with 1 mL of supernatant from bacterial cultures. After 30 min of dark incubation at room temperature and the absorbance was measured at 530 nm. Development of a pink colour indicates IAA production. Pure indole-3-acetic acid was used as a standard.

3.3.5 Amplification of 16S rDNA, Purification of PCR products and sequencing

For amplification of 16S rDNA, following primers were used: Forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-ACGGGCGGTGTGTTC-3' (Weisburg *et al*. 1991). Reaction mixture for the PCR contained 1× PCR buffer (Fermentas, USA), each dNTPs at a concentration of 200

μM, 1.5 mM $MgCl_2$, each primer at a concentration of 0.1 μM and 2.5U of Taq DNA polymerase (Fermentas, USA) in a final volume of 50 μl. PCR conditions were as follows: Preheating at 92°C for 2 min, 36 cycles of 92°C for 1 min, 48°C for 30 sec and 72°C for 2 min and final extension 72°C for 6 min. The products were purified using PCR purification kit (Valencia, CA) and partially sequenced using an ABI PRISM 3730XL capillary sequencer (Applied Biosystem, Foster City, USA).

Amplified 16S rDNA was purified using the QIAquick PCR purification kit (Qiagen, USA), followed the instructions of the manufacturer. Purified PCR products were eluted from the purification columns by the addition of 50 μl 10 mM Tris buffer (pH 8.0). The 16S rDNA products were subjected for partially sequenced using an Applied Biosystem automated sequencer (Delhi University South Campus, Delhi, India).

3.3.5.1 Analysis of sequence data

The 16S rDNA sequences of all the 30 bacterial isolates were subjected to the CHECK_CHIMERA program of the RDP (Maidak *et al.* 2001), in order to detect the presence of possible chimeric artifacts generated by PCR. Similarity of 16S rDNA sequences of all the isolates were compared with those available in GenBank databases using BLAST program (Altschul *et al*. 1997). The sequences were edited with BioEdit 5.0.6 (Hall 1999) and aligned using MAFFT v 6.240 with other sequences obtained from GenBank. All sequences were submitted to NCBI database

3.3.6 Phylogenetic analysis

Maximum parsimony analysis was performed using PAUP v4.0.b10 (Swofford 2002). One thousand heuristic search replicates were performed with starting trees generated by stepwise addition with random addition sequences followed by Tree Bisection Reconnection branch swapping. Gaps were treated as missing data. To assess the relative support for each clade, bootstrap values were calculated from 1000 replicates. Optimal models of DNA substitution was inferred using the Akaike

information criterion (AIC) (Akaike 1981) as implemented in MrModelTest ver. 2.3. Maximum likelihood (ML) analysis was performed in PAUP* with a GTR+I+G model of nucleotide substitution.

Bayesian analysis was performed using the Metropolis-coupled Markov Chains Monte Carlo search algorithm as implemented in the program MrBayes v 3.1.2 (Ronquist and Heulsenbeck 2003). Two simultaneous independent replicates of six were run for 5 million generations with sampling at every 100 generation, and the convergence of the runs visualized using Tracer ver. 1.4 (Rambaut and Drummond 2007). The first 20% of the trees were considered as burn-in (burn in = 2500) and the remaining 40,000 trees were summarized. The Bayesian approach of phylogenetic analysis was repeated five times to test the independence of the results from topological priors (Huelsenbeck et al 2002). Only Bayesian posterior probabilities (PP) greater than or equal to 50% are considered significant. To compare topologies resulting from the different search criteria, unconstrained trees (MP, ML) were compared in PAUP* using the Kishino-Hasegawa test (Kishino and Hasegawa 1989) in order to determine whether trees were significantly different. Trees were figured in Treeview (Page 1996).

3.3.7 Statistical analysis

All the experiments were performed in triplicate. The result was expressed as mean values and standard deviation (SD) and the data were analyzed by analysis of variance (ANOVA) using Graphpad prism (4.1) software followed by the Tukey's honestly significant difference test.

Chapter 4

Diversity and characterization of Cantharellaceae from Western Himalayas, India

4.1 Survey

Different locations mentioned in Fig 4.1 and Table 4.1 were surveyed for Cantharellaceae basidiomes under different forests. The field experience suggested that members of Cantharellacae are frequently associated with *Cedrus deodara* and *Quercus* sp. The results also confirmed that Cantharellaceae was confined to some of the low altitude regions (Mandi forest) but mainly to high altitude areas (Kalatop and Khajjiyar forests) of Himalayan region.

4.2 Classical taxonomy of Cantharellaceae

The present study includes the descriptions of the 17 species of Cantharellaceae from the Westen Himalayas, India, including 13 species of *Cantharellus* and 4 species of *Craterellus*. All the specimens were deposited at the Herbarium**,** Department of Botany, Punjabi University and Herbarium of the Directorate of Mushroom Research, Solan, India. The basidiospores of family Cantharellaceae are thin walled, smooth, granulated, monogutullate to multigutullate, apiculate, without germ pore or callus and exhibiting different shapes *viz*. elliptical, ellipsoidal, elongate, globose, subglobose and ovoid. Most of the species of *Cantharellus* have yellowish exsiccata, however, genus *Craterellus* have grayish black to blackish exsiccata and spores are smooth, inamyloid and broadly ellipsoid to elongate. The presence of clamp connections is not restricted to *Cantharellus* but also observed in *Craterellus*. The hollow stipe seems to be a synapomorphy for *Craterellus*, however, some of species recently reported within *Craterellus* having solid stipe and infundibuliform cap. So, these characters did not provided useful character for separating both genera.

Fig. 4.1 Map of Himachal Pradesh and Uttarakhand, India, showing the distribution of Cantharellaceae collection sites

Table 4.1 List of Cantharellcaeae species and their collection locations from different regions of Himachal Pradesh and Uttarakhand region of Western Himalayas, India

S. No	Collection No*	Locality	Host species
1	43-07, 121-08	Karshog forest (H.P.), Jageshwar forest (U.K.)	*Pinus roxburghii*
2	39-07, 84-08, 95-08	Chail (H.P.), Jageshwar forest (U.K.)	*Cedrus deodara, Quercus dilatata*
3	MSR1-08, MSR3-09 90-09	Solan (H.P.), Bageshwar (U.K.), Devidhura forest (U.K.)	*Q. incana, Q. dilatata*
4	184-08, 295-09	Bhasar forest (U.K.)	*P. wallchiana, Abies pindrow deodara*
5	236-06, 113-07, 17-08	Khada Pathar, Dhalli forest (H.P.)	*C. deodara, P. wallchiana*
6	43-06, 169-07, 32-09	Khada Pathar (H.P.), Kufari (H.P.), Jageshwar forest (U.K.)	*C. deodara*
7	MSR2-07, MSR4-08, 45-0	Kufri forest, Solan forest (H.P.)	*Q. leuotrichophora*
8	119-05, 333-06, 161-07	Khada Pathar, Solan forest (H.P.)	*C. deodara, P. roxburghii*
9	65-07	Dhalli forest (H.P.)	*C. deodara*
10	354-05, 251-09	Khada Pathar (H.P.)	*C. deodara, Q. dilatata*
11	106-08, 93-09 35-09	Chail forest (H.P.), Jageshwar forest (U.K.)	*C. deodara*, *Q. dilatata*
12	272-07, 281-07, 282-09	Khajjiyar, Kalatop forest (H.P.)	*C. deodara*
13	348-07, 316-06, 217-07	Khada Pathar (H.P.)	*C. deodara*
14	107-07, 134-08	Khada Pathar (H.P.)	*Quercus* species
15	322-05, 268-06	Pithoragarh forest (U.K.)	*Quercus* species
16	149-08, 19-09	Mandi forest (H.P.)	*Quercus* species
17	159-07, 211-08, 65-09	Chindi forest (H.P.)	*C. deodara*

*The collection numbers shown indicate number and year of collection of corresponding fruit body, for example, 43-07 (43 is collection number, 07 is collection year i.e., 2007). H.P. and U.K. denotes Himachal Pradesh and Uttarakhand, respectively.

4.2.1 Genus: *Cantharellus*

1. *Cantharellus applanatus* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig. 4.2. A-F)

MycoBank: MB519516

Etymology: from the *Latin* word *applanatus- applanate,* referring to the shape of cap.

Pileus 3-6.5 cm latus, applnatus ad lentiter depressus in centro, aureus, laevis, margine regulare, aureus, fissuratus, contextus 3-7 mm crassus, flavidus. Hymenium decurrentes, anastomosanes. Stipe centralis, 2.5-5 cm longus, 0.4-0.7 cm crassus, aequalis in diametrum, superficies glabrum vel tenuis capillatus. Sapor incognitus. Basidiosporae 7-8.5 (-9) × 4.5-5.5 µm, laevis, ellipsoideae, inamyloideae. Basidia ± 55-78 × 6.0-7.5 µm, clavata, 4-vel-5 sporifera; sterigmatis usque 2.5-4 µm longis. Basidiola numerosa. Trama hymenophorale irregularis vel intertextum, 3-7.5 µm crassus. Pleuro-vel cheilocystidia nulla, Fibulae praesentes.

Pileus upto 3-6.5 cm, applanate to shallow depressed; golden yellow (9L-6), smooth; margin regular, split. ***Context*** 3-7 mm thick, yellowish, confluent, unchanging on exposure to air, surface smooth, glabrous. ***Lamellae*** decurrent, folded, anastomosing, forked up to 2 mm broad, golden yellow (9L-6). ***Stipe*** central, 2.5-5 × 0.4-0.7 cm, cream (9D-2), equal in the diameter throughout, surface glabrous to thin hairy. Spore deposit white. ***Basidiospores*** [55/2/2], 7-8.5 (-9) × 4.5-5.5 µm, L= 7.62 µm, W= 4.49, Q= 1.54, ellipsoid, inamyloid, smooth, contents monoguttulate to granulated. ***Basidia*** ± 55-78 × 6.0-7.5 µm, clavate, sterigmata 2.5-4 µm diam, cornuted 4-5 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Hymenophoral trama*** irregular to interwoven, branched, hyaline to faint yellowish, slightly constricted at septa which are frequently clamped, 3-7.5 µm wide. ***Pileipellis***

consisting of compactly arranged projecting end hyphae, cylindric to filamentous, yellowish to yellowish brown, branched, thin to thick walled, frequently clamped, non-amyloid hyphae, 3-8µm wide, contents granulose. ***Pleurocystidia*** and ***cheilocystidia*** absent. ***Stipe cuticle*** made up of yellowish brown, cylindric to filamentous, 2.5-7 µm wide, branched, hyphae frequently clamped.

Habitat and distribution**:** caespitose to gregarious, on soil among green grass with *Pinus roxburghii*.

Materials examined**:** INDIA, Himachal Pradesh- Shimla- Karsog forest, N31°24' E77°.12' 1900m, 5 August 2007, collected by Deepika Kumari, 43-07; same location, 21 August 2008, collected by Deepika Kumari 121-08. Holotype: PUN 3964.

Notes: Wholly beautiful yellow *Cantharellus*. The present specimen matches well with the characters given for *C. viscosus* Berk and Corner (1966) which has similar spores range and Q values but in present specimen's pileus is applanate however the type specimen is prominently infundibuliform. Although Corner did not specify full description of the specimen. The variation in pileus shape and stem size can be confusing at times and these variations cannot be separated with any consistency, so number of population is examined in subsequently 3 years of collections.

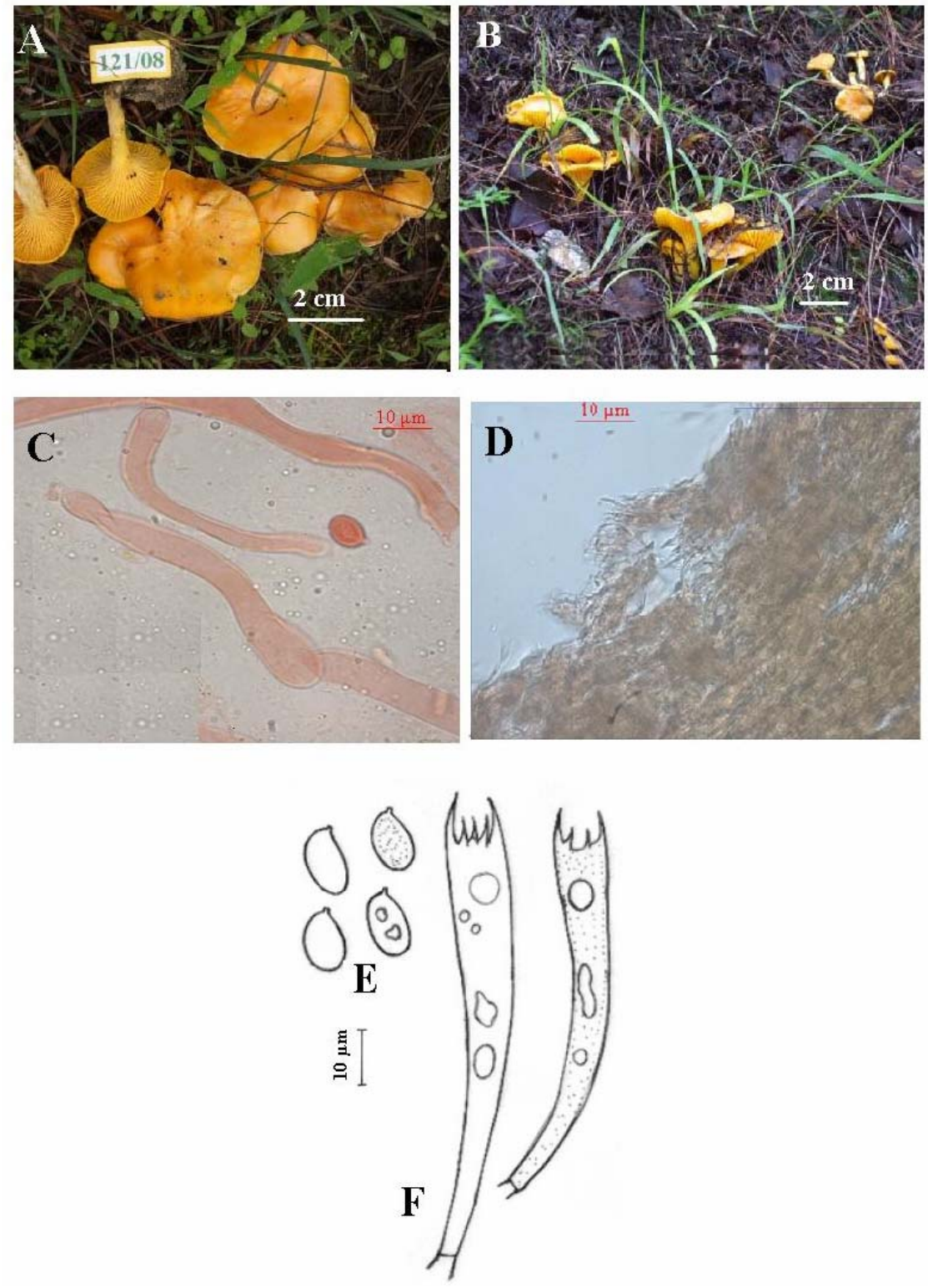


Fig. 4.2 A & B. Basidiocarps; C & D. Pileipellis and E &F. Basidiospores and Basidia of *C. applanatus*

2. *Cantharellus appalachiensis* Petersen, Svensk Bot, Tidsker. 65:402. 1971. (Fig. 4.3 A-E)

Pileus 3-7 cm wide, plano convex to shallow depressed, pinkish yellow (10I-4) to yellowish (10G-2), appressed to faintly squarose, surface glutinous, non-hygrophanous, covered with indistinct scales; margin irregular, non-striate, lobed, split, uplifted with age. ***Context*** up to 1 cm thick, yellowish. ***Lamellae*** decurrent, folded, interveined, hymeniform folded up to 2 mm broad, Apricot yellow (9K-5). ***Stipe*** 3.5-7 × 0.4-1.1 cm, terete with slightly expnded apex, lacunose, stipe surface fibrous pinkish yellow to citron yellow (10J-2); taste very pleasant (like other chanterelles). Spore deposit creamish white ***Basidiospores*** [45/2/2], 7.5-9.5 × 4.5-5.8 µm, L= 8.4 µm, W= 5.2, Q= 1.62, ellipsoid to elongate, inamyloid, smooth, faintly yellowish in 3% KOH, contents monoguttulate to granulated. ***Basidia*** ± 52-77 × 7.5-9 µm, cylindrical to narrowly clavate, strerigmata 4.5-7 µm long, cornuted 4-6 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Pileipellis*** epicutis made up of subclavate to clavte cells with projecting cystidiod end, ± 70-100 µm long, made up of radially to sub-radially arranged hyphae, 4-12 µm diam, granulated wall thin to slightly thick and pale yellowish in 3% KOH, context hyphae cylindric ±3-17 µm diam, slightly thick walled. ***Hymenophoral trama*** interwoven with cylindric hyphae ±2.5-7 µm wide. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of subclavate to clavate hyphae, thin to slightly thick walled, ± 3-11 µm wide, contents similar to suprapellis, septa frequently clamped.

Habitat and distribution: gregarious to caespitose; on soil under the trees of *Cedrus deodara*.

Materials examined: INDIA, Himachal Pradesh, Shimla- Chail forest, N31°06' E77°.10' 1800 m, 30 July 2007, collected by Deepika Kumari, 39-07, same location, 12 August 2008, collected by Deepika Kumari, 84-08; Uttarakhand, Jageshwar forest N29°00' E79°.17' 1646 m, 24 August 2008, collected by Deepika Kumari, 95-08. Holotype: PUN 3959.

Notes: The overall morphology of the basidiomata resembles with *Cantharellus appalachiensis* as described by Bigelow (1978). The distinguishing features of present collection; such as matted cap with appressed fibrils, non-hygrophanous, similar colour tones, folded lamellae, intervenose, subacute edges, similar spore size, and pileipellis hyphae non-encrusted with cystidiod end cells, shows similarity with *C. appalachiensis*. Dhancholia et al (1991) reported this species from the Uttarakhand, however the taxonomic details to support the identity of *C. appalachiensis* was not mentioned.

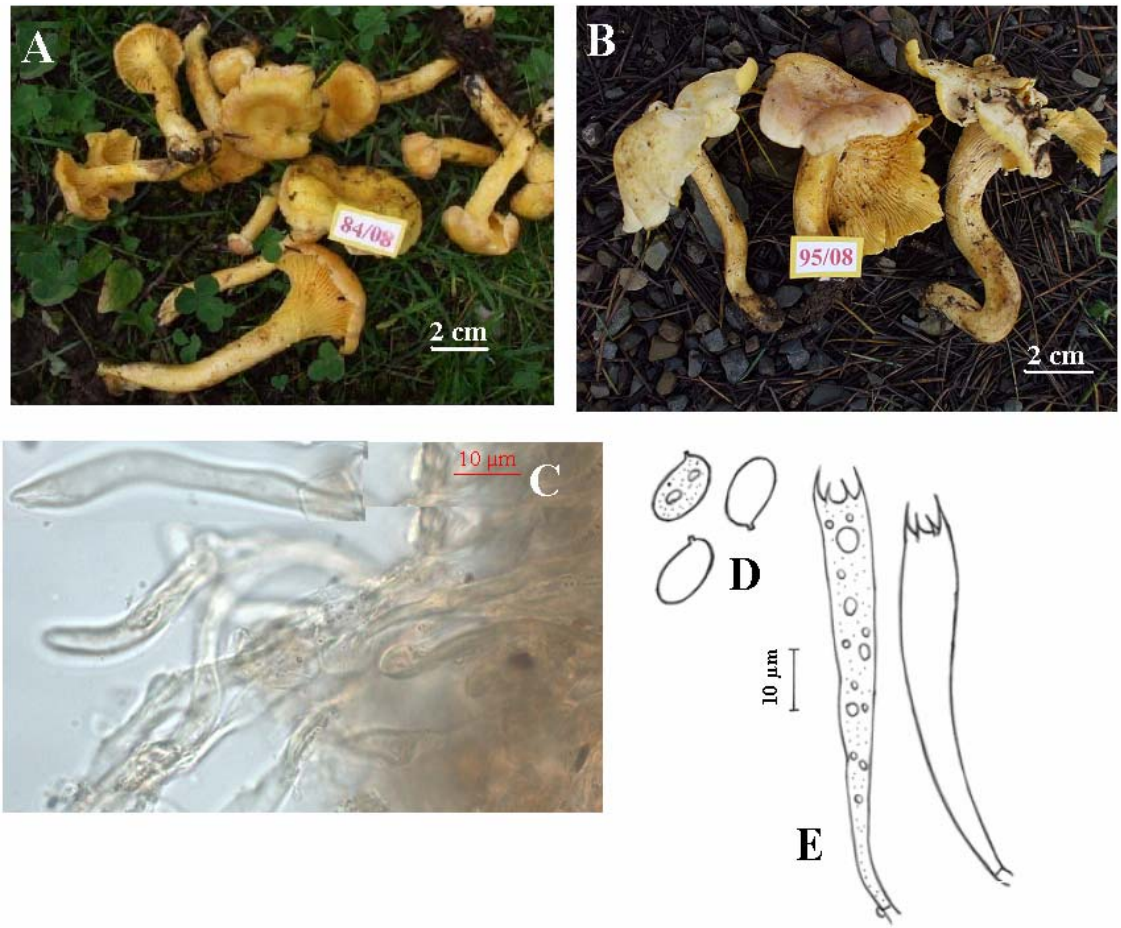


Fig. 4.3 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *C. appalachiensis*

3. *Cantharellus cibarius* Fries, Syst. Mycol. I: 318. 1821. (Fig. 4.4 A-E)

Himalayas and easily ***Pileus*** 3-15 cm wide, convex to becoming plane or depressed to funnel shaped, egg yellow to pale ochraceous yellowish, appressed to faintly squarose, surface glutinous, non-hygrophanous, covered with indistinct scales; margin incurved at first. ***Context*** up to 1.5 cm thick, whitish to yellowish. ***Lamellae*** decurrent, folded, interveined, anastomosing, hymeniform folded up to 2 mm broad, usually paler than the pileus. ***Stipe*** 3-8 × 0.4-0.8 cm, attenuate downwards, solid, range at the base on bruising, smell fruity of apricots. Spore deposit creamish white ***Basidiospores*** [45/2/2], 8.5-10.5 × 4.5-5.8 µm, L= 8.6 µm, W= 5.4, Q= 1.59, ellipsoid to elongate, inamyloid, smooth, faintly yellowish in 3% KOH, contents monoguttulate to granulated. ***Basidia*** ± 50-110 × 7.5-10 µm, cylindrical to narrowly clavate, strerigmata 5-8.5 µm long, cornuted 4-8 (mostely 6) per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Pileipellis*** filamentous, cylindric hyphae with end cells often often upturned or even forminf irregular turf**,** 4-12 µm diam, granulated wall thin to slightly thick and pale yellowish in 3% KOH, context hyphae cylindric ±4.5-18 µm diam, slightly thick walled. ***Hymenophoral trama*** interwoven with cylindric hyphae ±2.5-7 µm wide. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of filamentous to cylindic hyphae, thin to slightly thick walled, ± 3-14 µm wide, contents similar to suprapellis, septa frequently clamped.

Habitat and disstribution: On soil, gregarious to caespitose; under the trees of *Quercus incana*.

Materials examined: INDIA, Uttarakhand- Bageshwar forest, N29°45’ E79°.04’ 1696 m, 15 July 2007, collected by Deepika Kumari, MSR1-08; Devidhura forest N29°05’ E78°.00’1615 m, 12 August 2009, collected by Deepika Kumari MSR3-09; Himachal Pradesh- Karol forest N30°92’ E77°.15’ 2100 m, 22 September 2009, collected by M.S Reddy 90-09. Holotype: PUN 3973.

Notes: This is a most common species of the genus *Cantharellus,* frequently found in Indian recognized by the field characters, especially the fruit-like smell. Sohi *et al* (1991) already described the type specimen in detail from Kashmir region, India.

During research work, these collections were frequently collected from the forest of Bageshwar, Devidhura and Karol forest. After documentation and microscopic examination this specimen easily fits with the description of *C. cibarius* described by Sohi *et al* (1991). First the colour of pilei and stipe is ocharaceous brown to yellowish brown. Second, the decurrent hymenium forming blunt ridges or veins; basidia frequently 6 spored, slender clavate with basal clamp-connection. The spore 8.5-10.5 (-11) × 4.5-5.8 µm, L= 8.5 µm**,** W= 5.5 µm, Q= 1.54 ellipsoid, smooth, nonamyloid, wall hyaline, contents mono to multiguttulate are similar, which would also coincide to previously describes *C. cibarius*. So, the examined specimen was considered as a holotype, implicates in the original description and explicitly stated by Corner (1966).

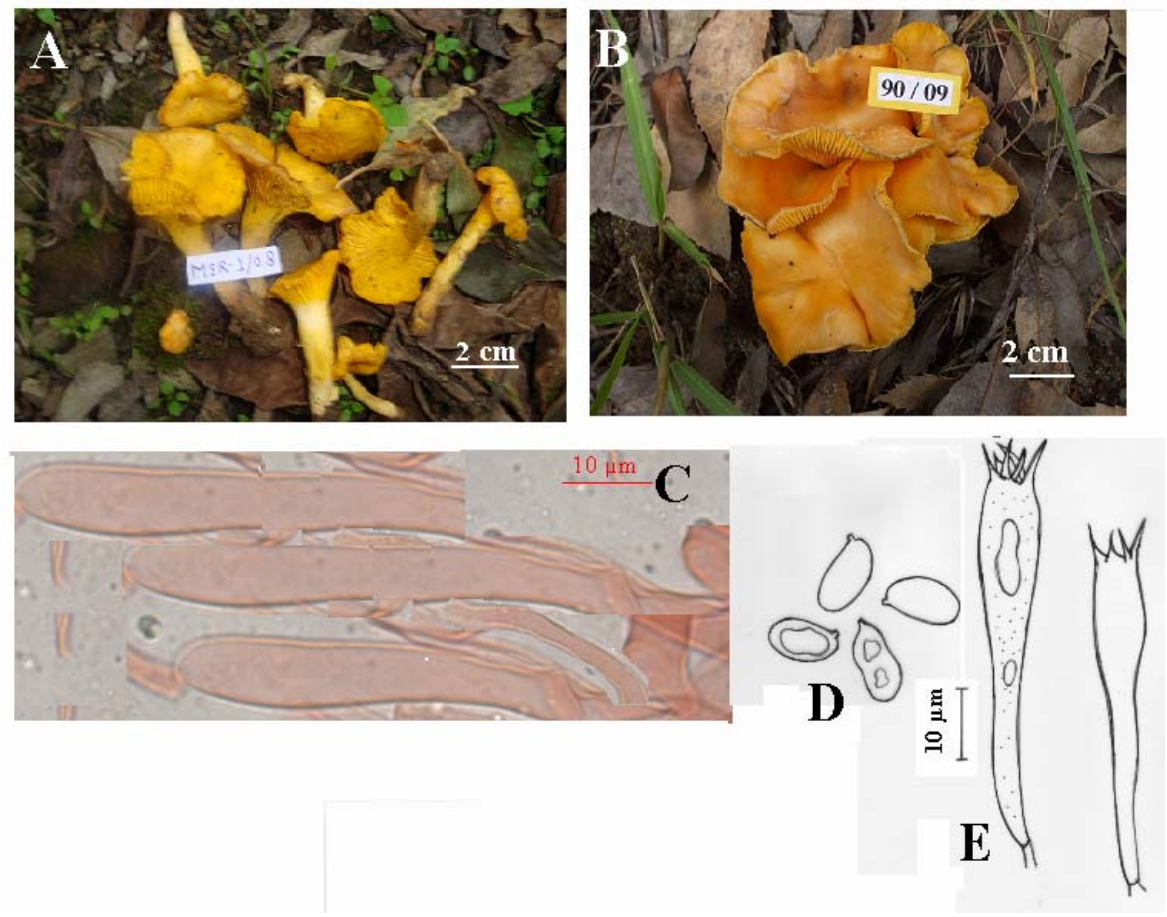


Fig. 4.4 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *C. cibarius*

4. *Cantharellus elongatipes* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig.4.5. A-D)

MycoBank: MB519521

Etymology: elongatipes - refer to long stipe

Pileus 1.5 cm latus, convexus vel planoconvexus cum leniter depressus, in centro. Margine regulare vel irregulare, fissuratus, contextus tenuis, aurantiacus ad flavus. Hymenium distinctus decurrentes, anastomosans vel longulus, aurantiacus; Stipe 3-3.5 cm longus, 0.4-0.7 cm crassus, centralis, aequalis in diametrum, superficies glaber. Sapor incognitus. Basidiosporae 5.9-7.2 × 4.5-5.4 µm, late ellipsoideae, vel ellipsoideae, laevis, *inamyloideae. Basidia ± 52-70 × 7-10 µm, anguste clavata vel clavata, 2-vel-4 sporifera, sterigmatis usque 2.5-4.5 µm longis. Basidiola numerosa. Trama hymenophorale intertextum. Pilei constatus subparallelis hyphosa. Pleuro-vel cheilocystidia nulla. Fibulae praesentes.*

Pileus upto 1.5 cm, convex to plano convex with slightly depressed in the centre; Mirabelle (10J-7), orangish yellow, smooth; margin regular to irregular, split. ***Context*** thin. ***Lamellae*** strongly decurrent, anastomosing to subdistant, hymeniform folded up to 1 mm high, orange. ***Stipe*** 3-3.5 × 0.4-0.7 cm, equal in diameter, lacunose, stipe surface glabrous, Mirabella (10J-7) to dirty orange. Spores deposit white. ***Basidiospores*** [55/2/2] 5.9-7.2 × 4.5-5.4 µm, L=6.6 µm, W=4.9 µm, Q=1.35, broadly ellipsoid to ellipsoid, nonamyloid, smooth, wall hyline, contents monoguttulate. ***Basidia*** ± 52-70 × 7-10 µm, narrowly clavate to clavate, strerigmata 2.5-4.5 µm long, cornuted 4-5 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Subhymenium*** made up of non inflated hyphal segments, branched, septate with frequently clamped. ***Hymenophoral trama*** interwoven with cylindric hyphae ± 2.5-6 µm wide. ***Pileipellis*** made up of sub parallel arranged filamentous hyphae, septate, clamped, hyaline to pale yellowish, 1.8-3.6 µm wide, followed by clavate to subclavate or irregularly arranged cylindrical elements, 9-18 µm wide, granulated. ***Pleurocystidia*** and ***Cheilocystidia***

absent. ***Stipe cuticle*** made up of hyaline to pale yellow, filamentous to cylindrical, followed by cystoids ends cells, branched, frequently clamped hyphae, 2.5-8 µm wide.

***Habitat and distribution*:** gregarious to caespitose; on soil under the trees of *Cedrus deodara*.

***Materials examined*:** INDIA, Uttarakhand, Pauri Garhwal- Bharsar forest, N29°45' E78°.55' 2350 m, 29 September 2008, collected by Deepika Kumari 184-08; same location, 8 September 2009, collected by Deepika Kumari 295-09. Holotype: PUN 3966.

***Notes*:** On the basis of macroscopic characters this species hardly fits within the description of other *Cantharellus,* but after microscopic examination, the presence of clamp connection throughout the fruit bodies, the cantharelloid strigmata and the almost cylindrical basidia, all would point to the placement of the present species in the genus *Cantharellus*. The morphological characteristics of the present species was initially mistaken for some species of the genus *Cantharellus viz. C. minor* Peck, but strongly different because of less fleshy stipe and less decurrent hymenium. The presence of small pileus as compared to stipe length (Pileus diam 0.8-1.5 cm, stipe length 2-4.5 cm), so, the name of assigned as *C. elongatipes*, The presence of distinctive taxonomic features supports the dissimilarity of the herewith described species with respect to other species of *Cantharellus*, verified that the present species is a new taxa to the world.

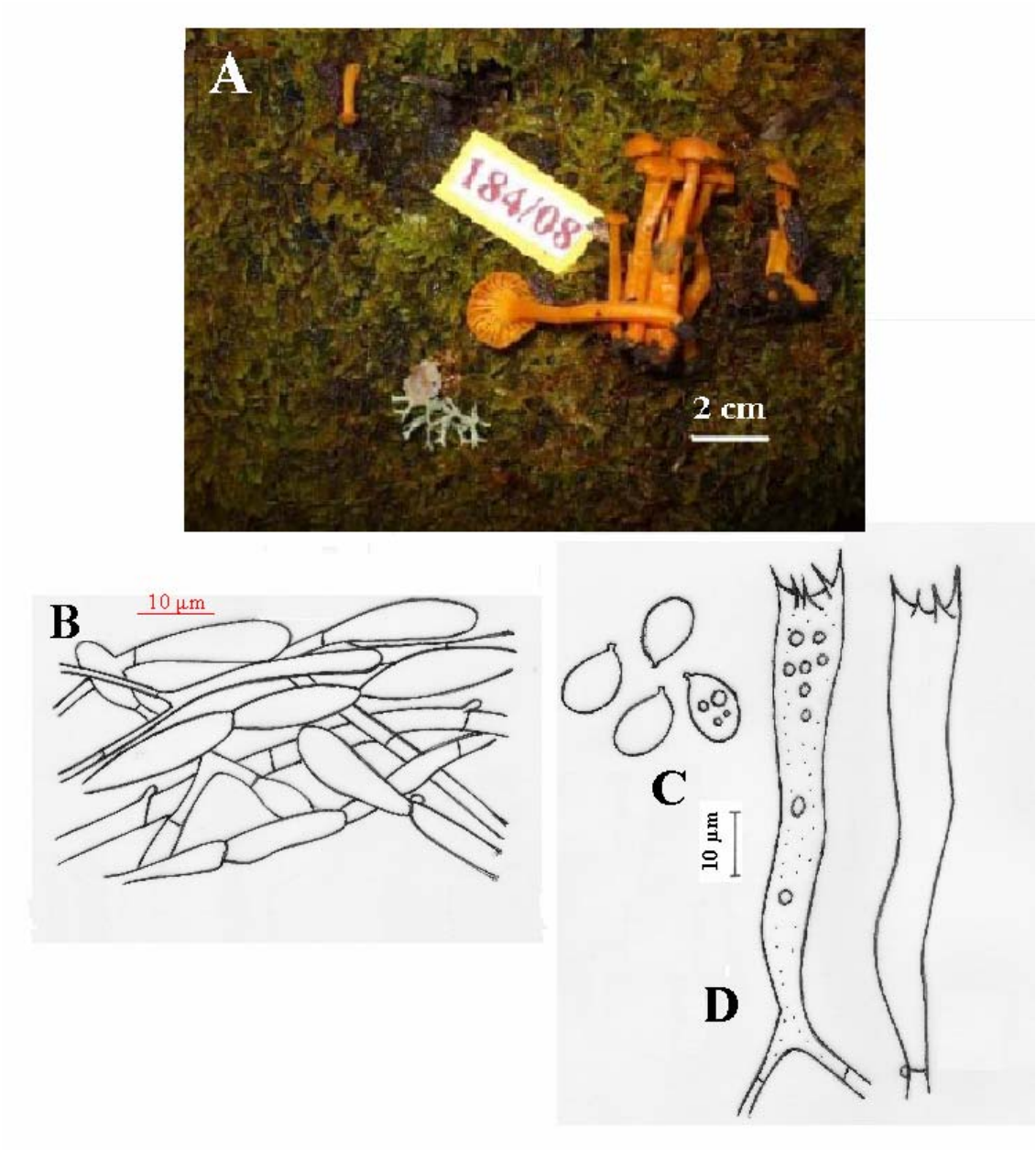


Fig. 4.5 A. Basidiocarp; B. Pileipellis and C & D. Basidiospores and Basidia of *C. elongatipes*

5. *Cantharellus fibrillosus* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig. 4.6.A-E)

MycoBank: MB519517

Etymology: from the Latin word *fibrillosus*- fibrillose, referring the pileus surface.

Pileus 5-8.2 cm latus, infunibuliformis, brunneus in centro, aureus wheat, compactus in centro, superficies sicco, non hygrophaneus, margine non-striato, irregulare, *contextus 2-3 mm crassus. Lamellae abundus decurrentes, anastomosans. Stipes 6-8 cm longus, 0.7-1.0 cm crassus, teres per leviter clavatus basi, superficies* laevis *ad capillatus. Sporae in cumulo albae ad luteo- albae. Odor et sapor fructus indistincti. Basidiosporae (7.8-) 8.5-12 (-12.5) × 5-6.2 (-6.6) µm, ellipsoideae vel cylidricus, inamyloideae. Basidia ± 60-90 × 9-11.5 µm, clavata;* sterigmatis *usque 5.9–7.2 µm longis. 4-vel-6 sporifera. Basidiola numerosa. Trama hymenophorale intertextum. Pleuro-vel cheilocystidia nulla. Epicutis pilei hyphosa. Fibulae praesentes.*

Pileus up to 5-8.2 cm wide, infundibuliform, Mustard brown (14D-10) in the center, golden wheat (11D-7) outwards, fibrillose, dense in the center, surface dry, hygrophanous; margin irregular, non-striate, decurved,. ***Context*** 2-3 mm thick, creamish, confluent, unchanging on exposure to air, surface smooth but not glabrous. ***Lamellae*** abundantly decurrent to sub decurrent, anastomosing, separable easily from the flesh. ***Stipe*** 6-8 × 0.7-1.0 cm thick, yellowish orange (10C- 4), terete with slightly swollen at base, surface smooth to hairy. Spore deposit white. ***Basidiospores*** [45/2/2] (7.8-) 8.5-12 (-12.5) × 5-6.2 (-6.6) µm, L=9.7 µm**,** W=5.9 µm, Q=1.64, ellipsoid to cylindrical, nonamyloid, noncyanophilic, wall hyaline, contents mono to multiguttulate with greenish referective oil droplets; spores deposit white. ***Basidia*** ± 60-90 × 9-11.5 µm, clavate, sterigmata 3.5-6.5 × 1.2-2 µm diam., cornuted 2-6 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Hymenophoral trama*** irregular to interwoven, branched, hyaline to faint yellowish, constricted slightly at septa, septa frequently clamped. ***Pileipellis***

consisting of filamentous and interwovenly arranged hyphae, hyaline, branched, thin to thick walled, frequently clamped, non-amyloid hyphae, 3-12 µm wide, contents granulose. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of hyaline to yellowish, branched, frequently clamped hyphae, 1.5-5 µm wide.

Habitat and distribution: caespitose to gregarious, on soil under the trees of *Cedrus deodara* and *P. wallchiana*.

Materials examined: INDIA, Himachal Pradesh- Shimla- Khada Pathar forest, N30°72' E78°.63.2' 1950 m, 30 September 2006, collected by R.C Upadhyay 236-06; Dhalli forest N 30°17' E78°.45' 1700 m, 18 September 2007, collected by Deepika Kumari 113-07; same location, 11 August 2008, collected by Deepika Kumari 17-08. Holotype: PUN 3957.

Notes: This species is described from deciduous and coniferous, both types of forest. Several collections of the described species were found from the forest of Khada pathar and Dhalli forest in consequently three years of collections. The described species resembles with *C. ianthinoxanthus* Kuhner (Corner 1966) as with pileus surface fibrillose, infundibuliform and similar spores size, however the stipe colour differentiated this species from present specimen. The stipe colour of *C. ianthinoxanthus* is clear yellow, whereas light orangish with brown stipe colour towards apex to lighter downwards and smaller basidia with 4–6 sterigmata, indicated that the present specimen is a new species, designted as *C. fibrillosus*.

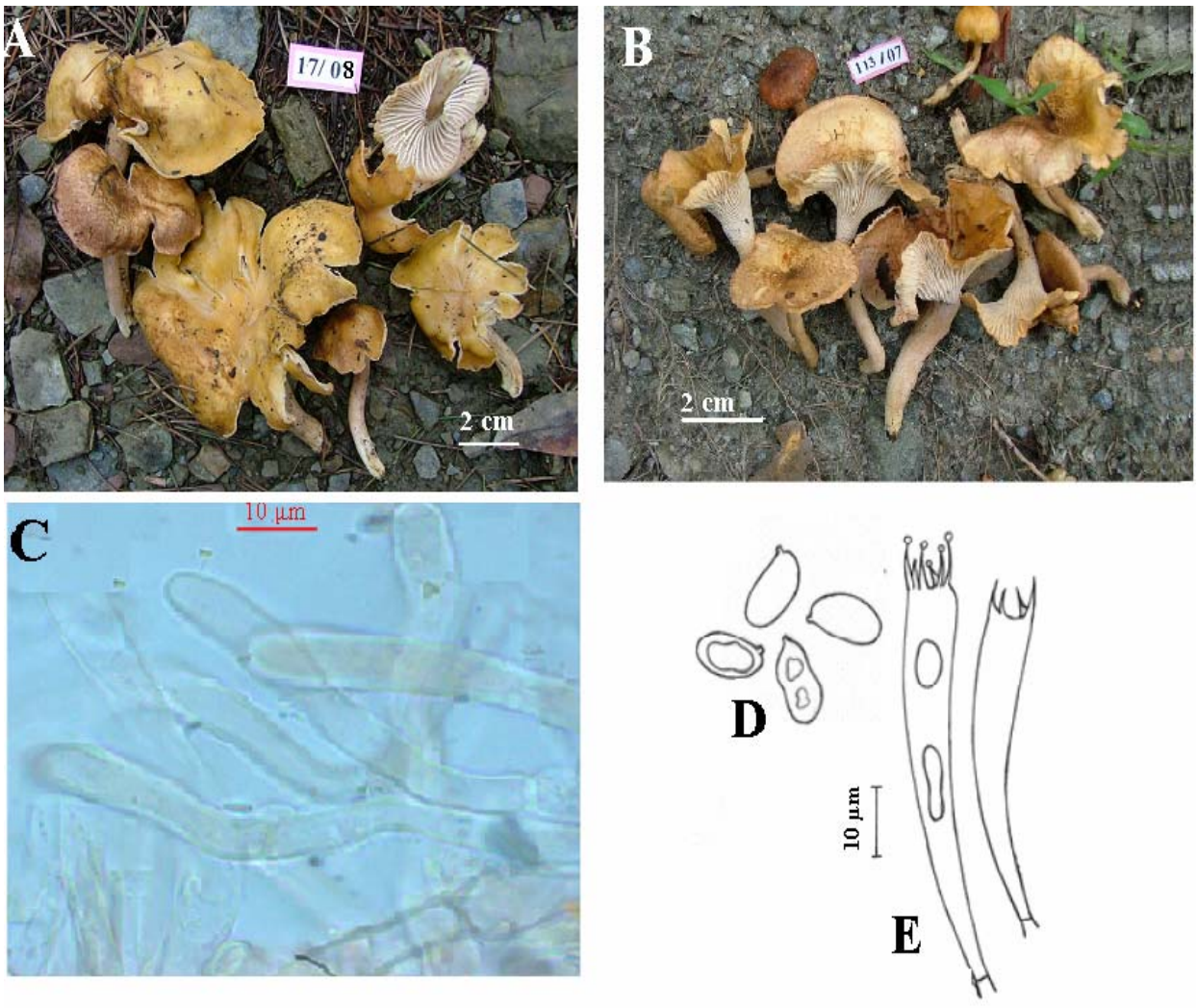


Fig. 4.6 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *C. fibrillosus*

6. *Cantharellus himalayensis* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig.4.7. A-F)

MycoBank: MB519518

Etymology: from Latin word *Himalayensis* -Himalaya, referring to locality.

Pileus 3-7 cm latus, infundibuliformis, margine undulates, irregulare, striato ad non-striato, flavidus, brunneus in centro propter squamula, superficies sicco; contextus 1.3 cm crassus. Lamellae distinctus, intertextus ad bifurcates. Stipes 4-7.5 x 0.8-1.1 cm longus, 0.7- 1.1 cm crassus, teres per leviter, superficies glaber ad capillatus. Sporae in cumulo albae ad luteo-albae. Odor et sapor fructus indistincti. Basidiosporae 6-8 × 4.5-6 µm, Q=1.34, ellipsoideae vel late ellipsoideae laevis, inamyloideae. Basidia 60-85 × 8-11µm, anguste clavata. vel teretiusculus; 2-vel-4 sporifera, sterigmatis usque 6-9.5 µm longis. Basidiola numerosa. Trama hymenophorale intertextum. Pleuro-vel cheilocystidia nulla. Fibulae praesentes.

Pileus up to 3-7 cm wide, infundibuliform, margin wavy, irregular, faintly striate to non striate, concolour yellowish, Pecan brown (14A-9) in the center due to scales, Champagne (11B-3) to Beige (11B-4) outwards, surface dry, non-hygrophanous. ***Context*** fleshy upto 1.3 cm wide, yellowish. ***Lamellae*** folded, hymeniform, interveined to bifurcate. Sugarcane (10B-6) to Cornhusk (10E-6) or Capuccine buff (9E-5), edges subacute. ***Stipe*** up to 4-7.5 × 0.8-1.1 cm, tapering downwards, stipe surface glabrous to thin hairy at below. Beige (11B-4) to Pecan brown (14A-9), darker at the base as leather brown. Smell of apricot; taste indistinct. Spore deposit creamish white. ***Basidiospores*** [45/1/1] 6-8 × 4.5-6 µm; L=5.91 µm, W=5.16 µm; Q=1.34, ellipsoid to broadly ellipsoid, inamyloid, smooth, hyaline, apiculus upto 0.5 µm long, contents mono gutullate to multigutullate and frequently granulated. ***Basidia*** 60-85 × 8-11 µm long, narrow clavate to subcylindric, sterigmata 4 per basidium, 6-9.5 µm long, contents granulated and with multi oil droplets, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Pileipellis*** made up of parallel to repent filamentous hyphae, 3-9 µm wide, followed

by cylindric hyphae, thin to thick walled yellowish, septate, branched, context hyphae compactly arranged. ***Hymenophoral trama*** irregular to interwoven, made up of thin walled, clamped, branched hyphae, 3-6 µm wide. ***Subhymenium*** made up of none inflated hyphal segments, septate, branched, clamped. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of longitudinally arranged thin walled, septate, branched, clamped hyphae, 3.-12 µm wide, contents similar to Pileipellis hyphae.

***Habitat and distribution*:** Caespitose to gregarious; among mosses on soil under the mixed forest dominated by *Cedrus deodara*.

***Materials examined*:** INDIA, Himachal Pradesh, Shimla- Khada Pathar forest, N30°72' E78°.63.2' 1950 m, 30 September 2006, collected by Deepika Kumari 43-06; Kufri forest, N30°00' E77°.15' 1700 m, 6 September 2007, collected by Deepika Kumari 169-07; same location, 23 August 2009, collected by Deepika Kumari 53-09. Holotype: PUN 3972.

***Notes*:** Several collections of the described specimens were recorded from different forest of Western Himalayas, India, and it has been confused, inexplicably with *Cantharellus cibarius* (Fr.), but after carefull microscopic examination, the present specimens is recognized by the presence of basidium with 4 spores, long sterigmata (up to 9.5 µm), smaller spore size (6-8 × 4.5-6 µm) and partial gelatinizes pileipellis, which differentiate it from the previously reported species *Cantharellus cibarius* (Fr.).

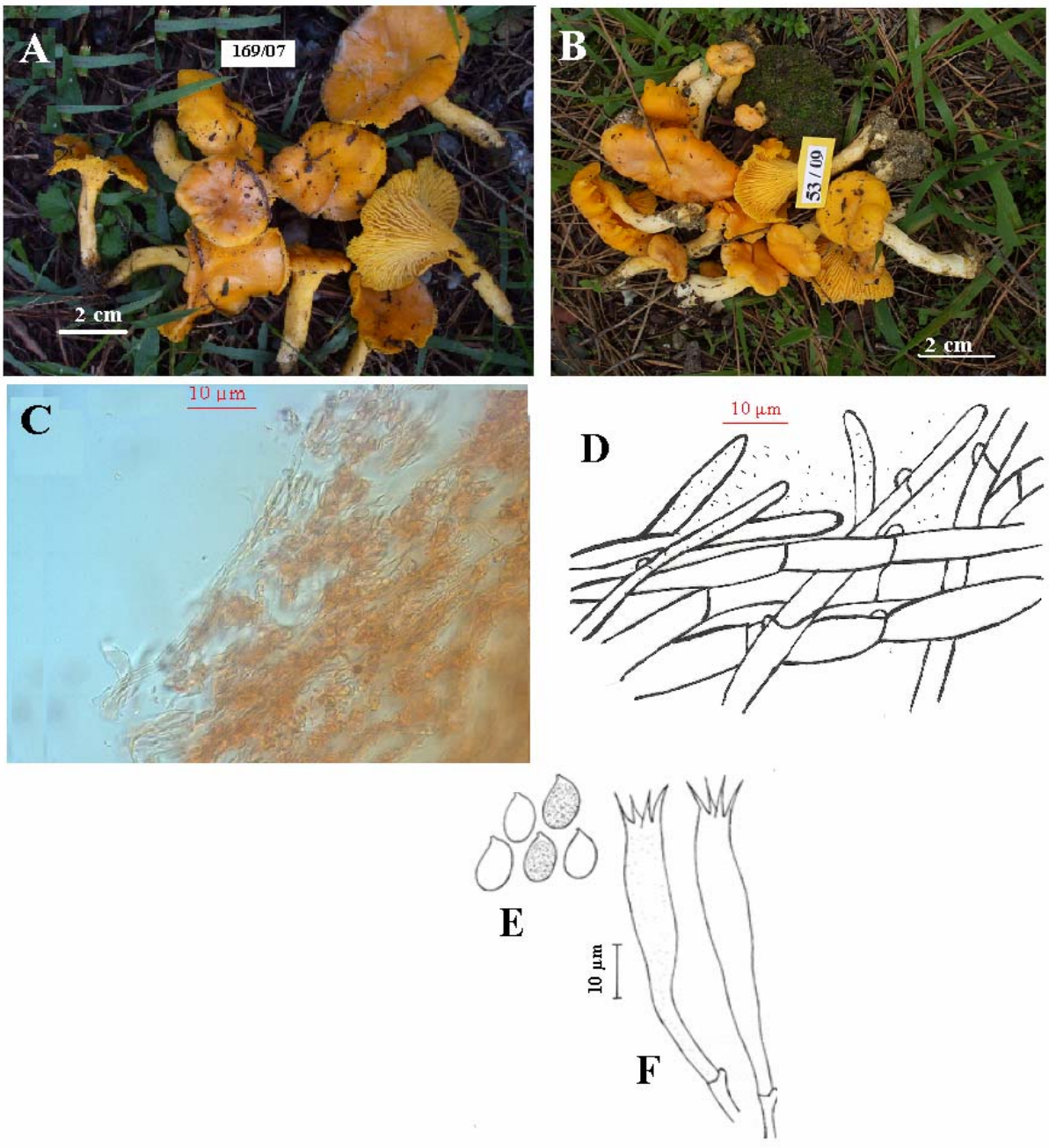


Fig. 4.7 A & B. Basidiocarps; C& D . Pileipellis and E & F. Basidiospores and Basidia of *C. himalayensis*

7. *Cantharellus indicus* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig.4.8. A-E)

Etymology: from the latin word Indicus - INDIA, referring to the region.

Pileus 3-7 cm latus, infundibuliformis ad applanatus, margine non-striato, irregulare, contextus 3-7 mm crassus, albus ad albolutescens. Lamellae decurrentes, anastomosans. Stipe centralis, 4-9 × 0.5-1 cm, superficies laevis ad capillatus, teres, basi leviter clavatus. Basidiosporae 7.2-10 × 4.5-5.4 µm, Q=1.5, ellipsoideae, laevis, inamyloideae, contento granulato. Basidia ±65-90 × 9.5-11 µm, clavatus, 4-6 sterigmatis, sterigmatis 4.5 × 1.2-2 µm, contento granulato. Basidiola numerosa. Trama hymenophorale intertextum. Pleuro-vel cheilocystidia nulla. Fibulae praesentes.

Pileus 3-7 cm broad, shallow depressed, infundibuliform to applanate; egg yellow to pale ochraceous yellow; margin invoute, split, irregular, lobed. ***Context*** 3-7 mm thick, pale yellow to creamish, confluent, unchanging on exposure to air, surface smooth but not glabrous. ***Lamellae*** decurrent, folded to anastomosing, crowded, separable easily from the flesh. ***Stipe*** 4-9 × 0.5-1 cm, Sunset (10C- 4), slightly swollen at the base, surface smooth to hairy. Spores deposit white. ***Basidiospores*** [35/2/2] 7.2-10 × 4.5-5.4 µm, L=7.9 µm, W=5.2 µm, Q=1.5, ellipsoid, smooth, nonamyloid, noncyanophilic,.wall hyaline, contents mono to multiguttulate with greenish referective oil droplets; spore deposit white. ***Basidia*** ±65-90 × 9.5-11 µm, clavate, sterigmata 3.5-4.5 × 1.2-2 µm diam., cornuted 4-6 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. **Hymenophoral trama** irregular to interwoven, branched, hyaline to faint yellowish, constricted slightly at septa, septa frequently clamped. ***Pileipellis*** consisting of filamentous and interwovenly arranged hyphae, hyaline, branched, thin to thick walled, frequently clamped, non-amyloid hyphae, 3-8 µm wide, contents granulose. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of hyaline to yellowish, cylindric, branched, frequently clamped hyphae, 1.8-3.5 µm wide.

Habitat and distribution: caespitose to gregarious, on soil under the trees of *Quercus leucotrichophora*.

Materials examined: INDIA, Himachal Pradesh, Shimla- Kufri forest, N30°00' E77°.15' 1700 m, 3 July 2007, collected by Deepika Kumari, 45-07; same location, 25 July 2008, collected by Deepika Kumari – MSR2-07; same location, 14 August 2009, collected by R.C. Upadhyay- MSR4-08; Holotype: PUN 3962.

Notes: This is an uncommon species, found from the high mountain forests of Karol and Kufri with *Q. leuotrichophora*. The morphological characters such as stem is long equaling the width of the pileus are resembles with *C. cibarius* var. *longipes* Peck (Corner 1966), but the pileus surface is smooth, hymenium more or less yellow, stem yellowish white and branched, as there are sufficient characteristics is uncommon that verified that the present species is new taxa and described as *C. indicus*.

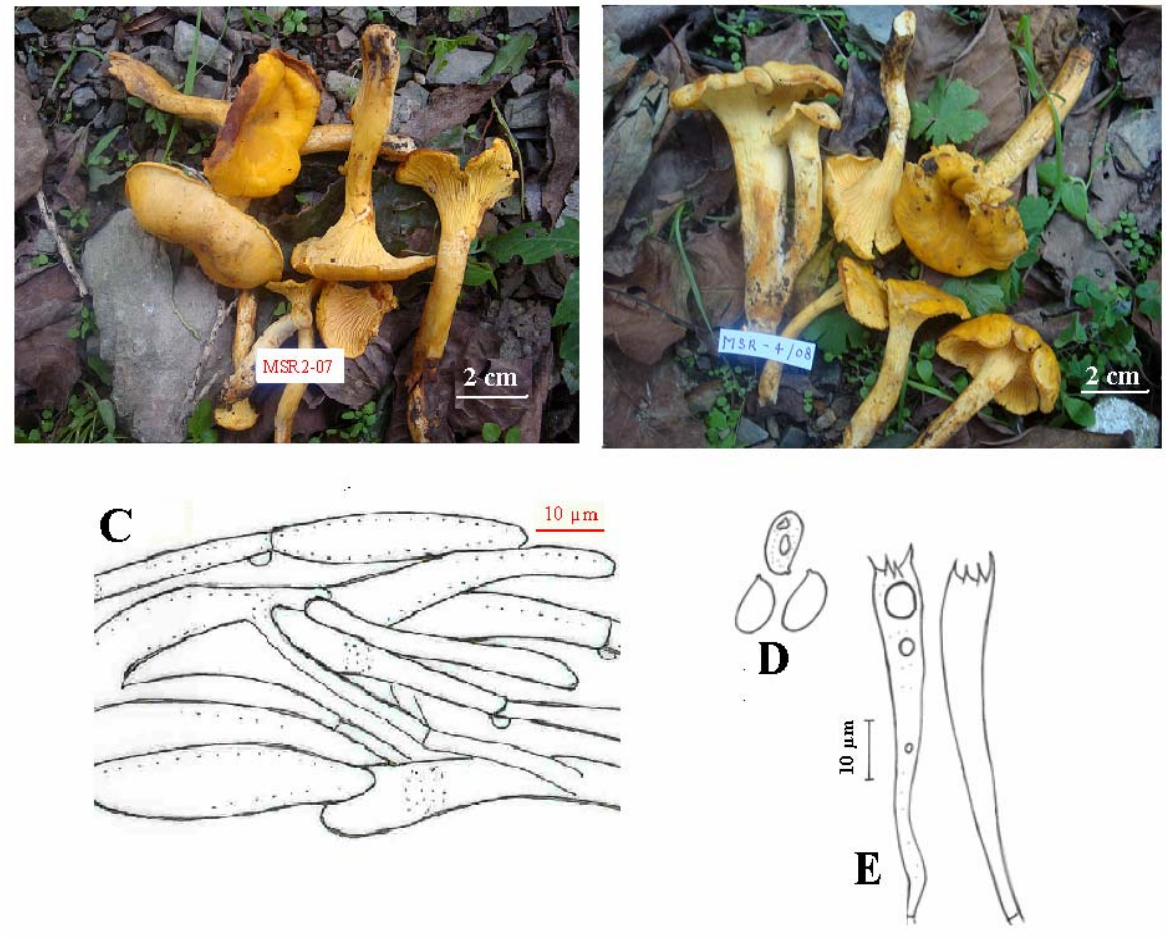


Fig. 4.8 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *C. indicus*

8. *Cantharellus lateritius* (Berk) Singer, Lilloa 22: 729. 1951. (Fig. 4.9.A-F)

Pileus upto 3-8 cm broad depressed in the middle to sub-infundibuliform, surface hygrophanous, moist, light yellow (10G-5) to golden ywllow (9I-6), sometime appressed to fibrillose; margin inrolled, irregular, split, sometime lobed. ***Hymenium*** smooth, without ridges or folds, Golden corn (9I-5). ***Stipe*** 3-7 × 0.4-1 cm, central to eccentric, tapering downwards, Raffia (11E-5), lacunose, glabrous, consistency cartilaginous, stuffed then hollow, context yellowish; taste mild pleasant when fresh. Spore deposits white. ***Basidiospores*** (45/2/2) 7-9 x 4.5-5.5 (-6) µm, L=7.93 µm, W=5.02 µm, Q'=1.57, broadly ellipsoid to ellipsoid, smooth, nonamyloid, noncyanophillic, wall hyline, contents monogutullate to multigutullate. ***Basidia*** ±29-60 × 4.5-6 µm, cylindro-clavate to clavate, sterigmata 2-4 per basidium, developing basidia with evenly granulose, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Pileipellis*** made up of repent to interwovenly arranged, 1.8-5.7µm wide, embedded in partially gelatinized matrix, branched, septa, clamped, contents granulated, context hyphae comparatively broader. ***Hymenophoral trama*** subparallel to interwovenly arranged, partially gelatinized at top, pale yellowish in 3% KOH, branched, clamped, 1.8-5.7 µm wide. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of hyaline, branched, thin to thick walled, granulated contents, 1.5-3.5 µm wide, clamp connections present.

***Habitat and distribution*:** caespitose to gregarious, on soil under the trees of *Cedrus deodara*.

***Materials examined*:** INDIA, Himachal Pradesh- - Shimla- Khada Pathar forest, N30°72' E78°.632' 1950 m, 13 September 2005, collected by R.C Upadhyay 119-05; same location, 28 September 2006, collected by Deepika Kumari 333-06; same location, 11 August 2007, collected by Deepika Kumari 161-07. Holotype: PUN 3958.

***Notes*:** The present specimen is similar to *Craterellus lateritius* as described by Bigelow (1978), with completely smooth hymenophore, sweet smell and clamped hyphae. Morphologically the described species showed similarities with *Cantharellus lateritius* (Berk), however, the Q values of the described specimen are

slightly smaller. This species is reported from Jageshwar forest, Uttarakhand by Dhancholia *et al* (1991), but they have not mentioned any taxonomical details to support their identification.

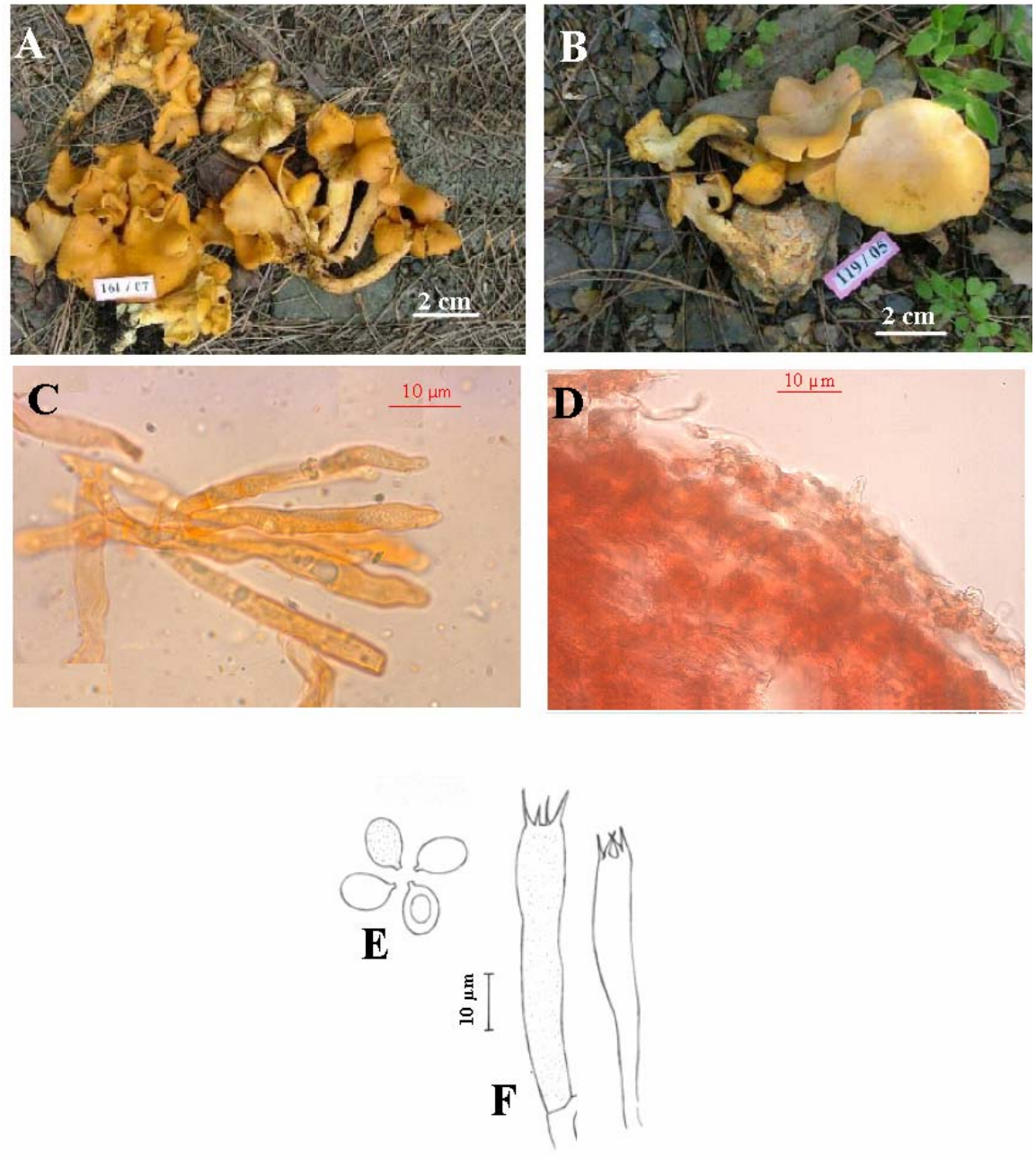


Fig. 4.9 A & B. Basidiocarps; C & D. Pileipellis and E & F. Basidiospores and Basidia of *C. lateritius*

9. *Cantharellus. miniatescens* Heinem., BULL. Jard. Bot. Etat Brux. 28, 393, 1958, f.36; Fl. Ic. Champ. Congo 8 (1958) 156. (Fig.4.10. A-D)

Pileus 3-5 cm wide, campanulate with papilla when young, later on more or less depressed, surface orange grey to grayish orange (6B-2 to 6B-3), appressed with light brown to brownish orange (7D-4 to 6C-4) fibrils in the center, grayish orange (5B-4 to 5B-3) outwards, cuticle not to half peeling; margin regular, non-striate. ***Context*** thin, whitish. ***Lamellae*** hymeniform not smooth, thick, decurrent, distant, light orange grey (5B-2), bifurcate at times, 2 mm broad, edges smooth, attachment to stipe distinct. ***Stipe*** central, 3.5-6.0 × 0.4-0.8 cm, cylindric, with a slightly broad base, concolourous to the pileus, not smooth, stuffed, context off white, exannulate. Spore deposit white. ***Basidiospores*** [58/1/1] 7.2-9 (-9.5) × (4.1-) 4.5-5.4 µm; L= 8.4 µm, W= 4.8 µm; Q= 1.74, ellipsoid, inamyloid, smooth, hyaline, apiculus upto 0.5 µm long, contents granulated. ***Basidia*** 50-75 × 7.2-9 µm, long clavate, 2-4(-6) spored, sterigmata 4.5-5.4 µm long, contents granulated and with multi oil droplets, basal septa with clamps. ***Hymenophoral trama*** irregular, made up of thin walled, clamped, and branched hyphae, 3.6-9 µm wide. ***Subhymenium*** made up of none inflated hyphal cells, septate, branched, clamped. ***Pileipellis*** made up of parallel to suberectely arranged hyphae, 3.6-9 µm wide, thin to thick walled (upto 0.9 µm thick), wall yellowish, septate, branched, hyphal ends as narrowly clavate or rounded and contents with multi small oil droplets to granulate. ***Pleurocystidia*** and ***Cheilocystidia*** none. All hyphae are gleopleorus. ***Stipe cuticle*** made up of longitudinally arranged thin walled, septate, branched, clamped hyphae 3.6-10 µm wide, contents similar to Pileipellis hyphae.

***Habitat and distribution*:** Solitary to gregarious; on soil under the mixed forest dominated by *Cedrus deodara*.

***Materials examined*:** INDIA, Himachal Pradesh Shimla- Dhalli forest N 30°17' E78°.45' 1700 m, 25 July 2007, collected by Deepika Kumari 65-07. Holotype: DMR 65-07

***Notes*:** The present specimen matches well with the characters given for *Cantharellus miniatescens* Heinem as the distinguishing features of *C. miniatescens*

are pileus widely campanulate, lamellae folded, decurrent, spore ellipsoid, stipe surface smooth but not glabrous, slightly swollen at base, colour dull ocher to dull ochraceous orange and Pileipellis hyphae are frequently clamped and end cells subclavate to lanceolate. However, the spore Q value is comparatively lower in the present described specimen (Q=1.74) which suggest that the spore are more cylindric than the type specimen (Q =1.66). The overall macroscopic and microscopic details of the present specimen are in conformity with *C. miniatescens* Heinem as given by Corner (1960) except for the slightly variation in spore shape.

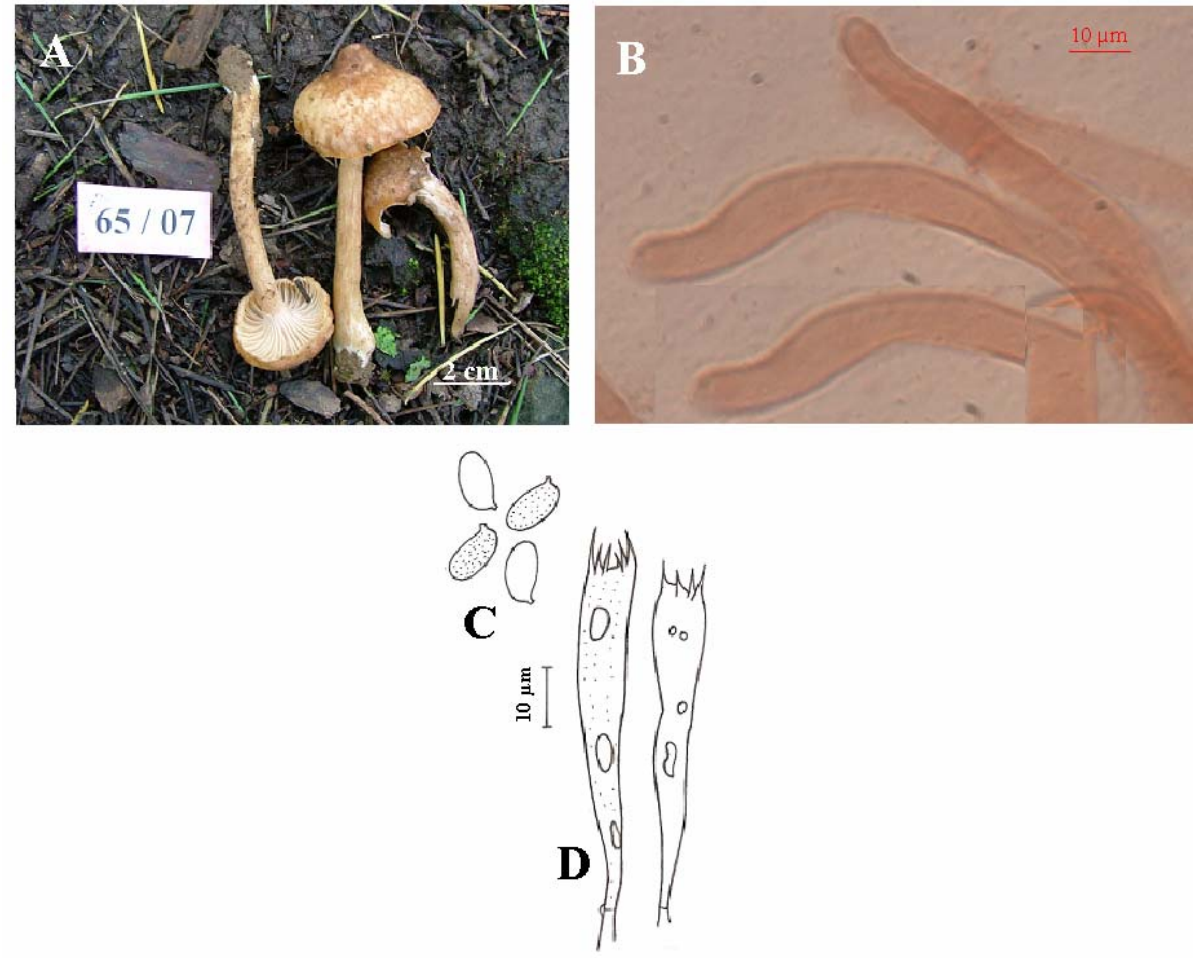


Fig. 4.10 A. Basidiocarp; B. Pileipellis and C & D. Basidiospores and Basidia of *C. miniatescens*

10. *Cantharellus minor* Peck, Annual Rep. New York State Cab 23: 122. 1872 (Fig.4.11 A-D)

Pileus 5-30 mm wide, obtuse to convex, campanulate, infundibuliform, dull yellow orange to orange, cuticle not to half peeling; margin incurred, becoming plan with narrowly decureved margin, non-striate. ***Context*** concolourous with pielus, thin. ***Lamellae*** decurrent, close to distant, very narrow, forked, not intervenose, concolourous with pielus, edge even, acute or obtuse. ***Stipe*** central, 1.5-2.5 × 0.3-0.5 cm, base attenuated, central, solid, concolourous to the pileus, surface glabrous. Spore deposit white. ***Basidiospores*** [48/1/1] 7.5-10 (-11.5) × (4.1-) 4.5-6 (-6.5) µm; L= 8.4 µm, W= 4.8 µm; Q= 1.75, broadly ellipsoid or ellipsoid to oblong, inamyloid, smooth, hyaline, contents granulated. Spore deposit light yellowish orange. ***Basidia*** (31-) 40-70 × 7.6-10.5 µm, long clavate, 4-5(-6) spored, sterigmata 3.5-5.0 µm long, contents granulated and with multi oil droplets, basal septa with clamps. ***Hymenophoral trama*** interwoven, made up of thin walled, clamped, and branched hyphae, 1.5-5.0 µm wide. ***Subhymenium*** made up of none inflated hyphal cells, septate, branched, clamped. ***Pileipellis*** made up of parallel to suberectely arranged hyphae, 3.6-8 (-9.5) µm wide, thin to thick walled (upto 0.9 µm thick), wall yellowish, septate, branched, hyphal ends cells protruding at times; context hypha cylindric, 2.5-6.3 µm diam. ***Pleurocystidia*** and ***Cheilocystidia*** none. ***Stipe cuticle*** made up of longitudinally arranged thin walled, septate, branched, clamped hyphae 2.6-8 µm wide, contents similar to pileipellis hyphae.

***Habitat*:** On soil, gregarious to caespitose; under the tree of *Cedrus deodara* and *Quercus dilatata*.

***Materials examined*:** INDIA, Himachal Pradesh- Shimla- Khada Pathar forest, N30°72' E78°.63.2' 1950 m, 15 July 2005, collected by R. C. Upadhyay 354-05; same location, 22 July 2009, collected by Deepika Kumari 251-09. Holotype: PUN 3971.

***Notes*:** *Cantharellus minor* is easily recognized by the field characters especially small basidiomata; yellow to orange yellow colour with decurrent hymenophore.

Sohi *et al* (1991) already described the type specimen in detail from Kashmir region, India. During research work, this specimen was also collected from forests of Chail and Khada Pathar. After documentation and microscopic examination this specimen easily fits with the description of *C. minor* described by Sohi *et al* (1991): first, the colour of pilei and stipe is olivaceous yellow to olivaceous and second, the gills are represented as crowed, not distant. Spore 6.5-9.2 × 4.5-5.5 µm, L= 8.2 µm**,** W= 5.5 µm**;** Q= 1.49, ellipsoid, smooth, nonamyloid, wall hyaline, contents mono to multiguttulate are similar, which would also coincide to previously describes *C. minor*. So, there is evidence that the describe specimen is the same collection type, previously described by Sohi *et al* (1991).

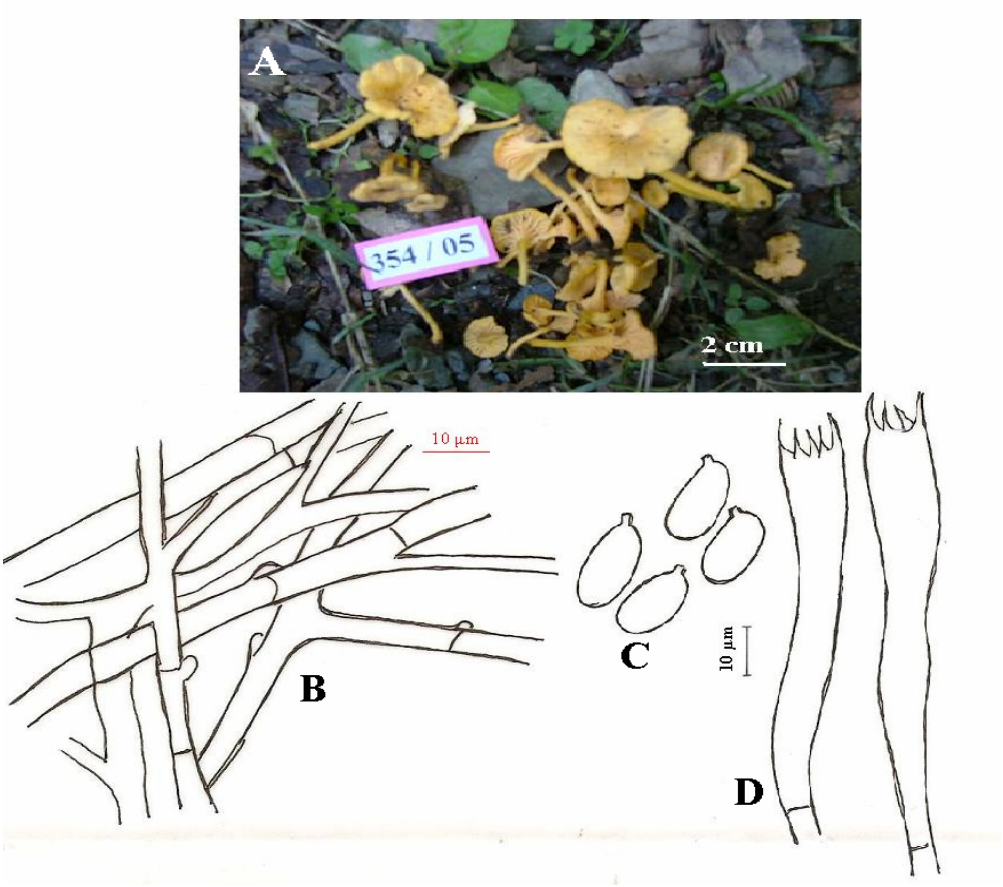


Fig. 4.11 A. Basidiocarp; B. Pileipellis and C & D. Basidiospores and Basidia of *C. minor*

11. *Cantharellus natarajanii* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig. 4.12.A-F)

MycoBank: MB519522

Etymology: Is named in honor of Professor K. Natarajan on account of his contribution towards Indian mycology.

Pileus 5-10 cm latus, hemisphaericus ad planoconvexus, postremo depressus, luteus ad sinico-flavus, laevis, margine non-striato, irregulare, undulates, lobutatus. Contextus 3.5 mm crassus, citrinus ad flavus, superficies laevis, glaber. Lamellae distinctus, decurrentus, anastomosans ad distinctus intervenatae. Stipe centralis, 3.5-6 cm longus, 0.4-1 cm crassus, lentiter clavatus basi. Sporae in cumulo albae. Basidiosporae 6.5-9 × 5.2-6.3 µm, Q=1.34, ellipsoideae, vel late ellipsoideae, laevis, inamyloideae. Basidia ±57-85 × 6.5-10.5 µm, clavata. 4-vel-5 sporifera; sterigmatis usque 4.5-8 µm longis, 1.2-2.5 µm crassus. Basidiola numerosa. Trama hymenophorale inaqualis/irregularis vel intertextum. Pilleipellis ex hyphis subparallelis distinctus subclavata vel subventricosus. Pleuro-vel cheilocystidia nulla. Fibulae praesentes.

Pileus 5-10 cm wide, hemispherical, plano-convex to finally depressed, Golden yellow (9K-4) to Chinese yellow (10K-6), smooth, margin irregular, wavy, lobed, non-striate. ***Context*** 3-5 mm thick, lemon yellow to yellow, confluent, unchanging on exposure to air, surface smooth, glabrous. ***Lamellae*** distinctly hymeniform, decurrent, anastomosing to distinctly interveined, golden yellow (9L-6). ***Stipe*** central, 3.5-6 × 0.4-1 cm, Sunset, slightly expanded at apex, surface glabrous to appressed fibrils. Spore deposit white. ***Basidiospores*** [54/2/2], 6.5-9 × 5.2-6.3 µm, L= 7.6 µm, W= 5.67, Q= 1.34, broadly ellipsoid to ellipsoid, thin walled, smooth, faintly yellowish in 3% KOH, contents monoguttulate to multi oil refractive gutullate, inamyloid. ***Basidia*** ± 57-85 × 6.5-10.5 µm, clavate, sterigmata 4.5-8 ×1.2-2.5 µm diam., cornuted 4-5 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH.

Hymenophoral trama irregular to interwoven, branched, hyaline to faint yellowish, slightly constricted at septa, septa frequently clamped. ***Pileipellis*** consisting of cylindric to filamentous and interwovenly arranged hyphae, end-cells are disctintly subclavate to subventricose, yellowish to yellowish brown, branched, thin to thick walled, frequently clamped, non-amyloid hyphae, 3-10 μm wide, followed by cystoids end hyphae, contents granulose. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of yellowish brown, cylindric to filamentous, branched, frequently clamped hyphae, 2.5-10 μm wide.

***Habitat and distribution*:** caespitose to gregarious, on soil among moss under *Cedrus deodara* and *Quercus dilatata*.

***Materials examined*:** INDIA, Himachal Pradesh- Shimla- Chail forest, N31°06' E77°.10' 1700 m, 30 July 2008, collected by Deepika Kumari 106-08; same location, 22 August 2009, collected by Deepika Kumari 35-09; same collection, Uttarakhand- Jageshwar forest N29°00' E79°.17' 29 August 2009, collected by Deepika Kumari, 93-09. Holotype: PUN 3963.

***Notes*:** The present species is characterized by its hemispherical, plano-convex basidiomes. Intially present spciemen was mistkan with *C. cibarius*, but *C. cibarius* is much larger species (pileus 15 cm broad, spores range 8.5-11.5 × 4.5-5.5 μm), moreover the colour morphology is dissimilar and an important character of *C. natarajanii* is "pileus hyphal end-cells which is distinctly subclavate to subventricose" clearly differentiates this from *C. cibarius* (composed of loosely interwoven hyphae), so the presence of distinctive taxonomic features supports the dissimilarity of the herewith described species with respect to other species of *Cantharellus*, verified that the present species is a new taxa and designated as *C. natarajanii*.

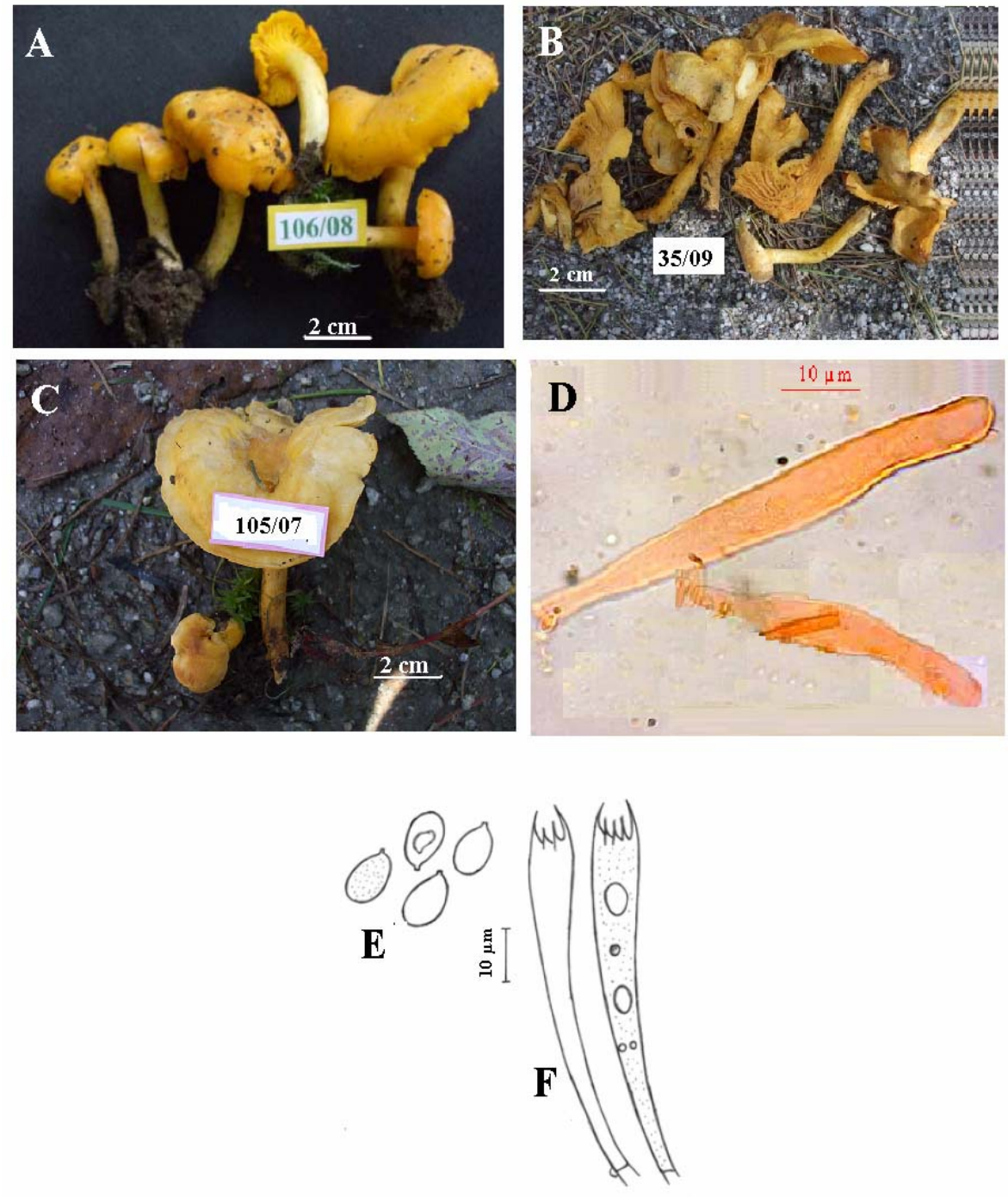


Fig. 4.12 A, B & C. Basidiocarps; D. Pileipellis and E & F. Basidiospores and Basidia of *C. natarajanii*

12. *Cantharellus pseudoformosus* Deepika, Upadhyay & Reddy, sp. nov. (Fig 4.13. A-E)

MycoBank: MB519030

Etymology: Pseudoformosus- refer to homology with *formosus*

Pileus 1–3 cm latus, applanatus, depressus, in centro squamis, brunneus, versus marginem brunneo-luteus vel bruneolo-aurantiacus, superficies sicco, non hygrophano. Lamellae decurrentes, infirme intervenatae, incarnato-aurantiacae vel pallide aurantiacae. Stipes2–4.5 cm longus, 0.3–0.6 cm crassus, teres, basi leviter clavatus, bruneolo-aurantiacus vel brunneus. Sporae in cumulo albae ad luteo-albae. Odor et sapor fructus indistincti. Basidiosporae7–9 × 5–6 µm, Q = 1.40, ellipsoideae, inamyloideae. Basidia ± 40–45 × 5.3–9 µm, cylindrica vel anguste clavata. sterigmatibus usque 5.9–7.2 µm longis. Basidiola numerosa. Trama hymenophorale intertextum. Pleuro-vel cheilocystidia nulla. Epicutis pilei hyphosa. Fibulae praesentes.

Pileus 1-3 cm wide, applanate with depressed center, pecan brown in the center due to scales, brownish light yellow to brownish orange outwards, surface dry, non-hygrophanous, covered with indistinct scales; margin decurved, regular, non-striate; context thin, yellowish, unchanging on brusing. ***Lamellae*** decurrent, folded, weakly interveined, pinkinsh orange to light orange or yellowish orange, edges sub acute, unchanging on bruising. ***Stipe*** 2–4.5 × 0.3–0.6 cm, terete with slightly clavate base, lacunose, stipe surface glabrous, brownish orange to pecan brown, darker at base as leather brown, unchanging on bruising. Spore print white to yellowish white. Odor and taste indistinct. *Basidiospores* [45/2/2] 7–9 × 5–6 µm, L=7.6 µm, W=5.4 µm Q=1.40, ellipsoid, inamyloid, smooth, faintly yellowish in 3% KOH, content monoguttulate to granulated. ***Basidia*** ± 40–65 × 5.3–9 µm, cylindrical to narrowly clavate, strerigmata 5.9–7.2 µm long, cornuted (1–) 2–5(–6) per basidium, immature basidia with evenly granulose, light yellowish content, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow content in 3% KOH. ***Pileipellis***

epicutis with projecting cystidiod end subclavate to clavate cells, ±50–128 µm long, made up of radially to sub-radially arranged hyphae, 2.5–13 µm diam, wall slightly thick and faintly yellowish, context hyphae cylindric ±3–17 µm diam, slightly thick walled. ***Hymenophoral trama*** interwoven with cylindric hyphae ±2.7–4.5 µm wide. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of subclavate to clavate hyphae, thin to slightly thick walled, ±2.5–3.5 µm wide, contains similar to suprapellis, septa frequently clamped.

***Habitat and distribution*:** Gregarious to caespitose; on soil under the trees of *Cedrus deodara*.

***Materials examined*:** INDIA, Himachal Pradesh, District-Chamba, Khajjiyar, N32°10' E75°.45' 6,400 m, 28 September 2007, collected by Deepika Kumari 281-07; Suala (25 km away from the Khajjiyar guest house);same collection, 28 September 2007, collected by Deepika Kumari 272-07; Bharmour (5 km away from the Bharmour bus stand), same collction, 12 September, 2009, collected by Deepika Kumari 282-09. Holotype: PUN 3883.

***Notes*:** This specimen is morphologically and microscopically well within the circumscriptions given for the *Cantharellus formosus* Corner. The colour of pileus, hymenium and stipe is similar, hymenium with forked, low ridges and furrows, cap surface with small dense, closely adhered scales which is slightly darker than cuticle, similar spores range and Q value, similar number of sterigmata on basidia, Pileipellis hyphae non-encrusted and end cells cystidiod and presence of clamp connections. But the molecular studies showed differences from other *C. formosus* reported previously, so concluded this as a new species and was identified as *C. pseudoformosus.*

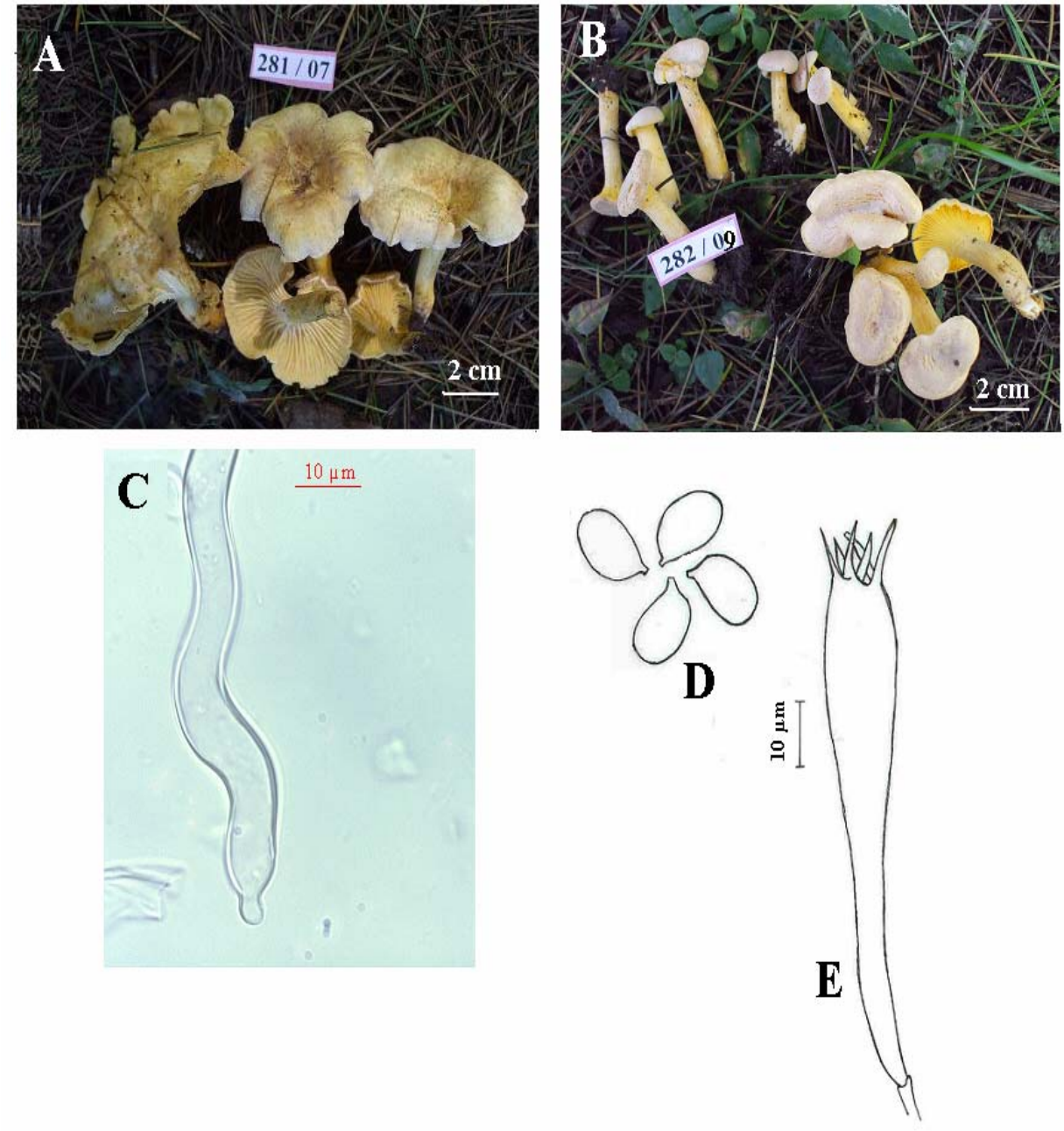


Fig. 4.13 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *C. pseudoformosus*

13. *Cantharellus umbonatus* Deepika, Upadhyay & Reddy, **sp. nov.** (Fig.4.14. A-E)

MycoBank: MB519523

Etymology: From latin word umbonatus- umbilicate, referring to the shape of the cap.

Pileus 4-5 cm latus, applanatus deinde umbonatus, aurantiacus ad flavus, laevis, margine non-striato, irregulare, lobulatus, contextus 2-3 mm crassus. Lamellae albae distinctus decurrentes, intervenatae vel anastomosans. Stipe centralis 9-11.5 cm aequalis in diametrum, teres, basi leviter clavatus. Sapor incognitus.. Basidiosporae (8-) 8.5-9.8 (-10) × 5-5.5 (-6) µm, Q=1.76, ellipsoideae, vel cylindricus, inamyloideae. Basidia ± 67-99 × 7-9.7 µm, clavata. vel angustus clavata.; 4-vel-6 sporifera, sterigmatis usque 3.5-6.5 µm longis, 1.2-2 µm crassus. Basidiola numerosa. Trama hymenophorale irregularis vel intertextum. Pleuro-vel cheilocystidia nulla. Fibulae praesentes.

Pileus 4-5 cm, applanate then umbilicate, Capuccine orange to yellow, smooth; margin irregular, hygrophanous, nonstriate, lobed. ***Context*** 2-3 mm thick, straw (10F-2), confluent, unchanging on exposure to air, surface smooth but not glabrous. ***Lamellae*** abundantly decurrent, interveined to anastomosing, white reaching up to the half of the stipe. ***Stipe*** 9-11.5 × 1-1.5 cm thick, central, Margurite yellow (10C-1), terete with slightly swollen apex, radicate, surface fibrous, stipe trama colonial buff (10G-2). Spore deposit white. ***Basidiospores*** [55/2/2] (8-) 8.5-9.8 (-10) × 5-5.5 (-6) µm, L=8.99 µm, W=5.08 µm, Q=1.76, ellipsoid to cylindrical, nonamyloid, noncyanophilic, wall hyaline, contents granulated to multiguttulate with greenish referective oil droplets. ***Basidia*** ± 67-99 × 7-9.7 µm, clubed shaped to sub clavate, sterigmata 3.5-6.5 × 1.2-2 µm diam., cornuted 4-6 per basidium, developing basidia with evenly granulose, light yellow contents, basal septa with clamps. ***Basidioles*** numerous with opaque light yellow contents in 3% KOH. ***Subhymenium*** made up of narrow to non inflated hyphal end cells. ***Hymenophoral trama*** irregular to

interwoven, branched, hyaline to faint yellowish, constricted slightly at septa, septa frequently clamped. ***Pileipellis*** consisting of filamentous and interwovenly arranged, hyaline, branched, thin to thick walled, frequently clamped, non-amyloid hyphae, 3-12 µm wide, followed by protruding end cells, contents granulose. ***Pleurocystidia*** and ***Cheilocystidia*** absent. ***Stipe cuticle*** made up of hyaline to yellowish, branched, frequently clamped hyphae, 2.5-6 µm wide.

***Habitat and distribution*:** caespitose to gregarious, on soil under the trees of *Cedrus deodara*.

***Materials examined*:** INDIA, Himachal Pradesh- Shimla- Khada Pathar forest, N30°72' E78°.63.2' 1950 m, 25 July 2006, collected by R. C. Upadhyay 316-06; same location, 29 July 2007, collected by R. C. Upadhyay 348-07; same location, 28 August 2009, collected by Deepika Kumari 217-09. Holotype: PUN 3968.

***Notes*:** Because of yellowish to yellowish brown fruit bodies, decurrent hymenium and the presence of clamp connections, this elegant species fits in genus *Cantharellus*. *C. cyanoxanthus* Heim, Corner (1966), is closely related, but the fruit bodies of describes specimen is more brightly coloured, spore is consistently wider. The main important character pileus is slightly umbonate pileus, hymenium is quite umber that reaches up to the half of the stipe and presence of clubed shape basidia. This variability reflected that the described species recorded as different species.

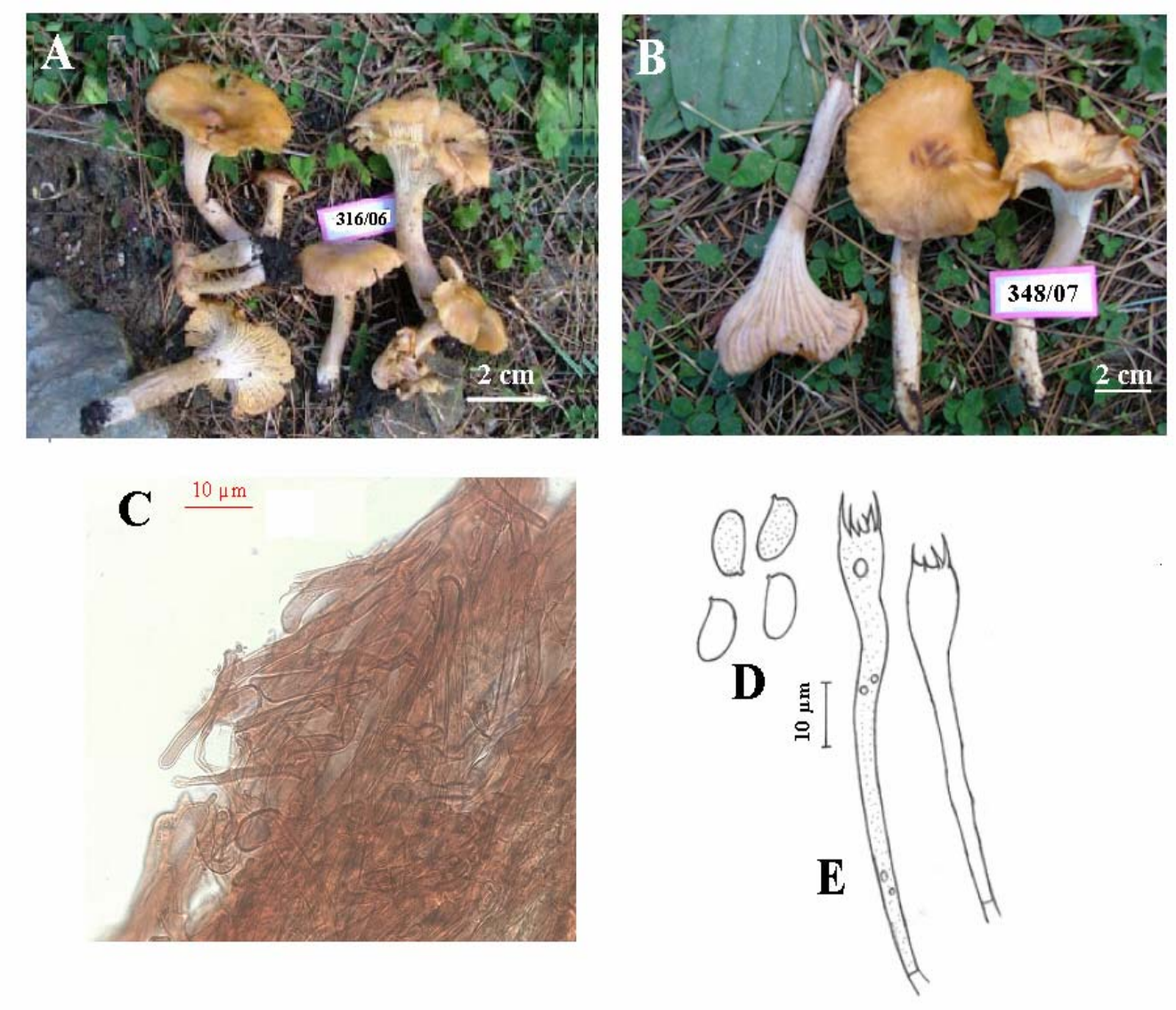


Fig. 4.14 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *C. umbonatus*

Table 4.2 Morphological characters of *Cantharellus* species collected from Western Himalayas region of India

	Morphological Characteristics					
Name of Species	**Pileus diam (cm)**	**Pileus surface**	**Stipe diameter (cm)**	**Basidia (µm)**	**Basidiospores (µm) Means; L;W;Q**	**Pileipellis hyphae**
C. applanatus	3-6.5	Golden yellow	2.5-5 × 0.4-0.7	55-78 × 6.0-7.5	7-8.5 × 4.5-5.5; 7.62; 4.49; 1.54	Projecting end hyphae
C. appalachiensis	3-7	Pinkish yellow to yellowish	3.5-7 × 0.4-1.1	52-77 × 7.5-9	7.5-9.5 × 4.5-5.8; 8.4; 5.2; 1.62	Subclavate to clavte cells with projecting cystidiod end
C. cibarius	3-15	Egg yellow to pale ochraceous	3-8 × 0.4-0.8	50-110 × 7.5-10	8.5-10.5 × 4.5-5.8; 8.6; 5.4; 1.59	Cylindric hyphae end cells
C. elongatipes	1.5	Orangish yellow	3-3.5 × 0.4-0.7	52-70 × 7-10	5.9-7.2 × 4.5-5.4; 6.6; 4.9;1.35	Sub parallel arranged filamentous hyphae
C. fibrillosus	5-8.2	Mustard brown	6-8 × 0.7-1.0	60-90 × 9-11.5	8.5-12 × 5-6.2; 9.7; 5.9;1.64	Filamentous and interwovenly arranged hyphae
C. himalayensis	3-7	Yellowish	4-7.5 × 0.8-1.1	60-85 × 8-11	6-8 × 4.5-6; 5.91; 5.16; 1.34	Parallel to repent filamentous hyphae
C. indicus	3-7	Egg yellow to pale ochraceous	4-9 × 0.5-1.0	65-90 × 9.5-11	7.2-10 × 4.5-5.4; 7.9; 5.2; 1.5	Filamentous and interwovenly arranged hyphae
C. lateritius	3-8	Light yellow to golden yellow	3-7 × 0.4-1.0	29-60 × 4.5-6	7-9 × 4.5-5.5; 7.93; 5.02; 1.57	Repent to interwovenly arranged
C. miniatescens	3-5	Orange grey to grayish orange	3.5-6.0 × 0.4-0.8	50-75 × 7.2-9	7.2-9 × 4.5-5.4; 8.4; 4.8; 1.74	Parallel to suberectely arranged hyphae
C. minor	0.5-3	Dull yellow to orange	1.5-2.5 × 0.3-0.5	40-70 × 7.6-10.5	7.5-10 × 4.5-6.0; 8.4; 4.8; 1.75	None inflated hyphal cells
C. natarajanii	5-10	Golden yellow	3.5-6 × 0.4-1.0	57-85 × 6.5-10.5	6.5-9 × 5.2-6.3; 7.6; 5.67; 1.34	Subclavate to subventricose hyphal cells
C. pseudoformosus	1-3	Light yellow to brownish orange	2-4.5 × 0.3–0.6	40–65 × 5.3–9	7-9 × 5.0-6.0; 7.6; 5.4; 1.40	Projecting cystidiod end subclavate to clavate cells
C. umbonatus	4-5	Capuccine orange yellow	9-11.5 × 1-1.5	67-99 × 7-9.7	8.5-9.8 × 5.5-5.8; 99; 5.08; 1.76	Filamentous and interwovenly arranged

Comparative photographs of *Cantharellus* species in their natural habitat and illustrations (microscopic details), collected from Western Himalayas, India, which indicate the differences among themselves are given below:

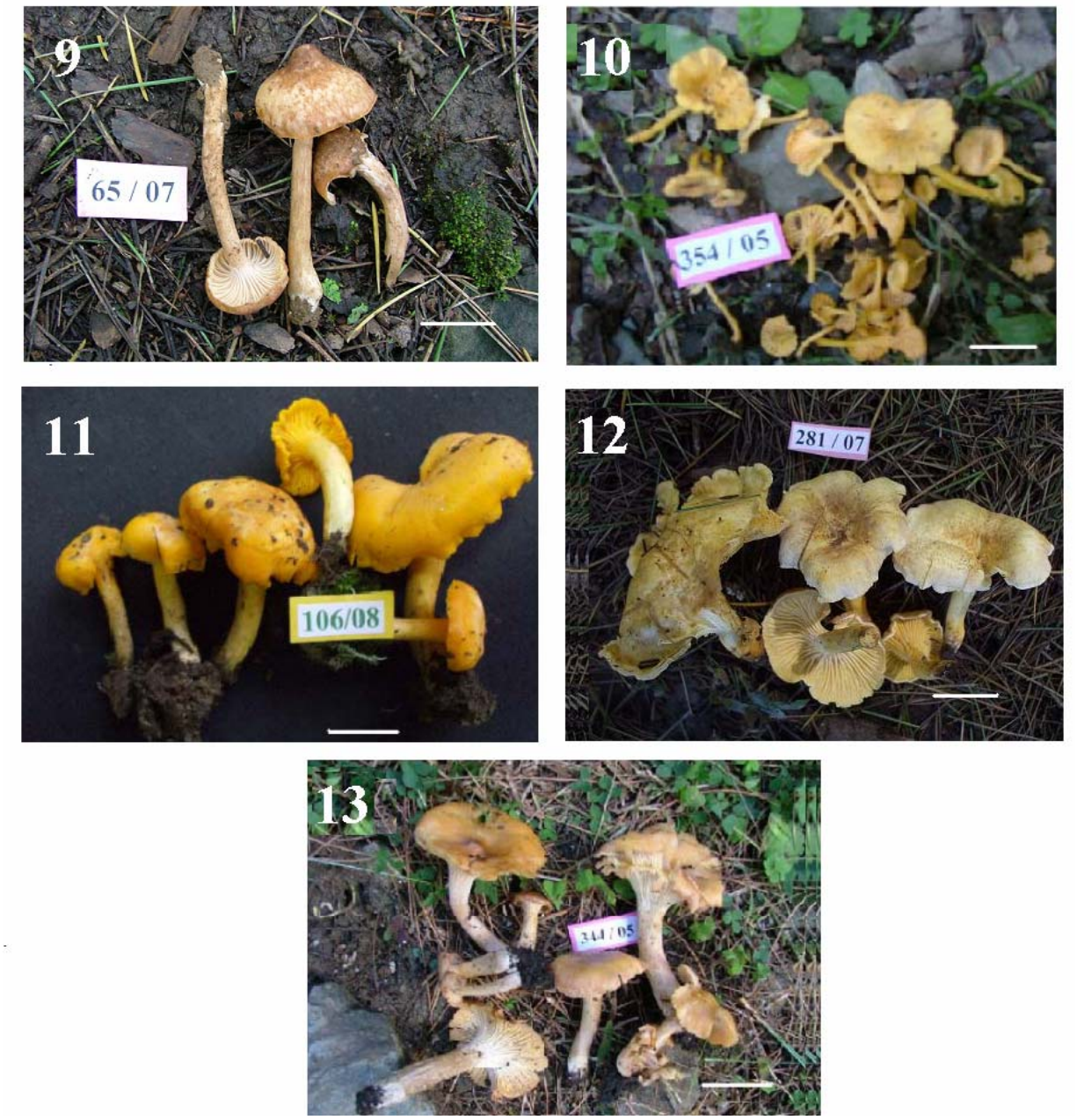
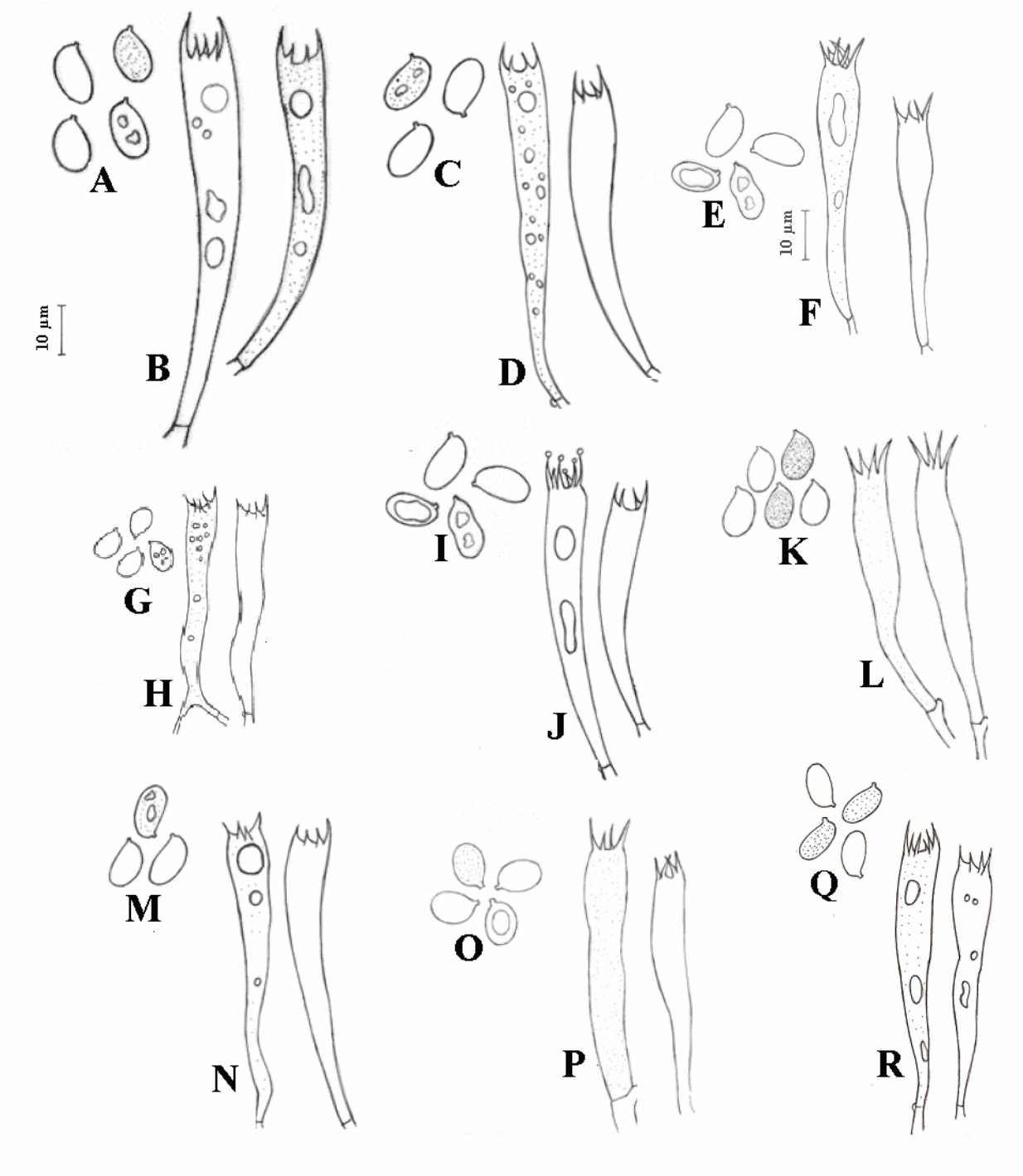


Fig. 4.15 Comparative photographs of *Cantharellus* species collected from Western Himalayas, India (**1.** *C. applanatus*; **2.** *C. appalachiensis*; **3**. *C. cibarius*; **4.** *C. elongatipes*; **5.** *C. fibrillosus*; **6.** *C. himalayensis*; **7.** *C. indicus*; **8.** *C. lateritius*; **9.** *C. miniatescens*; **10.** *C. minor*; **11.** *C. natarajanii*; **12.** *C. pseudoformosus* and **13.** *C. umbonatus*). All the photographs are the holotype and the bar represents 2 cm for basidiocarps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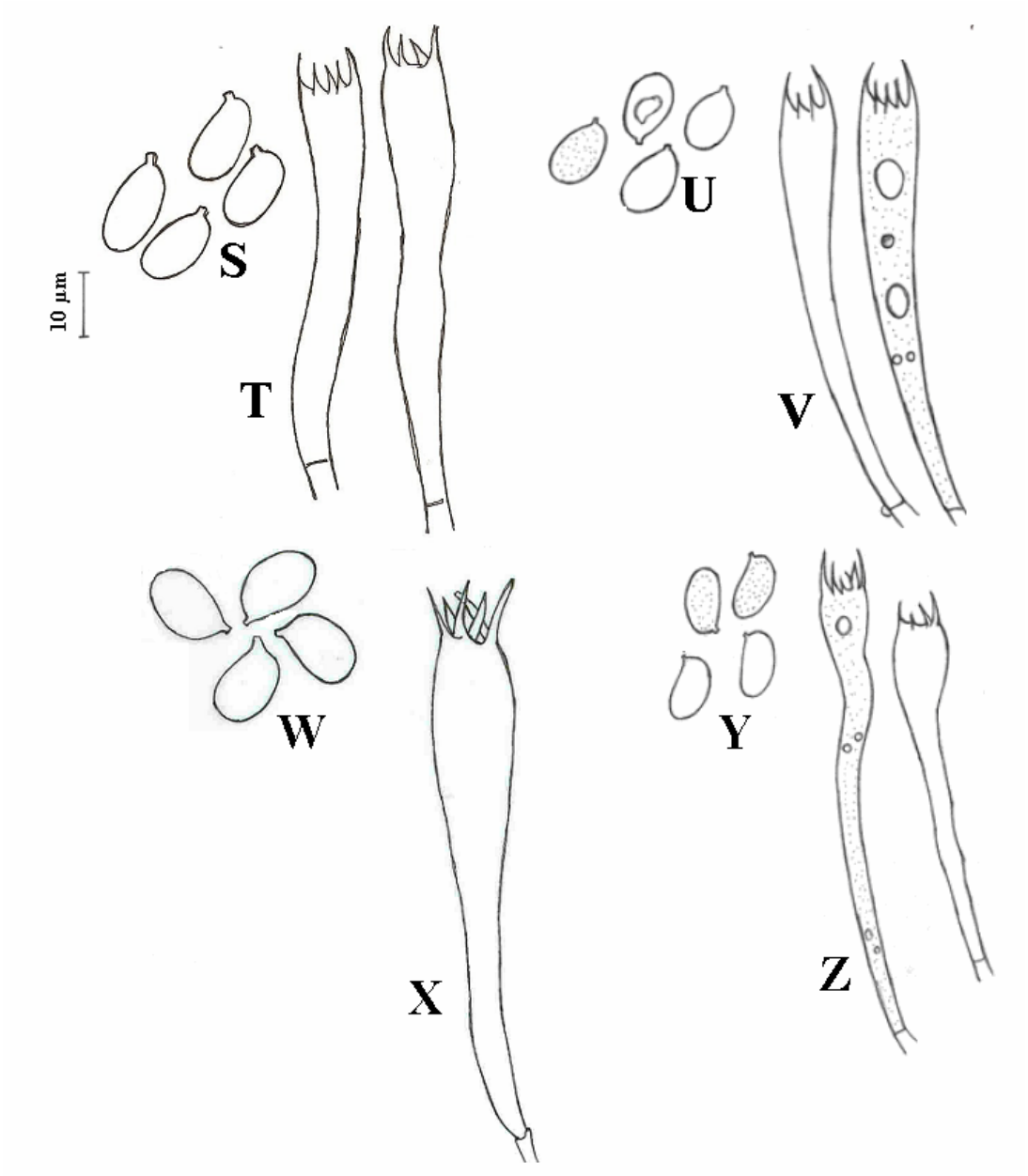
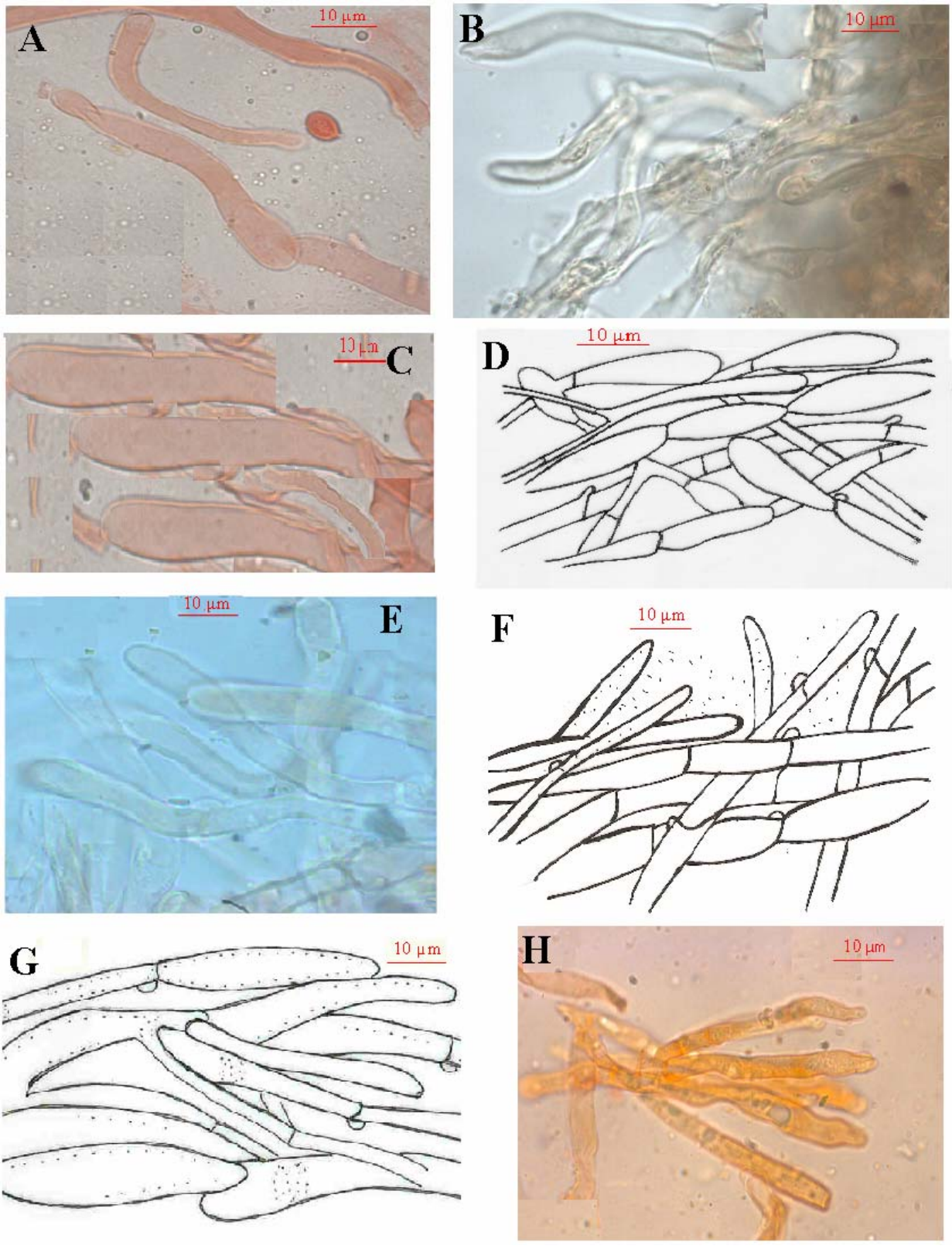


Fig. 4.16 Comparative illustrations of Basidiospores and Basidia of *Cantharellus* species collected from Western Himalayas, India. Basidiospores and Basidia of: **A & B** *C. applanatus*; **C & D**, *C. appalachiensis*; **E & F**, *C. cibarius*; **G & H** *C. elongatipes*; **I & J**, *C fibrillosus*; **K & L**, *C. himalayensis*; **M & N**, *C. indicus*; **O & P**, *C. lateritius*; **Q & R**, *C. miniatescen*; **S & T**, *C. minor*; **U & V**, *C. natarajanii*; **W & X**, *C. pseudoformosus* and **Y & Z**, *C. umbonatus*. (All the elements are drawn from the holotype and the bar represents 10 µm)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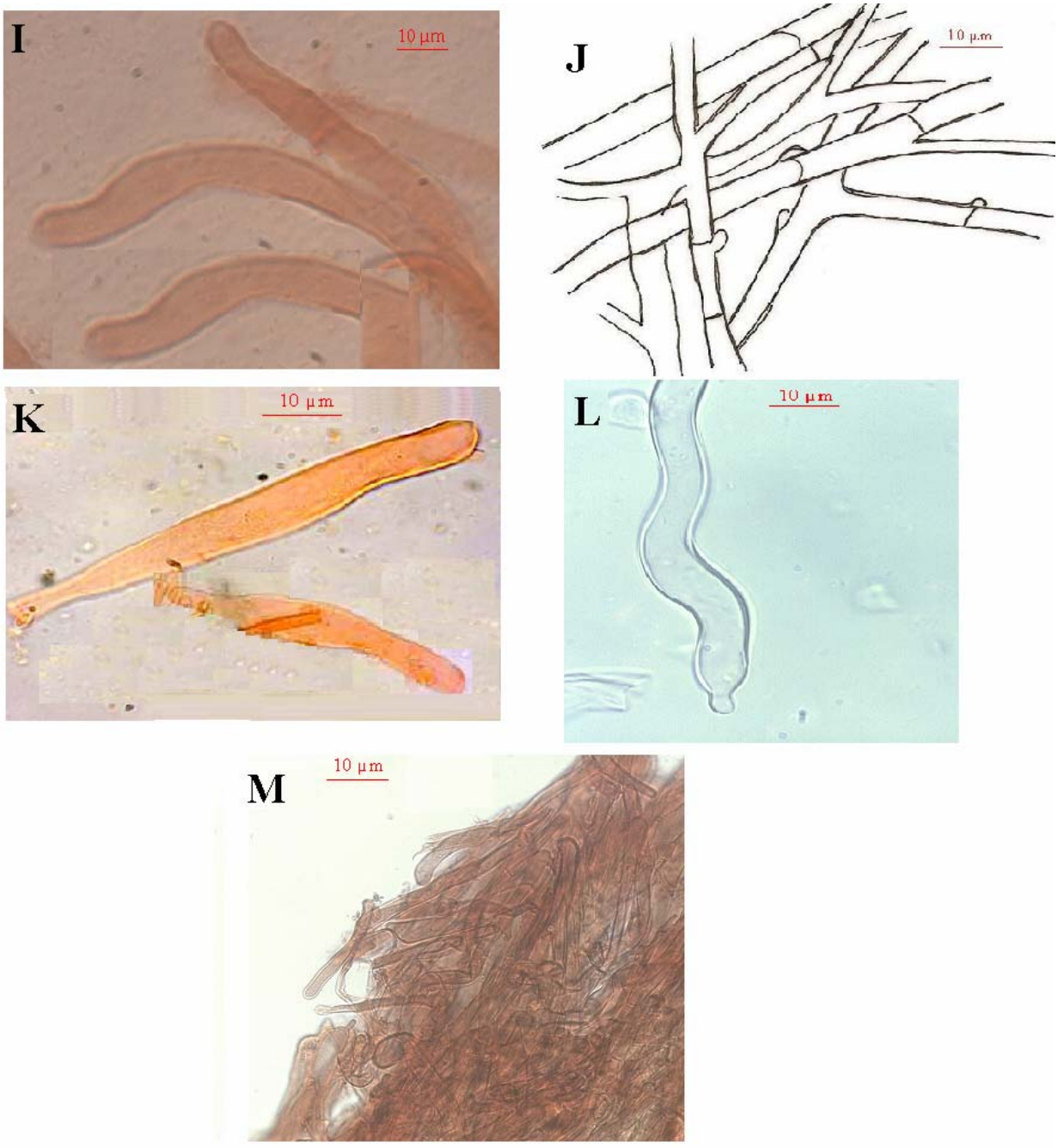

Fig. 4.17 Comparative illustrations of Pileipellis hyphae of *Cantharellus* species (Pileipellis hyphae of: **A.** *C. applanatus***; B.** *C. appalachiensis*; **C.** *C. cibarius*; **D.** *C. elongatipes*; **E.** *C. fibrillosus*; **F.** *C. himalayensis*; **G.** *C. indicus*; **H.** *C. lateritius*; **I.** *C. miniatescens*; **J.** *C. minor;* **K.** *C. natarajanii*; **L.** *C. pseudoformosus* and **M.** *C. umbonatus*.

Key to the Indian species of *Cantharellus*

1. Hymenophore smooth……***C. lateritius***

1.* Hymenophore with prominent folds…….2

2. Lamellae yellow, yellow orange or reddish orange when mature …….....3

2.* Lamellae whitish to off-white…….....***C. umbonatus*** sp. nov.

3. Fruit-bodies infundibuliform, ocharaceous brown to yellowish brown……4

3.* Fruit-bodies planoconvex to convex, hemispherical to applanate, egg yellow to yellowish range…….7

4. Pileus up to 7 cm broad, surface with prominent fibrils, squamules at center, usually surface uneven and rugose …….***C. fibrillosus*** sp. nov.

4.* Pileus < 7 cm broad, surface with matted fibrils or glabrous…….……5

5. Pileus ocharaceous yellow with gelatinizes pileipellis hyphae, 4 basidiospores per basidium…..... …….***C. himalayensis*** sp. nov.

5.* Pileus ocharaceous yellow without gelatinized hyphae, 4-6 basidiospores per basidium……6

6. Pileipellis epicutis with projecting cystidiod end subclavate to clavate cells, spore 7–9 x 5–6 µm……***C. pseudoformosus*** sp.nov.

6.* Pileipellis epicutis with elongate to broad clavate end cells, spores 8.5-11.5 × 4.5-5.8 µm……. ……...***C. cibarius***

7. Stipe more than the width of pileus……..8

7.* Stipe equal to width of pileus or smaller……...9

8. Fruit bodies plum yellow to organgish, pileus 1.5 cm broad, stipe up to 4.5 cm….. ……***C. elongatipes*** sp. nov.

8.* Fruit bodies egg yellow to pale ochraceous yellow, pileus 7cm broad, stipe up to 12 cm…….***C. indicus*** sp. nov.

9. Pileus < 3 cm broad ……. ……10

9.* Pileus > 3 cm broad ……11

10. Pileus yellowish orange to orange; context concolourous with pileus; spores 7-11.5 µm long …….....***C. minor***

10.* Pileus deep yellow, context pale yellow to yellow; spore 5-6 (-7) µm long ……....***C. friessi***

11. Lamellae decurrent, folded, interveined, hymeniform folded, yellowish, stipe surface fibrous, pinkish yellow to citron yellow……..12

11.* Stipe surface glabrous to thin hairy, yellowish…….....13

12. Pileus and stipe dull brown, becoming dingy yellowish to dingy orangish yellow with brownish tones remaining on the pileus disc and stipe base…… …….....***C. appalachiensis***

12.* Pileus and stipes colour not as in *C. appalachiensis* 14

13. Pileus applanate to shallow depressed; spore 7-8.5 × 4.5-5.5 µm ……***C. applanatus*** sp. nov.

13.*Pileus convex to shallow depressed; spore 10-13 × 6-8.5 µm……....***C. luteocomus***

14. Pileus cuticle made up of repent hyphae, context cells are distinctly subclavate to subventricose……..***C. natarajanii*** sp. nov.

14.*Pileus cuticle made up of parallel to suberectely arranged hyphae……. ……...***C. miniatescens***

Table 4.3 A list of Cantharellcaeae species collected from different regions of Himachal Pradesh and Uttarakhand region of Western Himalayas, India with their collection and voucher numbers

S. No	Species	Collection No*	Voucher No
1	*C. applanatus* sp. nov.	43-07, 121-08	PUN 3964
2	*C. appalachiensis*	84-08, 95-08	PUN 3959
3	*C. cibarius*	MSR1-08, 90-09	PUN 3973
4	*C. elongatipes* sp. nov.	184-08	PUN 3966
5	*C. fibrillosus* sp. nov.	113-07, 17-08, 236-06	PUN 3957
6	*C. himalayensis* sp. nov.	169-07	PUN 3972
7	*C. indicus* sp. nov.	MSR2-07, MSR4	PUN 3962
8	*C. lateritius*	119-05, 161-07	PUN 3958
9	*C. miniatescens*	65-07	DMR- 65/07
10	*C. minor*	354-05	PUN 3971
11	*C. natarajanii* sp. nov.	106-08	PUN 3963
12	*C. pseudoformosus* sp. nov.	272-07, 281-08	PUN 3883
13	*C. umbonatus* sp. nov.	348-07	PUN 3968
14	*Cr. cinerius*	107-07	PUN- 4145
15	*Cr. cornucopioides* var. *mediosporus*	268-06	PUN- 4143
16	*Cr. dubius*	149-08, 19-09	DMR-149/07
17	*Cr. indicus* sp. nov.	159-07	PUN 3884

*The collection numbers shown indicate number and year of collection of corresponding fruit body, for example, 43-07 (43 is collection number, 07 is collection year i.e., 2007). PUN- Punjabi University Herbarium; DMR- Harbarium of Directortae of mushroom Research

4.3 Molecular phylogeny of *Cantharellus*

After critically examined by both morphologically and microscopically of all the collected specimens of *Cantharellus* (Table 4.1), twenty different fruit bodies (Table 4.3) were selected to further confirm their identity based on molecular tools and also to examine the genetic variation among the fruit bodies. All the fruit bodies were analyzed for molecular characterization except *C. miniatescens* (65-07) due to insufficient fruit bodies

4.3.1 PCR amplification of ITS and LSU region

Genomic DNA was isolated from the 20 fruit bodies of *Cantharellus* (Table 4.3), 169-07, 113-07, 17-08, 161-07, 272-07, 281-07, 43-07, 121-08, 95-08, 84-08, 184-08, MSR1-08, MSR2-07, 90-09, MSR4-08, 106-08, 119-05, 236-06, 354-05 and 348-07. ITS region of the rDNA from genomic DNA was amplified by the PCR using ITS1 and ITS4 primers and LSU by ITS4R and LR5. Resultant PCR products were viewed after electrophoresis in agarose gel. The result showed that all ITS region of *Cantharellus* produced a single band of approximate 1.2 to 1.6 kb (Fig. 4.18) and LSU was ~ 950 bp (Fig. 4.19).

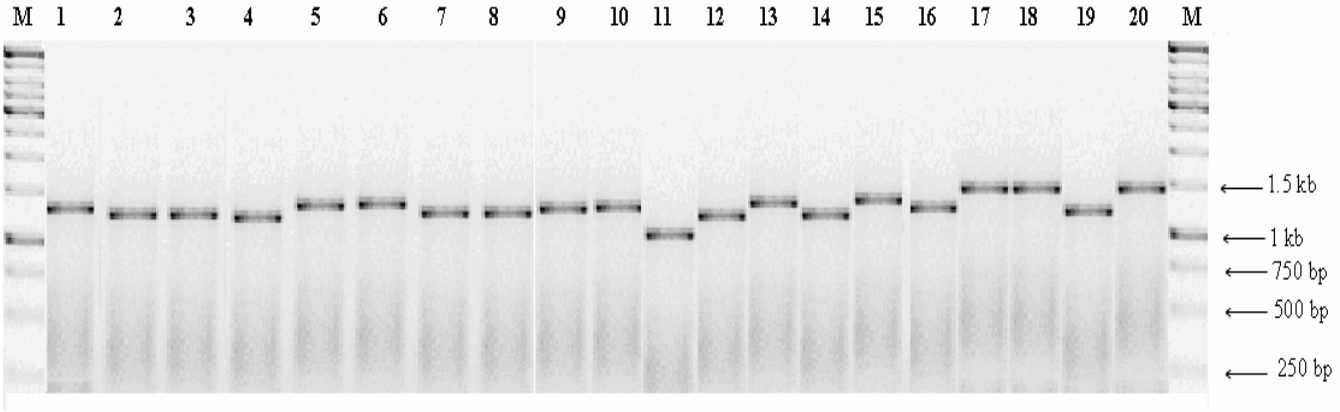

Fig. 4.18 ITS-PCR products of *Cantharellus* amplified with ITS1 and ITS4 primers. Lane M, DNA marker, lane 1-20 are species of 169-07, 113-07, 17-08, 161-07, 272-07, 281-07, 43-07, 121-08, 95-08, 84-08, 184-08, MSR1-08, MSR2-07, 90-09, MSR4-08, 106-08, 119-05, 236-06, 354-05 and 348-07 respectively. This is a composite figure from different gels.

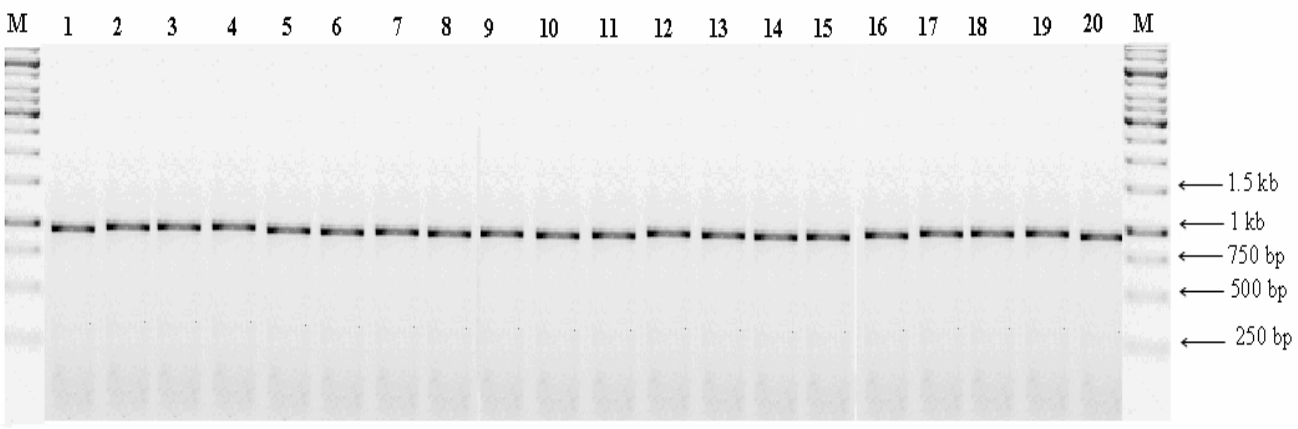

Fig. 4.19 LSU-PCR products of *Cantharellus* amplified with ITS4R and LR5 primers. Lane M, DNA marker, lane 1-20 are species of 169-07, 113-07, 17-08, 161-07, 272-07, 281-07, 43-07, 121-08, 95-08, 84-08, 184-08, MSR1-08, MSR2-07, 90-09, MSR4-08, 106-08, 119-05, 236-06, 354-05 and 348-07 respectively. This is a composite figure from different gels.

4.3.2 Restriction enzyme analysis of ITS and LSU products

Each collection sample of *Cantharellus* was found to have restriction sites of 5 restriction enzymes tested. To detect a wide range of polymorphism, the ITS-PCR amplified products ranged from 1.2 to 1.6 kb were cleaved with the restriction endonucleases. The three restriction endonucleases *viz*. *Hae III, Hinf I* and *Mbo I* produced 12 ITS-PCR banding pattern, while *Alu I* and *Taq I* generated 9 and 8 respectively (Fig 4.20). Several small fragments below 90 bp were ignored during RFLP comparisons. The restriction fragments obtained with all the endonucleases tested were used to determine genetic distance between different genotypes, and clustered them into specific groups. Those specimens which produced similar restriction patterns were further analyzed by digesting the LSU–PCR products with 4

different restrictions enzymes, *Alu I*, *Mbo I*, *Hae III* and *Hinf I*, but no difference was observed and results were similar to RFLP of ITS-PCR products (Fig. 4.21).

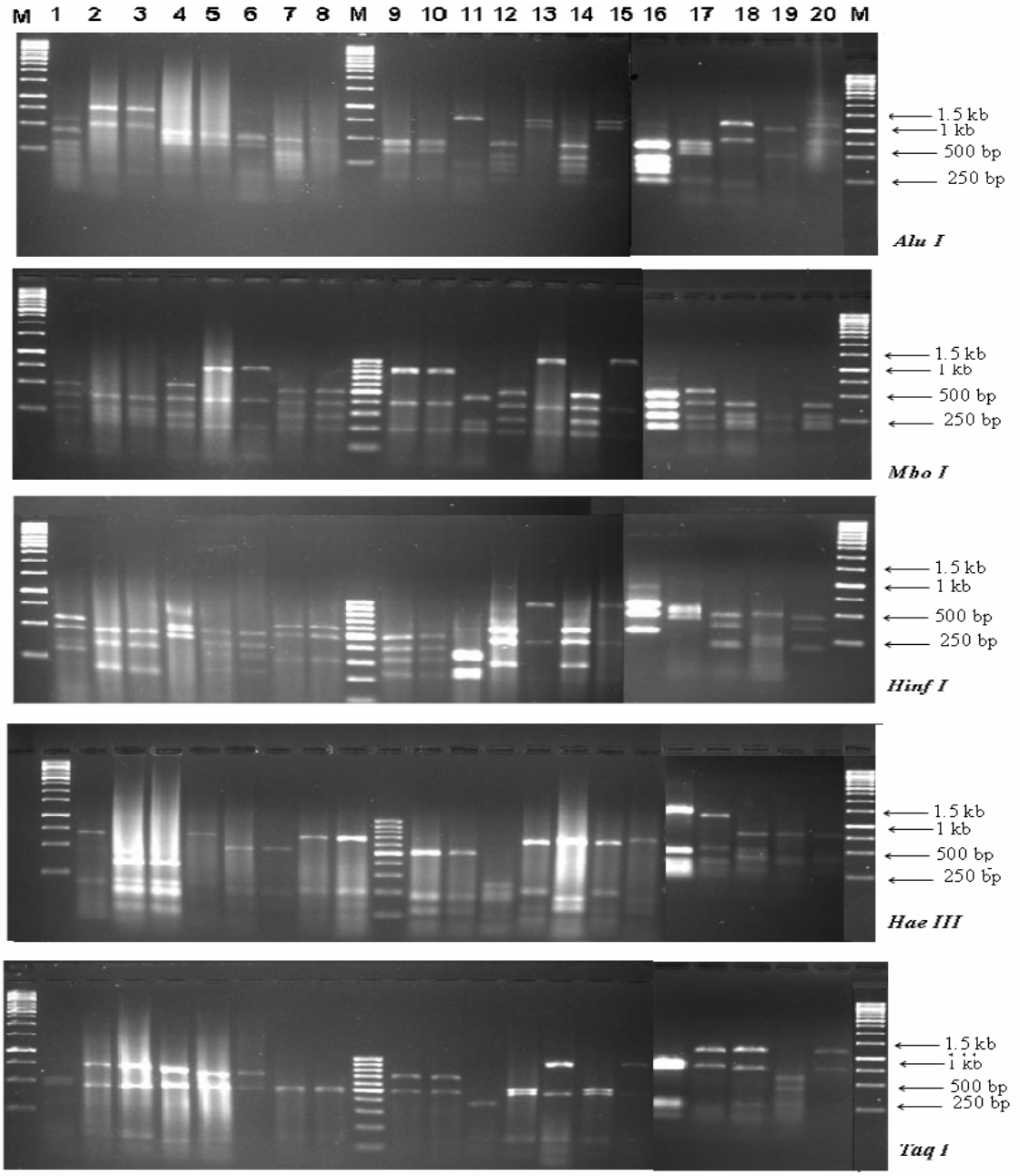

Fig. 4.20 ITS-RFLP analysis of *Cantharellus* digested with 5 different restriction enzymes. Lane M, DNA marker, lane 1-20 are species of 169-07, 113-07, 17-08, 161-07, 272-07, 281-07, 43-07, 121-08, 95-08, 84-08, 184-08, MSR1-08, MSR2-07, 90-09, MSR4-08, 106-08, 119-05, 236-06, 354-05 and 348-07 respectively. This is a composite figure from different gels.

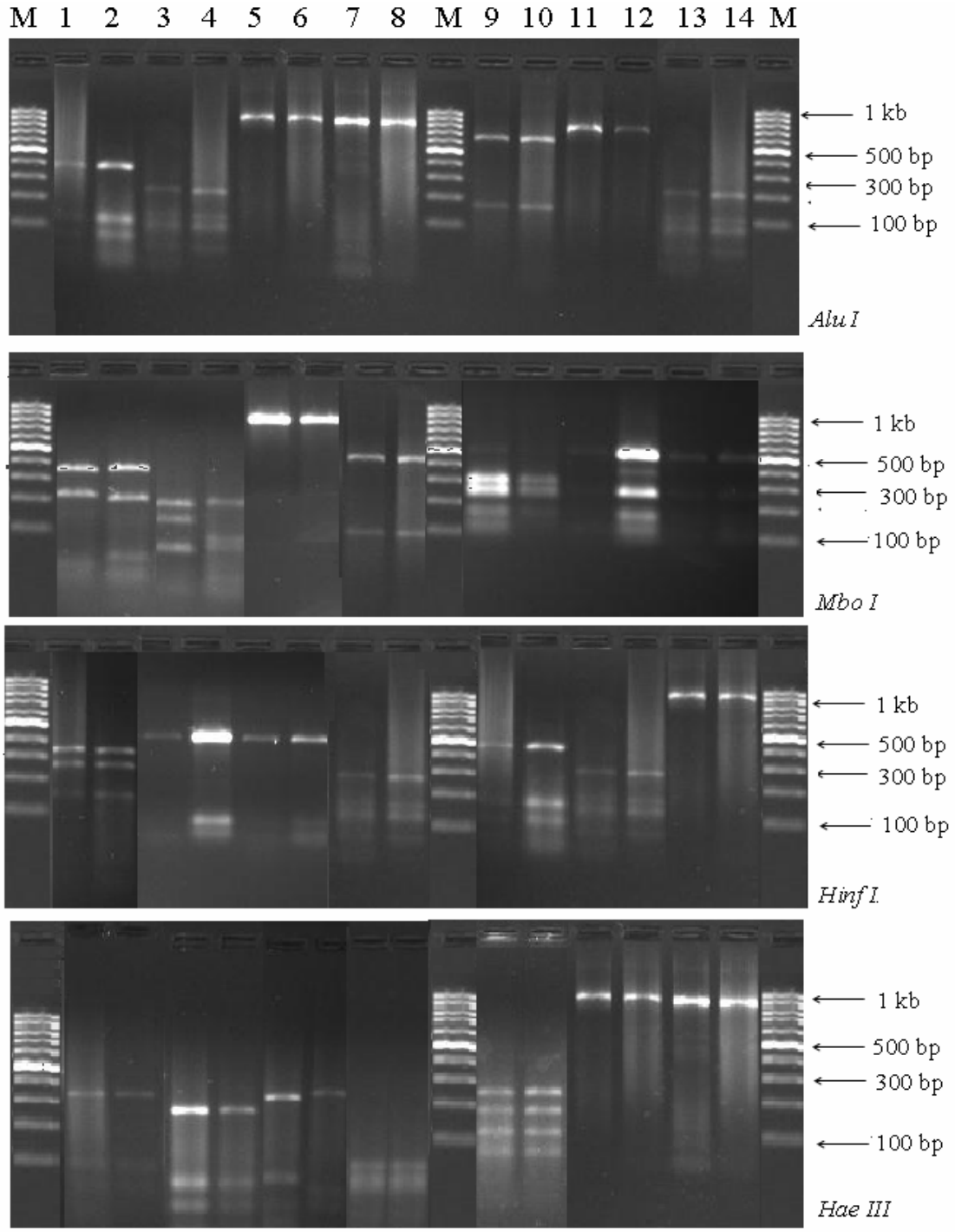

Fig. 4.21 LSU-RFLP analysis of *Cantharellus* digested with 4 different restriction enzymes. Lane M, DNA marker, lane 1-14 are species of 113-07, 17-08, 161-07, 119-05, 281-07, 272-07, 95-08, 84-08, MSR4-08, MSR2-07, 90-09, MSR1-08 and 121-08, 43-07 respectively. This is a composite figure from different gels.

4.3.3 Sequence analysis of *Cantharellus* species

Twenty fruit bodies of *Cantharellus* were subjected to ITS amplification using ITS1 and ITS4 primers and about 1.2 to 1.5 kb amplicons were observed (Fig 4.18). ITS products were cloned into pTZ57R/T vector. The 1.2 to 1.6 kb amplified products of different clones were subjected to restriction enzymes (*AluI, MboI, HinfI, HaeIII* and *TaqI*) digestion to see the variation in the ITS region. The ITS products of selected clones were then sequenced using Applied Biosystems automatic sequencer. Sequencing reactions were performed with M13 forward and M13 reverse prmiers followed by their inernal primers. The sequences were analyzed by multiple sequence alignment (ClustalW) to check similarity among species. The homologies among sequences were from 61 to 98% between species. Minimum of 61% similarity was found in *C. himalyensis* with *C. elongatipes* and maximum 98% similarity was found between *C. cibarius* with *C. applanatus.*

The LSU gene sequences of *Cantharellus* species were also obtained by amplification using ITS4R and LR5 primers, and about 950 bp amplicons were observed (Fig.4.19). The homologies among LSU sequences were found between 61 and 98% in different *Cantharellus* species. The minimum homology of 61% similarity was noticed in *C. himalyensis* with *C. elongatipes* while the maximum homology found was 98% between *C. cibarius* with *C. applanatus.*

Search for sequence identity in the GenBank DNA database using BlastN (NCBI) (Altschul *et al*., 1997), which revealed that ITS region of *Cantharellus* species had 84% to 97% similarity (Table 4.4) and LSU region had 93 to 99% similarity (Table 4.5) with the sequences of NCBI database. The ITS and LSU sequences of *Cantharellus* species determined in this study were deposited in the GenBank of NCBI data library under the accession numbers provided in Tables 4.4 and 4.5. The srquences of ITS and LSU regions of *Cantharellus* species were also analysed for restriction digestion with the help of Web cutter (www firatmarket.com/cutter) and

fiund that the restriction pattern obtained was same as obtained from the webcutter (Tables 4.6 and 4.7). The exact size of fragment was also deduced from the restriction patterns.

4.3.4 Phylogenetic analyses

The complete ITS and LSU region information procured were compared with the available sequence information in the GenBank with different *Cantharellus* species to derive the phylogenetic relationship. Three different datasets were assembled for the phylogenetic analyses. One dataset was composed of 23 ITS sequences and the other of 31 nLSU sequences, while the third dataset was combined ITS + nLSU of 18 sequences. *Craterellus* spp. was included as an outgroup taxon for rooting purposes. Three alignments were produced: ITS, LSU and ITS + nLSU and the alignments have been submitted to TreeBase (http://www.treebase.org) under the accession number S11503.

4.3.4.1 Phylogenetic analysis based on the ITS region

The two most widely used methods for molecular phylogenetic analyses (Maximum parsimony and Maximum Likelihood) were used to check the clades that were more consistently support by the sequence analysis. Phylogenetic analysis of the ITS region showed a reasonable degree of correlation with the morphological classification schemes of species within the genus.

PCR amplification with primers ITS1 and ITS4 produced 1.2 to 1.6 kb fragments. Phylogenetic analysis of ITS was performed with 23 sequences of *Cantharellus*. The sequences generated from the ITS contained 406 bp from the 5' end of the ITS 1 and 560 from the 3' end of the ITS 2 including the 5.8S nrDNA gene. The aligned dataset contained 1,105 characters and there were 521, 226 and 358 constant, parsimony-uninformative and parsimony-informative characters, respectively. Gaps were treated as missing data. Maximum parsimony analysis resulted in 5 equally

parsimonious trees with the branch-and-bound search yielded parsimony trees (TL = 1,653, CI = 0.85, RI=0.867, HI=0.141). Maximum likelihood analysis recovered as single topology (-ln L = 8812.2266). The resulting MP and ML topologies did not differ significantly. The ML and Bayesian topologies were almost identical. All taxa of *Cantharellus* formed a well-supported monophyletic lineage, sister to *Craterellus* species but with no significant support (Fig. 4.22). Most single species clades received moderate to strong support (55-100% BS, 56-100% PP), although the nodes indicating relationship amongst them generally received less support.

4.3.4.2 Phylogenetic analysis based on the LSU analysis

PCR amplification with the primers ITS4R and LR5 produced ~950 bp fragments from the 5' end of the nrDNA large subunit gene. Phylogenetic analysis of LSU was performed with 31 sequences of *Cantharellus*, resulting in a total of 792 nucleotides that were included. The aligned dataset contain 846 characters and there were 522, 55 and 269 constant, parsimony-uninformative and parsimony-informative characters, respectively. Gaps were treated as missing data. Maximum parsimony analysis resulted in 9 equally parsimonious trees with the branch-and-bound search yielded parsimony trees (TL = 567, CI = 0.73, RI=0.906, HI=0.269). Maximum likelihood analysis recovered as single topology (-ln L = 3755.6299). The resulting MP and ML topologies did not differ significantly. The Kishino-Hasegawa tests among topologies obtained from ML and UP indicated that the ML tree was significantly better and one of the Maximum likelihood trees is shown in Fig 4.23. Most single species clades received moderate to strong support (65-100% BS, 56-100% PP).

4.3.4.3 Phylogenetic analysis based on the combine ITS+LSU dataset

Results of the partition homogeneity test indicate no significant differences between the ITS and LSU data sets. The combined ITS+LSU dataset includes 19 sequences

and *Cr. tubaeformis* as the outgroup taxon for rooting purposes. The combine data matrix consists of 1,951 total characters and there were 956, 380 and 615 constant, parsimony-uninformative and parsimony-informative characters respectively. Gaps were treated as missing data. Maximum parsimony analysis resulted in 4 equally parsimonious trees with the branch-and-bound search yielded parsimony trees (TL = 1991, CI = 0.775, RI=0.782, HI=0.224). Maximum likelihood analysis recovered as single topology (-ln L = 11773.3369). The resulting MP and ML topologies did not differ significantly. The Kishino-Hasegawa test confirmed that two trees were not statistically different and one of the Maximum likelihood trees is shown in Fig 4.24. Most single species clades received moderate to strong support (59-100% BS, 62-100% PP). Bayesian analyses recovered topologies similar to ML topology, differing only in the position of *C. elongatipes*.

For the most part the higher-level topology within *Cantharellus*, based on ITS sequences, was congruent with the topology based on the LSU data set. There were few minor differences in both ITS and LSU based trees but those were not critical. *Cantharellus elongatipes* clustered in LSU data set with *C. natarajanii, C. applanatus* and *C. cibarius* in one clade with a weak support (BS = 55 % and PP = 61%), but the relationship among these species was resolved in ITS phylogeny. In another clade, the position of *C. lateritius*, formed an unresolved trichotomy with *C. pseudoformosus* and *C. umbonatus* with ITS sequence data with weak support (BS = 66%), but LSU data set produced strong support (BS = 98% and PP =100%) with previously reported *C. lateritius* (DQ898694).

Table 4.4 Examined *Cantharellus* species and their closest relative species inferred from ITS gene sequences of existing database

Species	Accession no	Nearest match	Query coverage	Maximum Identity
C. applanatus (121-08)	HQ270118	*C. cibarius* (DQ200926)	98%	97%
		C. cibarius (AF044689)	61%	93%
C. appalachiensis (95-08)	HQ386220	*C. cibarius* (EF546767)	98%	95%
		C. pallens (AF044690)	40%	92%
C. cibarius (90-09)	HQ270123	*C. cibarius* var. *roseocanus* (AY041173)	94%	95%
		C. cibarius var. *cibarius* (AY041177)	94%	95%
C. elongatipes (184-08)	HQ270115	*C. cibarius* (AB509586)	76%	90%
C. fibrillosus (113-07)	HQ270125	*C. appalachiensis* (HQ386220)	93%	90%
C. himalyensis (169-07)	HQ270129	*C. cibarius* (DQ200926)	97%	97%
C. indicus (MSR2-07)	HQ270122	*C. cibarius* (EF546767)	98%	84%
		C. minor (HQ270119)	78%	95%
C. lateritius (161-07)	HQ270121	*C. cibarius* (EF546767)	88%	85%
C. minor (354-05)	HQ270119	*C. cibarius* (EF546767)	86%	90%
C. natarajanii (106-08)	HQ270120	*C. cibarius* (EF546767)	96%	98%
C. pseudoformosus (281-07)	FJ769255	*C. cibarius* (EF546767)	58%	92%
C. umbonatus (348-07)	HQ270116	*C. cibarius* (EF546767)	96%	97%

Table 4.5 Examined *Cantharellus* species and their closest relative species inferred from LSU gene sequences of existing database

Species	Accession no	Nearest match	Query coverage	Maximum Identity
C. applanatus (121-08)	HM750918	*C. subalbidus* (AY041150)	98%	99%
		C. cascadensis (AY041162)	98%	99%
C. appalachiensis (95-08)	HQ342887	*C .appalachiensis* (DQ898690)	98%	97%
		C. cibarius (AY745708)	98%	97%
C. cibarius (90-09)	HM750927	*C. cibarius* (AY745708)	98%	99%
		C. cibarius (DQ898693)	92%	99%
C. elongatipes (184-08)	HM750929	*C. cibarius* (AY745708)	99%	99%
		C. subalbidus (AY041146)	99%	99%
C. fibrillosus (113-07)	HM750917	*C. appalachiensis* (HM582120)	100%	96%
		C. cinnabarius (AY041168)	100%	92%
C. himalyensis (169-07)	HM750928	*C. appalachiensis* (HM582121)	100%	96%
		C. minor (DQ898691)	100%	97%
C. indicus (MSR2-07)	HM750924	*C. appalachiensis* (HM594682)	100%	97%
		C. persicinus (AY041169)	100%	97%
C. lateritius (161-07)	HM750919	*C. lateritius* (DQ898694)	100%	99%
		C. cibarius (AY745708)	100%	94%
C. minor (354-05)	HM750923	*C. appalachiensis* (HM594682)	100%	96%
C. natarajanii (106-08)	HM750926	*C. cibrius* (HM594682)	100%	97%
		C. subalbidus (AY041150)	100%	98%
C. pseudoformosus (281-07)	GU237071	*C. cibarius* (DQ898693)	91%	96%
C. umbonatus (348-07)	HM750916	*C. appalachiensis* (HM582121)	100%	97%
		C. cinnabarius (AY041168)	100%	93%

Table 4.6 Differentiation of *Cantharellus* species into RFLP types according to the size of restriction fragments produced following digestion of the ITS PCR product with restriction enzymes. Exact size of fragment was determined using web programme Web-Cutter 2.0 (www.firstmarket.com/cutter)

Species	**Restriction enzymes**				
	Alu I	*Mbo I*	*Hinf I*	*Hae III*	*Taq I*
	Size of fragments (bp)				
C. applanatus (121-08)	436, 303, 253, 208,	489, 364, 269, 190, 21	573, 458, 294, 8	660, 264, 124, 92, 79, 55, 45, 14	509, 460, 14 122, 75, 18
C. appalachiensis (95-08)	434, 355, 254, 210,	542, 381, 266, 190, 21	622, 475, 295, 8	677, 249, 121, 90, 78, 69, 45, 56, 15	562, 477, 14 119, 75, 18
C. cibarius (90-09)	607, 428, 158, 146	502, 377, 329, 131	574, 479, 286	674, 283, 261, 121	501, 473, 24 122
C. elongatipes (184-08)	440, 303, 253, 208,	493, 364, 269, 190, 21	577, 458, 294, 8	660, 197, 124, 96, 79, 70, 55, 45, 13	513, 460, 12 149, 75, 18
C. fibrillosus (113-07)	809, 437, 115	545, 262, 257, 189, 76, 20, 12	914, 439, 8	311, 279 169, 140, 120, 109,92, 79, 34, 28	572, 441, 14 120, 66, 18
C. himalyensis (169-07)	618, 435, 304	597, 490, 270	575, 489, 293	684, 430, 243	746, 509, 12
C. indicus (MSR2-07)	848, 668	945, 375, 196	1033, 483	744, 416, 253, 103	706, 433, 37
C. lateritius (161-07)	579, 442, 411	696, 451, 190, 95	924, 408	704, 368, 247, 113	725, 502, 20
C. minor (354-05)	556, 427, 354, 74	801, 274, 190, 99, 26, 21	380, 313, 301, 200, 90, 88, 24, 15	360, 233, 184, 122, 115, 82, 76, 60, 45, 36, 22, 18, 15	680, 206, 14 121, 95, 75, 27, 16
C. natarajanii (106-08)	435, 303, 253, 274,	489, 269, 261, 190, 100, 20	573, 455, 293, 8	657, 197, 149, 123, 92, 55, 45, 11	508, 457, 14 122, 75, 18
C. pseudoformosus (281-07)	465, 356, 256, 167,	799, 391, 190, 21	484, 376, 311, 221,	520, 168, 198, 120, 111, 72, 43, 33, 22	673, 487, 11 75, 39, 17
C. umbonatus (348-06)	427, 419, 396, 144	615, 378, 372, 21	596, 472, 310, 8	184, 175, 155, 119, 72, 60, 36, 15	655, 474, 17 70, 18

Table 4.7 Differentiation of *Cantharellus* species into RFLP types according to the size of restriction fragments produced following digestion of the LSU PCR product with restriction enzymes. Exact size of fragment was determined using web programme Web Cutter 2.0 (www.firstmarket.com/cutter)

Species	Restriction enzymes			
	Alu I	*Mbo I*	*Hinf I*	*Hae III*
	Size of fragments (bp)			
C. fibrillosus (113-07)	600, 150, 100, 75, 50	385, 245, 95, 79, 65, 56	425, 320, 150, 30	390, 150, 95, 9 74, 67, 59
C. lateritius (161-07)	350, 180, 120, 90, 74, 85, 43	200, 190, 90, 85, 79, 70, 67, 58, 47, 33, 22	500, 90, 80, 75, 63, 51, 43, 30, 10	330, 95, 87, 85 74, 69, 52, 42,
C. pseudoformosus (281-07)	ND	ND	550, 95, 80, 72, 32	350, 98, 87, 81 68, 41, 32
C. appalachiensis (95-08)	ND	450, 100, 83, 75, 64, 28	320, 150, 190, 75, 65	115, 100, 93, 9 85, 81, 73, 69, 40
C. indicus (MSR2-07)	650, 150, 90, 33	330, 280, 150, 120, 43	500, 150, 130, 90, 53	350, 250, 190, 48
C. cibarius (90-09)	ND	500, 300, 50	300, 200, 180, 90	ND
C. applanatus (121-08)	240, 170, 130, 95, 80, 74, 69, 53, 6	500, 300, 82, 45	ND	ND

Notes: ND- restriction site not detected

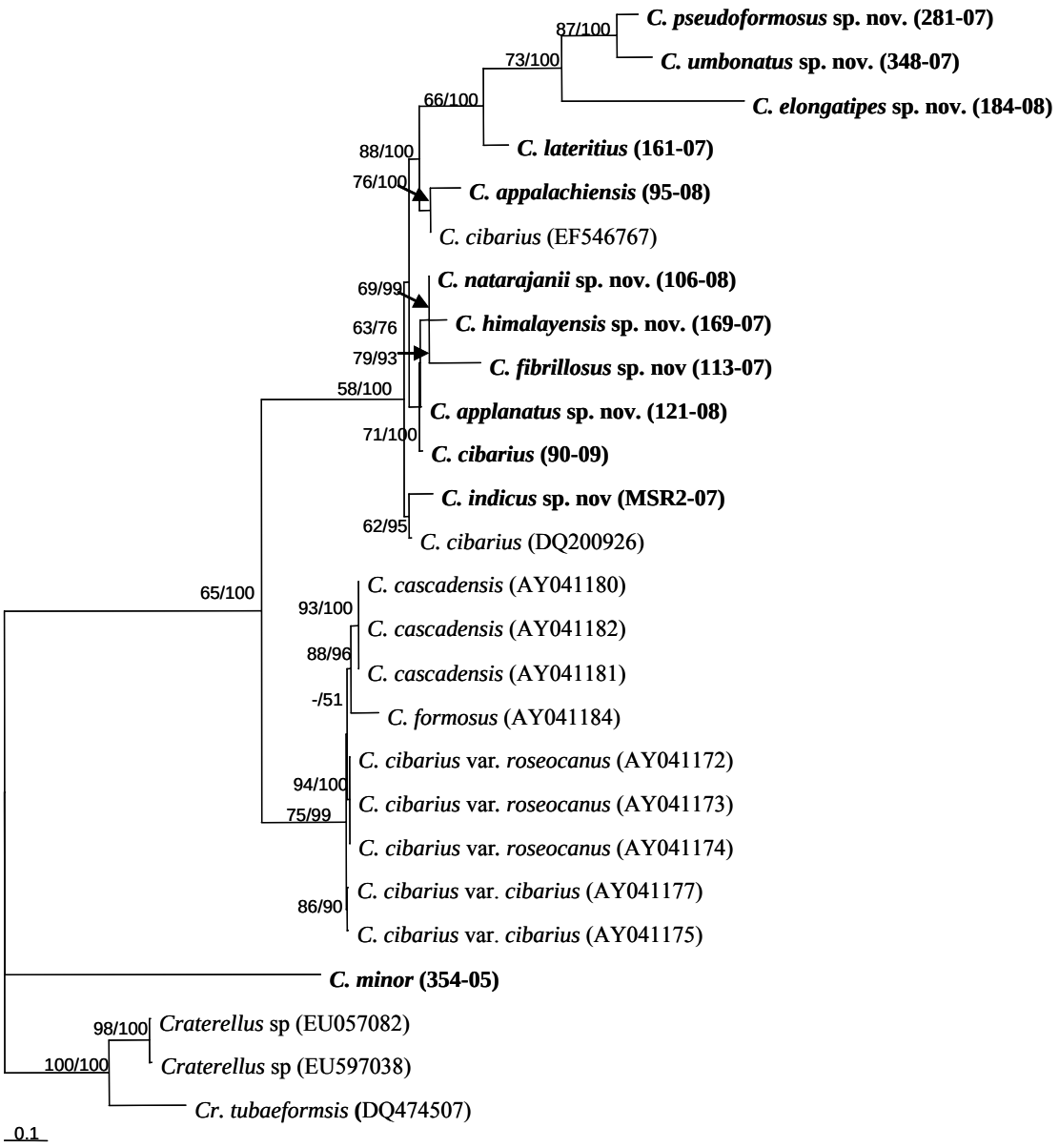

Fig. 4.22 Phylogeny of *Cantharellus* generated from Maximum Likelihood analysis of ITS sequences, rooted with *Craterellus species.* Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). The scale gives the substitution rate.The bold species represent the Indian collections.

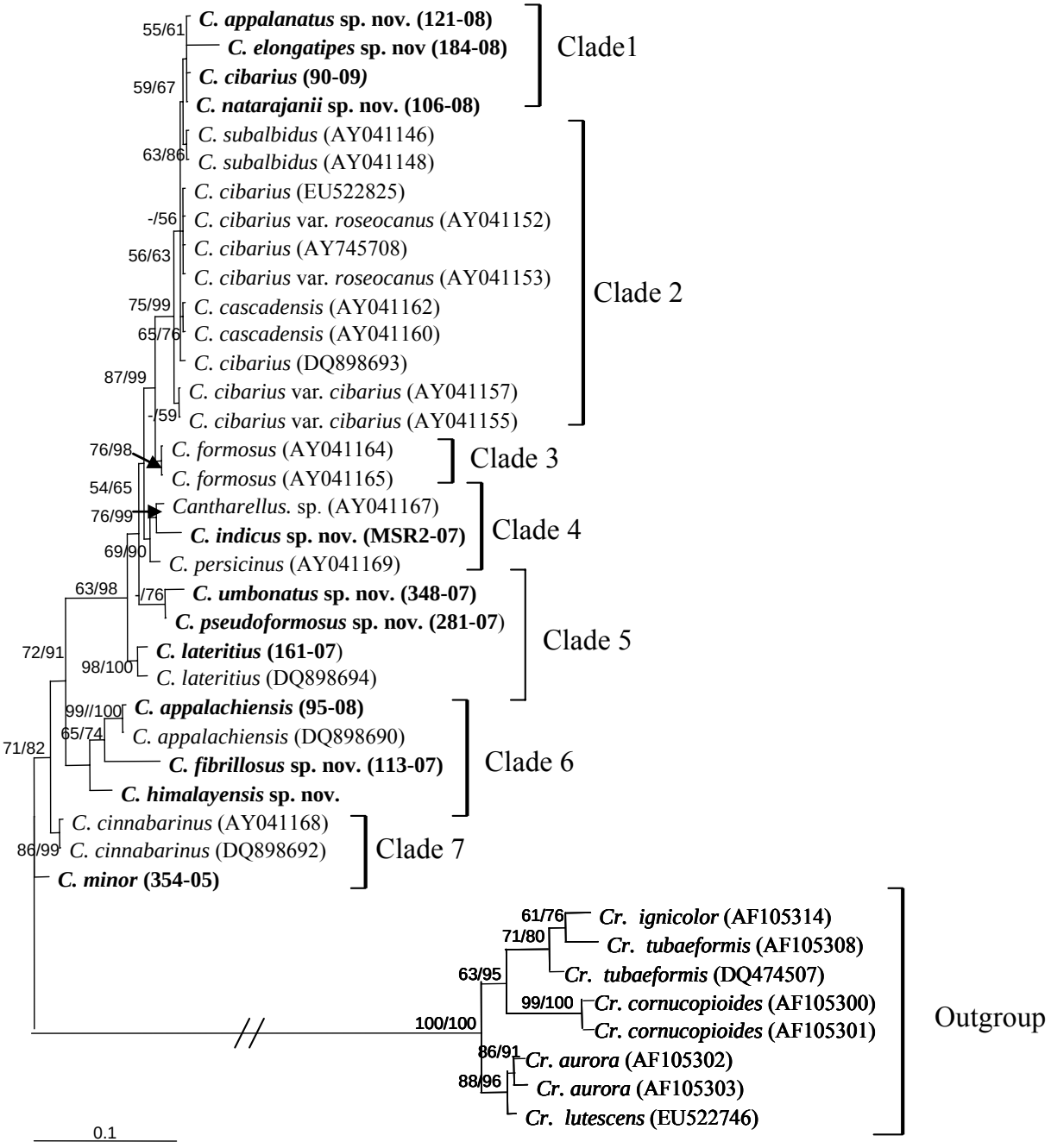

Fig. 4.23 Phylogeny of *Cantharellus* generated from Maximum Likelihood analysis of LSU sequences, rooted with *Craterellus species.* Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). The scale gives the substitution rate.The bold species represent the Indian collections.

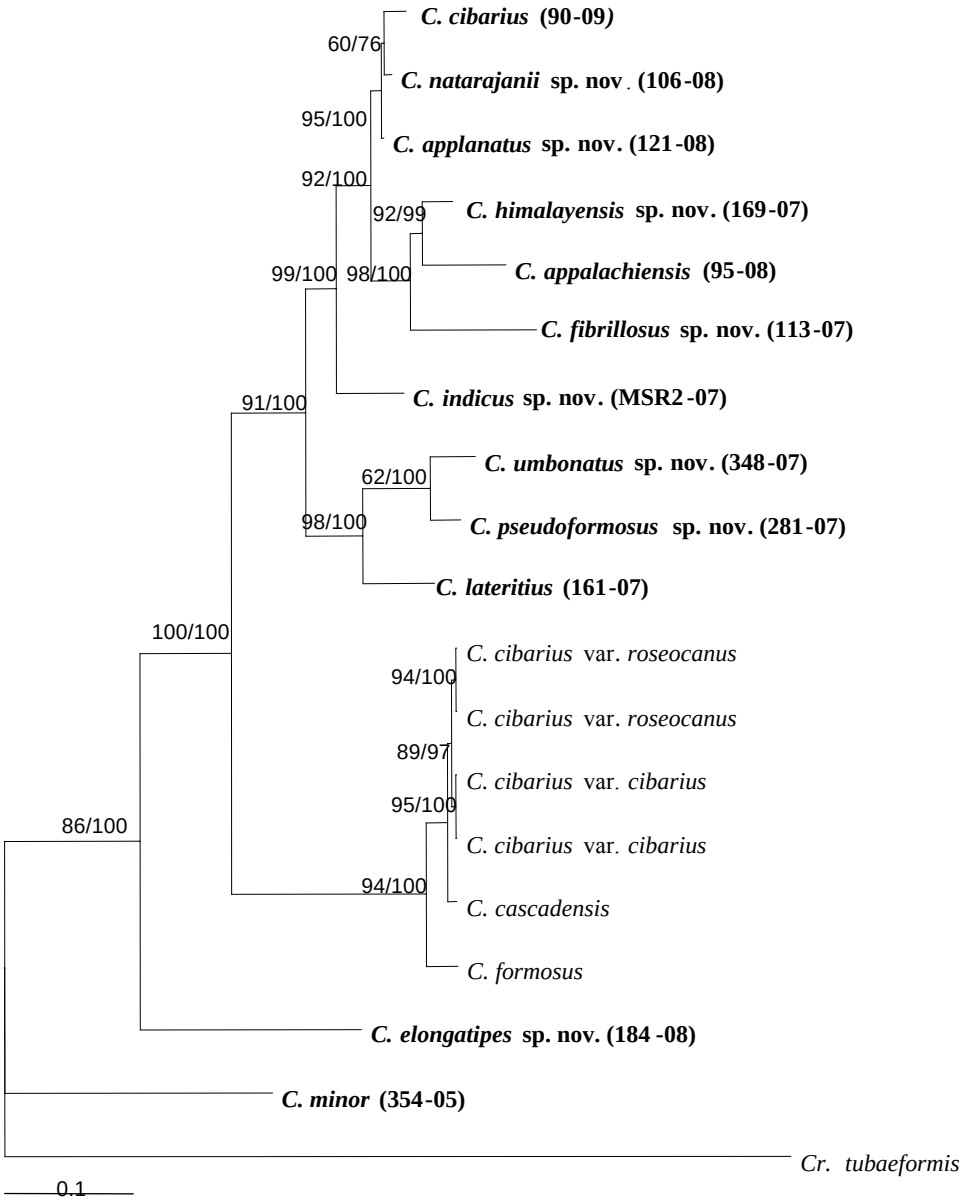

Fig. 4.24 Phylogeny of *Cantharellus* generated from Maximum Likelihood analysis of ITS+LSU sequences dataset, rooted with *Cr. tubaeformsis.* Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). The scale gives the substitution rate.The bold species represent the Indian collections.

4.4 Genus: *Craterellus*

1. *Craterellus cinerius* (Fries) Quelet, Fl. Mycol.p.36.1888. (Fig 4.25.A-E)

Pileus 1-5 cm wide, infundibuliform, Copra (7C-8) to pony brown (15A7), surface appressed grayish black to blackish fibrillose to squarose, dry, hygrophanous; margin slightly striate towards margin, crenulate, split. ***Context*** thin. ***Lamellae*** decurrent, with folded hymeniform, interveined, Elephant skin (7A-2). ***Stipe*** central or some specimen with eccentric stipe, 2-5.5 x 0.2-1 cm, terete, Mauve taupe (7C-8), glabrous to thin hairy, compressed and appear as groove in mature specimen; context blackish grey. Spores deposit white. Odor and taste indistinct. ***Basidiospores*** [35/2/2] 7-9 × 4-5.8 (7-) µm, L= 7.0 µm, W= 5.2 µm, Q= 1.34, broadly ellipsoid, smooth, nonamyloid, noncyanophilic, content a single large guttule, faintly yellowish in 3% KOH. ***Basidia*** 40-55 × 5.5-8 µm, clavate to subcylindric, developing basidia with granulose, light yellow content, mostly 6-spored but a few 4- or 5- spored, sterigmata 5-6 µm long. ***Basidioles*** numerous, content light yellowish in 3% KOH. ***Hymenophoral trama*** irregular to interwoven, hyphae branched, hyaline to faint yellowish colour, cylindric constricted. Clamp absent. ***Pileipellis*** made up of septate, branched cylindric hyphae, 3.5-12.5 µm wide, hyphal ends rounded, upright and protruding beyond surface, larger hyphae often slightly constricted at septa, wall and cross walls thin to thick walled (0.5-1.2 µm thick), some hyphae encrusted with brownish yellow encrustations; clamp connection absent from all hyphae. ***Pleurocystidia*** and ***Cheilocystidia*** absent.

***Habitat and distribution*:** Caespitose to gregarious; among moss on soil under the mixed forest dominated by *Quercus* species.

***Materials examined*:** INDIA, Himachal Pradesh- Shimla- Khada Pathar forest, N30°72' E78°.63.2' 1950 m, 25 August 2007, collected by Deepika Kumri 107-07; same location, 19 August 2008, collected by Deepika Kumari 134-08. Holotype: PUN 4145

Notes: The present specimen is show similar to the characters given for *Cr. cinerus* by Bigelow (1978). The distinguishing features of *C. cinerus* is the pileus surface made up of cylindric hyphae, recumbent or sometimes upright and protruding

beyond surface, cell usually short and appearing finely encrusted hyphae, spores are ellipsoid, basidia mostly 6-spored, clamp connections absent. However, the Q value is slightly lower in the present specimen, which shows the spores comparatively broader in type specimen, similar number of sterigmata on basidia, Pileipellis hyphae encrusted and clamp connections absent. The overall macroscopic and microscopic details of the present specimen are conformity with *Cr. cinerus* Fries.

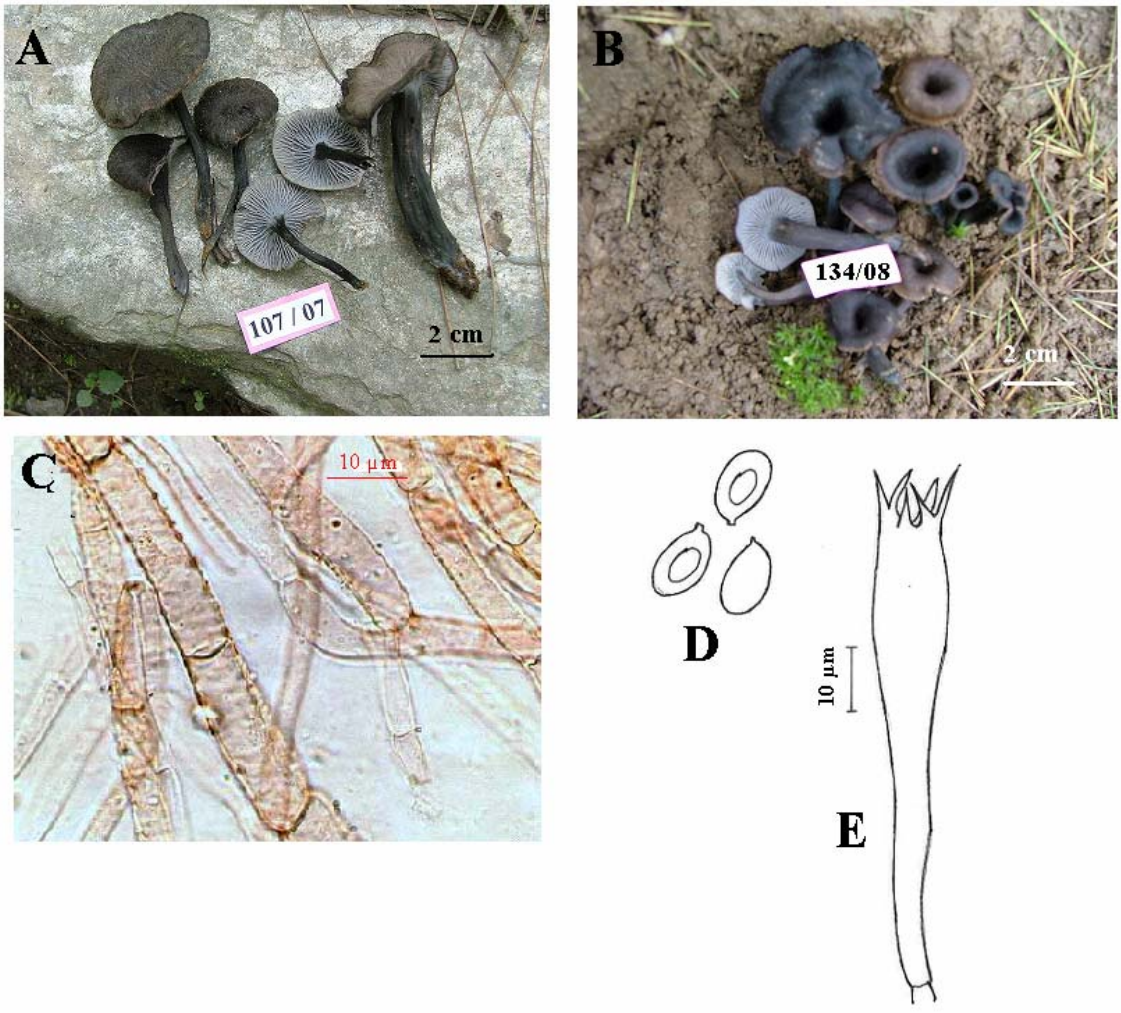

Fig. 4.25 A & B. Basidiocarps; C. Pileipellis and D & E. Basidiospores and Basidia of *Cr. cinerus*

2. *Craterellus cornucopioides* var. *mediosporus*; Corner, New phytol. 47 (1948)
(Fig. 4.26 A-F.)

Pileus 2.5-4.5 cm wide, umbilicate to infundibuliform, Kara Dagh (5A-2) to Sepia (8A-10), surface squarose to squamulous, dry, hygrophanous; margin wavy and split. ***Context*** up to 0.2 cm thick, grayish black. ***Lamellae*** smooth, Rose grey (7A-2). ***Stipe*** central, 1.5-2.8 x 0.3-0.7 cm, terete, Rose grey (7A-2), glabrous to smooth, hollow; blackish grey. Spore deposit white. ***Basidiospores*** [35/2/2] 8-10.2 x 6.5-7.5 µm, L= 9.5 µm**,** W= 7.1 µm, Q= 1.33, broadly ellipsoid to ellipsoid, smooth, nonamyloid, wall hyline, nonamyloid, noncyanophilic, content a single large guttule, faintly yellowish in 3% KOH. ***Basidia*** 50-70 x 8-8.5 µm, clavate to subcylindric, developing basidia with granulose, light yellow content, mostly 6-spored but a few 4- or 5- spored, sterigmata 5-6 µm long. ***Basidioles*** numerous, content light yellowish in 3% KOH. ***Hymenophoral trama*** irregular to interwoven, hyphae branched, hyaline to faint yellowish colour, cylindric constricted. ***Pileipellis*** made up of septate, branched cylindric hyphae, 3.5-22.3 µm wide, hyphal ends rounded, upright and protruding beyond surface, larger hyphae often slightly constricted at septa. Clamp connection absent from all hyphae. ***Pleurocystidia*** and ***Cheilocystidia*** absent.

***Habitat and distribution*:** Gregarious to caespitose; on soil under the mixed forest dominated by *Cedrus deodara*.

***Materials examined*:** INDIA, Himachal Pradesh-Shimla- Khada Pather forest, N30°72’ E78°.63.2’ 1950 m, 11 August, 2005, collected by R. C. Upadhyay 322-05, same location, 29 September 2006, collected by Deepika Kumari 268-06. Holotype: PUN 4143

***Notes*:** This specimen is morphologically and microscopically well within the circumscriptions given for the Corner (1966) monograph, *Cr. cornucopioides* var. *mediosporus*. The spore Q value is similar in the present described specimen (1.33) which suggests the spores are less ellipsoid than the type specimen (Q =1.45). The minor variation may be due to the ecological conditions.

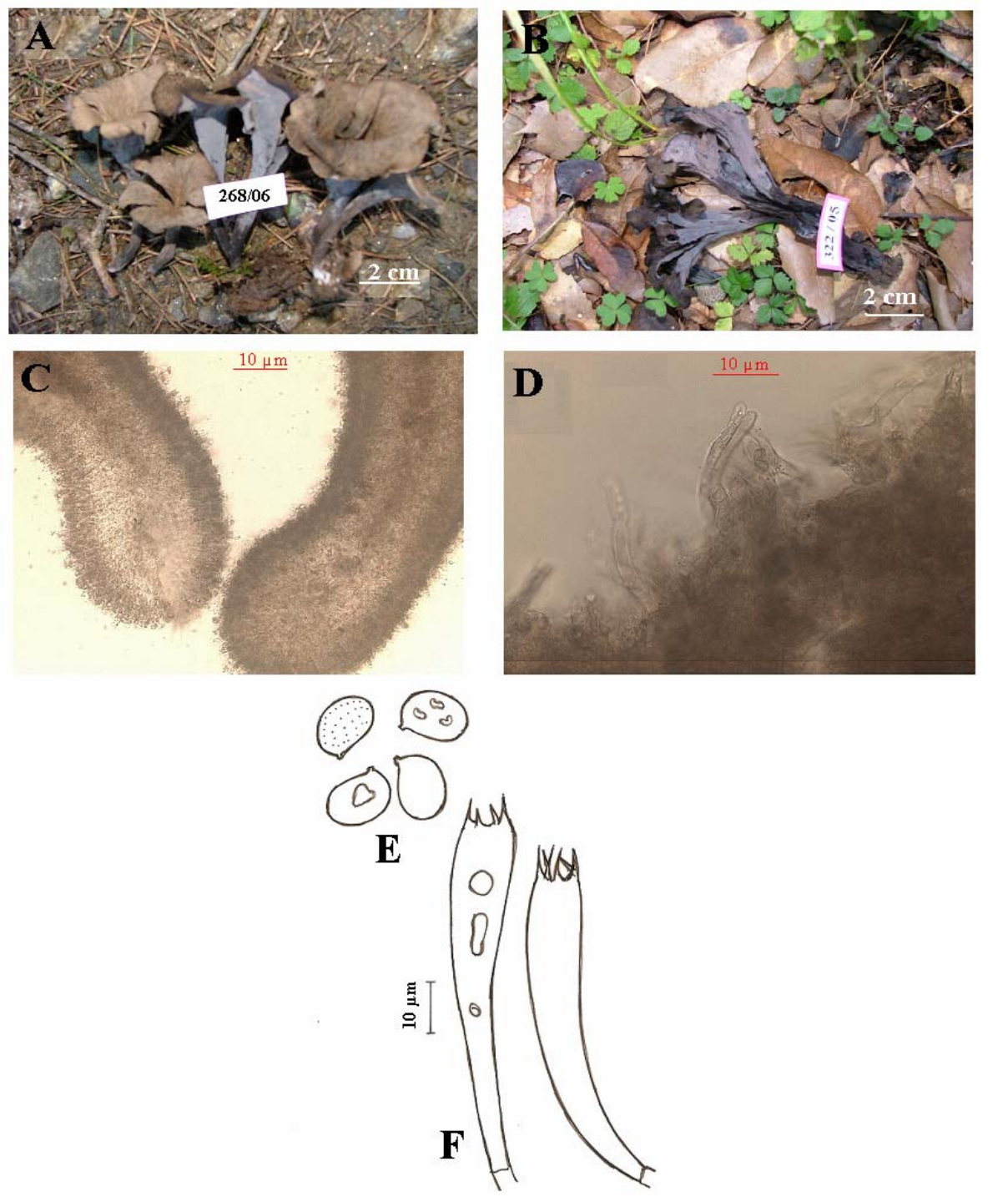

Fig. 4.26 A & B. Basidiocarps; C. Hymenophoral trama hyphae; D. Pileipellis and E & F. Basidiospores and Basidia of *Cr. cornucopioides* var. *mediosporus*

3. *Craterellus dubius* Peck, Ann. Rep. N.Y. St. Mus. 31, 38, 1879; Burt, Ann. Mo. Bot. Gdn (1914) 335; Lloyd, Mycol. Letters 63, note 494. (Fig. 4.27.A-D)

Pileus up to 2-5 cm wide, infundibuliform, Copra (7C-8) to pony brown (15A-7), surface appressed grayish black fibrils to innate in the middle, dry, hygrophanous; margin wavy and lobed, crenulate, split. ***Context*** thin. ***Lamellae*** anastomosing, with folded hymeniform, interveined, Elephant skin (7A-2). ***Stipe*** central, 2-3 x 0.2-1 cm, terete, Mauve taupe (7C-8), glabrous to thin hairy, compressed and appear as groove in mature specimen; context blackish grey. Spore deposit white. ***Basidiospores*** [42/2/2] 5.8-7.2 × 4.5-5.4 µm, L= 6.34 µm, W= 4.61 µm, Q= 1.37, broadly ellipsoid to subglobose, smooth, nonamyloid, noncyanophilic, light yellowish in 3% KOH. ***Basidia*** 37-55 × 6-8 µm, narrowly clavate to clavate, developing basidia with granulose, light yellow content, 4-6 spored, sterigmata 3-5 µm long. ***Hymenophoral trama*** irregular to interwoven, hyphae branched, hyaline to faint yellowish colour, cylindric constricted. Clamp absent. ***Subhymenium*** made up of noninflated, branched, septate and filamentous hyphae. ***Pileipellis*** made up of septate, branched cylindric hyphae, 3.5-14 µm wide, hyphal ends rounded, upright and protruding beyond surface, larger hyphae often slightly constricted at septa, wall and cross walls thin to thick walled (0.5-1.2 µm thick), some hyphae encrusted with brownish yellow encrustations; clamp connection absent from all hyphae. ***Pleurocystidia*** and ***Cheilocystidia*** none. ***Stipe cuticle*** made up of yellowish to brownish in 3% KOH, cylindric to filamentous, thin to thick walled, branched, 3-8 µm wide.

***Habitat and distribution*:** Solitary to gregarious; on soil under the mixed forest dominated by *Quercus* species.

***Materials examined*:** INDIA, Himachal Pradesh: Shimla, Mandi forest, N31°13'50" and E76°37'20" 1700 m, 15 August, 2008, collected by Deepika Kumari 149-08, same collection 3 September 2009, collected by Deepika Kumari 19-09. Holotype: DMR 149-08.

***Notes*:** This specimen is morphologically and microscopically well within the circumscriptions given for the Corner monograph (1966), *Cr. dubius* Peck. However, the spore Q value is comparatively lower in the present described specimen (Q=1.37)

which suggests the spores are less ellipsoid than the type specimen (Q =1.45). The minor variation may be due to the ecological conditions. This species is edible in North- America and sold in market but the edibility of Indian specimen is not known.

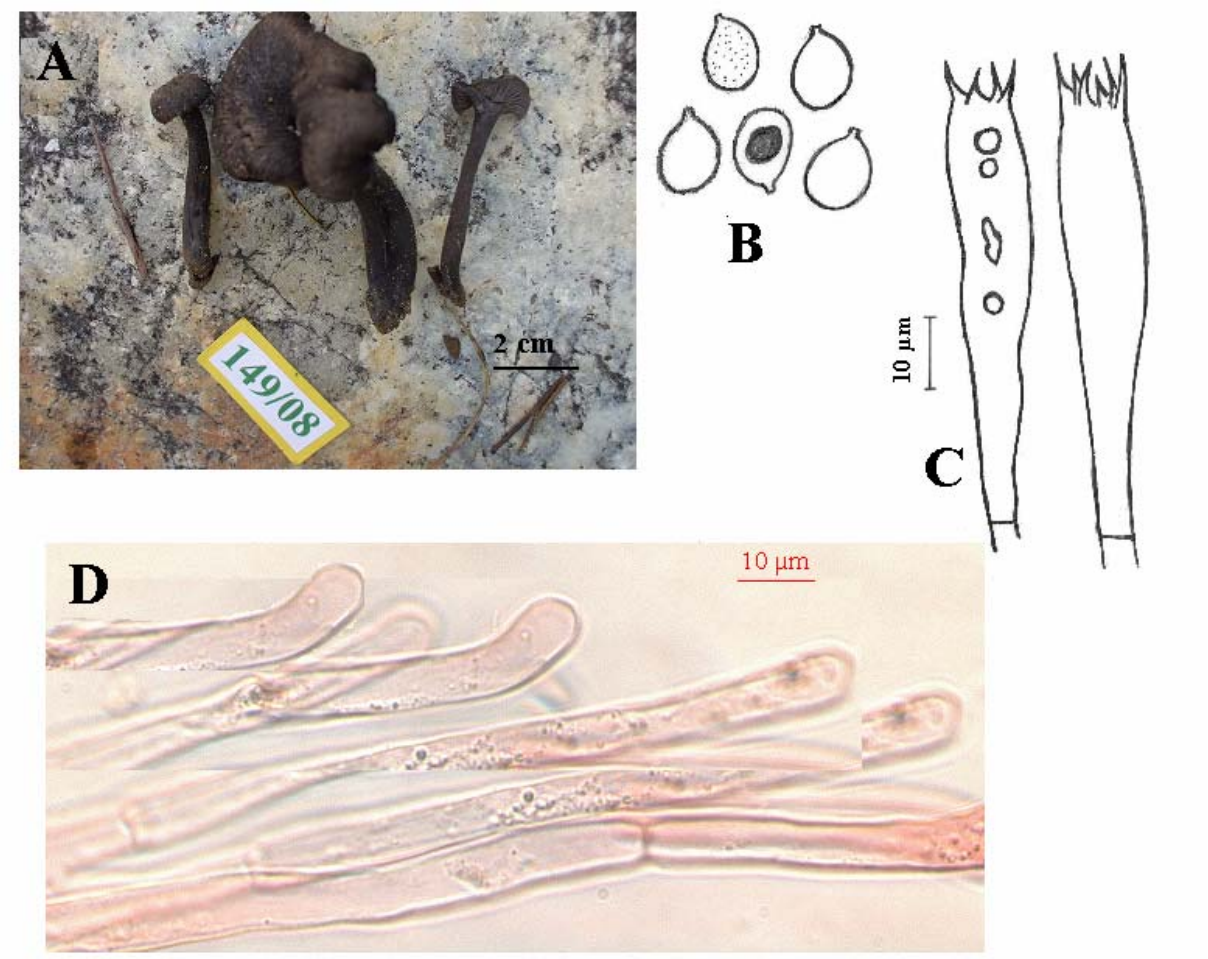

Fig. 4.27 A & B. Basidiocarps; B & C. Basidiospores and Basidia and D. Pileipellis of *Cr. dubius*

4. *Craterellus indicus* Deepika, Upadhyay & Reddy **sp. nov.** (Fig.4.28. A-E)

MycoBank: 519679

Etymology: from the latin word Indicus - INDIA, referring to the region.

Basidiocarpus parvus, Pileus 2 cm in diam, infundibuliformis, arenosus vel (13B-2) leviter brunneae, superficie sicco, non hygrophano, margin non-striatus, irregularis, contextus albus. Hymenium laveis, distinctus separatus ad margin, albus. Stipes centralis, 1-2.5 cm longus, 0.1-0.2 cm crassus, lacunosus, superficie glabrum. Basidiosporae (45/2/2) 7.5-10.5 × 6-7 µm, L=10.5 µm, W=8.22 µm, Q=1.32, ellipsoideae, inamyloideae. Basidia 48-85 × 6-12 µm, cylindrica vel anguste clavata, sterigmatibus (2-4), 4-6.5 µm, longis,1.2-2 µm crassus, Basidiola numerosa. Sporae in cumulo albus. Superficies pilei hyphis 4.5-9 µm latis, cellulis cylindrica vel subclavatus, 22-40 µm longus, 5-10 µm crassus, contextus hyphis comparatis, latus. Fibulae absentes. Pleuro- vel cheilocystidia nulla.

Pileus up to 2 cm diam, infudibuliform, light brownish to sand (13B-2) coloured, surface dry, non-hygrophanous, innate; margin nonstriate, irregular. ***Context*** off white, thin, unchanging coloured. ***Hymenium*** smooth to low folded, distinctly detached at margin, creamish (11C-3). ***Stipe*** 1-2.5 × 0.1-0.2 cm, central, equal in the diameter throughout, yellow gray (12B-2), lacunose, surface glabrous. Taste not recorded. Odour pleasant when fresh. Spore print white. ***Basidiospores*** {45/2/2} 7.5-10.5 × 6-7 µm, L= 10.5 µm, W= 8.22 µm, Q= 1.32, ellipsoid to broadly ellipsoid, inamyloid, noncyanophilic, wall hyaline in 3% KOH solution, contents monoguttulate. ***Basidia*** 48-85 × 6-12 µm, clavate to narrowly clavate, 2-4 sterigmata, 4-6.5 × 1.2-2 µm, contents granulated, basal septa clampless. ***Hymenophoral trama*** sub regular, made up of cylindric cells in the middle stratum, 6-11 µm wide, narrower towards subhymenium 3.5-7 µm wide, thin to slightly thick walls, branched, septate. ***Subhymenium*** consisting of non-inflated, branched, septate hyphal segments, 3-6 µm wide. ***Pileipellis*** composed of sub parallel arranged

hyphae, 4.5-9 µm wide, embedded in partially gelatinized matrix, branched, septa clampless, contents granulated, hyphal ends cylindric to subclavate, suberrectly arranged, 22-40 × 5-10 µm with thin to slightly thick walled; context hyphae comparatively broader. ***Stipe cuticle*** composed by hyaline, branched, thin to thick walled, 0.5-1 µm diam, slightly pigmented hyphae, 2.5-8 m wide, clamp connection absent. ***Pleurocystidia*** and **C*heilocystidia*** are absent.

***Habitat and distribution*:** Caespitose to gregarious, on soil under the trees of *Cedrus deodar*, coniferous type forest.

***Materials examined*:** INDIA. Himachal Pradesh: Shimla, Chindi (1700 m), 28 August 2007, 159-07 (Holotype); same location, 19 August 2008, 211-08 (Paratype); 6 September 2009, 65-09 (Paratype). Holotype: PUN 3884.

***Notes*:** The morphological character of present study specimen was compared with already described species to confirm it as a new species (Table 4.8). This species showed close similarity with the hymenium color of *Craterellus neotubaeformis* nom. prov. (Pilz et al. 2003) and *Craterellus sinuosus* Fries. Morphological features like pileus and stipe color, attachment of hymenium and some icroscopical features like basidiospores, basidia and the absence of clamp-connections differed with *Cr. neotubaeformis*. *Craterellus sinuosus* has larger basidiocarps (pileus 2-5 cm broad) and larger spores (range is 9.5-12 x 7-8 µm) (Bigelow 1978) than the present study specimen. There has not been any *Craterellus* species reported having cream-color with whitish hymenium that is distinctly detached from the stipe apex, which is a distinctive taxonomic feature of the present specimen compared to other species of *Craterellus*.

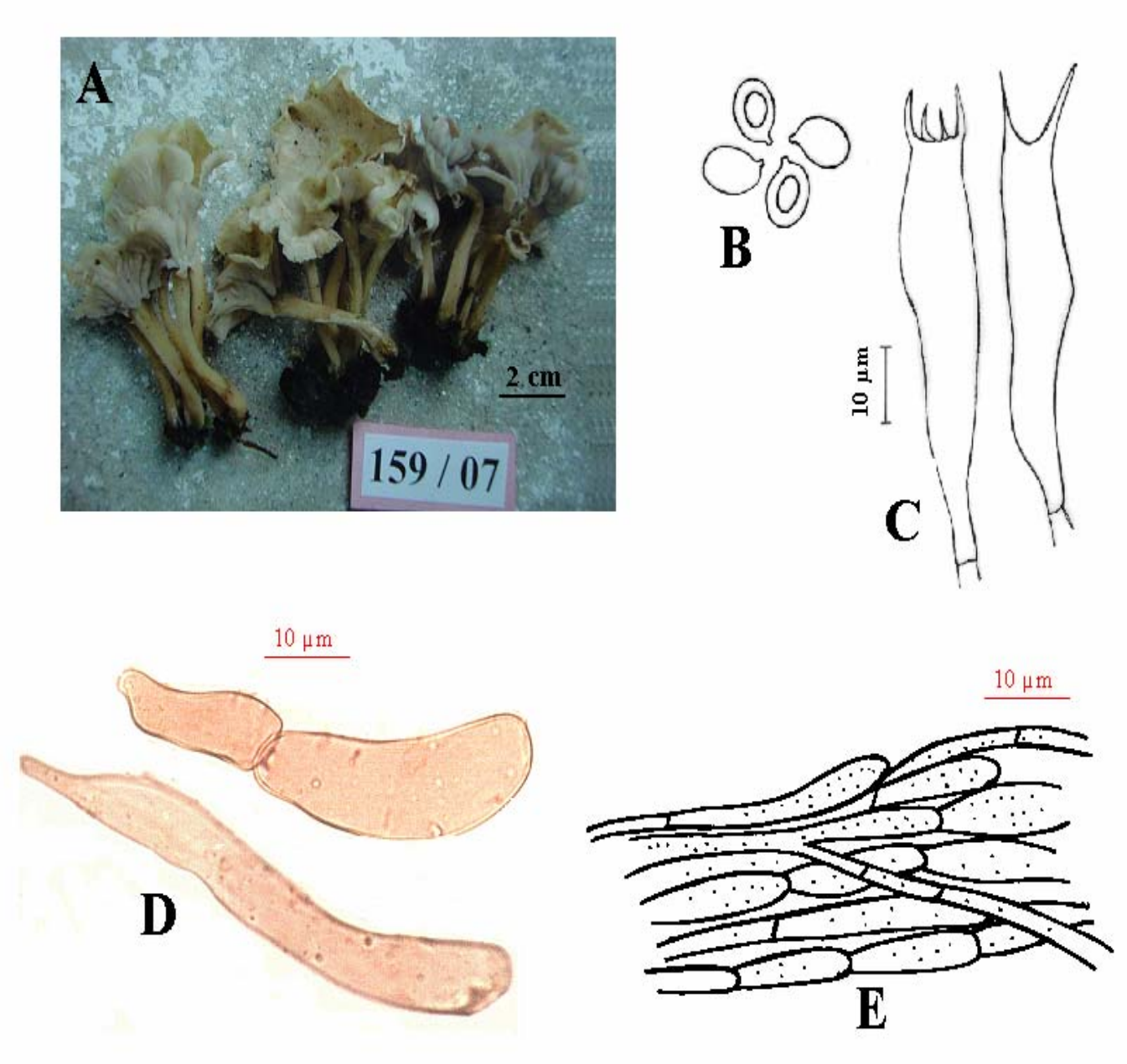

Fig. 4.28 A. Basidiocarp; B & C. Basidiospores and Basidia and D & E. Pileipellis of *Cr. indicus*

Table 4.8 Morphological characters of *Craterellus* species collected from Western Himalayas, India

Name of Species	Morphological Characteristics					
	Pileus diameter (cm)	**Pileus surface**	**Stipe diameter (cm)**	**Basidia (µm)**	**Basidiospores (µm) Mean; L; W; Q**	**Pileipellis hypahe**
Cr. cinereus	1-5	Dark brown to grayish	2-5.5 x 0.2-1.0	40-55 x 5.5-8	7-9 × 4-5.8; 7.0; 5.2; 1.34	Hyphal ends rounded, upright protruding beyond surface, mixed with encrusted hyphae
Cr. *cornucopioides* var. *mediosporus*	2.5-4.5	Dark grey to blackish	1.5-2.8 x 0.3-0.7	50-70 x 8-8.5	8-10.2 x 6.5-7.5; 9.5; 7.1; 1.33	Hyphal ends rounded, upright protruding beyond surface
Cr. dubius	2-5	Grayish black	2-3 x 0.2-1.0	37-55 x 6-8	5.8-7.2 x 4.5-5.4; 6.34; 4.61; 1.37	Hyphal ends rounded, upright protruding beyond surface
Cr. indicus	1.5-2	Light brownish to sand coloured	1-2.5 × 0.1-0.2	48-85 x 6-12	7.5-10.5 x 6-7; 10.5; 8.22; 1.32	Sub parallel arranged hyphae

Comparative photographs of *Craterellus* species in their natural habitat and illustrations (microscopic details), collected from Western Himalayas, India, which indicate the differences among themselves are given below:

Fig 4.29 Comparative photographs of *Craterellus* species collected from Western Himalayas, India **(1.** *Cr. cinerius*; **2.** *Cr. cornucopioides* var. *mediosporus*; **3.** *Cr. dubius* and **4.** *Cr. Indicus*). All the photographs are the holotype and the bar represents 2 cm.

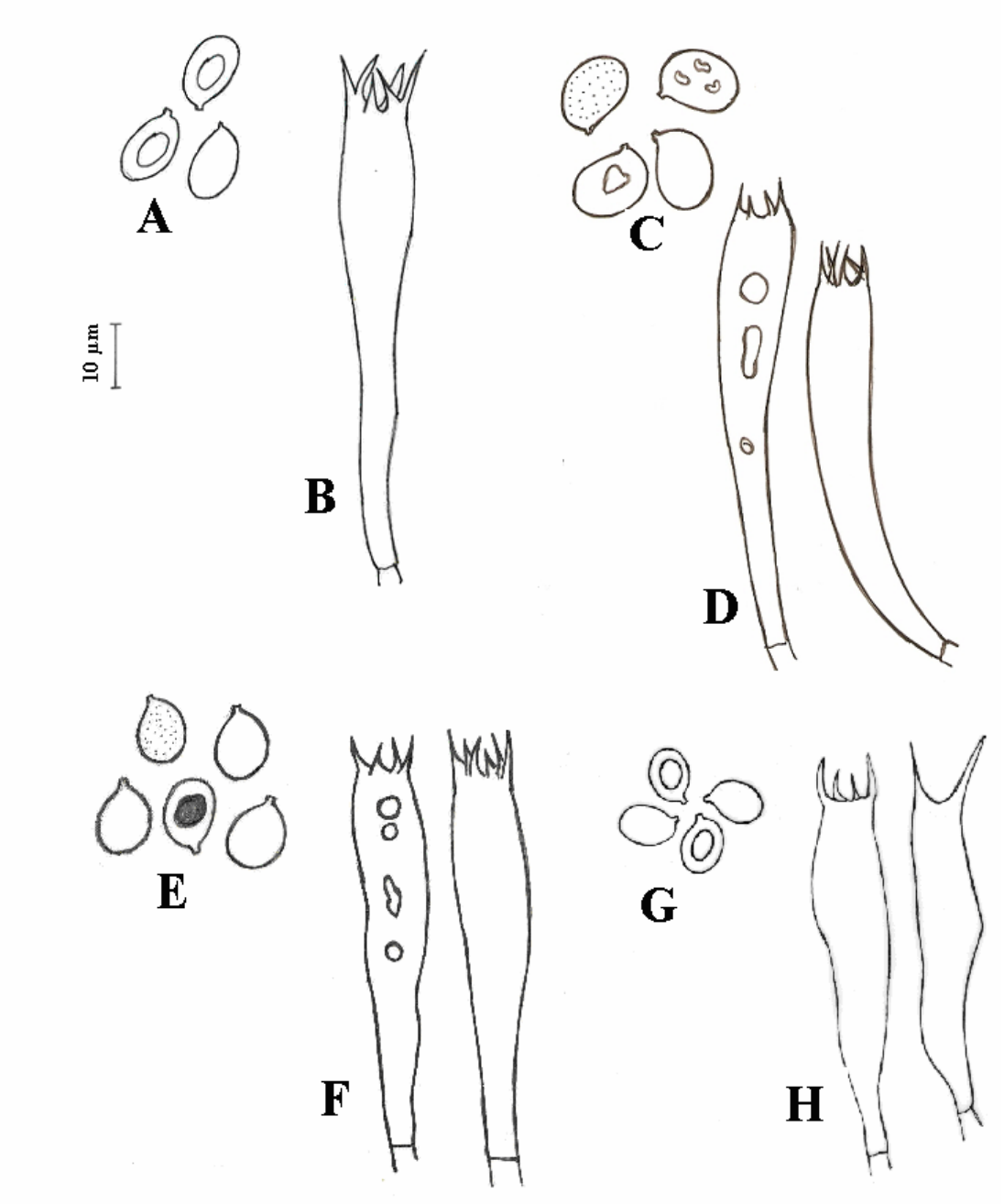

Fig. 4.30 Comparative illustrations of Basidiospores and Basidia of *Craterellus* species collected from Western Himalayas, India. Basidiospores and Basidia of: **A & B** *Cr. cinerius***; C & D** *Cr. cornucopioides* var. *mediosporus*; **E & F** *Cr. dubius* and **G & H** *Cr. indicus*. (All the elements are drawn from the holotype and the bar represents 10 µm).

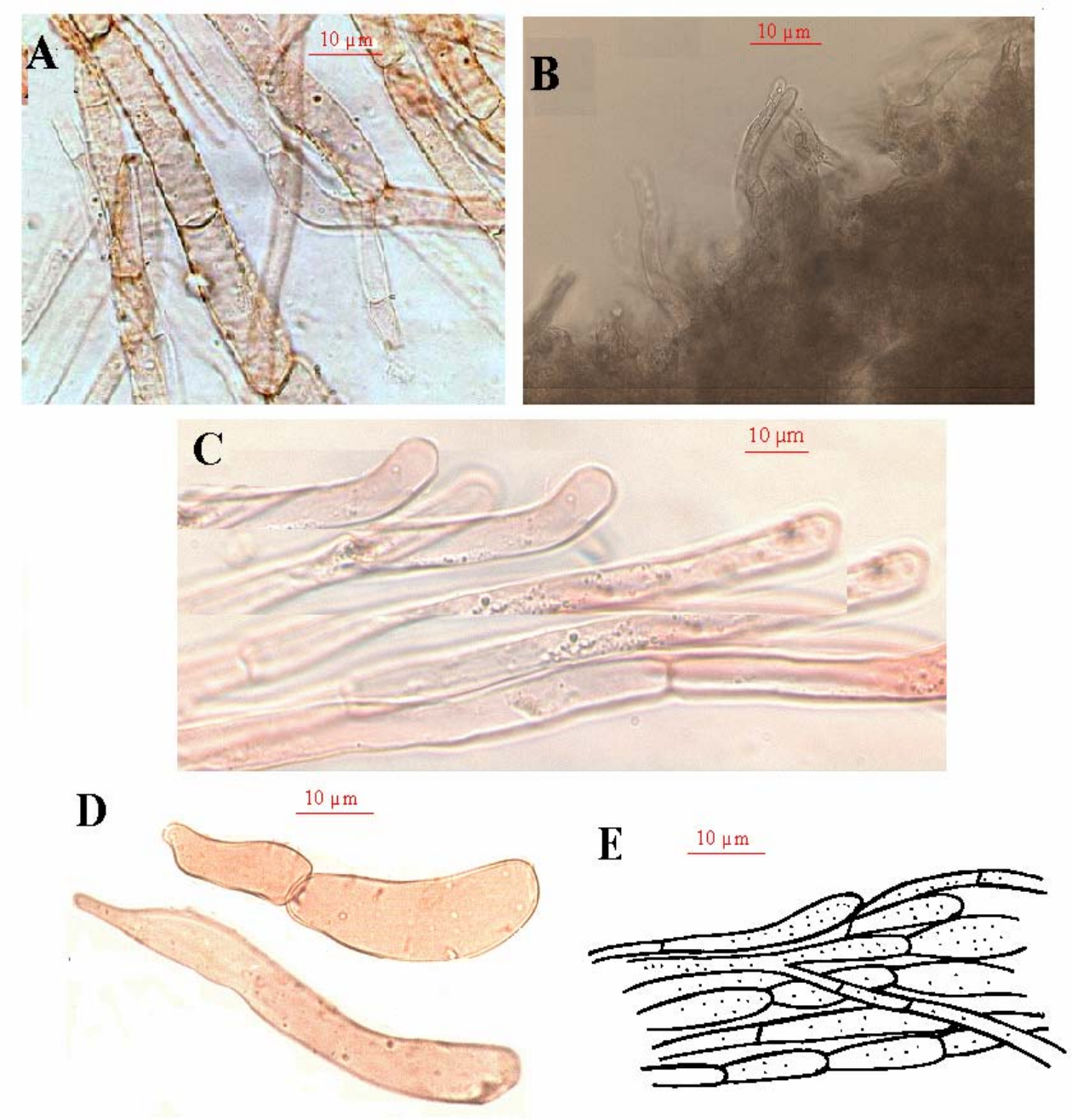

Fig. 4.31 Comparative illustrations of Pileipellis hyphae of *Craterellus* species (Pileipellis hyphae of: **A.** *Cr. cinerius***; B.** *Cr. cornucopioides* var. *mediosporus* **C.** *Cr. dubius* and **D & E** *Cr. indicus*).

Key to the Indian species of *Craterellus*:

1. Hymenophore smooth……. 2

1.* Hymenophore with prominent folds……..***Cr. cinerius***

2. Fruit bodies dark brownish to blackish, infundibuliform……..3

2* Fruit bodies light brown to sand, distinctly detached hymenium from the stipe apex ……...***Cr. indicus* sp nov.**

3. Pileus < 5 cm broad, margin lobed, spore 5.8-7.2 × 4.5-5.4 µm ……..***Cr. dubius***

3* Pileus < 5 cm broad, margin wavy, spore 8-10.2 x 6.5-7.5µm …….....***Cr. cornucopioides* var. *mediosporus***

4.5 Molecular phylogeny of *Craterellus*

The fruit bodies of *Craterellus* collected from different parts of Western Himalaya, India (Table 4.3) were collected and analyzed to see the genetic variation among the different fruit bodies except *Craterellus dubius* (149-08) due to insufficient fruit bodies. Although till date 20 species of *Craterellus* (Kirk et al., 2008) are recognized, unfortunately few sequences of ITS and LSU of *Craterellus* species are avialable in GenBank.

4.5.1 PCR amplification of ITS and LSU region

Genomic DNA was isolated from the three different fruit bodies of *Craterellus* (mentioned in Table 4.3), 159-07, 107-07 and 268-06. The ITS region was amplified by using ITS1 and ITS4 primers and LSU by ITS4R and LR5 prmiers. Resultant PCR products were viewed after electrophoresis in agarose gel. The result showed that all ITS region of *Craterellus* produced a single band of approximate 700 to 752 bp (Fig. 4.32) and LSU of ~ 950 bp (Fig. 4.33).

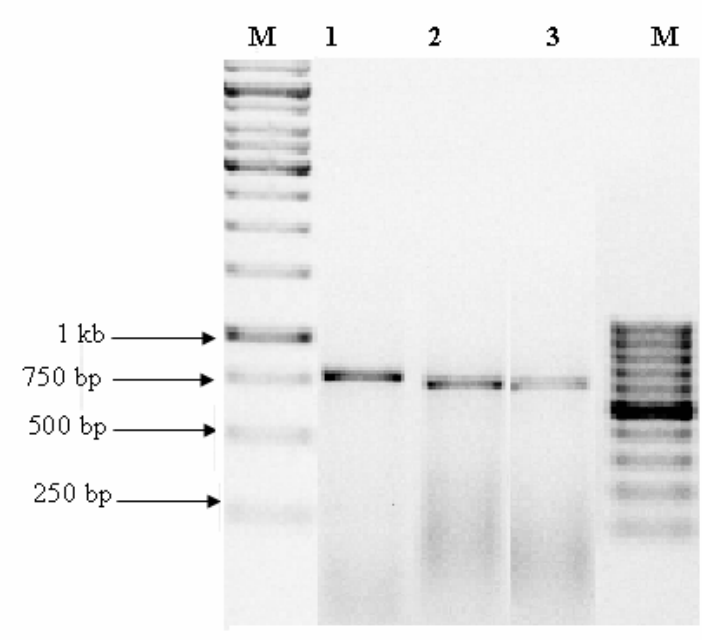

Fig. 4.32 ITS-PCR products of *Craterellus* amplified with ITS1 and ITS4 primers. Lane M, DNA marker, lane 1-3 are species of 159-07, 107-07, 268-06 respectively.

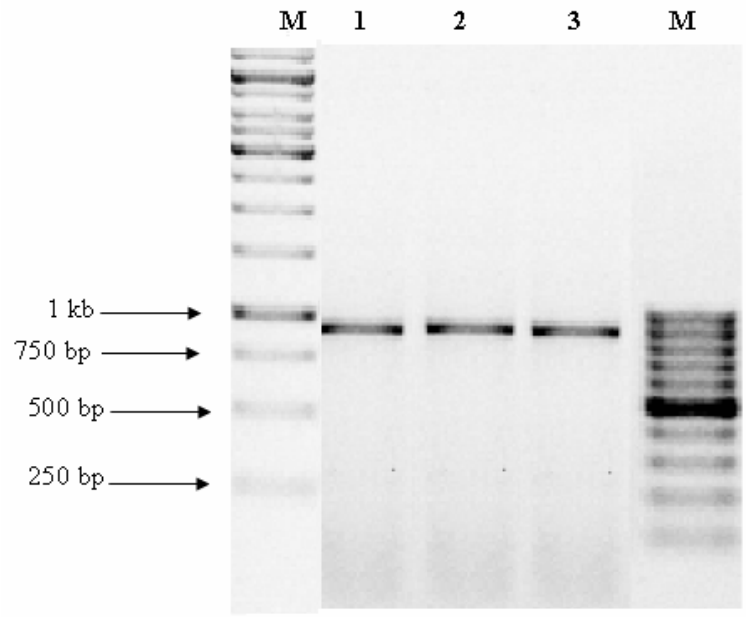

Fig. 4.33 LSU-PCR products of *Craterellus* amplified with ITS4R and LR5 primers. Lane M, DNA marker, lane 1-3 are species of 159-07, 107-07, 268-06 respectively

4.5.2 Restriction enzyme analysis of ITS products

ITS-PCR products obtained after PCR were subjected for Restriction Fragment Length Polymorphism (RFLP) analysis with restriction enzymes *Alu I*, *Mbo I*, *Hinf I* and *Hae III*. Restriction fragments were electrophoretically separated on 2.5% agarose gels stained with ethidium bromide, and scored against 1 kb and 100 bp ladders.

All the *Craterellus* species were found to have restriction sites for *Alu I* and *Hinf I* restriction enzymes (Fig. 4.34). *MboI* site was absent in all the 3 species (Table 4.11), whereas other enzyme *Hae III* showed only restriction site for 268-06. Based on RFLP patterns obtained, it was found that the 3 different species of *Craterellus*. The representative species was selected for sequencing and further studies.

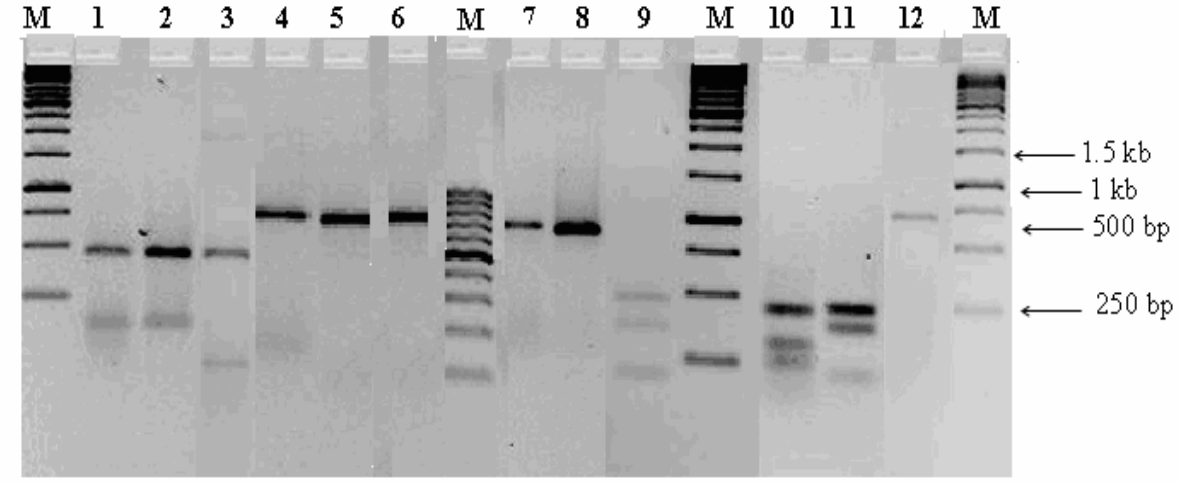

Fig. 4.34 ITS–RFLP analysis of *Craterellus* digested with 4 different restriction enzymes. Lane M, DNA marker, lane 1-3 are species of 159-07, 107-07, and 268-06 digested with *AluI*, lane 4-6 are digested with *Mbo I*, lane 7-9 digested with *Hae III*, and lane 10-12 digested with *Hinf I* respectively. This is a composite figure from different gels.

4.5.3 Sequence analysis of *Craterellus* species

The presence of ambiguous alignments in ITS region was excluded and aligned all the sequences with complete 5.8 and ITS2 regions of *Craterellus* species available in GenBank. The sequences were analyzed by multiple sequence alignment (ClustalW) to check similarity among species. The homologies among ITS sequences were from 74 to 82% between species. Minimum of 74% similarity was found in *Cr. indicus* with *Cr. cinerius* and maximum 82% similarity was found between *Cr. indicus* with *Cr. cornucopioides* var. *mediosporus*.

The homologies among LSU sequences were found between 86 and 92% in different *Craterellus* species. Minimum of 86% similarity was found in *Cr. indicus* with *Cr. cinerius* and maximum 92% similarity was found between *C. indicus* with *Cr. cornucopioides* var. *mediosporus.*

Sequences were compared for the similarity in the GenBank DNA database using BlastN (NCBI) (Altschul *et al*, 1997), which revealed that ITS region of *Craterellus* species had 94% to 99% similarity (Table 4.9) and LSU region had 93 to 99% similarity (Table 4.10) with the sequences of NCBI database.

The closest ITS sequence to *Craterellus* sequence with a new species *Cr. indicus* found in GenBank was *Pseudocraterellus sinuosus* (voucher TFB12700; TENN062865) with 92% similarity for a 96% coverage. The following closest sequence was *Cr. lutescens* with 91% similarity covering 48%. Other two new species from India; *Cr. cinerius*, which showed 100% similarity for 97% coverage with a *Craterellus sp.* (GU590930) and 94% similarity for 74% coverage with *Cr. fallax* (GU590928); *Cr. cornucopioides* var. *mediosporus* was showed similarity with *Cr cornucopioides* (GU590930) and *Cr. fallax* (GU590928) with 98% similarity for a 100% coverage.

The LSU sequence of *Cr. indicus* showed the closest match was *Cr. cornucopioides* with 93% similarity, covering 92% of the region. *Cr. cornucopioides* var. *mediosporus* showed match with *Cr. cornucopioides* (AF105298, AF105300) with 99% similarity, covering 100% of the sequence region and *Cr. cinerius* showed match with *Cr.*

lutescens (EU522786) and *Cr. aurora* with 97% similarity, covering 100% and 96% similarity, covering 99% respectively. All the gene sequences of *Craterellus* determined in this study were deposited in the NCBI data library (Tables 4.9 and 4.10). The ITS region of *Craterellus* species were also analyzed for restriction digestion with the help of web cutter (www.firstmarket.com/cutter) and found that restriction pattern obtained were same as obtained from the webcutter and exact size of fragments was also deduced (Table 4.11).

4.5.3.1 Phylogenetic analysis based on the ITS2+5.8 regions

The parsimony analysis of ITS sequences was performed with 19 taxa including *Hydnum* as outgroup taxon. The ITS sequence of *Hydnum* sp. was aligned to *Cantharellus*/*Craterellus* after taking consideration into partial sequences of 5.8 and complete sequences of ITS2 region. The aligned data set contains 641 characters thereof were 297, 107 and 237 constant, parsimony-uninformative and parsimony-informative characters, respectively. Gaps were treated as missing data. The branch-and-bound search returned two equally parsimonious trees (TL = 592, CI = 0.81, RI= 0.86, RC = 0.70). The Kishino-Hasegawa test confirmed that two trees were not statistically different and one of the parsimonious trees is shown in Fig. 4.35. The bootstrap consensus showed that *Cr. indicus* is part of a poorly resolved clade with other *Craterellus* sequences.

4.5.3.2 Phylogenetic analysis based on the LSU analysis

The parsimony analysis of LSU was performed with 20 taxa including *Hydnum umbilicatum* as outgroup taxon. The LSU sequence of *Hydnum umbilicatum* was aligned to *Cantharellus*/*Craterellus* by removing 50 nucleotides from the start and 320 nucleotides from the end of the each sequence. The aligned data set contains 580 characters thereof were 294, 71 and 215 constant, parsimony-uninformative and parsimony-informative characters, respectively. Gaps were treated as missing data. The branch-and-bound search returned two equally parsimonious trees (TL = 461, CI = 0.83, RI= 0.91, RC = 0.76). The MP and Bayesian analyses resulted in identical phylogenetic

trees. The phylogenetic reconstructions grouped all sequences into two clades differentiating. *Craterellus* from *Cantharellus* in a way strongly supported by both Bayesian and bootstrap values. The Kishino-Hasegawa test confirmed that two trees were not statistically different and one of the parsimonious trees is shown in Fig. 4.36. The bootstrap consensus showed that *Cr. indicus* is part of a poorly resolved clade with other *Craterellus* sequences.

The topology within *Craterellus*, based on ITS sequences, was congruent with the topology based on the LSU data set. There were few minor differences in both ITS and LSU based trees but those were not critical.

Table 4.9 Examined *Craterellus* species and their closest relative species inferred from ITS gene sequence of existing database

Species	Accession no	Nearest match	Query coverage	Maximum Identity
Cr. cinerius (107-07)	JF412278	*Cr. species* (GU590930)	98%	99%
Cr. cornucopioides var. *mediosporus* (268-06)	JF412277	*Cr. cornucopioides* (DQ205680)	100%	99%
Cr. indicus (159-07)	HM113530	*Pseudocraterellus sinuosus* (GU590932)	93%	94%

Table 4.10 Examined *Craterellus* species and their closest relative species inferred from LSU gene sequence of existing database

Species	Accession no	Nearest match	Query coverage	Maximum Identity
Cr. cinerius (107-07)	JF412276	*Cr. lutescens* (EU522746)	100%	97%
		Cr. aurora (AF105304)	99%	96%
Cr. cornucopioides var. *mediosporus* (268-06)	JF412275	*Cr. cornucopioides* (AF105296)	100%	99%
Cr. indicus (159-07)	HM113529	*Cr. cornucopioides* (AF105305)	98%	93%

Table 4.11 Differentiation of *Craterellus* species into RFLP types according to the size of restriction fragments produced following digestion of the ITS PCR product with restriction enzymes. Exact size of fragment was determined using web programme Web Cutter 2.0 (www.firstmarket.com/cutter)

Species	**Restreiction enzymes**			
	Alu I	*Mbo I*	*Hinf I*	*Hae III*
	Size of fragments (bp)			
Cr. cinerius (107-07)	200, 95, 85, 76, 68, 59, 55, 34, 28	ND	ND	190, 150, 100, 91, 83, 74, 56, 42, 14
Cr. cornucopioides var. *mediosporus* (268-06)	200, 84, 70, 68, 61, 59, 55, 40, 35, 28	ND	220, 170, 95, 81, 71, 63	450, 90, 80, 53, 27
Cr. indicus (159-07)	200, 90, 85, 80 75, 63, 54, 43, 32, 23, 17	ND	ND	190, 150, 100, 98, 87, 81 46

Notes: ND- restriction site not detected

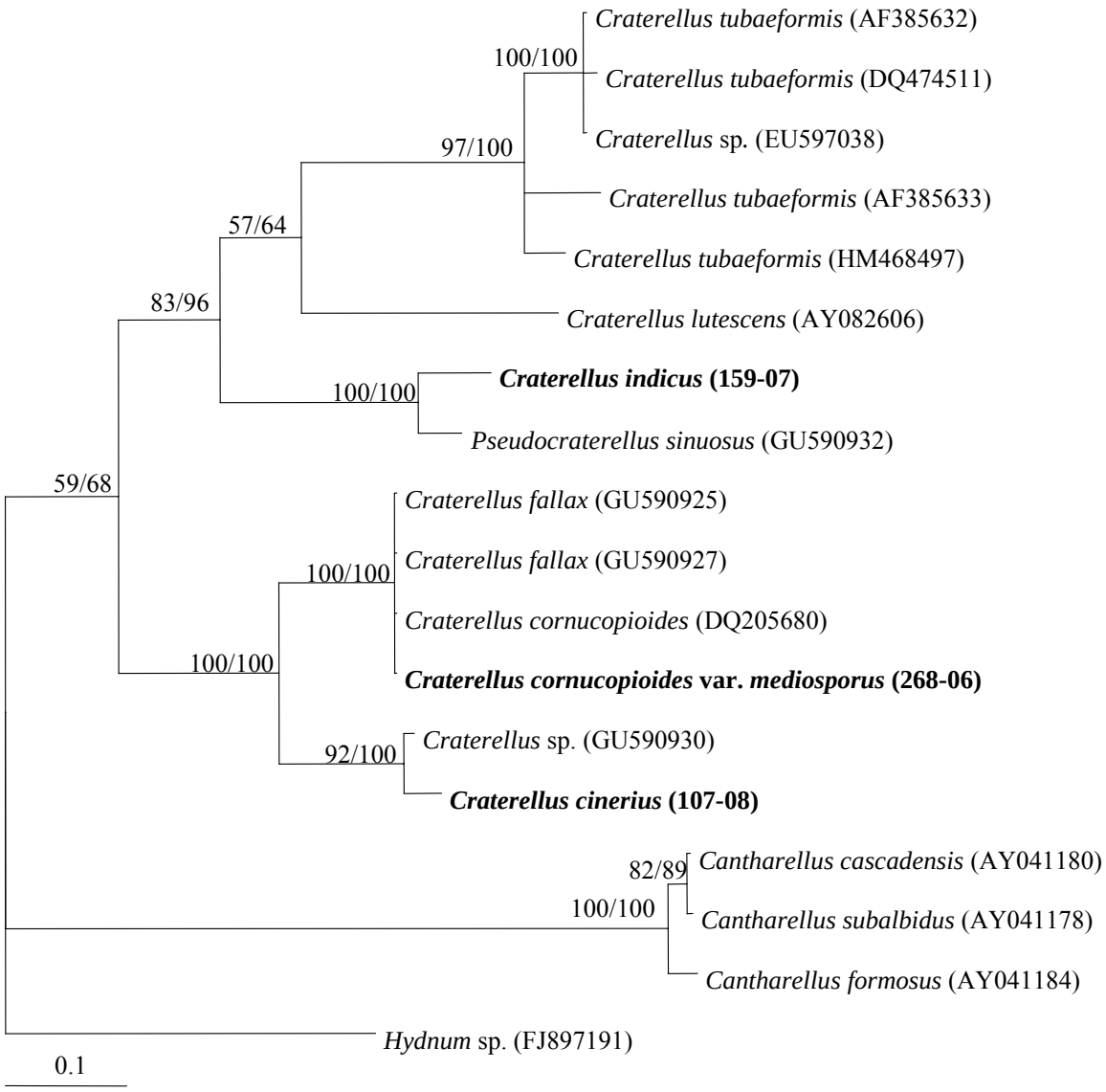

Fig. 4.35 Phylogeny of *Craterellus* generated from Maximum Likelihood analysis of ITS2+5.8 regions, rooted with *Cantharellus species.* Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). The scale gives the substitution rate. The bold species represent the Indian collections.

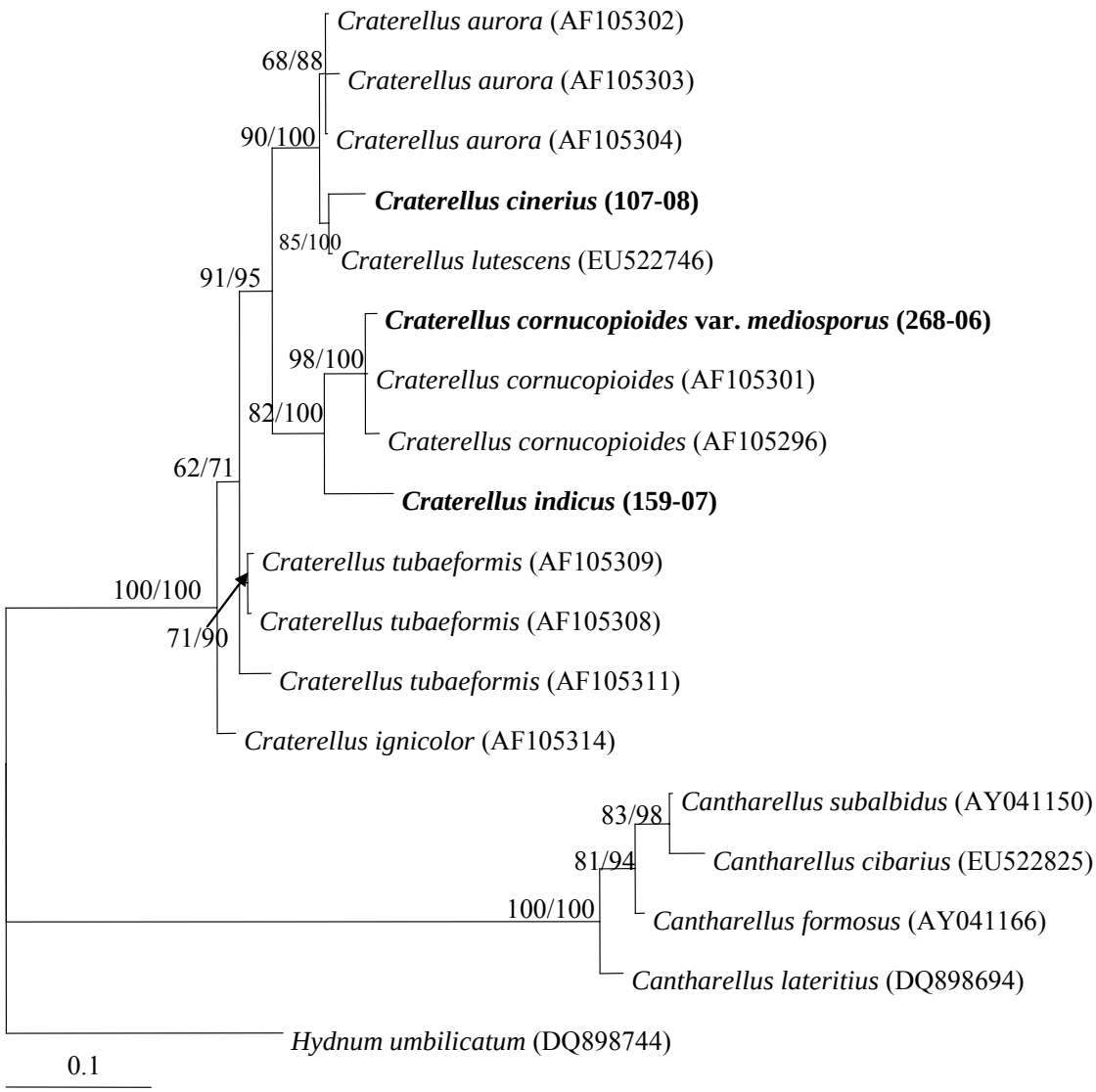

Fig. 4.36 Phylogeny of *Craterellus* generated from Maximum Likelihood analysis of LSU sequences, rooted with *Cantharellus species.* Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). The scale gives the substitution rate. The bold species represent the Indian collections.

Salient finding

- The phylogenetic analyses revealed clades corresponding to circumscribed morphological species and aided in distinguishing different taxa of Cantharellaceae. Morphogenetic studies based on macromophological and microscopical characters alongwith both ITS and LSU dataset confirmed the identity of these fungi. The two gene approaches resulted in a well supported monophyletic group containing different clades and the resolution for the inter-clade relationship between these groups was well supported
- The present study described 8 new taxa of *Cantharellus* to the world, namely *C. applanatus, C. elongatipes, C. fibrillosus, C. himalayensis, C. indicus, C. natarajanii, C. pseudoformosus* and *C. umbonatus.* Further *C. miniatescens* is reported first time from the Indian subcontinent
- Among *Craterellus*, *Cr. cornucopioides* var. *mediosporus* Corner, *Cr. dubius* Peck and *Cr. cinerius* Fries, described as new taxa from the Indian subcontinent. *Cr. indicus* sp. nov is new report from the world

Chapter 5

Nutritional properties of Cantharellaceae

5.1 Nutritional value

Fruit bodies of Cantharellaceae species were collected from the forests of Western Himalayas, India (Fig. 4.1 and Table 4.1). The identification of the species was made according to criteria obtained from macroscopic, microscopic and molecular examinations as described in chapter 4. Mushroom samples were brought to the laboratory in an ice bath and stored deep-frozen at –20°C until used. All of the fruit bodies of Cantharellaceae species were analyzed for their nutritional properties except *Craterellus indicus* (159-07) and *Craterellus dubius* (149-08), as there was not sufficient fruit bodies available for further analysis.

The chemical composition and calculated energy values (expressed on dry weight basis) of the wild edible Cantharellaceae species are shown in Table 5.1. It was observed that all the mushroom samples had high moisture content, ranged from 925 to 955 mg/g fresh weight. The fresh fruit bodies of *Cr. cornucopioides* var. *mediosporus* (268-06) and *C. natarajanii* (106-08) contained the highest amount of moisture (955 mg/g). This was followed in order by fruit bodies of *C. applanatus* (121-07), *C. pseudoformosus* (281-07), and *C. umbonatus* (348-07), containing moisture contents of 952, 951 and 948 mg/g, respectively. The fruit bodies of *C. lateritius* (161-07) contained minimum amount of moisture (925 mg/g) among all mushrooms studied. Ash content varied between 1.2 mg/g in *C. appalachiensis* (84-08) and 6.8 mg/g in *Cr. cornucopioides* var. *mediosporus* (268-06). Carbohydrates were an abundant macronutrient and ranged from 10 mg/g in both *C. appalachiensis* (84-08) and *Cr. cornucopioides* var. *mediosporus* (268-06), and 26.5 mg/g in *C. minor* (354-05). The highest energy value obtained was 132 kJ/g for *C. lateritius* (161-07), while *C. natarajanii* (106-08) gave the lowest energetic contribution (70 kJ/g).

Protein was found in high levels and varied between 21.6 mg/g in *C. natarajanii* (106-08) and 43.2 mg/g in *C. miniatescens* (65-07) of dry weight in different species of

Cantharellaceae (Table 5.1 and Fig. 5.1). The fat contents were generally low. The maximum content of crude fat recorded in *C. fibrillosus* (113-07) was 8.7 mg/g, followed by 7.8 mg/g in *C. cibarius* (90-09), while *C. natarajanii* (106-08) contained lowest amount (1.9 mg/g) of fat.

Table 5.1 Proximate chemical composition (mg/g) and energetic value (kJ/g) of different Cantharellaceae species

Collection No.	Moisture (mg/g)	Proteins (mg/g)	Ash (mg/g)	Total Fat (mg/g)	Carbohydrates (mg/g)	Energy (kJ/g)
MSR2-07	934 ± 2.1ef	40 ± 0.05a	4.3 ± 1.41ef	3.0 ± 0.61hi	18.0 ± 0.60ab	110 ± 0.42cd
65-07	931 ± 2.1fgh	43 ± 0.09a	2.1 ± 1.32hi	5.8 ± 0.55cde	18.0 ± 1.41ab	125 ± 0.60b
84-08	940 ± 1.1de	42 ± 0.03a	1.2 ± 1.42j	6.8 ± 0.36bc	10.0 ± 1.41e	112 ± 0.35cd
90-09	935 ± 2.1ef	39 ± 0.08ab	1.8 ± 1.32ij	7.8 ± 0.55ab	16.4 ± 0.42c	123 ± 0.70c
106-08	955 ± 1.2a	22 ± 0.03de	5.9 ± 1.13bc	1.9 ± 0.31j	15.6 ± 0.49cd	70 ± 0.70gh
107-07	939 ± 1.4de	28 ± 0.11c	6.8 ± 1.21a	5.5 ± 0.45de	20.6 ± 0.35ab	104 ± 0.50de
113-07	932 ± 1.2fg	38 ± 0.04ab	2.5 ± 1.33gh	8.7 ± 0.42a	19.0 ± 0.28ab	129 ± 0.90ab
121-08	952 ± 0.1ab	24 ± 0.05e	4.5 ± 1.23ef	6.4 ± 0.06cd	13.0 ± 0.56de	87 ± 0.42ef
161-07	925 ± 1.31h	38 ± 0.01ab	5.2 ± 1.41d	6.8 ± 0.30bc	25.0 ± 1.41ab	132 ± 0.50a
169-07	945 ± 0.2cd	26 ± 0.14cd	5.5 ± 1.33cd	2.7 ± 0.25hij	20.7 ± 0.49ab	89 ± 0.04ef
184-08	941 ± 0.4de	36 ± 0.04b	5.2 ± 1.09d	3.9 ± 0.41fgh	14.0 ± 0.49de	99 ± 0.40e
268-06	955 ± 2.1a	28 ± 0.09c	3.1 ± 1.37g	4.6 ± 0.55efg	9.9 ± 0.25ef	80 ± 0.49g
281-07	951 ± 0.8abc	27 ± 0.02c	4.5 ± 1.11ef	3.1 ± 0.23hi	15.0 ± 0.42cd	82 ± 0.16g
348-07	948 ± 0.3bc	26 ± 0.04cd	6.2 ± 1.41ab	4.9 ± 0.25ef	15.0 ± 0.91cd	88 ± 0.36fg
354-05	926 ± 0.5gh	28 ± 0.05ab	3.1 ± 1.41g	3.4 ± 0.46gh	26.5 ± 0.46a	127 ± 0.50ab

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3).

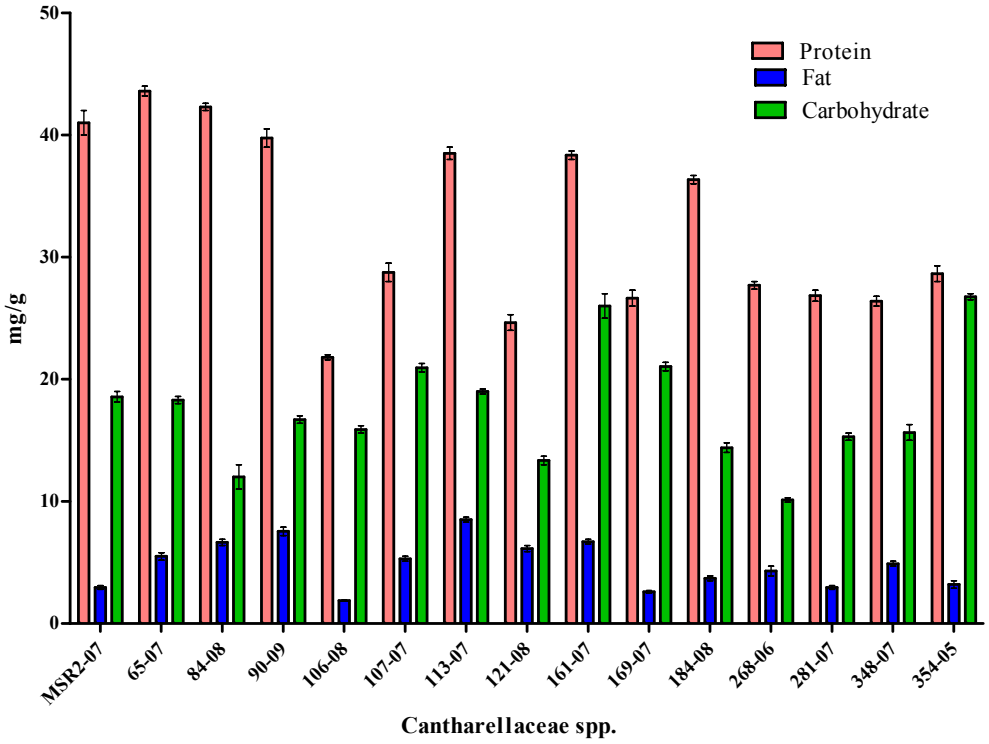

Fig. 5.1 Contents of protein, fat and carbohydrate of different Cantharellaceae species

5.2 Sugar composition

The results of total sugar composition, reducing sugar and non reducing sugar of the 15 different Cantharellaceae species are shown in Table 5.2 and Fig. 5.2. The maximum amount of total sugar was observed in *C. lateritius* 161-07 (20.7 mg/g) followed by *C. himalayensis* 169-07 (20.5 mg/g) and *C. cibarius* 90-09 (20.4 mg/g) and minimum amount in the fruit bodies of *C. fibrillosus* 113-07 (12.6 mg/g). The maximum non reducing sugar content was obtained in *C. lateritius* 161-07 (16.2 mg/g) followed by *C. cibarius* 90-09 (15.6 mg/g) and the minimum was in *C. fibrillosus* 113-07 (7.15 mg/g). The maximum reducing sugar contents were recorded in fruit bodies of *C. himalayensis* 169-07 (9.23 mg/g) while *Cr. cornucopioides* var. *mediosporus* 268-06 had minimum amount (2.35 mg/g). In present study non-reducing sugar were found to be relatively more than the reducing sugar (Fig. 5.2).

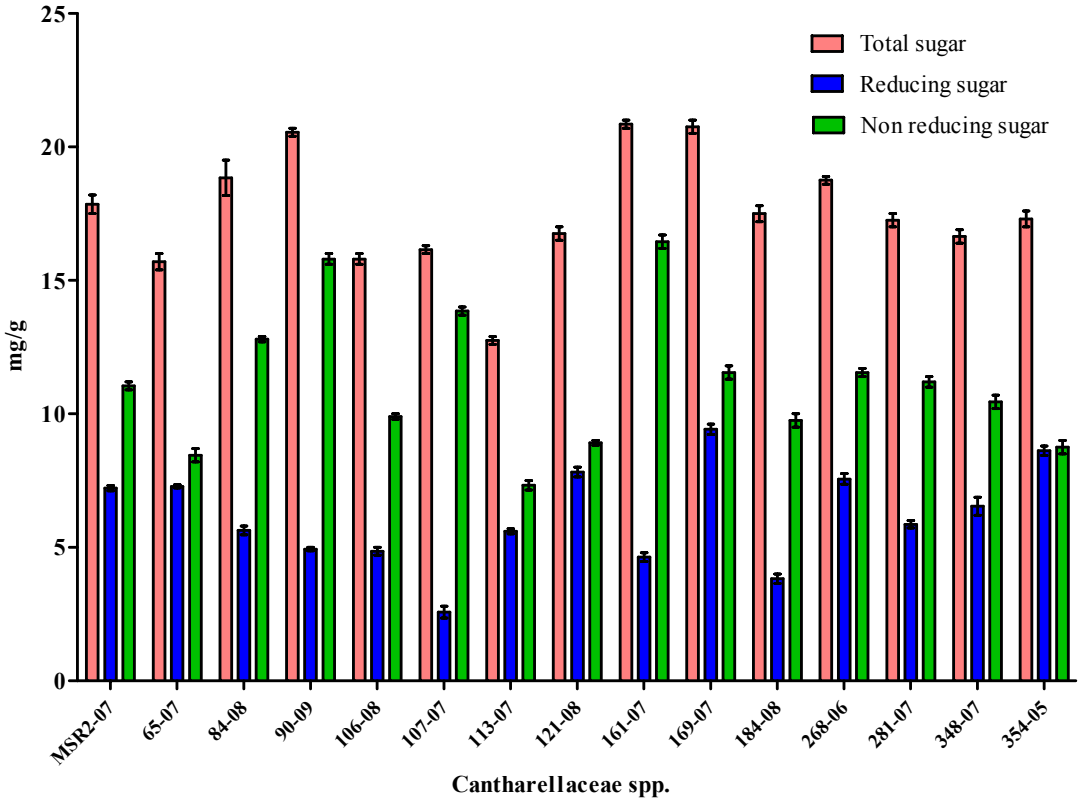

Fig. 5.2 Contents of total sugar, reducing sugar and non reducing sugar of different Cantharellaceae species

Table 5.2 Sugar composition (mg/g) of different Cantharellaceae species

Collection No.	Total Sugar (mg/g)	Reducing Sugar (mg/g)	Non reducing Sugar (mg/g)
MSR2-07	18.6 ± 1.72b	7.36 ± 3.84cd	11.4 ± 1.72c
65-07	15.4 ± 3.13def	7.23 ± 1.72cd	8.2 ± 3.93fgh
84-08	18.1 ± 1.72b	5.47 ± 1.72efgh	12.7 ± 1.72b
90-09	20.4 ± 2.43a	4.87 ± 1.72fgh	15.6 ± 2.43a
106-08	15.6 ± 2.43def	4.71 ± 3.13fghi	9.8 ± 5.34de
107-07	16.4 ± 1.01cde	5.00 ± 1.72fgh	11.0 ± 3.93c
113-07	12.6 ± 1.01h	5.51 ± 1.72efgh	7.15 ± 1.72h
121-08	16.5 ± 3.13cde	7.64 ± 2.43bc	8.8 ± 4.63efg
161-07	20.7 ± 2.00a	4.47 ± 1.86hi	16.2 ± 3.98a
169-07	20.5 ± 1.81a	9.23 ± 1.72a	11.3 ± 3.22c
184-08	17.2 ± 0.59bc	3.65 ± 3.13i	9.5 ± 4.39de
268-06	16.2 ± 1.72cde	2.35 ± 1.72j	13.7 ± 3.13b
281-07	17.2 ± 1.01bcd	5.73 ± 3.13ef	11.0 ± 3.93c
348-07	16.4 ± 2.43cde	6.21 ± 2.43de	10.2 ± 1.81cd
354-05	17.2 ± 2.43bcd	8.44 ± 2.43ab	8.5 ± 3.22efg

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3).

5.3 Amino acid composition

Sixteen known amino acids, including the essential ones, were identified and quantified in the different species of Cantharellaceae. The quantity of amino acids was calculated by internal normalization of the chromatographic peak area obtained from HPLC. Amino acid identification was made by comparing the relative retention times of sample peaks with standards. The representative chromatogram has been shown in Appendix II. The most abundant amino acid present in most of Cantharellaceae species was glutamic acid and the lowest levels were those of cystine and methionine (Figs. 5.3a and 5.3b). Of the total 16 types of amino acids estimated, the glutamic acid was in the range between 9.34 mg/g to 33.8 mg/g in *C. elongatipes* (184-08) and *Cr. cornucopioides* var. *mediosporus* (268-06) respectively. The aspartic acid was in the range between 7.3 mg/g in *C. elongatipes* (184-08) and 20.5 mg/g in *C. miniatescens* (65-07) while arginine ranged from 6.47 mg/g in *C. minor* (354-05) to 14.22 mg/g in *C. miniatescens* (65-07). Maximum amount of alanine (11.8 mg/g) was observed in *C. indicus* (MSR2-07) while *C. natarajanii* (106-08) contained maximal serine (12.9 mg/g). The proline concentration was not found to vary significantly among different members of Cantharellaceae, the contents ranged from minimum 6.8 mg/g in *C. fibrillosus* (113-07) to maximum 12.5 mg/g in *Cr. cinerius* (107-07).

All of the investigated Cantharellaceae contained significant amount of essential amino acids except methionine and cystine (Table 5.3b). Lysine contents ranged between 5.2 mg/g in *C. appalachinesis* (84-08) and 11.4 mg/g in *C. pseudoformosus* (281-07), while amount of leucine varied between 4.1 mg/g in *C. himalayensis* (169-07) and 14.8 mg/g in *C. miniatescens* (65-07). The amount of cystine was least among all the amino acids studied and ranged between 0.15 mg/g in *C. miniatescens* (65-07) and 1.14 mg/g in *C. fibrillosus* (113-07).

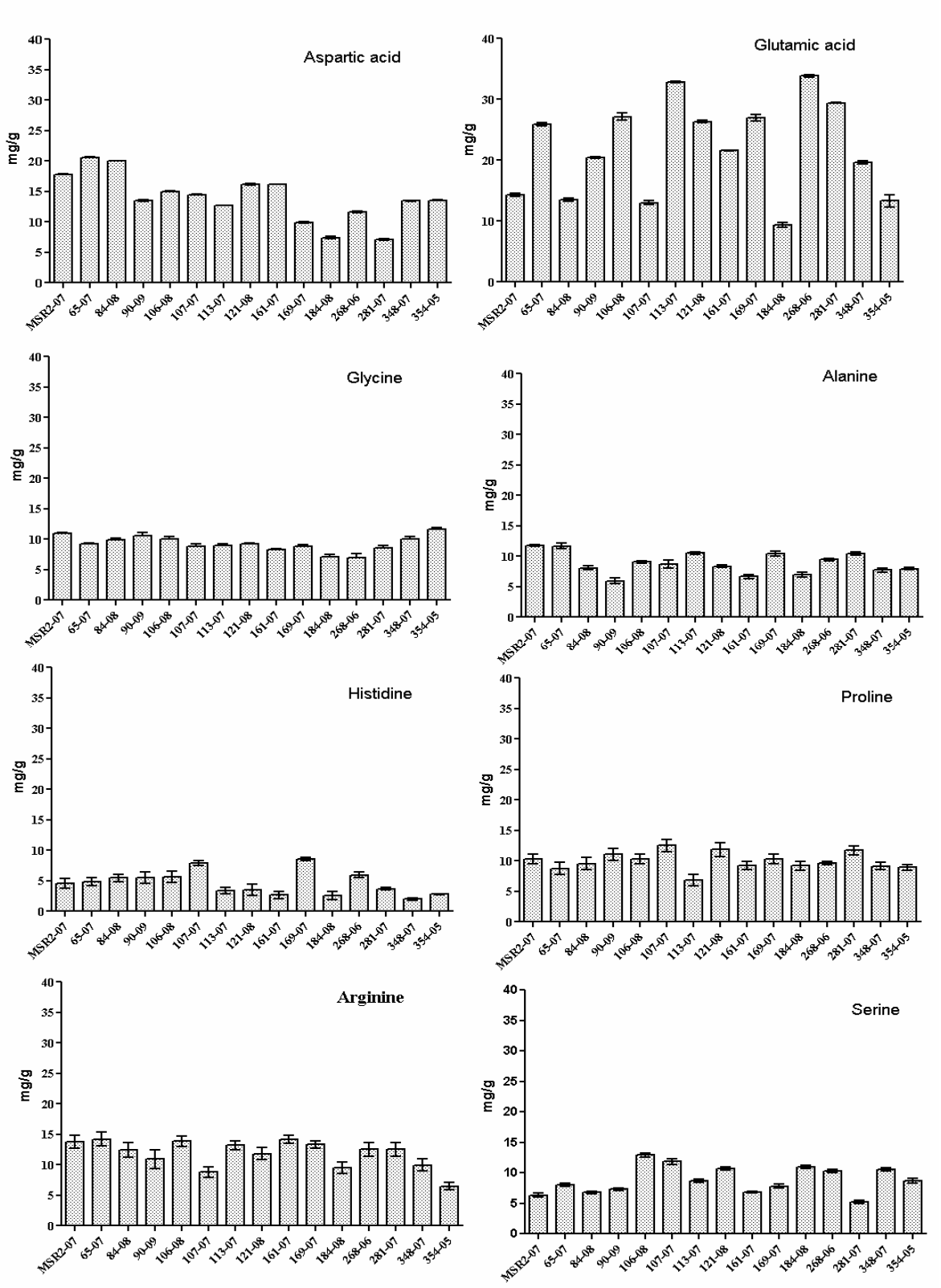

Fig. 5.3a Contents of non-essential amino acids (mg/g) (specific name provided with the graphs) present in different Cantharellaceae species

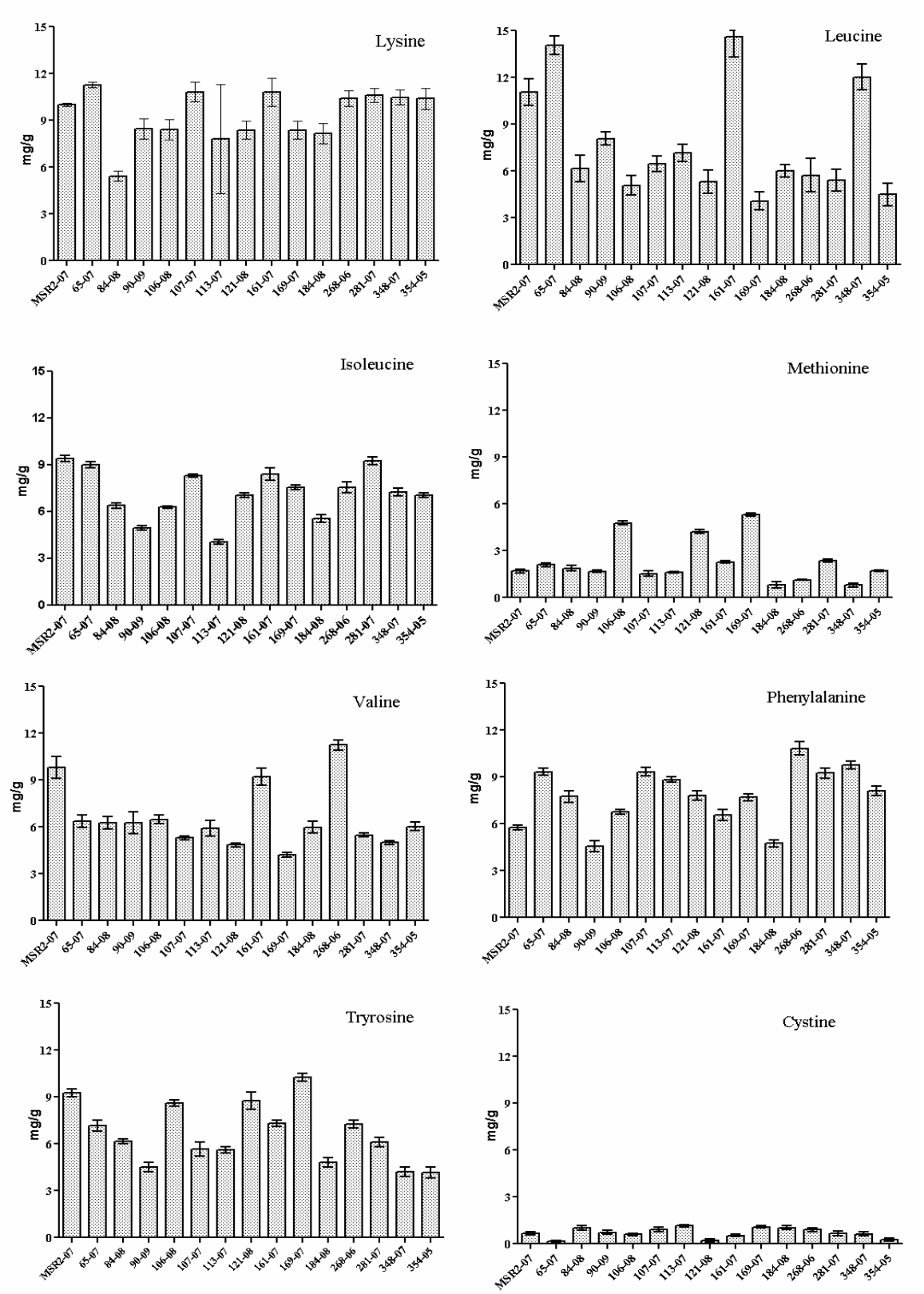

Fig. 5.3b Contents of essential amino acids (mg/g) (specific name provided with the graphs) present in different Cantharellaceae species

Table 5.3a Contents of non-essential amino acids (mg/g) of different Cantharellaceae species

Collection No.	Aspartic acid	Glutamic acid	Glycine	Alanine	Histidine	Proline	Arginine	Serine
MSR2-07	17.7 ± 0.06c	14.2 ± 0.88h	10.9 ± 0.19ab	11.8 ± 0.26a	4.6 ± 1.38ab	10.3 ± 1.34ab	13.8 ± 1.81ab	6.3 ± 0.41hi
65-07	20.5 ± 0.07a	25.8 ± 0.25d	9.2 ± 0.08efg	11.7 ± 0.76ab	4.8 ± 1.12ab	8.7 ± 1.69ab	14.2 ± 1.85a	8.1 ± 0.37efg
84-08	20.0 ± 0.01b	14.3 ± 0.04h	9.8 ± 0.31cdef	7.7 ± 0.47efg	5.7 ± 1.63ab	9.6 ± 0.41ab	12.0 ± 2.11abc	6.8 ± 0.29gh
90-09	13.5 ± 0.13h	20.4 ± 0.12f	10.5 ± 0.48abc	5.9 ± 0.80h	5.5 ± 1.56ab	11.0 ± 1.67ab	10.9 ± 2.65abcd	7.3 ± 0.32fgh
106-08	15.0 ± 0.72e	27.1± 0.58c	9.9 ± 0.49bcde	9.0 ± 0.35cde	5.9 ± 0.86ab	10.3 ± 1.33b	13.8 ± 1.56ab	12.9 ± 0.53a
107-07	14.4 ± 0.03f	13.5 ± 0.27hi	8.8 ± 0.43fgh	8.1 ± 0.55efg	7.9 ± 0.68a	12.5 ± 1.79a	8.8 ± 1.44bcd	11.8 ± 0.62ab
113-07	12.6 ± 0.04i	32.8 ± 0.13a	8.9 ± 0.29fgh	10.5 ± 0.35bc	3.4 ± 0.94b	6.8 ± 1.55b	13.2 ± 1.35abc	8.7 ± 0.32ef
121-08	16.0 ± 0.73d	26.3 ± 0.21cd	9.1 ± 0.12efg	8.7 ± 0.45de	4.7 ± 0.48ab	11.9 ± 1.91a	11.8 ± 1.73abc	10.6 ± 0.38bcd
161-07	16.1 ± 0.01d	21.6 ± 0.07e	8.2 ± 0.20ghi	6.7 ± 0.61gh	2.6 ± 0.98b	9.2 ± 1.10ab	14.1 ± 1.15a	6.8 ± 0.25gh
169-07	9.8 ± 0.12l	26.9 ± 0.52c	8.8 ± 0.33fgh	10.4 ± 0.51bc	8.7 ± 1.97a	10.3 ± 1.32ab	13.3 ± 1.03abc	7.8 ± 0.45fgh
184-08	7.3 ± 0.11b	9.3 ± 0.41j	7.1 ± 0.29ij	6.9 ± 0.75fgh	3.0 ± 1.05ab	9.2 ± 1.22ab	9.4 ± 1.62abcd	10.9 ± 0.32bc
268-06	11.6 ± 0.11k	33.8 ± 0.28a	7.0 ± 0.66j	9.8 ± 0.35cd	5.4 ± 1.00ab	9.5 ± 1.70ab	12.5 ± 1.92abc	10.3 ± 0.49cd
281-07	7.3 ± 0.11g	29.8 ± 0.60b	8.5 ± 0.40gh	10.6 ± 0.47bc	3.9 ± 0.78 ab	11.7 ± 1.23a	12.5 ± 1.59abc	5.0 ± 0.37i
348-07	13.4 ± 0.02h	19.6 ± 0.22f	10.0 ± 0.34bcd	7.7 ± 0.55efg	2.0 ± 0.33b	9.1 ± 0.97ab	9.9 ± 1.73abcd	10.6 ± 0.33bcd
354-05	13.5 ± 0.02h	13.0 ± 0.31i	11.6 ± 0.34a	7.9 ± 0.35efg	2.7 ± 1.48b	8.2 ± 1.56ab	6.5 ± 1.04d	8.7 ± 0.53ef

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3)

Table 5.3b Contents of essential amino acids (mg/g) of different Cantharellaceae species

Collection No.	Lysine	Leucine	Isoleucine	Methionine	Valine	Phenylalanine	Tyrosine	Cystine
MSR2-07	10.3 ± 0.27cde	11.0 ± 1.4de	9.2 ± 0.28a	1.7 ± 0.20def	9.7 ± 1.2ab	5.8 ± 0.25fg	9.4 ± 0.40ab	0.66 ± 0.17bcd
65-07	11.2 ± 0.22ab	14.8 ± 1.8a	9.3 ± 0.41a	2.1 ± 0.22cde	6.4 ± 0.68c	9.3 ± 0.35abc	7.4 ± 0.55de	0.15 ± 0.10d
84-08	5.2 ± 0.35i	6.4 ± 0.8d	6.3 ± 0.22def	1.8 ± 0.28cde	6.2 ± 0.71c	7.7 ± 0.68de	6.4 ± 0.45ef	1.00 ± 0.23ab
90-09	9.0 ± 0.62f	8.0 ± 0.7abc	5.2 ± 0.33f	1.6 ± 0.10def	6.3 ± 1.2cd	4.6 ± 0.61g	4.7 ± 0.50gh	0.71 ± 0.21c
106-08	8.1 ± 0.22gh	5.0 ± 1.1de	6.4 ± 0.13de	4.8 ± 0.20ab	6.4 ± 0.48c	6.8 ± 0.25ef	8.8 ± 0.40abcd	0.59 ± 0.15bcd
107-07	11.1 ± 0.26ab	6.1 ± 1.4de	8.4 ± 0.14ab	1.6 ± 0.08ef	4.9 ± 0.26cd	9.3 ± 0.50abc	5.6 ± 0.45fg	0.92 ± 0.27ab
113-07	9.1 ± 0.42f	7.2 ± 0.9cd	3.9 ± 0.286g	1.5 ± 0.32ef	5.9 ± 0.88cd	8.8 ± 0.30bcd	5.8 ± 0.35fg	1.14 ± 0.14a
121-08	8.8 ± 0.14fg	5.3 ± 1.3de	7.2 ± 0.24cd	4.2 ± 0.23b	4.9 ± 0.21cd	7.8 ± 0.50cde	8.8 ± 0.77abc	0.21 ± 0.15cd
161-07	10.3 ± 0.43cde	14.6 ± 2.2a	7.7 ± 0.28bc	2.2 ± 0.10cd	9.2 ± 0.91b	6.6 ± 0.60ef	7.6 ± 0.50cde	0.53 ± 0.09bcd
169-07	7.4 ± 0.20h	4.1 ± 1.0de	7.3 ± 0.45bcd	5.3 ± 0.19a	4.2 ± 0.25d	7.7 ± 0.40de	10.4 ± 0.50a	1.08 ± 0.15ab
184-08	7.3 ± 0.22h	6.0 ± 0.7de	5.8 ± 0.41ef	0.8 ± 0.34g	5.5 ± 0.22cd	4.7 ± 0.40g	5.0 ± 0.50fgh	1.02 ± 0.18ab
268-06	11.2 ± 0.23a	5.6 ± 1.5ab	7.1 ± 0.28cd	1.1 ± 0.03fg	11.2 ± 0.57a	10.8 ± 0.76a	7.5 ± 0.55de	0.90 ± 0.21ab
281-07	11.4 ± 0.13a	5.4 ± 1.2de	9.2 ± 0.20a	2.4 ± 0.16c	5.4 ± 0.36cd	9.2 ± 0.55bcd	6.4 ± 0.55ef	0.67 ± 0.26bcd
348-07	10.9 ± 0.20bcd	12.0 ± 1.4de	7.2 ± 0.20cd	0.8 ± 0.24g	5.0 ± 0.20cd	9.8 ± 0.45ab	4.4 ± 0.45gh	0.62 ± 0.23bcd
354-05	9.6 ± 0.33ef	4.5 ± 1.2de	7.3 ± 0.37bcd	1.7 ± 0.07def	6.0 ± 0.48cd	8.1 ± 0.50cde	3.9 ± 0.51h	0.26 ± 0.14cd

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3)

5.4 Antioxidant potential

The current study was also undertaken to measure the antioxidant potential from aqueous and alcoholic extracts of fruit bodies of different Cantharellaceae naturally grown in forests of western Himalayan region of India. The maximum hydroxyl radical scavenging activity observed for the alcoholic extract in the fruit bodies of *Cr. cornucopioides* var. *mediosporus* (268-06) was 40.7% followed by 39.6% in *C. miniatescens* (65-07) and minimum (22.7%) was in *C. himalayensis* (169-07) (Table 5.4 and Fig. 5.4). Among aqueous extracts, maximum hydroxyl radical scavenging activity was observed in *Cr. cornucopioides* var. *mediosporus* (268-06) *i.e*. 32.6% and minimum was in *C. himalayensis* (169-07) *i.e*. 14.4%.

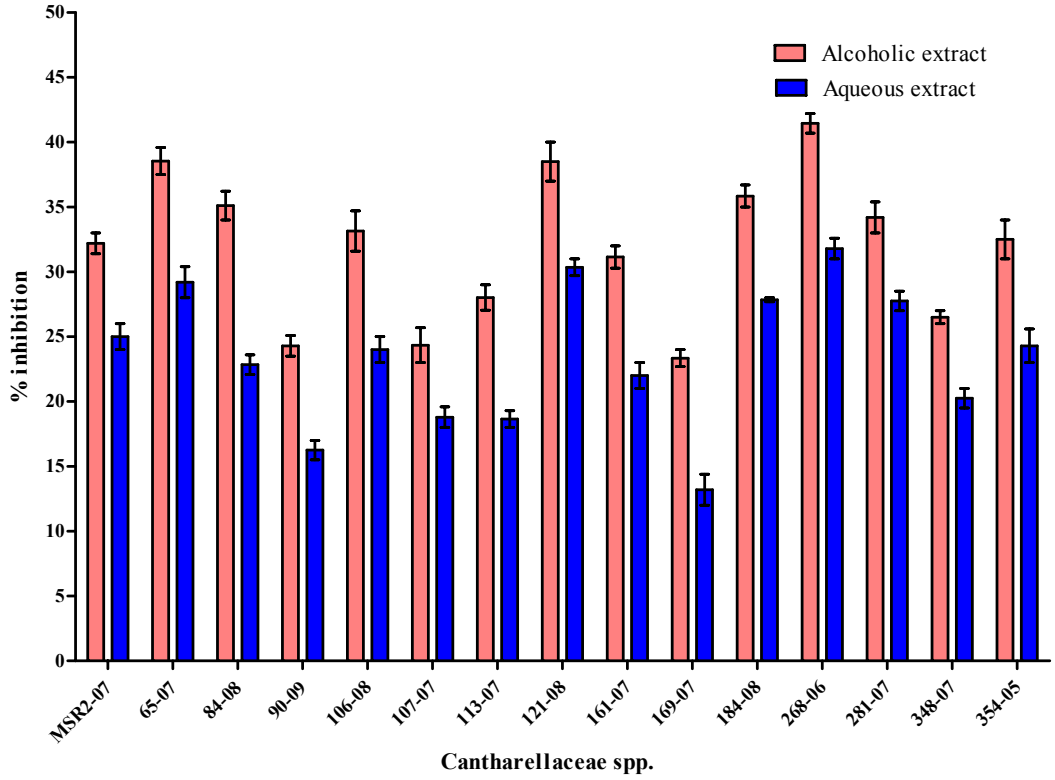

Fig. 5.4 Inhibition (%) of hydroxyl radical scavenging for antioxidant activity by different Cantharellaceae species

The methanolic and aqueous extracts of dried mushroom species were analyzed for antioxidant activity. The differences between water and alcoholic extracts of all the Cantharellaceae species on peroxidation in linoleic acid emulsion are shown in Fig. 5.5a. The antioxidant activity of both extracts increased significantly at the end of second hours compared to first and third hours in all investigated mushrooms. The alcoholic extracts were most effective and maximum activity was observed in fruit bodies of *Cr. cornucopioides* var. *mediosporus* (268-06). The inhibition of peroxidation in the linoleic acid in *Cr. cornucopioides* var. *mediosporus* (268-06) was 41.6%, 54.2% and 17.5% after incubation of one, two and three hours, respectively (Table 5.4 and Fig. 5.5a). The minimum inhibition observed in *C. himalayensis* (169-07) was 20% after one hour of incubation that increased to 35% after 2 hours of incubation and decreased to 16% at the end of 3 hours.

The inhibition of peroxidation in the linoleic acid in aqueous extract of fruit bodies of Cantharellaceae was significantly less effective compared to alcoholic extract. The maximum inhibition was observed in the aqueous extract of *C. pseudoformosus* (281-07) as 9% after one hour of incubation. The inhibition values were increased up to 15% after 2 hours while decreased to 5.5% after 3 hours of incubation. The minimum inhibition was observed in the aqueous extract of *C. elongatipes* (3.8%) after one hour of incubation. The antioxidant activity proved the ability to inhibit the peroxidation of linoleic acid. Studies thus provided the precise antioxidant status of indigenous species of Cantharellaceae, which can serve as useful database for the selection of mushrooms for the function of preparation of mushroom-based nutraceutical foods.

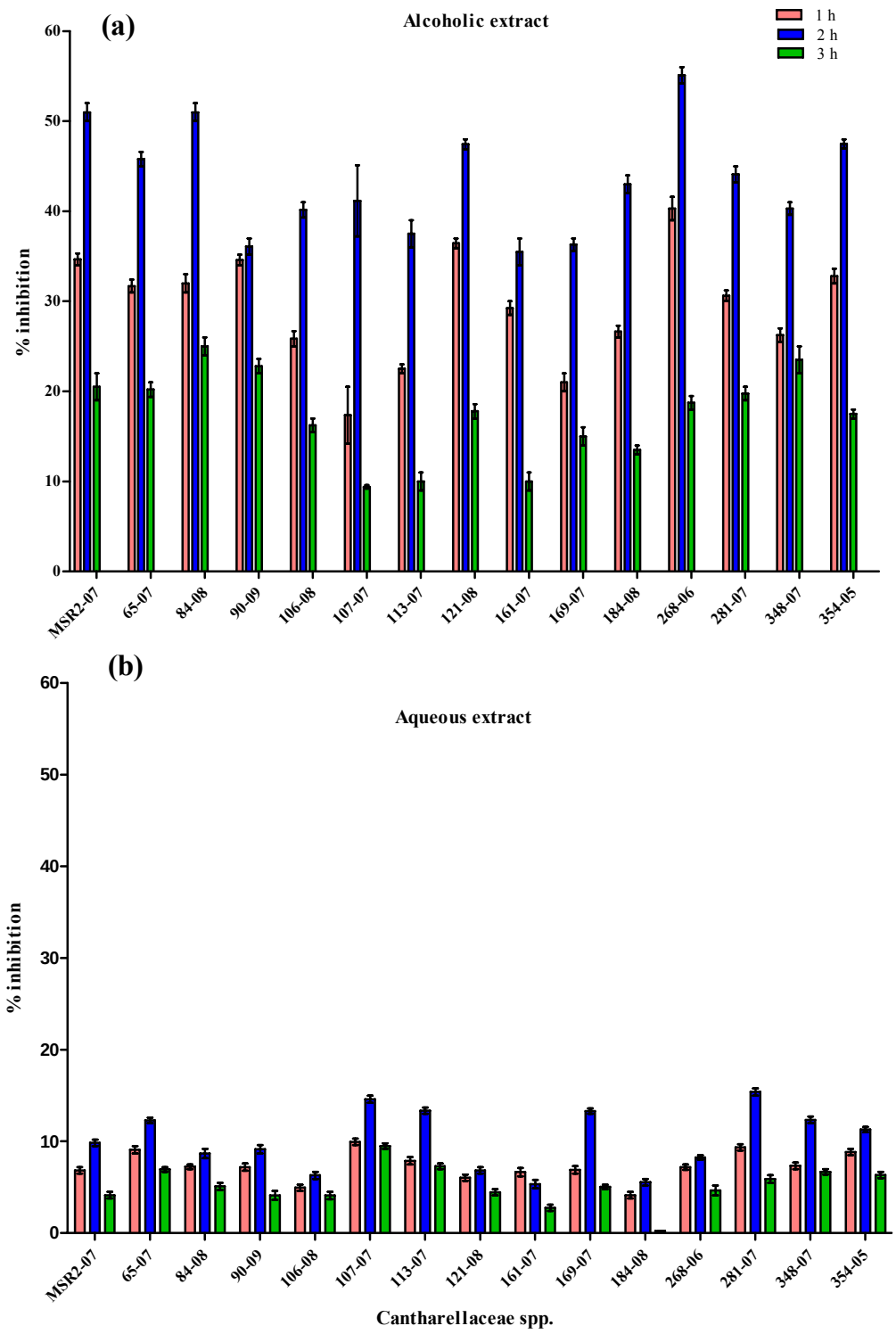

Fig. 5.5 Inhibition (%) of linoleic acid peroxidation for antioxidant activity in (a) alcoholic and (b) aqueous extracts by different Cantharellaceae species

The results for ABTS radical scavenging capacity are given in Table 5.4 and Fig. 5.6. It was observed that maximum inhibition (66%) was found in alcoholic extract of *Cr. cornucopioides* var. *mediosporus* (268-06) and minimum (57.2%) was in the extract of *C. himalayensis* (169-07). Among the aqueous extracts, maximum activity was observed in the fruit body of *Cr. cornucopioides* var. *mediosporus* (268-06) as 64% and minimum in *C. fibrillosus* (113-07) as 42.5%. Both extracts showed significant amount of free radical scavenging activity in all the species of Cantharellaceae.

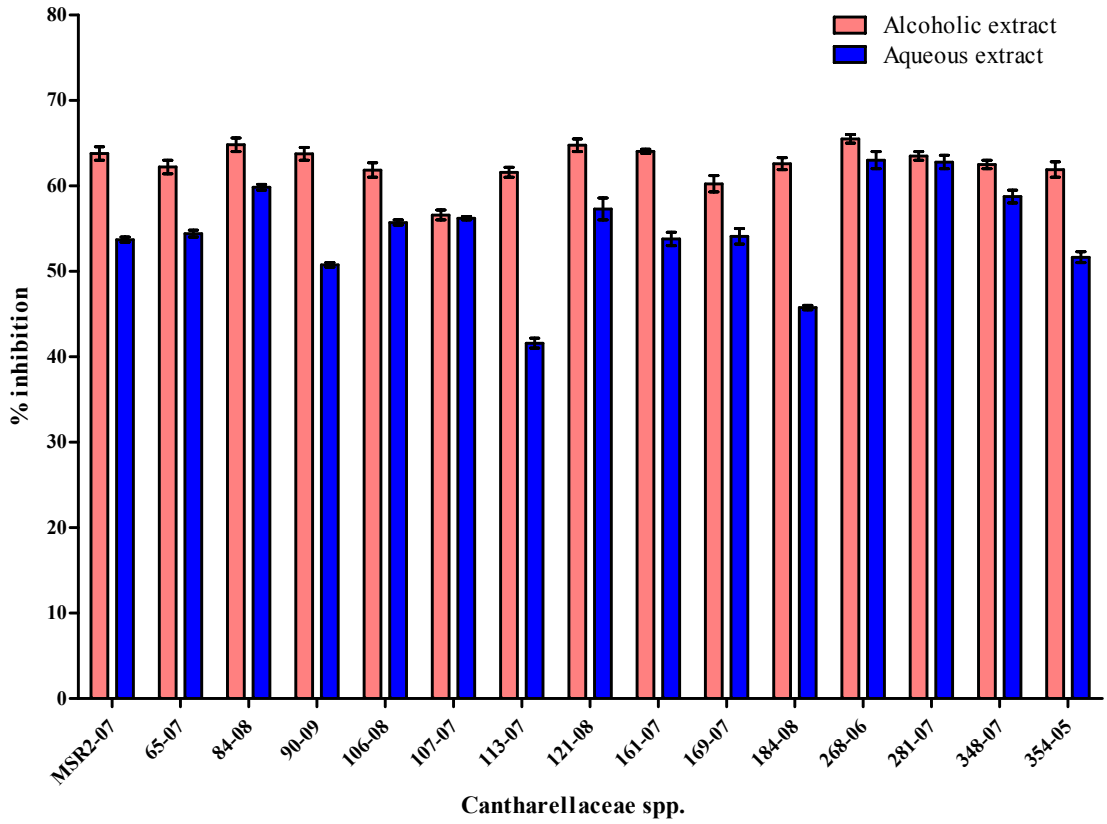

Fig. 5.6 Inhibition (%) of ABTS radical decolourization assay for antioxidant activity by different Cantharellaceae species

Table 5.4 Antioxidant potential study of extracts of Cantharellaceae species

Collection No.	Inhibition (%) of linoleic acid peroxidation						Inhibition (%) of Hydroxyl radical scavenging		Inhibition (%) of ABTS radical decolonization assay	
	Alcoholic Extract			Aqueous Extract			Alcoholic Extract	Aqueous Extract	Alcoholic Extract	Aqueous Extract
	1 hr	2 hrs	3 hrs	1 hr	2 hrs	3 hrs				
MSR2-07	35.3bc	50.0ab	22.0abcde	7.2efg	9.5ef	3.8fg	31.4cdef	26.0bcd	64.6abc	54.0ef
65-07	32.4cd	46.6c	19.4abcd	9.5bcd	12.0cd	6.7bc	39.6ab	30.4ab	61.4abc	54.8def
84-08	33.0cd	50.0ab	26.0a	7.5def	8.2efg	4.7def	36.2bcd	23.6cde	65.6ab	60.2bcd
90-08	35.2bc	43.5fg	23.6abc	7.6def	8.7ef	3.6fg	25.1g	17.0fg	64.5abc	51.0f
106-08	26.7fg	39.3def	17.0efg	4.6gh	5.9hi	3.7fg	34.7bcde	25.0cd	62.7abc	55.4cdef
107-07	37.2ab	45.1cd	20.5bcdef	9.6ef	14.2a	9.2a	25.7g	19.6ef	57.2d	56.4cdef
113-07	22.0ghi	36.0ef	9.0h	7.5cdef	13.0bc	7.0b	27.1efg	19.3ef	62.2abc	42.5h
121-08	35.9ab	46.9bc	18.6cdef	5.7fgh	6.5ghi	4.1efg	40.0ab	29.7a	65.5ab	58.6cde
161-07	28.5def	37.0fg	11.0h	6.2fg	4.9i	2.4g	30.3cdef	23.0de	64.3abc	54.6ef
169-07	20.0hi	35.6fg	16.0fgh	6.5efg	13.0bc	4.8def	22.7g	14.4g	59.3cd	53.2f
184-08	27.3efg	42.0d	14.0gh	3.8h	5.2i	0.2h	36.7abc	27.7abc	63.3a	45.5g
268-06	41.6a	54.2a	17.5cdef	6.9def	8.0fgh	4.1efg	40.7a	32.6a	66.0a	64.0a
281-07	31.2cde	43.6cd	20.5bcdef	9.0bc	15.0a	5.5cdef	35.4bcd	28.5abc	64.0abc	63.6b
348-07	25.5efg	39.6def	25.0ab	7.0def	12.0c	6.4bcd	27.0fg	19.5def	63.0abc	59.5bcde
354-05	33.6bcd	48.0bc	18.0defg	8.5cde	11.0cde	6.0cdef	34.0bcde	25.6bcd	62.8abc	52.3bc

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3).

5.5 Bioactive compounds

The three most important bioactive compounds *viz*. phenol, flavonoids and carotene in the extracts of Cantharellaceae fruit bodies were presented in Fig. 5.7. The content of total phenol was calculated on the basis of the calibration curve of gallic acid. The maximum total phenolic compounds (13.34 mg/g) were recorded in *C. appalachiensis* (84-08) followed by 12.46 mg/g in *C. fibrillosus* (113-07) and minimum (7.67 mg/g) in *C. natarajanii* (106-08) (Table 5.5).

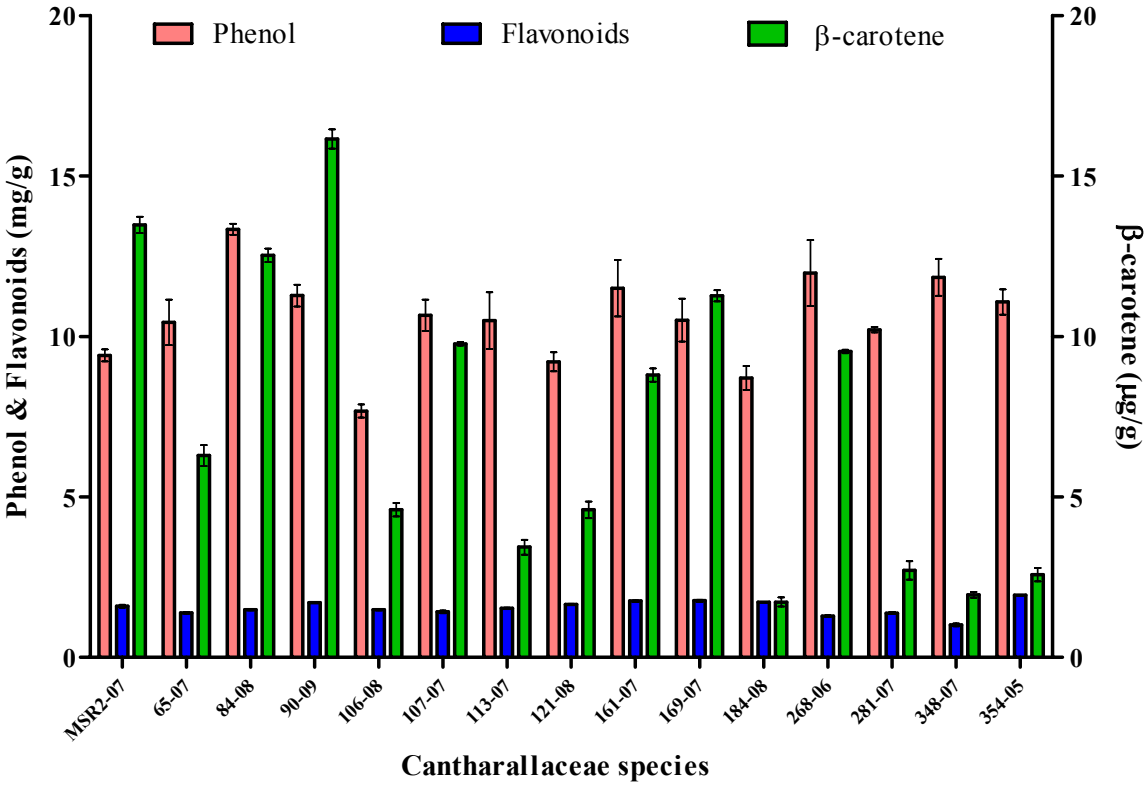

Fig. 5.7 Productions of bioactive compounds *i.e*., phenol (mg/g), flavonoids (mg/g) and β-carotene (µg/g) by different Cantharellaceae species

Flavonoid was found in small amounts, ranging between 0.95 mg/g in *C. umbonatus* (348-07) and 1.94 mg/g in *C. minor* (354-05). The β-carotene content was found in very low amount in different Cantharellaceae species ranging from 1.49 μg/g in *C. elongatipes* (184-08) to 15.67 μg/g in *C. cibarius* (90-09). Phenol was the major antioxidant component detected in all species of Cantharellaceae.

Table 5.5 Bioactive compounds of different Cantharellaceae species

Collection No.	Phenols (mg/g)	Flavonoids (mg/g)	β-carotene (μg/g)
MSR2-07	9.40 ± 0.31bcd	1.68 ± 0.13abcd	13.02 ± 0.24b
65-07	10.44 ± 0.22abcd	1.35 ± 0.17cde	5.68 ± 0.16g
84-08	13.34 ± 0.30a	1.45 ± 0.05bcde	12.18 ± 0.20bc
90-08	11.94 ± 1.10ab	1.72 ± 0.07abc	15.67 ± 0.20a
106-08	7.67 ± 0.35d	1.46 ± 0.14bcde	4.20 ± 0.29h
107-07	11.40 ± 1.05abc	1.34 ± 0.12bcde	9.88 ± 0.39d
113-07	12.46 ± 0.52bcd	1.51 ± 0.04bcdc	3.00 ± 0.201i
121-08	9.20 ± 0.51bcd	1.62 ± 0.11abcd	4.11 ± 0.28h
161-07	11.50 ± 1.52abc	1.72 ± 0.04abc	8.40 ± 0.28f
169-07	10.50 ± 0.15abcd	1.71 ± 0.20abc	11.20 ± 0.33d
184-08	8.70 ± 0.64cd	1.73 ± 0.07ab	1.49 ± 0.19k
268-06	11.97 ± 1.77ab	1.24 ± 0.17ef	9.43 ± 0.53e
281-07	10.20 ± 0.15bcd	1.32 ± 0.12def	2.15 ± 0.17jk
348-07	11.84 ± 1.10ab	0.95 ± 0.09f	1.78 ± 0.25jk
354-05	11.07 ± 0.68abc	1.94 ± 0.12a	2.16 ± 0.11ijk

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3).

5.6 Vitamins

The amount of vitamin A, vitamin B-complex, vitamin C, vitamin D2 (ergocaliferol) and vitamin E (tocopherol) in the different species of Cantharellaceae was presented in Table 5.6. The results have been expressed in µg/g for all vitamins except vitamin C (in mg/g) of fresh weight, calculated by internal normalization of the chromatographic peak area. A representative chromatogram is shown in Appendix III.

All the Cantharellaceae fruit bodies produced significant amount of vitamin A (Fig 5.9). The maximum content of vitamin A (11.6 µg/g) in the analyzed mushrooms was observed in *C. minor* (354-05) and minimum (1.4 µg/g) in *C. fibrillosus* (113-07). It has been reported that, the edible mushrooms are good sources of vitamin B complex including thiamine (B1), riboflavin (B2), niacin (B3) and cynocobalmine (B12). The thiamine contents ranged from 1.9 µg/g in *C. lateritius* (161-07) to 6.2 µg/g in *C. minor* (354-05). The riboflavin content was maximum (63 µg/g) in *C. minor* (354-05) and minimum (13 µg/g) in *C. cibarius* (90-09). Niacin was very high among vitamin B-complex in all different species of Cantharellaceae (Fig 5.8). The niacin contents varied from 26 µg/g in *C. lateritius* (161-07) to 73 µg.g in *C. minor* (354-05). The low amount of cynocobalmine was observed in all the Cantharellaceae species. The maximum content of cynocobalmine (0.67 µg/g) was found in *C. natarajanii* (106-08) and minimum (0.34 µg/g) in *Cr. cornucopioides* var. *mediosporus* (268-06). The result showed that *C. minor* (354-05) is a rich source of vitamin B complex.

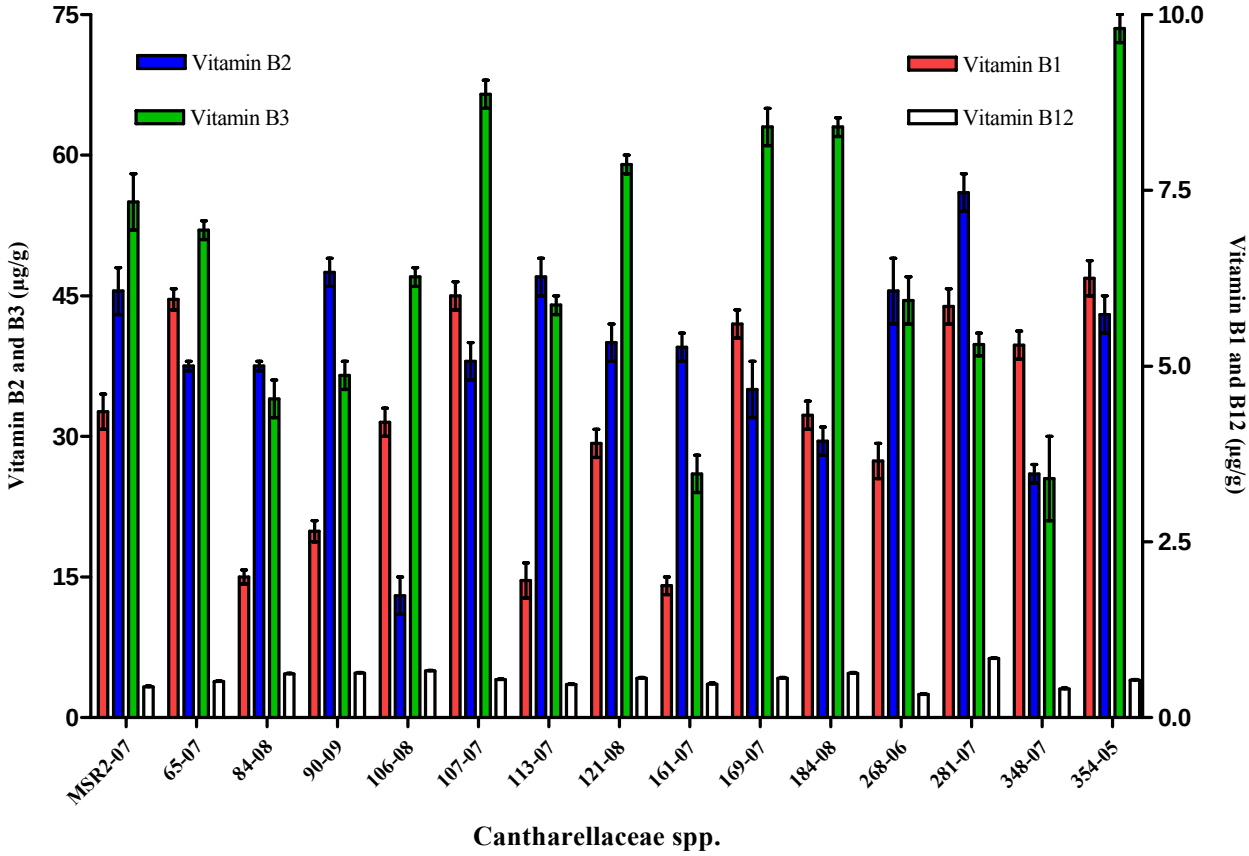

Fig. 5.8 Contents of vitamins B1, B2, B3 and B12 in different Cantharellaceae species

Vitamin C (ascorbic acid) was in the range of 0.41 mg/g in *C. lateritius* (161-07) to 1.2 mg/g in *Cr. cornucopioides* var. *mediosporus* (268-06). The fruit bodies of *C. cibarius* (90-08) contained 0.94 mg/g vitamin C (Fig 5.9). Out of all the Cantharellaceae species, the maximum concentration of vitamin D (1.68 µg/g) was observed in *C. umbonatus* (317-07) and minimum (0.87 µg/g) in *Cr. cinerius* (107-08).

The maximum amount of vitamin E (0.93 µg/g) was observed in *C. natarajanii* (106-08) followed by 0.75 µg/g in *C. lateritius* (161-07) and minimum (0.63 µg/g) in *C. himalayensis* (169-07) (Table 5.6 and Fig. 5.9).

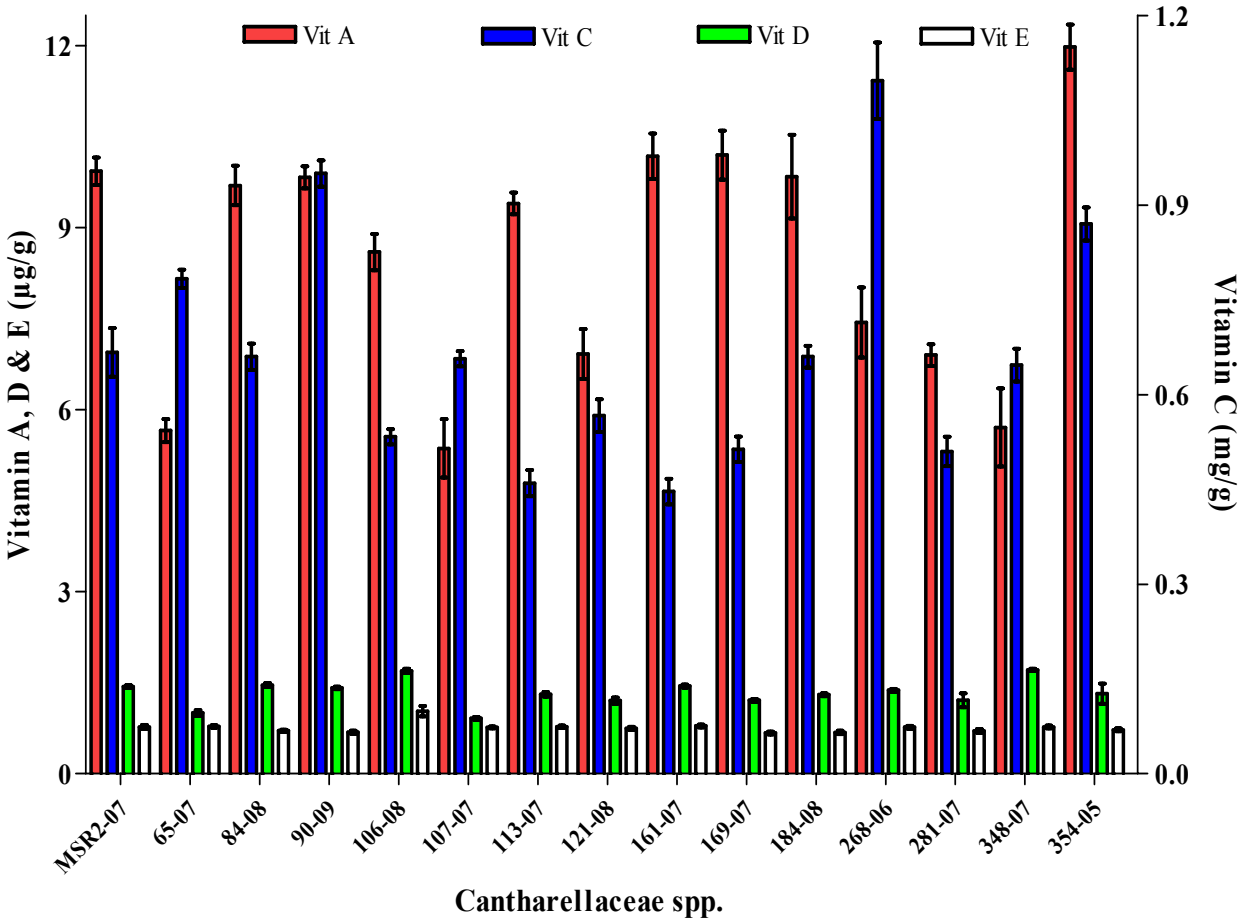

Fig. 5.9 Contents of vitamins A, C, D and E in different Cantharellaceae species

Table 5.6 Contents of various vitamins in different species of Cantharellaceae

Collection No.	Vitamin A (µg/g)	Thiamin (µg/g)	Riboflavin (µg/g)	Niacin (µg/g)	Cynocobalmine (µg/g)	Vitamin C (mg/g)	Vitamin D (µg/g)	Vitamin E (µg/g)
MSR2-07	10.8 ± 0.13abcd	4.3 ± 0.35b	46.5 ± 2.1bc	55 ± 4.2cde	0.45 ± 1.2cd	0.61 ± 0.03fgh	1.39 ± 0.01abcd	0.73 ± 0.09ab
65-07	8.1 ± 0.17cde	5.9 ± 0.02b	37.5 ± 0.7ef	52 ± 0.1def	0.52 ± 2.1 bc	0.78 ± 0.06cde	0.93 ± 0.07f	0.74 ± 0.13b
84-08	8.7 ± 0.05bcde	2.0 ± 0.14f	47.5 ± 2.1bc	34 ± 2.8jkl	0.63 ± 0.4 ab	0.62 ± 0.02fg	1.40 ± 0.12bcd	0.68 ± 0.06ab
90-08	10.3 ± 0.07abc	2.6 ± 0.21def	13.0 ± 2.3k	36 ± 2.1ijk	0.64 ± 0.2ab	0.94 ± 0.03b	1.38 ± 0.13bcd	0.64 ± 0.08ab
106-08	8.8 ± 0.14bcde	3.8 ± 0.84cd	47.0 ± 2.8bc	47 ± 1.4efgh	0.67 ± 0.1ab	0.51 ± 0.05ghi	1.63 ± 0.10ab	0.93 ± 0.04a
107-07	8.5 ± 0.12bcde	6.0 ± 0.28b	38.0 ± 2.8ef	66 ± 2.1ab	0.55 ± 0.8bc	0.65 ± 0.03def	0.87 ± 0.03f	0.73 ± 0.06ab
113-07	1.4 ± 0.16cde	5.6 ± 0.28b	58.5 ± 2.1°	50 ± 3.5def	0.54 ± 0.1bc	0.79 ± 0.06bcd	1.24 ± 0.10def	0.68 ± 0.01ab
121-08	8.3 ± 0.11abcd	3.9 ± 0.28cd	40.0 ± 2.8e	60 ± 1.4bcd	0.57 ± 1.1bc	0.52 ± 0.05ghi	1.11 ± 0.13def	0.70 ± 0.04ab
161-07	10.3 ± 0.04abc	1.9 ± 0.17a	39.5 ± 2.1ef	26 ± 2.8l	0.49 ± 0.2cd	0.41 ± 0.02i	1.40 ± 0.12abcd	0.75 ± 0.03ab
169-07	10.2 ± 0.20abc	5.6 ± 0.28b	35.0 ± 4.4fg	63 ± 2.8bc	0.57 ± 1.6 bc	0.48 ± 0.04ghi	1.17± 0.16cdef	0.63 ± 0.07ab
184-08	10.3 ± 0.07ab	4.3 ± 0.28bc	29.5 ± 2.1gh	63 ± 1.4bc	0.64 ± 0.1ab	0.63 ± 0.05ef	1.26 ± 0.01cde	0.63 ± 0.03ab
268-06	7.4 ± 0.17ef	3.6 ± 0.35cde	47.0 ± 2.8bc	44 ± 3.5fghi	0.34 ± 0.3cde	1.2 ± 0.10a	1.33 ± 0.09bcd	0.73 ± 0.09ab
281-07	7.9 ± 0.12def	5.8 ± 0.35b	56.0 ± 2.8ab	40 ± 1.7ghijk	0.85 ± 0.2a	0.47 ± 0.04hi	0.98 ± 0.03f	0.65 ± 0.04ab
348-07	5.7 ± 0.09f	5.3 ± 0.28b	26.0 ± 1.4hi	31 ± 2.1kl	0.42 ± 1.3cd	0.60 ± 0.40fgh	1.68 ± 0.04a	0.73 ± 0.05ab
354-05	11.6 ± 0.12a	6.2 ± 0.35b	43.0 ± 2.8de	73 ± 0.2a	0.54 ± 0.1bc	0.82 ± 0.04bc	1.00 ± 0.03ef	0.68 ± 0.04ab

Values bearing different letters in the same column are significant at $P<0.05$. All values are Mean ± SD (n = 3).

5.7 Free cholesterol

The potentiality of free cholesterol production by all the investigated Cantharellaceae mushrooms was also studied. Total cholesterol was obtained by high-performance liquid chromatography (HPLC) without any derivatization of cholesterol. The content of free cholesterol was recorded in µg per 100 gram dry weight of mushroom. The concentration of cholesterol was recorded in the range from 1.5 µg in *C. elongatipes* (184-08) to 15.7 µg in *C. cibarius* (90-09). *Cantharellus umbonatus* (348-07) contained only 1.8 µg free cholesterol per 100 gram of dry weight while *Craterellus cinerius* (107-07) had 2.5 µg free cholesterol in its fruit body. The two *Cantharellus* species, *C. minor* (354-05) and *C. pseudoformosus* (281-07) recorded nearly equal cholesterol amount (2.1 µg). *Cantharellus fibrillosus* (113-07) and *C. applanatus* (121-08) also produced very low amount of free cholesterol, 3 µg and 4.1 µg, respectively. *Cantharellus appalachiensis* (84-08) had 12.2 µg free cholesterol in its fruit body while *C. indicus* (MSR2-07) contained 13 µg of free cholesterol. Fig. 5.10 shows that all the species of Cantharellaceae have very low or negligible amount of free cholesterol.

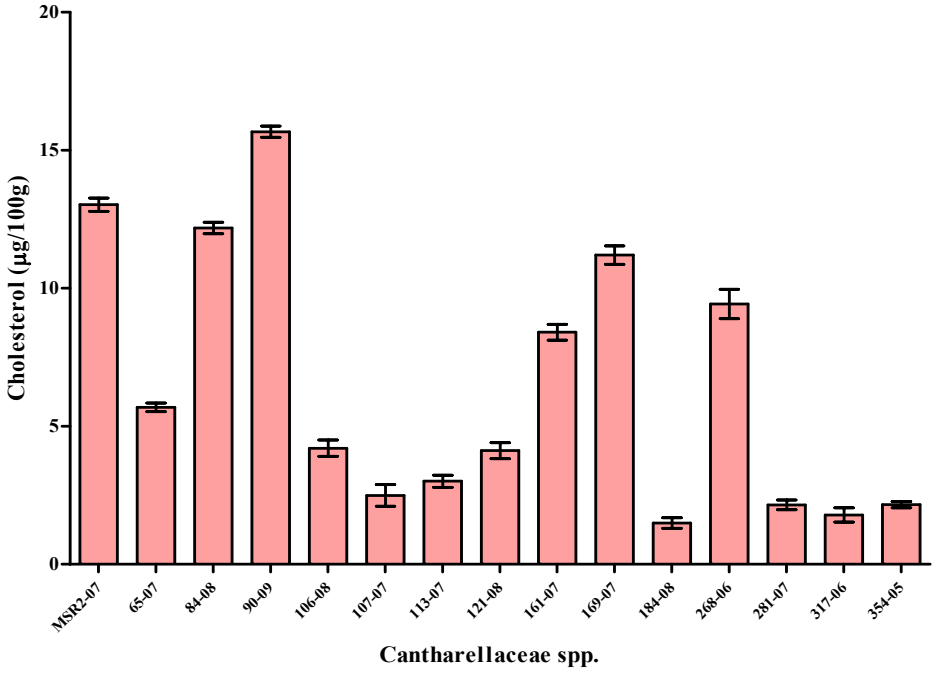

Fig. 5.10 Cholesterol content in different Cantharellaceae species

Salient findings:

- All the Cantharellaceae species have high nutritive value, specific aroma and supplement to a healthy diet. The significant levels of proteins, lipids, amino acids, vitamins, bioactive compounds and some neutraceuticals in their fruit bodies are of considerable value.
- Among all the studied Cantharellaceae species, protein was the principal macronutrient and all mushrooms also contained good amount of carbohydrates.
- The low fat or lipid contents also suggest that persons those with heart or weight problems can consume Cantharellaceae mushrooms. The presence of low fat content and energy value in different species of Cantharellaceae can be concluded good alternative for low fat/energy diets.
- *Craterellus cornucopioides* var. *mediosporus* and *Cantharellus miniatescens* are good sources of amino acids and *Cantharellus minor* and *C. natarajanii* are good sources of vitamins.
- All the investigated mushrooms also showed significant antioxidant activities. The nutritional and nutraceutical properties of these mushrooms are not reported before.

Chapter 6

Diversity of bacteria associated with Cantharellaceae

6. 1 Isolation of bacteria, screening and selection

Bacteria were isolated from internal tissue of sporocarps of Cantharellaceae species that were collected from the forests of Western Himalayas, India. The collected fruit bodies of present study contained 0.5-1.1 × 10^3 *cfu* (*c*olony *f*orming *u*nit) of bacteria per g of fresh weight at pH 4.5-5 on Nutrient agar media. Most of the bacterial colonies were appeared at 10^{-1} dilution, however no colonies were observed when samples were diluted three or four fold. Fifty endophytic bacteria were randomly selected out of 76 isolates from ten sporocarps (*Cantharellus appalachinesis*, *C. elongatipes* sp. nov., *C. lateritius*, *C. natarajanii* sp. nov., *C. cibarius*, *C. indicus* sp. nov., *C. pseudoformosus* sp. nov., *C. himalyensis* sp. nov., *C. umbonatus* sp. nov. and *Craterellus cornucopioides* var. *mediosporus*) based on their morphology and growth. All the isolates were sub-cultured at least three times to confirm purity. These isolates were designated as CB1 to CB50 (Table 6.1).

6.2 REP- and BOX-PCR based fingerprinting

These isolates were examined by REP- and BOX-PCR analysis and differentiated on the basis of different DNA banding patterns (Fig. 6.1). Amplification of the regions between highly conserved repetitive DNA sequences of the REP and BOX element of isolate-specific DNA fingerprints was generated. The number of bands obtained was between 5 and 30, with average 17.5 bands for each of the 50 isolates analyzed. Among 50 isolates, 28 revealed complex banding patterns while 22 isolates exhibited less than 9 bands. The isolates yielded a complex genomic fingerprint ranged from 0.22 to 2.99 kb and from 0.18 to 2.68 kb amplicons for REP- and BOX-PCR respectively. Visual observation of the DNA fingerprints clearly differentiated similarity and dissimilarity among bacterial strains. These results showed complex banding patterns, which reflected a high degree of inter or intra specific genetic unrelatedness among isolates. The dendrogram generated divided bacteria into 30 distinct banding patterns, which were later identified as different bacterial strains.

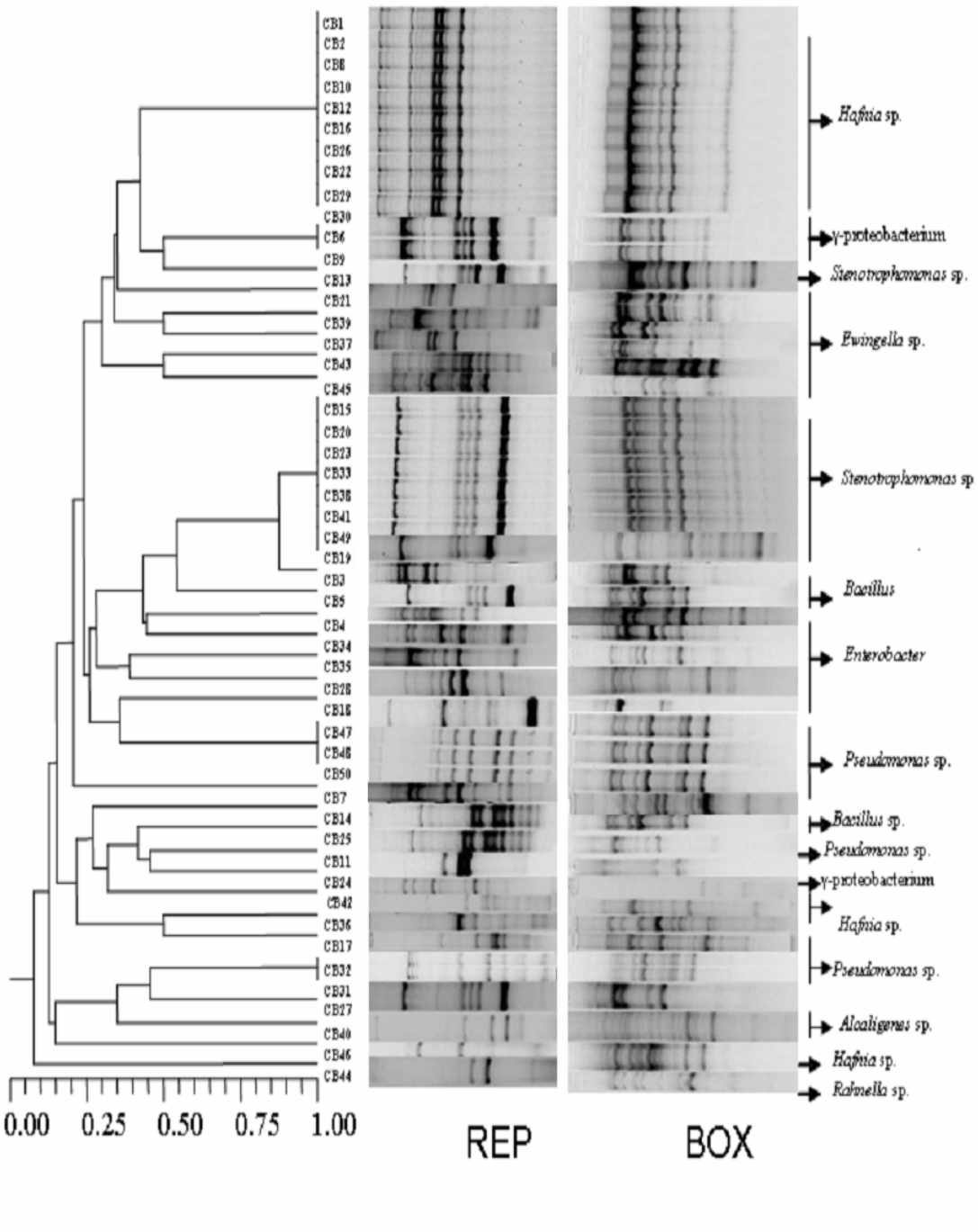

Fig. 6.1 Dendrogram generated from BOX- and REP-PCR fingerprints of endophytic bacterial isolates from fruit bodies of Cantharellaceae species.

Table 6.1 Location of different Cantharellaceae spp. and their associated bacteria

Species name	Locality (forest)	Strain name
C. appalachinesis	Chail (H.P.)	CB-1, 2
		CB-3
		CB-4
		CB-5
		CB-6
		CB-7
C. elongatipes sp. nov.	Bharsar (U.K.)	CB-8, 10
		CB-9
		CB-11
C. lateritius	Kadha Pathar (H.P.)	CB-12, 16
		CB-13
		CB-14
		CB-15
		CB-17
C. natarajanii sp. nov.	Jageshwar (H.P.)	CB-18
		CB-19, 20
		CB-21
		CB-22
		CB-23
C. cibarius	Karol (H.P.)	CB-24
		CB-25
		CB-26
		CB-27
		CB-28
C. indicus sp. nov.	Karol (H.P.)	CB-29, 30
		CB-31, 32
		CB-33
C. himalyensis sp. nov.	Kufri (H.P.)	CB-34, 35
		CB-36
		CB-37
		CB-38
C. pseudoformosus sp. nov.	Khajjiar (H.P.)	CB-39
		CB-40
		CB-41
		CB-42
		CB-43
C. umbonatus sp. nov	Khada Pathar (H.P.)	CB-44
		CB-45
		CB-46
Cr. cornucopioides var. *mediosporus*	Pithoragarh (U.K.)	CB-47, 48, 50
		CB-49

Note: H.P. and U.K. denotes Himachal Pradesh and Uttarakhand, respectively

6.3 Biochemical characterization of endophytic isolates

Majority of isolates showed negative to Gram reaction except four isolates (CB3, CB5, CB14 and CB25) which showed positive reaction. All isolates were catalase positive; most of them were oxidase positive, except strains CB21, CB37, CB39, CB43 and CB45. Esculin was hydrolyzed by all isolates. All the strains were able to hydrolyze starch except three strains (CB13, CB15 and CB19) while casein was not hydrolyzed by 9 strains (CB9, CB21, CB24, CB27, CB37, CB39, CB40, CB43 and CB45). Gelatin was not hydrolyzed by CB9, CB24, CB27, CB40 and CB44. Citrate was utilized by all the bacterial strains except CB3, CB5, CB14, CB25 and CB44. Similarly, malonate was utilized by all the bacterial strains except CB4, CB28, CB34, CB35 and CB44 (Table 6.2). A positive result for phenylalanine deamination and nitrate reduction was observed with most of the bacterial strains. Most of the bacterial isolates were able to grow between 25 and 30°C (Table 6.2).

Most of the isolates were able to ferment different carbon substrates like glucose, glycerol, lactose, ONPG, ribose, fructose, sucrose, D-arabinose, xylose and urease. Four strains (CB3, CB13, CB14 and CB15) were able to utilize maximum number (10) of carbon substrates. Antibiotic profiling study showed that many isolates were susceptible to most of the antibiotics used (Table 6.3).

6.4 Quantification of indoleacetic acid (IAA) production

All bacterial strains produced the plant growth hormone IAA (ranging from 8.00 to 37 µg/ml). CB1, CB8, CB29 and CB42 produced higher amount of IAA (28.9 to 37 µg/ml) compared to other isolates (Table 6.2). CB9, CB21, CB24, CB37, CB39, CB43 and CB45 produced least amount of IAA (8.00 to 12.00 µg/ml).

Table 6.2 Physiological characteristics of endophytic bacterial isolates from fruit bodies of Cantharellaceae spp.

Bacterial Isolates (number)	Gram Staining	Nitrate Reduction	Citrate Utilization	Malonate Utilization	Tyrosine degradation	Phenylalanine deamination	IAA (µg/ml)	Hydrolysis of				Optimum temp (°C)
								S	C	G	E	
Hafnia spp. (4)	-	-	+	+	-	+	†††	+	+	+	+	28-30
Pseudomonas spp. (5)	-	-	+	+	-	-	††	+	+	+	+	25-30
Ewingella spp. (5)	-	+	+	+	-	+	†	+	-	+	+	25-30
Enterobacter spp. (4)	-	-	+	-	+	+	††	+	+	+	+	25-37
Alcaligenes spp. (2)	-	-	+	+	-	-	††	+	-	-	+	28-30
γ-proteobacterium (2)	-	-	+	+	-	-	†	+	-	-	+	25-30
Stenotrophomonas spp. (3)	-	+	+	+	+	-	†††	-	+	+	+	25-30
Bacillus spp. (4)	+	+	-	+	+	+	†††	+	+	+	+	25-37
Rahnella sp. (1)	-	+	-	-	-	+	†	+	+	-	+	25-30

+: positive reaction; -: negative reaction (S = Starch, C = Casein, G = Gelatin, E = Esculin) († = 5-15, †† = 15-25, ††† =25-35)

Table 6.3 Carbon substrate fermentations by endophytic bacterial isolates from fruit bodies of Cantharellaceae species.

Bacterial isolates	Carbon substrate fermentation	Antibiotic Resistance*
CB1, CB8, CB29, CB42 (All *Hafnia* sp.)	Glucose, D- Arabinose, Rhamnose, Urease, Trehalose, Raffinose, Citrate	Ca, Am/Cx
CB4, CB18 (Both *Enterobacter* sp.)	Glucose, Lactose, Mannitol, Maltose, Xylose, ONPG, Citrate	Ca, Ce
CB21, CB37, CB39, CB43 (All *Ewingella* sp.)	Glucose, Lactose, Mannitol, Maltose, Xylose, ONPG, Citrate	-
CB34, CB35 (Both *Enterobacter* sp.), CB25 (*Bacillus* sp.)	Glucose, Lactose, Mannitol, Maltose, Xylose, ONPG, Citrate	S
CB27, CB40 (Both *Alcaligenes* sp.)	Lactose, Fructose, Trehalose, Sucrose, D-Arabinose, Citrate	Cp
CB9, CB24 (Both γ-proteobacterium)	Lactose, Sucrose, Dextrose, Citrate	P
CB45 (*Ewingella* sp.)	Mannitol, Sucrose, Trehalose, Citrate, D-Arabinose, Xylose, Maltose, Glycerol, Glucose	-
CB7, CB11, CB32, CB31 (*Pseudomonas* sp.)	Mannitol, Sucrose, Trehalose, Citrate, D-Arabinose, Xylose, Maltose, Glycerol, Glucose	P, A, Cp, G
CB47 (*Pseudomonas* sp.)	D-Arabinose, Xylose, Maltose, Glycerol, ONPG, Citrate	P, G
CB19 (*Stenotrophomonas* sp.)	D-Arabinose, Xylose, Maltose, Glycerol, ONPG, Citrate	Cm, N, Cp
CB13, CB15 (Both *Stenotrophomonas* sp.)	Xylose, Fructose, Raffinose, Trehalose, Glycerol, Sorbitol, Mannitol, ONPG, Ribose	Cm
CB3, CB14 (Both *Bacillus* sp.)	Xylose, Fructose, Raffinose, Trehalose, Glycerol, Sorbitol, Mannitol, ONPG, Ribose	-
CB5 (*Bacillus* sp.)	Lactose, Fructose, D-Arabinose, Trehalose, Insulin, Cellobiose, Urease	P
CB44 (*Rahnella* sp.)	Dextrose, Lactose, Trehalose	Cm, Cf

* Ca: Ceftazidime (30 μg), Am/Cx: Ampicillin/Cloxacillin (10/10 μg), Ce: Cephotaxime (30 μg), S: Sparfloxacin (5 μg), Cp: Ciprofloxacin (5 μg), P: Penicillin (10 units), A: Amikacin (30 μg), G: Gentamicin (10 μg), Cm: Chloramphenicol (30 μg), N: Norfloxacin (10 μg), Cf: Cefuroxime (30 μg)

6.5 Identification of the bacterial isolates

All bacterial isolates (CB1-CB30) were subjected to 16S rDNA amplification using universal primers, and about 1.5 kb amplicon was observed in all isolates (Fig. 6.2). The sequences were analyzed by multiple sequence alignment to check the similarity among the isolates. The homology among were from 54-99% between isolates. Minimum of 54% similarity was found in CB11 with CB14 and maximum 99% similarity was found between CB1 and CB8.

Sequences were compared for the similarity in the GenBank DNA database using BlastN (NCBI) (Altschul et al., 1997). Pairwise alignment revealed that 16S rDNA of bacterial isolates had 97 to 100% similarity with the sequences of NCBI database (Table 6.4). CB1, CB8, CB31 and CB42 showed 98-100% similarity with *Hafnia alvei* which was inhabited in 4 different *Cantharellus* species. CB4, CB18, CB28 and CB34 had 99-100% similarity with *Enterobacter* sp., while CB45, CB39, CB37, CB43 and CB21 showed 97-99% similarity with *Ewingella americana*. The isolates CB7, CB11, CB17, CB32 and CB47 were 98-100% similar with different species of *Pseudomonas* such as *P. tolaasii, P. brenneri*, *P. putida,* and *P. fluorescens*, while CB3, CB5, CB14 and CB25 showed 99-100% similarity with *Bacillus* sp., *B. cereus* and *B. pumilus*, these isolates were previously reported from *C. cibarius*. The isolates CB13, CB15 and CB19 showed 99-100% similarity with *Stenotrophomonas* sp., these isolates were also persistent like *Hafnia* in Indian *Cantharellus* species. CB44 had shown 100% similarity with *Rahnella* sp., CB9 and CB24 showed 99-100% similarity γ-proteobacterium, while CB27 and CB40 showed 98-100% similarity with *Alcaligenes faecalis*. These isolates were also reported from other edible mushrooms.

The 16S rDNA gene sequences of all 30 bacterial isolates determined in this study were deposited in the GenBank of NCBI data library under accession numbers GU944485 to GU944514 (Appendix IV).

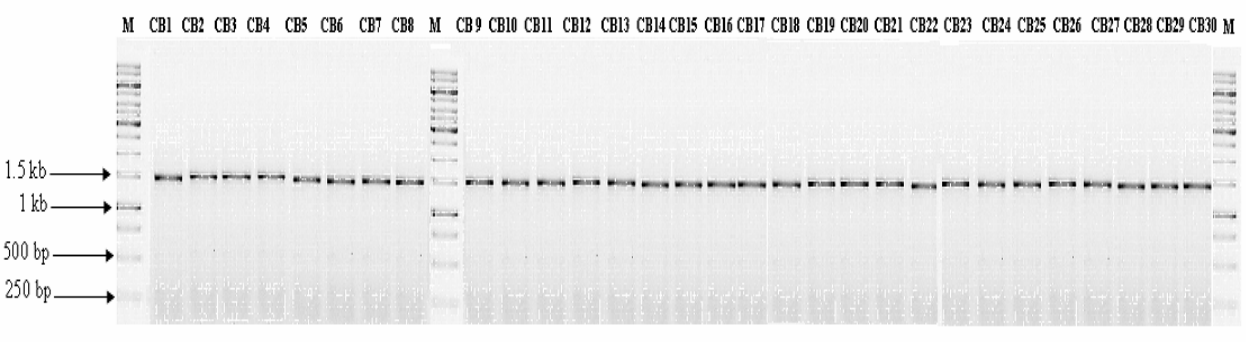

Fig. 6.2 16S rDNA amplification of bacteria isolates from Cantharellaceae. Lane 1-30: different bacterial isolate. Lane M, DNA marker (1 Kb marker; Fermentas). This is a composite figure from different gels.

6.6 Analyses of the 16S rDNA dataset

The aligned dataset contain 987 characters and there were 419, 107 and 458 constant, parsimony-uninformative and parsimony-informative characters, respectively. Maximum parsimony analysis resulted in 5 equally parsimonious trees with the branch-and-bound search yielded parsimony trees (TL = 1,308, CI = 0.65, RI=0.88, HI=0.31). Maximum likelihood analysis recovered as single topology (-ln L = 11769.3371). The resulting MP and ML topologies did not differ significantly. The ML and Bayesian topologies were almost identical. All taxa of endophytic bacteria formed a well-supported monophyletic lineage (Fig. 6.3). Most single species clades received moderate to strong support (48-100% BS, 68-100% PP), although the nodes indicating relationship amongst them generally received less support.

The phylogenetic analysis differentiated bacteria into nine different groups (A to I). Group A constituted four different strains of *Hafnia* (CB1, CB8, CB29 and CB42), four different strains (CB4, CB28, CB34 and CB35) of *Enterobacter* constituted group B, group C consisted of five different strains of *Ewingella* (CB21, CB37, CB39, CB43 and CB45), one strain (CB44) of *Rahnella* comprised group D, group E constituted into two different strains of γ-proteobacterium (CB9 and CB24), group F constituted five different strains of *Pseudomonas* (CB7, CB11, CB31, CB32 and CB47), group G formed three different strains of *Stenotrophomonas* (CB13, CB15 and CB19), group H comprised two different strains of *Alcaligenes* (CB27 and CB40) while four different strains of *Bacillus* (CB3, CB5, CB14 and CB25) constituted group I.

Table 6.4 Bacterial isolates from Cantharellaceae and their closest relative species inferred from 16S rRNA gene sequence of existing database

Bacterial isolates	Nearest match	Query coverage	Maximum identity
CB1, CB8, CB31, CB42	*Hafnia alvei* (FM179942)	100%	98-99%
CB4, CB18, CB28, CB34	*Enterobacter* sp. (AM989324)	100%	99-100%
CB45, CB39, CB37, CB43, CB21	*Ewingella americana* (DQ383802)	100%	97-99%
CB44	*Rahnella* sp. (EF693795)	100%	100%
CB9, CB24	γ-proteobacterium (HQ529454)	100%	99-100%
CB7	*Pseudomonas tolaasii* (EF154274)	100%	98%
CB11	*Pseudomonas brenneri* (FN393787)	100%	99%
CB17	*Pseudomonas putida* (DQ481475)	100%	98%
CB32, CB47	*Pseudomonas fluorescens* (EU169164)	100%	98-99%
CB13	*Stenotrophomonas mltophilia* (FJ772057)	100%	99%
CB15, CB19	*Stenotrophomonas* sp. (FJ545751)	100%	99-100%
CB27, CB40	*Alcaligenes faecalis* (FJ982933)	100%	98-99%
CB3, CB5	*Bacillus* sp. (DQ117544)	100%	99-100%
CB14	*Bacillus cereus* (FJ649682)	100%	99%
CB25	*Bacillus pumilus* (FJ808722)	100%	99%

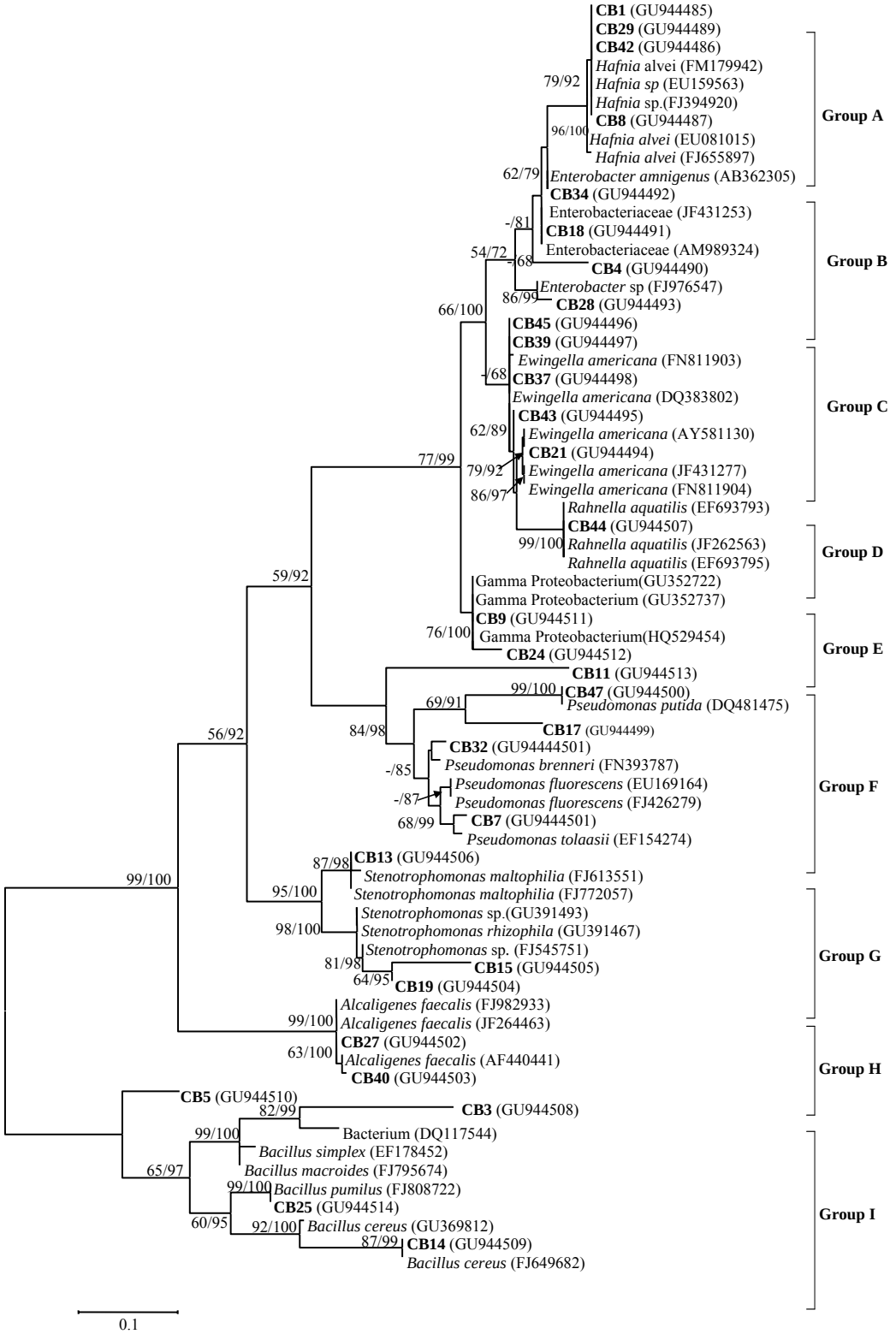

Fig. 6.3 Phylogeny of endophytic bacteria generated from Maximum Likelihood analysis of 16S rRNA gene sequences. Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). The scale gives the substitution rate.

Salient findings

- The present study demonstrated the existence of significant strain-level variation between endosymbiont of different *Cantharellus* hosts collected from geographically separated area and concerning the association of different bacterial species with various Cantharellaceae.
- The detailed diversity of bacteria associated with sporocarps of Cantharellaceae was studied for the first time. The study resulted in the isolation and characterization of 50 different bacterial isolates from different *Cantharellus* species. These isolates were differentiated into 30 different species by BOX- and REP-PCR based finger printing. Biochemical and 16S rRNA profile revealed characterization of the isolates belonging to nine different groups which includes *Hafnia* sp., *Enterobacter* sp., *Ewingella* sp., *Rahnella* sp., Gamma proteobacterium, *Pseudomonas* sp., *Stenotrophomonas* sp., *Alcaligenes* sp. and *Bacillus* sp.
- The *Hafnia* and *Stenotrophomonas* were the most persistent species associated to different Cantharellaceae. *Hafnia* spp. were found in 9 different fruit bodies whereas *Stenotrophomonas* was associated with 6 different fruit bodies of Cantharellaceae.

Chapter 7

Discussion

7.1 Diversity of Cantharellaceae from Western Himalayas, India

The diversity of Cantharellaceae in Western Himalayas, India, one of the word's hotspots of biodiversity, was estimated in the present study. Both phylogenetic and biological species recognition criteria were used (Taylor *et al* 2000) to identify different species of Cantharellaceae. Seventeen species of Cantharellaceae were reported in this study, out of which, 9 taxa are new to the world as described by both morphological characters and molecular analysis. Fungal identification is more reliable when classical and molecular approaches are combined (Hyde and Soytong 2007; Than *et al* 2008).

Maximum parsimony and Maximum Likelihood analyses of rDNA ITS and LSU yielded consistent topologies in different taxa of Cantharellaceae. A comparison of the concatenated ITS and LSU dataset showed that the combination of two rDNA regions resulted in a better resolution of the relationship of higher clades.

The phylogenetic tree also indicated that the present infrageneric classification is composed of non-monophyletic taxa. The comments on some of the infrageneric clades that were discovered during present study are discussed and data are supported by morphological characters. These numbered clades are marked in Fig. 4.23 and discussed below.

Clade 1: Phylogeny placement of *C. natarajanii, C. applanatus, C. cibarius* and *C. elongatipes*:

Cantharellus natarajanii and *C. applanatus* were clustered in same clade, but these were morphologically dissimilar to each other, suggesting these are distinct species. Although this clade is poorly supported in ML tree (60% bootstrap) and also not supported in Bayesian analysis (Posterior probability 76%), the conspicuous morphological differences suggest that they belong to two separate species. *Cantharellus natarajanii* is maintained as distinct taxa and distinguished by the shape of pileus, ellipsoid to broadly

ellipsoid basidiospores and the presence of subventricose to subclavatus hyphal system present in pileus cuticle, however, pileipelis of *C. applanatus* consisting of compactly arranged, cylindric to filamentous projecting end hyphae. On other hand *C. cibarius* is much larger species (pileus 15 cm broad, spores range 8.5-11.5 × 4.5-5.5 µm). The presence of distinctive taxonomic features supports the dissimilarity of described species with respect to *C. natarajanii* and *C. applanatus*. The observed consistency in the microscopic characterization (the primary character) is used to delimit the two species within collection (Mueller 1991). The phylogenetic structure of these species may be clarified when more materials from other gene loci are available.

A monophyletic subclade of *C. elongatipes* makes a clear differences with *C. natarajanii, C. applanatus* and *C. cibarius*; the fruit bodies of *elongatipes* are characteristics features, having small, dark orange to red brown basidiomata, basidiospores 4–5 per basidium and the presence of less pileus diameter as compared to stipe length (Pileus diam 0.8-1.5 cm, stipe length 2-4.5 cm) indicated that *C. elongatipes* is a new species.

Clade 2: Phylogeny placement of *C. subalbidus*, *C. cascadensis*, *C. cibarius* var. *roseocanus* and *C. cibarius* var. *cibarius*.

The phylogenetic analyses of *C. subalbidus*, *C. cibarius* var. *roseocanus*, *C. cibarius* var. *cibarius* and *C. cascadensis* formed an unresolved clade in the strict consensus tree with moderate bootstrap values. The analyses of the nrDNA large subunit and ITS regions show that *C. cascadensis*, along with two other yellow chanterelle taxa (*C. cibarius* var. *roseocanus* and European *C. cibarius* var. *cibarius*), are more closely related to white chanterelles (*C. subalbidus*) compared to the most common yellow species in the pacific western forest. These three species are co-occurring species fit into biological species concepts congruent with nrDNA phylogenetic species concepts (Dunham *et al.* 2003).

Clade 3: Phylogeny placement of *C. formosus*:

It is worth emphasizing that the interspecies diverse nature of the LSU of *C. formosus* is very high, though these two collections form a unique clade with maximum bootstrap

value ranging from 76-98%. These sequences have divergence with other sequences, suggesting that these are likely represented an independent taxon.

Clade 4: Phylogeny placement of *C. indicus* and *C. persicinus*:

Cantharellus indicus and *C. persicinus* are morphologically very dissimilar to each other, but were clustered in same groups, although this clade is poorly supported in MP and Bayesian tree with 69% and 90%, respectively. Morphologically the *C. indicus* was compared with *C. persicinus* and data showed that *C. persicinus* is a peach or pink color *Cantharellus*, pileus 2-4.5 cm wide; convex, becoming broadly convex or nearly flat; spores 10.5-11.5 x 6-7 µm, basidia with 4-sterigmata. However, *C. indicus* is egg yellow color, pileus 3-7 cm broad, infundibuliform, spores 7.2-10 × 4.5-5.4 µm, basidia with 4-6 sterigmata. So, the conspicuous morphological differences suggest that they belong to three separate species.

Clade 5: Phylogeny placement of *C. umbonatus, C. pseudoformosus* and *C. lateritius*:

Cantharellus umbonatus is a new report from the western Himalayas, due to its umblicate pileus surface, whitish hymenium that reaches up to the half of the stipe, supported the new placement of *C. umbonate* (Clade 8). However, the result of molecular analysis from both ITS and LSU showed its close relation with *C. pseudoformosus* with low bootstrap support 62% and 76%, respectively. The LSU sequences divergence between these two species is very low (LSU sequences difference between *C. umbonatus* and *C. pseudoformosus* was 10 base-pair). It is conceivable that the segregation of these taxa occurred recently or may be due to the fact that the two analyzed genes did not produced reasonable information to assume that much genetic difference between them, it may be revealed when multi-locus DNA analysis is applied. In most cases, genetic isolation and divergence seem to precede the differentiation of morphological characters (Taylor *et al* 2000).

Although the monophyletic clade of *C. lateritius* is still unclear; the fruit bodies of *C. lateritius* are totally different from other species of *Cantharellus* (smooth hymenium surface). Other morphological evidences (2-4 sterigmata per basidium) and also LSU data analysis (Fig. 4.23) further differentiate *C. lateritius* to *C. umbonatus* and *C. pseudoformosus*.

Clade 6: Phylogeny placement of *C. himalayensis*, *C. appalachiensis* and *C. fibrillosus*.

These three specimens of *Cantharellus* were collected from different locations of Western Himalayas, India, formed a supported clade with 91% bootstrap support linking with *C. fibrillosus* with bootstrap 74%, suggesting a relationship between these species. Among the three species, *C. fibrillosus* possess fibrils pileus surface, light orange to pinkish brown stipe color and basidia posses 4-6 sterigmata, however *C. himalayensis* produced yellowish fruit bodies, lacking fibrils on pileus surface, pileipellis partially gelatinized and sterigmata 2-4 per basidium, differentiated from other examined species. The biometric studies of spores confirm the opinion of previous work (Corner 1966) that the spore size and number of spores per basidia is useful for the delimitation of many Cantharellaceae species, because the intraspecific size variation is too high to characterize a specific group. In this study, obvious morphological differences were found between *C. appalachiensis*, *C. fibrillosus* and *C. himalayensis* (Table 4.2).

Clade 7: Phylogeny placement of *C. minor* and *C. cinnabarinus*

C. minor and *C. cinnabarinus* are grouped in a same clade, but make a distinct subclade with poorly supported in MP (less than 50% bootstrap) and Bayesian analysis (posterior probability 62%). So, the sequences divergence suggesting that these are two different species. Morphologically *C. cinnabarinus* is orangish red to red colour *Cantharellus*, pileus 1-5 cm broad, spore 6-11 x 4-6 µm compared to *C. minor* that is dull yellow to orange, pileus 5-30 mm broad and spore 7.5-10 x 4.5-6 µm. These morphological differences suggest that they belong to two separate species.

So, the phylogenetic analyses of LSU rDNA sequences revealed clades with statistical support, corresponding to circumscribed morphological characters and identified eight new species within *Cantharellus.* The discovery of new *Cantharellus* species of present research from the Indian Himalaya brings the total number of species of Cantharellaceae reported from India to15. The detailed characters of eight new species of *Cantharellus* are further discussed as following:

Cantharellus applanatus is recognized in the field by wholly yellow beautiful *Cantharellus*. The morphological characteristics of the present species was initially mistaken with the characters given for *C. viscosus* Berk and Corner (1966), but the

presence of large basidiocarps with prominent folded hymenium and applanate pileus surface compared to infundibuliform in type specimens differ it from *C. viscosus*. The ITS and LSU region was aligned with all the sequences present in GenBank. The closest ITS sequence of a new species *C. applanatus* found in GenBank was *C. cibarius* (DQ200926) with 97% similarity for 98% coverage. The following another closet sequences was *C. cibarius* (AF044689) with 93% similarity for 61% coverage. The LSU sequences of present specimen showed closest similarity with *C. subalbidus* (AY041150) with 99% similarity for 100% coverage and another closest sequence was *C. cascadensis* (AY041162) with 99% similarity for 100% coverage. The morphological features like pileus and stipe color, hymenium surface and other microscopical features like basidiospores, basidia, width of hymenium differed in described species from *C. cascadensis*. *Cantharellus subalbidus* (white *Cantharellus*) which have larger basidiocarps (pileus up to 14 cm broad) and moreover the color morphology is dissimilar with other microscopic characters. So the presence of distinctive taxonomic features supports the dissimilarity of the described species with respect to other species of *Cantharellus*. The phylogenetic analyses (Figs. 4.22, 4.23 and 4.24) also provided statistical support to make it as new species.

Most of the species mentioned in this study were common and wide spread in forest of Western Himalayas, India, except *C. elongatipes* that seems to be very rare and has only been found in the area of Bharsar forest (Uttarakhand) from the mixed type of forest. *Cantharellus elongatipes* was initially mistaken with small fruit bodies of *C. minor* but strongly different because of less fleshy stipe and less decurrent hymenium. The characteristics features, having small, dark orange to red brown basidiomata, basidiospores 4–5 per basidium and the presence of less pileus diameter as compared to stipe length (Pileus diam 0.8-1.5 cm, stipe length 2-4.5 cm) indicated that *C. elongatipes* is a new species. The long ITS (1200 bp) region supported its placement in the genus *Cantharellus*. The RFLP generated dendrogram and ITS sequence analyses formed a well-supported clade of *C. elongatipes*, which showed its difference from other species of *Cantharellus* studied. The combined ITS and ITS +LSU (Figs. 4.22 and 4.24) data finally resolved that the described species as a new record for world.

Cantharellus fibrillosus resembled morphologically with *C. ianthinoxanthus* Kuhner (Corner 1966) with pileus surface fibrillose, infundibuliform. After careful examination, it was observed that the presence of large spore size (up to 12.5 µm), 6 spored basidia (primary character), and stipe color of light orangish with brown towards apex. However, the stipe color of *C. ianthinoxanthus* was clear yellow. The closest ITS sequence of *C. fibrillosus* found in GenBank was *C. appalachiensis* (HQ386220) with 90% similarity for 93% coverage followed by *C. cibarius* (EF546767) with 90% similarity for 93% coverage. The LSU sequences of present specimen showed closest similarity with *C. appalachiensis* (HM582120) with 93% similarity for 100% coverage followed by *C. cinnabarius* (AY041168) with 92% similarity for 100% coverage. Based on microscopic characters, a new species, *C. fibrillosus* having mustard brown to pinkish brown basidiocarps, pileus surface with dense fibrillose at centre, long narrow basidia bearing 2-6 curved sterigmata was designated as new species. The phylogenetic analyses of ITS and LSU (Figs 4.22 and 4.23) also formed a well-supported clade of *C. fibrillosus* which differed with other species of previously reported *Cantharellus*.

Several collections of the *C. himalayensis* were recorded from different forests of Western Himalayas, India, and it has been confused, inexplicably with *Cantharellus cibarius* (Fr.), but after careful microscopic examination, the present specimens is recognized by the presence of basidium with 4 spores, long sterigmata (up to 9.5 µm), smaller spore size (6-8 × 4.5-6 µm) which differentiate it from the previously reported species of *C. cibarius* (Fr.). The closest ITS sequence of *C. himalayensis* was found in GenBank was *C. cibarius* (DQ200926) with 97% similarity for 97% coverage and another closet sequences was *C. cibarius* (EF546767) with 97% similarity for 97% coverage. The LSU sequences of present specimen showed closest similarity with *C. appalachiensis* (HM582121) with 96% similarity for 100% coverage and another closet sequences was *C. minor* (DQ898691) with 97% similarity for 100% coverage. *Cantharellus appalachiensis* has larger spores (range is 7.5-9 × 4.5-5.8 µm), ellipsoid to elongate, and basidia bearing 4-6 curved sterigmata (4.5-7 µm long) as compare to *C. himalayensis* spore was small (ranged from 6-8 × 4.5-6 µm), ellipsoid to broadly ellipsoid and sterigmata 4 per basidium (6-9.5 µm long). The size of basidiocarps of *C. himalayensis* is too large as compared to *C. minor* Peck. and other microscopic

examination data; spore size, pileipellis hyphae, showed that *C. himalayensis* is totally different from *C. minor*. Further phylogenetic analysis based on ITS and LSU sequences (Figs. 4.22 and 4.23) data indicated that this specimen is reported as a new record and frequently found in Western Himalayas region, so named assigned as *C. himalayensis*.

Cantharellus indicus is recognized in the field bearing long stem, equaling the width of the pileus or more. The morphological characteristic of the present species was initially mistaken with *C. cibarius* var. *longipes* peck (Corner, 1966), but the pileus surface is smooth, hymenium more or less yellow, stem yellowish white and branched, as there are sufficient microscopic characteristics are uncommon that verified that the present species is new taxa and described it as *C. indicus*. The closest ITS sequence of *C. indicus* found in the GenBank was *C. cibarius* (EF546767) with 84% similarity for 98% coverage and followed by *C. minor* (HQ270119) with 95% similarity for 78% coverage. The LSU sequences of present specimen showed closest similarity with *C. appalachiensis* (HM594682) with 97% similarity for 100% coverage and another closet sequence was *C. persicinus* (AY041169) with 97% similarity for 100% coverage area. Morphologically the *C. indicus* was compared with *C. persicinus* and data showed that *C. persicinus* is a peach or pink color *Cantharellus*, pileus 2-4.5 cm wide; convex, becoming broadly convex or nearly flat; spores 10.5-11.5 x 6-7 µm, basidia with 4-sterigmata. However, *C. indicus* is egg yellow color, pileus 3-7 cm broad, infundibuliform, spores 7.2-10 × 4.5-5.4 µm, basidia with 4-6 sterigmata. The taxonomic distinctness of *C. indicus* from other species is confirmed further by phylogenetic analyses based on ITS and LSU (Figs. 4.22 and 4.23) sequences that clearly separated the present collection from all other species of *Cantharellus* reported previously and describe it as a new species.

After detailed analysis of previously reported *Cantharellus*, it is confirmed that *C. natarajanii* is a new species. The main characteristic feature of the present specimen is plano-convex to hemispherical and this is confirmed a new record by the presence of pileus hyphal end-cells which is distinctly subclavate to subventricose. The closest ITS sequence of *C. natarajanii* found in GenBank was *C. cibarius* (EF546767) with 98% similarity for 96% coverage followed by *C. cibarius* (DQ200926) with 98% similarity for 96% coverage. The LSU sequences of present specimen showed closest similarity

with *C. cibarius* (HM594682) with 97% similarity for 100% coverage and *C. subalbidus* (AY041150) with 99% similarity for 100% coverage. Morphologically, present species was compared with *C cibarius* and *C. subalbidus* and found that the color of basidiocarps of *C. subalbidus* is whitish to cream, spore range is 7.5-9.0 × 5-6 µm, elliptical and pileus hyphal end cells is cylindrical to filamentous which is differed from the described species. *Cantharellus cibarius* is much larger species (pileus 15 cm broad, spores range 8.5-11.5 × 4.5-5.5 µm), moreover the color morphology is dissimilar and an important character of *C. natarajanii* is "pileus hyphal end-cells which is distinctly subclavate to subventricose" which clearly differentiated this from *C. cibarius*, so the presence of distinctive taxonomic features supports the dissimilarity of the described species with respect to other species of *Cantharellus*.

Cantharellus pseudoformosus is morphologically and microscopically well within the circumscriptions given for the *C. formosus* Corner (1960). Hymenium with forked, low ridges, cap surface with small dense, closely adhered scales which is slightly darker than cuticle, similar spores range, similar number of sterigmata on basidia, pileipellis hyphae contains cystidiod end hyphae and presence of clamp connections, but after molecular examination of the ITS region of *C. pseudoformosus* is varied with the ITS regions of *C. formosus* Corner (1,690 bp), *C. cibarius* Fr. (1,490 bp), *C. cascadensis* Dunham, O'Dell & R. Molina (1,490 bp), and *C. subalbidus* A.H. Smith & Morse (1,490 bp). BLAST analysis results showed no close matches with existing sequences of *Cantharellus*. Bootstrap consensus showed splits between *C. pseudoformosus* and a poorly resolved clade containing *C. cibarius*, *C. cascadensis*, *C. subalbidus*, and *C. formosus* with ITS and LSU sequences (Figs. 4.22 and 4.23). So, the molecular analysis of LSU and ITS sequences showed *C. pseudoformosus* is distinct from *C. formosus*. The maximum-parsimony analysis of the LSU and BLAST results of ITS sequences demonstrated that *C. pseudoformosus* as reported as a new species to the world.

A new species, *C. umbonatus* matched well with the characters given for *C. cyanoxanthus* Heim. by Corner (1966). Pileus color, surface slightly fibrillose, hymenium prominent anastomosing but different with prominently convex to infundibuliform pileus and violaceous color hymenium surface present in *C.*

cyanoxanthus, but in *C. umbonatus* pileus was found to be umbonate and hymenium whitish color. The closest ITS sequence of *C. umbonatus* was found in GenBank was *C. cibarius* (EF546767) with 97% similarity for 96% coverage and another closet sequences was *C. minor* (HQ270119) with 95% similarity for 79% coverage. The LSU sequences of *C. umbonatus* showed closest similarity with *C. appalachiensis* (HM582121) with 97% similarity for 100% coverage and *C. cinnabarius* (AY041168) with 93% similarity for 100% coverage. Morphologically, *C. umbonatus* was compared with *C. appalachiensis* and *C. cinnabarinus* also, and found that the *C. umbonatus* is dark orange to yellow in color, pileus surface smooth, spore ellipsoid to elongate (Q= 1.62), which differs from *C. appalachiensis* mentioned above. *Cantharellus cinnabarinus* is recognized as small mushroom (pileus up to 1.5 cm broad) with distinctive flamingo-pink colors, pileus up to 1.5 cm broad. In phylogenetic analyses, *C. ambonatus* showed close similarity with *C. pseudoformosus*, however the genetic distant between these two species is separated by RFLP generated restriction digestion of ITS region (Fig. 4.20) although they are maintained as a distinct taxa on the basis of primary characters and distinguished by the shape of pileus and whitish hymenium color, reaching up to the half of the stipe. This variability reflected that the described species recorded as new species.

Among the genus *Craterellus*, 4 taxa described here were also distinguished on the basis of macromorphological and micromorphological characters. The intraspecific variation of the ITS and LSU regions could classify the morphogenetic species of genus.

Corner (1966) found morphologically acceptance of *Cr. cornucopioides* var. *mediosporus*, on the basis of only one discontinuous character *i.e*., the spore size is rather smaller as *Cr. cornucopioides*. In the present study, the collected sample (268-06) resembled with *Cr. cornucopioides* var. *mediosporus*. The phylogenetic analyses of ITS and LSU region of described specimen also showed high supported clade with 100% bootstrap similar for species-level-recognition (Figs. 4.35 and 4.36).

Craterellus cinerius was characterized by having small basidiomes, often with folded hymenium and subglubose to broadly ellipsoid basidiospores. In the phylogenetic analysis of LSU sequence data (Fig. 4.36), *Cr. cinerius* formed an unresolved clade with *Cr. lutescens* (EU522746). However, morphological characteristics of *Cr. lutescens* were dark brownish colored and hymenium almost smooth or slightly veined and pink colored. So the presence of distinctive taxonomic features supports the dissimilarity with *Cr. cinerius* (the primary character used to delimit the two species) within collections (Muller 1991).

The present study reported a new *Craterellus* species; *Cr. indicus*. Described specimen showed some similarity with the hymenium color of *Cr. neotubaeformis* nom. prov. (Pilz *et al*. 2003) and *Cr. sinuosus* Fries. But the morphological features of *Cr. neotubaeformis* like pileus and stipe color, attachment of hymenium and some microscopical features like basidiospores, basidia and the presence of clamp connection differed with *Cr. indicus*. *Craterellus sinuosus* is much larger species (pileus 2-5 cm broad, spores ranged is 9.5-12 × 7-8 μm), moreover the color morphology is dissimilar and an important character of *Cr. indicus* was "distinctly detached hymenium from the stipe apex" differentiated it from *Cr. sinuosus*. There has not been any *Craterellus* species reported having creamy basidiome with whitish gills. So, the presence of distinctive taxonomic features supports the dissimilarity of the described species with respect to other species of *Craterellus*. The taxonomic distinctness of *Cr. indicus* from other species was confirmed further by molecular analysis. The combination of LSU and ITS data for the inference of the phylogenetic position of *Cr. indicus* with well-resolved phylogeny clade (Figs. 4.35 and 4.36) treated as new species.

In conclusion, the present work represents a contribution to the knowledge of some aspects of the taxonomy and phylogenetic identification of Cantharellaceae. The results are consistent with the monophyletic character of the species of Cantharellaceae. In addition to its contribution to the study of the taxonomy of Cantharellaceae, this work would be useful to design the probes in the identification of new species, or as a database for comparing sequences of unidentified species.

7.2 Nutritional properties of Cantharellaceae

The use of mushrooms as a food item is probably as old as civilization. They were preferred only for their culinary characteristics while the nutritive value of mushrooms was recognized much later (Crisan and Sands 1978; Chang 1980; Khanna and Garcha 1981). Edible mushrooms are grown and consumed all over the world according to the trade interest, culinary customs, subtle flavour (Priestly 1984), nice aroma, special taste appeal and climatic conditions (Bhatti *et al*. 1989). Mushrooms now days are not only an essential part of good cooking, which provide valuable protein, carbohydrates and calories but they are also highly valued for their varied texture and flavors (Colin and Lucas 1979). By keeping the interest in the nutritive values of Cantharellaceae species, in the present study 15 different species of these mushrooms were studied from Western Himalayan region of India. The primary interest was to conclude that the investigated wild edible mushrooms of Cantharellaceae are good nutritional sources in terms of protein, carbohydrate, amino acids composition, antioxidant activity and energy values.

The wild edible mushrooms of Cantharellaceae contained significant level of chemical composition compared with other edible mushrooms (Degreef *et al*. 1997; Caglarirmak *et al*. 2002; Mdachi *et al*. 2004 and Barros *et al*. 2008). The present nutritional data indicate that 100 g of these mushrooms provide on an average 25 kcal (104 kJ) of energy. *Cantharellus lateritius* contained highest values while *C. natarajanii* contributed the lowest energy value (Table 5.1).

The moisture content of the collected Cantharellaceae mushrooms was high, which indicate that fresh mushrooms cannot be kept for long time for study. This is because high water activity enhances microbial growth (Brock *et al*. 1986). Similar observations were made for other edible mushrooms (Sanmee *et al*. 2003; Leon-Guzman *et al*. 1997; Fasidi 1996).

Among all the studied Cantharellaceae species, protein was the principal macronutrient. The total protein content is based on the determination of nitrogen content by semi micro

Kjeldahl method. It has to be considered that the wall of fungi is partially built of chitin, a nitrogen containing molecules, which cannot be considered as a food source. In order to overcome the error introduced by the chitin nitrogen during protein estimation, the ratio of nitrogen/ dry weight is multiplied by a factor 6.25 in case of higher plants, while multiplied by a factor 4.38 in case of fungi (Delmas 1989). Protein contents varied among investigated mushrooms of Cantharellaceae. However, it is known that the protein contents of mushrooms are affected by various factors like the stage of development, the part sampled, level of nitrogen available and location (Flegg and Maw 1976). The average content of protein present in all 15 Cantharellaceae species was 31.8 mg/g of fresh weight. Hence, the fruit bodies of wild edible Cantharellaceae mushrooms can be added in diet as a protein supplement or as an alternative to fish and meat (Degreef *et al*. 1997). Vegetarians could also eat mushrooms because it served as alternative protein supplements in their diet. Mushroom proteins are generally higher than those of green vegetables and some fruits (Chan 1981; Jonathan 2002).

Beside proteins, mushrooms also contained good amount of carbohydrates. The mushrooms were not a good source of fat and only minor quantities of fats were present in them. Generally, high protein and carbohydrate contents, and low fat levels in mushrooms were also described by previous authors (Diéz and Alvarez 2001). The low fat or lipid contents also suggest that persons those with heart or weight problems can consume Cantharellaceae mushrooms.

The wild mushrooms are known as rich sources of protein and low amount of fat make it an ideal snack material (Barros *et al*. 2007). These high protein and low fat characteristics of the edible wild mushrooms have been previously reported by many workers (Aletor 1995; Longvah and Deosthale 1998; Diez and Alvarez 2001). The presence of low fat content and energy value in different species of Cantharellaceae can be concluded good alternative for low fat/energy diets.

The total protein or carbohydrate contents do not give sufficient information to determine nutritional value of food. Human beings need their alimentation of essential amino acids for maintenance of their food diets. The presence of essential amino acids in Cantharellaceae is responsible for maintenance of nitrogen equilibrium. If all the amino aids are provided, the human body can form the remaining amino acids necessary for the composition of its synthesis. A qualitative food must show the right equilibrium between the eight essential amino acids (Degreef *et al*. 1997). The other amino acids were well represented in the different species of Cantharellaceae. Among 15 different species, *Cr. cornucopioides* var. *mediosporus*, *C. miniatescens*, *C. himalayensis* and *C. natarajanii* are good sources of all the essential amino acids. Except cystine, all the mushrooms contained good amount of essential amino acid. Glutamic acid was most abundant amino acid among different species of Cantharellaceae. The maximum amount of glutamic acid was also reported in *Pleurotus ostreatus* (Kim *et al*. 2009; Patil *et al*. 2010). Mushrooms proved to be good sources of almost all essential amino acids when compared with common vegetables (Matilla *et al*. 2002). Further these results serve as a basis for encouraging local communities in especially in developing countries to harness the nutritive potential of wildly occurring edible mushrooms for abating nutritional deficiencies.

The antioxidant potential of the prepared extract from different Cantharellaceae fruit bodies was studied by three different methods *i.e*., hydroxyl radical scavenging potential, antioxidant activity in linoleic acid emulsion and improved ABTS radical decolorization assay. The present inhibition values were obtained in all the methods and it was observed that the alcoholic extracts were most effective than aqueous extracts (Table 5.4). Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a standard assay in antioxidant activity study and offers a rapid technique for screening the radical-scavenging activity of specific compounds or extracts (Amarowicz *et al*. 2004). The results showed that all the

Cantharellaceae mushrooms had significant hydroxyl radical scavenging activity ranging from 22.7 to 40.7% in alcoholic extracts and 14.4 to 36.6% in aqueous extracts. The results of the investigations reveal that Cantharellaceae mushroom extracts have potent hydroxyl radical scavenging, lipid peroxidation inhibition and ABTS decolorization activities. The methanol extract possesses higher antioxidant activity than the aqueous extract. The significant antioxidant activities of Cantharellaceae mushroom extracts thus suggest the therapeutic value of these mushrooms.

The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha *et al*. 2001). The Cantharellaceae mushrooms also showed an excellent antioxidant activity in terms of linoleic acid peroxidation in alcoholic extracts.

Mushrooms also accumulate a variety of secondary metabolites, including phenolic compounds, flavonoids, β-carotene and steroids. Naturally occurring antioxidants components, including tocopherol, β-carotene, flavonoids and total phenols were found maximum in methanolic extracts of all the Cantharellaceae species (Tables 5.5 and 5.6). However, β-carotene was detected in very less amounts. The maximum content of ascorbic acid was found in *Cr. cornucopioides* var. *mediosporus* (1.2 mg/g). Mushrooms have been shown to produce several biologically active compounds that are usually associated with cell wall, and these have been suggested to contribute to enhancement of immunity and tumor-retarding effects. Numerous data prove that the edible mushrooms may be potential sources of natural antioxidants. Because of their scavenging abilities on free radicals and chelating abilities on ferrous ions, phenol might possess good antioxidants, antimutagenic and anticancerous properties (Ramesh and Pattar 2010). Also, in mushrooms, the phenolic compound has been found to be an excellent

antioxidant and synergist that is not mutagenic (Ishikawa *et al*. 1984). It had been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds (Velioglu *et al*. 1998). In fact, phenols such as BHT (butylated hydroxytoluene) and gallate, are known to be effective antioxidants (Ferreira *et al*. 2007). Total phenols were the major naturally occurring antioxidant components found in the mushrooms of present study. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Komali *et al*. 1999; Moller *et al*. 1999). Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen *et al*. 1993; Gulcin *et al*. 2003). The phenolic compounds may contribute directly to antioxidative action (Duh *et al*. 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables (Tanaka *et al*. 1998). Total phenol productions by all different Cantharellaceae species were significantly higher than several mushroom species such as *Lycoperdon perlatum, Clavaria vermiculris, Marasmius oreades*, *Russula delica*, *Russula delica*, *Morchella conica*, *Pleurotus pulmonarius*, *Lactarius deliciosus*, *Tricholoma portentosum*, *Ganoderma lucidum* and *Coriolus versicolour* of previous reports (Mau *et al*. 2002; Turkoglu *et al*. 2006; Aziz *et al*. 2007; Ferreira *et al*. 2007; Ramesh and Pattar 2010). The higher content of total phenols in the Cantharellaceae mushroom extracts might account for the better results found in their radical scavenging effect. The amounts of ascorbic acid, β-carotene and flavonoids found in the Cantharellaceae mushroom extracts were very low, which emphasizes the idea that phenolic compounds could make a significant contribution to the Cantharellaceae mushrooms antioxidant activity. The similar findings were also observed by Barros *et al* (2007) in Portuguese wild edible mushrooms.

An important factor in the overall nutritional values of a food is its vitamin contents. Vitamin contents in the fruit bodies of Cantharellaceae were estimated and significant

amount of vitamin A was found in all species. Vitamin A, synthesized from β-carotene, (Jensen and Salisbury 1984) is essential for good night vision (Stryer 1988) a fact that might explain the use of chanterelles by Chinese herbalists to treat night blindness. All of the Cantharellaceae mushrooms contained good amount of vitamins although vitamin C content was higher than other vitamins. Vitamin C is one of the major contributors to the antioxidant activity of fruit vegetables and mushrooms (Caglarlrmak 2002). The content of vitamin C in the mushrooms of present study was significantly higher than various mushrooms such as *Lycoperdon perlatum, Clavaria vermiculris, Marasmius oreades*, *Russula delica*, *Russula delica*, *Morchella conica* and *Pleurotus pulmonarius* previous reports (Turkoglu *et al*. 2006; Aziz *et al*. 2007; Ramesh and Pattar 2010).

Cantharellaceae were also found to be good sources of vitamin B complex which helps in break down of protein, fat and carbohydrates. These species contained good amounts of niacin (up to 73 µg/g) and riboflavin (up to 63 µg/g). *Pleurotus ostreatus* has also been reported to contain higher amount of vitamin C followed by niacin and riboflavin (Patil *et al*. 2010). Niacin acts as a co-enzyme in the metabolism of carbohydrate and fatty acids, and is required for energy metabolism whereas, riboflavin is an essential enzyme required for the metabolism of carbohydrates and amino acids. It also supports antioxidant protection (Linder 1985). Mushrooms are known to best sources of niacin, as it also promotes healthy skin and ensure proper functioning of digestive and nervous system. Riboflavin (vitamin B2) helps to keep healthy R.B.C. in blood. Thiamine (vitamin B1) is essential for neural functioning and carbohydrate metabolism and the deficiency results in beriberi.

Chanterelles contain high level of vitamin D (Mattila *et al*. 1994), a vitamin synthesized from ergosterol when tissues are illuminated by sunlight or ultraviolet irradiation. The high concentration of Vitamin D might play a role in chanterelle ultraviolet protection and resistance to insect predation. Next to the cod liver oil, chanterelles are one of the most concentrated natural dietary sources of vitamin D. Rangel-Castro *et al* (2002)

reported the presence of ergocalciferol (vitamin D2) content in the individual fruit bodies of the edible mushroom *Cantharellus cibarius*. They also concluded that ergocalciferol content depends on the exposure of fruit bodies to sunlight. In addition, Cantharellaceae have also been found to contain rich amount of vitamin E (tocopherol), which has proven to be an antioxidant and cellular protector against oxidative damage. The nutrient value of Vitamin E is also high in chanterelles compared to many edible mushrooms (Rangel-Castro 2001). The high concentration of vitamin E protects the skin

allergen or other allergic reactions. The low level of cholesterol was recorded in all the species of Cantharellaceae. The negligible amount of cholesterol in wild edible mushrooms has been previously reported by other workers (Chang and Miles 2004).

The present research study analyzed the total antioxidative status, employing multimechanistic antioxidative assays such determination of total phenolics, flavonoids, ascorbic acid and β-carotene of each 15 species, and data showed that the *Cr. cornucopioides* var. *mediosporus* followed by *C. applanatus* and *C. pseudoformo*sus are good source of various antioxidant compounds among all the 15 different species.

In conclusion, the chemical composition and energy values of the wild edible mushrooms of Cantharellaceae species clearly indicate that they provide key nutrients such as protein and carbohydrates. Being a good source of protein and carbohydrate, they fall between most legumes and meat (FAO/WHO, 1989), and prove to be excellent foods that can be used in low caloric diets for their low contents of fat and energy. Also, the analyzed mushrooms contain very useful nutraceuticals such as phenolics, ascorbic acid, and carotenoids that could be extracted for the purpose of being used as functional ingredients against microbial infections. Nevertheless, the high nutritional quality and unique flavours of these mushrooms are likely to be lost if these wild edibles are not documented. Therefore, it is now imperative that a nutritional database of these mushrooms is set up to retain the information on these unique species and for a better management and conservation of this natural resource and habitats related to them. These edible mushrooms also represent a growing segment of today's food industry. Besides, these mushroom to be used directly in diet and promote health, taking

advantage of the additive and synergistic effects of all the bioactive compounds present. Searching wild sources may bring new natural products into the food industry with safer and better nutrition as well as antioxidants that provide good protection against the oxidative damage, which occurs both in the body and our daily foods. Therefore, wild edible mushrooms such as Cantharellaceae mushroom could be introduced for the purpose as natural nutrition sources.

7.3 Diversity of bacteria associated with Cantharellaceae

Endophytic bacteria can contribute to the health, growth, and development of fungal fruit bodies (Feng *et al*. 2006). It is very important to explore the diversity of indigenous bacteria of Cantharellaceae fruit bodies. The use of repetitive DNA sequences for bacterial classification is becoming frequent, and has allowed comparisons of possible intra-specific comparisons and genetic similarities among different bacterial genomes (Versalovic *et al*. 1991; Louws *et al*. 1998). REP- and BOX-PCR based fingerprinting was used to differentiate the bacterial species isolated from Cantharellaceae fruit bodies followed by 16S rRNA gene sequencing analysis for their identification.

The structure and dynamics of the endophytic bacterial community of different Cantharellus species has not been investigated previously except *Pseudomonas fluorescens*, *P. putia*, *Streptomyces* sp. *Xanthomonas* sp and *Bacillus* sp. which were identified in the fruit body of *C. cibarius*, and among these bacteria *P. fluorescens* were most dominant (Danell et al. 1993). The characterization of the bacterial isolates in present work shows that population is diverse, redundant, and dynamic among different species of *Cantharellus* from different types of coniferous forest of western Himalayan region, India. The genetic similarity between the isolates obtained from different forest within *Cantharellus* spp. may be due to the same nutritional correlation with endophytic bacteria and their respective fruit bodies. Phylogenetic analysis of representative isolates indicated that most of the isolates were related to the phylum Enterobacteriaceae. In the present study, Gram-negative isolates obtained were found to be more diverse and

consistent. *Pseudomonas fluorescens* was the predominant bacterium isolated from the sporocarps of *C. cibarius* (Danell and Camacho, 1997; Dunham *et al*. 2003), however, in this study *Hafnia* and *Stenotrophomonas* were the most persistent species associated with different *Cantharellus* spp.

IAA production is known to associate with many endophytic bacteria that help in plant growth promotion (Rosenblueth *et al*., 2006). All the isolates of present study produced IAA which might serve as growth promoting factor for fungi. This trait is considered one of the major mechanisms involved in fructification of *Cantharellus* spp. by the presence of dominant species such as *Hafnia*, *Pseudomonas*, *Ewingella, Stenotrophomonas* and *Bacillus*.

Many studies have noted beneficial interactions between bacteria and other mushrooms (Garbaye *et al*. 1991; Garbaye 1994). The amino acids, organic acids, and sugars released by *Cantharellus* served as nutrient sources for these endophytic bacteria (Rangel-Castro, 2001; Rangel-Castro *et al*., 2002). Role of *Cantharellus* fruit bodies associated bacteria in fructification by breakdown of organic matter has been studied by Rangel-Castro (2001). Bacterial populations in the host appear to be under the influence of both the host and the environment. Mycorrhizal fungi have been shown to provide a physical and nutritional substrate for many soil microbes (Tisdall 1991). The significance of the endophytic bacteria for a fungus (as well as for a plant) is still not completely described. Bacteria associated with mycorrhizal fungi could play an important role in sporocarp formation (Sbrana *et al*. 2000) and in promotion of mycorrhizal symbiosis (Garbaye 1994; Frey-Klett *et al*. 2005).

Dahm *et al* (2005) studied the diversity of the culturable bacteria associated with fruit bodies of ectomycorrhizal fungi such as *Suillus luteus*, *S. grevillei*, *Lycoperdon* sp., *Xerocomus* sp., *Hebeloma crustuliniforme*, *Thelephora terrestris*, *Scleroderma* sp., *Laccaria laccata*, *L. amethistina*, *L. proxima* and *Amanita muscaria*, and reported *Sphingomonas paucimobilis*, *Chromobacterium violaceum*, *Pseudomonas aeruginosa* and *Pseudomonas pickettii* as most dominant bacteria, however, their studies were

confined to bacterial population of limited species in the surrounding soil and in the fungal sporocarps. Varese *et al* (1996) isolated different bacteria from both the sporocarps of *Suillus grevillei* (Klotzsch) Sing. and the ectomycorrhizae of *S. grevillei – Larix deciduas* Mill in which genera *Pseudomonas, Bacillus* and *Streptomyces* were predominant. They suggested that some bacterial isolates exhibit very high fungus specificity at the intraspecific level.

In the present study it was found that among ten different species of *Cantharellus* fruit bodies, *Hafnia* spp. were most persistent, found in 9 different fruit bodies whereas *Stenotrophomonas* and *Pseudomonas* species were associated with 6 and 5 different fruit bodies, respectively collected from different diverse forests of Himalayan region. Although a correlation of highly frequent bacteria associated with *Cantharellus* fruit bodies doesn't prove any direct relationship, but this result strongly suggested that such bacteria make symbiotic combination and helping the fungal growth by producing enzymes, growth promoting hormones and utilization of different carbon sources (predominantly citrate and malonate). Such metabolites represent good sources for the fungal growth (Garbaye 1990). Nevertheless, these bacteria are especially important whenever the fructification of *Cantharellus* is considered. It may be hypothesized that the results tend to indicate some sort of tropic stimulation (involving nutritional relations) of the fungal growth by bacterium is the main mechanism involving in endophytic association effects (Rangel-Castro 2001). This interpretation could also partly explain why some of the endophytic bacteria are fungal-selective.

The present study demonstrated the existence of significant strain-level variation between endosymbiont of different *Cantharellus* hosts collected from geographically separated area and concerning the association of different bacterial species with various *Cantharellus* spp.

Summary

Studies on the fungal diversity have increased over the past decade partly due to the fact that fungi have great potential in industrial and biotechnological applications. Although much has been said and written about the biodiversity of fungi, little is known about their existence from different geographical areas and many fungi in different regions are yet to be discovered.

Cantharellaceae is one of the important families of wild edible mushrooms with most delicious taste, harvested from Europe, Africa, Asia and North America. The economical importance and the evolutionary significance of the Cantharellaceae have resulted in considerable research on their ecology, physiology and phylogenetic analyses. In the present research work, several fruit bodies of Cantharellaceae were collected from different parts of Western Himalayan region of India, analyzed to see the morphological and genetic variation among the species. All the species are morphologically well described. All the species are morphologically well described based on the size, color and habitat of fruit bodies and their spores.

As complete identification of fungal species can not be relied upon classical approach, in the present work classical taxonomy was further confirmed by molecular characterization to provide justification to identified species. Genomic DNA was extracted from 20 collected fruit bodies of *Cantharellus* (169-07, 113-07, 17-08, 161-07, 272-07, 281-07, 43-07, 121-08, 95-08, 84-08, 184-08, MSR1-08, MSR2-07, 90-09, MSR4-08, 106-08, 119-05, 236-06, 354-05 and 348-07) and 3 fruit bodies of *Craterellus* (159-07, 107-07 and 268-07). The ITS region of the rDNA was amplified by PCR using the primers ITS1 and ITS4. All species produced a single ITS fragment of approximately 1.2 to 1.6 kb in *Cantharellus* and 752 to 800 bp in *Craterellus*. ITS-PCR products obtained were subjected for restriction analysis with enzymes such as *Alu I*, *Mbo I*, *Hinf I*, *Hae III* and *Taq I*. All the examined species showed different restriction pattern for each endonuclease

tested, While *C. fibrillosus* (113-07, 17-07 and 236-06), *C. applanatus* (43-07 and 121-08), *C. indicus* (MSR-2 and MSR-4), and *C. applachinesis* (84-08 and 95-08) which were collected from different forests with different host association, showed same restriction pattern with all the 5 restriction enzymes (cluster with high support value, 100%). The LSU region was further amplified by using with ITS4R and LR5 primers of all the above collected fruit bodies and results showed that all the amplified PCR products size of LSU was ~900 bp. All different amplified products were selected for further ITS and LSU sequencing. The sequences were aligned with ITS and LSU sequences of different Cantharellaceae species from GenBank nucleotide database. A phylogenetic analysis for nuclear LSU and ITS region was based on parsimony using PAUP v4.0 b10 with the present study sequences and those registered in the database.

Phylogenetic analyses of rDNA ITS and LSU sequences revealed clades corresponding to circumscribed morphological species and aided in distinguishing traits useful for reliably identifying the different taxa of Cantharellaceae. Macromorphological and microscopical characters followed by molecular characterization (both the ITS and LSU dataset) *i.e.*, morpho-genetic studies confirmed the identity of these fungi. The present research work described 13 species of *Cantharellus*, among these 8 were new taxa, which includes *C. applanatus* sp. nov., *C. elongatipes* sp. nov., *C. fibrillosus* sp. nov; *C. himalayensis* sp. nov., *C. indicus* sp. nov., *C. natarajanii* sp. nov., *C. pseudoformosus* sp. nov., and *C. umbonatus* sp. nov. as new to the world while *C. miniatescens* Heinem was new record from the Indian subcontinent. Three species of *Craterellus* (*Cr. cornucopioides* var. *mediosporus* Corner, *Cr. dubius* Peck and *Cr. cinerius* Fries) were reported to new from the Indian subcontinent. *Craterellus indicus* sp. nov. was new report from the world. Two previously undescribed (*C. applachinesis* and *C. lateritius*) were also reported for the first time.

These Cantharellaceae species are consumed as a food due to its high nutritive value, specific aroma and supplement to a healthy diet. The significant levels of

proteins, lipids, minerals, vitamins, and some neutraceuticals in their fruit bodies are of considerable value. The nutritional benefits of wild edible Cantharellaceae have not been fully explored, so all of the fruit bodies of Cantharellaceae species were analyzed for their nutritional properties. The results of the chemical composition and estimated energetic value of the wild edible Cantharellaceae species were analyzed and the macronutrient profile in general revealed that the wild mushrooms were rich sources of protein and carbohydrates and had low amounts of fat. The presence of low fat content and energy value in 15 different species of Cantharellaceae can be concluded good alternative for low fat/energy diets. The total protein contents in different *Cantharellus* species were quite variable as the value was somewhere between 21.6 to 43.2 mg/g of dry weight in different species. So the Cantharellaceae species were high protein and low fat characteristics making it an ideal food material. These species also showed antioxidant activity, which was not reported before, thus needed to document. The nutritional data of present study confirmed that wild Cantharellaceae are highly nutritious and a suitable alternative for well-known foodstuffs.

Different bacterial species were isolated from all collected Cantharellaceae fruit bodies and were characterized on biochemical and molecular basis. REP and BOX-PCR fingerprints analysis revealed strains level genetic variation among isolated bacterial species. A total of 50 isolates were isolated from Cantharellaceae fruit bodies, which were further identified as 30 different strains of nine different genera (*Hafnia* sp., *Rahnella* sp., *Ewingella* sp., *Enterobacter* sp., γ-proteobacterium, *Stenotrophomonas* sp., *Pseudomonas* sp., *Alcaligenes* sp. and *Bacillus* sp.) based on 16S rRNA sequencing analysis. All the isolated bacteria produced the plant regulator hormone indoleacetic acid (IAA) ranging from 8.00 to 37.01 µg/ml. This hormone is considered as major mechanism involved in fungi growth promotion. The different fruit bodies of Cantharellaceae species which, growing in different habitats contained similar type number of bacteria. Significant variation in the types of indigenous bacteria isolated from diverse host species depends on host

specificity, geographical distribution. Cultural analysis of endophytes showed existence of limited morphotypes, suggesting that the genetic structure in such bacterial populations is restricted on account of the protection provided by the habitat and is highly conserved.

In conclusion, the present work documented 17 different species of Cantharellaceae collected from western Himalayan region of India. Nevertheless, the Himalayan mycota might be one of the best documented, however, detailed diversity of Cantharellaceae have not been reported previously. Moreover, with ongoing forest degradation in the Himalaya, we may lose many species without knowing them. It is important to have some database on ectomycorrhizal fungi such as Cantharellaceae before their diversity is depleted as a result of anthropogenic pressure and climatic change. All the wild edible fungi of the study are of high nutritional quality and establish a complete nutritional database, which could be used in the nutritional intervention program.

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Appendix I

Luria-Bertani (LB) medium

Ingredients	Quantity (g/L)
Nacl	10.0
Beef extract	5.0
Tryptone	10.0
Agar	10.0

pH 7 adjusted with 5N NaOH. Sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min. (Added filter sterilized ampicillin 50 µg/ml to prepare LB - Ampicillin plates)

Nitrate agar

Ingredients	Quantity (g/L)
Agar	12.0
Beef extract	3.0
Peptic digest of animal tissue	5.0
Potassium nitrate	1.0

Suspended 21 g of nitrate agar in 1000 ml distilled water. Boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min.

Phosphate buffer

Stock solution A

2 M monobasic sodium phosphate, monohydrate (276 g/L)

Stock solution B

2 M dibasic sodium phosphate (284 g/L).

Mixing an appropriate volume (ml) of A and B as shown in the table below and diluting to a total volume of 200 ml, a 1 M phosphate buffer of the required pH at room temperature.

A (mL)	B (mL)	pH
39.0	61.0	7.0
33.0	67.0	7.1
28.0	72.0	7.2
23.0	77.0	7.3
19.0	81.0	7.4
16.0	84.0	7.5

TBE buffer (10x)

Tris-HCl	0.09 M (pH 8)
Boric acid	0.9 M
EDTA	0.02 M (pH 8)

Plasmid extraction solution I (10X)
Tris-HCl 25 mM (pH 8.0)
Glucose 50 mM
Na_2EDTA 10mM

Plasmid extraction solution II

NaOH	5M
SDS	10%

Plasmid extraction solution III
5.0 M K-acetate (pH 4.5)

Agarose gel loading dye (6X)

Bromophenol blue	0.25%
Xylene cyanol FF	0.25%
Glycerol in water	30.0%

Ligation reaction of amplicon in pTZ57R/T

Plasmid pTZ57R/T (50ng/µl)	3µl
Amplicon (75ng/µl)	4µl
Buffer (10X)	3µl
T4 Ligase	1µl
H_2O	19µl

Melzer's reagent

Ingredients	**Quantity (g/100ml)**
KI	5.0 g
Chloral hydrate	100 g
Iodine	1.5 g
Distilled water	100 mL

Lacto phenol Cotton Blue

Ingredients	**Quantity (per litre)**
Phenol	200 g
Cotton Blue	5.00 g
Glycerol	400 mL
Latic Acid	200 mL
Deionized Water	200 mL

Primers

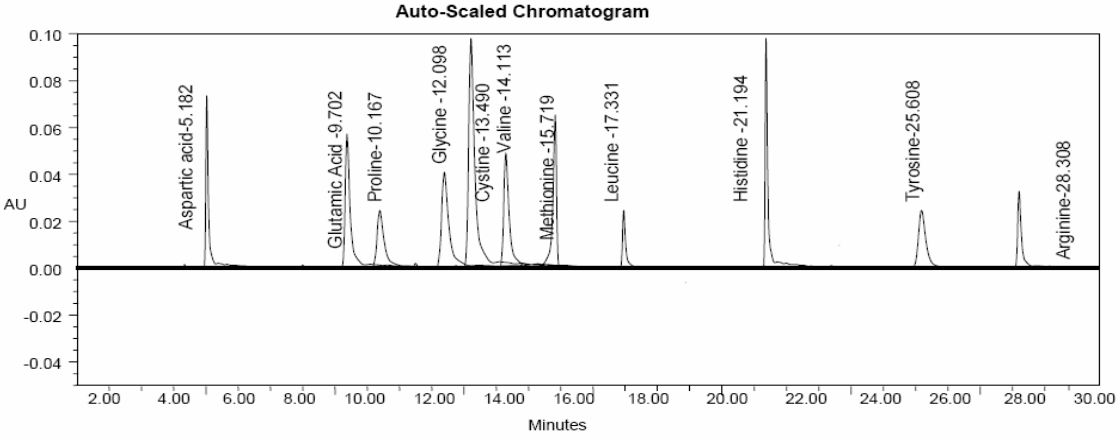
M13 forward primer	5''-GTAAAACGACGGCCAGT-3’
M13 reverse primer	5’-CAGGAAACAGCTATGAC-3’
Rep forward primer	5’-IIIICGICGICATCIGGC -3’
Rep reverse primer	5’-ICGICTTATCIGGCCTAC-3’
BOXA1R primer	5’CTACGGCAAGGCGACGCTGACG-3’
ITS1 forward primer	5’-TCCGTAGGTGAACCTGCGG-3’
ITS4 reverse primer	5’-TCCTCCGCTTATTGATATGC-3’
ITS4R forward primer	5’-GCATATCAATAAGCGGAGGA-3’
LR5 reverse primer	5’-ATCCTGAGGGAAACTTC-3’
16S rDNA forward primer	5’-AGAGTTTGATCCTGGCTCAG-3’
16S rDNA reverse primer	5’-ACGGGCGGTGTGTTC-3’

Appendix II

Chromatogram of standard solution of 16 amino acids obtained from HPLC

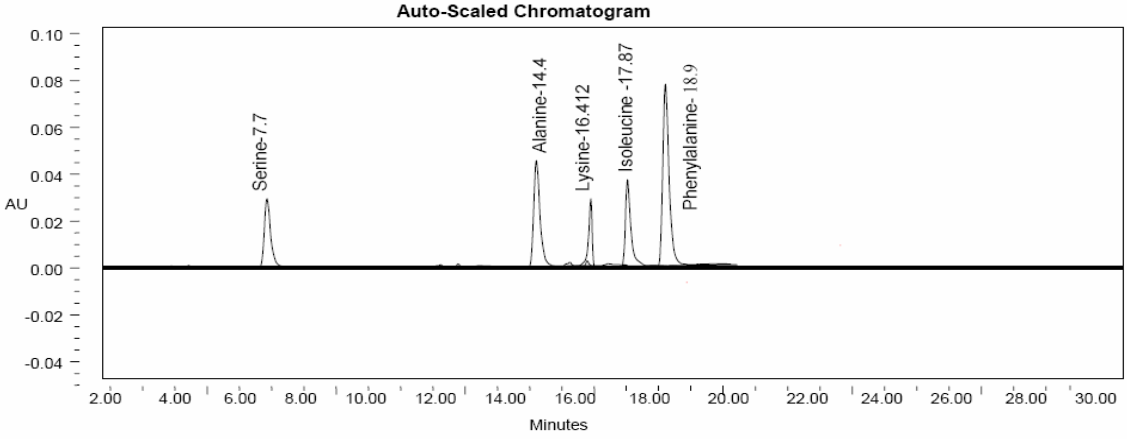
SAMPLE INFORMATION

Sample Name:	Amino Acid	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	Amino Acid
Vial:	14	Acq. Method Set:	Amino Acid
Injection #:	2	Processing Method:	Amino Acid
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	30.0 Minutes		
Date Acquired:	03/28/2010 17:09:31 IST		
Date Processed:	03/29/2010 10:30:39 IST		



SAMPLE INFORMATION

Sample Name:	Amino Acid	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	Amino Acid
Vial:	15	Acq. Method Set:	Amino Acid
Injection #:	2	Processing Method:	Amino Acid
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	30.0 Minutes		
Date Acquired:	03/26/2010 15:00:39 IST		
Date Processed:	03/27/2010 17:39:30 IST		



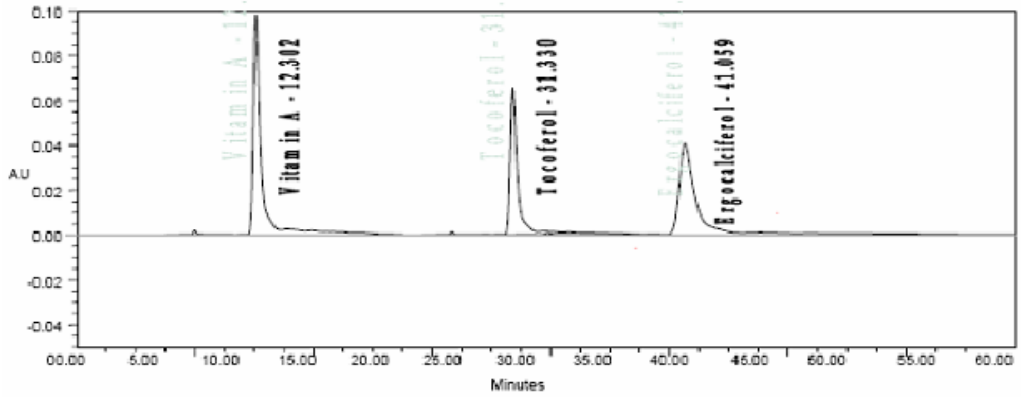
Appendix III

Chromatogram of starndard solution of vitamins obtained from HPLC

SAMPLE INFORMATION

Sample Name:	STD Vitamin A,D2,E	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	Vitamin,A,D,E
Vial:	10	Acq. Method Set:	Vitamin,A,D,E
Injection #:	1	Processing Method:	Vitamin,A,D,E
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	60.0 Minutes	Proc. Chnl. Descr.:	256
Date Acquired:	01/13/2010 12:45:02 IST		
Date Processed:	01/13/2010 17:50:51 IST		

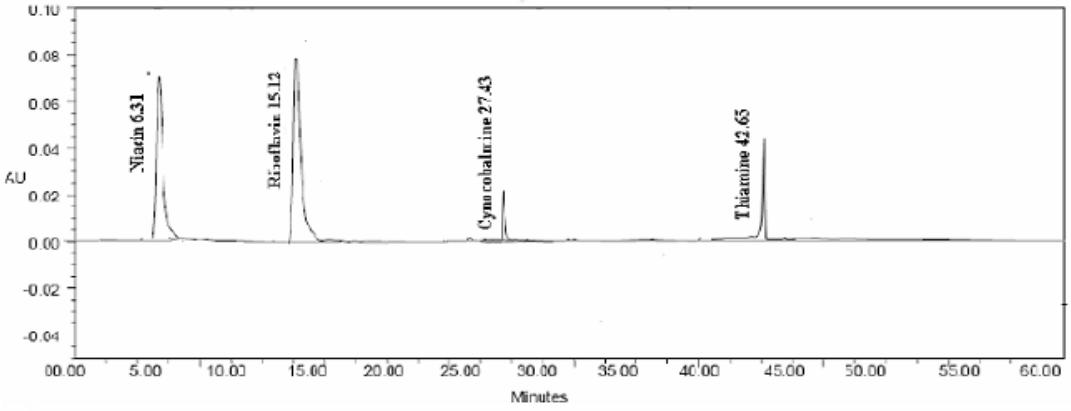
Auto-Scaled Chromatogram



SAMPLE INFORMATION

Sample Name:	B1, B2, B3, B12	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	Vitamin B1, B2, B3, B12
Vial:	2	Acq. Method Set:	Vitamin B1, B2, B3, B12
Injection #:	1	Processing Method:	Vitamin B1, B2, B3, B12
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	60.0 Minutes	Proc. Chnl. Descr.:	226
Date Acquired:	02/13/2010 14:47:27 IST		
Date Processed:	02/13/2010 17:54:28 IST		

Auto-Scaled Chromatogram



Appendix IV

LSU sequences of collection no. 348-07

LOCUS HM750916 926 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus sp. DK-2010b 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750916
VERSION HM750916
KEYWORDS .
SOURCE Cantharellus appalachiensis
ORGANISM Cantharellus appalachiensis
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 926)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 926)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..926
/organism="Cantharellus appalachiensis"
/mol_type="genomic DNA"
/strain="84-08"
/db_xref="taxon:409893"
rRNA <1..>926
/product="25S ribosomal RNA"
ORIGIN

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      121 gtagtccatc tggactgcat ggcgtccaag tacaataggc cgtcagtggg acatcataga
      181 gggtgacaat cccgtccttg atgccaatgt gcaagtccgt tacgatggct atctcagaga
      241 gtcgagcagt ttgggatttg ctgcttaaac atgggaggta gattccttct aaagctaaat
      301 acaggcgaga gaccgataga gaacaagtac cgcaagggaa agatgaaata gaactctgtg
      361 aagggagtca aaaggcgtga aattgttgcg agagaagcga ttcaagtcag cgtagctagg
      421 cccatcaagc gtctccgctt tggactagct ttgccagcgg tcttgtcctt agagacttga
      481 cataatatac gtgtccagcc tggtatgcac aggtagcccc tctgggggcc agtggcacgg
      541 ctggtggaat ggcttgcaat cgacccgtct tgaaacacgg accaaggagt ctaacatgta
      601 tgcgagtata agggtggcaa acccgtatgc gcaatgaaag tgattttcgg tgattaagaa
      661 tggcctgatg accagacaag caatacatgt ccctcggacg gccattgccc aagttccgtg
      721 gtagatccca tgaacatgga gttggagcat acatgctagg acccgaaaga tggtgaacta
      781 tgcctggaca aagcgaagcc aaaggaaact ctggtggagg cttgtagcga ttctgacgtg
      841 caaatcgatc gtctgatctg ggtatagggg cgaaagacta atcgaaccat ctcatagctg
      901 gttcctgccg aagtttccct caggat
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LSU sequences of collection no. 113-07

LOCUS HM750917 925 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus sp. DK-2010b 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750917
VERSION HM750917
KEYWORDS .
SOURCE Cantharellus sp. DK-2010b
ORGANISM Cantharellus sp. DK-2010b
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 925)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 925)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar
University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..925
/organism="Cantharellus sp. DK-2010b"
/mol_type="genomic DNA"
/strain="113"
/db_xref="taxon:885892"
rRNA <1..>925
/product="25S ribosomal RNA"
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     121 gagtgggcca tctggactgc gtggcgtcca agtacaatag gccgtcagtg ggacatcata
     181 gagggtgaca atcccgtcct tgatgccaac gtacgagtcc tttacgatgc cttatctcgg
     241 agagtcaagc agtttgggat ttgctgctta aacatgggag gtagattcct tctaaagcta
     301 aatacaggca agagaccgat agagaacaag taccgcaagg gaaagatgaa atagaactca
     361 gtgaagggag tcaaaaggcg tgaaattgtt gcgagagaag cgattcaagt cagcgtagct
     421 gggcccatta agcctctgcg ctttggacca gctttgccag cggtcttgtc ttagagactt
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     541 gtggaatggc ttgcaatcga cccgccttga aacacggacc aaggagtcta acatgcatgc
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     661 cctgatgacc agacaagcaa tattatgtcc ctcgtacggt cattgcccaa gtcccatggt
     721 cagatcccat ggacatggag ttggagcatg catgctagga cccgaaagat ggtgaactat
     781 gcctggacaa agcgaagcca aaggaaactc tggtggaggc ttgtagcgat tctgacgtgc
     841 aaatcgatcg tctgatctgg gtataggggc gaaagactaa tcgaaccatc tcattgctgg
     901 ttcctgccga agtttccctc aggat
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LSU sequences of collection no. 121-08

LOCUS HM750918 927 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus sp. DK-2010a 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750918
VERSION HM750918
KEYWORDS .
SOURCE Cantharellus sp. DK-2010a
ORGANISM Cantharellus sp. DK-2010a
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 927)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 927)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar
University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..927
/organism="Cantharellus sp. DK-2010a"
/mol_type="genomic DNA"
/strain="121"
/db_xref="taxon:885894"
rRNA <1..>927
/product="25S ribosomal RNA"

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     181 ggtgacaatc ccgtccttga cgccaatgtg caagtctatt acgatggcta tctcagagag
     241 tcgagcagtt tgggatttgc tgcttaaaca tgggaggtag attccttcta aagctaaata
     301 caggcgagag accgatggag aacaagtacc gcaagggaaa gatgaaatag cactctgtga
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     661 atggcctgat gaccagacaa gcaatatatg tccctcgtac ggccattgcc caagttccgt
     721 ggtagatccc acgaacatgg agttggagca tacatgctag gacccgaaag atggtgaact
     781 atgcctggac aaagcgaagc caaaggaaac tctggtggag gcttgtagcg attctgacgt
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     901 ggttcctgcc gaagtttccc tcaggat
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LSU sequences of collection no. 161-07

LOCUS HM750919 942 bp DNA linear PLN 15-SEP-2010
DEFINITION Craterellus cantharellus var. intermedius strain 161 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750919
VERSION HM750919
KEYWORDS .
SOURCE Craterellus cantharellus var. intermedius
ORGANISM Craterellus cantharellus var. intermedius
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 942)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 942)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..942
/organism="Craterellus cantharellus var. intermedius"
/mol_type="genomic DNA"
/strain="161"
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/db_xref="taxon:885901"
rRNA <1..>942
/product="25S ribosomal RNA"
ORIGIN

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      721 ttgcccaagt tccgtggtag atcccatgaa catggagttg gagcatacat gctaggaccc
      781 gaaagatggt gaactatgcc tggacaaagc gaagccaaag gaaactctgg tggaggcttg
      841 tagcgattct gacgtgctaa tcgatcgtct gatctgggta taggggcgaa agactaatcg
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LSU sequences of collection no. 354-05

LOCUS HM750923 950 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus minor strain 354 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750923
VERSION HM750923
KEYWORDS .
SOURCE Cantharellus minor
ORGANISM Cantharellus minor
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 950)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 950)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..950
/organism="Cantharellus minor"
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/strain="354"
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rRNA <1..>950
/product="25S ribosomal RNA"
ORIGIN

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     121 gagtaggtca tctggactgc gtggtgtcca agtacaatag gccgtcagtg ggacatcata
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LSU sequences of collection no. MSR2-07

LOCUS HM750924 923 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus cibarius var. longipes strain MSR2 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750924
VERSION HM750924
KEYWORDS .
SOURCE Cantharellus cibarius var. longipes
ORGANISM Cantharellus cibarius var. longipes
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 923)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 923)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..923
/organism="Cantharellus cibarius var. longipes"
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/strain="MSR2"
/variety="longipes"
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rRNA <1..>923
/product="25S ribosomal RNA"

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LSU sequences of collection no. 106-08

LOCUS HM750926 927 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus sp. DK-2010f 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750926
VERSION HM750926
KEYWORDS .
SOURCE Cantharellus sp. DK-2010f
ORGANISM Cantharellus sp. DK-2010f
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 927)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 927)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar
University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..927
/organism="Cantharellus sp. DK-2010f"
/mol_type="genomic DNA"
/strain="106"
/db_xref="taxon:885905"
rRNA <1..>927
/product="25S ribosomal RNA"

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ORIGIN
       1 gcatatcaat aagcggagga aaagaaacta actaggattc ccccagtaac tgcgagtgaa
      61 gagggaagag ctcatcattg gaatctggca gtgttgcact gtccgagttg tagatgagga
     121 gtagtccgtc tggactgcat ggcgtccaag tacaataggc cgtcagtggg acatcataga
     181 gggtgacaat cccgtccttg acgccaatgt gcaagtctat tacgatggct atctcagaga
     241 gtcgagcagt ttgggatttg ctgcttaaac atgggaggta gattccttct aaagctaaat
     301 acaggcgaga gaccgataga gaacaagtac cgcaagggaa agatgaaata gcactctgtg
     361 aagggagtca aaaggcgtga aattgttgcg agagaagcga ttcaagtcag cgtagctagg
     421 cccatcaagc gtcttcgctt tggactagct ttgccagcgg tcttgtccct tgaagagact
     481 tgacttgtaa tatgtccagc ctggcgtgca caggcagccc cttctggggg ccagtggcat
     541 ggctgtggaa tggcttgcaa tcgacccgtc ttgaaacacg gaccaaggag tctaacatgt
     601 atgcgagtat aagggtggca aacccgtatg cgcaatgaaa gtgattttcg gtgattgaga
     661 atggcctgat gaccagacaa gcaatatatg tccctcgtac ggccattgcc caagttccgt
     721 ggtagatccc acgaacatgg agttggagca tacatgctag gacccgaaag atggtgaact
     781 atgcctggac aaagcgaagc caaaggaaac tctggtggag gcttgtagcg attctgacgt
     841 gcaaatcgat cgtctgatct gggtataggg gcgaaagact aatcgaacca tctcatagct
     901 ggttcctgcc gaagtttccc tcaggat
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LSU sequences of collection no. 90-09

LOCUS HM750927 928 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus cibarius strain 90 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750927
VERSION HM750927
KEYWORDS .
SOURCE Cantharellus cibarius (chanterelle)
ORGANISM Cantharellus cibarius
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 928)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 928)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..928
/organism="Cantharellus cibarius"
/mol_type="genomic DNA"
/strain="90"
/db_xref="taxon:36066"
rRNA <1..>928
/product="25S ribosomal RNA"
ORIGIN

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       1 gcatatcaat aagcggagga aaagaaacta actaggattc ccccagtaac tgcgagtgaa
      61 gagggaagag ctcatcattg gaatctggca gtgttgcact gtccgagttg tagatgagga
     121 gtagtccgtc tggactgcat ggcgtccaag tacaataggc cgtcagtggg acatcataga
     181 gggtgacaat cccgtccttg acgccaatgt gcaagtctat tacgatggct atctcagaga
     241 gtcgagcagc ttgggatttg ctgcttaaac atgggaggta gattccttct aaagctaaat
     301 acaggcgaga gaccgataga gaacaagtac cgcaagggaa agatgaaata gcactctgtg
     361 aagggagtca aaaggcgtga aattgttgcg agagaagcga ttcaagtcag cgtagctagg
     421 cccatcaagc gtcttcgctt tggactagct ttgccagcgg tcttgtccct tgaagagact
     481 tgacttgtaa tatgtccagc ctggcgtgca caggcagccc cttctggggg ccagtggcat
     541 ggctggtgga atggcttgca atcgacccgt cttgaaacac ggaccaagga gtctaacatg
     601 tatgcgagta taagggtggc aaacccgtat gcgcaatgaa agtgattttc ggtgattgag
     661 aatggcctga tgaccagaca agcaatatat gtccctcgta cggccattgc ccaagttccg
     721 tggtagatcc cacgaacatg gagttggagc atacatgcta ggacccgaaa gatggtgaac
     781 tatgcctgga caaagcgaag ccaaaggaaa ctctggtgga ggcttgtagc gattctgacg
     841 tgcaaatcga tcgtctgatc tgggtatagg ggcgaaagac taatcgaacc atctcatagc
     901 tggttcctgc cgaagtttcc ctcaggat
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LSU sequences of collection no. 169-07

LOCUS HM750928 837 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus sp. DK-2010c 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750928
VERSION HM750928
KEYWORDS .
SOURCE Cantharellus sp. DK-2010c
ORGANISM Cantharellus sp. DK-2010c
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 837)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 837)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar
University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..837
/organism="Cantharellus sp. DK-2010c"
/mol_type="genomic DNA"
/strain="169"
/db_xref="taxon:885902"
rRNA <1..>837
/product="25S ribosomal RNA"

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ORIGIN
       1 actgcgagtg agagggaaga gctcatcatt ggaatctggc agcgttgcgc tgtccgagtt
      61 gtagatgaga agagtaggac atctggactg catggtgtcc aagtacaata ggccgtcagt
     121 gggacatcat agagggtgac aatcccgtcc ttgacaccaa tgtacgagtc ctttacgatg
     181 cctatctcgg agagtcgagc agtttgggat ttgctgctta aacacgggag gtagattcct
     241 tccaaagcta aatacaggca agagaccgat agtgaacaag taccgcaagg gaaagatgaa
     301 atagaactct gtgaagggag tcaaaaagac gtgaaattgt tgcgagagaa gcgattcaag
     361 tcagcgtagc tgggcccatc aagcctcgcg ctttggacca gctttgccag cggtcttgtc
     421 cctagagact tgactaaggt tgccctgccc ggtctaacgg acagacctcc cctctgggga
     481 ggccagtggc atggctggtg gaatggcttg caatcgaccc gtcttgaaac acggaccaag
     541 gagtctaaca tgtacgcgag tataagggtg gcaaacccgt atgcgcaaag aaagtgatag
     601 cctatggctg agaatgacct gacgacctga caagaaatat catgtccctc gtacggtcat
     661 tgcccaagtc ccatggtcag atcccatgga catggagttg gagcgtgcat gctaggaccc
     721 gaaagatggt gaactatgcc tggacaaagc gaagccaaag gaaactctgg tggaggcttg
     781 tagcgattct gacgtgcaaa tcgatcgtct gatctgggta taggggcgaa agactaa
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LSU sequences of collection no. 184-08

LOCUS HM750929 834 bp DNA linear PLN 15-SEP-2010
DEFINITION Cantharellus sp. DK-2010e 25S ribosomal RNA gene, partial sequence.
ACCESSION HM750929
VERSION HM750929
KEYWORDS .
SOURCE Cantharellus sp. DK-2010e
ORGANISM Cantharellus sp. DK-2010e
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 834)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 834)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-JUL-2010) Department of Biotechnology, Thapar
University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..834
/organism="Cantharellus sp. DK-2010e"
/mol_type="genomic DNA"
/strain="184"
/db_xref="taxon:885904"
rRNA <1..>834
/product="25S ribosomal RNA"
ORIGIN

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      1 tactgcgagt gagagggaag agctcatcat tggaatctgg cagtgttgca ctgtccgagt
     61 tgtagatgag gagtagtccg tctggactgc atggcgtcca agtacaatag gccgtcagtg
    121 ggacatcata gagggtgaca atcccgtcct tgacgccaat gtgcaagtct attacgatgg
    181 ctatctcaga gagtcgagca gtttgggatt tgctgcttaa acatgggagg tagattcctt
    241 ctaaagctaa atacaggcga gagaccgata gagaacaagt accgcaaggg aaagatgaaa
    301 tagcactctg tgaagggagt caaaaggcgt gaaattgttg cgagagaagc gattcaagtc
    361 agcgtagcta ggcccatcaa gcgtcttcgc tttggactag ctttgccagc ggtcttgtcc
    421 cttgaagaga cttgacttgt aatatgtcca gcctggcgtg cacaggcagc cccttctggg
    481 ggccagtggc atggctggtg gaatggcttg caatcgacct gtcttgaaac acggaccaag
    541 gagtctaaca tgtatgcgag tataagggtg gcaaacccgt atgcgcaatg aaagtgattt
    601 tcggtgattg agaatggcct gatgaccaga caagcaatat atgtccctcg tacggccatt
    661 gcccaagttc cgtggtagat cccacgaaca tggagttgga gcatacatgc taggacccga
    721 aagatggtga actatgcctg gacaaagcga agccaaagga aactctggtg gaggcttgta
    781 gcgattctga cgtgcaaatc gatcgtctga tctgggtata ggggcgaaag acta
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LSU sequences of collection no. 95-08

LOCUS HQ342887 800 bp DNA linear PLN 28-MAR-2011
DEFINITION Cantharellus appalachiensis strain 39-07 large subunit ribosomal RNA gene, partial sequence.
ACCESSION HQ342887
VERSION HQ342887
KEYWORDS .
SOURCE Cantharellus appalachiensis
ORGANISM Cantharellus appalachiensis
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 800)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 800)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (06-OCT-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..800
/organism="Cantharellus appalachiensis"
/mol_type="genomic DNA"
/strain="39-07"
/db_xref="taxon:409893"
/country="India: north-western Himalayan region"
/collected_by="Deepika Kumari"
/identified_by="M. S. Reddy"
rRNA <1..>800
/product="large subunit ribosomal RNA"
ORIGIN

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        1 ctggcagcgt ctcgctgtcc gagttgtaga tgagaagagt gggccatctg gactgcatgg
       61 tgtccaagta caataggccg tcagtgggac atcatagagg gtgacaatcc cgtccttgac
      121 accaatgtac gagtcctttt acgatgccta tctcggagag tcgagcagtt tgggatttgc
      181 tgcttaaaca tgggaggtag attccttcca aagctaaata caggcaagag accgatagag
      241 aacaagtacc gcaagggaaa gatgaaatag aactctgtga agggagtcaa aagacgtgaa
      301 attgttgcga gagaagcgat tcaagtcagc gtagctgggc ccatcaagcc tcgcgctttg
      361 gaccagcttt gccagcggtc ttgtcctaga gactcgacta aggtgccctg cccggtctga
      421 cgggcagacc ttccccccctg ggggaggcca gcggcacggc tggtggaatg gcttgcaatc
      481 gacccgtctt gaaacacgga ccaaggagtc taacatgcat gcgagtataa gggtggcaaa
      541 cccgtatgcg caaagaaagt gatagccttt acggctgtga atgacctgat gaccagacaa
      601 gcgatatcat gtccctcgta cggtcattgc ccaagtcccg tggtcagatc ccatggacat
      661 ggagttggag cgtgcatgct aggacccgaa agatggtgaa ctatgcctgg acaaagggaa
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      781 ctgggtatag gggcgaaaga
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LSU sequences of collection no. 281-07

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LOCUS       GU237071               829 bp    DNA     linear   PLN 28-DEC-2009
DEFINITION  Cantharellus sp. SMR-2009a large subunit ribosomal RNA gene,
            partial sequence.
ACCESSION   GU237071
VERSION     GU237071.1  GI:281490066
KEYWORDS    .
SOURCE      Cantharellus sp. SMR-2009a
  ORGANISM  Cantharellus sp. SMR-2009a
            Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
            Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE   1  (bases 1 to 829)
  AUTHORS   Reddy,S.M., Kumari,D. and Upadhyay,R.C.
  TITLE     Cantharellus pseudoformosus a new species associated with Cedrus
            deodara from India
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 829)
  AUTHORS   Reddy,S.M., Kumari,D. and Upadhyay,R.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-NOV-2009) Department of Biotechnology, Thapar
            University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES             Location/Qualifiers
     source          1..829
                     /organism="Cantharellus sp. SMR-2009a"
                     /mol_type="genomic DNA"
                     /strain="MSR-4"
                     /specimen_voucher="PUN3883"
                     /db_xref="taxon:701518"
     rRNA            <1..>829
                     /product="large subunit ribosomal RNA"
ORIGIN
        1 gagtgaagag ggaagagctc atcattggaa tctggcagtg ttgcactgtc cgagttgtag
       61 atgaggagta gtccatctgg actgcatggc gtccaagtac aataggccgt cagtgggaca
      121 tcatagaggg tgacaatccc gtccttgatg ccaatgtgca agtccgttac gatggctatc
      181 tcagagagtc gagcagtttg ggatttgctg cttaaacatg ggaggtagat tccttctaaa
      241 gctaaataca ggcgagagac cgatagagaa caagtaccgc aagggaaagg tgaaatagaa
      301 ctctgtgaag ggagtcaaaa ggcgtgaaat tgttgcgaga gaagcgattc aagtcagcgt
      361 agctaggccc atcaagcgtc tccgctttgg actagctttg ccagcggtct tgtccttaga
      421 gacttgacat aatatacgtg tccagcctgg tatgcacagg tagcccctct gggggccagt
      481 ggcacggctg gtggaatggc ttgcaatcga cccgtcttga aacacggacc aaggagtcta
      541 acatgtatgc gagtataagg gtggcaaacc cgtatgcgca atgaaagtga ttttcggtga
      601 ttaagaatgg cctgatgacc agacaagcaa tacatgtccc tcggacggcc attgcccaag
      661 ttccgtggta gatcccatga acatgagttg gagcatacat gctaggaccc gaaagatggt
      721 gaactatgcc tggacaaagc gaagccaaag gaaactctgg tggaggcttg tagcgattct
      781 gacgtgcaaa tcgatcgtct gatctgggta taggggcgaa agactaatc
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ITS sequences of collection no. 184-08

LOCUS HQ270115 1337 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus sp. DK-2010e isolate 184 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270115
VERSION HQ270115
KEYWORDS .
SOURCE Cantharellus sp. DK-2010e
ORGANISM Cantharellus sp. DK-2010e
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1337)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1337)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1337
/organism="Cantharellus sp. DK-2010e"
/mol_type="genomic DNA"
/isolate="184"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:885904"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..110
/product="18S ribosomal RNA"
misc_RNA 111..777
/product="internal transcribed spacer 1"
rRNA 778..956
/product="5.8S ribosomal RNA"
misc_RNA 957..1293
/product="internal transcribed spacer 2"
rRNA 1294..>1337
/product="28S ribosomal RNA"
ORIGIN

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        1 ccgtaggtga acctgcggaa ggatcaaccc ctgtgggtat agtgagatgg tttttccaac
       61 ccaacccgtg cgcacatcca ttggactgga tgcacgggtg tgtgtggtct tcttggtggg
      121 gaggcctggt tagggcctga cttggtgggg gtgactaggc tggacggtgt actgacaaag
      181 ccgtttttcc agttatagag ccgttccggt cgggcccaag ggcatgtcca gtcccattgg
      241 tggtggacgc ccctccgtgg gctctgaggc cttgtcaccc tcagtgtccg tcggagtcta
      301 caaaggggca cttgtgcccc ggcgtaacgt ggatgtgtac atacatacat acatattccg
      361 gttggccttg tgccaacaac atgccagtga agacgtgtcc ttgtccactc caacgttttt
      421 acctacggga caaggcctag ctggtccgca ggcggcggga tgacttgggt tggaagaggg
      481 gggttccaga aggagaagga gactacactt ggtcgatctc tctctgctgg acttgttggg
      541 tgtaaggtag gcatccacag ttatttggcg tgcaaccccg atgtgtacac aatgccctcc
      601 ttccgttgtg cccaatcgtc tgaagttatg ggcctcgacg aattgatgta agtcgagggg
      661 gtcatatctg tttttggccc ggcatggacc tagacctaca cgcggtgccc ctgttcttga
      721 ctgattaggt ctataacatc aagctgagtt ttccaatggg tttttaaata actctcaaca
      781 atggatctct aggctcttgc atcgatgaag aacgcagtga actgcgataa ctggtgtgag
      841 ttgcatggca cttctaatct aacaccaagc gaatcatcga gtctttgaac gcaaacggca
      901 cccttccagt ccattccaaa gcggtggcgg aggatgaaga caagggtatc tctggctgag
      961 ggtaatgaga actgatccag tgtaaccgtt ggttagctgg ggattgggct tgcttggggc
     1021 agttgctctg gcttgcctca aaatcaagcg ttgtgtggat tggactttca agcatgcatt
     1081 ggggacgcag gctctgcgct atggcaagcc cttgaccgtc ataggtgctt tgattggggt
     1141 tttcagtcta gccaacaagg ctgggttgaa ggactttggg gctgcattgg gggcgcaagg
     1201 gcagctctgt tcgtggcgtc cgatgaccgt catgatgcat gcgtgattgg acttcaacta
     1261 gcaatttatc attatcatca tcattactat gggttacctc aggtcagaga agactacccg
     1321 ctggacttaa gcatatc //
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ITS sequences of collection no. 348-07

LOCUS HQ270116 1386 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus sp. DK-2010h isolate 317 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270116
VERSION HQ270116
KEYWORDS .
SOURCE Cantharellus sp. DK-2010h
ORGANISM Cantharellus sp. DK-2010h
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1386)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1386)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..1386
/organism="Cantharellus sp. DK-2010h"
/mol_type="genomic DNA"
/isolate="317"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:885907"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..105
/product="18S ribosomal RNA"
misc_RNA 106..857
/product="internal transcribed spacer 1"
rRNA 858..991
/product="5.8S ribosomal RNA"
misc_RNA 992..1340
/product="internal transcribed spacer 2"
rRNA 1341..>1386
/product="28S ribosomal RNA"
ORIGIN

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       1 ccgtaggtga acctgcggaa ggatcaaccc ctgtaatagt ttgtgggcaa gtctccccgt
      61 gttgtaagtc cattggactg gatgcgcggt ggtggatgcc ggtctccttg gtggggaggc
     121 ctggcttagt taggcctttc ttggcttggt gggggcggtg gctaggctgg acggtgcact
     181 gacaaagccg ttttccagtt atagggccgt ttttccaagt cgggccaggg catgtccagt
     241 cccatccatt ggtggtggtg gggcgcccct ccttggctct gaggccttgt cgccctcagt
     301 tgtccgtcgg agtctagaaa gaaacctggc gtgggccaga tggattggat tggccttgtg
     361 ccaataacat gccggcgaag atgtccttgt ccgctccatt ctactatacc tacaggacaa
     421 gacctagctg gtccgcaggt ggcgggatga cttgggttgg gagagagggg ttccggatgg
     481 aagactgggt agcatagcat aggtggcgtt gctagtgcgc tgctgaggag caaccggcca
     541 gttggtccgc gctacttggt tgatatctgg acttgctttt ggatgtaagg taggcatcca
     601 caagtgttgg tgtgtgcacc cagtgtgctc ccaatggatc tctaggctct tgcatcgatg
     661 aagaacgcag tgaactgtcc ttccgttgtg cccaatcgtc tgaagttatg ggcctcgacg
     721 aattgatgta agtcgagggg gtcatatctg tttttggccc ggcatggacc tagacctaca
     781 cgcggtgccc ctgttcttga ctgattaggt ctataacatc aagctgagtt ttccaatggg
     841 tttttagata actctcaacc gataactggt gtgagttgca cggcacttct aatctaacac
     901 caagcgaatc atcgagtctt tgaacgcaaa cggcaccctt ccagtccatt ccaaagcggt
     961 ggcggaggat gaagacaagg gtatctctgg ctgagggtaa tggaaactga tccagcgtgc
    1021 cactctggtc actggggatt gggcttgctt ggggcaacgt tgctctggct tgccttaaaa
    1081 tcaagcgttg tgtggattgg actttcaagc atgcattggg gacgcaggct ctgcgctatg
    1141 gcaagccctt gaccgtcata ggtgctttga ttggggtttc agtctagcca acaaggctgg
    1201 gttggagact ttggggctgc atttgggggg gccgtagggc agctctgttc gtggcgtccg
    1261 atgaccgtca tggtgcatga ttggaccttc ttcaactagc aatttatcat ctgatatcat
    1321 atcatatcat cattaccatg ggttacctca ggtcagagaa gactacccgc tggacttaag
    1381 catatc
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//

ITS sequences of collection no. 121-08

LOCUS HQ270118 1333 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus sp. DK-2010a isolate 121 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270118
VERSION HQ270118
KEYWORDS .
SOURCE Cantharellus sp. DK-2010a
ORGANISM Cantharellus sp. DK-2010a
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1333)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1333)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..1333
/organism="Cantharellus sp. DK-2010a"
/mol_type="genomic DNA"
/isolate="121"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:885894"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..110
/product="18S ribosomal RNA"
misc_RNA 111..777
/product="internal transcribed spacer 1"
rRNA 778..952
/product="5.8S ribosomal RNA"
misc_RNA 953..1289
/product="internal transcribed spacer 2"
rRNA 1290..>1333
/product="28S ribosomal RNA"
ORIGIN

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     121 gaggcctggt tagggcctga cttggtgggg gtgactaggc tggacggtgt actgacaaag
     181 ccgtttttcc agttatagag ccgttccggt cgggcccaag ggcatgtcca gtcccattgg
     241 tggtggacgc ccctccgtgg gctctgaggc cttgtcaccc tcagcgtccg tcggagtcta
     301 caaaggggca cttgtgcccc ggcgtaacgt ggatgtgtac atacatacat attccggttg
     361 gccttgtgcc aacaacatgc cagtgaagac gtgtccttgt ccactccaac gtttttacct
     421 acgggacaag gcctagctgg tccgcaggcg gcgggatgac ttgggttgga agaggggggt
     481 tccagaagga gaaggagact acacttggtc gatctctctc tgctggactt gttgggtgta
     541 aggtaggcat ccacagttat ttggcgtgca accccgatgt gtacacaatg ccctccttcc
     601 gttgtgccca atcgtctgaa gttatgggcc tcgacgaatt gatgtaagtc gaggggggtca
     661 tatctgtttt tggcccggca tggacctaga cctacacgcg gtgcccctgt tcttgactga
     721 ttaggtctat aacatcaagc tgagttttcc aatgggtttt taaataactc tcaacaatgg
     781 atctctaggc tcttgcatcg atgaagaacg cagtgaactg cgataactgg tgtgagttgc
     841 atggcacttc taatctaaca ccaagcgaat catcgagtct ttgaacgcaa acggcaccct
     901 tccagtccat tccaaagcgg tggcggagga tgaagacaag ggtatctctg gctgagggta
     961 atgagaactg atccagtgta accgttggtt agctggggat tgggcttgct tggggcagtt
    1021 gctctggctt gcctcaaaat caagcgttgt gtggattgga ctttcaagca tgcattgggg
    1081 acgcaggctc tgcgctatgg caagcccttg accgtcatag gtgctttgat tggggttttc
    1141 agtctagcca acaaggctgg gttgaaggac tttggggctg cattgggggc gcaagggcag
    1201 ctctgttcgt ggcgtccgat gaccgtcatg atgcatgcgt gattggactt caactagcaa
    1261 tttatcatta tcatcatcat tactatgggt tacctcaggt cagagaagac tacccgctgg
    1321 acttaagcat atc//
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ITS sequences of collection no. 354-05

LOCUS HQ270119 1411 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus minor isolate 354 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270119
VERSION HQ270119
KEYWORDS .
SOURCE Cantharellus minor
ORGANISM Cantharellus minor
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1411)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1411)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1411
/organism="Cantharellus minor"
/mol_type="genomic DNA"
/isolate="354"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:57195"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..107
/product="18S ribosomal RNA"
misc_RNA 108..816
/product="internal transcribed spacer 1"
rRNA 817..983
/product="5.8S ribosomal RNA"
misc_RNA 984..1295
/product="internal transcribed spacer 2"
rRNA 1296..>1411
/product="28S ribosomal RNA"
ORIGIN

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      121 ggcctggctt agttaggcct ttcttggctt ggtgggggcg gtggctaggc tggacggtgc
      181 actgacaaag ccgttttcca gttatagggc cgtttttcca agtcgggcca gggcatgtcc
      241 agtcccatcc attggtggtg gtggggcgcc cctccttggc tctgaggcct tgtcgccctc
      301 agttgtccgt cggagtctag aaagaaacct ggcgtgggcc agatggattg gattggcctt
      361 gtgccaataa catgccggcg aagatgtcct tgtccgctcc attctatacc tacaggacaa
      421 gacctagctg gtccgcaggt ggcgggatga cttgggttgg gagagagggg ttccggatgg
      481 aagactgggt agcatagcat aggtggcgtt gctagtgcgc tgctgaggag gagcaaccgg
      541 ccagttggtc cgcgctactt ggttgatatc tggacttgct tttggatgta aggtaggcat
      601 ccacaagtgt tggtgtgtgc acccagtgtg ctccacgccc ccccttccgt cgcggcccaa
      661 tcatctgaag ttatgggcct cgacgatgat gtgagtcgag ggggtcatca tctgttggcc
      721 cggcatggac ctaacctaca cgcgtgccct gttcttgact gattaggtct ataacatcaa
      781 gctgagtttt ccaatgggtt atcgagataa ctctcaacaa tggatccgct ccatacacgg
      841 ggggcaaaga tcgagctggt ccgcaggtgg cggggtggtc ttgagaggag aggagaggag
      901 gaggaggagg ggaggtcctt cgcgttcccc ttccatgcat gcatgctcgg gccatctgta
      961 gtaagtgggc ctcgacgatt tgatgtgagg gttgagtcga gggggtcttt cgtggacctg
     1021 gcatggacct aaaccttcac gcggtgcccc tgttcgtact gattaggtct ataacatcaa
     1081 tcctaggtgt tccaatgggt tatgagataa ctctcaacaa tggatctcta ggctcttgca
     1141 tcgatgaaga acgcagtgaa ctgcgataac tggtgtgagt tgcacggcac ctctaatcta
     1201 acaccaagcg aatcatcgag tctttgaacg caaacggcac ccttccagtc cattccaaag
     1261 cggtggcggc ggatgaagac aagggtatct ctggttgagg gcaatgagaa tcgatccagg
     1321 ctgaatcggc ctggggattg ggcttgcttg ggggagtgag ggtgcactct tggcttgcct
     1381 taaaatcact gttgtgttga ttgaactttc a//
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ITS sequences of collection no. 106-08

LOCUS HQ270120 1329 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus sp. DK-2010f isolate 106 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270120
VERSION HQ270120
KEYWORDS .
SOURCE Cantharellus sp. DK-2010f
ORGANISM Cantharellus sp. DK-2010f
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1329)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1329)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1329
/organism="Cantharellus sp. DK-2010f"
/mol_type="genomic DNA"
/isolate="106"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:885905"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..109
/product="18S ribosomal RNA"
misc_RNA 110..772
/product="internal transcribed spacer 1"
rRNA 773..940
/product="5.8S ribosomal RNA"
misc_RNA 941..1231
/product="internal transcribed spacer 2"
rRNA 1232..>1329
/product="28S ribosomal RNA"
ORIGIN

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     121 aggcctggtt agggcctgac ttggtggggg tgactaggct ggacggtgta ctgacaaagc
     181 cgtttttcca gttatagagc cgttccggtc gggcccaagg gcatgtccag tcccattggt
     241 ggtggacgcc cctccgtggg ctctgaggcc ttgtcaccct cagtgtccgt cggagtctac
     301 aaaggggcac ttgtgccccg gcgtaacgtg gatgtgtaca tacatacata ttccggttgg
     361 ccttgtgcca acaacatgcc agtgaagacg tgtccttgtc cactccaacg tttttaccta
     421 cgggacaagg cctagctggt ccgcaggcgg cgggatgact tgggttggaa gaggggggtt
     481 ccagaaggag aaggagacta cacttggtcg atctctctct gctggacttg ttgggtgtaa
     541 ggtaggcatc cacagttatt tggcgtgcaa ccccgatgtg tacacaatgc cctccttccg
     601 ttgtgcccaa tcgtctgaag ttatgggcct cgacgaattg atgtaagtcg agggggtcat
     661 atctgttttt ggcccggcat ggacctagac ctacacgcgg tgcccctgtt cttgactgat
     721 taggtctata acatcaagct gagttttcca atgggttttt aaataactct caacaatgga
     781 tctctaggct cttgcatcga tgaagaacgc agtgaactgc gataactggt gtgagttgca
     841 tggcacttct aatctaacac caagcgaatc atcgagtctt tgaacgcaaa cggcaccctt
     901 ccagtccatt ccaaagcggt ggcggaggat gaagacaagg gtatctctgg ctgagggtaa
     961 tgagaactga tccagtgtaa ccgttggtta gctggggatt gggcttgctt ggggcagttg
    1021 ctctggcttg cctcaaaatc aagcgttgtg tggattggac tttaatcgga tccctcgggg
    1081 taattgctct ggcttgcctt aaaatcaagc gttgtgtgga ttggactttc aagcatgcat
    1141 tggggacgca ggctctgcgc tatggcaagc ccttgaccgt cataggtgct ttgattgggg
    1201 ttttcagtct agccaacaag gctgggttga aggactttgg ggctgcattg ggggcgcaag
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ITS sequences of collection no. 161-07

LOCUS HQ270121 1432 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus lateritius isolate 161 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270121
VERSION HQ270121
KEYWORDS .
SOURCE Cantharellus lateritius
ORGANISM Cantharellus lateritius
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1432)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1432)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India source 1..1432
/organism="Cantharellus lateritius"
/mol_type="genomic DNA"
/isolate="161"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:57194"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..155
/product="18S ribosomal RNA"
misc_RNA 156..202
/product="internal transcribed spacer 1"
rRNA 203..381
/product="5.8S ribosomal RNA"
misc_RNA 382..734
/product="internal transcribed spacer 2"
rRNA 735..>1432
/product="28S ribosomal RNA"
ORIGIN

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      61 cgtgtgtcca ttggatctgg atgcgcggcg gttgggggggg cctggtcttc tggagagaag
     121 gtctgggtta ggttagttgg ttttaggcct ggcttggggg cgaccaaggc tggggcggtg
     181 tactgacaaa gccgtttctt tccagtcata ggagcctgtt ccggtcaggc caaggccaaa
     241 gaagggcatg ttccagtccc ataatcggtg gggcgcccct ccgtggctct gaggccttgt
     301 cgccctcagt gtccatcgga gcataatgcg tataaataat tgggcattcc gatatatata
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     421 attcctacag gacaagacct agctggtccg caggtggcgg gatgacttgg gttgggagag
     481 aggggttccg gatggaagac tgggtagcat agcataggtg gcgttgctag tgcgctgctg
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     601 atgtaaggta ggcatccaca agtgttggtg tgcaccccag tgtgtacact cctcttccgt
     661 cgtgcccaat catctgaagt tatgggcctc gacgattgat gtgaggtcga gggggccatc
     721 ttgtttggcc tggcatggac ctaaacctac acgcggtgcc cctgttcttg actgattagg
     781 tctataacat caagctgagt tttccaaacg ggttatcgag ataactctca acaatggatc
     841 tctaggctct tgcatcgatg aagaacgcag tgaactgcga taactggtgt gagttgcacg
     901 gcacttctaa tctaacacca agcgaatcat cgagtctttg aacgcaaacg gcacccttcc
     961 agtccattcc aacgcggtgg cggaggatga agacaagggt atctctggct gagggtaatg
    1021 aaaactgatc cagtgtgccg ctttttttt ggtcactggg gattgggctt gcttggggta
    1081 atgtcgttgc tctggcttgc tttaaaatca agcgttgtgt ggattgggct ttcaagcatg
    1141 cattggggac gcaggctctg cgctatggca agcccttgac cgtcataggt gctttgattg
    1201 gggtttcagt ctagccaaca aaggctgggt tggagacttt ggggctgcat tgggggcgt
    1261 agggcagctc tgttcgtggc gtccgatgac cgtcatggtg catgattgga ccttcaacca
    1321 gcaattatca ttatctgata taattataat cattactatg ggttacctca ggtcagagaa
    1381 gactacccgc tggacttaag catatcaata agcggaggaa atctagatgc at//
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ITS sequences of collection no. MSR2-07

LOCUS HQ270122 1516 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus cibarius var. longipes isolate MSR4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270122
KEYWORDS .
SOURCE Cantharellus cibarius var. longipes
ORGANISM Cantharellus cibarius var. longipes
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1516)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1516)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1516
/organism="Cantharellus cibarius var. longipes"
/mol_type="genomic DNA"
/variety="longipes"
/isolate="MSR4"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:885895"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..85
/product="18S ribosomal RNA"
misc_RNA 86..939
/product="internal transcribed spacer 1"
rRNA 940..1126
/product="5.8S ribosomal RNA"
misc_RNA 1127..1448
/product="internal transcribed spacer 2"
rRNA 1449..>1516
/product="28S ribosomal RNA"

ORIGIN
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  61 cacggtggta gggtagaccc ggtcttctct tggtgggggg aggcctgggc gctagttagg
 121 cctggctttg ggggtggtaa ctagactgga cggtgtactg acaaagccgt tttccagtca
 181 tagagccgtt ccagtcaggc cagggcatgt ctggtcccat ttggtggtgg ggcgcccctc
 241 tgtggctctg aggccttgtc atcctcagtg tccgtcggag ttatgggtgt tgggtgctgc
 301 gtgggggggat ttgctctgat gtgtctgatt aacacgctag gaggcaagcc tttttttgc
 361 agagccctgt gtacttggac ttcatcaccc aagtagacga attagcctct ttatggcccc
 421 tctagggtcg ggcaagcatt ctgagggacg ccgatacgaa tgcaagccac tgcggagcct
 481 accacaagta ggtaaagaaa ggtgacgagg aattacatgt gtgtgtgtgt gtgtgttttt
 541 aggggccctt gggctctata tttatttagg tactgctgca aggcagttcc gattggcctt
 601 gtgccaataa catgccagtg aagacgtcct tgtccccact ccattacaca ctacaggaca
 661 agacctagct ggtccgcagg cggcgggatg acttgggttg gtgagtcgag ggggtcatca
 721 tctgttggcc cggcatggac ctaacctaca cgcgtgccct gttcttgact gattaggtct
 781 ataacatcaa gctgagtttt ccaatgggtt atcgagataa caggggtca tctgttttga
 841 cccggcatgg acctaaacct acacgcggtg cccctgttct tgactgatta ggtctataac
 901 atcaagccga gttttccaaa atgggtgaga taactctcaa caatggatct ctaggctctt
 961 gcatcgatga agaacgcagt gaactgcgat aactggtgtg agttgcatgg cacttctaat
1021 ctaacaccaa gcgaatcatc gagtctttga acgcaaacgg cacccttcca gtccattcca
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1261 gcgctatatg gcaagccctt gaccgtcata ggtgctttga ttggggtttt cagtctagcc
1321 aacaaaggct gggttggact ttggggctgc attgggggcg tagggcagct ctgttcgtgg
1381 cgtccgatga ccgtcatggt gcatgattgg acttcaacca gcaattatca tattatcatt
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1501 gaggaaatcg gatccc//
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ITS sequences of collection no. 90-09

LOCUS HQ270123 1339 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus cibarius isolate MSR1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270123
VERSION HQ270123
KEYWORDS .
SOURCE Cantharellus cibarius (chanterelle)
ORGANISM Cantharellus cibarius
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1339)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1339)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1339
/organism="Cantharellus cibarius"
/mol_type="genomic DNA"
/isolate="MSR1"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:36066"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..107
/product="18S ribosomal RNA"
misc_RNA 108..826
/product="internal transcribed spacer 1"
rRNA 827..994
/product="5.8S ribosomal RNA"
misc_RNA 995..1242
/product="internal transcribed spacer 2"
rRNA 1243..>1339
/product="28S ribosomal RNA"
ORIGIN

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       61 acccgtgcgc acatccattg gactggatgc acgggtgtgt gtggtcttct tggtggggag
      121 gcctggttag ggatccgatt gggtgactag gctggacggt gtactgacaa agccgttttt
      181 ccagttatag agccgttccg gtcgggccca agggcatgtc cagtcccatt ggtggtggac
      241 gcccctccgt gggctctgag gccttgtcac cctcagtgtc cgtcggagtc tacaaagggg
      301 cacttgtgcc ccggcgtaac gtggatgtgt acatacatac atattccggt tggccttgtg
      361 ccaacaacat gccagtgaag acgtgtcctt gtccactcca acgtttttac ctacgggaca
      421 aggcctagct ggtccgcagg cggcgggatg acttgggttg gaagaggggg gttccagaag
      481 gagaaggaga ctacacttgg tcgatctctc tctgctggac ttgttgggtg taaggtaggc
      541 atccacagtt atttggcgtg caaccccgat gtgtacacaa tgccctcctt ccgttgtgcc
      601 caatcgtctg aagttatggg cctcgacgaa ttgatgtaag tcgagggggt catatctgtt
      661 tttggccccg gcatggacct agacctacac gcggtgcccc tgttcttgac tgattaggtc
      721 tataacatca agctgagttt tccaatgggt ttttaaataa ctctcaacaa tggatctcta
      781 ggctcttgca tcgatgaaga acgcagtgaa ctgcgataac tggtgtgagt tgcatggcac
      841 ttctaatcta acaccaagcg aatcatcgag tctttgaacg caaacggcac ccttccagtc
      901 cattccaaag cggtggcgga ggatgaagac aagggtatct ctggctgagg gtaatgagaa
      961 ctgatccagt gtaaccgttg gttagctggg gattgggctt gcttggggca gttgctctgg
     1021 cttgcctcaa aatcaagcgt tgtgtggatt ggactttcaa gcatgcattg gggacgcagg
     1081 ctctgcgcta tggcaagccc ttgaccgtca taggtgcttt gattggggtt ttcagtctag
     1141 ccaacaaggc tgggttgaag gactttgggg ctgcattggg ggcgcaaggg cagctctgtt
     1201 cgtggcgtcc gatgaccgtc atgatgcatg cgtgattgga cttcaactag caatttatca
     1261 ttatcatcat cattactatg ggttacctca ggtcagagaa gactacccgc tggacttaag
     1321 catatcaata agcggagga
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```

ITS sequences of collection no. 113-07

LOCUS HQ270125 1361 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus sp. MSR-2010a isolate 113 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270125
VERSION HQ270125
KEYWORDS .
SOURCE Cantharellus sp. MSR-2010a
ORGANISM Cantharellus sp. MSR-2010a
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1361)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1361)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1361
/organism="Cantharellus sp. MSR-2010a"
/mol_type="genomic DNA"
/isolate="113"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:907774"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..107
/product="18S ribosomal RNA"
misc_RNA 108..863
/product="internal transcribed spacer 1"
rRNA 864..1039
/product="5.8S ribosomal RNA"
misc_RNA 1040..1258
/product="internal transcribed spacer 2"
rRNA 1259..>1361
/product="28S ribosomal RNA"
ORIGIN

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       1 cgtaggtgaa cctgcggaag gatcaacccc tgtgggtata gtgagatggt ttcaacccaa
      61 cccgtgcgca catccattgg actggatgca cgggtgtgtg tggtcttctt ggtggggagg
     121 ccaggttata gagggcctgg cttggtgggg tgactaggct ggacggtgta cacgggtgtg
     181 gccgtttttc cagttataga gccgttccag tcgggcccca gggcatgtcc agtcccattg
     241 gtggtggggc gcccctccgt gggctctgag gccttgttct gaggccttgt caccgtcgga
     301 gaagggcagg ggcacttgtg ccccggcgta atgtggatgt gtgtatatac atgttccggt
     361 tggccttgta ccaacaacat gccagtgaag acgtgtcctt gtccactcca atattttacc
     421 tacgggacaa ggcctagctg gtccgcaggc ggcgggatga cttgggttgg aagagggaag
     481 gagaagggga ctgggttagc ataggcggcg ttgccagtgc gcgctgctga ggagcaaccg
     541 gccagttggt ccgcgctact tggtcgatct ctctgctgga cttgttgggt gtaaggtagg
     601 catccacggt tattttggcg tgcaccctcc tttttggcgt gcaccctcct tccgttgtgc
     661 ccaatcgtct gaagttatgg gcctcgacga attgatgtaa gtcgaggggg tcatatctgt
     721 ttttggcccg gcatggacct aaacctttcg cgcggtgccc gtgttgttac tgattaggtc
     781 tataacatca ctagtggttc caatgggcga gataactctc aacaatggat ctctaggctc
     841 ttgcatcgat gaagaacgca gtgaactgcg ataactggtg tgagttgcac ggcactctaa
     901 tctaacgcca agtgaatcat cgagtctttg aacgcaaacg gcacccttcc agtccattcc
     961 aaagcggtgg cggaggatga agacaagggt atctctgact gagggcaacg agaactgatc
    1021 caggctggct ttgctggcct ggggattggg cttgcttgga gcgggtaccc cgcttttggc
    1081 ttgccttaaa atgatcgttg tgtggatcgg actttcaagc acgcgctggg gacgcaggct
    1141 ctgcgctaaa gcaagccctt gaccgtcaat ggtgtcttga ttggagttct tcagccttat
    1201 ttaaggccgg gttgaacttt ggggctgcac tgggtgcgca ggacagctct ctttgtggcg
    1261 tccattgacc gcaatggtgc atgcaaggat ttcaacggcc aaaaaatAAC ccatcgtggt
    1321 ataattgcct caggtcagag aaggctaccc gctgggacta a//
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ITS sequences of collection no. 169-07

LOCUS HQ270129 1357 bp DNA linear PLN 20-OCT-2010
DEFINITION Cantharellus sp. MSR-2010c isolate 169 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ270129
VERSION HQ270129
KEYWORDS .
SOURCE Cantharellus sp. MSR-2010c
ORGANISM Cantharellus sp. MSR-2010c
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1357)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Diversity of Cantharellaceae from north western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1357)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (18-SEP-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..1357
/organism="Cantharellus sp. MSR-2010c"
/mol_type="genomic DNA"
/isolate="169"
/isolation_source="North western Himalayan forest"
/db_xref="taxon:907775"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..109
/product="18S ribosomal RNA"
misc_RNA 110..774
/product="internal transcribed spacer 1"
rRNA 775..953
/product="5.8S ribosomal RNA"
misc_RNA 954..1291
/product="internal transcribed spacer 2"
rRNA 1292..>1357
/product="28S ribosomal RNA"
ORIGIN

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        1 tccgtaggtg acctgcggag gatcaacccc tgtgggtata gtgagatggt ttttccaacc
       61 caacccgtgc gcacatccat tggactggat gcacgggtgt gtgtggtctt cttggtgggg
      121 aggcctggtt agggcctgac ttggtggggg tgactaggct ggacggtgta ctgacaaagc
      181 cgtttttcca gttatagagc cgttccggtc gggcccaagg gcatgtccag tcccattggt
      241 ggtggacgcc cctccgtggg ctctgaggcc ttgtcaccct cagtgtccgt cggagtctac
      301 aaaggggcac ttgtgccccg gcgtaacgtg gatgtgtaca tacatacata ttccggttgg
      361 ccttgtgcca acaacatgcc agtgaagacg tgtccttgtc cactccaacg tttttaccta
      421 cgggacaagg cctagctggt ccgcaggcgg cgggatgact tgggttggaa gagggggggt
      481 tccagaagga gaaggagact acacttggtc gatctctctc tgctggactt gttgggtgta
      541 aggtaggcat ccacagttat ttggcgtgca accccgatgt gtacacaatg ccctccttcc
      601 gttgtgccca atcgtctgaa gttatgggcc tcgacgaatt gatgtaagtc gaggggggtca
      661 tatctgtttt tggcccggca tggacctaga cctacacgcg gtgcccctgt tcttgactga
      721 ttaggtctat aacatcaagc tgagtttttc caatgggttt ttaaataact ctcaacaatg
      781 gatctctagg ctcttgcatc gatgaagaac gcagtgaact gcgataactg gtgtgagttg
      841 catggcactt ctaatctaac accaagcgaa tcatcgagtc tttgaacgca aacggcaccc
      901 ttccagtcca ttccaaagcg gtggcggagg atgaagacaa gggtatctct ggctgagggt
      961 aatgagaact gatccagtgt aaccgttggt tagctgggga ttgggcttgc ttggggcagt
     1021 tgctctggct tgcctcaaaa tcaagcgttg tgtggattgg actttcaagc atgcattggg
     1081 gacgcaggct ctgcgctatg gcaagccctt gaccgtcata ggtgctttga ttggggtttt
     1141 cagtctagcc aacaaggctg ggttgaagga ctttggggct gcattggggg cgcaagggca
     1201 gctctgttcg tggcgtccga tgaccgtcat gatgcatgcg tgattggact tcaactagca
     1261 atttatcatt atcatcatca ttactatggg ttacctcagg tcagagaaga ctacccgctg
     1321 gacttaagca tatcaataag cggaggaaat cggatcc
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ITS sequences of collection no. 95-08

LOCUS HQ386220 1400 bp DNA linear PLN 05-NOV-2010
DEFINITION Cantharellus appalachiensis isolate 95-08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION HQ386220
VERSION HQ386220
KEYWORDS .
SOURCE Cantharellus appalachiensis
ORGANISM Cantharellus appalachiensis
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1400)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Biodiversity of Cantharellaceae from Western Himalaya, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1400)
AUTHORS Kumari,D., Reddy,M.S. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (08-OCT-2010) Department of Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
source 1..1400
/organism="Cantharellus appalachiensis"
/mol_type="genomic DNA"
/isolate="39-07"
/db_xref="taxon:409893"
/country="India: forests of North-western Himalayan region"
/collected_by="Deepika Kumari"
/identified_by="M. S. Reddy"
rRNA <1..107
/product="18S ribosomal RNA"
misc_RNA 108..818
/product="internal transcribed spacer 1"
rRNA 819..1002
/product="5.8S ribosomal RNA"
misc_RNA 1003..1313
/product="internal transcribed spacer 2"
rRNA 1314..>1400
/product="28S ribosomal RNA"
ORIGIN

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       1 ccgtaggtga acctgcggaa ggatcaaccc ctgtgggtat agtgagatgg tttcaaccca
      61 acccgtgcgc acatccattg gactggatgc acgggtgtgt gtggtcttct tggtggggag
     121 gccaggttat agagggcctg gcttggtggg gtgactaggc tggacggtgt actgacaaag
     181 ccgtttttcc agttatagag ccgttccagt cgggccccag ggcatgtcca gtcccattgg
     241 tggtggggcg cccctccgtg ggctctgagg ccttgtcgcc ctcagtgtcc gtcggagtct
     301 acaaaggggc acttgtgccc cggcgtaatg tggatgtgtg tatatacatg ttccggttgg
     361 ccttgtacca acaacatgcc agtgaagacg tgtccttgtc cactccaata ttttacctac
     421 gggacaaggc ctagctggta ccgcaggcgg cgggatgact tgggttggaa gagggaagga
     481 gaaggggact gggttagcat aggcggcgtt gccagtgcgc gctgctgagg agcaaccgcg
     541 cagttggtcc gcgctacttg gtcgatctct ctgctggact tgttgggtgt aaggtaggca
     601 tccacggtta ttttccctgc accctccttt ttggcgtgca ccctccttcc gttgtgccca
     661 atcgtctgaa gttatgggcc tcgacgaatt gatgtaagtc gaggggtca taacggtttt
     721 tggcccggca tggacctaga cctacacgcg gtgcccctgt tcttgactga ttaggtctat
     781 aacatcaagc tgagttttcc aatgggtttt tagataactc tcaacaatgg atctctaggc
     841 tcttgcatcg atgaagaacg cagtgaactg cgataactgg tgtgagttgc atggcacttc
     901 taatctaaca gatagcgaat catcgagtct ttgaacgcaa acggcaccct tccagtccat
     961 tccaaagcgg tggcggagga tgaagacaag ggtatctctg gctgagggta atgagaactg
    1021 atccagtgtc taccgttggt tagctgggga ttgggcttgc ttggggtaat attgctctgg
    1081 cttgccttaa aatcaagcgt tgtgtggatt ggactttcaa gcatgcattg gggacgcagg
    1141 ctctgcgcta tttcaagccc ttgaccgtca taggtgcgca gattggggtt ttcagtctag
    1201 ccaacaaggc tgggttgaag gactttgggg ctgcattggg ggcgcaaggg cagctctgtt
    1261 cgtggcgtcc gatgaccgtc atgatgcatg cgtgattgga gggtcaacta gcaatttatc
    1321 attatcatca tcattactat gggttacctc aggtcagaga agactacccg ctggacttaa
    1381 gcatatcaat aagcggagga
```

ITS sequences of collection no. 281-07

LOCUS FJ769255 1401 bp DNA linear PLN 01-APR-2010
DEFINITION Cantharellus sp. SMR-2009a 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
ACCESSION FJ769255
VERSION FJ769255.1 GI:225348360
KEYWORDS .
SOURCE Cantharellus sp. SMR-2009a
ORGANISM Cantharellus sp. SMR-2009a
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Cantharellus.
REFERENCE 1 (bases 1 to 1401)
AUTHORS Reddy,S.M., Kumari,D. and Upadhyay,R.C.
TITLE Cantharellus pseudoformosus, a new species associated with Cedrus deodara from India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1401)
AUTHORS Reddy,S.M., Kumari,D. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (20-FEB-2009) Dept. Biotechnology, Thapar University, Patiala, Punjab 147004, India
source 1..1401
/organism="Cantharellus sp. SMR-2009a"
/mol_type="genomic DNA"
/isolate="MSR-4"
/db_xref="taxon:701518"
rRNA <1..105
/product="18S ribosomal RNA"
misc_RNA 106..814
/product="internal transcribed spacer 1"
rRNA 815..1001
/product="5.8S ribosomal RNA"
misc_RNA 1002..1342
/product="internal transcribed spacer 2"
rRNA 1343..>1401
/product="28S ribosomal RNA"
ORIGIN

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        1 tccgtaggtg acctgcggaa ggatcaaccc ctgtaatagt ttgtgggcaa gtctccccgt
       61 gttgtaagtc cattggactg gatgcgcggt ggtggatgcc ggtctccttg gtgggggagg
      121 cctggcttag ttaggccttt cttggcttgg tgggggcggt ggctaggctg gacggtgcac
      181 tgacaaagcc gttttccagt tatagggccg tttttccaag tcgggccagg gcatgtccag
      241 tcccatccat tggtggtggt ggggcgcccc tccttggctc tgaggccttg tcgccctcag
      301 ttgtccgtcg gagtctagaa agaaacctgg cgtgggccag atggattgga ttggccttgt
      361 gccaataaca tgccggcgaa gatgtccttg tccgctccat tctataccta caggacaaga
      421 cctagctggt ccgcaggtgg cgggatgact tgggttggga gagaggggtt ccggatggaa
      481 gactgggtag catagcatag gtggcgttgc tagtgcgctg ctgaggagga gcaaccggcc
      541 agttggtccg cgctacttgg ttgatatctg gacttgcttt tggatgtaag gtaggcatcc
      601 acaagtgttg gtgtgtgcac ccagtgtgct ccacgccccc ccttccgtcg cggcccaatc
      661 atctgaagtt atgggcctcg acgatgatgt gagtcgaggg ggtcatcatc tgttggcccg
      721 gcatggacct aacctacacg cgtgccctgt tcttgactga ttaggtctat aacatcaagc
      781 tgagttttcc aatgggttat cgagataact ctcaacaatg gatctctagg ctcttgcatc
      841 gatgaagaac gcagtgaact gcgataactg gtgtgagttg cacggcactt ctaatctaac
      901 accaagcgaa tcatcgagtc tttgaacgca aacggcaccc ttccagtcca ttccaaagcg
      961 gtggcggagg atgaagacaa gggtatctct ggctgagggt aatggaaact gatccagcgt
     1021 gccactctgg tcactgggga ttgggcttgc ttggggcaac gttgctctgg cttgccttaa
     1081 aatcaagcgt tgtgtggatt ggactttcaa gcatgcattg gggacgcagg ctctgcgcta
     1141 tggcacgccc ttgaccgtca taggtgcttt gattggggtt tcagtctagc caacaaggct
     1201 gggttggaga ctttggggct gcatttgggg gggccgtagg gcagctctgt tcgtggcgtc
     1261 cgatgaccgt catggtgcat gattggacct tcttcaacta gcaatttatc atctgatatc
     1321 atatcatatc atcattacca tgggttacct caggtcagag aagactaccc gctggactta
     1381 agcatatcaa taagcggagg a
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LSU sequences of collection 159-07

LOCUS HM113529 977 bp DNA linear PLN 08-JUN-2010
DEFINITION Craterellus sp. SR-2010 25S ribosomal RNA gene, partial sequence.
ACCESSION HM113529
VERSION HM113529
KEYWORDS .
SOURCE Craterellus sp. SR-2010
ORGANISM Craterellus sp. SR-2010
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 977)
AUTHORS Reddy,M.S., Kumari,D. and Upadhyay,R.C.
TITLE Craterellus indicus sp. nov., a new species associated with Cedrus
deodara from Western-Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 977)
AUTHORS Reddy,M.S., Kumari,D. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-APR-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..977
/organism="Craterellus sp. SR-2010"
/mol_type="genomic DNA"
/isolation_source="Chindi forest, Himachal Pradesh"
/specimen_voucher="PUN3884"
/db_xref="taxon:795949"
/collection_date="28-Aug-2007"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..>977
/product="25S ribosomal RNA"
ORIGIN

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       1 gcatatcaat aagcggagga aaagaaacta acaaggattc cctcagtaac ggcgagtgaa
      61 gagggaagag ctcagttttg gaatctggca gtgattcgct gtccgagttg tggattgaag
     121 agaatgtcat ccacgctgga ccatgtctaa attgtaatga tccacagtcc tttgaaggag
     181 tatcagagag ggtaataatc ccgtctttaa catggactta ccagtgtttc tgtgatgcac
     241 tctctcagag tcgagtagtt tgggaatgct gctctaatgg gaggtaaact ccttctaaag
     301 ctaaatattg gcgggagacc gatagcgaac aagtaccgtg agggaaagat gaaaagaact
     361 ctggaaagag agtcaaatag aacgtgaaat tgctgaaagg gaagcgcttg gagtcagacg
     421 catctttcat gctataccac tcagcggcat gaaggctgag agcggttact tgaagatggg
     481 ccagcactca cgaagaccat tgaagaaggg gtcatggaat gtagctcagt cttgagtgat
     541 tatagccttg attcagaagc aatgtgtctt tgtgtgagat tgacagcacc aacttgagtg
     601 cttgattgag actgacataa tggctctaag cgacccgtct tgaaacacgg accaaggagt
     661 ctaacatgta tgcgagtatt agagtgattg aaactctgaa tgcgcgatga aagtgttctg
     721 actgagaatc ccccctttg gagaggcatc agtaccccga gtctttgtta gtctttgact
     781 aaagatttgg aggtagagca tacatgttgg gacccgaaag atggtgaact atgcctgaat
     841 agggtgaagt cagaggaaac tctgatggag gcccgtagcg attctgacgt gcaaatcgat
     901 cgtagaattt gggtataggg gcgaaagact aatcgaacca tctagtagct ggttcctgcc
     961 gaagtttccc tcaggat
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//

LSU sequences of collection no. 268-06

LOCUS JF412275 511 bp DNA linear PLN 25-APR-2011
DEFINITION Craterellus cornucopioides var. mediosporus isolate 268-06 25S ribosomal RNA gene, partial sequence.
ACCESSION JF412275
VERSION JF412275
KEYWORDS .
SOURCE Craterellus cornucopioides var. mediosporus
ORGANISM Craterellus cornucopioides var. mediosporus
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 511)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Diversity of Cantharellaceae from Western Himalayan region of India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 511)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-FEB-2011) Crop Improvement, Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh 173213, India
FEATURES Location/Qualifiers
source 1..511
/organism="Craterellus cornucopioides var. mediosporus"
/mol_type="genomic DNA"
/variety="mediosporus"
/isolate="268-06"
/db_xref="taxon:1010608"
/country="India: Pithoragarh forest, Himalayan region"
/collected_by="Deepika Kumari"
/identified_by="Deepika, Upadhyay & Reddy"
rRNA <1..>511
/product="25S ribosomal RNA"
ORIGIN

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        1 ctggcagtga ttcactgtcc gagttgtaaa ttgaagagaa tgtcatccac gctggaccac
       61 gtctaaattg taatgatcca cagtcctttg atggagtatc agagagggta ataatcccgt
      121 ctttaaagtg gactgccagt gtttctgtga tgcactctct cagagtcgag tagtttggga
      181 atgctgctct aatgggaggt aaactccttc taaagctaaa tattggcgag agaccgatag
      241 cgaacaagta ccgtgaggga aagatgaaaa gaactctgaa aagagagtca aatagaacgt
      301 gaaattgctg aaagggaagc gtttggagtc agacgcgtct tcatgcaata cagctcagtg
      361 gttgagactt tgagacaagt tacttgaaga cgggccagca ctcacgaaga ccattgaaga
      421 aggggtcatg gaatgtagct cagtgctgag tgattatagc cttgattcaa aagcaatgtg
      481 tcctttgtga gagatggaca gcacggacaa c
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LSU sequences of collection no. 107-07

LOCUS JF412276 513 bp DNA linear PLN 25-APR-2011
DEFINITION Craterellus cinereus isolate 107-08 25S ribosomal RNA gene, partial sequence.
ACCESSION JF412276
VERSION JF412276
KEYWORDS .
SOURCE Craterellus cinereus
ORGANISM Craterellus cinereus
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 513)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Diversity of Cantharellaceae from Western Himalayan region of India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 513)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-FEB-2011) Crop Improvement, Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh 173213, India
FEATURES Location/Qualifiers
source 1..513
/organism="Craterellus cinereus"
/mol_type="genomic DNA"
/isolate="107-08"
/db_xref="taxon:1010609"
/country="India: Khada Pathar, Himachal Pradesh"
/collected_by="Deepika Kumari"
/identified_by="Deepika, Upadhyay & Reddy"
rRNA <1..>513
/product="25S ribosomal RNA"

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ORIGIN
        1 ctggcagtca ttccactgtc cgagttgtaa attgaagaga atgtcatcca cactggacca
       61 tgtctaaatt gtaataatcc acagtccttt gatggagtat cagagagggt aataatcccg
      121 tctttaatgg tggaccacca gtgttacgta agatgcactc tctcagagtc gagtaatttg
      181 ggaatgctgc tctaatggga ggtaaactcc ttctaaagct aaatattggc gagagaccga
      241 tagcgacaaa gtaccgtgag ggaaagatga aaagaactct gaaaagagag tcaaacagaa
      301 cgtgaaattg ctgaaaggga agcgtttgga gtcagatgca tcaagtatgt aatacggctc
      361 agttggcaga gactgagagt cagtgacttg aagatgggcc agcactcacg tagatcattg
      421 gagaaggggt catggaatgt agctcaattc ttgggtgttt atagccttga ttcatataca
      481 atgtgtctta tgtgtgttaa ttgacagcac aag
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ITS sequences of collection no. 159-07

LOCUS HM113530 752 bp DNA linear PLN 08-JUN-2010
DEFINITION Craterellus sp. SR-2010 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence.
ACCESSION HM113530
VERSION HM113530
KEYWORDS .
SOURCE Craterellus sp. SR-2010
ORGANISM Craterellus sp. SR-2010
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 752)
AUTHORS Reddy,M.S., Kumari,D. and Upadhyay,R.C.
TITLE Craterellus indicus sp. nov., a new species associated with Cedrus deodara from Western-Himalayas, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 752)
AUTHORS Reddy,M.S., Kumari,D. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (12-APR-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..752
/organism="Craterellus sp. SR-2010"
/mol_type="genomic DNA"
/isolation_source="Chindi forest, Himachal Pradesh"
/specimen_voucher="PUN3884"
/db_xref="taxon:795949"
/collection_date="28-Aug-2007"
/collected_by="Deepika Kumari"
/identified_by="Deepika Kumari"
rRNA <1..54
/product="18S ribosomal RNA"
misc_RNA 55..304
/product="internal transcribed spacer 1"
rRNA 305..496
/product="5.8S ribosomal RNA"
misc_RNA 497..723
/product="internal transcribed spacer 2"
rRNA 723..>752
/product="25S ribosomal RNA"
ORIGIN

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       1 cctttcttct ctaagtgggc tcagatgggc tcactgtgat actgagatgg gtctgagaag
      61 aagccctttt atgatttgct ttcgggtgtc tcatcctatc tctcagcctt tttcattcaa
     121 ctgtgagttg gatgcgctgt atcactcagt tggtcatgtt tgaaagcaat cggactcttg
     181 ggagagggaa tcatcttatc caagatctta ttccccccct ttgagtgtca atgagctttg
     241 ggctcaaaca gattctgtcc ataaatgatt gttttgatca tgggcttcat ttgacaactt
     301 tcagcaatgg atctcttggt tctcgcatcg atgaagaacg cagtgaaatg cgataactgg
     361 tgtgaattgc atccaagtaa ctctaattta cgaataacac caagtgaatc atcgagtctt
     421 tgaacgcaat tgcgccctct cggtcgcttc caattggggg ttgactcata gggggtacat
     481 ttgttcgagg gtcatttgag tctctcaaaa gaggtttatc actcctttgg ggttttgggt
     541 cttgctgtga aatttatctc ggcttacctt gaaagcatta gcaagtatct caagtctttt
     601 gtgatgaggg cgttcttaat tggtcaagtt ctcgcaaact ggtctgtgta gccagtgtaa
     661 aagacgtcta aacatcatta agtgactttt aaagtgaagc tagcttccaa tcatccttat
     721 gggatgggca ttgagctcct tcaatgttta tc

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ITS sequences of collection no. 268-06

LOCUS JF412277 672 bp DNA linear PLN 25-APR-2011
DEFINITION Craterellus cornucopioides var. mediosporus isolate 268-06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence.
ACCESSION JF412277
VERSION JF412277
KEYWORDS .
SOURCE Craterellus cornucopioides var. mediosporus
ORGANISM Craterellus cornucopioides var. mediosporus
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 672)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Diversity of Cantharellaceae from Western Himalayan region of India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 672)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-FEB-2011) Crop Improvement, Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh 173213, India
FEATURES Location/Qualifiers
source 1..672
/organism="Craterellus cornucopioides var. mediosporus"
/mol_type="genomic DNA"
/variety="mediosporus"
/isolate="268-06"
/db_xref="taxon:1010608"
/country="India: Pithoragarh forest, Himalayan region"
/collected_by="Deepika Kumari"
/identified_by="Deepika, Upadhyay & Reddy"
rRNA <1..74
/product="18S ribosomal RNA"
misc_RNA 75..208
/product="internal transcribed spacer 1"
rRNA 209..551
/product="5.8S ribosomal RNA"
misc_RNA 552..607
/product="internal transcribed spacer 2"
rRNA 608..>672
/product="25S ribosomal RNA"
ORIGIN

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       1 tgtgagattg ggaccatatt ataatatcac tcagttgttg gtcaagctga aaagcaatct
      61 actcttggag gggagagaag atatgattga gggttttgta gccccaaggt tgtattctat
     121 cttgtctctc ttgagtctag agaacttgag ataatttgtc catatggaat tatcataaca
     181 tgggctcatt tacaactttc agcaatggat ctcttggttc tcgcatcgat gaagaacgca
     241 gtgaaatgcg ataactggtg tgaattgcat ccaagtaact ctaatttacg aataacacca
     301 agtgaatcat cgagtctttg aacgcaattg cgccctctcg gtcgcttcca attgggggtt
     361 gactcatagg gggtacgtct gttcgagggt catttgaacc tctcaaaggg tttttagatt
     421 ttaaaccctt tggatttggg tcttgccgtg aaattctatc ttggcttacc ttgaaagcat
     481 tagcaaagct tttcagcact tgaaagtctt tttgaatgag ggcgttgtta atttggtcag
     541 gcctccttga gaggaatctc atacgtctct cagtcatgga aagtctcagt gatttttgaa
     601 acttgcttcc aatgatcctt attaaaggat aaggccccc tcctggcctt tttcaatgta
     661 tatcaatgac ct
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ITS sequences of collection no. 107-07

LOCUS JF412278 870 bp DNA linear PLN 25-APR-2011
DEFINITION Craterellus cinereus isolate 107-08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence.
ACCESSION JF412278
VERSION JF412278
KEYWORDS .
SOURCE Craterellus cinereus
ORGANISM Craterellus cinereus
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Cantharellales; Cantharellaceae; Craterellus.
REFERENCE 1 (bases 1 to 870)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Diversity of Cantharellaceae from Western Himalayan region of India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 870)
AUTHORS Upadhyay,R.C., Kumari,D. and Reddy,M.S.
TITLE Direct Submission
JOURNAL Submitted (20-FEB-2011) Crop Improvement, Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh 173213, India
FEATURES Location/Qualifiers
source 1..870
/organism="Craterellus cinereus"
/mol_type="genomic DNA"
/isolate="107-08"
/db_xref="taxon:1010609"
/country="India: Khada Pathar, Himachal Pradesh"
/collected_by="Deepika Kumari"
/identified_by="Deepika, Upadhyay & Reddy"
rRNA <1..102
/product="18S ribosomal RNA"
misc_RNA 103..419
/product="internal transcribed spacer 1"
rRNA 420..617
/product="5.8S ribosomal RNA"
misc_RNA 618..815
/product="internal transcribed spacer 2"
rRNA 816..>870
/product="25S ribosomal RNA"
ORIGIN

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      1 aaagtcgtaa caaggtttcc gtaggtgaac ctgcggaagg atcattcact tgataaaagg
     61 gcttgtgaag cctttctctt tcttaaccaa gtgggggttc ttgaatcacc actgtgatgc
    121 tcggatatgg gtctttgaag ccctttgtga ttgctttcag gcatctgatc ttatccagca
    181 aaaaaaaggt tcatggtaaa cccgactgtg agtctgggag atggacccta ttataatatc
    241 actcagttgt tgttggtcaa gttgaaaagc aatccactct tgggggggaag gatacgattt
    301 tgagggtttg taacccaaag gctgtattct atctatctct tgagtctcaa tgagttgtta
    361 gaaactcgag acaattgtcc atatggattt atcataacat gggctcattt acaactttca
    421 gcaatggatc tcttggttct cgcatcgatg aagaacgcag tgaaatgcga taagtggtgt
    481 gaattgcatc caagtaactc taatttacga ataacaccaa gtgaatcatc gagtctttga
    541 acgcaattgc gccctctcgg tcgcttccaa ttgggggttg acggcaaggg ggtacatctg
    601 ttcgagggtc attttgaacc tctcaaaggg ttttgcttta acccttgga tttgggtgtc
    661 ttgccgtgaa attctatctt ggctcacctt gaaagcatta gcaaggtttt caataaagga
    721 aagtcttttt gaatgaggtt ggaagttaat ttggtcaagc ctcctcctta agagagccat
    781 tcatctcatt cggcaaactg gtttgtgcag ccagtggtgg atgtgataaa caaaaagcgc
    841 ctcaatgact tttgaaactt gcttccaatg
```

16S rDNA sequences of isolate CB1

LOCUS GU944485 701 bp DNA linear BCT 27-SEP-2010
DEFINITION Hafnia sp. CB1(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944485
VERSION GU944485
KEYWORDS .
SOURCE Hafnia sp. CB1(2010)
ORGANISM Hafnia sp. CB1(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Hafnia.
REFERENCE 1 (bases 1 to 701)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 701)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..701
/organism="Hafnia sp. CB1(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885277"
/country="India: Kufari forest"
rRNA <1..>701
/product="16S ribosomal RNA"
ORIGIN

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       1 ggtgagtaat gtctgggaaa ctgcctgatg gaggggggata actactggaa acggtagcta
      61 ataccgcatg acgtcttcgg accaaagtgg gggaccttcg ggcctcacgc catcagatgt
     121 gcccagatgg gattagctag taggtggggt aatggctcac ctaggcgacg atctctagct
     181 ggtctgagag gatgaccagc cacactggaa ctgagacacg gtccagactc ctacgggagg
     241 cagcagtggg gaatattgca caatgggcgc aagcctgatg cagccatgcc gcgtgtatga
     301 agaaggcctt cgggttgtaa agtactttca gcgaggagga aggcattgtg gttaataacc
     361 acagtgattg acgttactcg cagaagaagc accggctaac tccgtgccag cagccgcggt
     421 aatacggagg gtggcaagcg ttaatcggaa ttactgggcg taaagcgcac gcaggcggtt
     481 gattaagtca gatgtgaaat ccccgagctt aacttgggaa ctgcatttga aactggtcag
     541 ctagagtctt gtagaggggg gtagaattcc aggtgtagcg gtgaaatgcg tagagatctg
     601 gaggaatacc ggtggcgaag gcggcccccct ggacaaagac tgacgctcag gtgcgaaagc
     661 gtggggagca aacaggatta gataccctgg gtagtccacg c
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//

16S rDNA sequences of isolate CB42

LOCUS GU944486 704 bp DNA linear BCT 27-SEP-2010
DEFINITION Hafnia sp. CB42(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944486
VERSION GU944486
KEYWORDS .
SOURCE Hafnia sp. CB42(2010)
ORGANISM Hafnia sp. CB42(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Hafnia.
REFERENCE 1 (bases 1 to 704)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 704)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..704
/organism="Hafnia sp. CB42(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885278"
/country="India: Khajjiar forest"
rRNA <1..>704
/product="16S ribosomal RNA"
ORIGIN

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        1 gcgctagcac aggagagctt gctctctcag gtgacgagcg gcggacgggt gagtaatgtc
       61 tgggaaactg cctgatggag ggggataact actggaaacg gtagctaata ccgcatgacg
      121 tcttcggacc aaagtggggg accttcgggc ctcacgccat cagatgtgcc cagatgggat
      181 tagctagtag gtggggtaat ggctcaccta ggcgacgatc tctagctggt ctgagaggat
      241 gaccagccac actggaactg agacacggtc cagactccta cgggaggcag cagtggggaa
      301 tattgcacaa tgggcgcaag cctgatgcag ccatgccgcg tgtatgaaga aggccttcgg
      361 gttgtaaagt actttcagcg aggaggaagg cattgtggtt aataaccaca gtgattgacg
      421 ttactcgcag aagaagcacc ggctaactcc gtgccagcag ccgcggtaat acggagggtg
      481 caagcgctta atcggaatta ctgggcgtaa agcgcacgca ggcggttgat taagtcagat
      541 gtgaaatccc cgagcttaac ttgggaactg catttgaaac tggtcagcta gagtcttgta
      601 gaggggggta gaattccagg tgtagcggtg aaatgcgtag agatctggag gaataccggt
      661 ggcgaaagcg gccccctgga caaagactga cgctcagtgc gaaa
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16S rDNA sequences of isolate CB8

LOCUS GU944487 704 bp DNA linear BCT 27-SEP-2010
DEFINITION Hafnia sp. CB8(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944487
VERSION GU944487
KEYWORDS .
SOURCE Hafnia sp. CB8(2010)
ORGANISM Hafnia sp. CB8(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Hafnia.
REFERENCE 1 (bases 1 to 704)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 704)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..704
/organism="Hafnia sp. CB8(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885279"
/country="India: Bharsar forest"
rRNA <1..>704
/product="16S ribosomal RNA"
ORIGIN

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       1 tgagtaatgt ctgggaaact gcctgatgga gggggataac tactggaaac ggtagctaat
      61 accgcatgac gtcttcggac caaagtgggg gaccttcggg cctcacgcca tcagatgtgc
     121 ccagatggga ttagctagta ggtggggtaa tggctcacct aggcgacgat ctctagctgg
     181 tctgagagga tgaccagcca cactggaact gagacacggt ccagactcct acgggaggca
     241 gcagtgggga atattgcaca atgggcgcaa gcctgatgca gccatgccgc gtgtatgaag
     301 aaggccttcg ggttgtaaag tactttcagc gaggaggaag gcattgtggt taataaccac
     361 agtgattgac gttactcgca gaagaagcac cggctaactc cgtgccagca gccgcggtaa
     421 tacggagggt gcaagcgtta atcggaatta ctgggcgtaa agcgcacgca ggcggttgat
     481 taagtcagat gtgaaatccc cgagcttaac ttgggaactg catttgaaac tggtcagcta
     541 gagtcttgta gaggggggta gaattccagg tgtagcggtt gaaatgcgta gagatctgga
     601 ggaataccgg tggcgaaggc ggcccctgg acaaagactg acgctcagtg cgaaagcgtg
     661 gggagcaaac aggattagat accctggtag tccacgctgt aaac
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//

16S rDNA sequences of isolate CB31

LOCUS GU944488 697 bp DNA linear BCT 27-SEP-2010
DEFINITION Gamma proteobacterium CB31(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944488
VERSION GU944488
KEYWORDS .
SOURCE gamma proteobacterium CB31(2010)
ORGANISM gamma proteobacterium CB31(2010)
Bacteria; Proteobacteria; Gammaproteobacteria.
REFERENCE 1 (bases 1 to 697)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 697)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..697
/organism="gamma proteobacterium CB31(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885304"
/country="India: Karol forest"
rRNA <1..>697
/product="16S ribosomal RNA"
ORIGIN

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       1 cttgcttctc ttgagagcgg cggacgggtg agtaatgcct aggaatctgc ctggtagtgg
      61 gggataacgt tcggaaacgg acgctaatac cgcatacgtc ctacgggaga aagcacgggg
     121 accttcgggc cttgggtaat ggctcaccaa ggcgacaatc cgtaactggt ctgagaggat
     181 gcaccaaggc gacgatccgt aactggtctg agaggatgat cagtcacact ggaactgaga
     241 cacggtccag actcctacgg gaggcagcag tggggaatat tggacaatgg gcgaaagcct
     301 gatccagcca tgccgcgtgt gtgaagaagg tcttcggatt gtaaagcact ttaagttggg
     361 aggaagggca gttgcctaat acgtaactgt tttgacgtta ccgacagaat aagcaccggc
     421 taactctgtg ccagcagccg cggtaataca gagggtgcaa gcgttaatcg gaattactgg
     481 gcgtaaagcg cgcgtaggtg gtttgttaag ttggatgtga aatccccggg ctcaacctgg
     541 gaactgcatt caaaactgac tgactagagt atggtagagg gtggtggaat ttcctgtgta
     601 gcggtgaaat gcgtagatat aggaaggaac accagtggcg aaggcgacca cctggactga
     661 tactgacact gaggtgcgaa agcgtgggga gcaaaca
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16S rDNA sequences of isolate CB29

LOCUS GU944489 704 bp DNA linear BCT 27-SEP-2010
DEFINITION Hafnia sp. CB29(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944489
VERSION GU944489
KEYWORDS .
SOURCE Hafnia sp. CB29(2010)
ORGANISM Hafnia sp. CB29(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Hafnia.
REFERENCE 1 (bases 1 to 704)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 704)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..704
/organism="Hafnia sp. CB29(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885280"
/country="India: Karol forest"
rRNA <1..>704
/product="16S ribosomal RNA"
ORIGIN

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       1 tgagtaatgt ctgggaaact gcctgatgga gggggataac tactggaaac ggtagctaat
      61 accgcatgac gtcttcggac caaagtgggg gaccttcggg cctcacgcca tcagatgtgc
     121 ccagatggga ttagctagta ggtggggtaa tggctcacct aggcgacgat ctctagctgg
     181 tctgagagga tgaccagcca cactggaact gagacacggt ccagactcct acgggaggca
     241 gcagtgggga atattgcaca atgggcgcaa gcctgatgca gccatgccgc gtgtatgaag
     301 aaggccttcg ggttgtaaag tactttcagc gaggaggaag gcattgtggt taataaccac
     361 agtgattgac gttactcgca gaagaagcac cggctaactc cgtgccagca gccgcggtaa
     421 tacggagggt gcaagcgtta atcggaatta ctgggcgtaa agcgcacgca ggcggttgat
     481 taagtcagat gtgaaatccc cgagcttaac ttgggaactg catttgaaac tggtcagcta
     541 gagtcttgta gaggggggta gaattccagg tgtagcggtg aaatgcgtag agatctggag
     601 gaataccggt ggcgaaggcg gccccctgga caaagactga cgctcagtgc gaaagcgtgg
     661 ggagcaaaca ggattagata ccctggtagt ccacagctgt aaac
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16S rDNA sequences of isolate CB4

LOCUS GU944490 617 bp DNA linear BCT 27-SEP-2010
DEFINITION Enterobacter sp. CB4(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944490
VERSION GU944490
KEYWORDS .
SOURCE Enterobacter sp. CB4(2010)
ORGANISM Enterobacter sp. CB4(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter.
REFERENCE 1 (bases 1 to 617)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 617)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..617
/organism="Enterobacter sp. CB4(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885281"
/country="India: Kufari forest"
rRNA <1..>617
/product="16S ribosomal RNA"

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ORIGIN
       1 ggggtgagaa aagtctggga aactgcctga tggaggggga taactactgg aaacggtagc
      61 taataccgca taacgtctca agaccaaaga gggggacctt cgggcctctt gccatcacat
     121 gtgcccagat gggattagct agtaggtggg gtaatggctc acctaggcga caatccctag
     181 ctggtctgag aggatgacca gccacactgg aactgagaca cggtccacac tcctacggga
     241 ggcagcagtg gggaatattg cacaatgggc gcaagcctga tgcagccatg ccgcgtgtgt
     301 gaagaaagcc ttctggttgt aaagtacttt cagcgaggag gaaggcgttg aggttaataa
     361 ccttattgat tgacgttact cgcagaaaaa acaccggcta actccgtgcc agcagccgcg
     421 gtaatacaga gggtgcaagc gttaatcgca attactgggc gtaaagcgca cgcacgcggt
     481 ctgtcaagtc tgatgtgaaa tccccgggct ctacctggga actgcattct aaactggcac
     541 gctagagtct tgtatagagg ggtataattc caggtgtatc gctgaaatgc gtatagatct
     601 ggagaaatac cggtgtc
//
```

16S rDNA sequences of isolate CB18

LOCUS GU944491 692 bp DNA linear BCT 27-SEP-2010
DEFINITION Enterobacter sp. CB18(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944491
VERSION GU944491
KEYWORDS .
SOURCE Enterobacter sp. CB18(2010)
ORGANISM Enterobacter sp. CB18(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter.
REFERENCE 1 (bases 1 to 692)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 692)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..692
/organism="Enterobacter sp. CB18(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885282"
/country="India: Karsog forest"
rRNA <1..>692
/product="16S ribosomal RNA"
ORIGIN

```
        1 gacgggtgag taatgtctgg gaaactgcct gatggagggg gataactact ggaaacggta
       61 gctaataccg cataacgtcg caagaccaaa gaggggacc ttcgggcctc ttgccatcag
      121 atgtgcccag atgggattag ctagtaggtg gggtaatggc tcacctaggc gacgatccct
      181 agctggtctg agaggatgac cagccacact ggaactgaga cacggtccag actcctacgg
      241 gaggcagcag tggggaatat tgcacaatgg gcgcaagcct gatgcagcca tgccgcgtgt
      301 atgaagaagg ccttcgggtt gtaaagtact ttcagcgagg aggaaggcat tgtggttaat
      361 aacctcagtg attgacgtta ctcgcagaag aagcaccggc taactccgtg ccagcagccg
      421 cggtaatacg gagggtgcaa gcgttaatcg gaattactgg gcgtaaagcg cacgcaggcg
      481 gtctgtcaag tcggatgtga aatccccggg ctcaacctgg gaactgcatt cgaaactggc
      541 aggctagagt cttgtagagg ggggtagaat tccaggtgta gcggtgaaat gcgtagagat
      601 ctggaggaat accggtggcg aaggcggccc cctggacaaa gactgacgct caggtgcgaa
      661 agcgtgggga gcaaacagga ttagataccc tg
```

//

16S rDNA sequences of isolate CB35

LOCUS GU944492 696 bp DNA linear BCT 27-SEP-2010
DEFINITION Enterobacter sp. CB35(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944492
VERSION GU944492
KEYWORDS .
SOURCE Enterobacter sp. CB35(2010)
ORGANISM Enterobacter sp. CB35(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter.
REFERENCE 1 (bases 1 to 696)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 696)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..696
/organism="Enterobacter sp. CB35(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885283"
/country="India: Solan forest"
rRNA <1..>696
/product="16S ribosomal RNA"
ORIGIN

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        1 cggtaacagg aagcagcttg ctgcttcgct gacgagtggc ggacgggtga gtaatgtctg
       61 ggaaactgcc tgatggaccg tcataactac tggaaacggt agctaatacc gcataacgtc
      121 gcaagaccaa agaccgtcac cttcgggcct cttgccatcg gatgtgccca gatgggatta
      181 gctagtaggt ggggtaacgg ctcacctagg cgacgatccc tagctggtct gagaggatga
      241 ccagccacac tggaactgag acacggtcca gactcctacg ggaggcagca gtggggaata
      301 ttgcacaatg ggcgcaagcc tgatgcagcc atgccgcgtg tatgaagaag gccttcgggt
      361 tgtaaagtac tttcagcggg gaggaaggtg atgaggttaa taacctcggc aattgacgtt
      421 acccgcagaa gaagcaccgg ctaactccgt gccagcagcc gcggtaatac ggagggtgca
      481 agcgttaatc ggaattactg ggcgtaaagc gcacgcaggc ggtctgtcaa gtcggatgtg
      541 aaatccccgg gctcaacctg ggaactgcat tcgaaactgg caggctagag tcttgtagag
      601 gggggtagaa ttccaggtgt agcggtgaaa tgcgtagaga tctggaggaa taccggtggc
      661 gaaggcggcc cccttccgat ctcctcgact atctac
```

//

16S rDNA sequences of isolate CB34

LOCUS GU944493 677 bp DNA linear BCT 27-SEP-2010
DEFINITION Enterobacter sp. CB34(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944493
VERSION GU944493
KEYWORDS .
SOURCE Enterobacter sp. CB34(2010)
ORGANISM Enterobacter sp. CB34(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter.
REFERENCE 1 (bases 1 to 677)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 677)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..677
/organism="Enterobacter sp. CB34(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885284"
/country="India: Solan forest"
rRNA <1..>677
/product="16S ribosomal RNA"
ORIGIN

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        1 caggcctaac acatgcaagt cgagcggtag cacagagagc ttgttttcgg gtgacgagcg
       61 gcggacgggt gagtaatgtc tgggaaactg cctgatggag ggggataact actggaaacg
      121 gtagctaata ccgcataacg tcgcaagacc aaagaggggg accttcgggc ctcttgccat
      181 cagatgtgcc cagatgggat tagctagtag gtggggtaat ggctcaccta ggcgacgatc
      241 cctagctggt ctgagaggat gaccagccac actggaactg agatggaggg ggataactac
      301 tggaaacggt aggacacggt ccagactcct acgggaggca gcagtgggga atattgcaca
      361 atgggcgcaa gcctgatgca gccatgccgc gtgtatgaag aaggccttcg ggttgtaaag
      421 tactttcagc gaggaggaag gcattgtggt taataaccgc agtgattgac gttactcgca
      481 gaagaagcac cggctaactc cgtgccagca gccgcggtaa tacggagggt gcaagcgtta
      541 atcggaatta ctgggcgtaa agcgcacgca ggcggtctgt caagtcggat gtgaaatccc
      601 cgggctcaac ctgggaactg cattcgaaac tggcaggcta gagtcttgta gaggggggta
      661 gaattccagg tgtagcg
//
```

16S rDNA sequences of isolate CB21

LOCUS GU944494 654 bp DNA linear BCT 27-SEP-2010
DEFINITION Ewingella sp. CB21(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944494
VERSION GU944494
KEYWORDS .
SOURCE Ewingella sp. CB21(2010)
ORGANISM Ewingella sp. CB21(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Ewingella.
REFERENCE 1 (bases 1 to 654)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 654)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..654
/organism="Ewingella sp. CB21(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885285"
/country="India: Karsog forest"
rRNA <1..>654
/product="16S ribosomal RNA"
ORIGIN

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        1 gccagatgga gggggataac tactggaaac ggtagctaat accgcatgac ctcgaaagag
       61 caaagtgggg gaccttcggg cctcacgcca tcggatgtgc ccagatggga ttagctagta
      121 ggtgaggtaa tggctcacct aggcgacgat ccctagctgg tctgagagga tgaccagcca
      181 cactggaact gagacacggt ccagactcct acgggaggca gcagtgggga atattgcaca
      241 atgggcgcaa gcctgatgca gccatgccgc gtgtgtgaag aaggccttcg ggttgtaaag
      301 cactttcagc gaggaggaag gcattaaggt taataacctt agtgattgac gttactcgca
      361 gaagaagcac cggctaactc cgtgccagca gccgcggtaa tacggagggt gcaagcgtta
      421 atcggaatta ctgggcgtaa agcgcacgca ggcggtttgt taagtcagat gtgaaatccc
      481 cgagcttaac ttgggaactg catttgaaac tggcaagcta gagtcttgta gaggggggt
      541 agaattccag gtgtagcggt gaaatgcgta gagatctgga ggaataccgg tggcgaaggc
      601 ggccccctgg acaaagactg acgctcaggt gcggaggcgt ggggagcaaa cagg
//
```

16S rDNA sequences of isolate CB43

LOCUS GU944495 706 bp DNA linear BCT 27-SEP-2010
DEFINITION Ewingella sp. CB43(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944495
VERSION GU944495
KEYWORDS .
SOURCE Ewingella sp. CB43(2010)
ORGANISM Ewingella sp. CB43(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Ewingella.
REFERENCE 1 (bases 1 to 706)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 706)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..706
/organism="Ewingella sp. CB43(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885286"
/country="India: Khajjiar forest"
rRNA <1..>706
/product="16S ribosomal RNA"
ORIGIN

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        1 gggtgagtaa tgtctgggaa actgcctgat ggaggggat aactactgga aacggtagct
       61 aataccgcat gacctcgaaa gagcaaagtg ggggaccttc gggcctcacg ccatcggatg
      121 tgcccagatg ggattagcta gtaggtgagg taatggctca cctaggcgac gatccctagc
      181 tggtctgaga ggatgaccag ccacactgga actgagacac ggtccagact cctacgggag
      241 gcagcagtgg ggaatattgc acaatgggcg caagcctgat gcagccatgc cgcgtgtgtg
      301 aagaaggcct tcgggttgta aagcactttc agcgaggagg aaggcgttaa ggttaataac
      361 cttggtgatt gacgttactc gcagaagaag caccggctaa ctccgtgcca gcagccgcgg
      421 taatacggag ggtgcaagcg ttaatcggaa ttactgggcg taaagcgcac gcaggcggtt
      481 tgttaagtca gatgtgaaat ccccgagctt aacttgggaa ctgcatttga aactggcaag
      541 ctagagtctt gtagaggggg gtagaattcc aggtgtagcg gtgaaatgcg tagagatctg
      601 gaggaatacc ggtggcgaaa gcggcccccc ggacaaagac tgacgctcat gtgcgaaagc
      661 gtggggagca aacaggatta gataccctgg tagtccacgc tgtaaa
//
```

16S rDNA sequences of isolate CB45

LOCUS GU944496 714 bp DNA linear BCT 27-SEP-2010
DEFINITION Ewingella sp. CB45(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944496
VERSION GU944496
KEYWORDS .
SOURCE Ewingella sp. CB45(2010)
ORGANISM Ewingella sp. CB45(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Ewingella.
REFERENCE 1 (bases 1 to 714)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 714)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..714
/organism="Ewingella sp. CB45(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885287"
/country="India: Dhalli forest"
rRNA <1..>714
/product="16S ribosomal RNA"
ORIGIN

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        1 ggtgagtaat gtctgggaaa ctgcctgatg gaggggggata actactggaa acggtagcta
       61 ataccgcatg acctcgaaag agcaaagtgg gggaccttcg ggcctcacgc catcggatgt
      121 gcccagatgg gattagctag taggtgaggt aatggctcac ctaggcgacg atccctagct
      181 ggtctgagag gatgaccagc cacactggaa ctgagacacg gtccagactc ctacgggagg
      241 cagcagtggg gaatattgca caatgggcgc aagcctgatg cagccatgcc gcgtgtgtga
      301 agaaggcctt cgggttgtaa agcactttca gcgaggagga aggcgttaag gttaataacc
      361 ttggcgattg acgttactcg cagaagaagc accggctaac tccgtgccag cagccgcggt
      421 aatacggagg gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg caggcggttt
      481 gttaagtcag atgtgaaatc cccgagctta acttgggaac tgcatttgaa actggcaagc
      541 tagagtcttg tagaggggggg tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg
      601 aggagtaccg gtgggcgaat gcggcccccct gggacacaga ctgacgctca ggtgcgaaag
      661 cgtggggagc aaacgggatt cacatacccc tggtagtcca cgctgtaaac gatg
```

//

16S rDNA sequences of isolate CB39

LOCUS GU944497 661 bp DNA linear BCT 27-SEP-2010
DEFINITION Ewingella sp. CB39(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944497
VERSION GU944497
KEYWORDS .
SOURCE Ewingella sp. CB39(2010)
ORGANISM Ewingella sp. CB39(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Ewingella.
REFERENCE 1 (bases 1 to 661)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 661)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..661
/organism="Ewingella sp. CB39(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885288"
/country="India: Khajjiar forest"
rRNA <1..>661
/product="16S ribosomal RNA"
ORIGIN

```
        1 gggtgagtaa tgtctgggaa actgcctgat ggaggggat aactactgga aacggtagct
       61 aataccgcat gacctcgaaa gagcaaagtg ggggaccttc gggcctcacg ccatcggatg
      121 tgcccagatg ggattagcta gtaggtgagg taatggctca cctaggcgac gatccctagc
      181 tggtctgaga ggatgaccag ccacactgga actgagacac ggtccagact cctacgggag
      241 gcagcagtgg ggaatattgc acaatgggcg caagcctgat gcagccatgc cgcgtgtgtg
      301 aagaaggcct tcgggttgta aagcactttc agcgaggagg aaggcgttaa ggttaataac
      361 cttggcgatt gacgttactc gcagaagaag caccggctaa ctccgtgcca gcagccgcgg
      421 taatacggag ggtgcaagcg ttaatcggaa ttactgggcg taaagcgcac gcaggcggtt
      481 tgttaagtca gatgtgaaat ccccgagctt aacttgggaa ctgcatttga aactggcaag
      541 ctagagtctt gtagaggggg gtagaattcc aggtgtagcg gtgaaatgcg tatagatctg
      601 gaggaatacc ggtggggaaa gcggcccccct ggacaaagac tgacgctcaa gtgcgaaaag
      661 c
```

//

16S rDNA sequences of isolate CB37

LOCUS GU944498 734 bp DNA linear BCT 27-SEP-2010
DEFINITION Ewingella sp. CB37(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944498
VERSION GU944498
KEYWORDS .
SOURCE Ewingella sp. CB37(2010)
ORGANISM Ewingella sp. CB37(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Ewingella.
REFERENCE 1 (bases 1 to 734)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 734)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..734
/organism="Ewingella sp. CB37(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885289"
/country="India: Solan forest"
rRNA <1..>734
/product="16S ribosomal RNA"
ORIGIN

```
       1 cctacacatg cagtcgagcg gcagcgggaa gtagcttgct actttgccgg cgagcggcgg
      61 acgggtgagt aatgtctggg aaactgcctg atggaggggg ataactactg gaaacggtag
     121 ctaataccgc atgacctcga aagagcaaag tgggggacct tcgggcctca cgccatcgga
     181 tgtgcccaga tgggattagc tagtaggtga ggtaatggct cacctaggcg acgatcccta
     241 gctggtctga gaggatgacc agccacactg gaactgagac acggtccaga ctcctacggg
     301 aggcagcagt ggggaatatt gcacaatggg cgcaagcctg atgcagccat gccgcgtgtg
     361 tgaagaaggc cttcgggttg taaagcactt tcagcgagga ggaaggcgtt aaggttaata
     421 accttggcga ttgacgttac tcgcagaaga agcaccggct aactccgtgc cagcagccgc
     481 ggtaatacgg agggtgcaag cgttaatcgg aattactggg cgtaaagcgc acgcaggcgg
     541 tttgttaagt cagatgtgaa atccccgagc ttaacttggg aactgcattt gaaactggca
     601 agctagagtc ttgtagaggg gggtagaatt ccaggtgtag cggtgaaatg cgtagagatc
     661 tggaggaata tgtgaaatcc ccaagcttaa cttgcgaact gcatttggga ctggctagct
     721 agagtcttgt agag
//
```

16S rDNA sequences of isolate CB32

LOCUS GU944499 720 bp DNA linear BCT 27-SEP-2010
DEFINITION Pseudomonas sp. CB32(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944499
VERSION GU944499
KEYWORDS .
SOURCE Pseudomonas sp. CB32(2010)
ORGANISM Pseudomonas sp. CB32(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.
REFERENCE 1 (bases 1 to 720)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 720)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..720
/organism="Pseudomonas sp. CB32(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885290"
/country="India: Karol forest"
rRNA <1..>720
/product="16S ribosomal RNA"
ORIGIN

```
       1 atgacgggag cttgctcctt gattcagcgg cggacgggtg agtaatgcct aggaatctgc
      61 ctggtagtgg gggacaacgt ttcgaaagga acgctaatac cgcatacgtc ctacgggaga
     121 aagcagggga ccttcgggcc ttgcgctatc agatgagcct aggtcggatt agctagttgg
     181 tggggtaatg gctcaccaag gcgacgatcc gtaactggtc tgagaggatg atcagtcaca
     241 ctggaactga gacacggtcc agactcctac gggaggcagc agtggggaat attggacaat
     301 gggcgaaagc ctgatccagc catgccgcgt gtgtgaagaa ggtcttcgga ttgtaaagca
     361 ctttaagttg ggaggaaggg cagtaagcta ataccttgct gttttgacgt taccgacaga
     421 ataagcaccg gctaactctg tgccagcagc cgcggtaata cagagggtgc aagcgttaat
     481 cggaattact gggcgtaaag cgcgcgtagg tggttcgtta agttggatgt gaaagccccg
     541 ggctcaacct gggaactgca tccaaaactg gcgagctaga gtacggtaga gggtggtgga
     601 atttcctgtg tagcggtgaa atgcgtagat ataggaagga acaccagtgg cgaaggcgac
     661 cacctggact gatactgaca ctgaggtgcg aaagcgtggg gagcaaacag gattagatac
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16S rDNA sequences of isolate CB47

LOCUS GU944500 699 bp DNA linear BCT 27-SEP-2010
DEFINITION Pseudomonas sp. CB47(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944500
VERSION GU944500
KEYWORDS .
SOURCE Pseudomonas sp. CB47(2010)
ORGANISM Pseudomonas sp. CB47(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.
REFERENCE 1 (bases 1 to 699)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 699)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..699
/organism="Pseudomonas sp. CB47(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885291"
/country="India: Kalatop forest"
rRNA <1..>699
/product="16S ribosomal RNA"
ORIGIN

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        1 cgagcggatg acgggagctt gctccttgat tcagcggcgg acgggtgagt aatgcctagg
       61 aatctgcctg gtagtggcta cgggagaaag caggggacct tcgggccttg cgctatcaga
      121 tgagcctagg tcggattagc tagttggttg cgctatcaga tgagcctagg tcggattagc
      181 tagttggtga ggtaatggct caccaaggcg acgatccgta actggtctga gaggatgatc
      241 agtcacactg gaactgagac acggtccaga ctcctacggg aggcagcagt ggggaatatt
      301 ggacaatggg cgaaagcctg atccagccat gccgcgtgtg tgaagaaggt cttcggattg
      361 taaagcactt taagttggga ggaagggcag taagttaata ccttgctgtt ttgacgttac
      421 cgacagaata agcaccggct aactctgtgc cagcagccgc ggtaatacag agggtgcaag
      481 cgttaatcgg aattactggg cgtaaagcgc gcgtaggtgg ttcgttaagt tggatgtgaa
      541 agccccgggc tcaacctggg aactgcatcc aaaactggcg agctagagta tggtagaggg
      601 tggtggaatt tcctgtgtag cggtgaaatg cgtagatata ggaaggaaca ccagtggcga
      661 aggcgaccac ctggactgat actgacactg aggtgcgaa
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16S rDNA sequences of isolate CB7

LOCUS GU944501 720 bp DNA linear BCT 27-SEP-2010
DEFINITION Pseudomonas sp. CB7(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944501
VERSION GU944501
KEYWORDS .
SOURCE Pseudomonas sp. CB7(2010)
ORGANISM Pseudomonas sp. CB7(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
Pseudomonadaceae; Pseudomonas.
REFERENCE 1 (bases 1 to 720)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 720)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..720
/organism="Pseudomonas sp. CB7(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885292"
/country="India: Kufari forest"
rRNA <1..>720
/product="16S ribosomal RNA"
ORIGIN

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        1 cctggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgagc ggtagagaga
       61 agcttgcttc tcttgagagc ggcggacggg tgagtaatac ctaggaatct gcctgatagt
      121 gggggataac gttcggaaac ggacgctaat accgcatacg tcctacggga gaaagcaggg
      181 gaccttcggg ccttgcgcta tcagatgagc ctaggtcgga ttagctagtt ggtgaggtaa
      241 tggctcacca aggctacgat ccgtaactgg tctgagagga tgatcagtca cactggaact
      301 gagacacggt ccagactcct acgggaggca gcagtgggga atattggaca atgggcgaaa
      361 gcctgatcca gccatgccgc gtgtgtgaag aaggtcttcg gattgtaaag cactttaagt
      421 tgggaggaag ggcatttacc taatacgtga ctgttttgac gttaccgaca gaataagcac
      481 cggctaactc tgtgccagca gccgcggtaa tacagagggt gcaagcgtta atcggaatta
      541 ctgggcgtaa agcgcgcgta ggtggtttgt taagttgaat gtgaaatccc cgggctcaac
      601 ctgggaactg catccaaaac tggcaagcta gagtacggta gagggtagtg gaatttcctg
      661 tgtagcggtg aaatgcgtag ataatcaatc gaggtgtgct cgctctccga ctcgggttcc
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```

16S rDNA sequences of isolate CB27

LOCUS GU944502 716 bp DNA linear BCT 27-SEP-2010
DEFINITION Alcaligenes sp. CB27(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944502
VERSION GU944502
KEYWORDS .
SOURCE Alcaligenes sp. CB27(2010)
ORGANISM Alcaligenes sp. CB27(2010)
Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Alcaligenes.
REFERENCE 1 (bases 1 to 716)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 716)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..716
/organism="Alcaligenes sp. CB27(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885293"
/country="India: Narkanda forest"
rRNA <1..>716
/product="16S ribosomal RNA"
ORIGIN

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       1 tctcttggta agcggcgagt ggcggacggg tgagtaatat atcggaacgt gcccagtagc
      61 gggggataac tactcgaaag agtggctaat accgcatacg ccctacgggg gaaaggggg
     121 gattcttcgg aacctctcac tattggagcg gccgatatcg gattagctag ttggtggggt
     181 aaaggctcac caaggcaacg atccgtagct ggtttgagag gacgaccagc cacactggga
     241 ctgagacacg gcccagactc ctacgggagg cagcagtggg gaattttgga caatggggga
     301 aaccctgatc cagccatccc gcgtgtatga tgaaggcctt cgggttgtaa agtacttttg
     361 gcagagaaga aaaggtatct cctaatacga gatactgctg acggtatctg cagaataagc
     421 accggctaac tacgtgccag cagccgcggt aatacgtagg gtgcaagcgt taatcggaat
     481 tactgggcgt aaagcgtgtg taggcggttc ggaaagaaag atgtgaaatc ccagggctca
     541 accttggaac tgcatttta actgccgagc tagagtatgt cagaggggg tagaattcca
     601 cgtgtagcag tgaaatgcgt agatatgtgg aggaataccg atggcgaagg cagccccctg
     661 ggataatact gacgctcaga cacgaaagcg tggggagcaa acaggattag atgcgc
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16S rDNA sequences of isolate CB40

LOCUS GU944503 670 bp DNA linear BCT 27-SEP-2010
DEFINITION Alcaligenes sp. CB40(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944503
VERSION GU944503
KEYWORDS .
SOURCE Alcaligenes sp. CB40(2010)
ORGANISM Alcaligenes sp. CB40(2010)
Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales;
Alcaligenaceae; Alcaligenes.
REFERENCE 1 (bases 1 to 670)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 670)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..670
/organism="Alcaligenes sp. CB40(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885294"
/country="India: Khajjiar forest"
rRNA <1..>670
/product="16S ribosomal RNA"

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ORIGIN
       1 cttgctctct tggtggcgag cgcggagacg ggtgagtaat atatcggaac gtgcccagta
      61 gcgggggata actactcgaa agagtggcta ataccgcata cgccctacgg gggaaaggtc
     121 gggattcttc ggaacctctc actagtggag cggccgatat cggattagct agttggtggg
     181 gtaaaggctc accaaggcaa cgatccgtag ctggtttgag aggacgacca gccacactgg
     241 gactgagaca cggcccagac tcctacggga ggcagcagtg gggaattttg gacaatgggg
     301 gaaaccctga tccagccatc ccgcgtgtat gatgaaggcc ttcgggttgt aaagtacttt
     361 tggcagagaa gaaaaggtat ctcctaatac gagatactgc tgacggtatc tgcagaataa
     421 gcaccggcta actacgtgcc agcagccgcg gtaatacgta gggtgcaagc gttaatcgga
     481 attactgggc gtaaagcgtg tgtaggcggt tcggaaagaa agatgtgaaa tcccagggct
     541 caaccttgga actgcatttt taactgccga gctagagtat gtcagagggg ggtagaattc
     601 cacgtgtagc agtgaaatgc gtagatatgt ggaggaatac cgatggcgaa ggcagccccc
     661 tagggctcaa
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//

16S rDNA sequences of isolate CB19

LOCUS GU944504 610 bp DNA linear BCT 27-SEP-2010
DEFINITION Stenotrophomonas sp. CB19(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944504
VERSION GU944504
KEYWORDS .
SOURCE Stenotrophomonas sp. CB19(2010)
ORGANISM Stenotrophomonas sp. CB19(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas.
REFERENCE 1 (bases 1 to 610)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 610)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..610
/organism="Stenotrophomonas sp. CB19(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885295"
/country="India: Karsog forest"
rRNA <1..>610
/product="16S ribosomal RNA"
ORIGIN

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        1 gttaacggaa tcggaatcta ccctgacgtg ggggataacg tagggaaact tacgctaata
       61 ccgcatacga ccttcgggtg aaagcagggg accttcgggc cttgcgcgga tagatgagcc
      121 gatgtcggat tagctagttg gcggggtaaa ggcccaccaa ggcgacgatc cgtagctggt
      181 ctgagaggat gatcagccac actggaactg agacacggtc cagactccta cgggaggcag
      241 cagtggggaa tattgaaggt tcagcgcaag cctgatccag ccataccgcg tgggtgaaga
      301 aggccttcgg gttgtaaagc ccttttgttg ggaaagaaaa gcaatcgatt aatactcggt
      361 tgttctgacg gtacccaaag aataagcacc ggctaacttc gtgccagcag ccgcggtaat
      421 acgaagggtg caagcgttac tcggaattac tgggcgtaaa gcgtgcgtag gtggttgttt
      481 aagtctgttg tgaaagccct gggctcaacc tgggaattgc agtggatact gggcgactag
      541 agtgtggtag agggtagttg gccatgccaa cgtgcagtga aaatgcgtag agatcgggaa
      601 cgtcacgtca
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```

16S rDNA sequences of isolate CB15

LOCUS GU944505 610 bp DNA linear BCT 27-SEP-2010
DEFINITION Stenotrophomonas sp. CB15(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944505
VERSION GU944505
KEYWORDS .
SOURCE Stenotrophomonas sp. CB15(2010)
ORGANISM Stenotrophomonas sp. CB15(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas.
REFERENCE 1 (bases 1 to 610)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 610)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..610
/organism="Stenotrophomonas sp. CB15(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885296"
/country="India: Chail forest"
rRNA <1..>610
/product="16S ribosomal RNA"
ORIGIN

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       1 gttaacggaa tcggaatcta ccttttcgtg ggggataacg tagggaaact tacgctaata
      61 ccgcatacga ccttcgggtg aaagcagggg accttcgggc cttgcgcgga aacggttcaa
     121 aaacctgcaa aaagaagttg gcggggtaaa ggcccaccaa ggcgacgatc cgtagctggt
     181 ctgagaggat gatcagccac actggaactg agacacggtc cagactccta cgggaggcag
     241 cagtggggaa tattgaaggt tcagcgcaag cctgatccag ccataccgcg tgggtgaaga
     301 aggccttcgg gttgtaaagc ccttttgttg ggaaagaaaa gcaatcgatt aatactcggt
     361 tgttctgacg gtacccaaag aataagcacc ggctaacttc gtgccagcag ccgcggtaat
     421 acgaagggtg caagcgttac tcggaattac tgggcgtaaa gcgtgcgtag gtggttgttt
     481 aagtctgttg tgaaagccct gggctcaacc tgggaattgc agtggatact gggcgactag
     541 agtgtggtag agggtagttg gccatgccaa cgtgcagtga aaatgcgtag agatcgggag
     601 gaacatccat
//
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16S rDNA sequences of isolate CB13

LOCUS GU944506 702 bp DNA linear BCT 27-SEP-2010
DEFINITION Stenotrophomonas sp. CB13(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944506
VERSION GU944506
KEYWORDS .
SOURCE Stenotrophomonas sp. CB13(2010)
ORGANISM Stenotrophomonas sp. CB13(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas.
REFERENCE 1 (bases 1 to 702)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 702)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..702
/organism="Stenotrophomonas sp. CB13(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885297"
/country="India: Chail forest"
rRNA <1..>702
/product="16S ribosomal RNA"

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ORIGIN
       1 gggtggcgag tggcggacgg gtgaggaata catcggaatc tacccgttcg tgggccataa
      61 cgtagggaaa cttacgctaa taccgcatac gacctacggg tgaaagcagg ggatcttcgg
     121 accttgcgcg attgaatgag ccgatgtcgg attagctagt tggcggggta aaggcccacc
     181 aaggcgacga tccgtagctg gtctgagagg atgatcagcc acactggaac tgagacacgg
     241 tccagactcc tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
     301 agccataccg cgtgggtgaa gaaggccttc gggttgtaaa gccctttgt tgggaaagaa
     361 atccagctgg ttaatacccg gttgggatga cggtacccaa agaataagca ccggctaact
     421 tcgtgccagc agccgcggta atacgaaggg tgcaagcgtt actcggaatt actgggcgta
     481 aagcgtgcgt aggtggtcgt ttaagtctgt tgtgaaagcc ctgggctcaa cctgggaact
     541 gcagtggaaa ctggacgact agagtgtggt agagggtagc ggaattcctg gtgtagcagt
     601 gaaatgcgta gagatccctg gtgtagcagt gaaatgcgta gagatcagga ggaacatcca
     661 tggcgaaggc agctacctgg accaacactg ataagcaaac ag
//
```

16S rDNA sequences of isolate CB44

LOCUS GU944507 693 bp DNA linear BCT 27-SEP-2010
DEFINITION Rahnella sp. CB44(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944507
VERSION GU944507
KEYWORDS .
SOURCE Rahnella sp. CB44(2010)
ORGANISM Rahnella sp. CB44(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Rahnella.
REFERENCE 1 (bases 1 to 693)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 693)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..693
/organism="Rahnella sp. CB44(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885298"
/country="India: Dhalli forest"
rRNA <1..>693
/product="16S ribosomal RNA"
ORIGIN

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        1 aagtccggga gcggcagcgg gaagtagatg cagtcgagcg gcancgggaa gtagcttgct
       61 actttgccgg cgagcggcgg acgggtgagt aatgtctggg aaactgcctg atggaggggg
      121 ataactactg gaaacggtag ctaataccgc atgacctcga aagagcaaag tgggggatct
      181 tcggacctca cgccatcgga tgtgcccaga tgggattagc tagtaggtga ggtaatggct
      241 cacctaggcg acgatcccta gctggtctga gaggatgacc agccacactg gaactgagac
      301 acggtccaga ctcctacggg aggcagcagt ggggaatatt gcacaatggg cgcaagcctg
      361 atgcagccat gccgcgtgtg tgaagaaggc cttagggttg taaagcactt tcagcgagga
      421 ggaaggcatc ayacttaata cgtgtggtga ttgacgttac tcgcagaaga agcaccggct
      481 aactccgtgc cagcagccgc ggtaatacgg agggtgcaag cgttaatcgg aattactggg
      541 cgtaaagcgc acgcaggcgg tttgttaagt cagatgtgaa atccccgagc ttaacttggg
      601 aactgcattt gaaactggca agctagagtc ttgtagaggg gggtagaatt ccaggtgtag
      661 cggtgaaatg cgtagagatc tggaggaata ccg
//
```

16S rDNA sequences of isolate CB3

LOCUS GU944508 574 bp DNA linear BCT 27-SEP-2010
DEFINITION Bacillus sp. CB3(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944508
VERSION GU944508
KEYWORDS .
SOURCE Bacillus sp. CB3(2010)
ORGANISM Bacillus sp. CB3(2010)
Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 574)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 574)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..574
/organism="Bacillus sp. CB3(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885299"
/country="India: Kufari forest"
rRNA <1..>574
/product="16S ribosomal RNA"
ORIGIN

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        1 aatcgatggg agcttgctcc ctgcgattag cggcggacgg gtgagtaaca cgtgggcaac
       61 ctgcctataa gactgggata acttcgggaa accggagcta ataccggata cgttcttttc
      121 tcgcatgaga gaagatggaa agacggtttt gctggccctt attgatgggc cccccgccca
      181 ataacttgtt tggggaggaa tggctcaccc aggggaccaa tcctaaccca actgaaaagg
      241 tgatccgcca ccctgggact gaaaccccgg ccaaaatccc accggaagga gccattagga
      301 atcttcccca atggaacaaa gtctgaccga accacccccc ctggaccaag aaaggcttcc
      361 ggtccgaaag ttttgttggt aagggagaac cagttccagg attaatgccg ggaccttgag
      421 gttcctaacc agaaaccacc ggctaacaca tgccagcgcc gcggtaatac gtggtggcaa
      481 gcgttgtccg gaataatggc gtaaagcgcc gcaggtgttc ttaaggctga tgtgaaagcc
      541 acggctcaac cgtgaaggtc attggaaact ggga
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16S rDNA sequences of isolate CB14

LOCUS GU944509 642 bp DNA linear BCT 27-SEP-2010
DEFINITION Bacillus sp. CB14(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944509
VERSION GU944509
KEYWORDS .
SOURCE Bacillus sp. CB14(2010)
ORGANISM Bacillus sp. CB14(2010)
Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 642)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 642)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..642
/organism="Bacillus sp. CB14(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885300"
/country="India: Chail forest"
rRNA <1..>642
/product="16S ribosomal RNA"
ORIGIN

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        1 gagtaacacg tgggtaacct gcccataaga ctgggataac tccgggaaac cggggctaat
       61 accggataac attttgaacc ggatggttcc aaattgaaag gcggcttcgg ctgtcactta
      121 tggatggacc cgcgtcgcat taactagttg gtgaggtaac ggctcaccaa ggcaacgatg
      181 cgtagaccac ctgagagggt gatcggccac actgggactg agacacggcc cagactccta
      241 cgagaggcag cagtagggaa tcttcctcaa tggacgaaag tctgacggag caacgccccg
      301 tgagtgatga aggctttcgg gtcgtaaact ctgttgtgag gcaagaacaa gtgctagttg
      361 aataagctgg caccttgacg gtacctaaca gaatgccacg gccactacga tgcagcagcc
      421 gcagtaatac gtaggagtca tgcgtatcag catttcgggc gtaatcgcgc gcaggggttt
      481 acttccgtct gatgtcaagg ccactgcctt agcgatgagg tcttggtacc tgggagactg
      541 agtgatacag gtacggaatt cctgtgtaat cgtgggatgc ctaaattgga ggaaccctgg
      601 catgcaactc tctgtctgac tgactggagc caagctggaa ca
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```

16S rDNA sequences of isolate CB5

LOCUS GU944510 620 bp DNA linear BCT 27-SEP-2010
DEFINITION Bacillus sp. CB5(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944510
VERSION GU944510
KEYWORDS .
SOURCE Bacillus sp. CB5(2010)
ORGANISM Bacillus sp. CB5(2010)
Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 620)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 620)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..620
/organism="Bacillus sp. CB5(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885301"
/country="India: Kufari forest"
rRNA <1..>620
/product="16S ribosomal RNA"
ORIGIN

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        1 gggtgagtaa cacgtgggca acctacccta tagtttggga taactccggg aaaccggggc
       61 taataccgaa taatctcttt tgcttcatgg tgaaagactg aaagacggtt tcggctgtcg
      121 ctataggatg ggcccgcggc gcattagcta gttggtgagg taacggctca ccaaggcgac
      181 gatgcgtagc cgacctgaga gggtgatcgg ccacactggg actgagacac ggcccagact
      241 cctacgggag gcagcagtag ggaatcttcc acaatgggcg aaagcctgat ggagcaacgc
      301 cgcgtgagtg aagaaggttt tcggatcgta aaactctgtt gtaagggaag aacaagtaca
      361 gtagtaactg gctgtacctt gacggtacct tattagaaag ccacggctaa ctacgtgcca
      421 gcagccgcgg ataatacgta ggtggcaagc gttgtccgga attattgggc gtaaagcgcg
      481 cgcaggcggt cctttaagtc tgatgtgaaa gcccacggct caaccgtgga gggtcattgg
      541 aaactggggg acttgagtgc agaagaggaa agtggaattt ccaagtgtag cggtgaaatg
      601 cgtagagatt tggaggaaca
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16S rDNA sequences of isolate CB9

LOCUS GU944511 693 bp DNA linear BCT 27-SEP-2010
DEFINITION Gamma proteobacterium CB9(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944511
VERSION GU944511
KEYWORDS .
SOURCE gamma proteobacterium CB9(2010)
ORGANISM gamma proteobacterium CB9(2010)
Bacteria; Proteobacteria; Gammaproteobacteria.
REFERENCE 1 (bases 1 to 693)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 693)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..693
/organism="gamma proteobacterium CB9(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885305"
/country="India: Bharsar forest"
rRNA <1..>693
/product="16S ribosomal RNA"
ORIGIN

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        1 gctcaagctc acaacagctc gcggtcccca tgacgtttgg tccaggctga gtaatgtctg
       61 ggaaactgcc tgatggaggg ggataactac tggaaacggt agctaatacc gcataacgtc
      121 ttcggaccaa agtgggggac cttcgggcct cacaccatcg gatgtgccca gatgggatta
      181 gctagtaggt ggggtaatgg ctcacctagg cgacgatccc tagctggtct gagaggatga
      241 ccagccacac tggaactgag acacggtcca gactcctacg ggaggcagca gtggggaata
      301 ttgcacaatg ggcgcaagcc tgatgcagcc atgccgcgtg tatgaagaag gccttcgggt
      361 tgtaaagtac tttcagcggg gaggaaggcg ataaggttaa taaccttgtc gattgacgtt
      421 acccgcagaa gaagcaccgg ctaactccgt gccagcagcc gcggtaatac ggagggtgca
      481 agcgttaatc ggaattactg ggcgtaaagc gcacgcaggc ggtctgtcaa gtcagatgtg
      541 aaatccccgg gcttaacctg ggaactgcat ttgaaactgg caggctagag tcttgtagag
      601 gggggtagaa ttccaggtgt agcggtgaaa tgcgtagaga tctggaggaa taccggtggc
      661 gaaggcggcc ccctggacaa agactgacgc tca
//
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16S rDNA sequences of isolate CB24

LOCUS GU944512 697 bp DNA linear BCT 27-SEP-2010
DEFINITION Gamma proteobacterium CB24(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944512
VERSION GU944512
KEYWORDS .
SOURCE gamma proteobacterium CB24(2010)
ORGANISM gamma proteobacterium CB24(2010)
Bacteria; Proteobacteria; Gammaproteobacteria.
REFERENCE 1 (bases 1 to 697)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 697)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (27-FEB-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..697
/organism="gamma proteobacterium CB24(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885306"
/country="India: Narkanda forest"
rRNA <1..>697
/product="16S ribosomal RNA"
ORIGIN

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      121 taccgcataa cgtcttcgga ccaaagtggg ggaccttcgg gcctcacacc atcggatgtg
      181 cccagatggg attagctagt aggtggggta atggctcacc taggcgacga tccctagctg
      241 gtctgagagg atgaccagcc acactggaac tgagacacgg tccagactcc tacgggaggc
      301 agcagtgggg aatattgcac aatgggcgca agcctgatgc agccatgccg cgtgtatgaa
      361 gaaggccttc gggttgtaaa gtactttcag cggggaggaa ggcgataagg ttaataacct
      421 tgtcgattga cgttacccgc agaagaagca ccggctaact ccgtgccagc agccgcggta
      481 atacggaggg tgcaagcgtt aatcggaatt actgggcgta aagcgcacgc aggcggtctg
      541 tcaagtcaga tgtgaaatcc ccgggcttaa cctgggaact gcatttgaaa ctggcaggct
      601 agagtcttgt agaggggggt agaattccag gtgtagcggt gaaatgcgta gagatctgga
      661 ggaataccgg tggcgaaggc ggcccccctgg acaaaga
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16S rDNA sequences of isolate CB11

LOCUS GU944513 674 bp DNA linear BCT 27-SEP-2010
DEFINITION Pseudomonas sp. CB11(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944513
VERSION GU944513
KEYWORDS .
SOURCE Pseudomonas sp. CB11(2010)
ORGANISM Pseudomonas sp. CB11(2010)
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.
REFERENCE 1 (bases 1 to 674)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 674)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Direct Submission
JOURNAL Submitted (02-MAR-2010) Biotechnology, Thapar University, Bhadson Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..674
/organism="Pseudomonas sp. CB11(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885302"
/country="India: Bharsar forest"
rRNA <1..>674
/product="16S ribosomal RNA"
ORIGIN

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       61 gcctggtagt gggggacaac gtttcgaaag gaacgctaat accgcatacg tcctacggga
      121 gaaagcaccg gaccttcggg ccttgcgcta tcagatgagt ttaggtcgga ttagctagtt
      181 ggtgaggtaa tggctcacca aggcgacgat ccgtaactgg tctgagagga tgatcagtca
      241 cactggaact gagacacggt ccagactcct acgggaggca gcagtgggga atattggaca
      301 atgggcgaaa gcctgatcca gccatgccgc gtgtgtgaag aaggtcttcg gattgtaaag
      361 cactttaagt tgggaggaag ggcagtaagt taataccttg ctgttttgac gttaccgaca
      421 gaataagcac cggctaactc tgtgccagca gccgcggtaa tacagagggt gcaagcgtta
      481 atcggaatta ctgggcgtaa agcgcgcgta ggtggttcgt taagttggat gtgaaatccc
      541 cgggctcaac ctgggaactg catccaaaac tggcgagcta gagtatggta gagggtggtg
      601 gaatttcctg tgtagcggtg aaatgcgtag atataggaag gaacaccagt ggcgaaggca
      661 aaacctgcat gcaa
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16S rDNA sequences of isolate CB25

LOCUS GU944514 744 bp DNA linear BCT 27-SEP-2010
DEFINITION Bacillus sp. CB25(2010) 16S ribosomal RNA gene, partial sequence.
ACCESSION GU944514
VERSION GU944514
KEYWORDS .
SOURCE Bacillus sp. CB25(2010)
ORGANISM Bacillus sp. CB25(2010)
Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 744)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay,R.C.
TITLE Molecular diversity of endophytic bacteria associated with
Cantharellus spp.
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 744)
AUTHORS Reddy,M.S., Deepika,K. and Upadhyay, R.C.
TITLE Direct Submission
JOURNAL Submitted (02-MAR-2010) Biotechnology, Thapar University, Bhadson
Road, Patiala, Punjab 147004, India
FEATURES Location/Qualifiers
source 1..744
/organism="Bacillus sp. CB25(2010)"
/mol_type="genomic DNA"
/isolation_source="sporocarp of Cantharellus"
/db_xref="taxon:885303"
/country="India: Narkanda forest"
rRNA <1..>744
/product="16S ribosomal RNA"
ORIGIN

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     121 taacacgtgg gtaacctgcc tgtaagactg ggataactcc gggaaaccgg agctaatacc
     181 ggatagttcc ttgaaccgca tggttcaagg atgaaagacg gtttcggctg tcacttacag
     241 atggacccgc ggcgcattag ctagttggtc cggtaatggc tcaccaaggc gacgatgcgt
     301 agccgacctg agagactgat cggccacact gggactgaga cacggcccag actcctacgg
     361 gaggcagcag tagggaatct tccgcaatgg acgaaagtct gacggagcaa cgccgcgtga
     421 gtgatgaagg ttttcggatc gtaaagctct gttgttaggg aagaacaagt gcgagagtag
     481 ctgctcgcac cttgacggta cctaaccaga aagccacggc taactacgtg ccagcagccg
     541 cggtaatacg taggtggcaa gcgttgtccg gaattattgg gcgtaaaggg ctcgcaggcg
     601 gtttcttaag tctgatgtga aagcccccgg ctcaacctgg gagggtcatt ggaaactggg
     661 aaacttgagt gcagaagagg agagtggaat tccacgtgta gcggtgaaat gcgtagagat
     721 gtttggccaa tcactgccaa actg
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