MUSHROOMS OF SWAT PAKISTAN-A STUDY BASED UPON MOLECULAR AND MORPHOANATOMICAL CHARACTERIZATION

PhD Thesis

BY

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CENTRE OF PLANT BIODIVERSITY
UNIVERSITY OF PESHAWAR
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DEDICATION

This sacred research endeavor is dedicated to my great teachers and mentors right from primary schooling till date and onward.


بسم الله الرحمن الرحيم

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Inocybaceae	*Inocybe*	*Inocybe kohistanensis*	105
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		Inocybe pallida	115
		Inocybe rimosa	120
		Inocybe swatica nom. prov.	124
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ABSTRACT

This dissertation elucidates first comprehensive attempt on morphological and molecular phylogenetic investigation of mushrooms of district Swat, a floristically rich and diverse Hindu Kush region of Pakistan. District Swat is an area of diverse vegetation and ecosystem types. For exploring mycobiotic diversity of the area field surveys were conducted from 2012–2015 during rainy seasons for collection and documentation of mushrooms. More than 300 fruiting bodies of mushrooms were collected and preserved from the study area. Among the collected mushrooms, 100 samples were subjected to DNA analysis. A total of 63 out of 100 specimens amplified successfully during Polymerase Chain Reaction, while only 46 among the amplified ones yielded editable nuclear ribosomal ITS sequences which were further subjected to morphological and molecular biosystematics studies.

Sum of 46 taxa belonging to 26 genera (15 families) are described in this work. Mushroom families like Strophariaceae and Russulaceae owe their leading positions by forming major assemblages of 6 species each in 3 genera and 1 genus, respectively. The Second largest family stands to be Inocybaceae with 5 species in 1 genus, followed by Amanitaceae, Mycenaceae and Tricholomataceae with 4 species each in 1, 2 and 4 genera respectively. Assemblages of 3 species each is revealed by Agaricaceae in 3 genera, Cortinareace in 1 genus and Marasmiaceae in 2 genera each. Hygrophoraceae and Boletaceae are represented by 2 species and 2 genera each, whereas, Bolbitiace, Hymenogastraceae, Pleurotaceae and Auriscalpiaceae are represented by 1 species each.

Out of these 2 species viz *Xerocomellus fulvus* Ahmad and Jabeen 2016 and *Inocybe kohistanensis* Jabeen and Ahmad 2016 are new to science whereas 21 species viz *Amanita aurumana* nom. prov., *Amanita longiformis* nom. prov., *Amanita superiora* nom. prov., *Cortinarius kohistanicus* nom. prov., *Gymnopus subcampanulatus* nom. prov., *Hygrocybe decoriae* nom. prov., *Inocybe Swatica* nom. prov., *Lentinellus horridus* nom. prov., *Lepiota granulata* nom. prov., *Macrocystidia obscurum* nom. prov., *Mycena bractea* nom. prov., *Mycena lacerata* nom. prov., *Phachylepyrium kalamicum* nom. prov., *Pholiota lanceolata* nom. prov., *Pholiota manga* nom. prov. and *Pseudoomphalina conifera* nom. prov. *Russula kalamica* nom. prov., *Russula deodara* nom. prov., *Russula umbra* nom. prov., *Russula mankialica* nom. prov. and *Xeromphalina flavens* nom. prov., seems previously undescribed.

Twenty species are new reports for Swat among which 8 species viz; *Cortinarius cistoglaucopus, Cortinarius elegantior, Gymnopus barbipes, Inocybe mimica, Lepista irina Leucocalocybe mongolicum* var. *perpura, Pholiota gummosa* and *Russula puellaris* are new records for Pakistan. Twelve species viz *Amanita flavipes, Boletus reticuloceps, Clitocybe gibba, Conocybe semiglobata var. campanulata, Cyclocybe erebia, Echinoderma asperum, Hebeloma theobrominum, Hygrophorus chrysodon, Inocybe rimosa, Leucocoprinus cretaceous, Pleurotus ostreatus and Russula sichuanensis* are already reported from Pakistan, Whereas 3 species viz *Inocybe pallida, Mycena pura* and *Pholiota spumosa* are already reported from Swat Pakistan.

This first attempt to explore the floristically rich area of District Swat for morphological and molecular phylogenetic assessment of Mushrooms has not only upgraded the existing

information/literature regarding the Mushrooms of Pakistan but has also established a standard bench mark for future research endeavors. The existence of 50 % species new to science in the region indicates that more concerted fungal assessment of the diverse floral region like District Swat is compulsory on urgent basis so that the rapidly growing taxonomic knowledge of fungal biodiversity could be boosted locally and globally for its application in food industry and forestry.

CAHPTER I INTRODUCTION

1.1 The study area

Swat is a floristically rich and diverse mountainous terrain of eastern Hindu Kush region of Pakistan, sometimes referred to as Swat Himalayas (Schickhoff, 2005; Ahmad *et al.* 2015). Swat is famous for its natural beauty and geographic location that offers enormous opportunities in the trans Hindu Kush-Himalayan region besides the socioeconomic development through multiple uses of biodiversity and ecosystem services. District Swat lies adjacent to the intersection point of the world's largest mountain ranges i.e Himalaya, Hindu Kush and Karakurram, which makes climatically more divise establishment of diverse forest types. Altitudinal variations of Swat (600 to 6000m) range from mild relief in the southern parts to very steep relief and high altitudes in the north. The valley spreads over 6226 km² of land (Ahmad, 1995; Ali *et al.* 2013; Ahmad *et al.* 2015). The ranges are predominantly directed towards the north-southern aspect. Swat valley is confined at all sides by the patronizing Hindu Raj Mountains and is drained up by the meandering Swat River originating in the highlands to the north. The picturesque Swat Valley is gifted with astonishing biodiversity due to the presence of a variety of phyto-geographical regions with heavy monsoon rains concentrated mostly in summer. The distinctive climatic and edaphic blend of the region make it unique for stringing a diversity of biotic communities stimulated by numerous temperature and precipitation influences (Ahmad and Ahmed 2004, Ahmad and Sirajuddin 1996, Ali and Qaiser 1986).

Swat is popular for its swift rivers, stunning mountains, variety of fruits, medicinal herbs and plant resources around the world (Bangash, 2012; Ali. *et al.* 2013). The forest type of

Swat ranges from subtropical chirr pine forest in south to alpine pastures and meadows to cold desert in the north (Sher, 2013; Ahmad *et al.* 2015).

1.2 Fungal Biodiversity

Fungi encompass the most prominent segment of Biodiversity. Presently, the mega-diverse lineage "fungi" consisting of 3 kingdoms, mostly belong to the Fungi known as Eumycota. No of fungi estimated in the world is about 5.1 - 13.5 M species (Abdel-Azeem 2010; Blackwell 2011).

Out of approximately, 120,000 described species of fungi, over 21,000 of 30,000 accepted species of Basidiomycota are Agaricomycetes and many of these live in soil (Webster and Weber, 2007; Kirk *et al.* 2008). Most of these are Agarics, Discomycetes and Gasteromycetes (Chang, 1987). Among these, higher fungi are featured by macroscopic sporocarps. It includes mushrooms, bracket fungi, puff balls and cup fungi. Credentials of these fungi are based on morphological characters of fruiting body (Alexopoulos *et al.* 1996).

1.3 Importance of Mushrooms

Gilled fungi (mushrooms) belong to the fungal order Agaricales (Basidiomycota). As forest mycological entities, mushroom forming fungi can be found playing important roles as 1) saprobes on wood and other organic material which is on the verge of decay 2) forming symbiotic associations with the living cells of plant roots known as mycorrhizal associations 3) they may be parasitic on living plants upto some extant (Bruns *et al.* 1991; O'Brien *et al.* 2005; Bjorn, *et al.* 2013). Mushrooms are known to be very amazing group

of fungi presumed their excellent adjustment skills to house settings of the environment where thesy have to act as natural lignocellulose slayers (Nam–Seok *et al.* 2009).

Mushrooms are used by scientists and the public from ancient times due to their significant roles in man's life; like their use in the medicine, in food industry (Lindequist *et al.* 2005) and their significant role in ecosystem service in the form biodegradation (De Boer *et al.* 2005). Mushrooms have been exploited commercially world over and may be cultivated or gathered from the wild (Boa, 2004). The rate of consumption of fleshy fungi in many countries has increased in recent years and hence it becomes imperative to explore the treasure of wild mushroom (Pandey and Singh, 1978).

Plentiful compounds have been extracted from mushroom-forming fungi which are of great utility if made as mushroom nutraceutical and pharmaceutical alternates (Alemu, 2013). These include some proteoglycans and water soluble polysaccharides, proteins, and various rudiments of small molecular mass known to have immune modulatory potential by regulating several types of immune cells that function in antitumor or antimicrobial activities, including dendritic cells, macrophages, cytolytic T cells, and NK cells (Ko *et al.* 1995; Hsu *et al.* 2004; Lindequist *et al.* 2005; Yu *et al.* 2009).

1.4 Significance of Mushrooms in increasing forest cover

Mushroom fungi has great role in maintaining plant biodiversity and boosting the forest economy in the form of mycorrhizal association. Mycorrhizal association is present almost in 85% of trees of world (Kirk *et al*. 2008). Under natural conditions, several hundred fungal species are associated with plant roots within forests (Ishida *et al*. 2007;

Opik *et al*. 2009; Jumpponen *et al*. 2010). These fungi are considered to be essential agents that determine the composition of plant communities (Booth 2004; Nara and Hogetsu 2004; Peay *et al*. 2010). For example, mycorrhizal fungi facilitate the soil nutrient acquisition of plants (Smith and Read 2008) and thereby enhance the competitive ability of their specific hosts in local communities (Nara 2006). Likewise, phylogenetically diverse fungal root endophytes not only promote the growth of plants but also enhance the pathogen resistance of their hosts (Upson *et al*. 2009; Newsham 2011), while some of them are known to negatively affect the fitness of host plants (Reininger and Sieber 2012). Thus, ecologically and phylogenetically diverse fungi differentially interact with plant species in the wild, potentially playing important roles in the dynamics of forest ecosystems (Klironomos 1999,2003; Fukami and Nakajima 2013).

The temperate forests of Swat Pakistan are generally dominated by trees of the Pinaceae and Fagaceae families (Ahmad et al. 2015). Members of these plant families form symbiotic associations with mycorrhizal fungi forming various groups of ectomycorrhizal mushroom fungi (Bahram *et al*. 2012; Tedersoo *et al*. 2012). Transport of nitrogen and phosphorus to the partner plant is achived by these fungi which spread extra-radical mycelia into soil (Finlay and Read 1986; Cairney 2005; Wu *et al*. 2012). Ectomycorrhizal fungi also help in protectection of their host roots from other pathogenic fungi or nematodes (Borowicz 2001). Through such impressions, ectomycorrhizal fungi are imperative in the development and existence of forests.

Ectomycorrhizal (ECM) mushrooms form symbiotic association with roots of various angiosperm families such as Moraceae, Betulaceae, Fagaceae and Pinaceae hence

becoming necessary component of forest ecosystem (Brundrett, 2004; Razaq *et al.* 2012b). ECM mushrooms help to transport water and nutrients; they mobilize nitrogen, magnesium, calcium and phosphorus from compact mineral source through organic acid digestion (Landeweert *et al.* 2001). Provision of nutrients to trees from a larger portion of soil through mycelial hyphae of mushrooms is a relatively low cost method (Alemu, 2013). In general, growth and survival of a palnt is enhanced by mycorrhizal fungi though nutrents transport for return of a small amount of carbohydrates (Hogberg and Hogberg 2002). Mycorrhizal colonisation of fungi has also been illustrated to influence plant defense against insect herbivores hence disturbing population fitness of insect (Wooley & Paine, 2007; Vannette & Hunter, 2011; Roger, *et al.* 2013).

1.5 Earlier works on Mushrooms of Pakistan

Pakistan is fertile and ecologically rich country. Its forests are not only rich in biodiversity of fauna and flora but also in mushroom flora (Burni *et al.* 2006; Niazi *et al.* 2006; Niazi, 2008; Razaq *et al.* 2012a). An initial survey of the mushroom diversity of district Swat has been conducted by Ahmad (1952, 1956, 1969, 1972, 1980a), Shibata (1992), Murakami (1993) and Iqbal and Khalid (1996). They reported 38 mushroom species from district Swat (Ahmad *et al.* 1997).

Butler and Bisby (1933) worked on Basidiomycetes of Pakistan for the first time. Noteworthy work has been done by Arthur and Cummins (1933), Malik and Khan (1944) on Basidiomycetes of Pakistan.

Dr. Sultan Ahmad (1910-1983) the pioneer and renowned mycologist of Pakistan, has the aid of more than 170 papers together with monographs to fungi of Pakistan. His inputs include description of 60 species (Ahmad, 1992) and "Fungi of Pakistan" listing 200 species (Ahmad, 1956) with a subsequent addition of 100 species to the list in the later years (Ahmad, 1962, 1969, 1972, 1976, 1980a). A chicklist of Agaricales was provided by Ahmad (1980b) including 183 species, 147 of which were new records for Pakistan.

Khan (1975) reported 91 species of mushrooms from different parts of Pakistan. Japanese scientists Izumi *et al.* (1992), Ono (1992), Ono and Kakishma (1992), Kakishma *et al.* (1993a, 1993b) Kaneko (1993) and Ono and Izumi (1995) reported Parasitic Basidiomycetes and some higher basidiomyctes during their cryptogamic expedition of Pakistan (Fiaz, 2013). Other Japanese scientests, Shibata (1992) reported 51 and Murakami (1993) reported 76 species of higher basidiomycetes from Pakistan.

Edible mushrooms of Pakistan have been worked out by Sultana *et al.* (1997). Gardezi (1998) and Gardezi and Ayub (2003) surveyed for mushrooms of Azad Jammu and Kashmir. A complete monograph of Fungi of Pakistan has been compiled by late Sultan Ahmad, Iqbal & Khalid, (1997).

In the past decade and recent years some of the eminent mycologists of Pakistan have made great contributions to the mushroom fungi of Pakistan. They include; Jabeen and Khalid (2013), Fiaz *et al.* (2013), Fiaz (2013), Razaq *et al.* (2013), Ilyas *et al.* (2013), Sarwar (2011, 2012), Saba *et al.* (2012, 2013), Denchev *et al.* (2012), Yousaf *et al.* (2012, 2013), Razaq *et al.* (2012a,b), Sultana *et al.* (2011), Niazi *et al.* (2010), Afshan (2009), Niazi (2008), Razaq (2007), Gardezi and Ayub (2003), Ahmad *et al.* (1997).

Sarwar and Khalid (2014) reported more than 280 new records of basidiomycetes for Pakistan along with some new species to science from different areas of Pakistan. Sarwar and Khalid (2012) provided a checklist of Boletales of Pakistan including 54 taxa. Around 1360 species of basidiomycetes with 700 species of homobasidiomycetes have been repoted from different parts of Pakistan (Fiaz, 2013).

1.6 Modern trends in mushrooms taxonomy

Taxonomy is that branch of biology which is concerned with the naming and grouping of organisms. Generally, classification is based on macroscopically and microscopically observable characteristics, the phenotypic approach. However, morphological characteristics differ greatly, depending on the species and environmental conditions. Furthermore, the characteristics accepted as typical for fungi have arisen polyphyletically, and there seems to be a definite trend toward asexual reproduction, reducing the spectrum of morphological characteristics (Lemke *et al.* 2001). The incorporation of molecular phylogenetic approaches particularly the analysis of DNA sequences, into modern systematics has caused imperative changes in the customary concepts of taxonomy (Katoch & Kapoor, 2014). The introduction of PCR has allowed the study of dried herbarium material in minute quantities (Carlile & Watkinson, 1994). The creation of universal primers specific to fungi has made access easy to related nucleotide sequences. These segments of nuclear ribosomal DNA are highly conserved (Hendriks *et al.* 1992Van de Peer, *et al.* 1997) and are extensively used in inferring evolutionary attributes of species. Comparisons of the 18S rRNA

sequences have been executed to evaluate the relationships of the major groups of living organisms.

Molecular phylogenetic analyses have been widley used in current mushroom taxonomy. Hibbett *et al*. (1997) reconstructed a comprehensive phylogenetic history of major groups of fungi like Agaricales, Aphyllophorales and gasteromycete using representative nuclear and mitochondrial ribosomal DNA sequences, their discovery demonstrates that morphology is not consistent with molecular phylogenetic analysis for these groups. Moncalvo *et al*. (2000) studied phylogenetic relationships of mushroom forming fungi through nuclear large subunit ribosomal (LSU) DNA sequences. Their findings uncovered polyphyletic status of Hygrophoraceae, Cortinariaceae and Tricholomataceae and the polyphyletic genera like *Omphalina, Clitocybe* and *Marasmius*. Another molecular phylogenetic analyses by Moncalvo *et al.* (2002), taking more (LSU) sequences of euagarics into account, revealed 117 monophyletics lineages. In other studies some non-gilled cyphelloid, resupinate, and gasteroid fungi were found affiliated to the Agaricales (Peintner *et al.* 2001; Binder and Bresinsky 2002; Bodensteiner *et al.* 2004; Binder *et al.* 2005; Larsson *et al.* 2004; Moncalvo *et al.* 2002; Hallen *et al.* 2003; Matheny and Bougher 2006).

These discoveries conclude that natural groupings should'nt be made only on morphological basis but other fetaures like molecular phylogenetic analyses should also be taken into consideration. In a molecular phylogenetic assessment of Agaricales, Matheny *et al*. (2006) proposed 6 clades labeled as Tricholomatoid, Agaricoid, Marasmioid, Plicaturopsidoid, Pluteoid and Hygrophoroid clades. Their dataset included

238 species of 146 genera while using 6 gene regions: nrITS (18S, 25S and 5.8S), rpb1, rpb2, rpb-intron2.

1.7 Objectives

The current study was aimed to achieve the following objectives:

1. To explore and document the diversity of mushroom flora of district Swat
2. To make additions to the "Fungi of Pakistan"
3. To provide an assessment of the mycorhizzal mushrooms associated with the forest trees of Swat necessary for conservation related issues
4. To submit nrITS sequences of the mushrooms of Swat in international database like NCBI GENEBANK for the accession of reference sequences from Pakistan for phylogenetic and evolutionary relationships among different groups of mushrooms globally
5. To present a phylogenetic framework for the discussion of evolution of mushrooms
6. To strengthen the sequence database of the Fungi of Pakistan
7. To name the mushrooms of Swat correctly for their application in ethnomycology, forestry, biodiversity conservation and other uses of mushrooms

CHAPTER 2 MATERIALS AND METHODS

2.1 Survey of the sampling sites

Extensive investigation of the floristically richer sites of Swat (34°30'-3°55' North, 71°45'-72°50'East) was made during rainy seasons of 2012-2015, for collection of mushrooms. These sites were visited from the time of appearance of sporocarps till their disappearance.

2.2 Collection of specimens

Fruiting bodies of mushrooms were collected while using sharp digger (Sarwar 2013). Collected mushrooms were photographed, vouchered and set aside in card boxes. Every specimen was assigned a collection number.

2.3 Drying and preservation of the specimens

Collections were rapidly dried to avoid rotting of the basidiomata. Larger specimens were sliced into two or more slices to ensure quik drying. Drying was carried out through fan heater. Each fully dried specimen was preserved in plastic grip seal bag supplied with label. The voucher specimens were deposited in the Herbarium, Centre of Plant Biodiversity, University of Peshawar (Catalague nos; UPBG-ISP001 to UPBG-ISP300) and Herbarium, Department of Botany, university of the Punjab, Quide-e-Azam Campus, Lahore (Catalague nos; LAH-ISP001 to LAH-ISP300).

2.4 Molecular Characterization

2.4.1 Extraction and amplification of genomic DNA

Genomic DNA was isolated from a small wedge of pileus having gills by a modified CTAB method (Bruns, 1995) and in some instances, where CTAB method proved unfruitful, by using 'DNeasy Plant Mini Kit' (Qiagen). Amplification of Internal Transcribed Spacers ITS1, ITS2 and 5.8S region of the nuclear ribosomal RNA gene was targeted using the primer pairs ITS1F/ITS4 (White *et al.* 1990; Gardes and Bruns, 1993). PCR operation included: 94 ^{o}C for 1 min, 35 × (94 ^{o}C for 1 min, 53 ^{o}C for 1 min, and 72 ^{o}C for 1 min), and final extension 72 ^{o}C for 8 mins in GeneAmp PCR System 9700; Applied Biosystems made in USA . Amplified PCR products were purified and were bidirectionaly sequenced at Macrogen Lab (Korea).

2.4.2 Editing of sequences and BLAST analysis of ITS sequences

The nrDNA ITS sequences were matched using Basic Local Alignment Search Tool (BLAST), National Center for Biotechnology Information (NCBI) for comparison and confirmation of identifications. BioEdit (Version 7.2.3) was used for cleaning and editing of the sequences where required. Newly generated sequences were aligned with other sequences of the GenBank, using muscle alignment tool (www.ebi.ac.uklTools/msalmuscle). In aligned dataset, all characters were equally pondered, while treating gap positions as missed data.

2.5 Phylogenetic analysis

Phylogenetic tree was made for each species sequence/sequences. Maximum Likelihood (ML) analysis was done using Molecular Evolutionary Genetic Analysis (MEGA ver-6.0) with default settings of program i.e. Jukes-Cantor Model and for ML Heuristic Nearest-Neighbor-Interchange (NNI) method was used (Tamura *et al.* 2011). About 1000 bootstrapping was implemented for analysis replicates.

2.6 Morpho-anatomical characterization

Techniques adopted by Nauta (2001) were employed for morpho-anatomical characterization of preserved specimens. Macroscopic data was noted in field and through photographs of the collected specimens. Macroscopic data involved the study of Pileus: size (diameter), shape, color, texture and ornamentation of the surface, color and bruising reaction (change in color) of the context, shape color and thickness of its margin. Lamellae/Gills: insertion, color, spacing, thickness, shape of margin, color of pore surface. Stipe: size, location, shape, color and bruising reaction, texture and ornamentation, consistency, presence of ring, nature of pith and attachment. Annulus**:** position and color. Volva: patches of universal veil and color.

Compound microscopes were used for assessment of the microstructures of preserved mushrooms. Important characters like size (length × width), presence of cytoplasmic contents, wall size, surface ornamentation and shape were recorded for basidiospores, basidia, cystidia, pileipellis and stiptipellis by preparing slides in KOH, phloxin and Meltzer reagent. Measurements of anatomical charcters are presented for at least 20

structers per sample using ocular micrometer with 100x oil-immersion objective this include, X = arithmetic mean of spore length and spore width for all spores measured and Q = spore length divided by spore width. Line drawings were made with LUCIDA camera. Color designations are from Munsell (1975).

2.7 Scanning Electron Microscopy

SEM was conducted at Centralized Resource Laboratories (CRL), University of Peshawar. Tissue of the lamellar region of mushrooms were pasted on metal stubs using double sided adhesive tape and coated by fine coating of gold while sputtering in a vacuum sputterer (JFC-1100 JEOL, Japan). Subjects were then observed in a Scanning Electron Microscope (JSM-6360 JEOL, Japan) at an accelerating voltage of 20 KV.

CHAPTER 3 RESULTS

3.1 Exploration and Collection

In the current study attempts were made during 2012-2015 to elucidate the morpho-anatomical and molecular characterization of mushrooms growing in district Swat, Pakistan. Different approaches were made to characterize the unkown mushroom forming fungi of the study area. In mropho-anatomical characterization, SEM technology was used for those fungi which appeared new to science (Yanaga *et al.* 2012). The molecluar chararization of all the collected sepcimens was made using nrITS primers. The evolutionary relationships of the species were inferred using conserved regions of nuclear ribosomal gene i.e internal transcribed spacer (nrITS) by means of Maximum likelihood via Neighbor-Join and BioNJ algorithms using the MCL (Maximum Composite Likelihood) approach. The treated species were analysed phylogenetically for placing them in their genuine locations. Authentic positioning of the species was sustained by traditional fungal taxonomy coupled with molecular phylogenetic analyses.

During this study more than 300 fruiting bodies of mushrooms were collected and preserved. Among the collected mushrooms 100 samples, which seemed morphologically different, were subjected to DNA analysis. Out of which only 63 were amplified successfully during polymerase Chain Reaction while only 46 of them yielded editable nuclear ribosomal ITS sequences through latest molecular phylogenetic protocols and were further subjected to morphological and molecular phylogenetic investigation.

These 46 described and identified taxa from different climatic combinations of district Swat belong to 26 genera viz *Echinoderma* (Locq. ex Bon) Bon, *Lepiota* (Pers.) Gray, *Leucocoprinus* Pat., *Amanita* Pers., *Lentinellus* P. Karst., *Conocybe* Fayod, *Boletus* L., *Xerocomellus* Šutara, *Cortinarius* (Pers.) Gray, *Hygrocybe* (Fr.) P. Kumm., *Hygrophorus* Fr., *Hebeloma* (Fr.) P. Kumm., *Inocybe* (Fr.) Fr., *Gymnopus* (Pers.), *Macrocystidia* Joss., *Mycena* (Pers.) Roussel, *Xeromphalina* Kühner & Maire, *Pleurotus* (Fr.) P. Kumm., *Russula* Pers., *Agrocybe* Fayod, *Pholiota* (Fr.) P. Kumm. *Pachylepyrium* Singer, *Infundibulicybe* Harmaja, *Lepista* S. Imai, *Pseudoomphalina* (Singer) Singer and *Leucocalocybe* X.D. Yu & Y.J. Yao (Table 3.1). These genera are circumscribed in 15 families.

In the current work Strophariaceae and Russulaceae owe their leading positions by forming major assemblages of 6 species each in 3 genera and 1 genus, respectively. The Second largest family is Inocybaceae with 5 species in 1 genus, followed by, Amanitaceae, Mycenaceae and Tricholomataceae with 4 species each in 1, 2 and 4 genera, respectively. Assemblages of 3 species each is revealed by Agaricaceae in 3 genera, Cortinareace in 1 genus and Marasmiaceae in 2 genera each. Hygrophoraceae and Boletaceae are represented by 2 species and 2 genera each, whereas, Bolbitiace, Hymenogastraceae, pleurotaceae and Auriscalpiaceae are represented by 1 species each.

Out of these 2 species viz *Inocybe kohistanensis* Jabeen, Ahmad and Khalid and *Xerocomellus fulvus* Sarwar, Ahmad and khalid have been published by the author as new species, whereas, 21 species viz *Amanita aurumana* nom. prov., *Amanita longiformis* nom. prov., *Amanita superiora* nom. prov., *Cortinarius kohistanicus* nom.

prov., *Gymnopus subcampanulatus* nom. prov., *Hygrocybe decoriae* nom. prov., *Inocybe swatica* nom. prov., *Lentinellus horridus* nom. prov., *Lepiota granulata* nom. prov., *Macrocystidia obscurum* nom. prov., *Mycena bractea* nom. prov., *Mycena lacerata* nom. prov., *Pachylepyrium kalamicum* nom. prov., *Pholiota lanceolata* nom. prov., *Pholiota manga* nom. prov. and *Pseudoomphalina conifera* nom. prov. *Russula kalamica* nom. prov., *Russula deodara* nom. prov., *Russula umbra* nom. prov., *Russula mankialica* nom. prov., *Xeromphalina flavens* nom. prov., seems previously undescribed.

This work designates 8 species viz; *Cortinarius cistoglaucopus, Cortinarius elegantior, Gymnopus barbipes, Inocybe mimica, Lepista irina, Leucocalocybe mongolicum, Pholiota gummosa* and *Russula puellaris* as new records for Pakistan.

Out of 20 species as new reports for Swat, 8 species viz; *Cortinarius cistoglaucopus, Cortinarius elegantior, Gymnopus barbipes, Inocybe mimica, Lepista irina, Leucocalocybe mongolicum* var. *purpura* var. nov., *Pholiota gummosa* and *Russula puellaris* are new records for Pakistan. Twelve species viz *Amanita flavipes, Boletus reticuloceps, Clitocybe gibba, Conocybe semiglobata var. campanulata, Cyclocybe erebia, Echinoderma asperum, Hebeloma theobrominum, Hygrophorus chrysodon, Inocybe rimosa, Leucocoprinus cretaceus, Pleurotus ostreatus* and *Russula sichuanensis* are already reported from Pakisan.Whereas 3 species viz *Inocybe pallida, Mycena pura and Pholiota spumosa* are already reprted from Swat Pakistan.

Table 3.1 Passport information of mushrooms reported from Swat Pakistan

S/No	Family	Botanical Name	Lcality in Swat	V-No	Mycorhizzal partner	Date of collection
1	Agaricaceae	*Echinoderma asperum*	Kalam and Mankial	ISP 028& 074	NA	03-09-2013
2	Agaricaceae	*Lepiota granulata* nom. prov.	Malam Jabba	ISP 002	NA	09-08-2012
3	Agaricaceae	*Leucocoprinus cretaceus*	Jura Matta	ISP 290	NA	15-06-2013
4	Amanitaceae	*Amanita aurumana* nom. prov.	Ushu kalam	ISP 050	NA	04-09-2013
5	Amanitaceae	*Amanita flavipes*	Kalam	ISP 049	*Pinus wallichia*	04-09-2013
6	Amanitaceae	*Amanita longiformis* nom. prov.	Miandam	ISP 116	NA	02-09-2013
7	Amanitaceae	*Amanita superiora* nom. prov.	Mankial	ISP 65	NA	04-09-2013
8	Auriscalpiaceae	*Lentinellus horridus* nom. prov.	Kalam	ISP 039	NA	03-09-2013
9	Bolbitiaceae	*Conocybe semiglobata* var. campanulata	Kalam	ISP 044	NA	03-09-2013
10	Boletaceae	*Boletus reticuloceps*	Mankial	ISP 080 & 105	*Cedrus deodara*	05-09-2013
11	Boletaceae	*Xerocomellus fulvus* Sp. Nov.	Ushu kalam	ISP 060	NA	04-09-2013
12	Cortinariaceae	*Cortinarius cistoglaucopus*	Mankial	ISP 077	*Cedrus deodara*	05-09-2013
13	Cortinariaceae	*Cortinarius elegantior*	Miandam	ISP 125	*Abies pidrow*	06-09-2013
14	Cortinariaceae	*Cortinarius kohistanicus* nom. prov.	Kalam	ISP 056	NA	03-09-2013
15	Hygrophoraceae	*Hygrocybe decoriae* nom. prov.	Yakh Tangay	ISP 009	NA	02-09-2013
16	Hygrophoraceae	*Hygrophorus chrysodon*	Miandam	ISP 124	*Cedrus deodara & Pinus wallichia*	06-09-2013
17	Hymenogastraceae	*Hebeloma theobrominum*	Ushu kalam	ISP 047	*Cedrus deodara & P. wallichia*	04-09-2013
18	Inocybaceae	*Inocybe kohistanensis*	Kalam	ISP 042	*Cedrus deodara*	04-09-2013

S/No	Family	Botanical Name	Lcality in Swat	V-No	Mycorhizzal partner	Date of collection
19	Inocybaceae	*Inocybe mimica*	Ushu kalam & Mankial	ISP 048& 069	*Cedrus*	04-09-2013
20	Inocybaceae	*Inocybe pallida*	Kalam	ISP 058	*Cedrus deodara*	02-09-2013
21	Inocybaceae	*Inocybe rimosa*	Kalam	ISP 037	*Cedrus deodara*	04-09-2013
22	Inocybaceae	*Inocybe swatica* nom. prov.	Mankial	ISP 079	NA	04-09-2013
23	Marasmiaceae	*Gymnopus barbipes*	Malam Jabba	ISP 008	NA	02-09-2013
24	Marasmiaceae	*Gymnopus subcampanulatus nom. prov.*	Kalam	ISP 041	NA	04-09-2013
25	Marasmiaceae	*Macrocystidia obscurum* nom. prov.	Malam Jabba	ISP 007	NA	04-09-2013
26	Mycenaceae	*Mycena bractea* nom. prov	Miandam	ISP 123	NA	09-09-2013
27	Mycenaceae	*Mycena lacerata* nom. prov.	Malam Jabba	ISP 04	NA	02-09-2013
28	Mycenaceae	*Mycena pura*	Kalam	ISP 016	NA	04-09-2013
29	Mycenaceae	*Xeromphalina flavens* nom. prov.	Kalam	ISP 026	NA	03-09-2013
30	Pleurotaceae	*Pleurotus ostreatus*	Matta	ISP 282	NA	14-09-2012
31	Russulaceae	*Russula mankialica* nom. prov.	Mankial	ISP 070	NA	04-09-2013
32	Russulaceae	*Russula umbra* nom. prov.	Miandam	ISP 113	NA	09-09-2013
33	Russulaceae	*Russula puellaris*	Kalam	ISP 034	*Cedrus deodara*	03-09-2013
34	Russulaceae	*Russula sichuanensis*	Yakh Tangay	ISP 003	*Pinus wallichia*	02-09-2013
35	Russulaceae	*Russula deodara* nom. prov.	Miandam	ISP 078	NA	08-09-2013
36	Russulaceae	*Russula kalamica* nom. prov.	Kalam	ISP 032	NA	03-09-2013
38	Strophariaceae	*Pachylepyrium kalamicum* nom. prov.	Kalam	ISP 027	NA	03-09-2013
37	Strophariaceae	*Cyclocybe erebia*	Kalam	ISP 021	NA	04-09-2013
39	Strophariaceae	*Pholiota lanceolata* nom. prov.	Kalam	ISP 022	NA	04-09-2013
40	Strophariaceae	*Pholiota gummosa*	Kalam	ISP 17	NA	04-09-2013

41	Strophariaceae	*Pholiota manga* nom. prov.	Malam Jabba	ISP 006	NA	02-09-2013
S/No	**Family**	**Botanical Name**	**Lcality in Swat**	**V-No**	**Mycorhizzal partner**	**Date of collection**
42	Strophariaceae	*Pholiota spumosa*	Malam Jabba	ISP 001	NA	02-09-2013
43	Tricholomataceae	*Clitocybe gibba*	Kalam	ISP 013	NA	02-09-2013
44	Tricholomataceae	*Lepista irina*	Yakh Tangay	ISP 005	NA	02-09-2013
45	Tricholomataceae	*Pseudoomphalina conifera* nom. Prov	Kalam	ISP 030	NA	
46	Tricholomataceae	*Leucocalocybe mongolica* var. *purpura*	Miandam	ISP 083	NA	

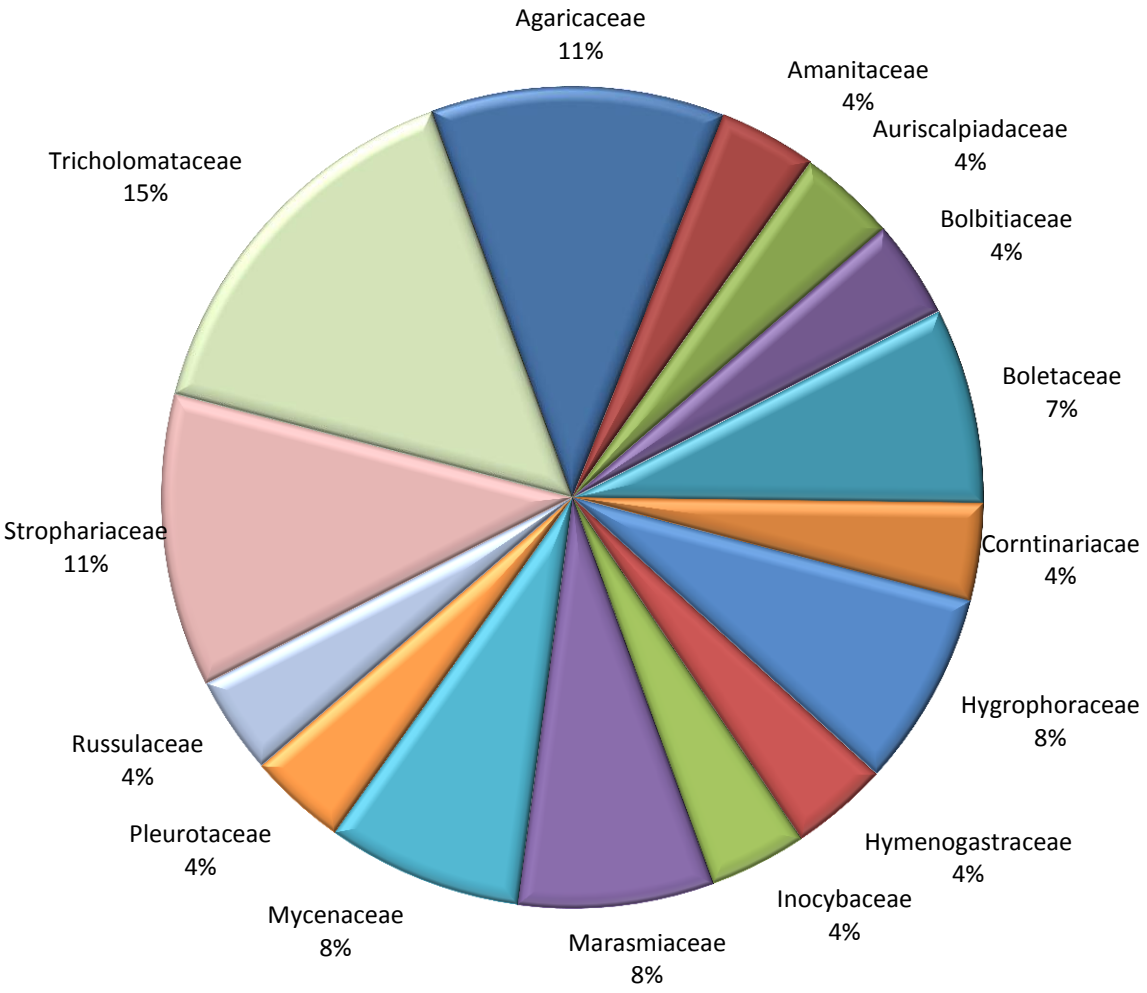


Fig 3.1 Chart showing genera wise abundance of the studied mushroom families of District Swat

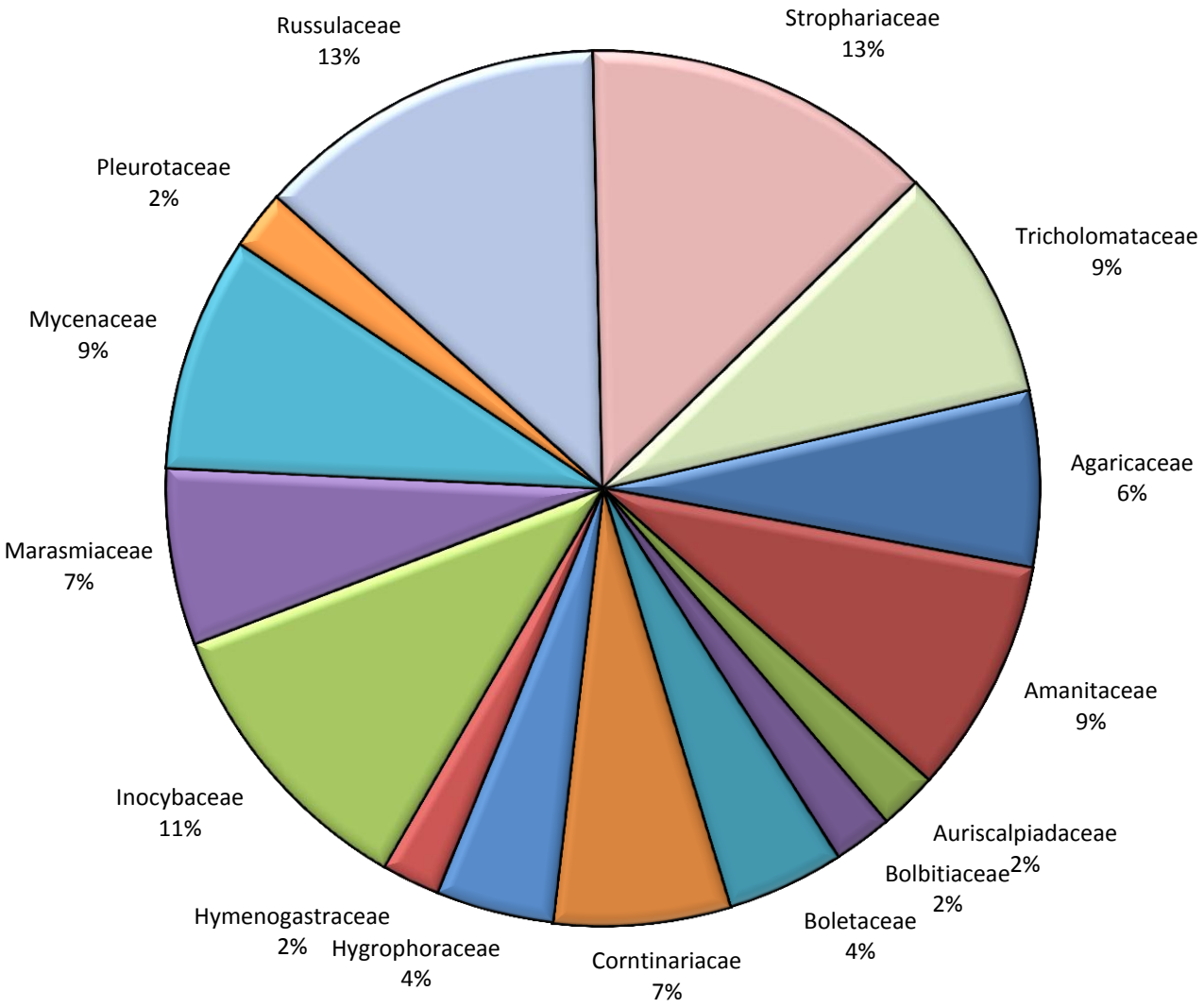


Fig 3.2 Chart showing species abundance of the studied mushroom families of District Swat

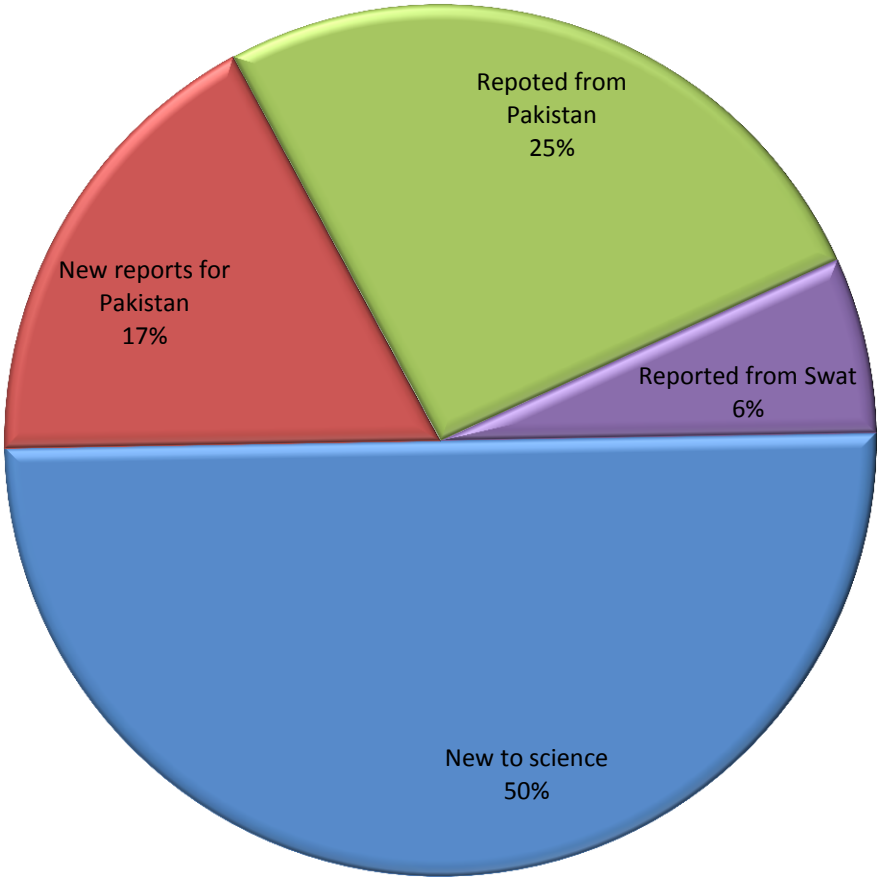


Fig 3.3 Chart showing status of mushrooms species novelty in District Swat

3.2 Morpho-anatomical and Molecular Characterization

The complete disription including morpho-anatomical and molecular characterization of each species is given below for each family circumscribed in this work.

Agaricaceae

Echinoderma

1. *Echinoderma asperum*

Lepiota

2. *Lepiota granulata* nom. prov.

Leucocoprinus

3. *Leucocoprinus cretaceus*

1. *Echinoderma asperum* (Pers.) Bon *Docums Mycol*. 21(82): 62 (1991)

Plate 01 – Figs. (A –F) & 3.4

Morpho-Anatomical characterization

PILEUS: 3 – 11 cm across, plane, dark orange yellow to dark yellowish brown (10YR 1/2 – 7.5YR 6/8 – 10YR 1/2); central disc umbonate; curvature deflexed; surface warty. LAMELLAE: free and approximte, light orangish when young become white with age, entire and crowded. STIPE: 3.8 – 8.5 × 0.7 – 1.6 cm, even and equal, centrally attached, moderate orange yellow (7.5YR 9/8). ANNULUS: superior with yellowish brown pigmentation.

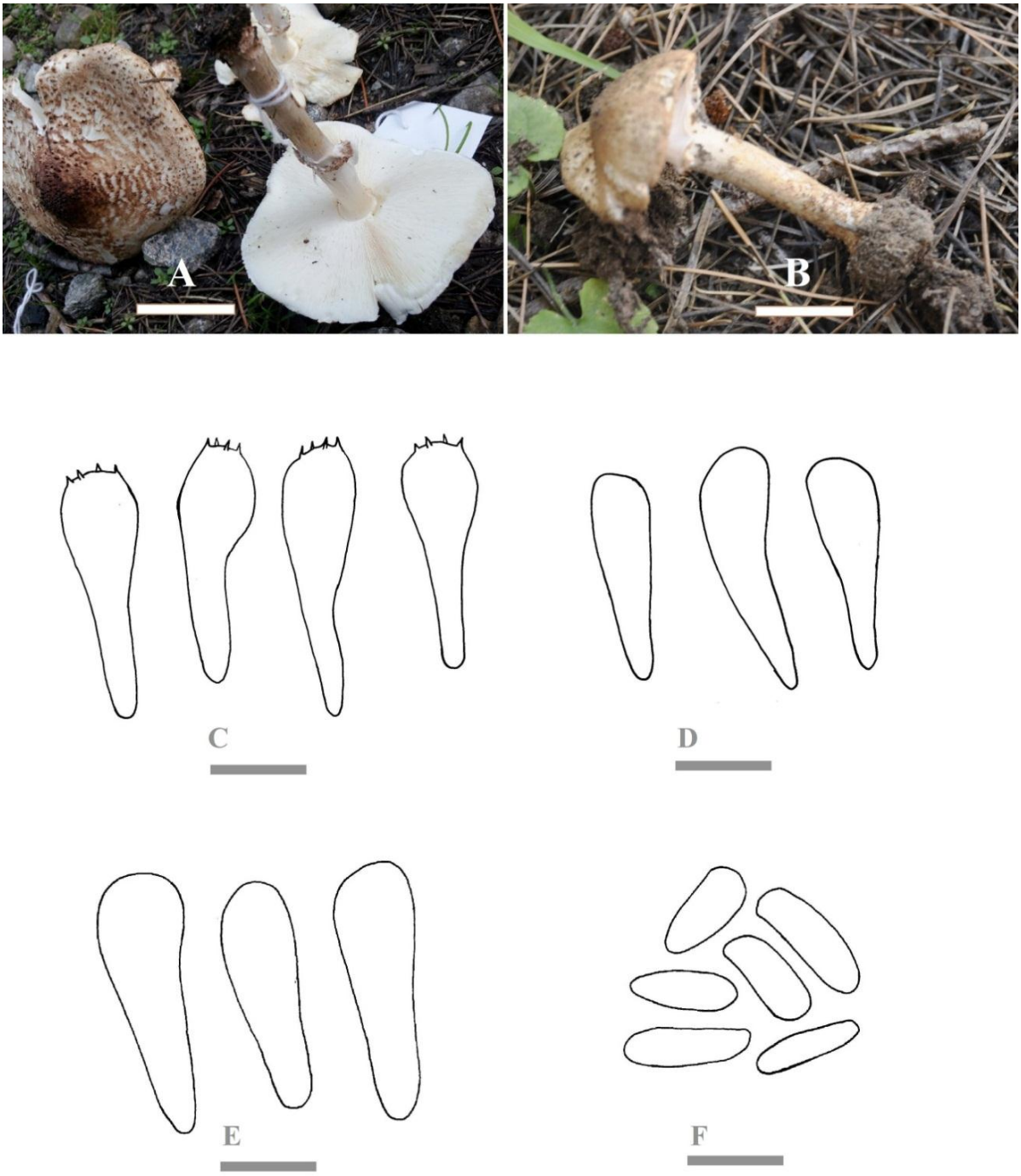
BASIDIOSPORES: 6.6 – 8.4 × 2.7 – 3.9 µm [X = 3.3 × 7.7 µm, Q = 2.119], ellipsoid, apiculate, smooth to slightly bullet shaped, hyaline in KOH and reddish in phloxin, anamoyloid. BASIDIA: 17.2 – 23.4 × 6.6 – 8.5 µm, clavate, quadrisporic, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 11.3 – 16.3 × 5.4 – 9.4 µm, clavate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: 11.5 – 24 × 5.4 – 10.1 µm.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Kalam and Mankial, solitary on soil in coniferous forests under *Cedrus deodara* (Roxb. ex D. Don) G. Don. 03 September, 2013, ISP # 028, 074 (LAH).

COMMENTS: This species has been described previously from Pakistan under its synonym *Lepiota acutesquamsa* (Weinm.) P. Kumm. from Abbottabad by Shibata (1992), Iqbal

and Khalid (1996) and Razzaq *et al.* (2013). This study reports its occurrence in district Swat Pakistan for first time.

Plate 01



Figures A-F Morpho-anatomical shapes of ***Echinoderma asperum.*** (A-B) Basidiomata (C) Basidia (C) Basidiospores (D) Cheilocystida (E) Pleurocystida (F) Basidiospores Scale bars: A = 3.5 cm, B = 1.5 cm C = µm, C = µm 7.5 D = µm 5.3 E = µm 3.8 F = 5.5 ɯm

2. ***Lepiota granulata*** nom. prov. Plate 02 – Figs. A-E & 3.4

Morpho-Anatomical characterization

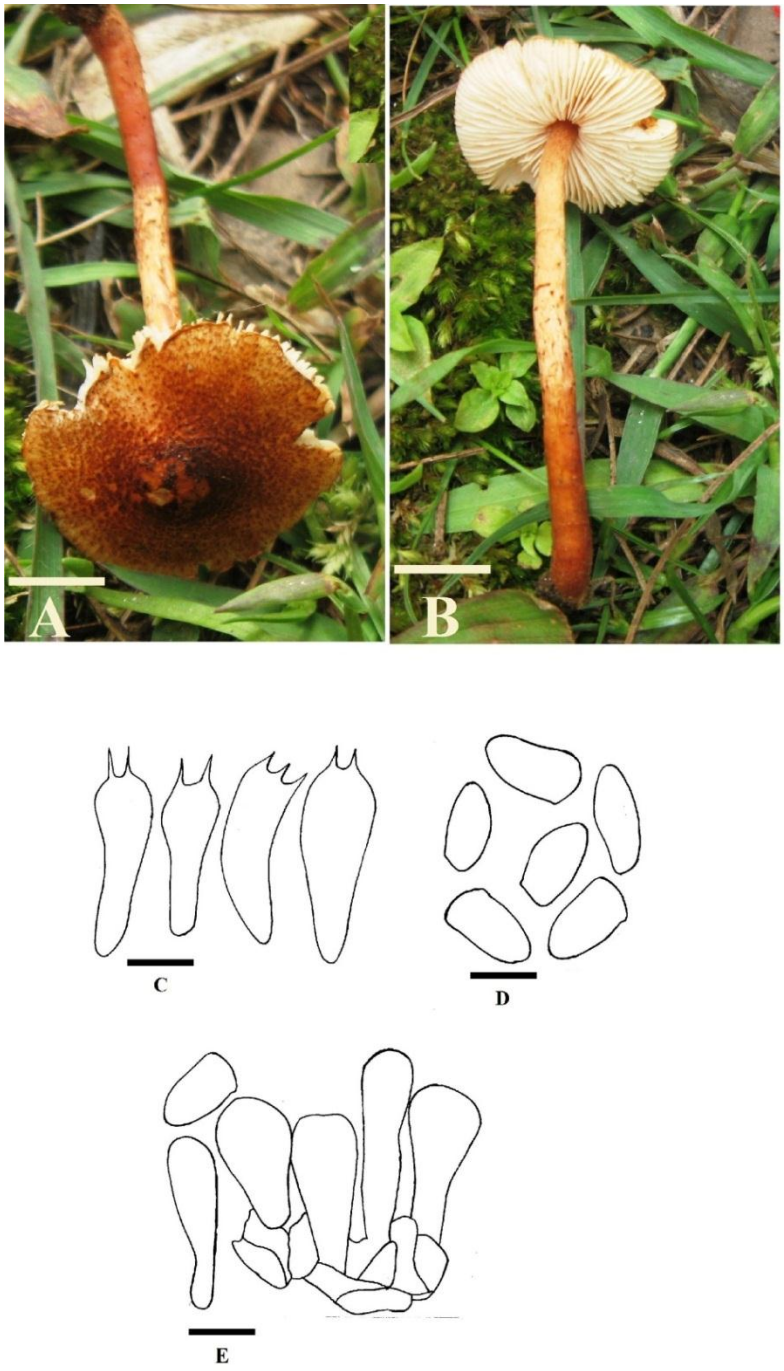
Etymology: The specific epithet may be referred to the granular cap and stem cuticle of this species.

PILEUS: 0.5 – 1.0 cm diam, plane, umbonate central disc, vivid yellowish pink to vivid redish orange (10R 7/16 – 10R 5/16); granular and dry surface; curvature straight to uplifted; margins plicate, eroded at maturity. LAMELLAE: free and approximate, close, thin, pale yellow (5Y 9/9); margins ragged eroded. STIPE: 6.0 – 7.0 cm in length, central, soft, equal and radiating, cylindrical moderate orange yellow in upper half (10YR 7/8) to strong reddish brown in lower half (10R 3/8); surface granular.

BASIDIOSPORES: 8.0 – 10.0 × 4.6 – 5.9 µm [x = 5.2 × 9.0 µm, Q = 1.731], with very indistinct spur, apiculus present, smooth, thin-walled, hyaline in KOH. BASIDIA: 17.4 – 21.6 × 5.9 – 7.0 µm, clavate, one to four spored basidia, thin-walled, hyaline in KOH, red in phloxin; Sterigmata 2.6 – 4.6 µm. CHEILOCYSTIDIA: 17.5 – 25.0 × 5.7 – 8.1 µm, thin-walled, hyaline in KOH, red in phloxin. PILEUS CUTICLE: 4.2 – 9.9 µm laxolattice, light brown in KOH. STIPE HYPHAE: parallel, cylindrical, 3.4 – 8.1 µm, hyaline in KOH to red in phloxin.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Malam Jabba, solitary on ground in mixed coniferous forest under *Pinus wallichiana* A. B. Jacks, 09 August, 2012, ISP # 002.

Plate 02



Figures– A-E Morpho-anatomical shapes of ***Lepiota granulata*** (A-B) Basidiomata; (C) Basidia (D) Basidiospores; (E) Cheilocystidia. Scale bars: A = 0.3 cm, B = 1.4 cm, C = 7.6 µm, D = 6 µm, E = 7.5 µm.

Molecular Phylogenetic Analysis

The target region copmrising of internal transcibed spacers (ITS1 and ITS2) and 5.8S of rDNA gave fragments of approximately 665 bp for *Echinoderma asperum* and about 603 for *Lepiota granulata* on amplification in polymerase chian reaction (PCR) using fungal universal primers pairs (ITS1 and ITS4). Initial BLAST analysis of nucleotide sequences revealed that *Echinoderma asperum* has maximum similarity with *Echinoderma asperum* (GenBank KC581336, Query coverage - 100%, expect value 0.0 and Identity index - 99%) collected from Canada followed by *Lepiota acutesquamosa* (GenBank FJ998400, Query coverage - 100%, expect value 0.0 and Identity index - 99%) of USA. *Lepiota granulata* has maximum similarity with *Lepiota pilodes* Vellinga & Huijser (GenBank accession # AY176476 and EF080865, Identity index 91%, Query coverage 98%) collected in Netherlands and USA respectively followed by *Lepiota castanea* Quél. (GenBank accession # AY176463 and EU416282, Identity index 89%, Query coverage 98%) collected in Netherlands and China respectively

Phylogenetic analysis of the studied *Lepiota* accessions was done by involving 27 closest sequences from Genbank using Maximum Likelihood method. *Limacella delicata* (Fr.) H.V. Sm = *Limacella glioderma* (Fr.) Maire was selected as outgroup taxon (Vellinga 2003). The data set contained 729 characters out of which 291 were conserved, 425 were variable, 272 were parsimony informative and 150 were singletons. Positions with gaps and unavailable data were not taken into account. The final dataset maintained 367 positions exclusively. Preliminary tree(s) for the experiential search were acquired automatically by application of Neighbor-Join and Bio NJ data alogrithms to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite

Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. The tree is figured out in accordance to the branch lengths measured for the number of substitutions per site. The bootstrap consensus tree is inferred from 1000 repeats. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.4).

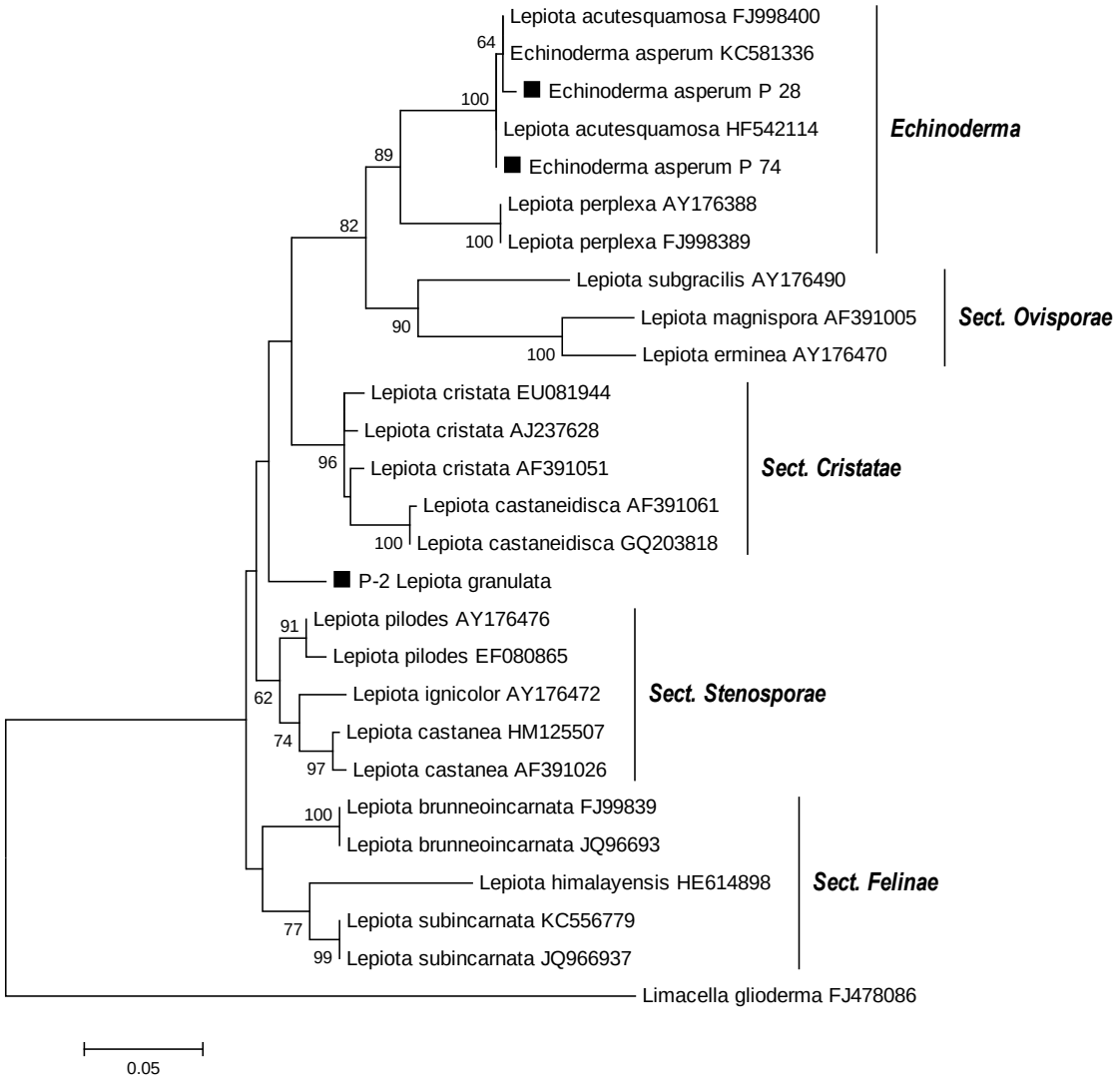


Figure – 3.4 Molecular phylogenetic analysis of ***Echinoderma asperum*** and ***Lepiota granulata*** by Maximum Likelihood method. The tree with the highest log likelihood (-2112.7842) is shown. Collections of Swat are represented by black cubes (■).

3. ***Leucocoprinus cretaceus*** **(Bull.) Locq.**, *Bull. mens. Soc. linn. Soc. Bot. Lyon* 14: 93 (1945) Plate 03 – Figs. A-E & 3.5

Morpho-Anatomical characterization

PILEUS: 4.5 – 5.5 cm in diameter, fleshy, hemispherical to companulate becoming plano convex to convex later with distinct umbo, fibrillose, white, thickly covered with concolorus floccose squamules, margins uplifted, plicate, incurved when immature. Context bright white. LAMELLAE: white, free, closed, thin with entire edges. LAMELLULAE: present, alternating with lamellae. STIPE: 7 – 9.8 × 2 – 15 mm, centrally attached, white context becoming cream at maturity, thin at apex becoming broad at base, covered with white squamules underneath annulus, smooth in the apical region, base bulbous. VOLVA: present. ANNULUS: white, thin, descending.

BASIDIOSPORES: 6.3 – 9.9 × 8.7 – 12.5 µm, [X = 8.5 × 11.24 µm, Q 1.27] moderately thick walled, transparent to pale in KOH, appiculate, guttulate, ellipsoid sub to globose, smooth, dextrinoid. BASIDIA: 15.4 – 30.6 × 10.9 – 13.7 µm, clavate, hyaline in KOH, thin walled. PLEUROCYSTIDIA: absent. CHEILOCYSTIDIA: 24.1 – 34.9 × 11.1 – 14.2 µm, hyaline in KOH, thin walled, smooth, broadly clavate to subclavate or ventricose, guttules present. PILEIPELLIS: 5.7 – 16.9 µm in diameter, laxolattice, thin walled, hyaline to pale in KOH, branched, irregular, clamps absent. STIPTIPELLIS: 5.9 – 11.8 µm in diameter, laxotrichoderm, hyaline to pale, irregular, thin walled, clamp connection present.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Matta, Jura, scattered to gregarious on soil in fruit orchards of *Prunus persica* (L.) Stokes 15 June, 2013, ISP # 290 (LAH).

COMMENTS: previously reported from Lahore Pakistan.

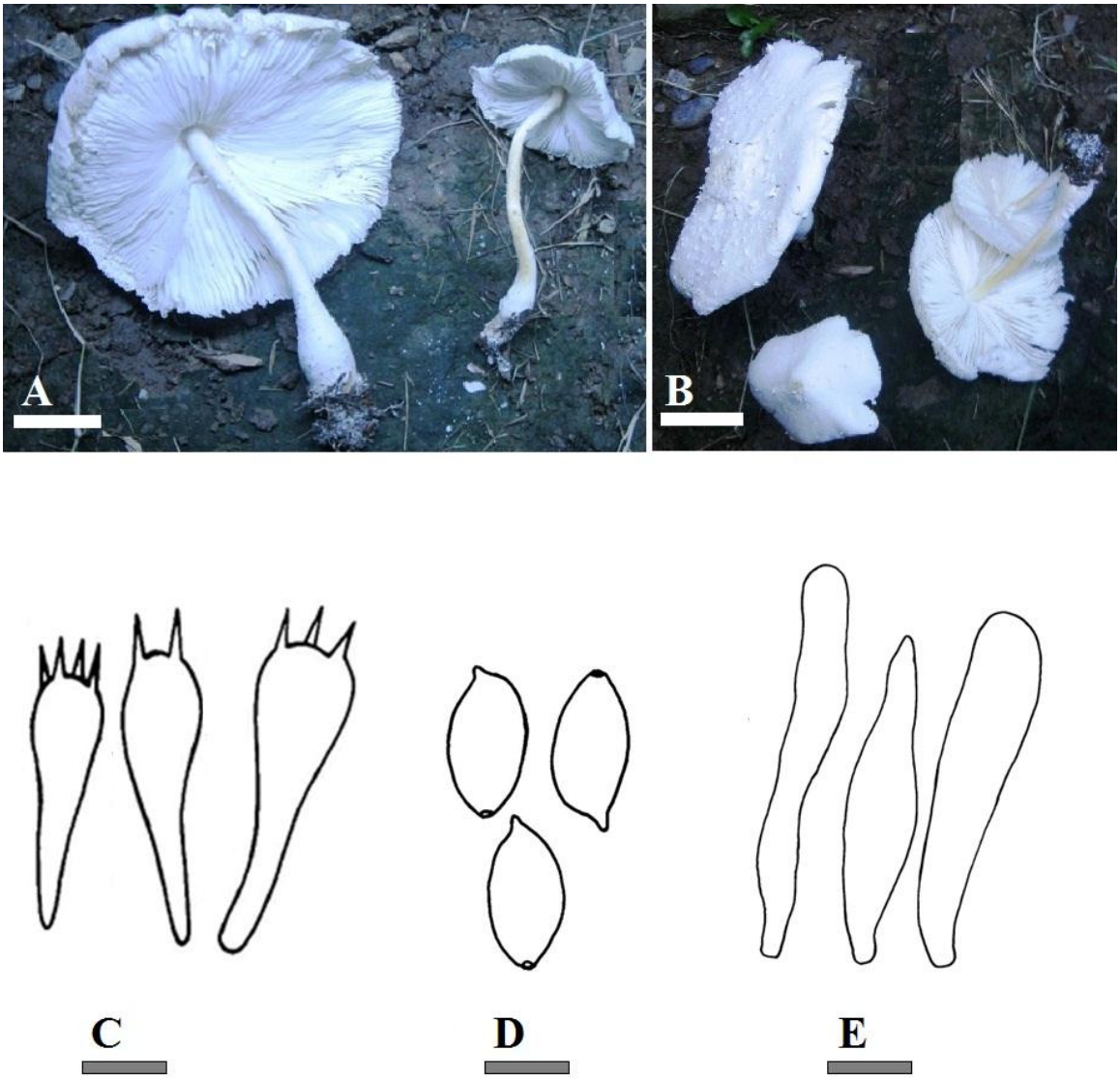
Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 617 base pairs. In initial BLAST search of GenBank database, the closest hit was *Leucocoprinus cretaceus* (GenBank JF899547; maximum identity 100%, Query coverage 99%) from USA.

The generated ITS sequence of this species along with those retrieved from GenBank based on the result from BLAST search and representative of other genera of Agaricaceae were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. The tree is figured out in accordance to the branch lengths calculated for the number of substitutions per site. Total characters in the analysis were 1476. Out of these 776 positions were included in the treatment after trimming the ambiguous ones. Among these 279 were conserved, 479 were variable, 341 were parsimony informative and 131 were singletons. The analysis included 34 nucleotide sequences. Those positions in which data was missing were not

taken into account. Final dataset had 246 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.5).

Plate 03



Figures – A-E Morpho-anatomical shapes of ***Leucocoprinus cretaceus*** (A-B) Basidiomata (C) Basidia (D) Basidiospores (E) Cheilocystidia. Scale bars: A = 3.5 cm, B = 1.5 cm, C = 7.5 **µm,** D = 6.6 **µm,** E = 8.8 **µm.**

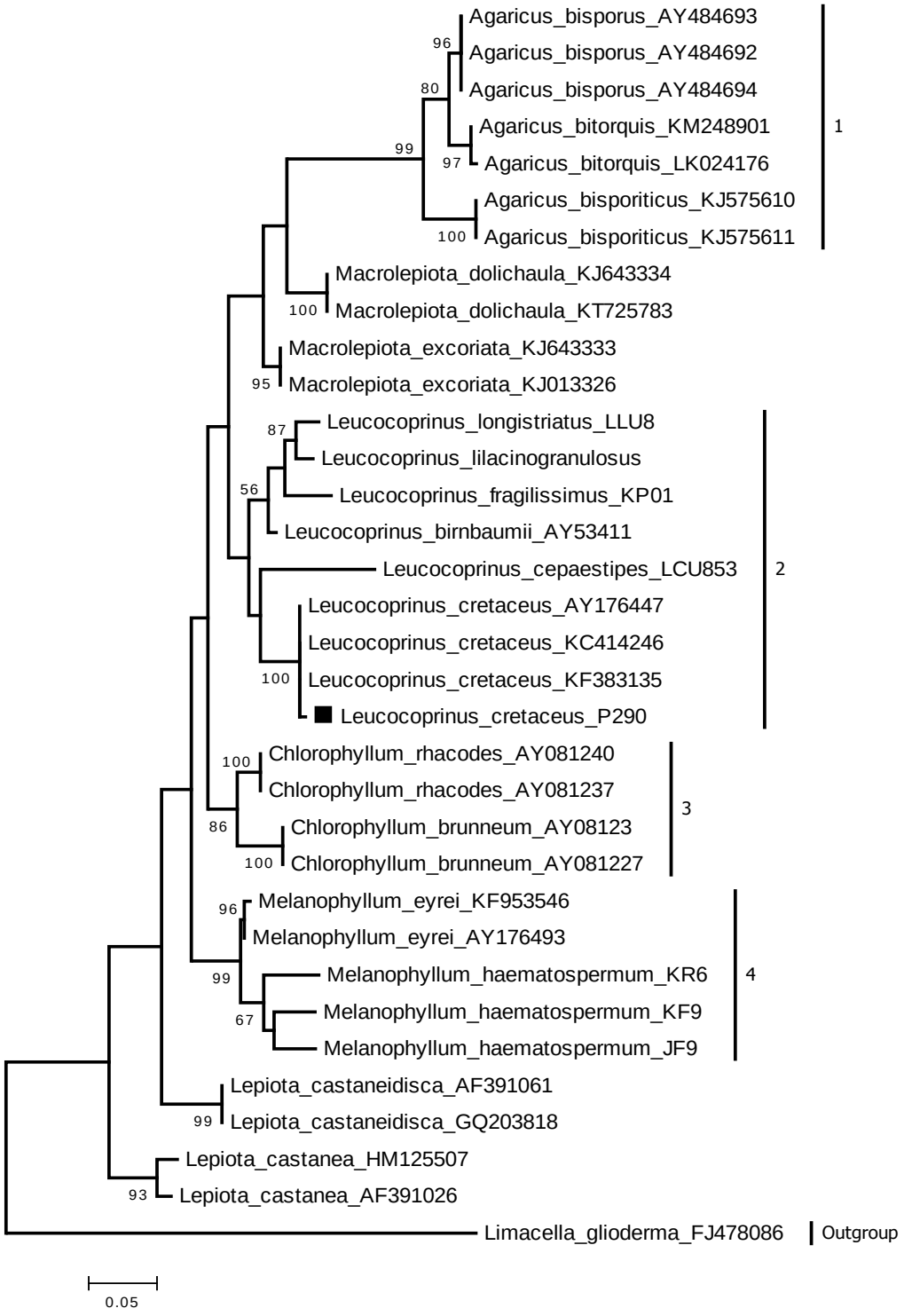


Figure – 3.5 Molecular Phylogenetic analyses of ***Leucocoprinus cretaceus*** by Maximum Likelihood method. Highest log likelihood = (-1743.7606). Collection of Swat is represented by black cube (■).

Amanitaceae

Amanita

4. *Amanita aurumana* nom. prov.
5. *Amanita flavipes*
6. *Amanita longiformis* nom. prov.
7. *Amanita superiora* nom. prov.

4. ***Amanita aurumana*** nom. prov. Plate 04 – Figs A-G & 3.6

Etymology: The specific epithet may be referred to the golden color of the cap of this species.

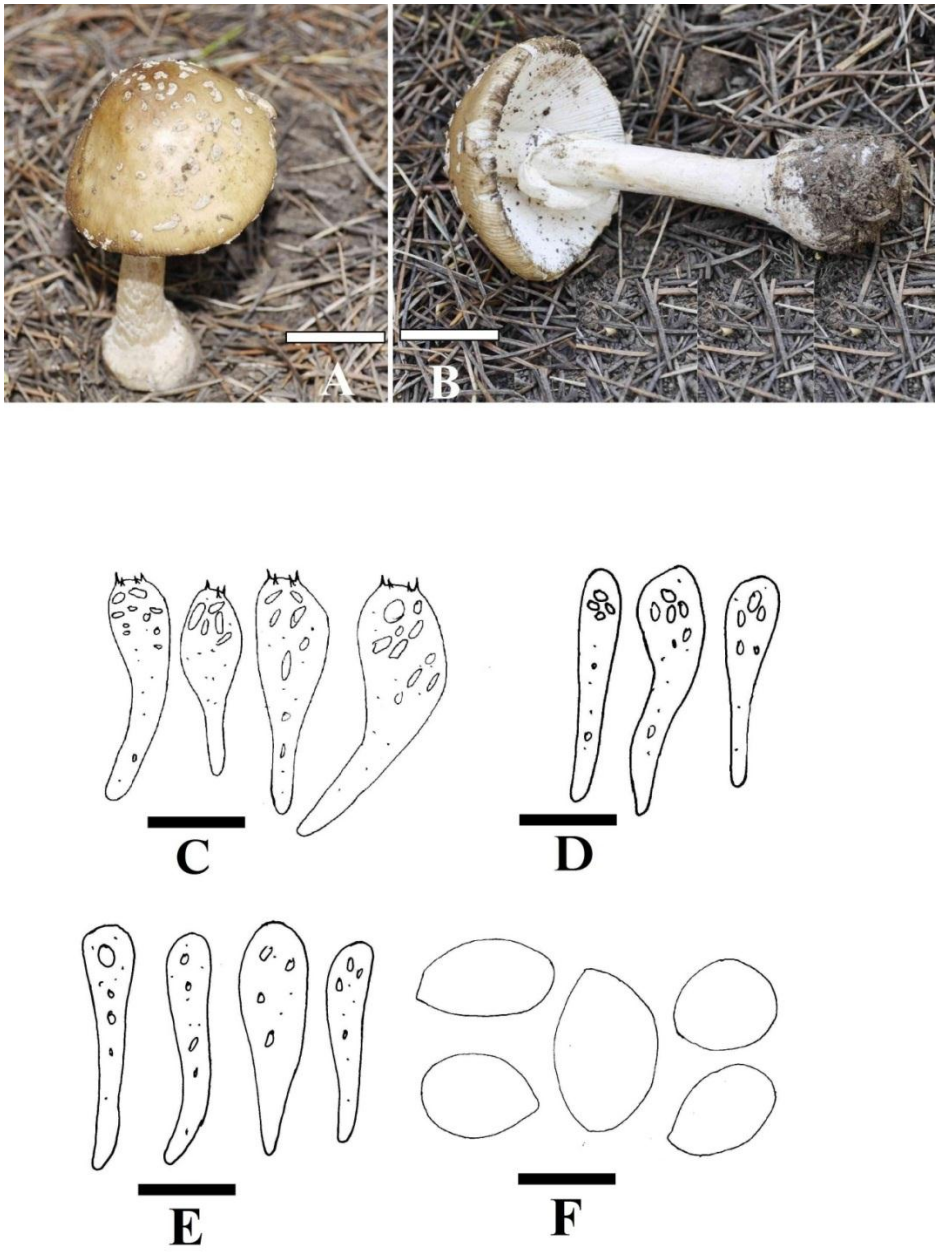
Morpho-Anatomical characterization

PILEUS: 4.5 – 5.5 cm across, pulvinate, warty, strong brown to golden brown (5YR 6/12 – 5YR 4/8); central disc obtuse; curvature deflexed; surface dry, glabrous with whitish scattered warts; flesh white; margin slightly inrolled and striate. LAMELLAE: free approximate, milky white, soft, crowded, even. STIPE**:** 8.5 – 10 × 0.5 – 1.5 cm, white, cylindrical, equal, smooth. ANNULS: milky white, superior, single edged. VOLVA: white, napiform.

BASIDIOSPORES: 9.5 – 15.5 × 6.0 – 13.0 µm [X = 12.6 × 8.7 µm, Q = 1.448], broadly ellipsoid to globose, apiculate, smooth, inamoyloid, yellow in KOH. BASIDIA: 32.5 – 40.5 × 10.5 – 14.5 µm, clavate, triporic to quadrisporic, thin-walled, guttiulate, hyaline in KOH. CHEILOCYSTIDIA: 38.5 – 60.0 × 10.5 – 14.0 µm, clavate, guttiulate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: 31.9 – 53.2 × 8.1 – 11.6 µm, clavate, guttiulate, thin-walled, hyaline in KOH.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Ushu, Kalam, solitary to gregarious on ground in dry temprate mixed forest of *Cedrus deodara* and *Quercus semicarpifolia* Smith 04 August, 2013, ISP # 050

Plate 04



Figures – A-G Morpho-anatomical shapes of ***Amanita aurumana*** (A-B) Basidiomata (C) Basidia (D) Pleurocystidia (E) Chielocystida (F) Basidiospores. Scale bars: A-B= 2.5 cm, C = 14 cm, D = 18 µm, E = 22µm, F = 9 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 645 base pairs. In initial BLAST search of GenBank database, the closest hits were *Amanita pantherina* (DC.) Krombh (GenBank JF899547 and EU909452; Query coverage 96 % with maximum identity 96 %,) of USA.

The generated ITS sequence of this species along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. The treatment contained 1379 position. 658 were selected for analysis after trimimg ambiguous letters. Among these 474 were conserved characters, 163 were variable, 76 were parsimony informative and 85 were singletons. Molecular phylogenetic analysis was done for 26 nucleotide sequences. Those positions in which data was missing were not taken into account. Final dataset had 468 positions in the concluding dataset. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.6).

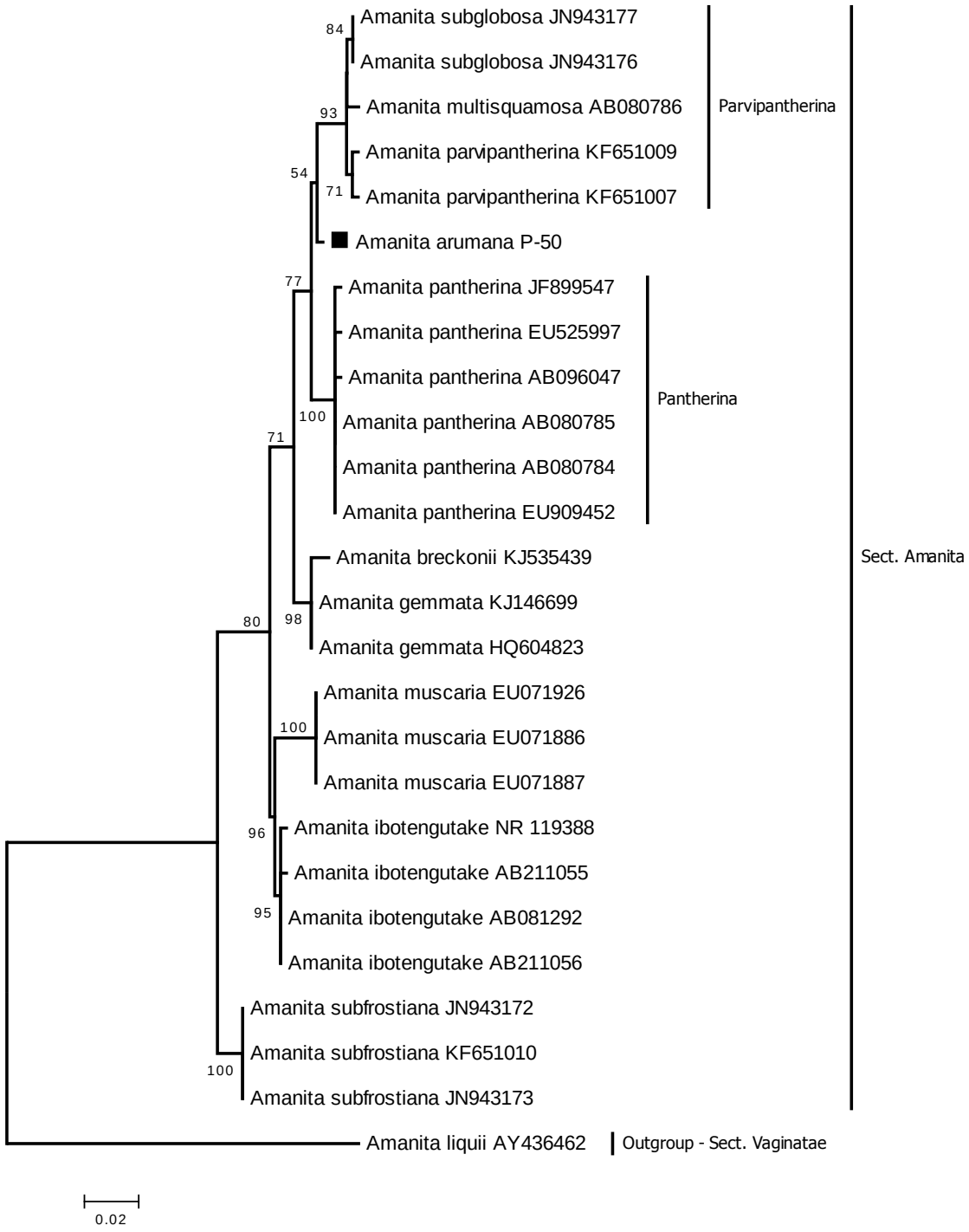


Figure – 3.6 Molecular phylogenetic analysis of ***Amanita aurumana*** by Maximum Likelihood method. Maximum log likelihood value = (-1314.5743) is shown. Black cube (■) represents collection of Swat.

5. ***Amanita flavipes*** **S. Imai,** *Bot. Mag.*, Tokyo 47: 428 (1933)

Plate 05 – Figs. A-E & 3.7

Morpho-Anatomical characterization

PILEUS: 1.5 – 4.0 cm across, pulvinate to ovate, warty, brilliant yellow to light brown (2.5Y 9/10); central disc obtuse; surface dry, glabrous with light yellow scattered warts margin pubescent, incurved to connivent. LAMELLAE: free and remote, light orange yellow to brilliant yellow (7.5YR 9/8 – 2.5Y 9/10), soft, close equal and curved. STIPE: 4.5 – 11 × 0.5 – 2 cm, milky white, cylindrical, slightly tapering toward apex, squamulose, squmules brilliant yellow (2.5Y 9/10). ANNULS: light yellow to pale yellow (7.5YR 9/8), superior to median. VOLVA: light yellow to pale yellow (7.5YR 9/8), napiform to sheathing when young.

BASIDIOSPORES: 7.8 – 11.1× 5.7 – 7.8 µm [X = 9.3 × 6.8 µm, Q = 1.367], broadly ellipsoid, apiculate, smooth, amoyloid, yellow in KOH. BASIDIA: 34.7 – 31.9 × 13.1 – 10.9 µm, clavate, bisporic to quadrisporic thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 43.2 – 26.0 × 13.7 – 7.6 µm, clavate, guttiulate thin walled, hyaline in KOH. PLEUROCYSTIDIA: 36.6 – 25.6 × 12.8 – 6.9 µm, clavate, guttiulate, thin-walled, hyaline in KOH.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Kalam, solitary to gregarious on ground in mix coniferous forest under *Pinus wallichiana*, *Cedrus deodara,* and *Abies pidrow* (Royle ex D.Don) Royle. 04 August, 2013, ISP # 049

COMMENTS: This species is previously reported by Murakami (1993) on the ground in *Pinus wallichiana-Quercus* dilatata forest at Muree and Shogran. This is first record of this species from Swat Pakistan.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 619 base pairs. In initial BLAST search of GenBank database, the closest hit was *Amanita flavipes* S. Imai, 1933 (GenBank KF245911; maximum identity 100%, Query coverage 99%) from South Korea.

Recently generated ITS sequence of the *Amanita flavipes* and other cloely related sequences retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Final dataset had 1236 charcters among which 710 were included in the analysis. Out of these 443 were conserved characters, 211 were variable, 94 were parsimony informative and 115 were singletons. Phylogenetic analysis was done for 22 closed taxa. 1st+2nd+3rd codon positions were included. Those positions in which data was missing were not taken into account. Final dataset had 533 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.7).

Plate 05

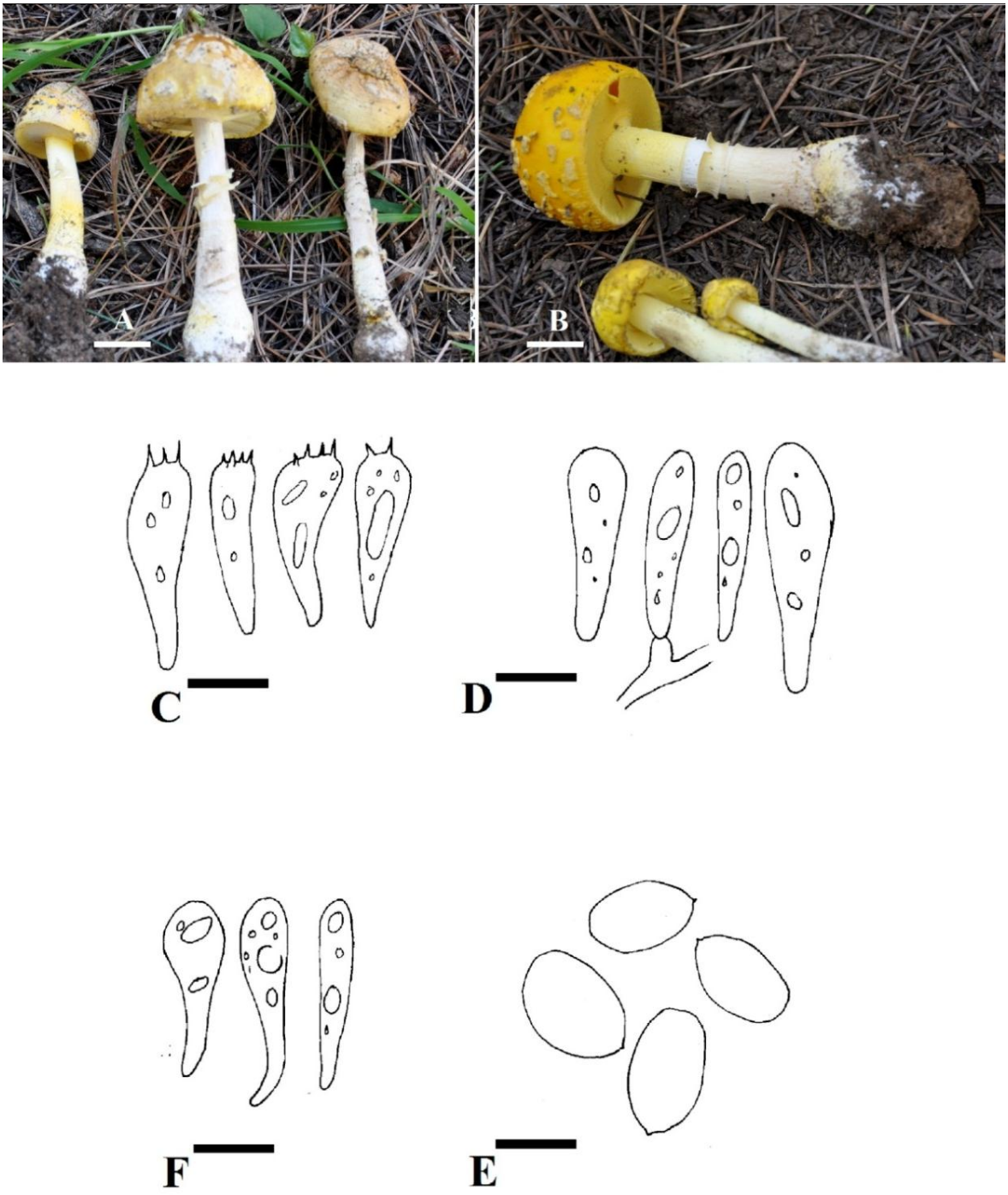


Figure A-E Morpho-anatomical shapes of ***Amanita flavipes*** (A-B) Basidiomata (C) Basidia (D) Pleurocystidia (E) Basidiospores (F) Chielocystida. Scale bars: A = 0.5 cm, B = 1 cm, C = 11 µm, D = 10 µm, E = 15 µm, F = 10 µm.

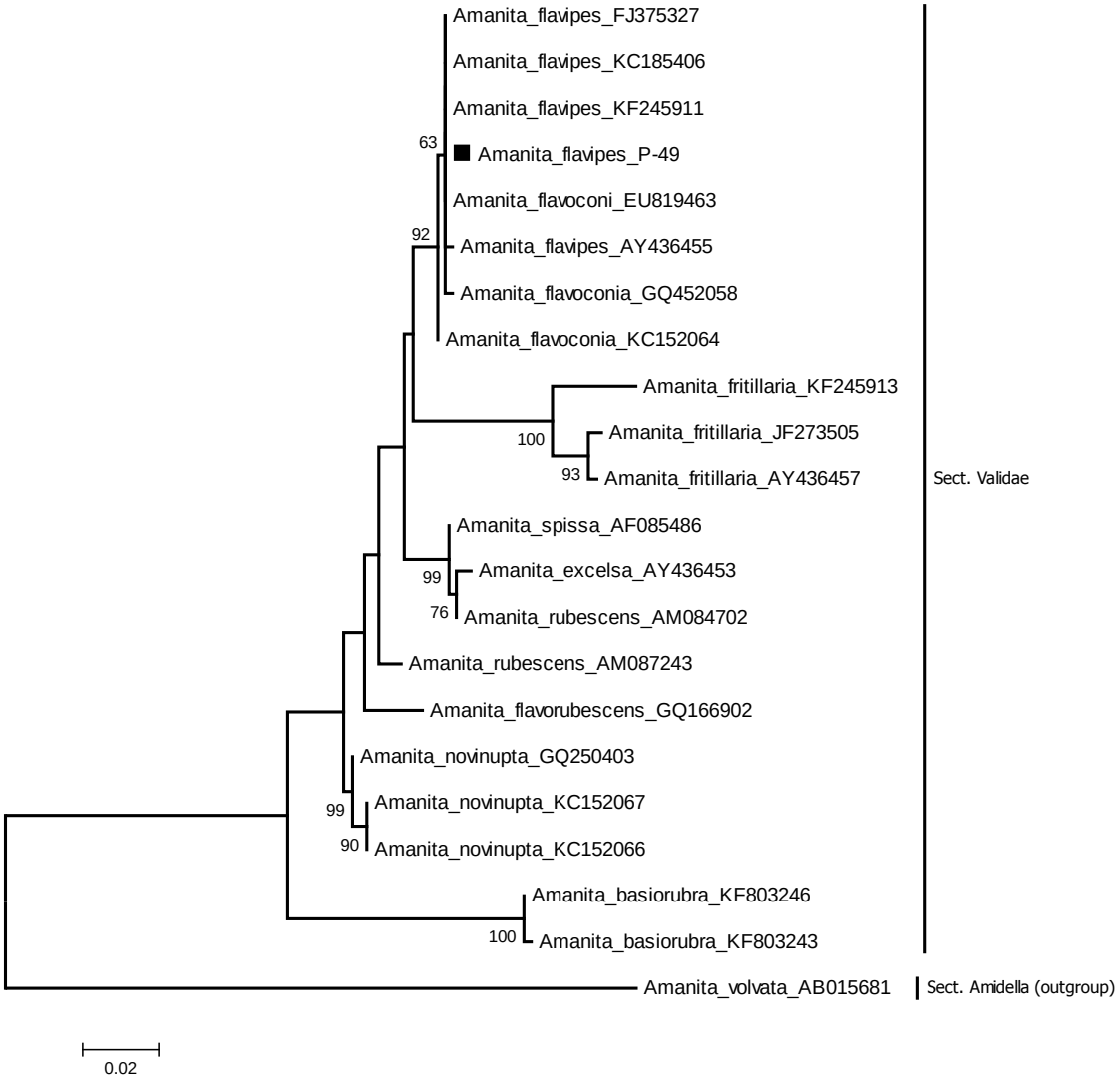


Figure 3.7 Molecular phylogenetic tree of ***Amanita flavipes*** by Maximum Likelihood method. Black cube (■) depicts the analyzed species. Highest log likelihood (-1758.0862) is shown.

6. ***Amanita longiformis*** nom. prov. Plate 06 – Figs. A-D & 3.8

Etymology: The specific epithet may be referred to the long stipe of this species.

Morpho-Anatomical characterization

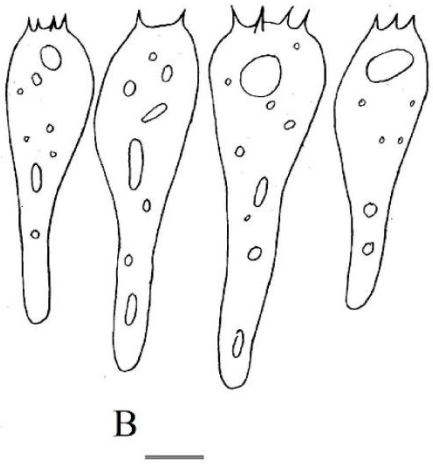
PILEUS: 1.9 – 2.2 cm across, pulvinate to ovate, smooth, moderate brown in center to light brown towards edges (7.5YR 3/4 – 7.5YR 5/4); central disc obtuse; curvature incurved; radially fibrillose, moist; margin plicate. LAMELLAE: free and remote, white, soft, equal and curved, close. STIPE: 9.5 – 9.9 × 0.8 – 1.0 cm, snow white, cylindrical, slightly tapering toward apex.

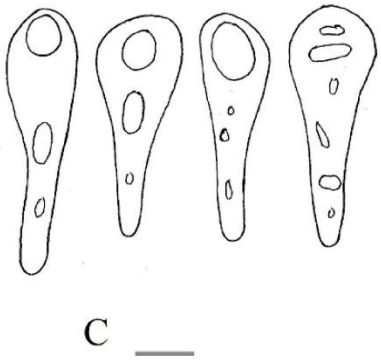
BASIDIOSPORES: 7.9 – 13.7 × 7.5 – 12.7 µm [X = 9.8 × 10.8 µm, Q = 1.102], spherical, apiculate, reticulated, hayline in KOH and phloxin, inamoyloid, blue in trypan blue. BASIDIA: 56.2 – 77.4 × 13.5 – 17.0 µm, clavate, bisporic, to trisporic, mostly quadrisporic thin-walled, hyaline in KOH. CHEILOCYSTIDIA: 51.4 – 75.5 × 14.6 – 22.2 µm, clavate, guttiulate, thin walled.

HABITAT AND DISTRIBUTION**:** PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Miandam, Solitary on ground in *Pinus wallichiana* and *Cedrus deodara* forest, 02 September, 2013; ISP # 116 (LAH).

Plate 06







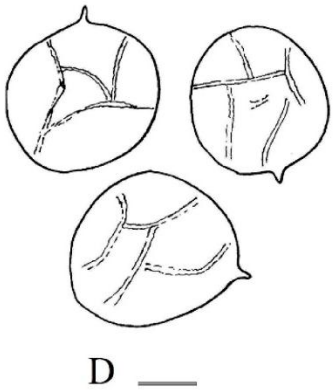


Figure A-D Morpho-anatomical shapes of ***Amanita longiformis*** (A) Basidiome (B) Basidia (C) Chielocystida (D) Basidiospores. Scale bars: A = 1.3 cm, B = 10 µm C = 14 µm, D = 4 µm,

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 745 base pairs. In initial BLAST search of GenBank database, the closest hit was *Amanita griseofolia* Zhu L. Yang 2004, (GenBank KF017936; maximum identity 93%, Query coverage 98%) from South Korea. Comparison of ITS sequence data obtained from the present *Amanita* species with the identical nucleotide sequences in GenBank approves the identity of our collection as sp. nov.

The generated ITS sequence of the new species, *Amanita longiformis* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Among these 180/705 were conserved characters, 416 were variable, 252 were parsimony informative and 161 were singletons. 38 nucleotide sequences were treated in the analysis. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 262 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.8).

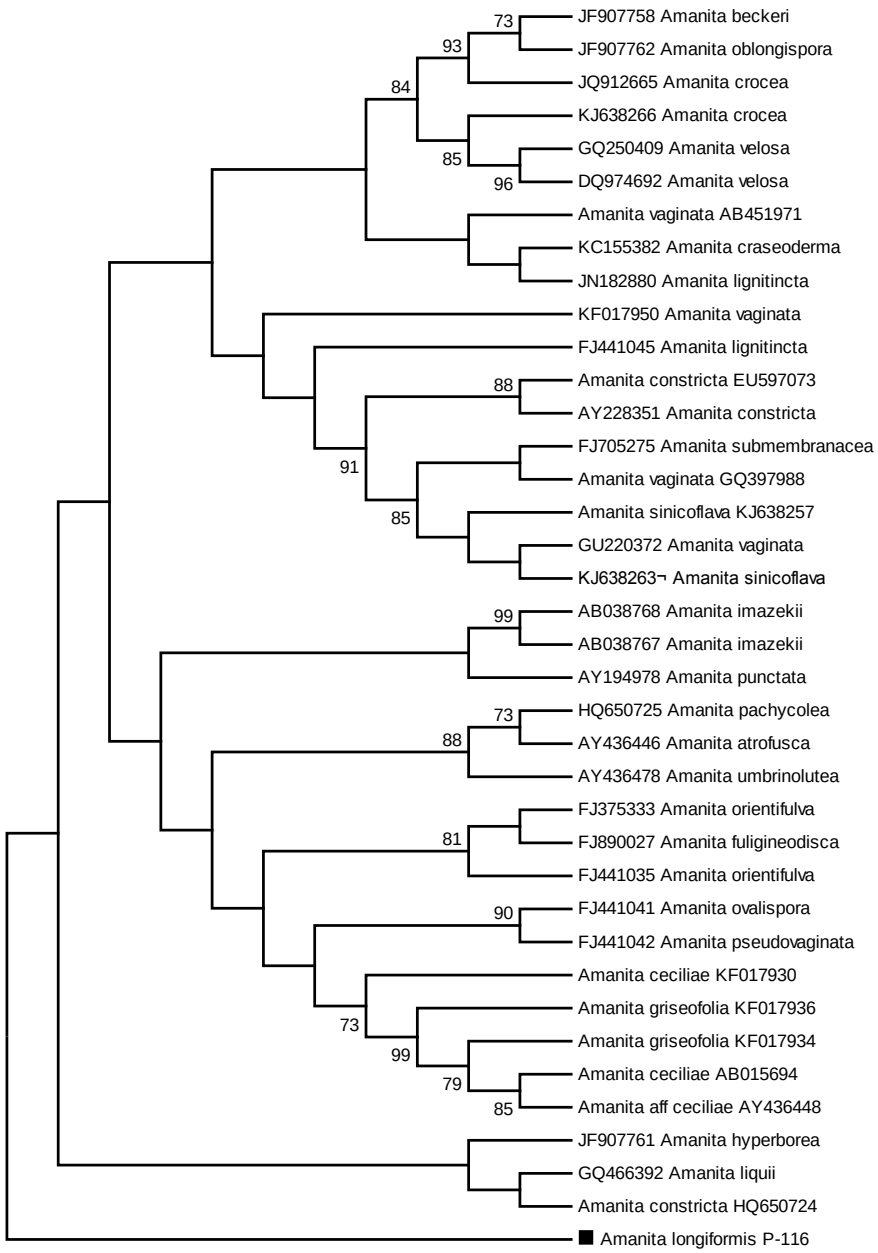


Figure 3.8 Molecular phylogenetic analysis of ***Amanita longiformis*** by Maximum Likelihood method. Maximum log likelihood value = (-1788.6457) is shown. Collection of Swat is represented by black cube (■).

7. ***Amanita superiora*** nom. prov. Plate 07– Figs A-G & 3.9

Etymology: The specific epithet may be referred to the habitat of this species which is hill tops.

Morpho-Anatomical characterization

PILEUS: 4.5 – 5.5 cm across, Plano convex, warty, light olive brown (2.5Y 5/2); central disc umbonate; curvature deflexed to straight and flaring; surface, dry, glabrous with dark olive (2.5Y 2/2) scattered warts; margin cottony, white. LAMELLAE: free approximate, white, crowded, curved to even. STIPE: 5.5 – 8 × 0.5 – 2 cm, whitish olive brown (10YR 5/2), cylindrical, tapering to the apex, smooth, bulbous. ANNULS: white, median to superior. VOLVA: whitish olive brown (10YR 5/2), napiform.

BASIDIOSPORES: 4.5 – 9.8 × 4.4 – 8.9 µm [X = 7.1 × 7.8 µm, Q = 1.098], globose to hemispheric to ovate, apiculate, smooth, yellow in KOH. BASIDIA: 34.2 – 50.3 × 9.4 – 12.6 µm, clavate, predominantly bisporic, thin-walled, guttiulate, hyaline in KOH. CHEILOCYSTIDIA: 37.1 – 66.4 × 16.4 – 24.4 µm, clavate, guttiulate, thin walled hyaline in KOH. PLEUROCYSTIDIA: 21.5 – 40.9 × 5.2 – 14.6 µm, clavate, guttiulate, thin-walled, hyaline in KOH. PARACYSTIDIA: 21.8 – 34.2 × 8 – 14.5 µm, clavate, guttiulate, thin-walled, hyaline in KOH. CAULOCYSTIDIA: 86.4 – 234.4 × 16.9 – 43.4, clavate, thin-walled, hyaline in KOH. PILEIPELLIS: hyphae cylindrical, cylindrical 2.8 – 9.2 µm, thin-walled, hyaline in KOH, red in Phloxin. STIPE HYPHAE: cylindrical and parallel 1.9 – 13.7 µm, hyaline yellow in KOH, CLAMP CONNECTIONS: present.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Mash koon, Mankial, solitary to gregarious on ground in dry temprate coniferous forest under, *Cedrus deodara,* and *Abies pidrow*. 04 August, 2013, ISP # 06

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 745 base pairs. In initial BLAST search of GenBank database, the closest hit was *Amanita franchetii* (Boud.) Fayod 1889 (GenBank JX515563; maximum identity 95%, Query coverage 100%) from Spain. Comparison of ITS sequence data obtained from the present Amanita species with the identical nucleotide sequences in GenBank approves the identity of our collection as sp. nov. The generated ITS sequence of the *Amanita superiora* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Among these 448/748 were conserved characters, 274 were variable, 191 were parsimony informative and 82 were singletons. The analysis was carried out for 45 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 442 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.9).

Plate 07



Figures A-G Morpho-anatomical shapes of ***Amanita superiora*** (A) Basidiomata (B) Pleurocystidia (C) Chielocystida (D) Caulocystidia (E) Basidiospores (F) Stipe hyphae (G) Pileipellis. Scale bars: A = 1 cm, B = 10 µm, C = 16 µm, D = 54, µm E = 8µm, F = 40 µm, G = 25 µm

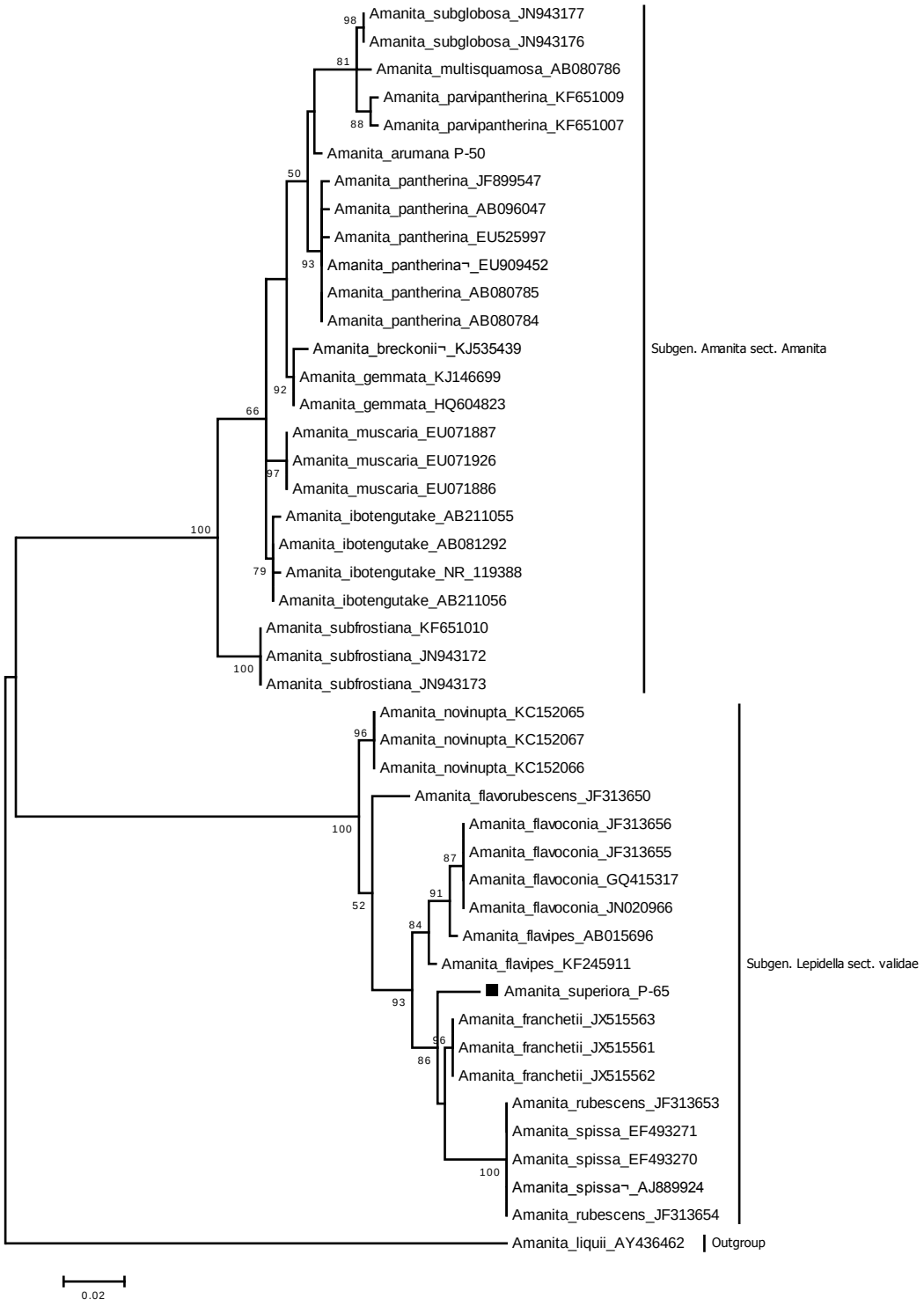


Figure 3.9 Molecular phylogenetic analysis of ***Amanita superiora*** by Maximum Likelihood method. Maximum log likelihood value = (-1737.5247) is shown. Black cube (■) represents collection of Swat.

Auriscalpiadaceae

Lentinellus

8. *Lentinellus horridus* nom. prov.

8. ***Lentinellus horridus*** nom. prov. Plates 08 & 09 – Figs. 3.5.1.1 – 3.10

Etymology: The specific epithet may be referred to the rough and grimy cap cuticle of this species.

Morpho-Anatomical characterization

PILEUS: 3.0 – 4.0 cm diam, depressed, umbillicate, light yellowish pink to pale yellowish pink (5YR 9/4 – 7.5YR 9/2); apressed fibrillose, dry velvety surface; curvature flaring; margins rimose to stigose; LAMELLAE: aruate to decurrent, close, thin, white; margins ragged eroded; STIPE: 3.0 – 3.4 × 0.3 – 0.6 cm, centrally to attached (omphaloid), pliable and soft, hollow, light yellowish brown (7.5YR 7/4); surface smooth, minutely pubescent.

BASIDIOSPORES: 3.2 – 6.4 × 3.6 – 5.4 µm [X = 4.5 × 4.9 µm, Q = 1.088], rounded to hemiglobose, apiculus present, warty, thin-walled, hyaline in KOH, amyloid, densly ornamented with smaller aculiae. BASIDIA: 5.4 – 22.9 × 4.0 – 7.8 µm, clavate, one to four spored basidia, thin-walled, hyaline in KOH; TRAMAL HYPHAE: 2.0 – 5.9 µm, thin walled, hyaline in KOH. CYSTIDIA: 16.8 – 32.9 × 5.9 – 12.1 µm, clavate, thin walled, hyaline in KOH. CLAMP CONNECTIONS: present in all tissues.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, Solitary on rotton logs in *Abies pindrow* forest, 03 September, 2013; ISP # 039 (LAH).

Plate 08



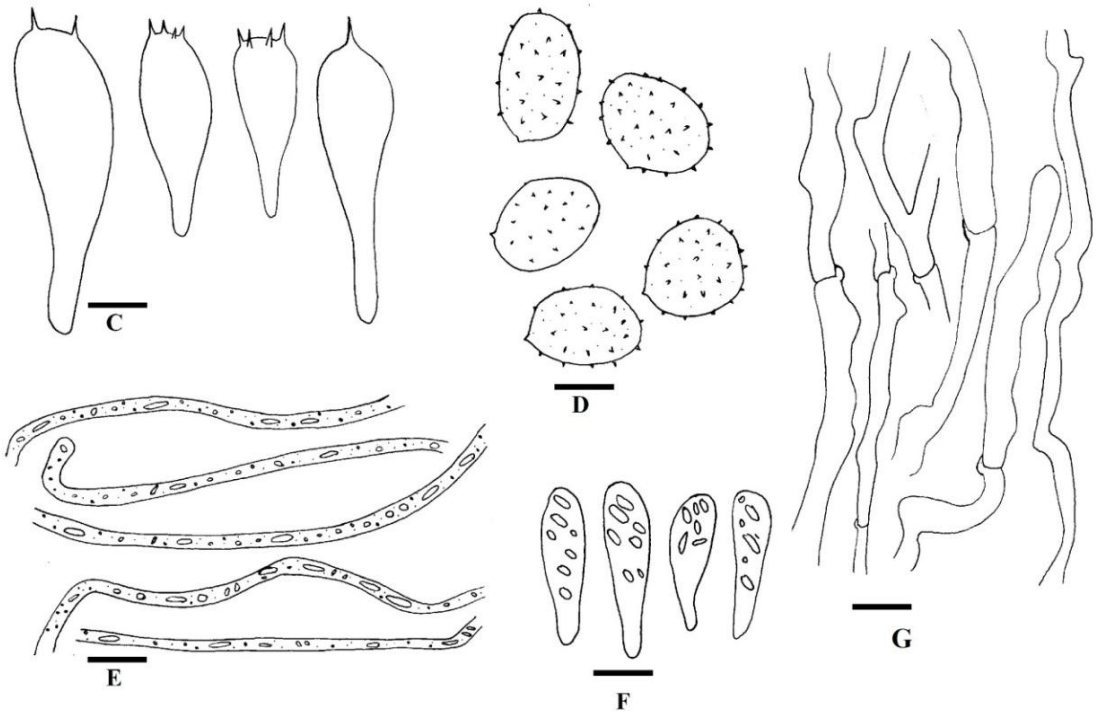
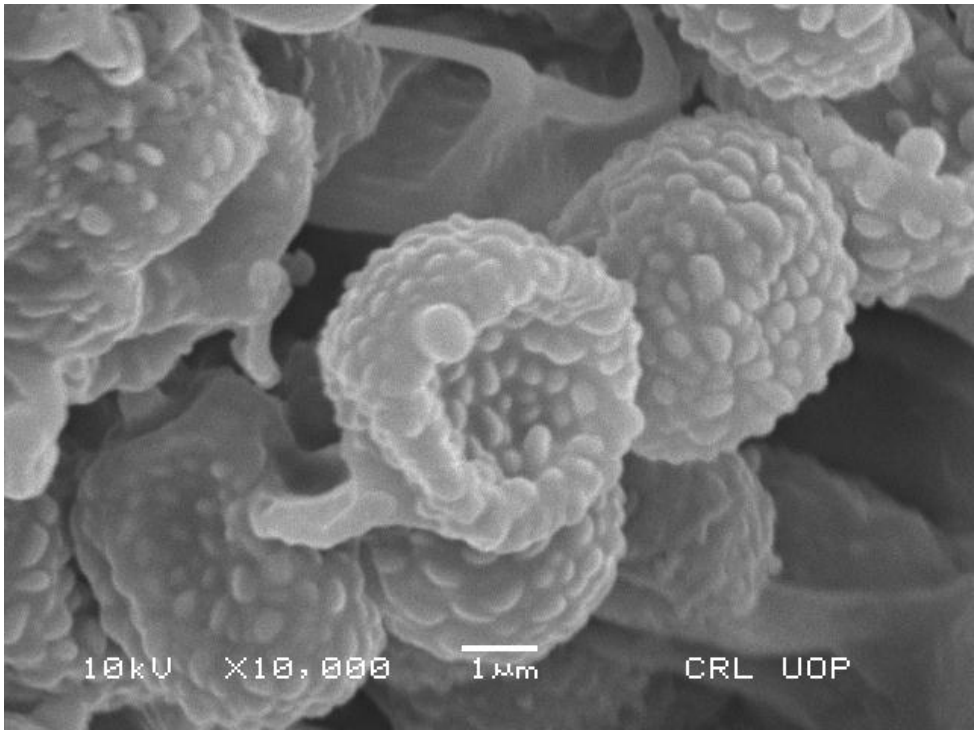


Figure (A-G) Morpho-anatomical shapes of ***Lentinellus horridus*** (A-B) Basidiomata; (C) Basidia (D) Basidiospores ; (E) Tramal hyphae; (F) Cystidia; (G) Tramal hyphae. Scale bars: A-B = 0.5 cm, C = 4 µm, D = 3 µm, E = 17 µm, F - G = 10 µm.

Plate 09



Scanning electron micrograph of basidiospores of ***Lentinellus horridus***.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 575 base pairs. In initial BLAST search of GenBank database, the closest hit was *Lentinellus cystidiosus* Petersen 2004 (GenBank AY513141; maximum identity 96%, Query coverage 100%) from France followed by *Lentinellus montanus* O.K. Mill. 1965 (GenBank AY513149; maximum identity 100%, Query coverage 95%) from USA and *Lentinellus flabelliformis* (Bolton) S. Ito 1959 (GenBank AY513164; maximum identity 100%, Query coverage 95%) from France.

The newly generated ITS sequence of the *L. horridus* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Initial tre(s) for the heuristic search were obtained automatically by applying Bio NJ & Neighbor-Join data algorithms to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Represention of the evolutionary history of the taxa analyzed was shown by inferring the bootstrap consensus tree inferred from 1000 replicates is shown. Final dataset had 736 characters exclusive. Among these 497/678 were conserved characters, 141 were variable, 63 were parsimony informative and 75 were singletons. The analysis was carried out for 22 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 526

positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.10).

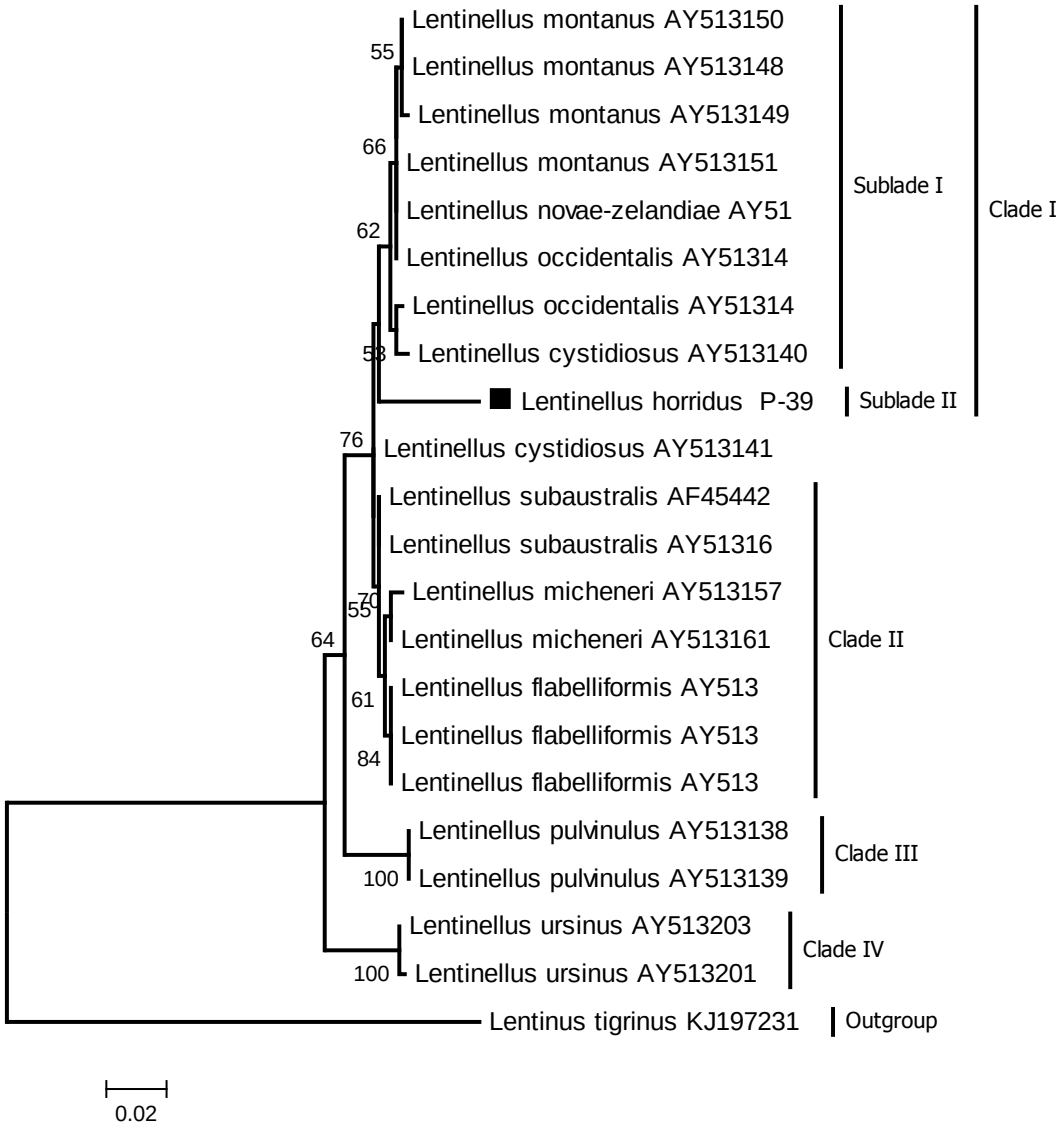


Figure 3.10 Molecular phylogenetic analysis of ***Lentinellus horridus*** by Maximum Likelihood method. Maximum log likelihood value = (-1526.2685) is shown. Collection of Swat is represented by black cube (■).

Bolbitiaceae

Conocybe

9. *Conocybe semiglobata* var. *campanulata*

9. ***Conocybe semiglobata*** **var.** ***campanulata*** **Hauskn** *Fungi europ.* (Alassio) 11: 167 (2009) Plate 10 – Figs.A-D & 3.11

Morpho-Anatomical characterization

PILEUS: 1.5 cm diam, conic to ovate with cuspidate central disc, pale orang yellow to strong yellowish brown (10YR8/4 -- 10YR 5/6); velvety, apressed fibrillose; curvature deflexed; margins plicate, pubiscent, LAMELLAE: free, distant, thin, strong yellowish brown (10YR 4/8); margin even; STIPE: 9.2 × 2 – 3 cm, centrally attached, soft, hollow, even and deep yellowish brown (10YR5/6); surface smooth.

BASIDIOSPORES: 7.4 – 9.4 × 10.7 – 14.0 µm [x = 12.6 × 8.4 µm, Q = 1.5], broadly ellipsoid, thick-walled, reddish olive in KOH, BASIDIA: 18.2 – 25.5 × 12.5 – 14.9 µm, clavate, four spored basidia, thin-walled, hyaline in KOH, STIPE HYPHAE: cylindrical, 4.8 – 14.6 µm, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, Solitary on ground in *Cedrus deodara* forest, 03 September, 2013; ISP # 044 (LAH).

COMMENTS: This Species is previously reported from Pakistan by Ahmad (1980b) on ground from Ladhar, Sheikhupura. This study reports new record of this species for Swat.

Plate 10

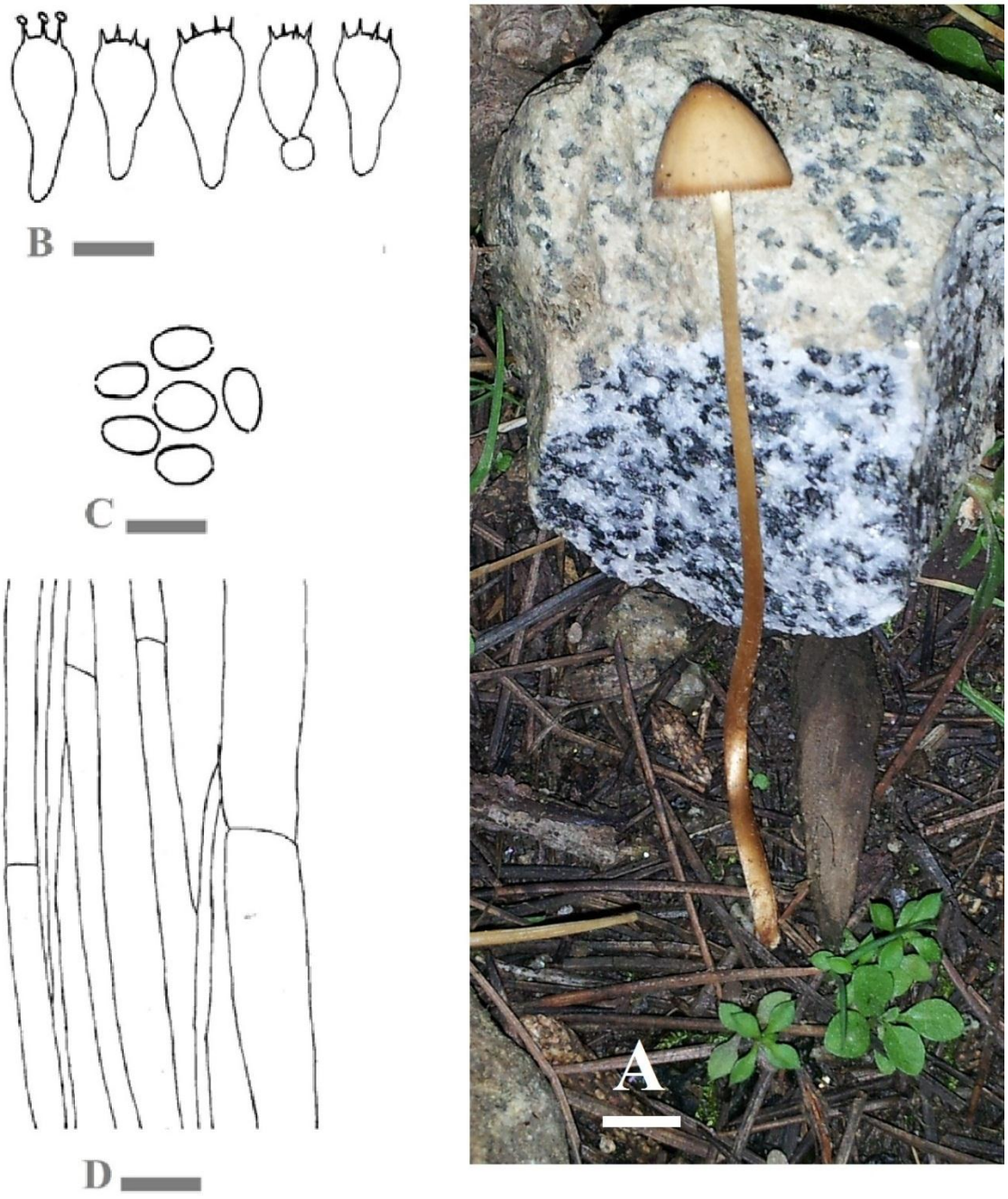


Figure A-D Morpho-anatomical shapes of ***Conocybe semiglobata*** var. *campanulata (*A) Basidiome (B) Basidia (C) Basidiospores (D) Stipe hyphae. Scale bar: A = 0.8 cm, B = 11 µm, C = 18 µm, D = 18 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 575 base pairs. In initial BLAST search of GenBank database, the closest hit was *Conocybe semiglobata* var. *campanulata* Hauskn (GenBank JX968169; maximum identity and Query coverage 100%) from Hungary, followed by *Conocybe subpubescens* (GenBank JX968169) maximum identity and Query coverage 100%, from Hungary.

The newly generated ITS sequence of the *Conocybe semiglobata* var. campanulata along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary history of the taxa was analyzed by selecting the bootstrap consensus tree inferred from 1000 replicates is taken. Among these 418/660 were conserved characters, 234 were variable, 145 were parsimony informative and 86 were singletons. The analysis was carried out for 20 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 555 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.11).

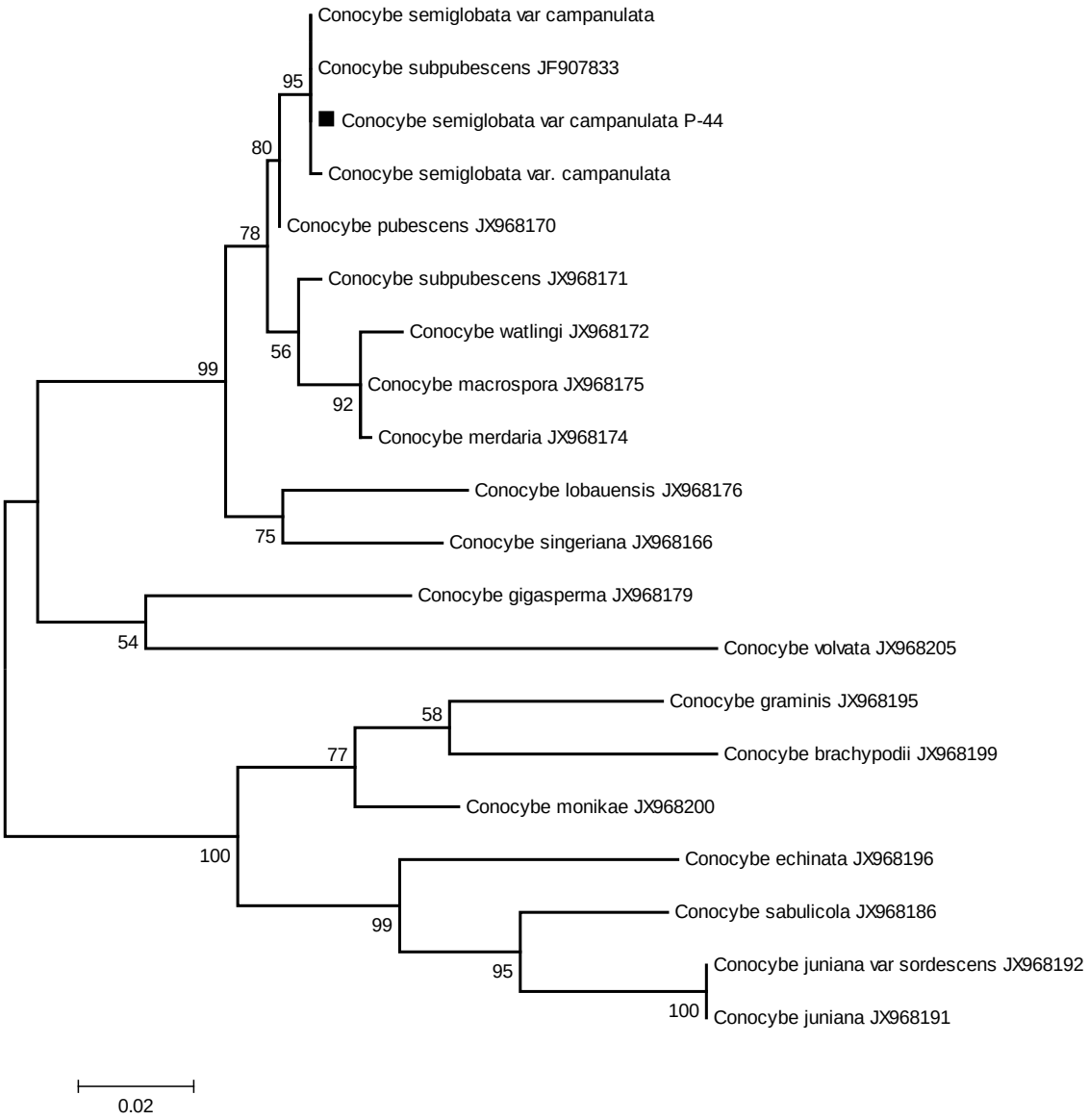


Figure 3.11 Molecular phylogenetic analysis of ***Conocybe semiglobata*** var. *campanulata* by Maximum Likelihood method. Maximum log likelihood value = (-2559.3403) is shown. Collection of Swat is represented by black cube (■).

Boletaceae

Boletus

10. *Boletus reticuloceps*

Xerocomellus

11. *Xerocomellus fulvus* nom. prov.

10. *Boletus reticuloceps* (Zang, Yuan & Gong) Wang & Yao. *Sydowia* 57(1): 132 2005. Plate 11 – Figs. A-H & 3.12

Morpho-Anatomical characterization

PILEUS: 4.0 – 8.5 cm across, depressed when young becoming convex to hemispheric, moderate orange to dark reddish orange (5YR 6/8 – 10R 6/12 – 10R 4/10); shiny, dry and aerolate glabrous; central disc obtuse; curvature faring to deflexed; context color milky white to snow white; margin cottony. TUBEPORES: free and remote, soft, upto 1.5 cm long, crowded, margins angular, light greenish yellow (7.5Y 9/6) to vivid greenish yellow (7.5Y 9/8) to light orange yellow (10YR 9/8). STIPE: 4.0 – 9.0 × 1.5 – 2.5 cm, net stipe (pseudo reticulation), ventricose, smooth, cylindrical, solid, reticulate, white with light to vivid yellow (2.5Y 8/8 – 2.5Y9/2 – 5Y 9/6) patches in the upper region.

BASIDIOSPORES: 4.7 – 9.3 × 14.2 – 19.6 µm [X = 5.4 × 16.5 µm, Q = 3.51], narrowly ellipsoid to oblong, apiculate, smooth, thin walled, hyaline in KOH. BASIDIA:, 12.1 – 19.2 × 29.3 – 41.5 µm, clavate, bisterigmate to tristerigmate, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 11.9 – 18.0 × 26.7 – 42.1 µm, clavate, guttiulate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: same to cheilocystidia. PILEIPELLIS: a cutis to hymeniform, cylindrical, 8.5 – 18.9 µm in diameter, thin-walled, hyaline in KOH. STIPTIPELLIS: hymeniform to laxolattice, cylindrical, 5.7 – 15.6 µm, hyaline in KOH. CLAMP CONNECTIONS: present.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Mashkoon Mankial, Solitary to gregarious on ground in *Cedrus deodara* forest, 05 September, 2013; ISP # 080 (LAH).

COMMENTS: previously reported from Pakistan.

Plate 11

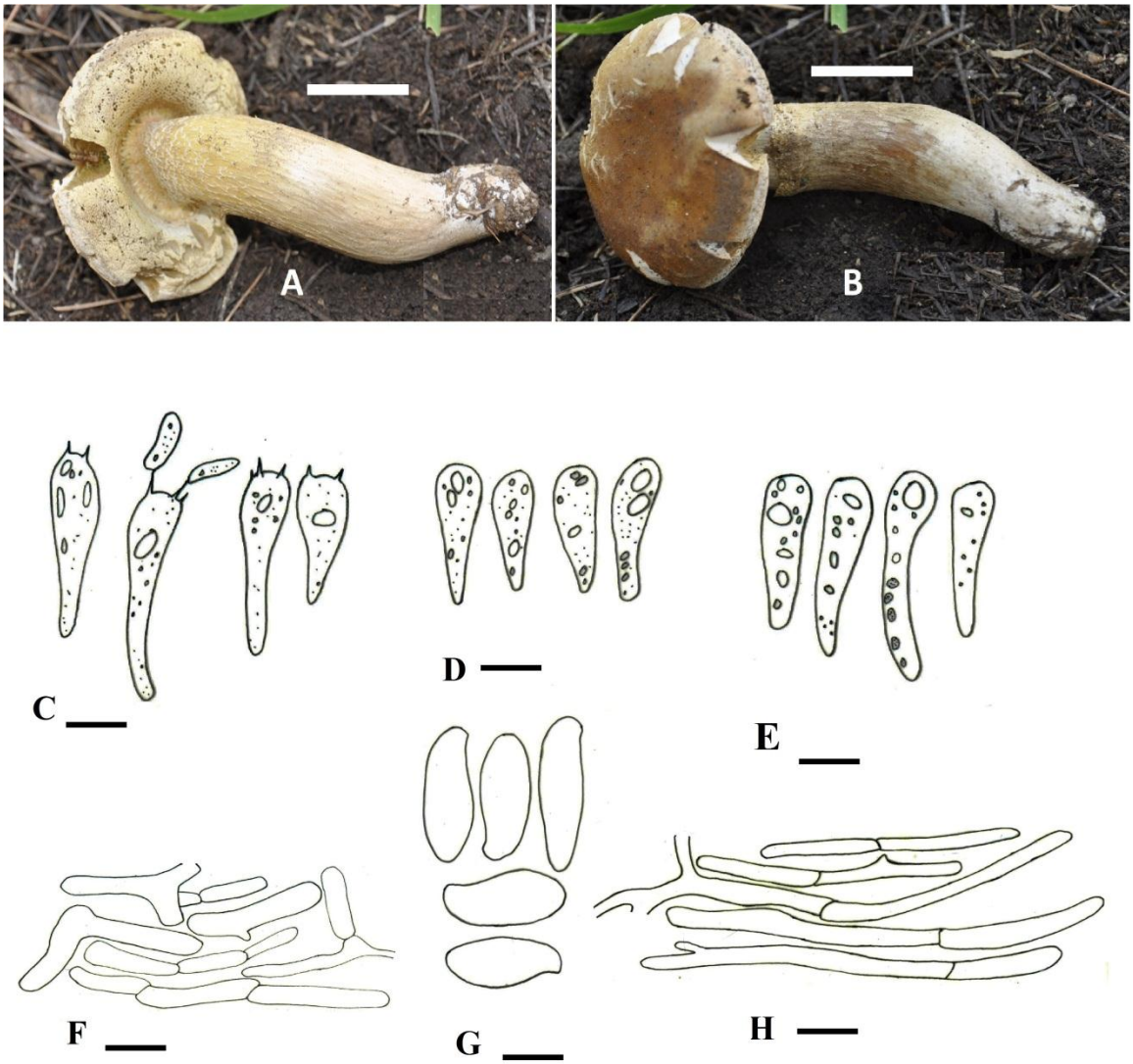


Figure (A-H) Morpho-anatomical shapes of ***Boletus reticuloceps*** (A-B) Basidiomata (C) Basidia (D) Pleurocystidia (E) Chielocystida (F) Pileipellis (G) Basidiospores (H) Stiptipileus; Scale bars: A = 2.3 cm, B = 1.6 cm, C = 11µm, D = 15 µm E = 12 µm, F = 41 µm G = 7 µm, H =18 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated sequences of 681-698 base pairs. In initial BLAST search of GenBank database, the closest hit was *Boletus reticuloceps* (GenBank KJ131225; maximum identity 99%, Query coverage 99%).

The newly generated ITS sequence of the *Boletus reticuloceps* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 497/1026 were conserved characters, 438 were variable, 335 were parsimony informative and 100 were singletons. The analysis was carried out for 25 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 378 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.12).



Figure 3.12 Molecular phylogenetic analysis of ***Boletus reticuloceps*** by Maximum Likelihood method. Maximum log likelihood value = (-1208.5071) is shown. Collection of Swat treated in present work is represented by black cube (■).

11. ***Xerocomellus fulvus*** Sarwar, I. Ahmad & Khalid, in Hernandez-Restrepo *et al.* Sydowia 68: 222 (2016) Plate 12 & 13 – Figs. A-H, A-H & 3.13

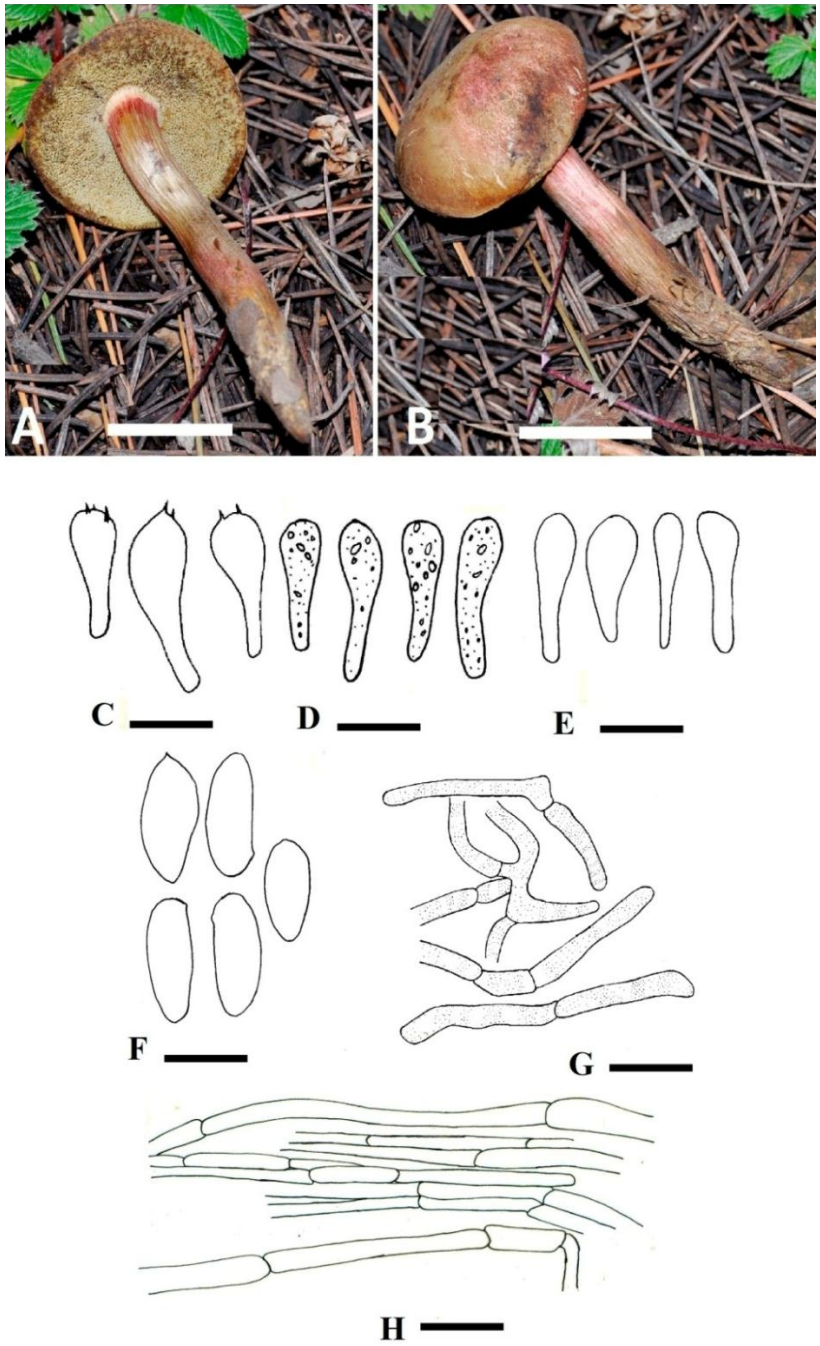
Etymology: The specific epithet may be referred to to the reddish brown color of basidiomata of this species.

PILEUS: 2.0 - 3.1 cm across, convex to hemispheric, deep pink (2.5R 6/14) to strong yellowish brown (10YR 5/8). CAP**:** dry and aerolate, central disc obtuse with deflexed curvature. CAP MARGIN: cottony. TUBEPORES: adnexed, soft, light greenish yellow (7.5Y 9/6) to vivid greenish yellow (7.5Y 9/8). STIPE: 5.0 – 6.3 × 1.5 – 1.7 cm, net stipe (pseudo reticulation), equal or slightly tapering toward base, smooth and cylindrical, light greyish red (2.5R 6/8) from top to center and dark yellowish brown (10YR 2/4) from center to base.

BASIDIOSPORES: 6.4 – 8.3 x 13.7 – 17.2 µm [x = 7.0 × 15.5 µm, Q = 2.214], ellipsoid to oblong, apiculate, smooth, yellow to green olive in KOH, amyloid. BASIDIA:, 13.0 – 15.3 × 37.5 – 39.6 µm, clavate, two to three spored basidia, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 11.6 – 13.5 x 29.2 – 36.6 µm, clavate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: 10.8 – 12.5 x 31.1 – 42.2 µm, clavate, guttiulate, thin-walled, hyaline in KOH. PILEIPELLIS: a cutis cylindrical, 8.9 – 13.2 µm in diameter, thin-walled, hyaline in KOH. STIPE HYPHAE: cylindrical, 11.0 – 12.5 µm, hyaline in KOH. CLAMP CONNECTIONS: not observed.

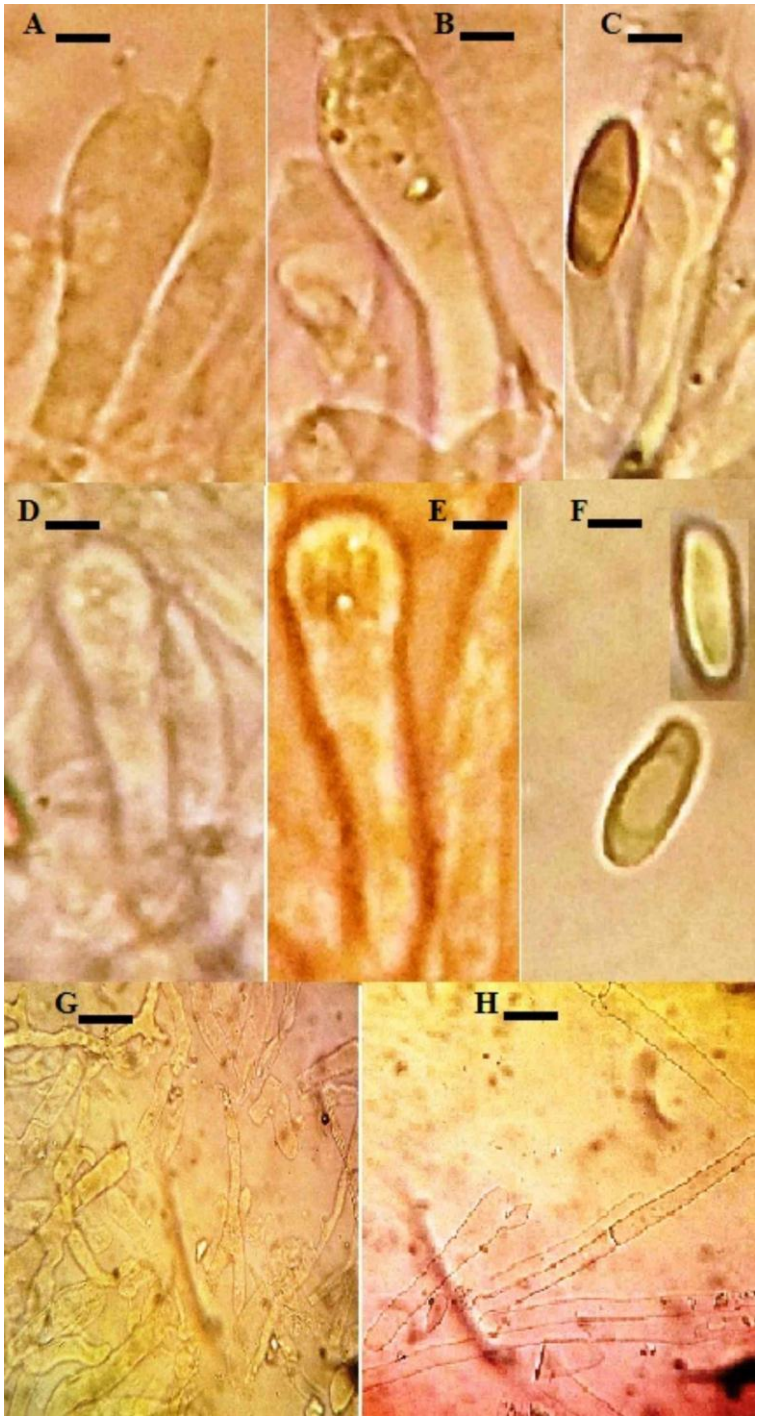
MATERIALEXAMINED: KHYBER PAKHTUNKHWA, Swat, Ushu Kalam, on soil in thick forest of *Cedrus deodara* (Roxb.) G.Don. 04 September, 2013, ISP # 060(LAH)

Plate 12



Figures A-H Morpho-anatomical shapes of ***Xerocomellus fulvus*** nom. prov.(A-B)Sporocarps(C) Basidia (D) Chielocystida (E) Pleurocystidia (F) Basidiospores (G) Pileipellis; (H) Stiptipileus; Scale bars: A-B = 1.8 cm C = 17 µm, D = 18 µm E = 22 µm, F = 11 µm (G) = 44 µm (H) = 34 µm

Plate 13



Figures A-H Morpho-anatomical shapes of ***Xerocomellus fulvus*** nom. prov. A & B Basidia, C Basidum and Basidiospore, D Cheilocystidia; E Pleurocystidia; F Basidiospores; G Pilepellius; H Stiptipellius. Scale Bar A, B, C, & F = 6 µm; D = 5 µm; E = 4 µm; G & H = 22 µm.

Molecular Phylogenetic Analysis

nrDNA sequence copmrising of internal transcibed spacers (ITS1 and ITS2) and 5.8S yielded 604 bp on amplification in polymerase chian reaction (PCR). BLAST execution of nucleotide sequences revealed that *Xerocomellus fulvus* showed highest similarity with *Boletus rubellus* Krombh. (EU819460) maximum identity 94%.

The newly generated ITS sequence of the *Xerocomellus fulvus* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 598/1844 were conserved characters, 948 were variable, 791 were parsimony informative and 70 were singletons. The analysis was carried out for 27 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 345 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.13).

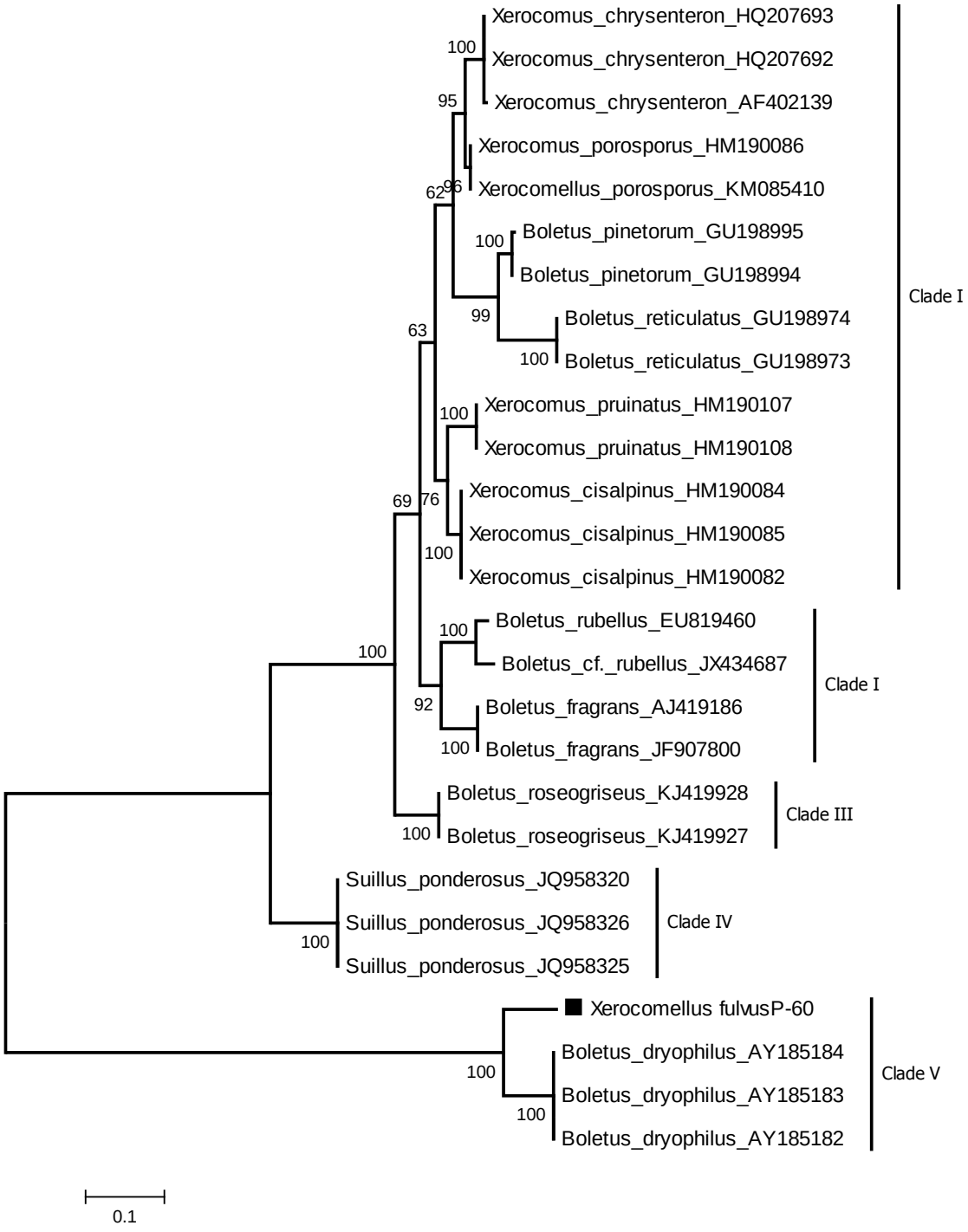


Figure 3.13 Molecular phylogenetic analysis of ***Xerocomellus fulvus*** nom. prov.by Maximum Likelihood method. Maximum log likelihood value = (-2277.2514) is shown. Collection of Swat is represented by black cube (■).

Cortinariaceae

Cortinarius

12 *Cortinarius cistoglaucopus*

13 *Cortinarius elegantior*

14 *Cortinarius kohistanicus* nom. prov.

12. *Cortinarius cistoglaucopus* Ortega, Vila, Campos & Fernandez-Brime, *Mycologia* 106(3): 499 (2014) Plate – 14 Figs. A-G & 3.14

Morpho-Anatomical characterization

PILEUS: 3 – 4 cm across, convex, white with light orange shades (2.5YR 8/8), central disc obtuse, curvature deflexed, surface apressed fibrillose and velvety. MARGIN: hirsute. LAMELLAE: sinnuate, light gray (2.5YR 6/2), even, crowded. STIPE: 2.1 – 2.5 × 1.1 – 1.6 cm, light gray (2.5YR 6/2) with very sparse strong orange shades.

BASIDIOSPORES: 8.0 – 10.8 × 4.5 – 7.0 µm [X = 8.9 × 5.8 µm, Q = 1.534], ellipsoid, apiculate, warty, golden brown in KOH and phloxin, anamoyloid. BASIDIA: 20.3 – 36.2 × 8.3 – 13.7 µm, clavate, mostly bisporic, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 25.5 – 36.2 × 7.8 – 12.1 µm, clavate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: same to cheilocystidia. PILEIPELLIS: 1.8 – 4.4 hyphae cylinder, parallel, hyaline in KOH. STIPTIPELLIS: 1.7 – 4.6 µm, hyphae cylinder, parallel, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Mashkoon Mankial, Solitary to gregarious on ground in *Cedrus deodara* forest, 05 September, 2013; ISP # 077 (LAH).

COMMENTS: This study reports new record of this species for Pakistan.

Plate 14

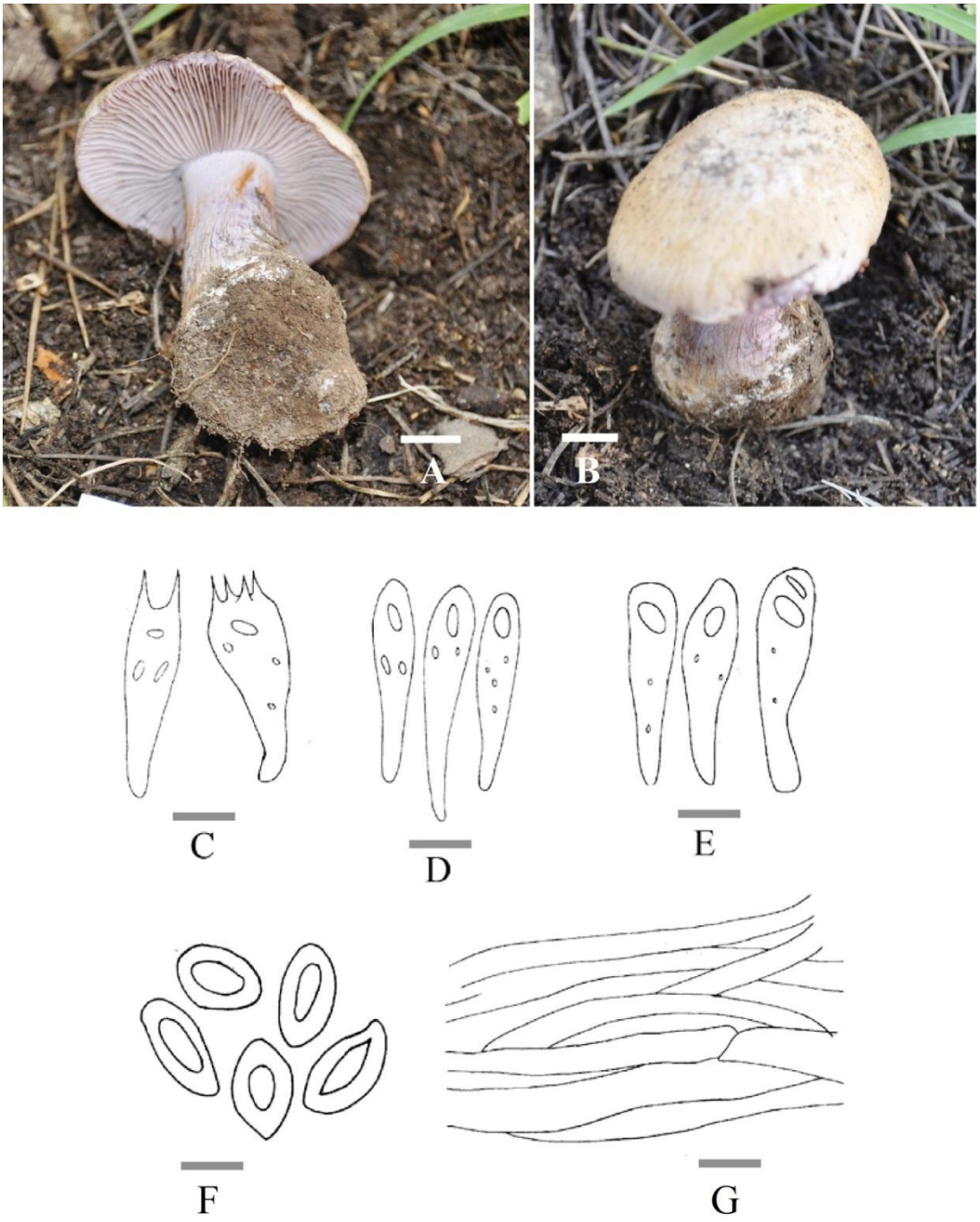


Figure A-G Morpho-anatomical shapes of ***Cortinarius cistoglaucopus*** (A-B) Basidiome (C) Basidia (D) Cheilocystida (E) Pleurocystidia (F) Basidiospores (G) Stipetipellus. Scale bars: A-B = 1 cm, C-E = 11 µm, F = 5 µm G = 7 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 608 base pairs. In initial BLAST search of GenBank database, closest hits were a series of *Cortiniarius* accessions from different parts of the world having the same expect value and same percentage of query coverage and identity index i.e 0.0, 100% and 99%, respectively. These included *Cortinarius cistoglaucopus* Ortega, Vila, Campos & Fernandez-Brime (KF738089), *Cortinarius misermontii* (GenBank KF732622, KF732621, NR_130230), *Cortinarius olidoamarus,* (GenBank KF732362) and *Cortinarius alticaudus* (GenBank KF732610, NR_130189).

The newly generated ITS sequence of the *Cortinarius cistoglaucopus* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 560/622 were conserved characters, 52 were variable, 23 were parsimony informative and 28 were singletons. The analysis was carried out for 22 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into

account. Final dataset had 554 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.14).

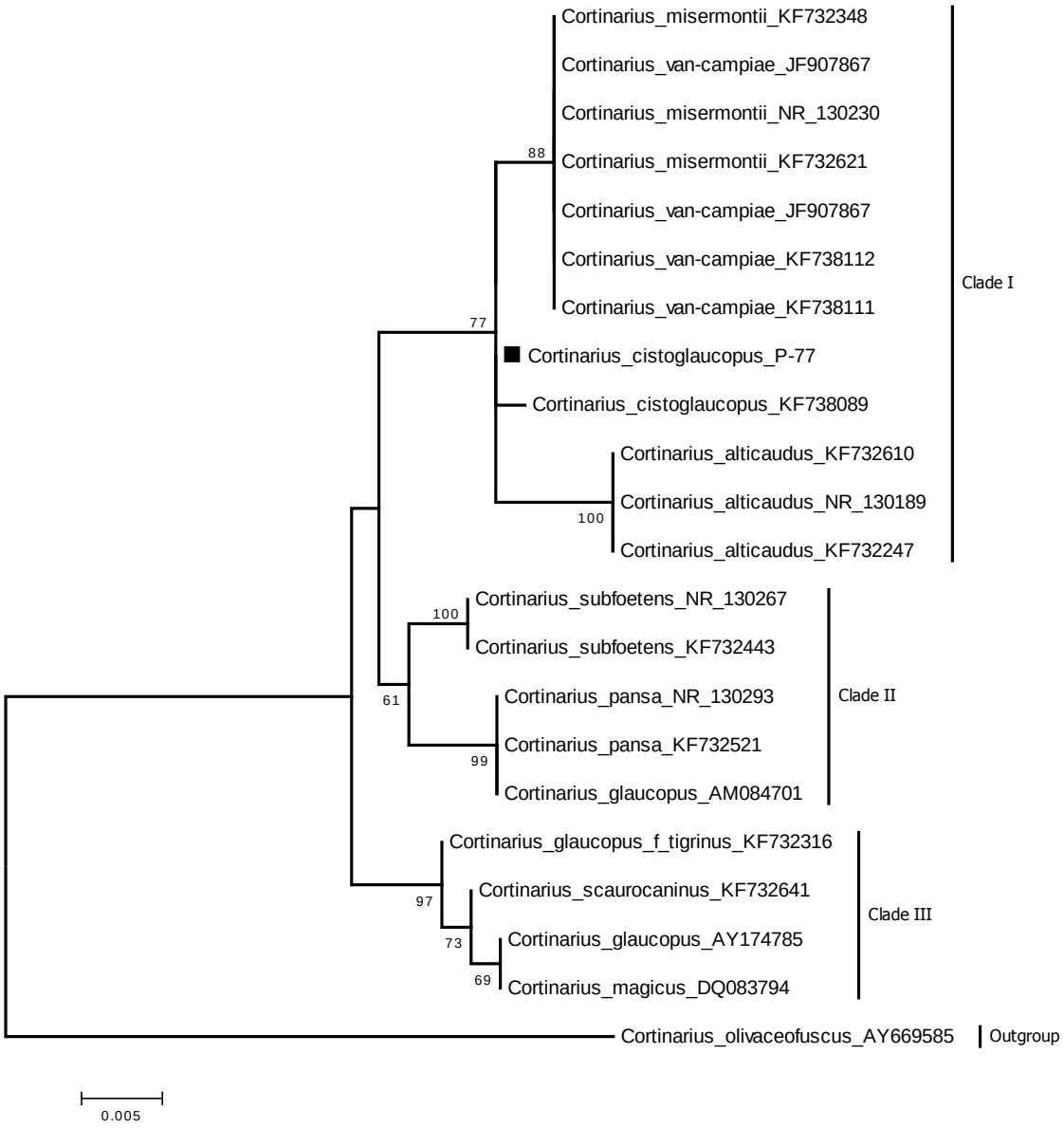


Figure 3.14 Molecular phylogenetic analysis of ***Cortinarius cistoglaucopus*** by Maximum Likelihood method. Maximum log likelihood value = (-1068.8658) is shown. Collection of Swat is represented by black cube (■).

13. *Cortinarius elegantior* (Fr.) Fr., *Epicr. syst. mycol.* (Upsaliae): 267 (1838)

Plates 15 & 16 – Figs. A-D & 3.15

Morpho-Anatomical characterization

PILEUS**:** 4 – 4.5 cm across, hemispheric, dark orange yellow to light orange yellow (7.5YR 6/8 – 7.5YR 9/8); central disc obtuse; curvature uplifted; surface glabrous, moist. MARGIN: cottony. LAMELLAE**:** adnate and horizental, pale yellow (7.5YR 9/4), even, thin, close. STIPE**:** 2 – 3 × 2 – 2.5 cm, ventricose, white.

BASIDIOSPORES: 7.5 – 12.0 × 13 – 15.5 µm [X = 9.5 × 14.5 µm, Q = 1.526], broadly ellipsoid, apiculate, warty with a distant urticle, golden brown in KOH, reddish brown phloxin, amoyloid,. BASIDIA: 29.3 – 38.5 × 7.1 – 11.1 µm, clavate, mostly bisporic, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 24.8 – 35.7 × 8.3 – 19.9 µm, clavate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: same to cheilocystidia.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Miandam, Solitary to gregarious on ground in *Pinus-Abies-Cedrus* forest, 06 September, 2013; ISP # 125 (LAH).

Plate 15

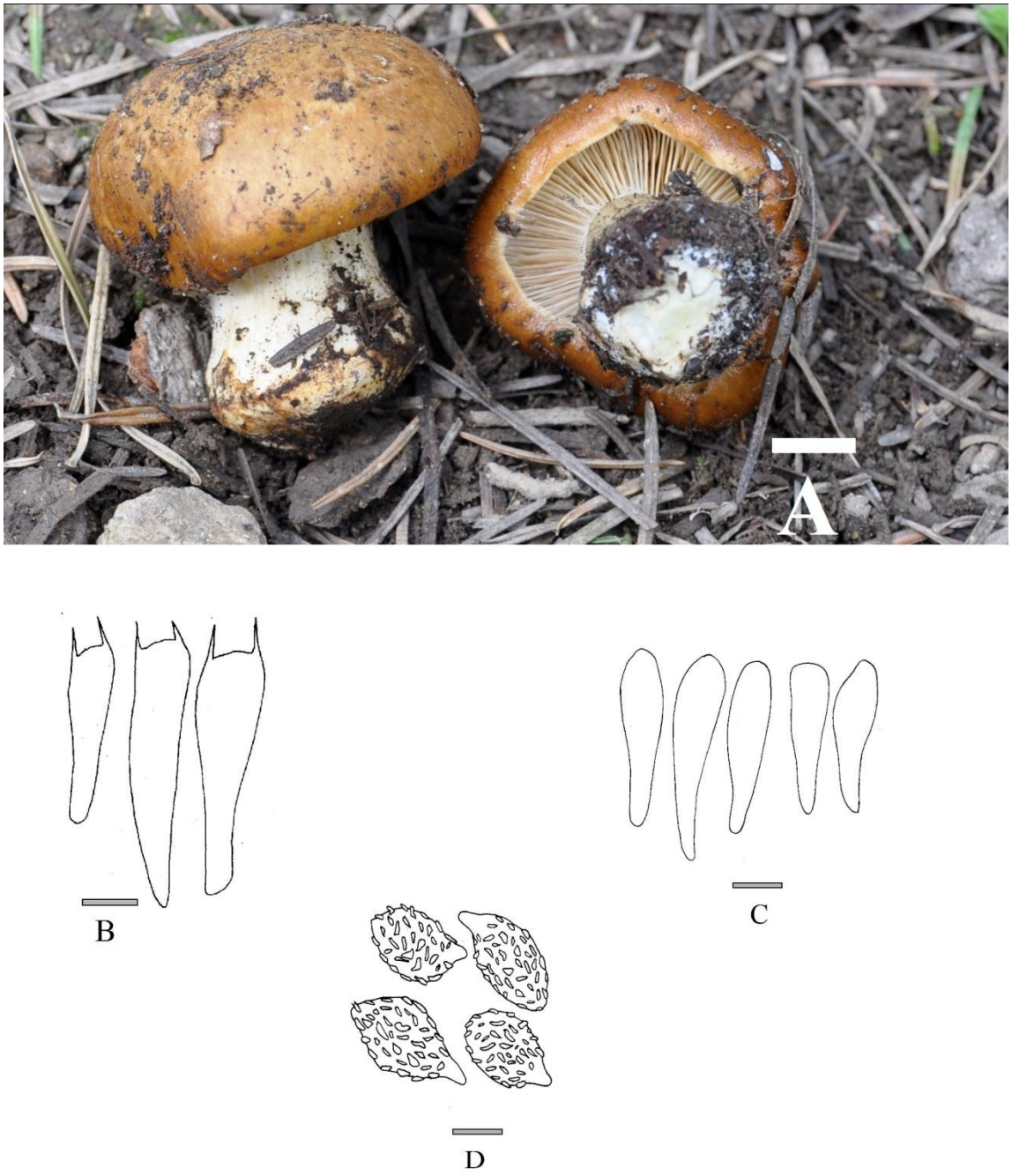
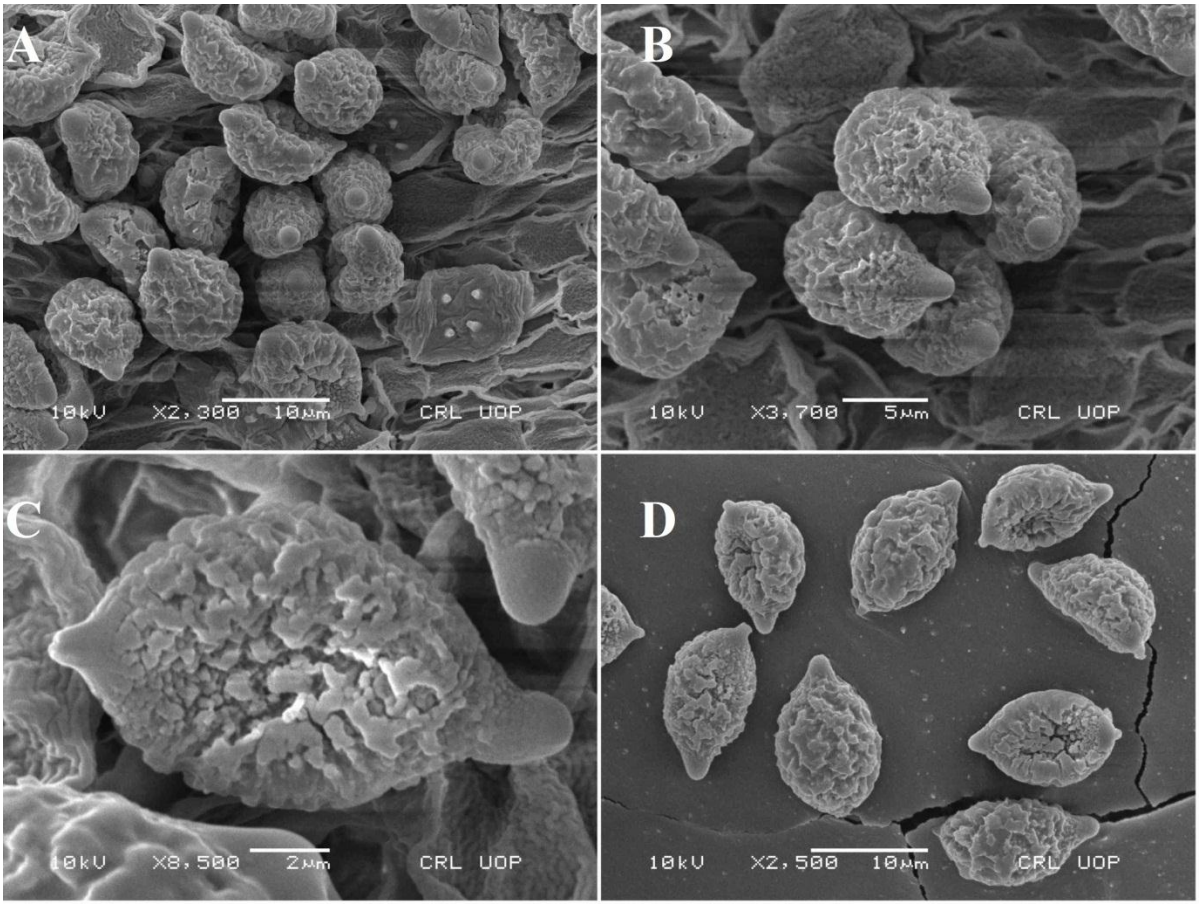


Figure A-D Morpho-anatomical shapes of ***Cortinarius elegantior*** (A) Basidiomata (B) Basidia (C) Basidiospores (D) Cystida. Scale bars: A = 1.4 cm, B = 10 µm, C-D = 5 µm.

Plate 16



Figures A-D Scanning electron micrograpes of ***Cortinarius elegantior***

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 579 base pairs. In initial BLAST search of GenBank database, the closest hit was *Cortinarius bergistanensis* Ballarà (= *Cortinarius elegantior* (Fr.) Fr.,) (GenBank JX219778 and JX219777; maximum identity 96%, Query coverage 100%) followed by *Cortinarius elegantior* (GenBank JX219779; maximum identity 96%, Query coverage 100%).

The newly generated ITS sequence of the *Cortinarius elegantior* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 411/717 were conserved characters, 221 were variable, 93 were parsimony informative and 126 were singletons. The analysis was carried out for 31 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 422 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.15).

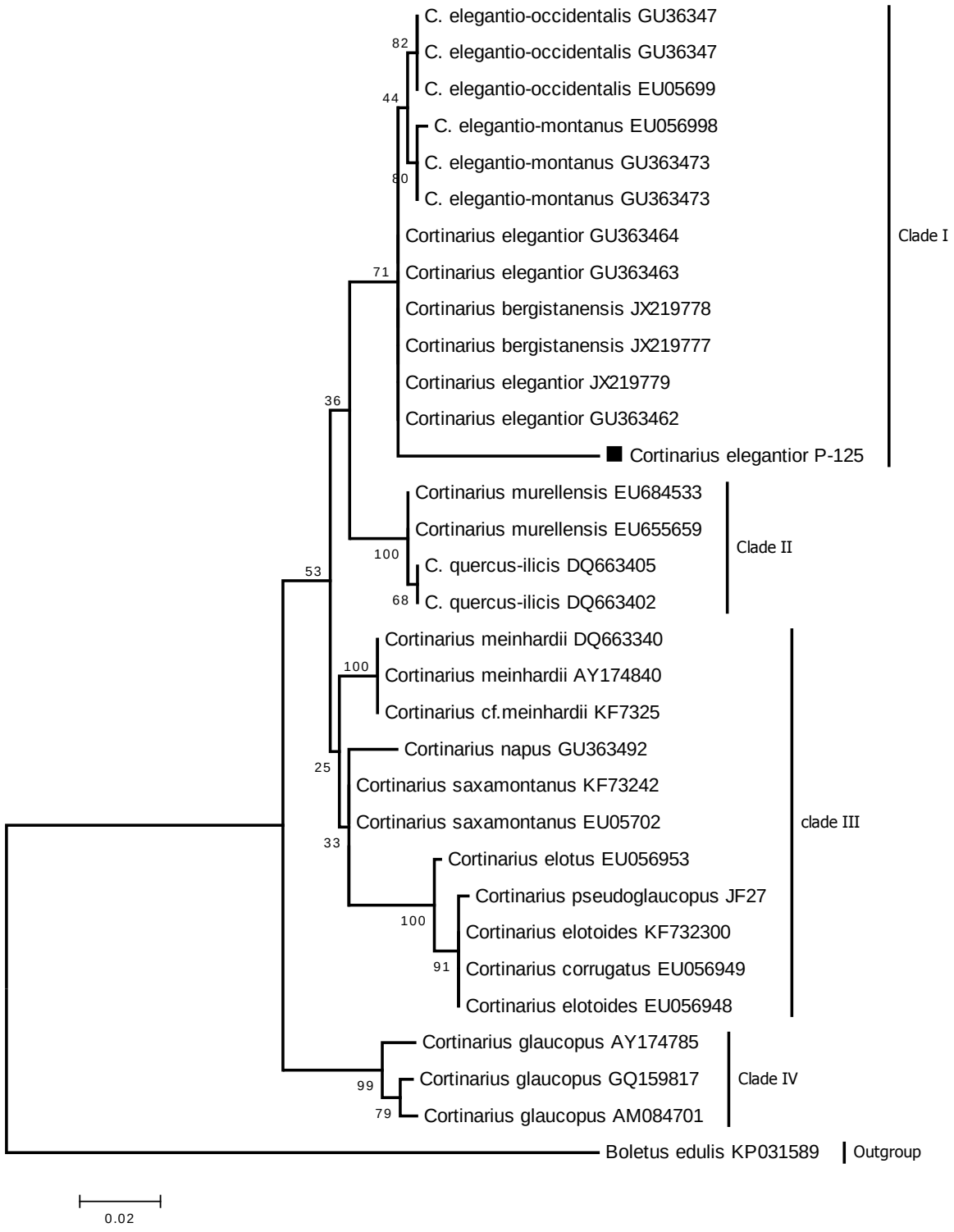


Figure 3.15 Molecular Phylogenetic analysis of ***Cortinarius elegantior*** by Maximum Likelihood method. Maximum log likelihood value = (-1375.8090) is shown. Collection of Swat is represented by black cube (■).

14. ***Cortinarius kohistanicus*** nom. prov. Plates 17 & 18 – Figs A-D & 3.16

Etymology: The specific epithet may be referred to Swat Kohistan, the type locality of this species.

Morpho-Anatomical characterization

PILEUS**:** 3.5 – 5.8 cm across, plane, dark orange yellow to light orange yellow (7.5YR 6/8 – 7.8YR 9/8); central disc umbillicate; curvature inrolled; surface apressed fibrillose, velvety, margin cottony. LAMELLAE**:** free and approximte, purple to greenish pink (10RP 7/2), ragged, close to crowded. STIPE**:** 3.0 – 4.5 × 0.5 – 1.8 cm, upper region like pileus covering, below very light orange yellow.

BASIDIOSPORES: 11.4 – 15.4 × 6.8 – 8.6 µm [X = 7.8 × 13 µm, Q = 1.619], broadly ellipsoid, apiculate, warty, reticulate, golden brown in KOH and reddish brown in phloxin, anamoyloid. BASIDIA: 31.6 – 39.8 × 9.9 – 13.9 µm, clavate, bisporic to quadrisporic, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 11.4 – 15.4 × 6.8 – 8.6 µm, clavate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: same to cheilocystidia. PILEIPELLIS: 3.8 – 7.5 hyphae cylinder, parallel, hyaline in KOH. STIPTIPELLIS: 4.5 – 9.2 µm, hyphae cylinder, parallel, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, Solitary to gregarious on ground in *Pinus-Abies-Cedrus* forest, 03 September, 2013; ISP # 056 (LAH).

Plate 17

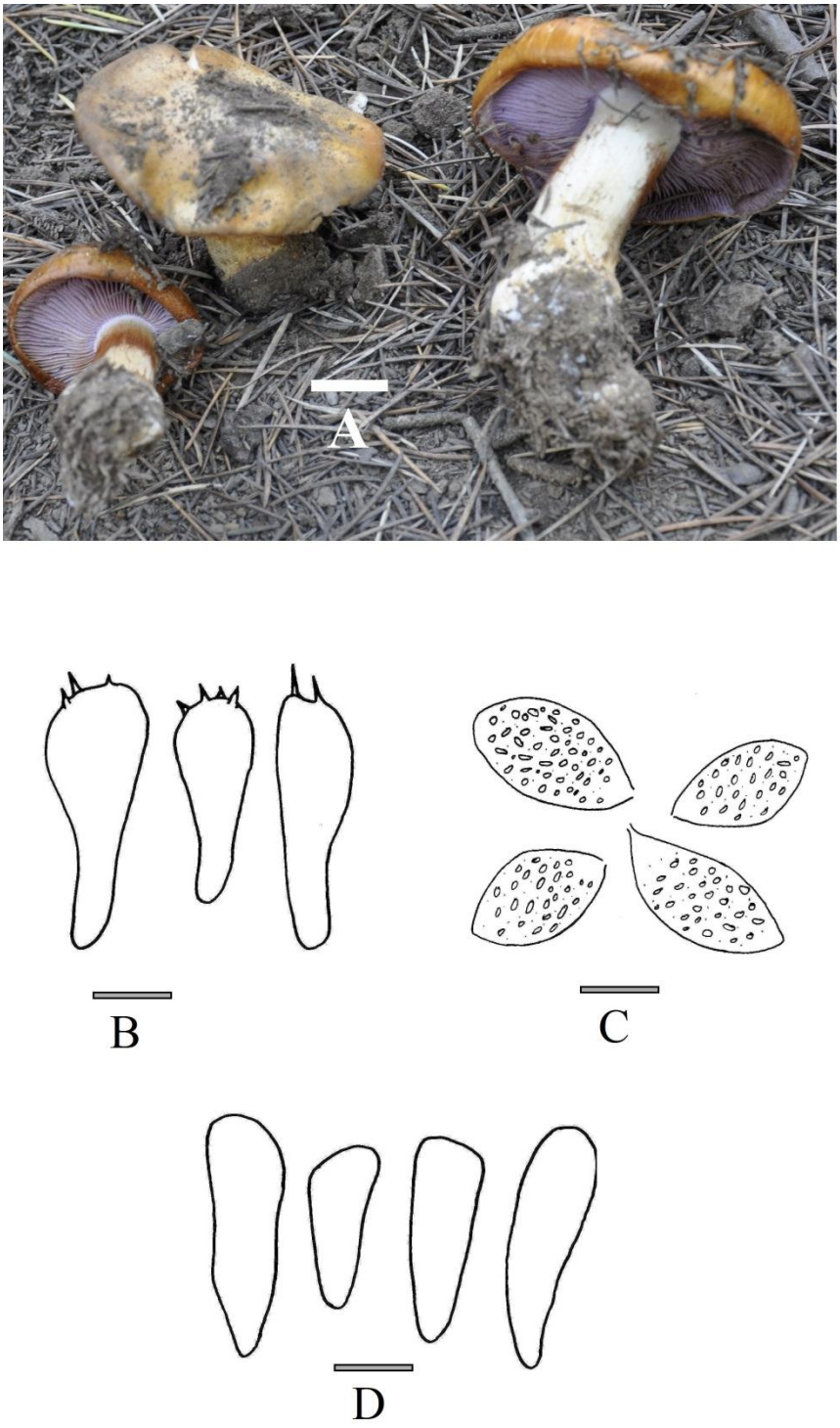
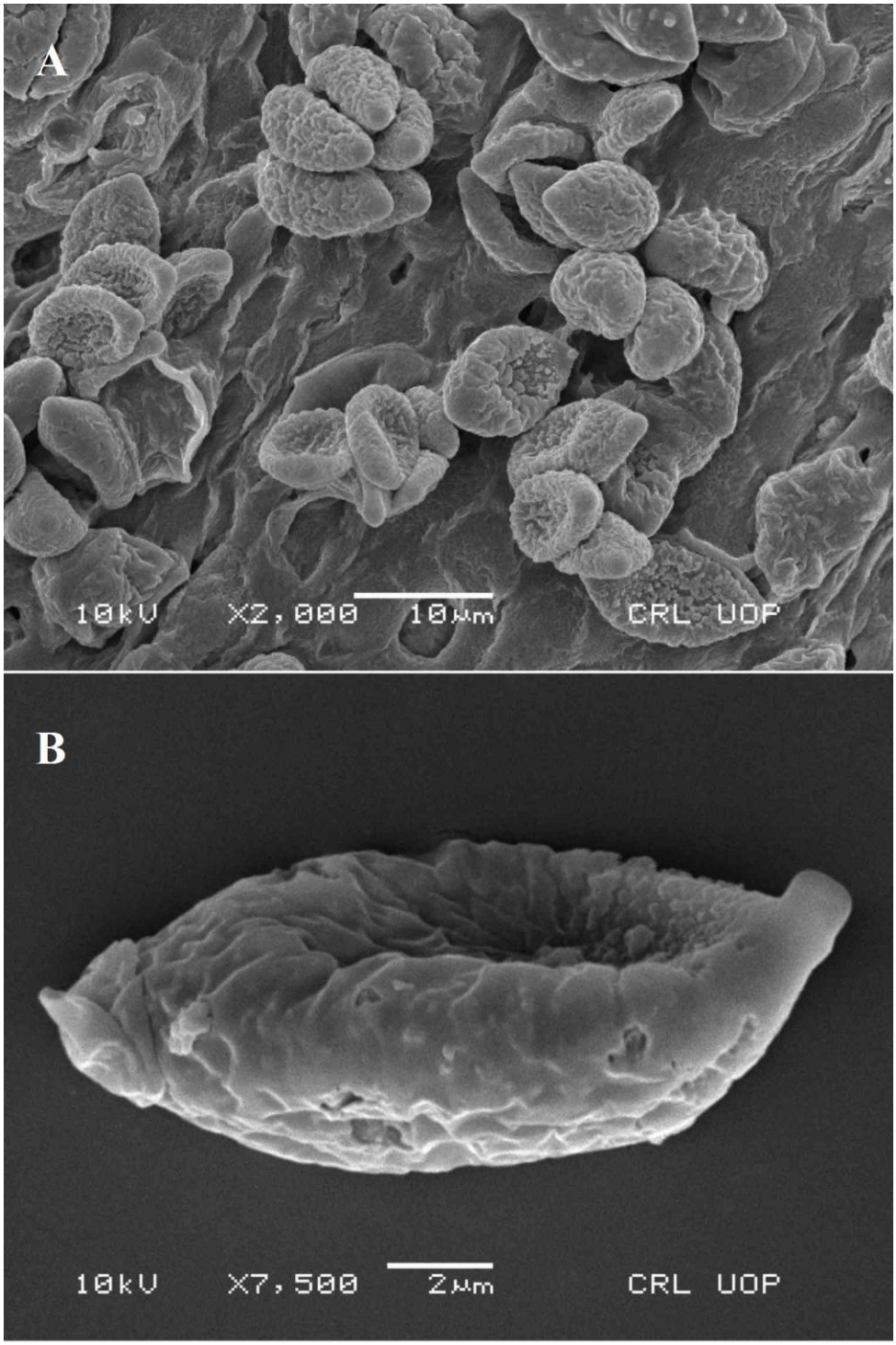


Figure A-D Morpho-anatomical shapes of ***Cortinarius kohistanicus*** nom. prov. (A) Basidiomata (B) Basidia (C) Basidiospores (D) Cystida. Scale bars: A = 1.4 cm, B = 10 µm, C-D = 5.

Plate 18



Scanning electron micrograpes of ***Cortinarius kohistanicus*** nom. prov. (A) basidiospore attached to basidia (B) Basidiospore.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 522 base pairs. In initial BLAST search of GenBank database, the closest hit was *Cortinarius elotus* Fr. (GenBank EU056953; maximum identity 82%, Query coverage 98%) followed by Lentin *Cortinarius elotoides* Moser & McKnight (GenBank KF732300; maximum identity 82%, Query coverage 98%).

The newly generated ITS sequence of the *Cortiniarius kohistanicus* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 364/699 were conserved characters, 248 were variable, 83 were parsimony informative and 165 were singletons. The analysis was carried out for 31 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 438 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.16).

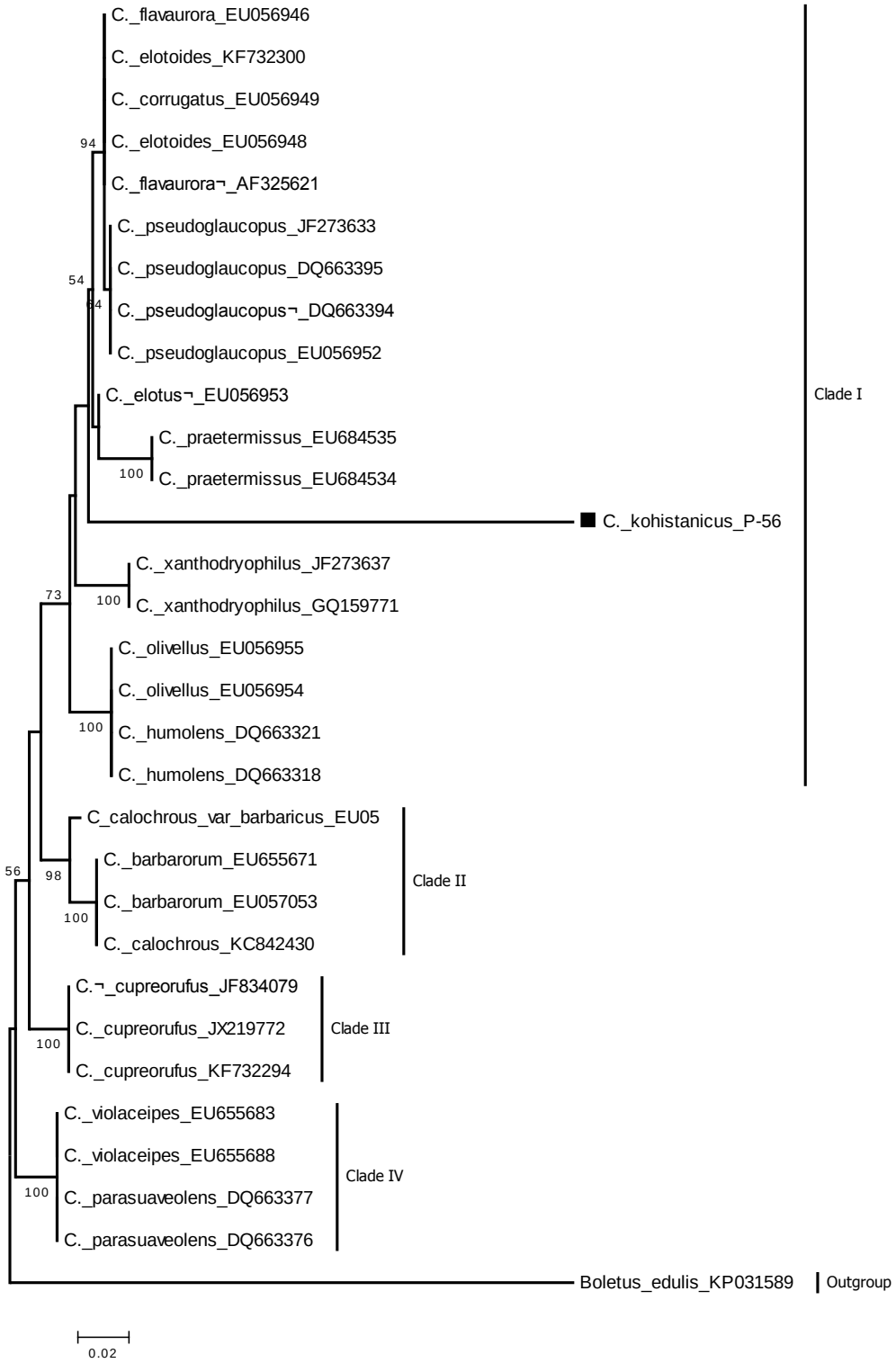


Figure 3.16 Molecular Phylogenetic analysis of ***Cortinarius kohistanicus*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1592.1245) is shown. Collection of Swat is represented by black cube (■).

Hygrophoraceae

Hygrocybe

15 *Hygrocybe decoriae* nom. prov.

Hygrophorus

16 *Hygrophorus chrysodon*

15. ***Hygrocybe decoriae*** nom. prov. Plate 19 – Figs. A-E & 3.17

Etymology: The specific epithet may be referred to to the attractive colors of this species.

Morpho-Anatomical characterization

PILEUS: 3.4 – 4.0 cm diam, conical, papillate central disc, deep yellow red to moderate yellow red (10R4/12 - 10R6/16) with glabrous slimy surface; curvature deflexed; margins slightly inrolled to cottony; LAMELLAE:: free and approximate, close, thick, off white (10R9/2); margins ragged eroded; breadth 1.5 – 1.8 cm. STIPE: 5.6 - 6.4 cm, centrally attached, pliable, hollow, cylindrical light yellow green to deep yellow green (5GY5/8 – 5GY7/10); surface striated and equal.

BASIDIOSPORES: 5.7 – 19.0 × 8.3 – 11.6 µm [X = 7.2 × 9.8 µm, Q = 1.361], ellipsoid to oblong to ovoid to rhomboid, apiculus present, smooth, thin-walled, hyaline in KOH, non-amyloid. BASIDIA: 13.1 – 15.0 (25.1) × 34.5 – 42.7 (51.3) µm, clavate, two to four spored basidia, thin-walled, hyaline in KOH; Sterigmata 1.9 – 2.9 µm. Tramal hyphae, cylindrical, regular, 4.5 – 7.3 µm, thin walled, hyaline in KOH. CheiloCYSTIDIA: 8.5 – 10.9 × 22.7 – 32.6 µm, clavate, thin walled. Pileipellis:: a cutis, hyphae cylindrical, (2.4–) 3.8 – 7.1 (–7.0) µm, thin-walled, hyaline in KOH. BASIDIOLE: 8.1 – 13.3 × 27.14 – 36.1 µm, thin-walled, hyaline in KOH. Pileal CYSTIDIA: 8.1 – 11.4 × 25.8 – 33.6 µm, thin-walled, hyaline in KOH. STIPE HYPHAE: cylindrical, 5.9 – 12.1 µm, hyaline in KOH. CLAMP CONNECTIONS: present in all tissues.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Yakh Tangay, **S**hangla and Shangla Top, found below *Pinus wallichiana* A. B. Jacks., 02 Sep. 2013; ISP # 009 (LAH).

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 647 base pairs. In initial BLAST search of GenBank database, closest hit was Hygrocybe conica (GenBank JN021030, Query coverage - 99%, expect value - 0.0 and Identity index - 90%) submitted by Dentinger, Didukh and Moncalvo from Canada.

The newly generated ITS sequence of the Hygrocybe decoriae along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 311/836 were conserved characters, 497 were variable, 333 were parsimony informative and 141 were singletons. The analysis was carried out for 24 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 334 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.17).

Plate 19

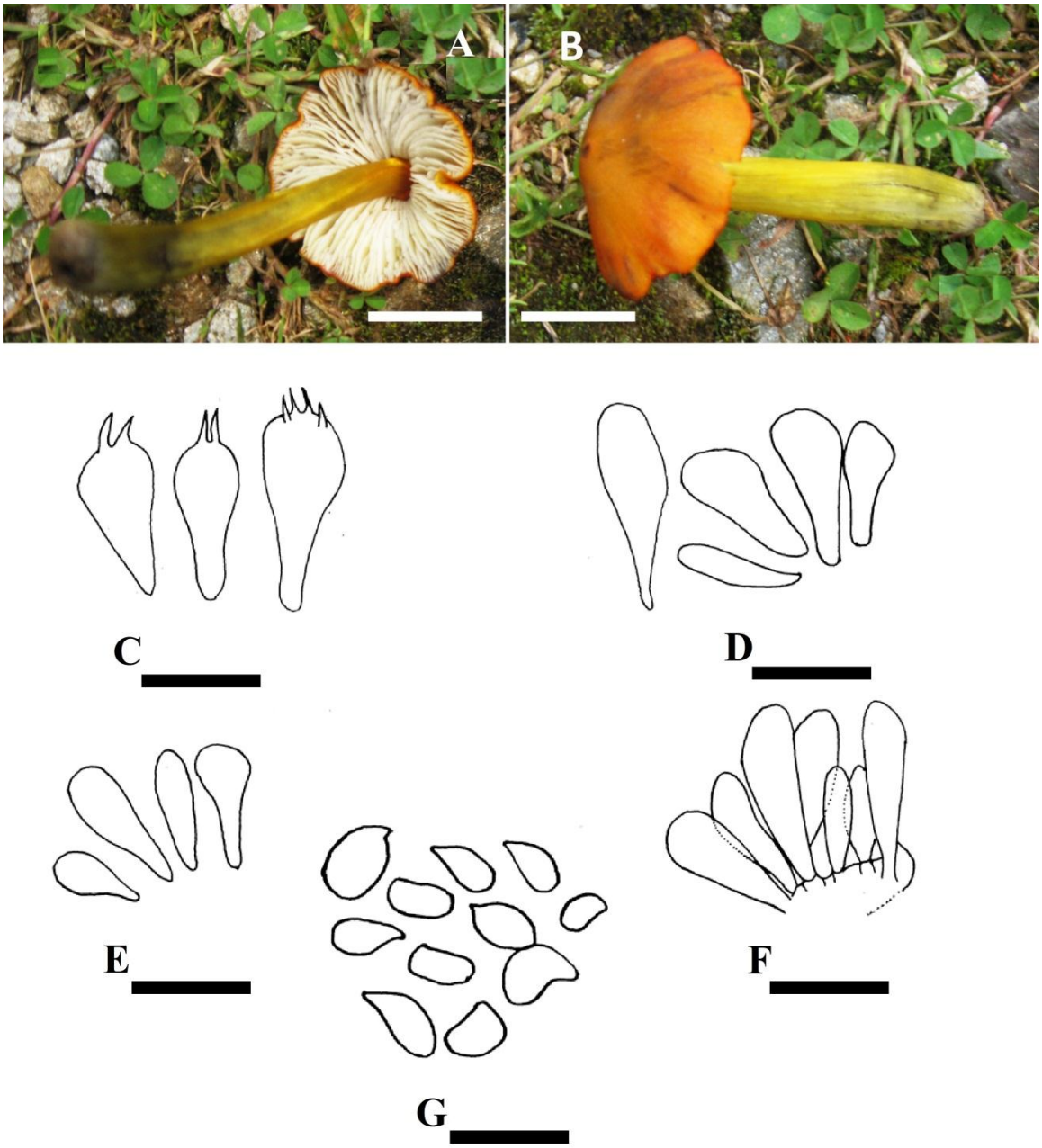


Figure A-E Morpho-anatomical shapes of ***Hygrocybe decoriae*** nom. prov. (A-B) Basidiomata (C) Basidia (D) Chielocystida (E) Basidiole (F) Pileal Cystidia (G) Basidiospores; Scale bars: A = 2 cm, B = 2.5 cm C = 9.5 µm, D- E = 8.0 µm, F = 20 µm, G = 16 µm

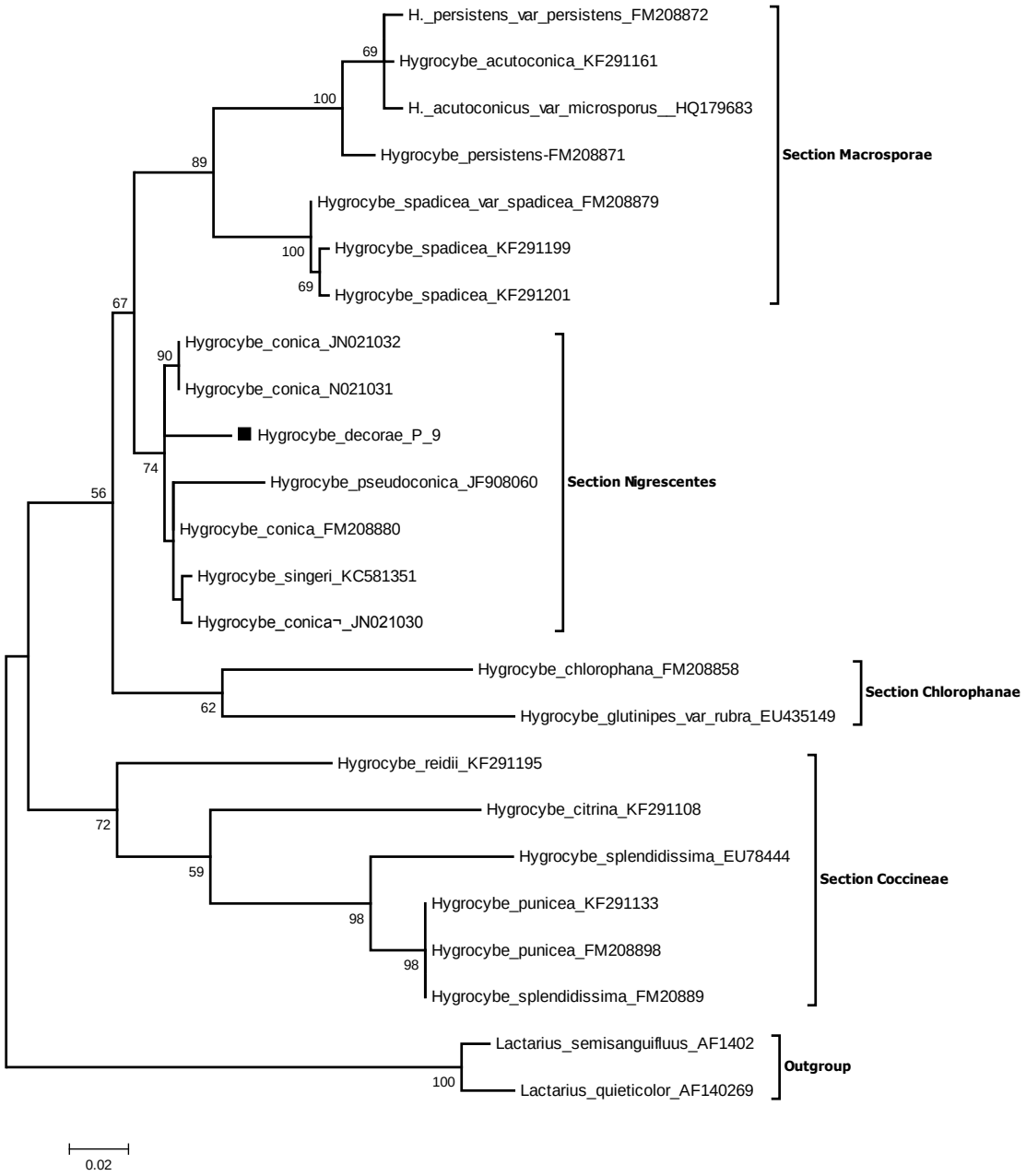


Figure 3.17 Molecular phylogenetic analysis of ***Hygrocybe decoriae*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1994.1608) is shown. Collection of Swat is represented by black cube (■).

16. *Hygrophorus chrysodon* (Batsch) Fr. *Epicr. syst. mycol.* (Upsaliae): 320 (1838)

Plate 20 – Figs. A-G & 3.18

Morpho-Anatomical characterization

PILEUS**:** 1.7 – 3.5 cm across, depressed, white with very sparse yellow orange (7.5YR 7/2) marks; central disc obtuse; curvature deflexed; surface, appressed fibrillose, velvety. MARGIN: slightly strigose with very sparse yellow orange (7.5YR 7/2) marks. LAMELLAE**:** areuate, white, equal, sub distant. STIPE**:** 3.4 – 5.5 × 0.3 – 0.6 cm, milky white, cylindrical, equal, soft.

BASIDIOSPORES: 10.0 – 12.2 × 4.0 – 5.6 µm [X = 4.7 × 11.0 µm, Q = 2.34], narrowly elliptic, apiculate, smooth, yellowish green in KOH. BASIDIA: 34.0 – 48.0 × 10.4 – 15.4 µm, clavate, quadrisporic thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 27.9 – 37.3 × 5.2 – 10.4 µm, clavate, thin walled, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Miandam, Solitary to gregarious on ground in *Pinus wallichiana* and *Cedrus deodara* forest, 02 September, 2013; ISP # 124 (LAH).

COMMENTS: This species is reported by A. Razzaq from Abbotabad, KP Pakistan, whereas this study records first report of this species from Swat.

Plate 20

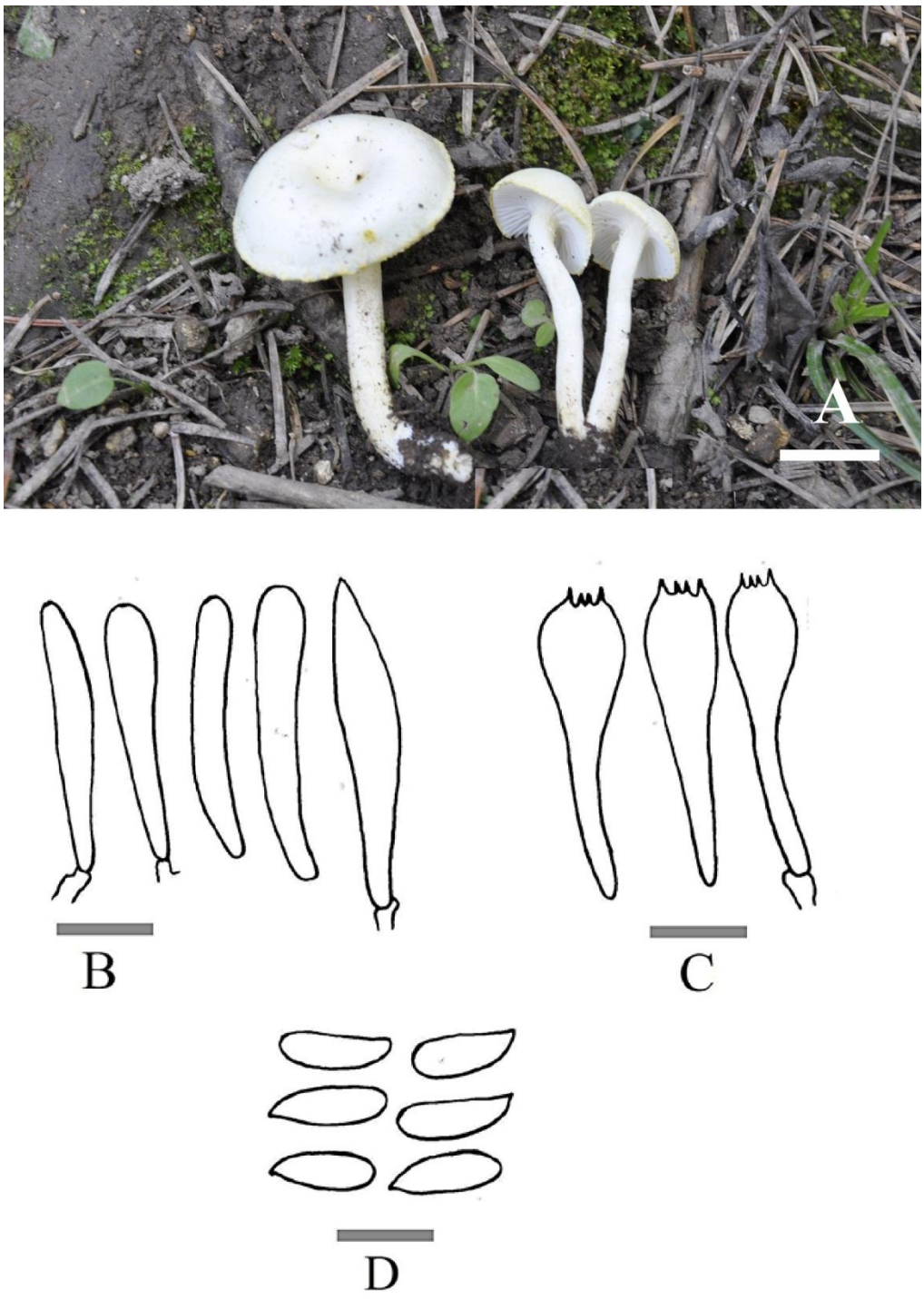


Figure A-G Morpho-anatomical shapes of ***Hygrophorus chrysodon*** (A) Basidiomata (B) Cystidia (C) Basidia (D) Basidiospores. Scale bars: A = 1.4 cm, B = C = 25 µm D = 8 µm

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 542 base pairs. In initial BLAST search of GenBank database, closest hit was *Hygrophorus chrysodon* (GenBank DQ974809, Query coverage - 100%, expect value - 0.0 and Identity index - 95%) from USA followed by *Hygrophorus chrysodon* (GenBank LK022682, Query coverage - 85%, expect value - 0.0 and Identity index - 99%) from Pakistan. The latter is submitted by S. Ilyas, A. Razaq and A. N. Khalid in 2014.

The newly generated ITS sequence of the *Hygrophorus chrysodon* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria and then selecting the topology with superior log likelihood value. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 322/698 were conserved characters, 324 were variable, 250 were parsimony informative and 74 were singletons. The analysis was carried out for 30 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 238 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.18).

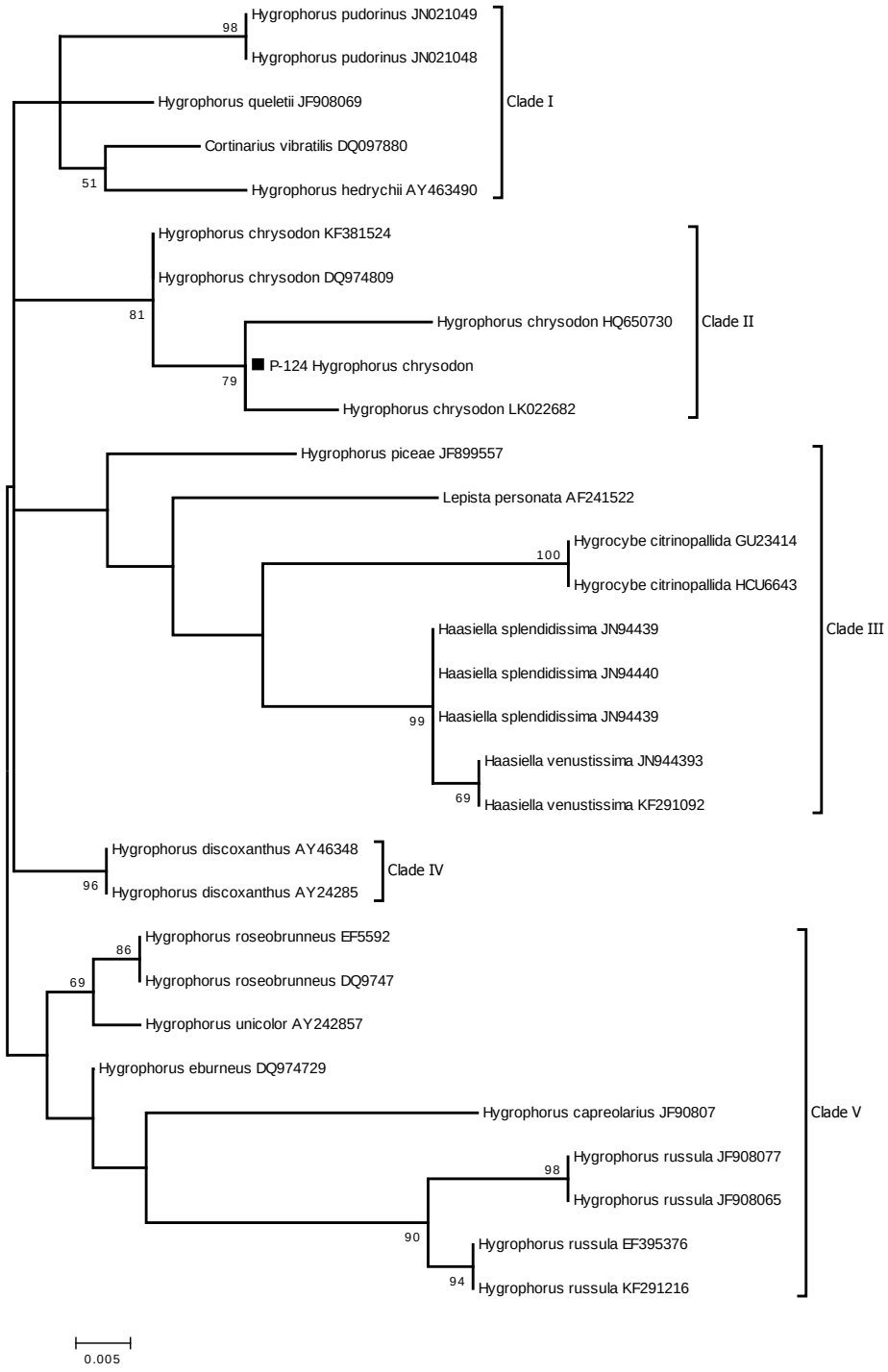


Figure 3.18 Molecular phylogenetic analysis of ***Hygrophorus chrysodon*** by Maximum Likelihood method. Maximum log likelihood value = (-783.3827) is shown. Collection of Swat is represented by black cube (■).

Hymenogastraceae

Hebeloma

17. *Hebeloma theobrominum*

17. ***Hebeloma theobrominum*** Quadr., *Mycotaxon* 30: 311 (1987)

Plate 21 – Figs. A-G & 3.19

Morpho-Anatomical characterization

PILEUS: 2 – 6 cm diam., convex to plano convex, slightly obtuse umbonate, plane upon expansion; margin incurved and eroded at places; surface dry and aerolate, margin torn and at times; light orangish yellow to pale orangish yellow (7.5YR 9/8 – 10YR 9/4); context white. LAMELLAE: adnate and ascending, crowded, lamellules present, edges ragged and eroded, pale orangish yellow (10YR 8/4). STIPE: 2.0 –5.0 cm × 0.5–1.0 cm, even and equal; pale yellow (5Y 9/4). Taste and spore deposit not recorded.

BASIDIOSPORES: 4.9 – 8.2 × 8.2 – 13.4 µm [X = 11.7 × 6.9 µm, Q = 1.695], amygdaliform, germ pore and appiculus present, thick-walled, reddish olive in KOH. BASIDIA: 19.8 – 24.1 × 7.5 – 10.0 µm, clavate, four spored basidia, thin-walled, hyaline in KOH. CHEILOCYSTODIA: 16.4 – 20.4 × 5.9 – 8.2, clavate to subclavate, thin walled, hyaline in KOH. . PLEUROCYSTODIA: 10.2 – 21.2 × 5.5 – 8.1, clavate to subclavate, thin walled, guttule present, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Ushu, Solitary to on ground in *Pinus wallichiana* and *Cedrus deodara* forest, 04 September, 2013; ISP # 047 (LAH).

COMMENTS: This species is reported by Razzaq (2013) from Abbotabad, KP Pakistan, whereas this study records first report of this species from Swat.

Plate 21

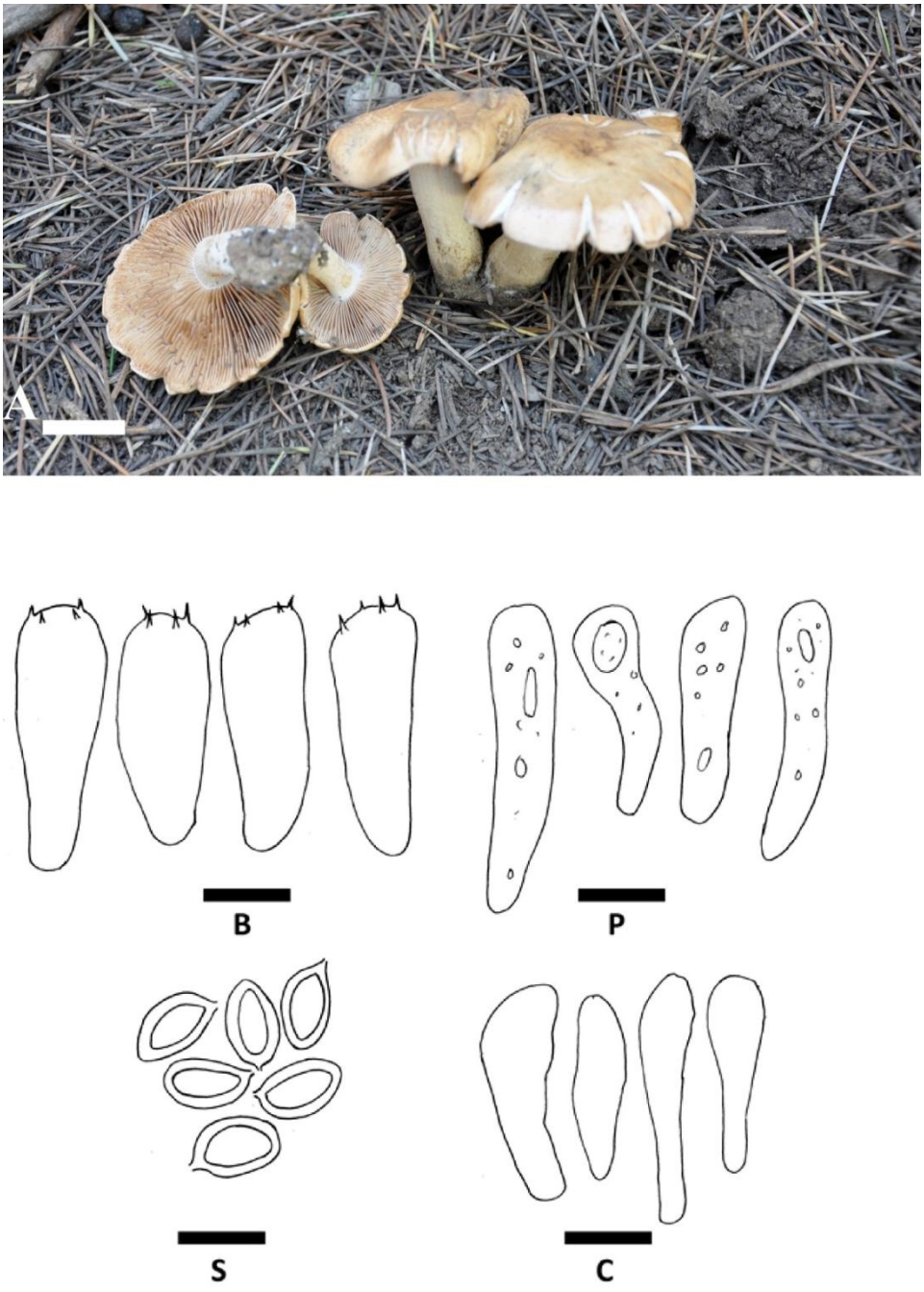


Figure 3A-G Morpho-anatomical shapes of ***Hebeloma theobrominum*** (A) Basidiomata (B) Basidia (P) Pleurocystidia (S) Basidiospores (C) Chielocystida. Scale bars: A = 1.8 cm, B = 3 cm, P = 6.8 µm, S = 12, µm, C = 5 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 624 base pairs. In initial BLAST search of GenBank database, closest hit was *Hebeloma truncatum* (Schaeff.) P. Kumm. = *Hebeloma theobrominum* Quadr (GenBank FJ168588, Query coverage - 99%, expect value - 0.0 and Identity index - 99%) and *Rhodocybe truncate* = *Hebeloma truncatum* = *Hebeloma theobrominum* (GenBank FJ770393, Query coverage - 98%, expect value - 0.0 and Identity index - 99%) from UK.

Sequences of reprentative infrageneric sections retrieved from GenBank with the newly generated ITS sequence of the *Hebeloma* were used for clarification of the phylogenetic placement of current collection of the *Hebeloma theobrominum* and aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 436/668 were conserved characters, 213 were variable, 146 were parsimony informative and 65 were singletons. The analysis was carried out for 4`19 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 447 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.19).

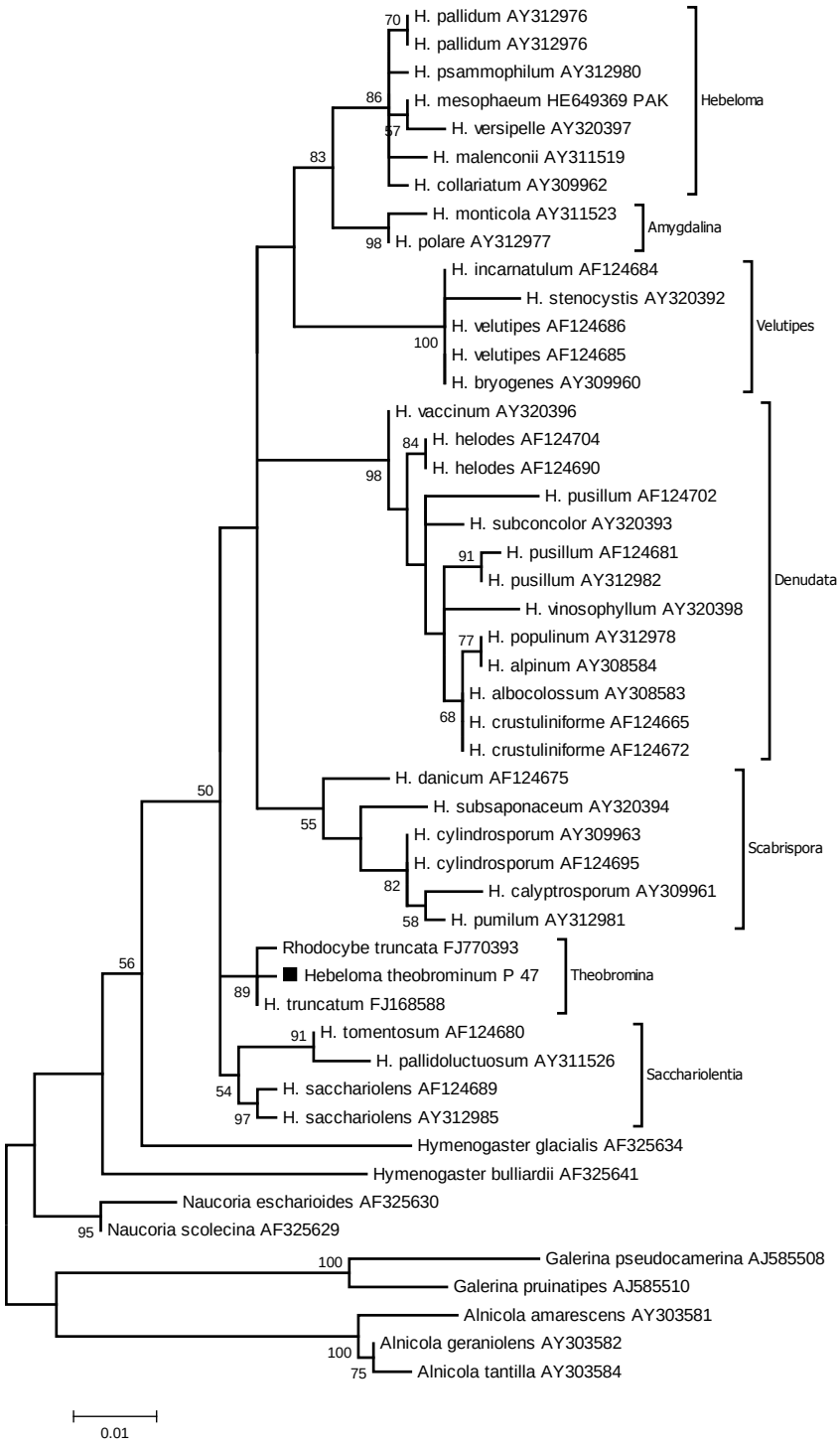


Figure 3.19 Molecular phylogenetic analysis of ***Hebeloma theobrominum*** by Maximum Likelihood method. Maximum log likelihood value = (-1824.2096) is shown. Collection of Swat is represented by black cube (■).

Inocybaceae

Inocybe

18. *Inocybe kohistanensis*
19. *Inocybe mimica*
20. *Inocybe pallida*
21. *Inocybe rimosa*
22. *Inocybe Swatica* nom. prov.

18. ***Inocybe kohistanensis*** Jabeen and Ahmad. *Turkish Journal of Botany*, 40(3), 312-318. (2016) Plate 22 – Figs. A-G & 3.20

Etymology: "kohistanensis" refers to the 'Kohistan'. Swat Kohistan is the type locality.

Morpho-Anatomical characterization

PILEUS: 3.1 – 4.8 cm, gobose to hemispherical to conical, umbonate, obtuse when young, becoming subcampanulate, dark brown (2.5YR4/10) or reddish brown (10R3/8), surface fibrillose to apperessed fibrillose, dry, margin incurved, rimose to rimulose. LAMELLAE: adnate to adnexed, close, edges even to slightly fimbriate, cream (2.5Y9/2) to greyish (5Y8/2) and pruinose when immature to dark brown or fulvus (2.5YR/10). LAMELLULAE: alternating with lamellae. STIPE: 0.4 – 0.9 × 3.5 – 10 cm, centrally attached, cylinderical, up to 1.5 cm in diameter swollen at base, surface smooth, white to cream (2.5Y9/2), pruinose and brownish (5YR6/8).

BASIDIOSPORES: (8.7) 9.4 – 12.5 (13.3) × (6.1) 6.6 – 9.2 (9.4) µm, Q = 1.19 – 1.69, X = 1.4, elongated heterodiametrical to globose, angular, nodulose, 8 – 9 nodules, guttulate, yellowish brown in 5% KOH,. BASIDIA: 4-spored, 25.3 – 31 × 9.5 – 12.6 µm, clavate, densely guttulate, yellowish, thin walled, clamp connections present at the base. CHEILOCYSTIDIA: (56.6) 67.2 – 72 (75.5) × (19.6) 23.4 – 26.2 (28.8) µm, pale yellow to hyaline, wall thickness up to 3 µm, crystalliferous. PARACYSTIDIA: 10 – 15.4 × 5 – 6.9 µm, broadly clavate, pale yellow to hyaline, thin-walled, catenate, clamp connections present at the base. PLEUROCYSTIDIA: (50.7) 65 – 76 (79) × (20.8) 21 – 28 (31.9) µm, wall up to 3 µm, apex densely crystalliferous, pale yellow to hyaline,. CAULOCYSTIDA: 45

– 50.3 × 9.5 – 15.8 µm, often in clusters, thin walled, mucronate, pale yellow to hyaline, clamp connections present at the base. PILEIPELLIS: 4.6 – 8.6 µm wide, branched, yellowish brown, incrusting of pigments observed on hyphal wall, fusiform terminals, clamp connections frequent. STIPITIPELLIS: a cutis 3 – 10 µm wide, pale yellow, rarely branched, clamp connections not observed.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, solitary to gregarious on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, September 4, 2013; ISP # 042 (LAH).

Plate 22

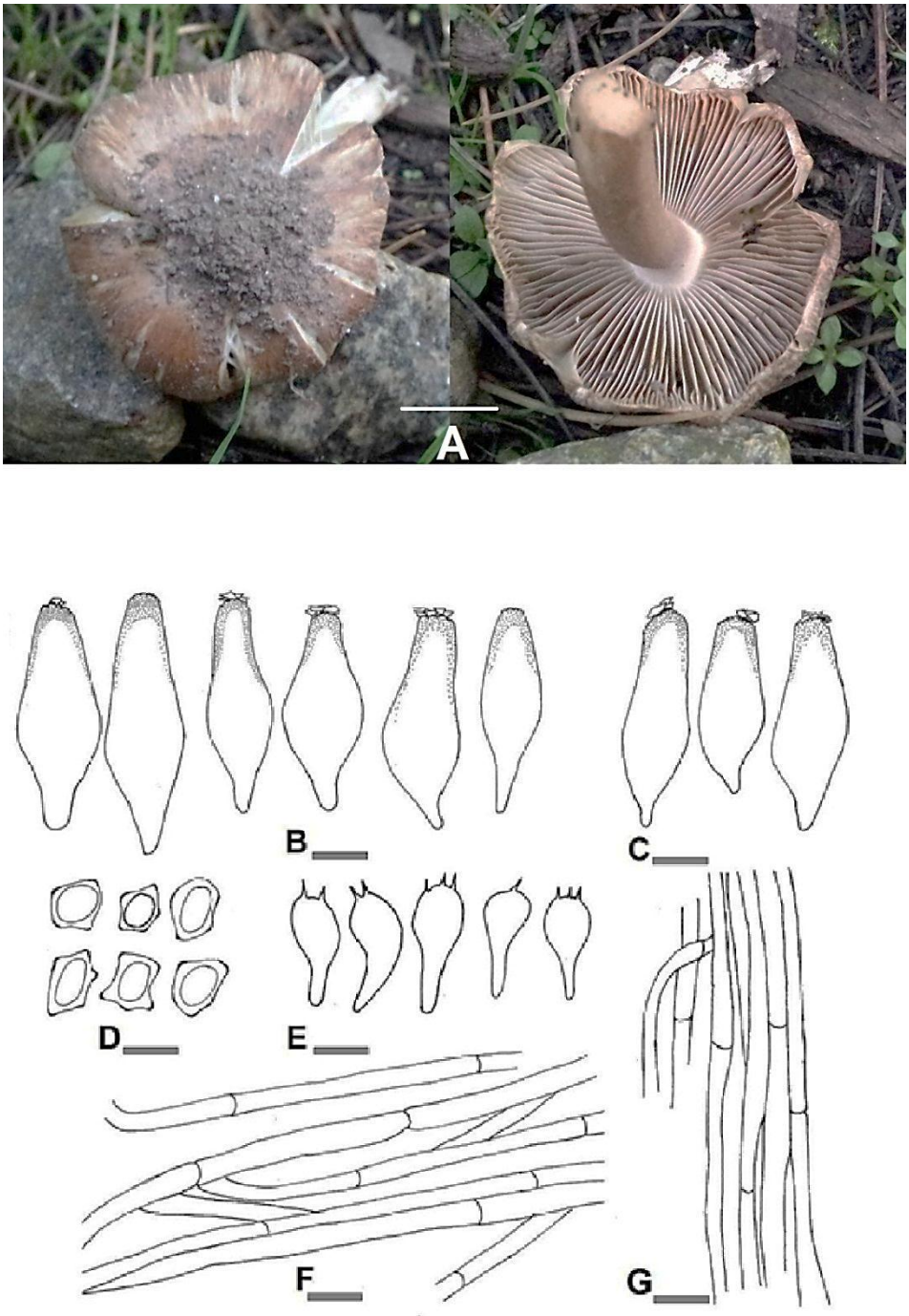


Figure – A-G Morpho-anatomical shapes of ***Inocybe kohistanensis*** A: Basidiomata B: Pleurocystidia C: Cheilocystidia. D: Basidiospores E: Basidia. F: Hyphae from pileipellis G: Hyphae from stipitipellis.*Scale Bars*: A = 1.3 cm B = 15 µm; C = 17 µm; D = 12 µm; E = 13 µm; F = 17 µm; G = 18 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 587 base pairs. In initial BLAST search of GenBank database, closest hit was *Inocybe dunensis* (GenBank JF908092, Query coverage - 100%, Identity index - 84%).

The newly generated ITS sequence of the *Inocybe kohistanensis* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 285/744 were conserved characters, 444 were variable, 368 were parsimony informative and 73 were singletons. The analysis was carried out for 51 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 745 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.20).

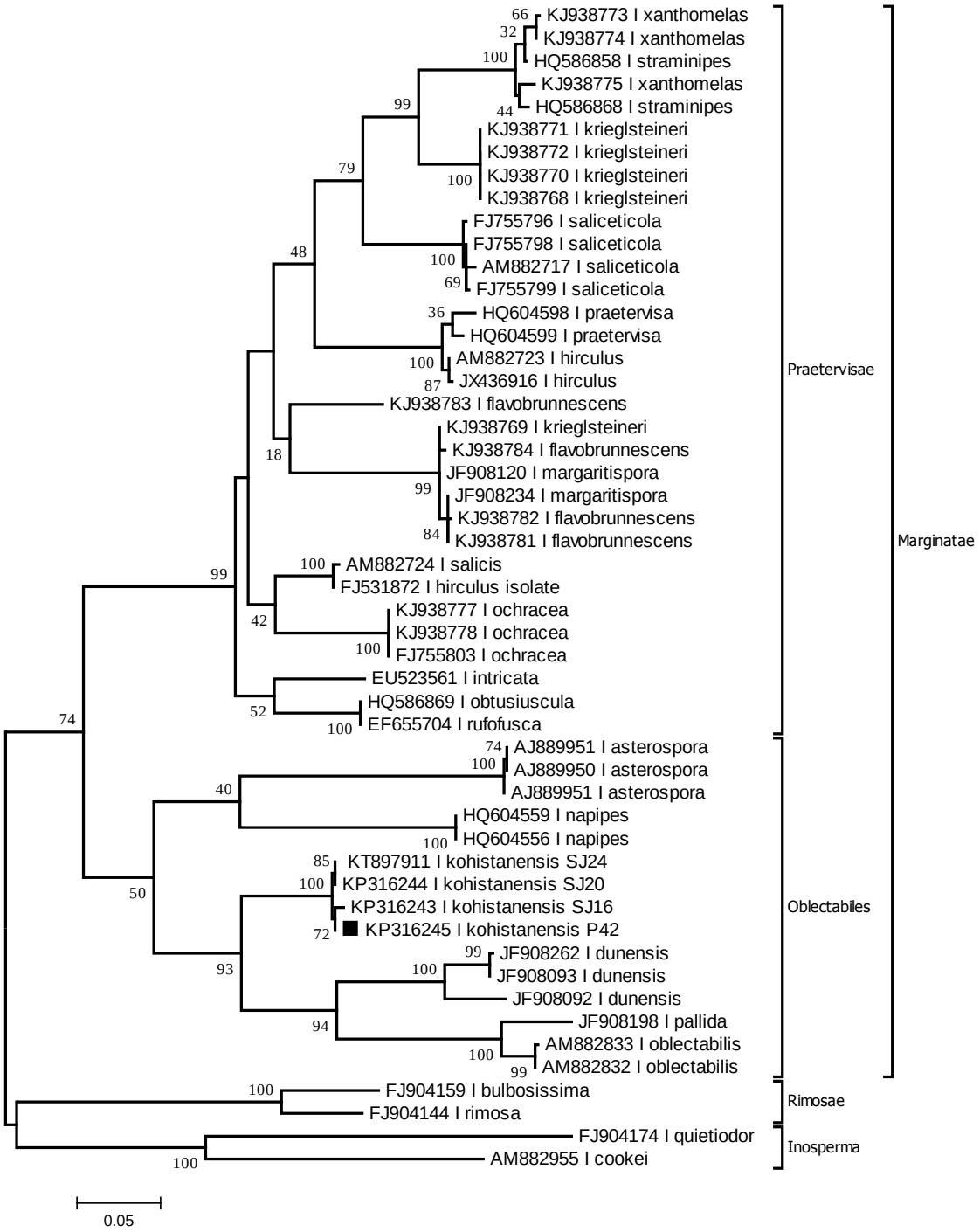


Figure 3.20 Molecular Phylogenetic analysis ***Inocybe kohistanensis*** by Maximum Likelihood method. Maximum log likelihood value = (-6969.6500) is shown. Collection of Swat is represented by black cube (■).

19. ***Inocybe mimica*** Massee, *Ann. Bot.,* Lond. 18: 492 (1904)

Plate 23 – Figs A-F & 3.21

Morpho-Anatomical characterization

PILEUS: 2-3 cm diam, conic to companulate, papillate central disc, moderate orang yellow when younger to dark orange yellow (10YR7/8-10YR6/8) with fibrillose to hirsute velvety surface; curvature cuspidate to obtuse; margins stigose and eroded; LAMELLAE: adnat ascending to adnexed, close to crowdy, thin, light yellow orange to dark orange yellow(7.5YR9/8-10YR6/8); margins ragged eroded; breadth 1.2-1.5 cm. STIPE: 6-8 cm, centrally attached, pliable, hollow, cylindrical white to yellowish white (5Y9/2-5Y9/4); surface equal and smooth.

BASIDIOSPORES: (7.3–) 7.9 – 10.1 × 13.6 – 17.6 (–18.4) µm [x = 8.1 × 16.0 µm, Q = 1.975], 3ellipsoid or ovoid, apiculus present, smooth, thin-walled, guttiulate, golden yellow in KOH, amyloid. BASIDIA: 14.5 – 16.5 × 335.4 –41.0 µm, [x = 16.2 × 38.9 µm], clavate to fusoid, mono- to four spored basidia, stegmata pointed, thin-walled, guttiulate, hyaline in KOH; CHEILOSYSTIDIA: 12.35–21.85 × 40.4–48.5 µm, [x = 18.1 × 45.1 µm], clavate to fusoid to aciculate to utriform, thin-walled, guttiulate, hyaline in KOH. PLEUROCYSTIDIA: 11.3–18.8 × 28.6–47.5 µm, [x = 17.3 × 39.3 µm], clavate, guttiulate, hyaline in KOH. PILEIPELLIS: a cutis, hyphae cylindrical, 1.7–2.85 µm, thin-walled, hyaline in KOH. STIPTIPELLIS: cylindrical, 2.7–3.3 µm, hyaline to in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Ushu and Mash-Koon Mankial, solitary to gregarious on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, September 4, 2013; ISP # 048 and ISP # 069 (LAH).

COMMENTS: This species is new record for Pakistan.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 652-654 base pairs. In initial BLAST search of GenBank database, closest hit was Inocybe mimica (GenBank KF056319, Query coverage - 99%, Expect value- 0.0 Identity index - 99%) from India.

The newly generated ITS sequence of the Inocybe mimica along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 387/745 were conserved characters, 340 were variable, 215 were parsimony informative and 120 were singletons. The analysis was carried out for 31 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into

account. Final dataset had 397 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.21).

Plate 23

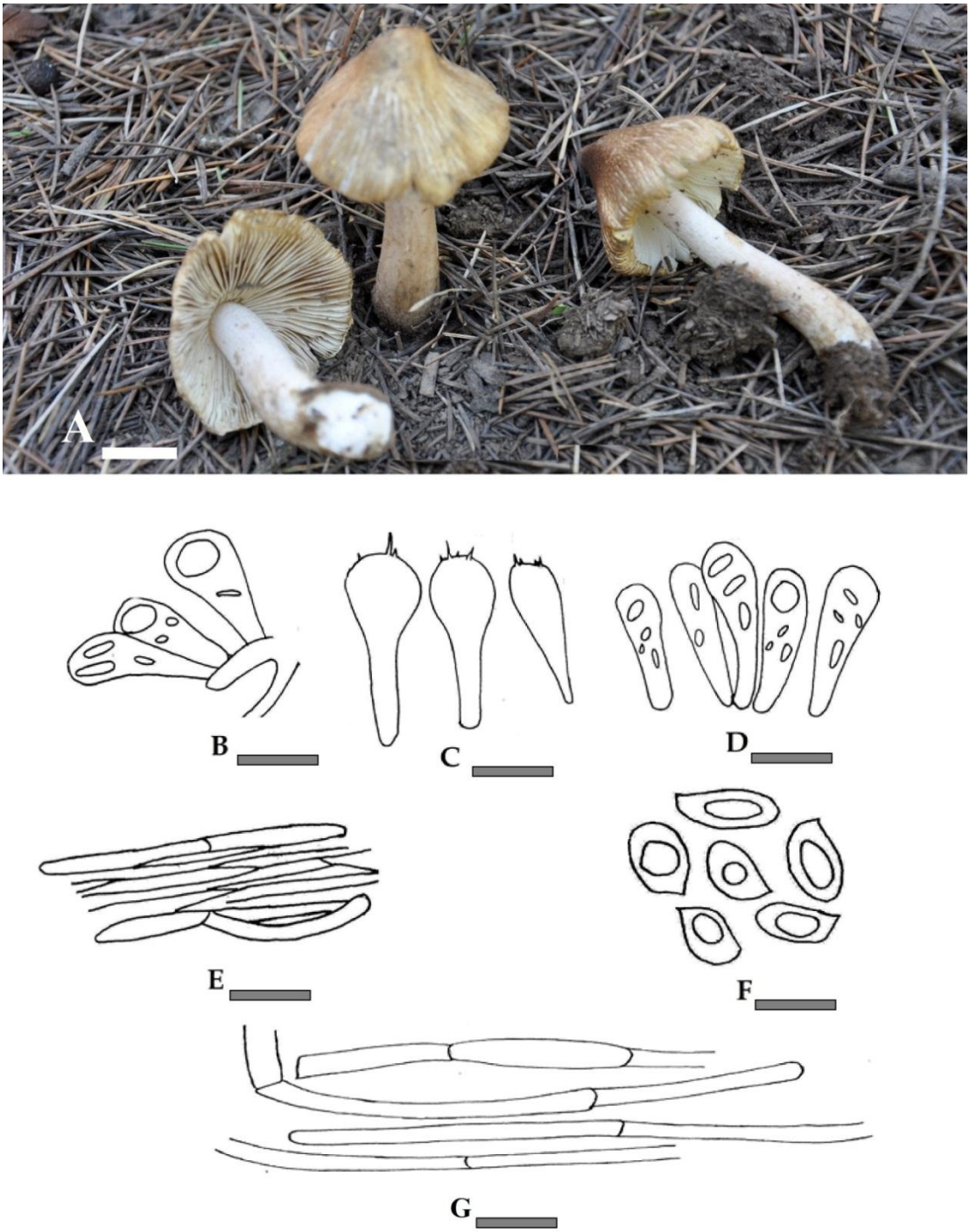


Figure – A-F Morpho-anatomical shapes of ***Inocybe mimica*** (A) Sporocarps; (B) Chielocystida (C) Basidia (D) Pleurocystidia (E) Stiptipellis (F) Basidiospores (G) Pileipellis ; Scale bars: A = Scale bar: 2.2 cm, B = 22 µm, C = 16.5 µm, D = 19 µm, E = 12 µm F = 12 µm, G = 6 µm

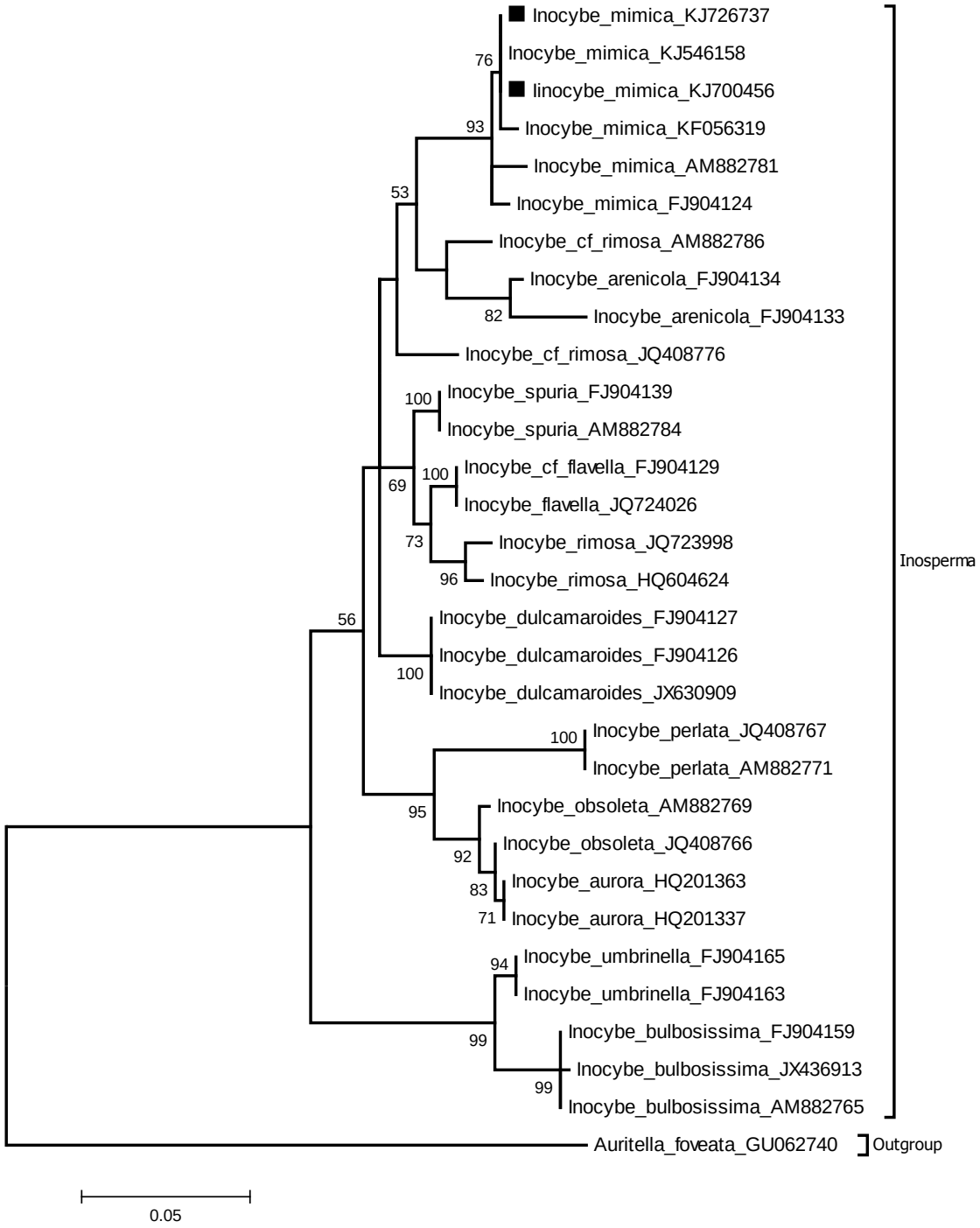


Figure 3.21 Molecular phylogenetic analysis of ***Inocybe mimica*** by Maximum Likelihood method. Tree with the highest log likelihood -1735.1446) is shown. Collection of Swat is represented by black cube (■).

20. ***Inocybe pallida*** Velen**,** *České Houby* 2: 366 (1920)

Plate 24 – Figs. A-H & 3.22

Morpho-Anatomical characterization

PILEUS: 1.5 – 2.5 cm diam., plane, umbonate; margin cottony, inrolled, often cracked; surface dry, fibrillose to appressed fibrillose, unbroken when young, later rarely torn at places; ochraceous reddish orange to moderate orange (2.5YR 5/6 – 5YR 6/8); context light orange yellow (10YR 9/8), not changing color where bruised. LAMELLAE: free and remote, close, ochraceous orange yellow (10YR 6/8); entire. STIPE: 3.2 – 3.8 cm × 0.2 – 0.6 cm, even and cylendrical; brilliant orange to light orange (5YR 5/6 – 10YR 9/8); context solid, mostly pallid. Taste and spore deposit not recorded.

BASIDIOSPORES: 10.8 – 15.8 × 10.8 – 13 [X = 11.8 × 13.5, Q = 1.472] gibbous (6-12 nodules), golden yellow, anamoyloid, guttiulate. BASIDIA: 28.3 – 34.7 × 10.8 – 17.0 μm, clavate, cylindric predominantly bisporic, rarely trisporic, sterigmata upto 9.5 μm. PLEUROCYSTIDIA: 61.8 – 74.1 × 55.9 – 71.3 × 18.8 – 28.8 μm metuloid, utriform to broadly fusiform or saccate and slightly tapered towards the apex, usually obtuse to rounded and crystalliferous, with a slender, basal pedicel; thick-walled, walls thick, hyaline. CHEILOCYSTIDIA: 22.6 – 32.3 × 61.8 – 74.1 μm metuloid, utriform to broadly fusiform or saccate and slightly tapered towards the apex, usually obtuse to rounded.CAULOCYSTIDIA: 63.7 – 68.0 × 21.2 – 25.9 same in shape to cheilocystidia. PILEAL CYSTIDIA: 47.2 – 68.0 × 21.2 – 25.9 same in shape to cheilocystidia. STIPITIPELLIS: cylindrical parallel, 4.5 – 11.8 μm; PILEIPELLIS: Hyphae cylindrical 5.1 – 13.0 μm width.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, 2 September 2013; ISP # 058 (LAH).

COMMENTS: This species is previously reported as *I. fastigata* from different regions of Pakistan including district Swat.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 583 base pairs. In initial BLAST search of GenBank database, closest hit was *Inocybe sp*. (GenBank JF927857, Query coverage - 98%, Identity index - 98%) submitted by Dr. Muhammad Hanif, from Pakistan. It is followed by *Inocybe pallida* (GenBank JF908198, Query coverage - 97%, Identity index - 98%) from USA.

The newly generated ITS sequence of the *Inocybe sp*. along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 310/748 were conserved characters, 431 were variable, 347 were parsimony informative and 76 were singletons. The analysis was carried out for 47 reprentative sequences. Both coding and non-coding positions were

included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 185 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.22).

Plate 24

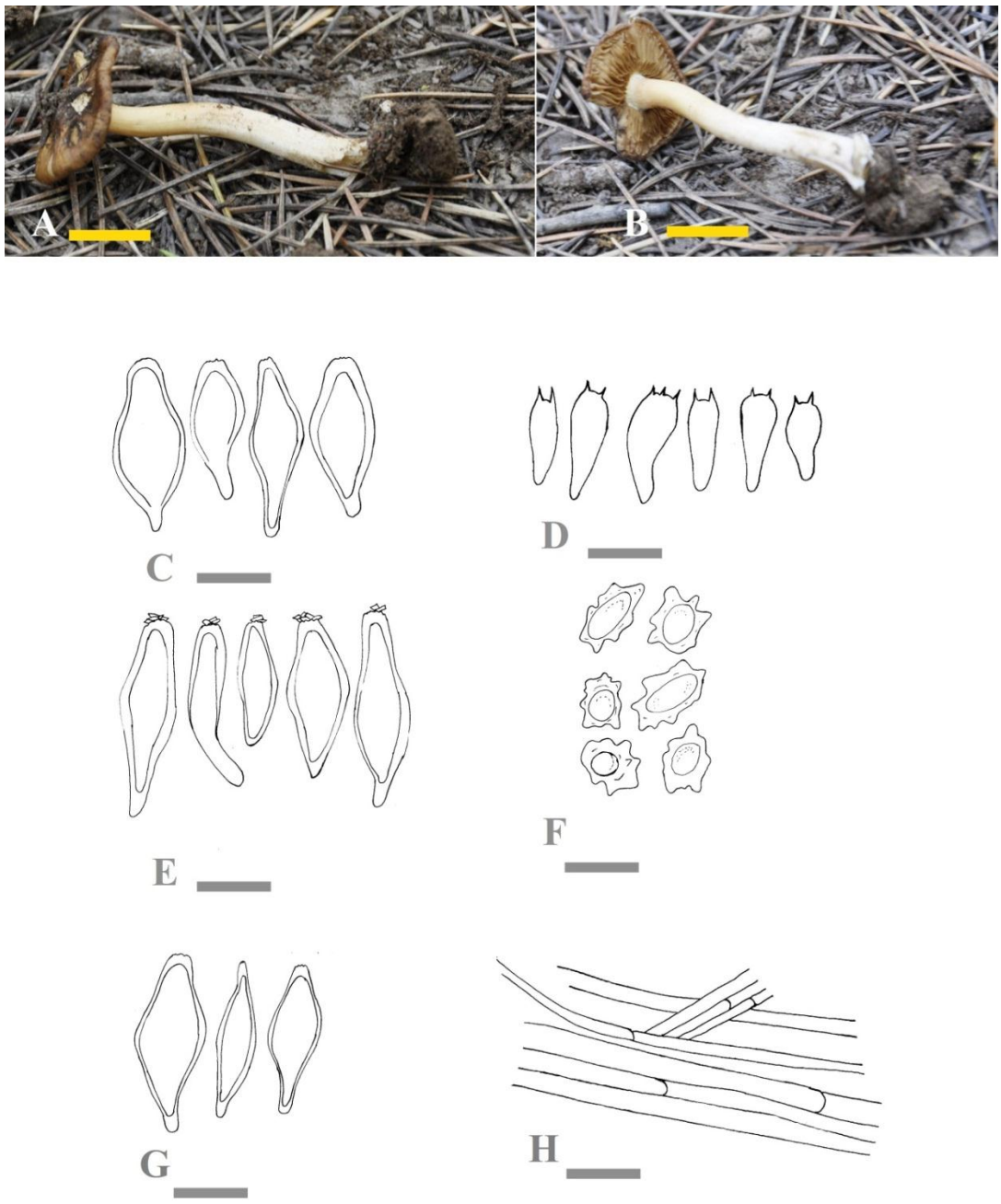


Figure A-H Morpho-anatomical shapes of ***Inocybe pallida*** (A-B) Basidiomata (C) Chielocystida (D) Basidia (E) Pleurocystidia (F) Basidiospores (G) Pileal Cystidia; (H) Stiptipellis. Scale bars: A-B = 1 cm, C = 28 µm, D = 21 µm, E = 27 µm, F = 9.5 µm, G = 33 µm.

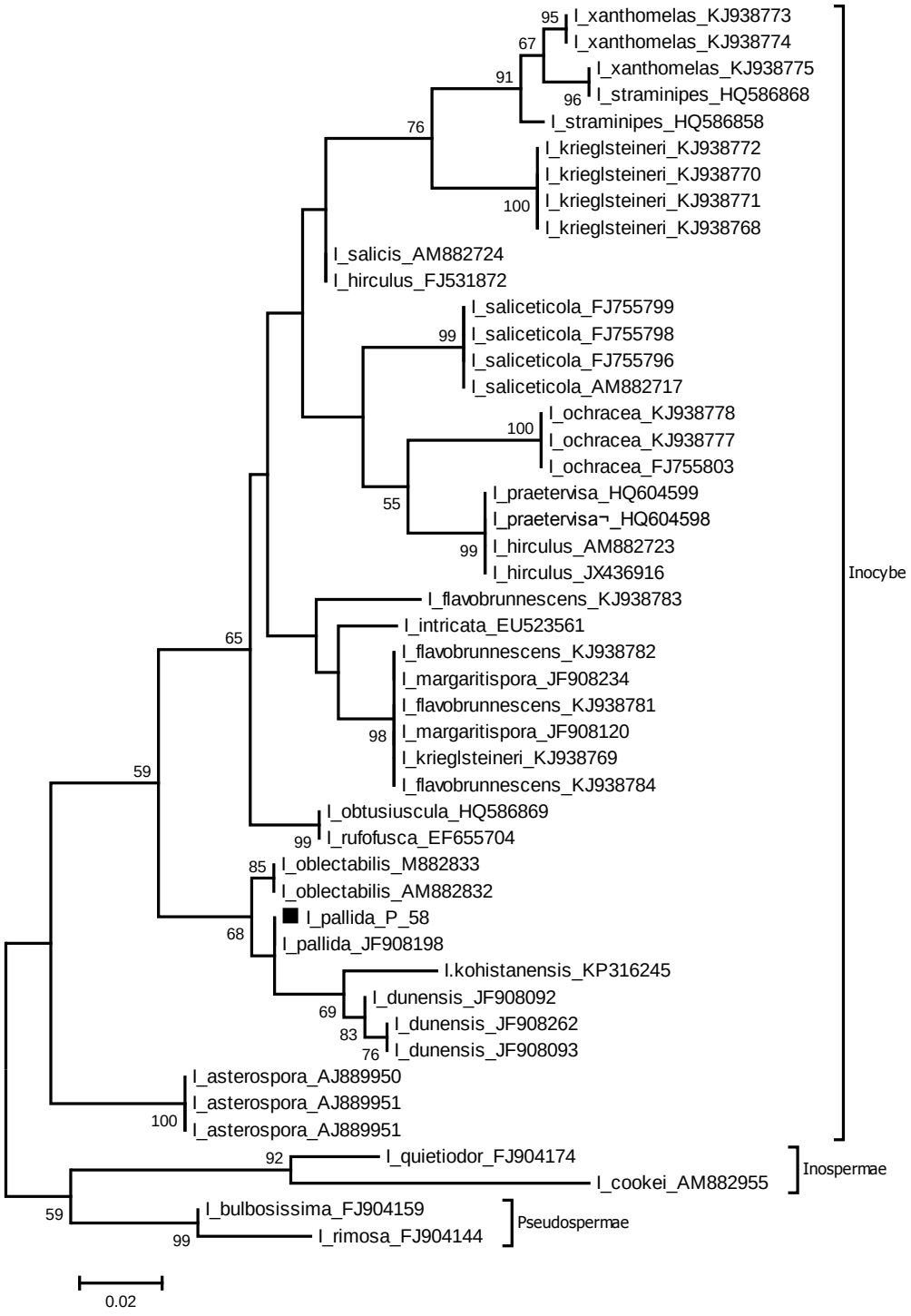


Figure 3.22 Molecular phylogenetic analysis of ***Inocybe pallida*** by Maximum Likelihood method. Maximum log likelihood value = (-937.3222) is shown. Collection of Swat is represented by black cube (■).

21. ***Inocybe rimosa*** **(**Bull.) P. Kumm. & Führ. Pilzk. 1871.

Plate 25 – Figs. A-G & 3.23

Morpho-Anatomical characterization

PILEUS**:** 4 – 5 cm across, plane, umbillicate, strong brown in center (5YR 4/6) to moderate orangish brown toward margins (5YR 7/8); margins plicate and recurrved; surface dry. FLESH**:** whitish, soft, not much thick. LAMELLAE**:** strong yellowish brown (10YR 5/6) to light yellowish brown (10YR 7/4) in color, fee and approximate, closed, edges raged and eroded, gills broad, lamellulae are also present. STIPE**:** 5.4 x 1.0 cm, milky white, hollow, equal slightly swollen at the base, dry, smooth.

BASIDIOSPORES**:** 11.3 – 14.9 × 7.1 – 10.1 µm [X = 8.3 × 13.2 µm Q = 1.5], broadly ellipsoid to elongated, thin walled, light yellowish brown in KOH, smooth margins without ornamentation. BASIDIA**:** one to-three spored, 20.3 – 227.6 × 8.3 – 10.4 µm, light yellowish brown in 5% KOH; sterigmata 2.9 – 5.3 µm. CHEILOCYSTIDIA: clavate, 23.9 – 27.9 × 8.4 – 8.7 µm, thin walled, hyaline to pale yellow in KOH. PLEUROCYSTIDIA: clavate, 22.0 – 39.3 × 9.4 – 10.2 µm, thin walled, hyaline to pale yellow in KOH. PILEIPELLIS: 4.3 – 9.2 µm, thin walled, yellowish brown in KOH. STIPE HYPHAE: 4.3 – 10.2 µm, thin walled, yellowish brown in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, September 4, 2013; ISP # 037 (LAH).

COMMENTS: Reported from Pakistan under its synonym, *I. fastigata*. However this is first report of this species from Swat.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 607 base pairs. In initial BLAST search of GenBank database, closest hit was *Inocybe mimica* (GenBank HQ604207, Query coverage - 100%, Expect value- 0.0 Identity index - 96%) from Canada

The newly generated ITS sequence of the *Inocybe mimica* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 389/710 were conserved characters, 310 were variable, 253 were parsimony informative and 56 were singletons. The analysis was carried out for 24 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 416 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Figs. 3.23).

Plate 25

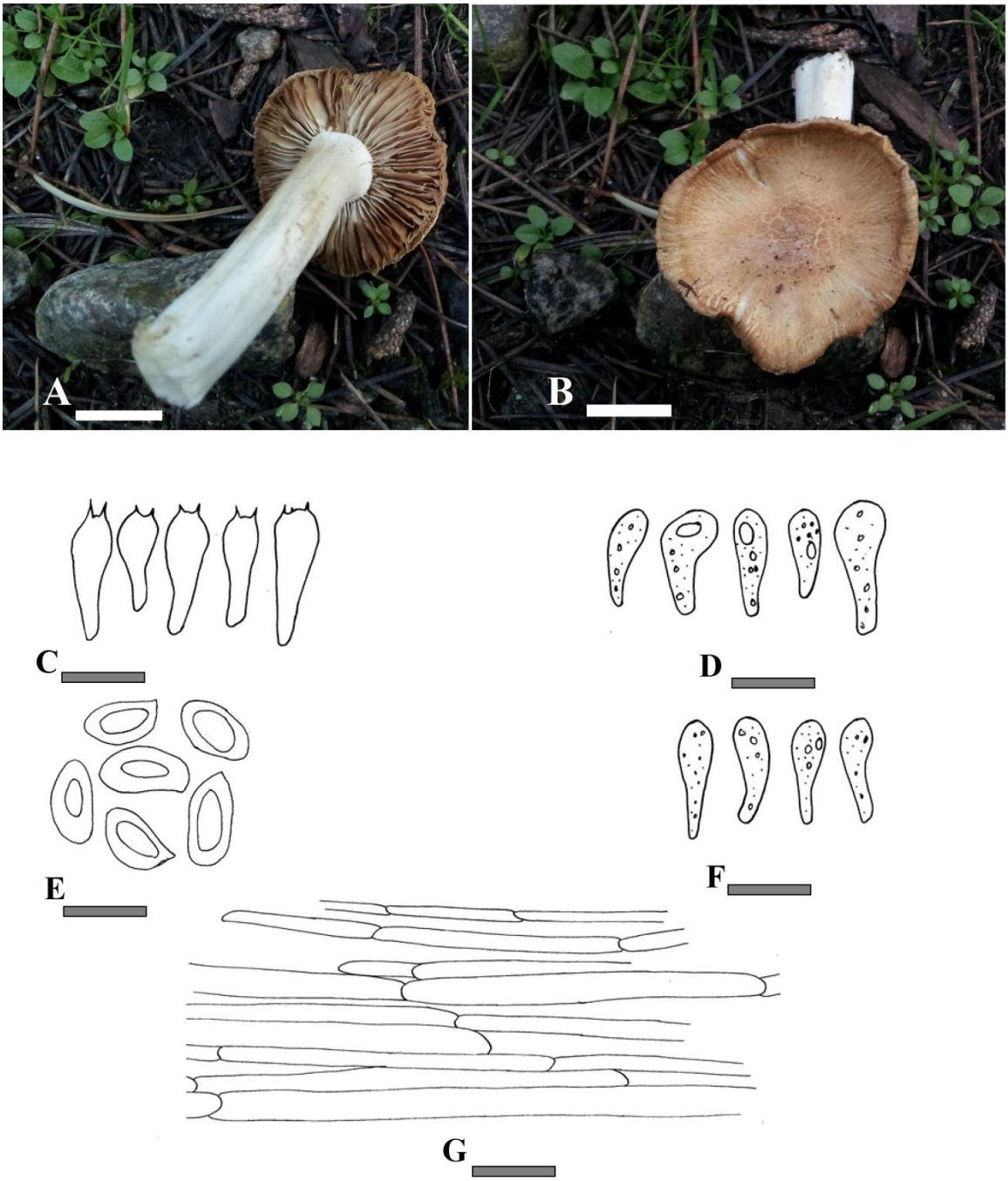


Figure –A-G Morpho-anatomical shapes of ***Inocybe rimosa*** (A-B) Basidiomata (C) Basidia (D) Chielocystida (E) Basidiospores. (F) Pleurocystidia (G) Stipe hyphae. Scale bars: A = 2 cm, B = 1.5 cm, C = 12 µm, D = 16, µm, E = 11 µm, F = 25 µm, G =

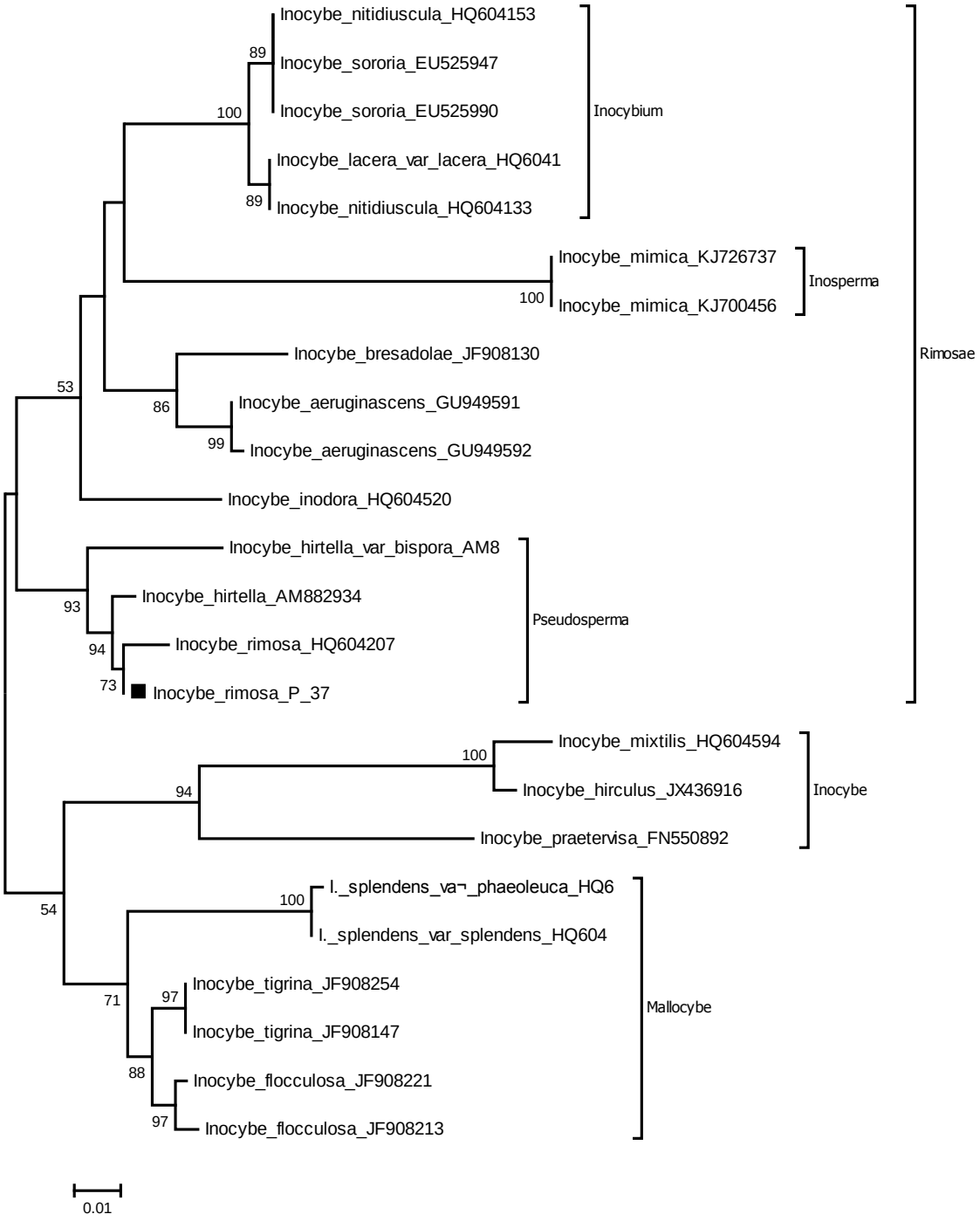


Figure 3.23 Molecular phylogenetic analysis of ***Inocybe rimosa*** by Maximum Likelihood method. Maximum log likelihood value = (-1778.5228) is shown. Collection of Swat is represented by black cube (■).

22. ***Inocybe swatica*** *nom*. prov. Plates 26 & 27 – Figs. A-I & 3.24

Etymology: "*Swatica*" refers to type locality.

Morpho-Anatomical characterization

PILEUS: 2 – 2.5 cm across., convex to hemispheric, slightly conical when young, becoming sub campanulate, more or less obtuse; moderate orange yellow to yellowish brown (10YR 7/8 – 10YR 5/8); margin sulcate to striate; surface dry, rugulose, rimose to rimulose; margin entire, rarely torn; context, not changing color where bruised. LAMELLAE: sinnuate, close, lamellules present; snow white to off white; edges pallid and entire. STIPE: 3.5 – 10.0 cm × 0.5 – 1.0 cm, equal and cylindrical, marginate base, hollow, soft, generally pallid; context solid, mostly pallid. Taste and spore deposit not recorded.

BASIDIOSPORES: (10.5) 11.4 – 12.76 (13) × (6.8)7.6 – 10.1 (11.4) µm, [X = 11 × 8.2, Q = 1.329], gibbous about a trapeziform outline, with 4–9 moderate to small-sized nodules, golden yellow in KOH. Basidia: *(*28.1) 32.6 – 33.1 (38.6) × (8.5) 10.2 – 14.6 (16.6) µm, clavate, hyaline in KOH, red in phloxin, bisporic to quadrisporic, mostly bisporic, sterigmata upto 4.7 – 8 µm, pointed. CHEILOCYSTIDIA: (94.4) 92.5 – 69.8 (59.9) × (25.9) 24.6 – 19.6 (18.4) µm, metuloid, utriform to broadly fusiform or saccate, usually obtuse to rounded and crystalliferous, with a slender, basal pedicel; thick-walled, walls thickness µm, hyaline, sterigmata 2 – 5.6 µm. PLEUROCYSTIDIA: (89.5) 88.4 – 75.6 × (31.6) 29.7 – 25.5 (24.6) µm. metuloid, utriform to broadly fusiform or saccate, usually obtuse to rounded and crystalliferous, with a slender, basal pedicel; thick-walled, walls thickness 4.0 – 5.4 µm, hyaline. CAUOLOCYSIDIA: 42.5 – 63.0 × 13.9 – 22.4 µm, metuloid, present

throughout stipe. PILEIPELLIS: a cutis, hyphae 5.9 – 14.8 μm wide, hayline, parallel cynindrical, septate, inter-woven, thin walled. STIPITIPELLIS: a cutis, hyphae 5.9 – 13.2 μm wide, hyaline, septate, thin walled. Clamp connections: present.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Mash-Koon Mankial, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, September 4, 2013; ISP # 079 (LAH).

Plate 26

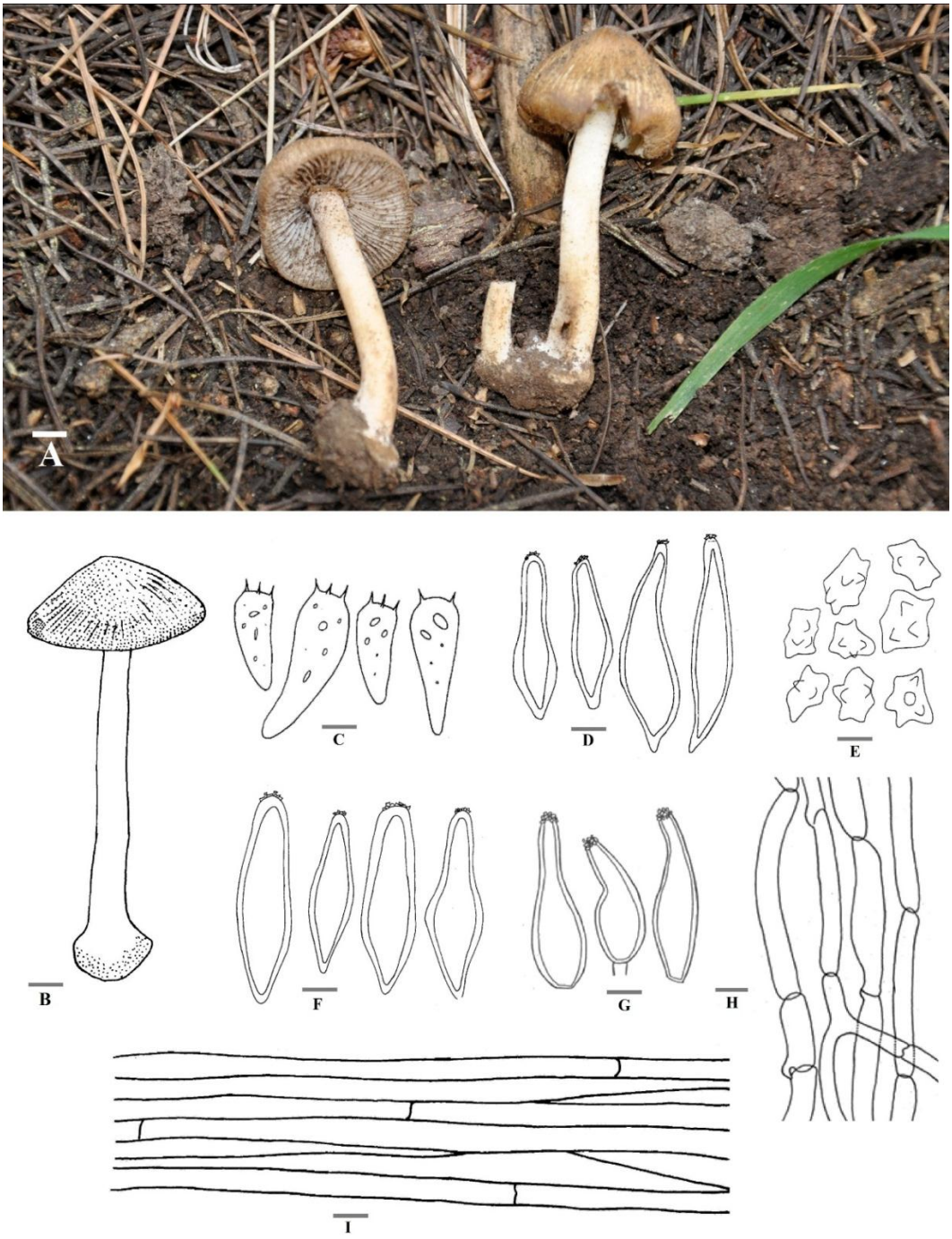
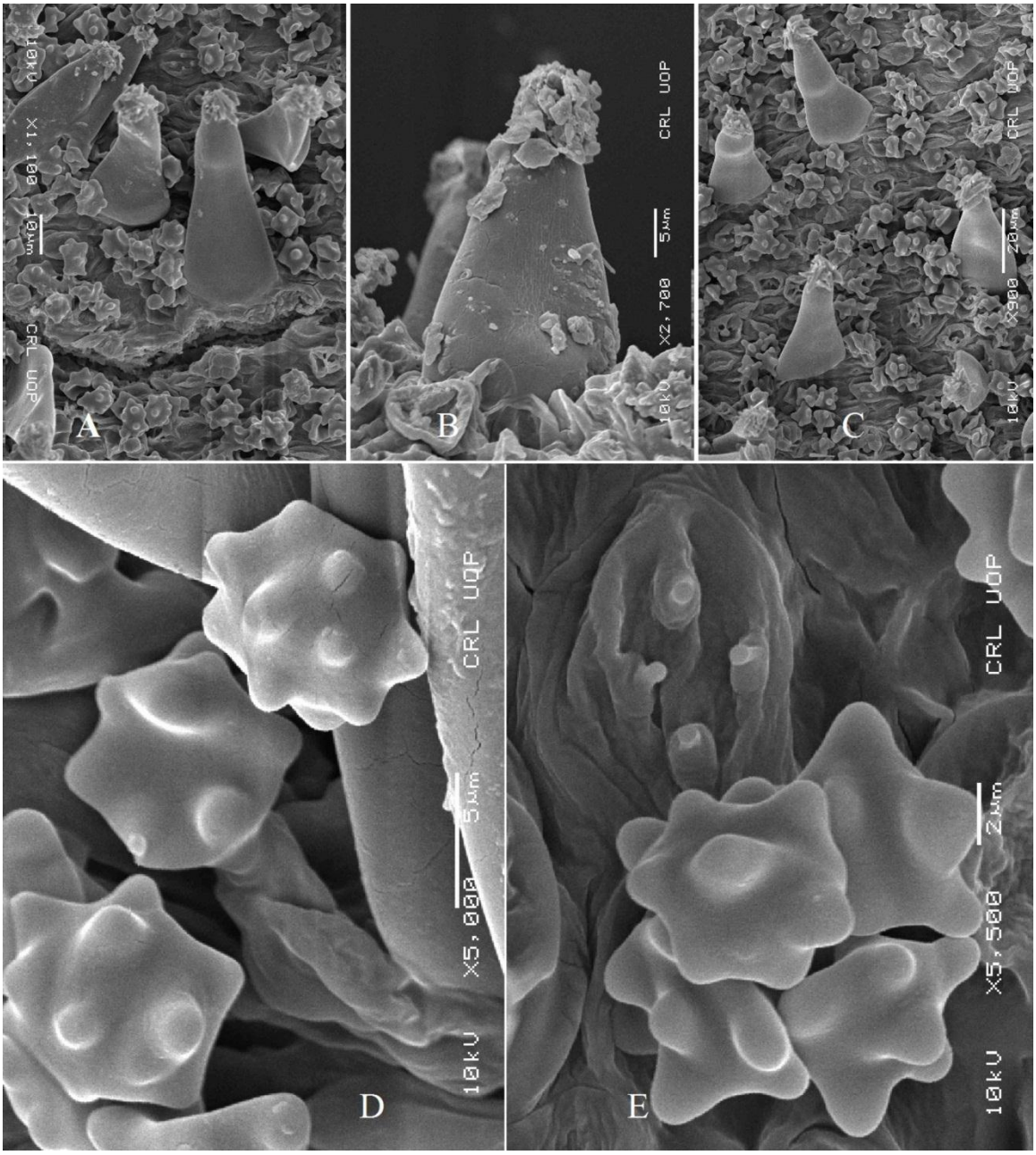


Figure A-I Morpho-anatomical shapes of ***Inocybe swatica*** nom. prov. (A) Basidiomata (B) Basidiome (C) Basidia (D) Cheilocystidia (E) Basidiospore (F) Pleurocystidia (G) Caulocystidia (H) Pileal hyphae (I) Stiptipellis. Scale bar: A = 0.3 cm, B = 0.5 cm C = 5 µm, D = 12 µm, E = 5 µm F = 11 µm G- I = 9 µm.

Plate 27



Scanning electron micrographs of ***Inocybe swatica*** nom. prov. (A-C) Pleurocystidia (D) Basidiospore (E) Basidium and basidiospores attached to basidium

Molecular Phylogenetic Analysis

Sequencing of the PCR products of nrITS region of *Inocybe swatica* generated 722–732 base pairs. Consensus sequence of 528 base pairs was subjected to BLAST. It has been revealed that these sequences were 86% identical to *I. praetervisa* (HQ604599), from Canada with 100% query cover.

The newly generated ITS sequence of the *Inocybe swatica* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 408/743 were conserved characters, 291 were variable, 179 were parsimony informative and 107 were singletons. The analysis was carried out for 24 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 379 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.24).

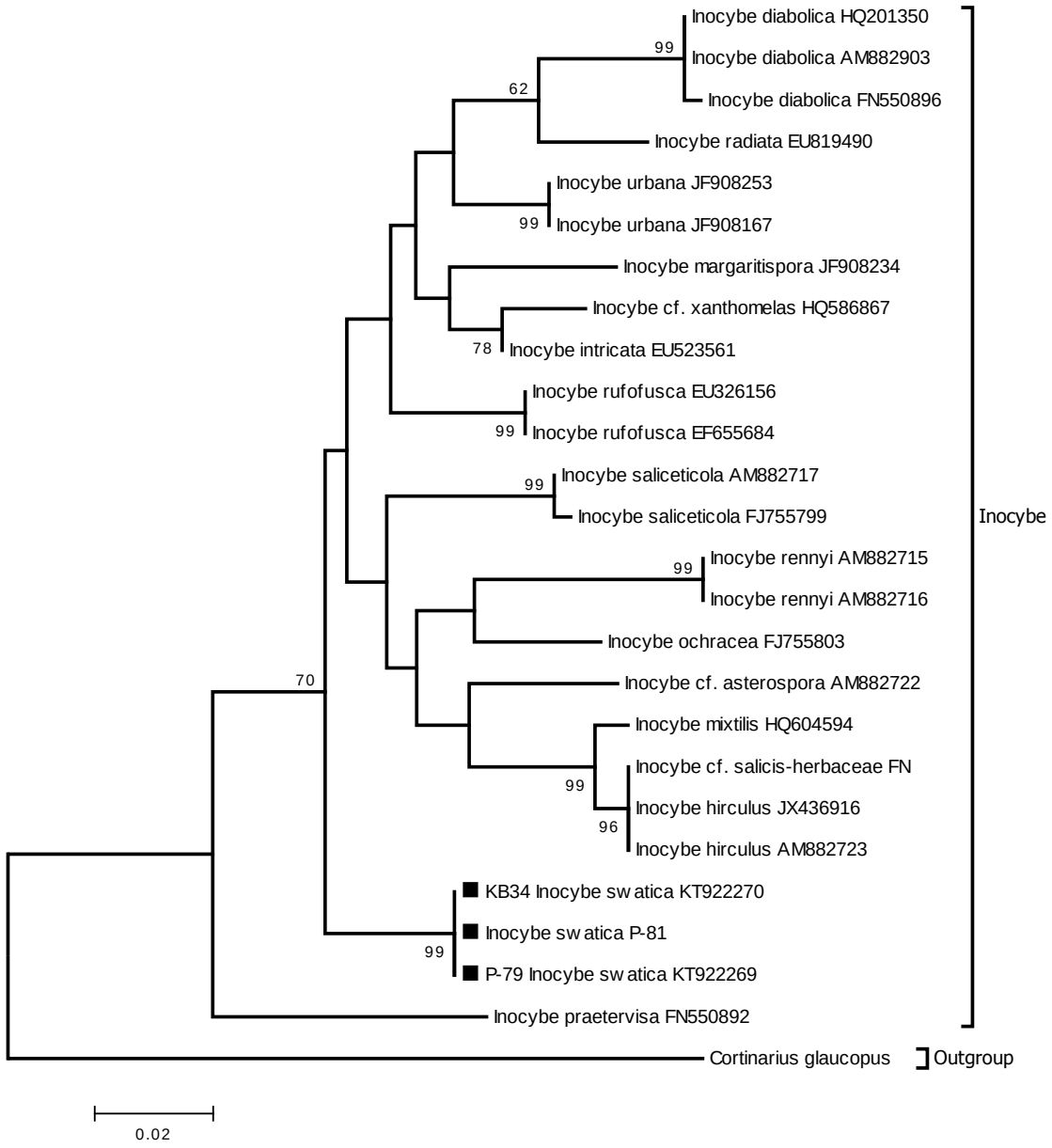


Figure 3.24 Molecular phylogenetic analysis of ***Inocybe swatica*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1559.5645) is shown. Collection of Swat is represented by black cube (■).

Marasmiaceae

Gymnopus

23 *Gymnopus barbipes*

24 *Gymnopus subcampanulatus* nom. prov.

Macrocystidia

25 *Macrocystidia obscurum* nom. prov.

23. *Gymnopus barbipes* Petersen & Hughes, *N. Amer. Fung.* 9(3): 2 (2014)

Plate 28 – Figs A-C & 3.25

Morpho-Anatomical characterization

PILEUS 1.5 – 2.0 cm across, depressed, more or less umbillicate; moderate orange to stronbrown (5YR 7/8 – 5YR 4/8); margin slightly cottony; surface dry, appressed fibrillose;; context, not changing color where bruised. LAMELLAE: free and approximate, close, lamellules present; pale yellow (2.5Y 9/6); edges pallid and entire. STIPE: 2 – 2.5 cm × 0.2 – 0.5 cm, equal and cylindrical, milky white in the base to pale yellow (2.5Y 9/4); context solid. Taste and spore deposit not recorded.

BASIDIOSPORES: 5.5 – 7 × 2 – 3.5 µm, ellipsoid, slightly flattened adaxially, smooth, thin-walled, aguttulate, hyaline in KOH, inamyloid. BASIDIA: 24 – 31 × 6 – 7.5 µm, clavate, 4-sterigmate, sterigma pointed. Cheilocystidia and pleurocystidia not observed.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Malam Jabba valey, solitary on logs in *Pinus wallichiana* forest, September 4, 2013; ISP # 008(LAH).

COMMENTS: This species is new record for Pakistan.

Plate 28

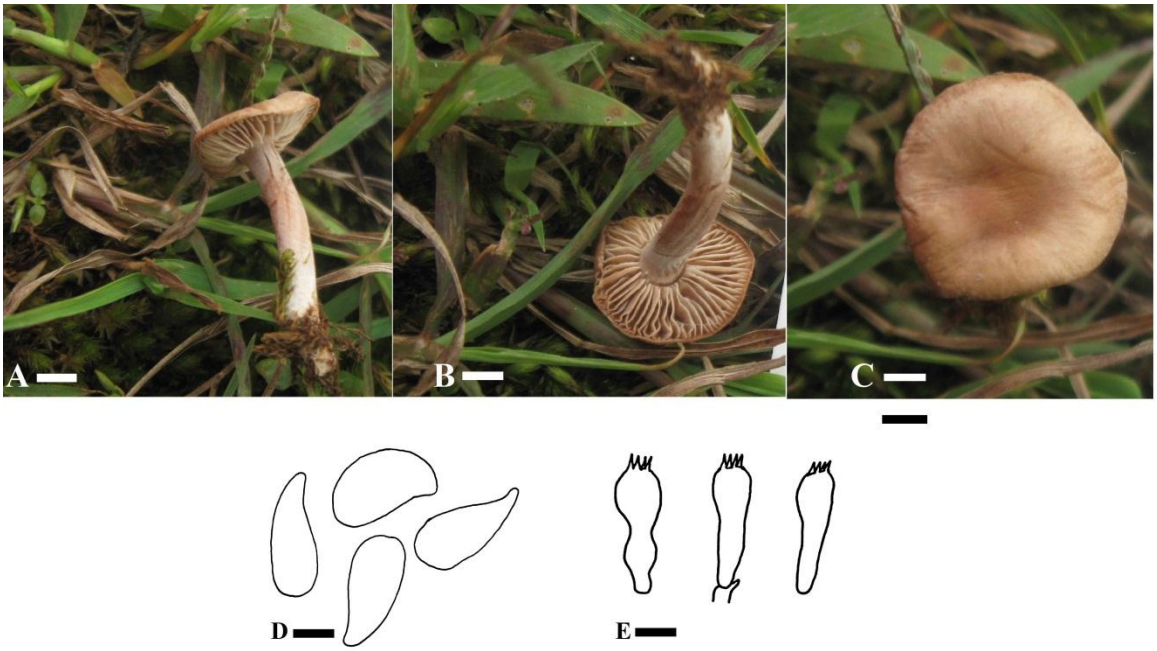


Figure (A-C) Morpho-anatomical shapes of ***Gymnopus barbipes*** Scale bar: A = 0.3 cm, B = 0.5 cm C = .4 cm, D = 2.8 **µm**, E = 9 µm

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 624 base pairs. In initial BLAST search of GenBank database, closest hit was *Gymnopus barbipes* (GenBank KJ416269, Query coverage - 100%, expect value - 0.0 and Identity index - 97%) from USA.

The newly generated ITS sequence of the *Gymnopus barbipes* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 497/797 were conserved characters, 291 were variable, 220 were parsimony informative and 70 were singletons. The analysis was carried out for 31 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 497 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.25).

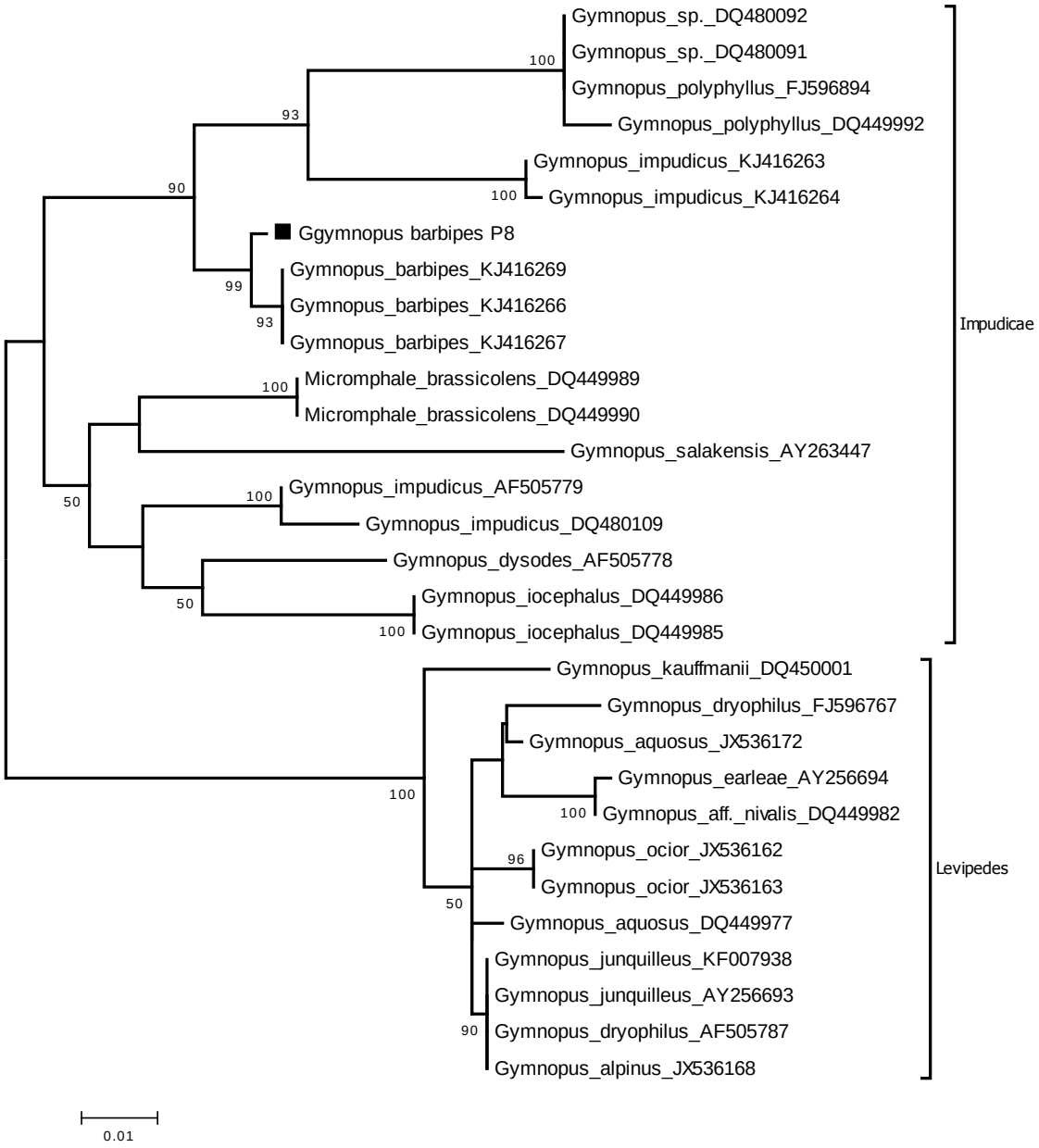


Figure 3.25 Molecular phylogenetic analysis of ***Gymnopus barbipes*** by Maximum Likelihood method. Maximum log likelihood value = (-1858.1347) is shown. Collection of Swat is represented by black cube (■).

24. ***Gymnopus subcampanulatus*** nom. prov. Plate 29 – Figs. A-G & 3.26

Etymology: "*subcampanulatus*" refers to pileus shape of this species.

Morpho-Anatomical characterization

PILEUS: 0.4 – 2 cm diam., conical to convex, reddish orange to light reddish orange (2.5YR 6/6 –2.5YR 6/4); slightly conical in youth, becoming sub campanulate, more or less umbonate convex upon expansion; margin plicate, strigose and incurved, often cracked; surface dry, fibrillose to appressed fibrillose, unbroken; greyish. LAMELLAE: adnate to adnexed, close, lamellules present; off white, edges pallid and entire. STIPE: 6 – 7.5 × .2 – .6 cm, even and cylendrical; light greyish orange (10Y 6/2) soft and hollow. Taste and spore deposit not recorded.

BASIDIOSPORES: 7.9 – 5.1 × 2.3 – 6.0 [mean 3.9 × 6.2 Q value 1.589] smooth, fusiform to sharply ovoid, hayline, anamyoloid, apiculus distinct. BASIDIA: 14.5 – 19.5 × 5.3 – 6.6 µm, clavate cylindric or ventricose (particularly when mono-sporic), monosporic to trisporic, sterigmata upto 4 µm, thin-walled hyaline CHEILOCYSTIDIA: 15.9 – 21.5 × 2.2 – 8.1 µm, clavate, thin-walled, hyaline. PLEUROCYSTIDIA: 16.5 – 24.3 × 2.7 – 5.1 µm clavate, thin-walled, hyaline. PILEIPELLIS: a cutis of cylindric hyphae 4.3 – 14.9 µm wide. CLAMP CONNECTIONS: not observed.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PUKHTUNKHWA, Kalam, Swat, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, 4 Sep. 2013; ISP # 041 (LAH).

Plate 29

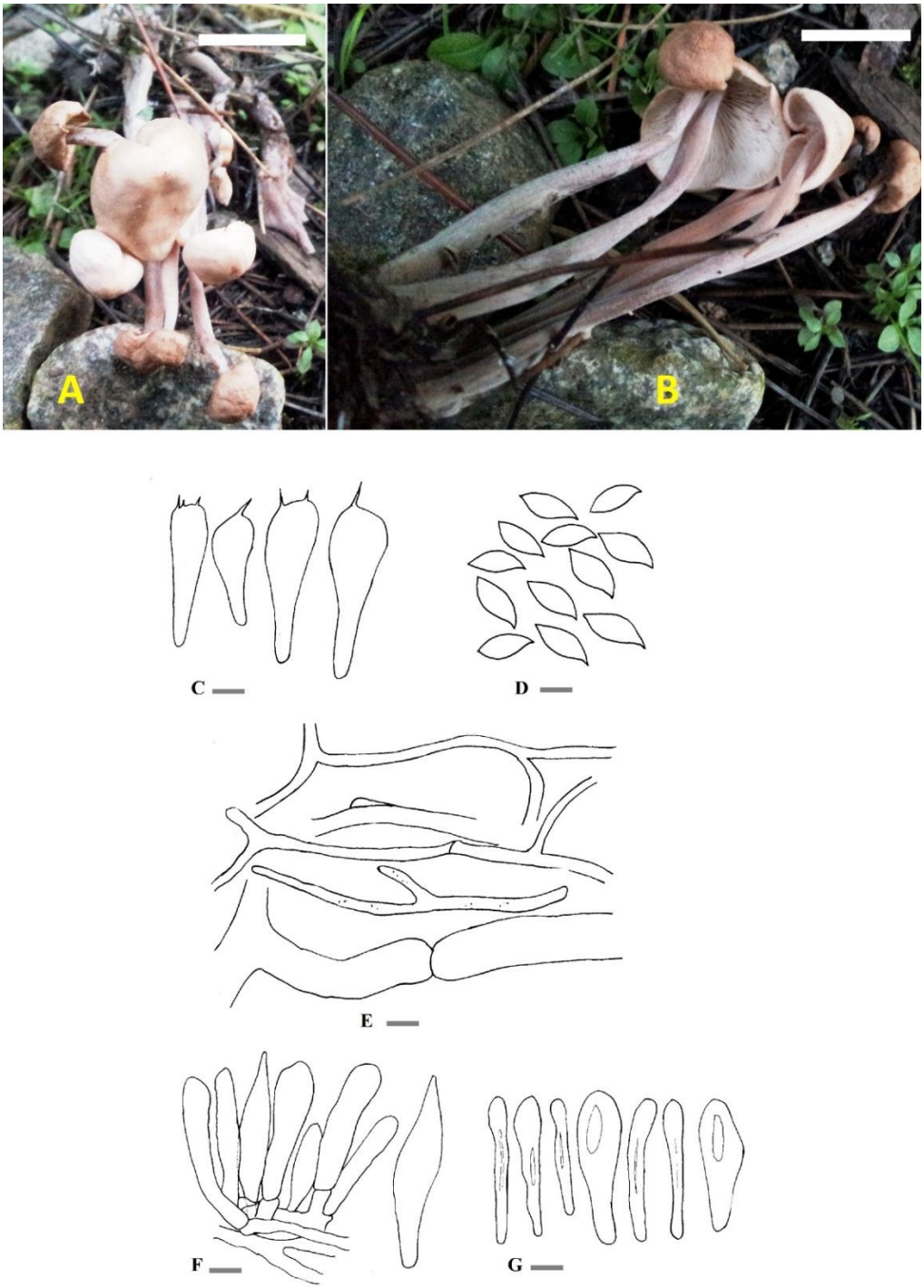


Figure A-G Morpho-anatomical shapes of ***Gymnopus subcampanulatus*** nom. prov. (A-B) Basidiomata (C) Basidia (D) Basidiospores (E) Pilepellis; (F) Pleurocystidia (G) Chielocystida. Scale bars: A = 1.7 cm, B = 1.5 cm, C = 3.3 µm, D = 2.8 µm, E = 3.8

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 624 base pairs. In initial BLAST search of GenBank database, closest hit was *Gymnopus readiae* (GenBank HQ533036, Query coverage - 90%, expect value - 0.0 and Identity index - 93%) from New Zealand The newly generated ITS sequence of the *Gymnopus subcampanulatus* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 528/962 were conserved characters, 379 were variable, 252 were parsimony informative and 115 were singletons. The analysis was carried out for 38 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 543 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.26).

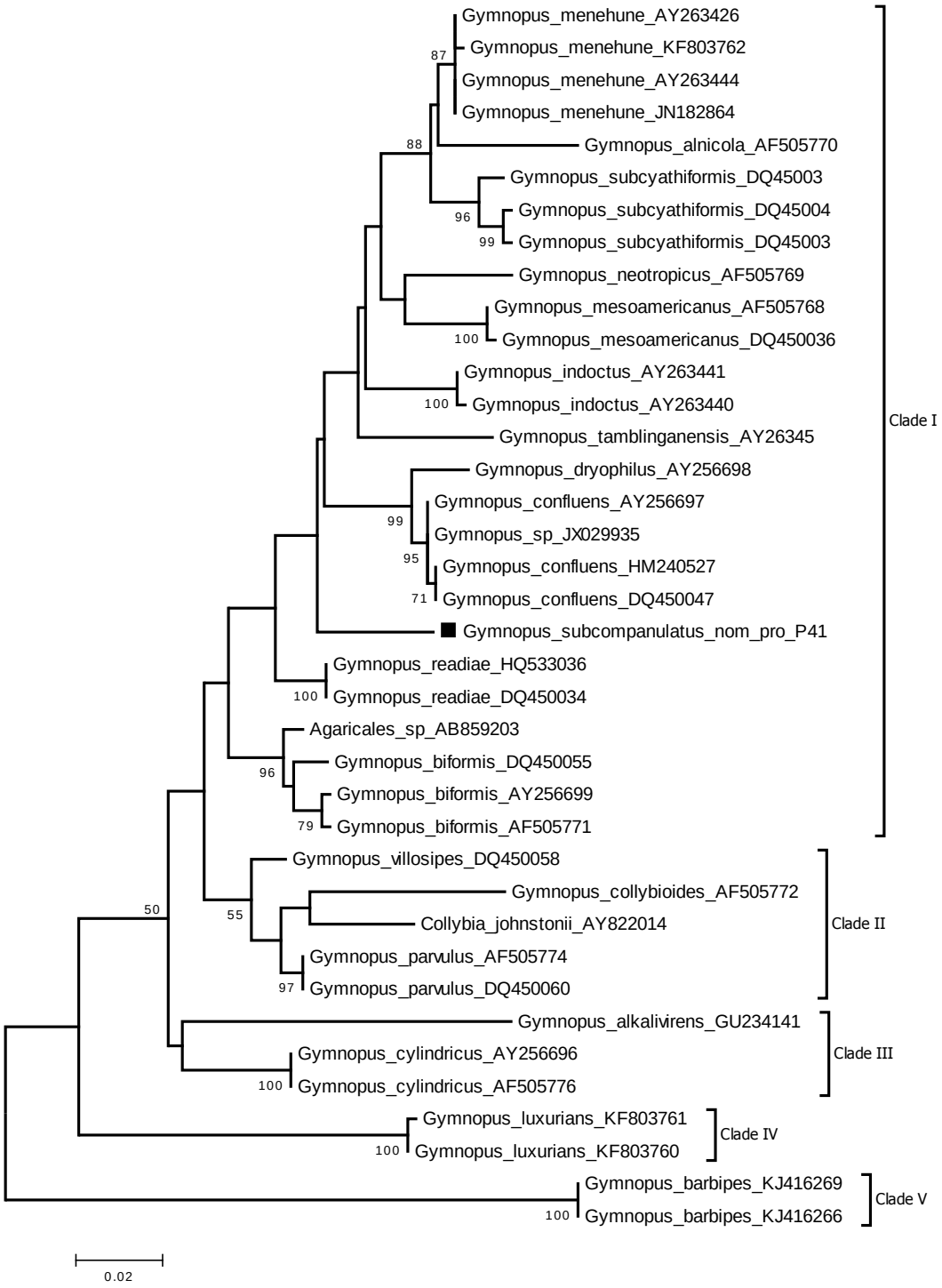


Figure 3.26 Molecular phylogenetic analysis of ***Gymnopus subcampanulatus*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-3217.33) is shown. Collection of Swat is represented by black cube (■).

25. ***Macrocystidia obscurum*** nom. prov. Plate 30– Figs. A-F & 3.27

Etymology: "*obscurum*" refers to the darkish complexion this species.

Morpho-Anatomical characterization

PILEUS: 1.5 – 2 cm diam., cuspidate conic; surface velvety, fibrillose to appressed fibrillose, even; margin plicate, irregular; curvature deflexed; light reddish brown () to brownish orange (2.5YR 5/4 – 5YR 5/8). LAMELLAE: sinuate, close, lamellules alternate, about 1/3 in length of lamellae; moderate orange to brownish orange (5YR 6/8 – 5YR 5/8); edges even. STIPE: 4.5 × 0.2 cm, even and cylindrical, moderate brown (7.5YR ¾) at base to strong brown (5YR 4/4) at apex. Taste and spore deposit not recorded.

BASIDIOSPORES: 6.0 –10 × 4.0 – 6.0 µm [X = 4.9 × 8.0 µm, Q = 1.632] smooth, ovoid to ellipsoid, hyaline in KOH and reddish in phloxin, apiculus distinct, thin walled. BASIDIA: 20 – 29 × 6.0 – 9.0 µm, clavate, cylindric, guttiulate, monosporic to quadrisporic, PLEUROCYSTIDIA: 32.4 – 58.9 × 14.7 – 21.8 µm, fusoid ventricose to mucronate, with a slender basal pedicel; thin-walled, hyaline. STIPITIPELLIS a cutis, 2.9 – 13.5 µm, cylindrical, parallel hyaline in KOH, thin wall.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Malam Jabba valley, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, September 4, 2013; ISP # 007 (LAH).

Plate 30

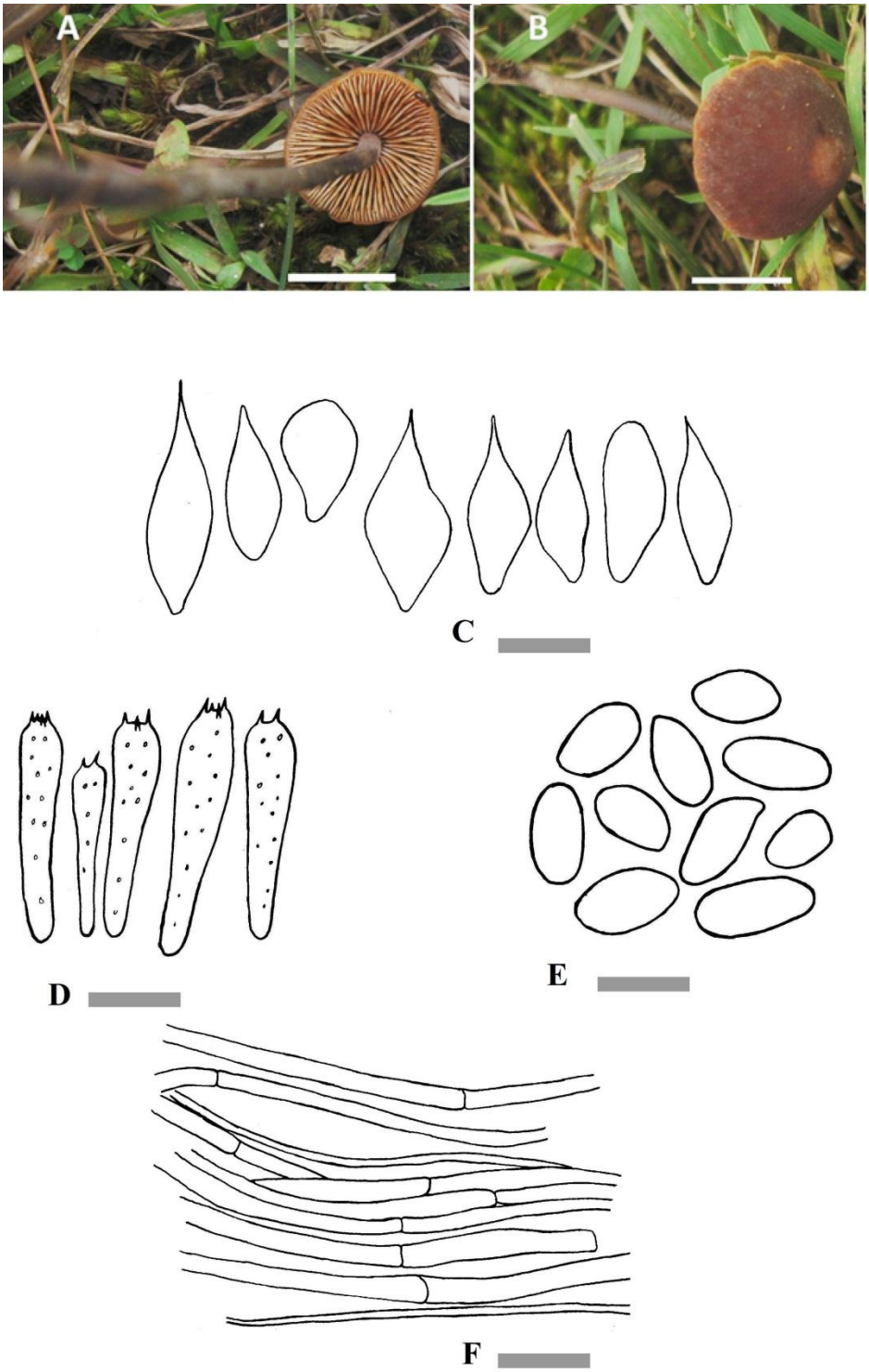


Figure (A-F) Morpho-anatomical shapes of ***Macrocystidia obscurum*** nom. prov. (A-B) Basidiomata; (C) Cystidia (D) Basidia (E) Basidiospores (F) Stipe hyphae. Scale bars: A-B = 1 cm, C = 23 µm, D = 10 µm, E = 7.5 µm, F = 38 µm

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 604 base pairs. In initial BLAST search of GenBank database, closest hit was *Macrocystidia reducta* E. Horak & Capellano (GenBank JQ694109, Query coverage - 91%, expect value - 0.0 and Identity index - 90%) from New Zealand, followed by *Macrocystidia cucumis* (Pers.) Joss. (GenBank HQ604758, Query coverage - 95%, expect value - 0.0 and Identity index - 88%) from Canada.

The newly generated ITS sequence of the *M. obscurum* nom. prov. along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 401/808 were conserved characters, 300 were variable, 221 were parsimony informative and 72 were singletons. The analysis was carried out for 24 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 412 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.27).

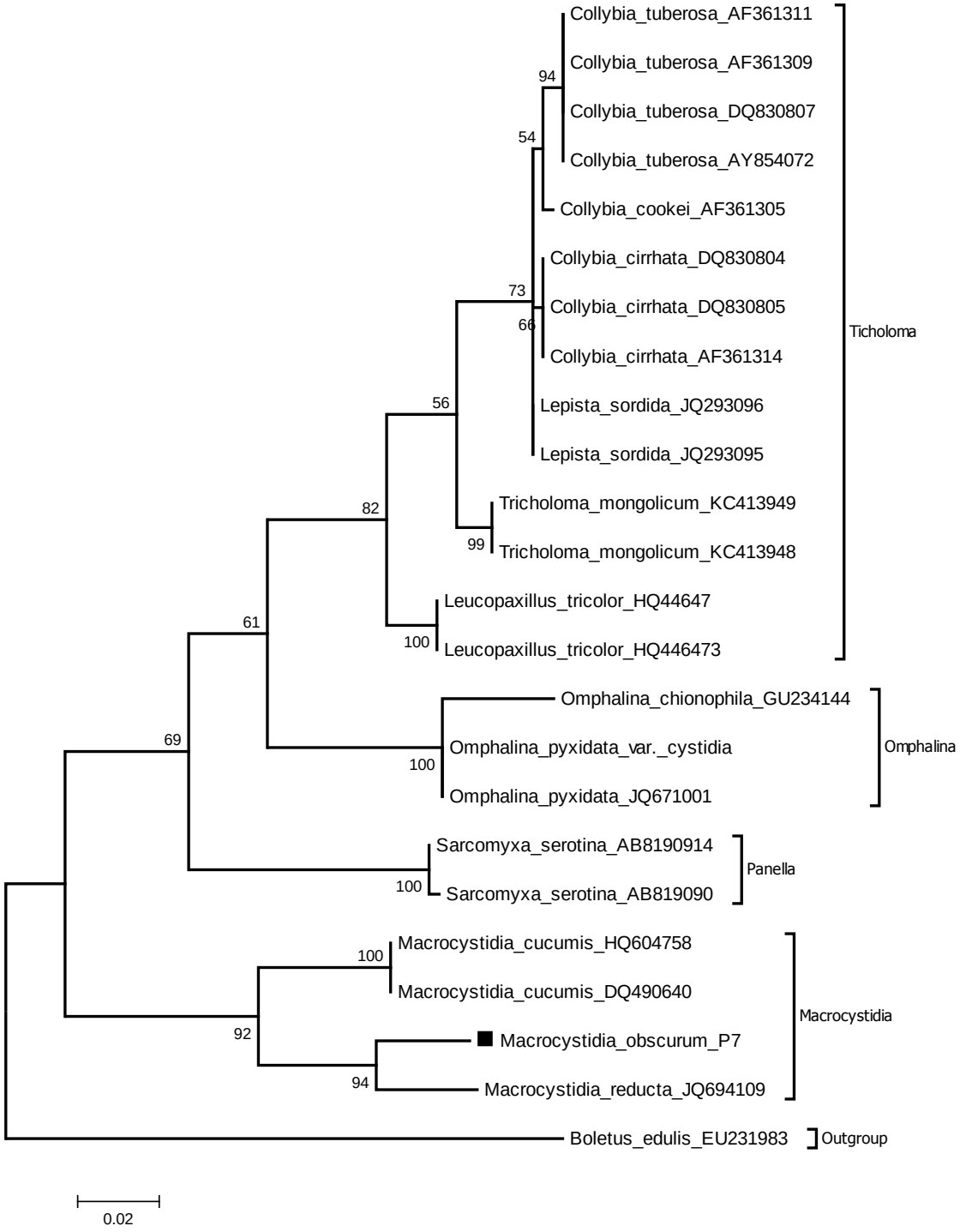


Figure 3.27 Molecular phylogenetic analysis of ***Macrocystidia obscurum*** nom. prov.by Maximum Likelihood method. Maximum log likelihood value = (-2295.9291) is shown. Collection of Swat is represented by black cube (■).

Mycenaceae

Mycena

26 *Mycena bractea* nom. prov.

27 *Mycena lacerata* nom. prov.

28 *Mycena pura*

Xeromphalina

29 *Xeromphalina flavens* nom. prov.

26. ***Mycena bractea*** nom. prov. Plate 31 – Figs A-F & 3.28

Etymology: "*bractea*" refers to the pate pileus of this species.

Morpho-Anatomical characterization

PLEUS: 1 – 2.5 cm diam, plane, umbonate central disc, white: surface appressed fibrillose; curvature straight and flaring; margin plicate. LAMELLAE: free and approximate, sub distant, colour same to pileus; margins entire; STIPE: 2.5 – 4.0× 0.2 – 0.3 cm, centrally attached, soft, hollow, cylindrical, equal,white.

BASIDIOSPORES: 5.1 – 8.4 × 3.7 – 5.1 µm [x = 4.6 × 6.5 µm, Q = 1.426], broadly ellipsoid, apiculus present, smooth, thin-walled, mono guttiuate, hyaline in KOH. BASIDIA: 22.1 – 33.0 × 6.5 – 9.8 µm, clavate, bisporic to quadrisporic, thin-walled, guttiulate, hyaline in KOH; CHEILOCYSTIDIA: 46.0 – 70.0 × 17.5 – 31.4 µm, broadly fusoid to fusoid-venticose, thin walled, hyaline in KOH. PLEUROCYSTIDIA: 51.4 – 64.4 × 15.1 – 22.7 µm, fusoid to fusoid-venticose, thin walled, hyaline in KOH. STIPE HYPHAE: 3.0 – 9.9 µm, cyledrical, parallel, thin walled, hayaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Miandam, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don and *Abies pidrow* forest, 9 September 2013; ISP # 123 (LAH).

Plate 31



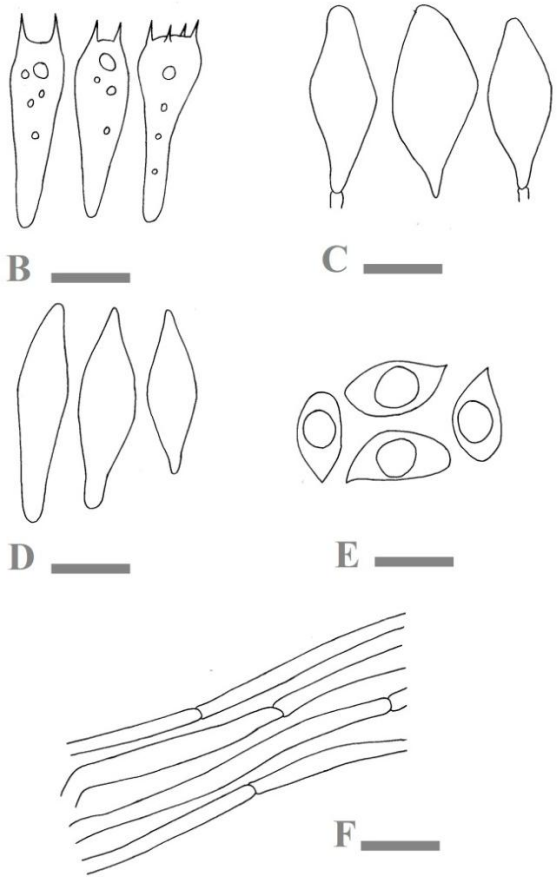


Figure (A-F) Morpho-anatomical shapes of ***Mycena bractea*** nom. prov. (A) Basidiomata (B) Basidia (C) Cheilocystidia (D) Pleurocystidia (E) Basidiospore (F) Stipe hyphae. Scale bar: A = 1.3 cm, B = 14 µm, C = 25 µm, D = 35 µm, E-F = 12

27. ***Mycena lacerata*** nom. prov. Plate 32 – Figs.A-E & 3.28

Etymology: "*lacerata*" refers to the ragged texture of gills of this species.

Morpho-Anatomical characterization

PLEUS: 2 – 3 cm diam, convex, obtuse, light yellowish pink to pale yellowish pink (2.5YR 9/4 – 7.5YR 9/2), surface, glabrous, dry; curvature, deflexed; margins, hirsute; context, pale pink (2.5YR 9/2). LAMELLAE: free and approximate, distant, thick, strong brown at attachment to pale yellowish pink at edges (5YR 4/6 – 7.5YR 9/2); marginate; STIPE: 4.0 – 4.6 × 0.2 – 0.4 cm, centrally to attached, fragile, soft, strong brown (5YR 4/6); smooth, equal (Fig. 3.14.1.1 A-B).

Basidiospores: 7.6 – 9.4 × 4.2 – 5.6 µm [x = 5.0 × 8.7 µm, Q = 1.74], ellipsoid, smooth, apiculate, thin-walled, germ pore present, hyaline in KOH, red in phloxin. Basidia: 17.5–26.3 × 5.9 – 7.3 µm, clavate, thin-walled, two to four spored, red in phloxin, hyaline in KOH, Sterigmata pointed 1.4 – 2.6 µm. PLEUROCYSTIDIA: 46.5 – 93.0 × 19.4 – 22.2 µm, thin walled, hyaline in KOH. Clamp connections: present in all tissues (Fig. 3.14.1.1 C-E).

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Malam Jabba, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don and *Abies pidrow* forest,02 September 2013; ISP # 04 (LAH).

Plate 32



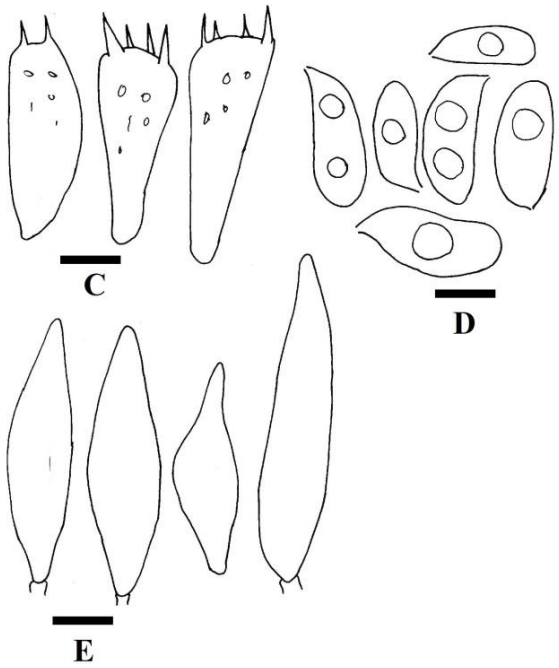


Figure (A-E) Morpho-anatomical shapes of ***Mycena lacerata*** *nom. prov.* (A-B) Basidiomata; (C) Basidia (D) Basidiospores (E) Pleurocystidia. Scale bars: A = 0.8 cm, B = 0.5 µm C = 7 µm, D = 4.5 µm, E = 14 µm.

28. ***Mycena pura*** **(Pers.) P. Kumm.** *Führ.Pilzk.* (Zerbst): 107 (1871)

Plate 33 – Figs. A-S & 3.28

Morpho-Anatomical characterization

PLEUS: 3.5 cm diam, hemispheric, umbonate central disc; very light violet to very pale violet (10BP 8/6 – 10BP 9/4) with smooth velvety surface; curvature deflexed; margin translucent. LAMELLAE: free and approximate, crowded, thin, colour same to pileus; margins curved; STIPE: 5.2 × 0.6 cm, centrally attached, soft, hollow, cylindrical dark greyish yellow (2.5Y 7/4) in the lower quarter to very light violet at upper places (10BP 8/8).

Basidiospores: 4.2 – 4.9 × 6.4 – 7.8 µm [x = 4.8 × 7.0 µm, Q = 1.458], oblong or ellipsoid, apiculate, smooth, hyaline in KOH, thin-walled. Basidia: 7.8 – 11.6 × 18.7 – 23.2 µm, clavate, bisporic to quadrisporic, hyaline in KOH, thin-walled. CHEILOCYSTIDIA: 15.9 – 20.1 × 40.8 – 55.0, fusoid to fusoid-venticose, thin walled, hyaline in KOH. PLEUROCYSTIDIA: same as cheilocystidia.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, solitary on ground under *Cedrus deodara* (Roxb. ex D. Don) G. Don, September 4, 2013; ISP # 016 (LAH).

COMMENTS: Reported from Pakistan by Shibata (1992), Murakami (1993) and Iqbal and Kalid (1996) on ground in mixed vegetation under Pine forests of Pirchin (AJK), Nathiagali and Malakundi. This species is also reported from Swat earlier.

Plate 33



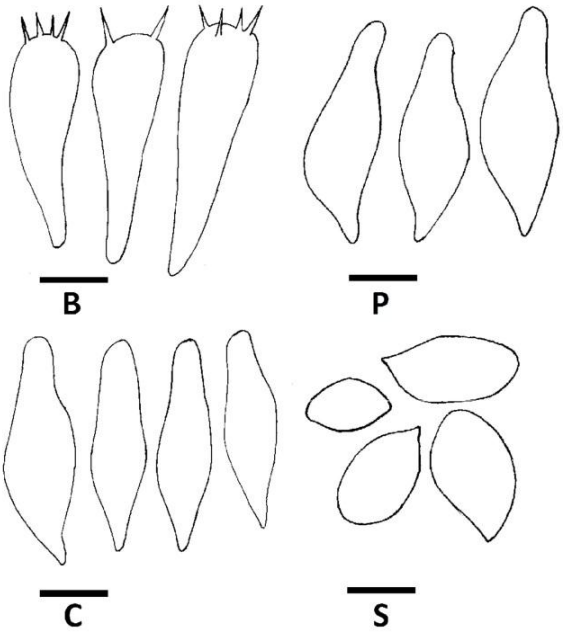


Figure (A-S) Morpho-anatomical shapes of ***Mycena pura*** (A) Basidiome (B) Basidia (C) Cheilocystidia (P) Pleurocystidia (S) Basidiospores. Scale bars: A = 1.5 cm, B = 6.3 µm, C = 15 µm, P = 15 µm, S = 4 µm.

Molecular Phylogenetic Analysis

ITS region of *Mycena bractea, Mycena lacerata* and *Mycena pura* generated sequences of 617, 613 and 567 base pairs respectively. In initial BLAST search of GenBank database, closest hit of *Mycena bractea* was *Mycena pura* (FN394588) from Denmark with 97% Identity, followed by *Mycena pura* (JF908472) from Italy with 96% Identity. Similarly closest hit for *Mycena lacerata* was *Mycena pura* (KJ705186) from Canada with 95% Identity, followed by *Mycena rosella* (JF908488) from Italy with 95% Identity index and closest hit for *Mycena pura* in initial BLAST search of GenBank database was *Mycena pura* (JF908472) from Italy with 100% Identity.

The newly generated ITS sequence of the *Mycena bractea* nom. prov.*, Mycena lacerata* nom. prov. and *Mycena pura* along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 444/732 were conserved characters, 187 were variable, 68 were parsimony informative and 117 were singletons. The analysis was carried out for 29 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 500

positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.28).

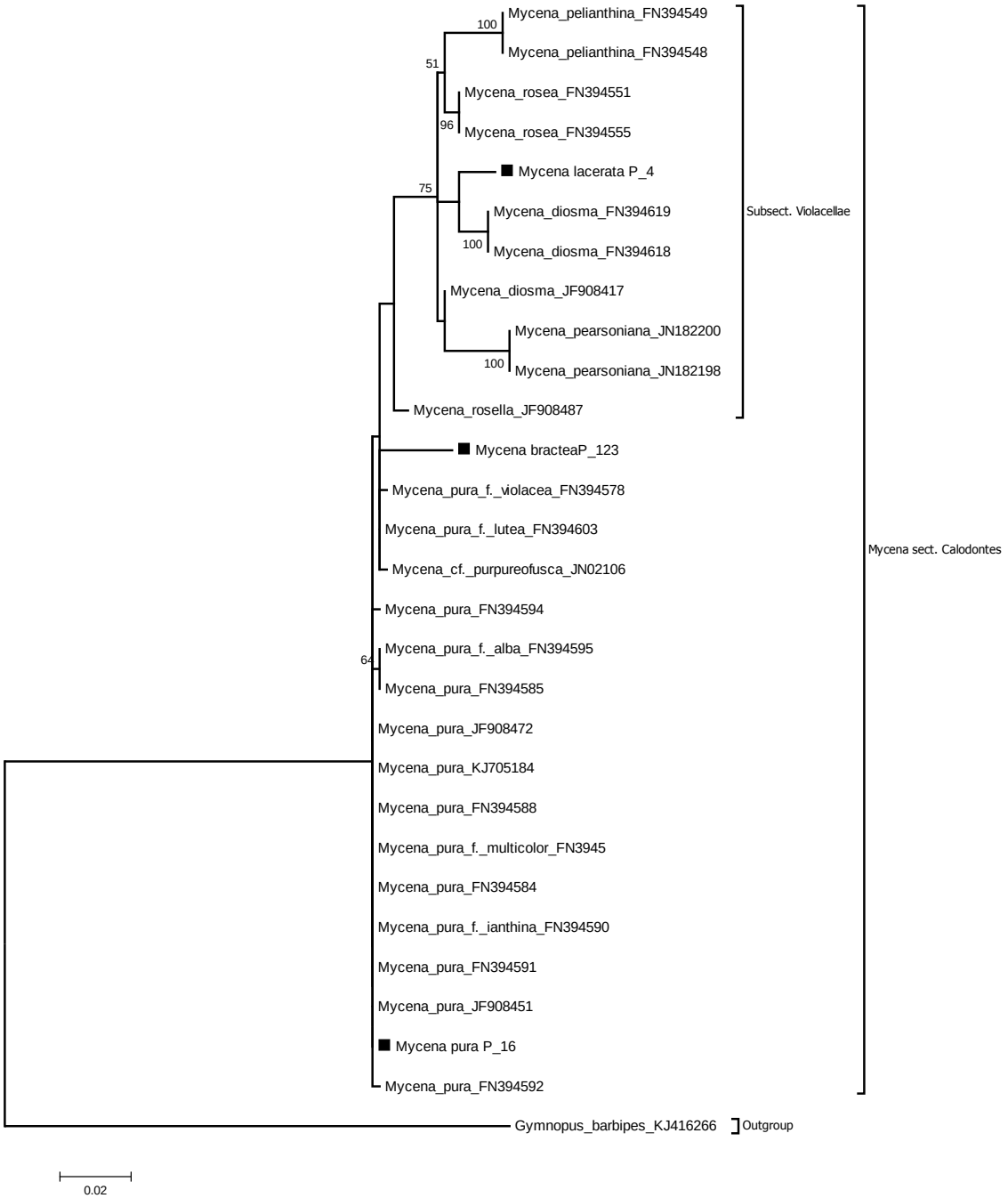


Figure 3.28 Molecular phylogenetic analysis of ***Mycena bractea*** nom. prov., ***Mycena lacerata*** nom. prov. and ***Mycena pura*** by Maximum Likelihood method. Maximum log likelihood value = (-1398.9627) is shown. Collection of Swat is represented by black cube (■).

29. ***Xeromphalina flavens*** nom. prov. Plate 34 – Figs. A-E & 3.29

Etymology: "*flavens*" refers to the yellowish golden color of this species.

Morpho-Anatomical characterization

PLEUS: 1.5 – 2.5 cm diam, depressed, umbillicate central disc, brilliant yellow to dark orange yellow (2.5YR 9/10 – 10YR 6/8) with aapreesed fibrillose, velvety surface; curvature uplifted; margins stited; LAMELLAE: decurrent, distant to sub distant, thin, light yellow (7.5YR 9/6); margins ragged, equql,; STIPE: 2 – 2.5 × 0.1 – 0.3cm, centrally attached, pliable, hollow, cylindrical white to yellowish white (5Y9/2-5Y9/4); surface equal and smooth.

BASIDIOSPORES: 2.4 – 3.4 × 2.5 – 3.8 µm [X = 3.0 × 3.2 µm, Q = 1.066], rounded germpore evident, smooth, thin-walled, golden yellow in KOH, amyloid. BASIDIA: 14 – 24.1 × 4.0 – 6.1 µm, clavate, bistergmate, stegmata pointed, thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 13.0 – 24.5 × 3.3 – 6.0 µm, clavate thin-walled, hyaline in KOH. PLEUROCYSTIDIA: same to cheilocystidia.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam,

Solitary on rotton logs in *Cedrus deodara* forest, 03 September, 2013; ISP # 026 (LAH).

Plate 34

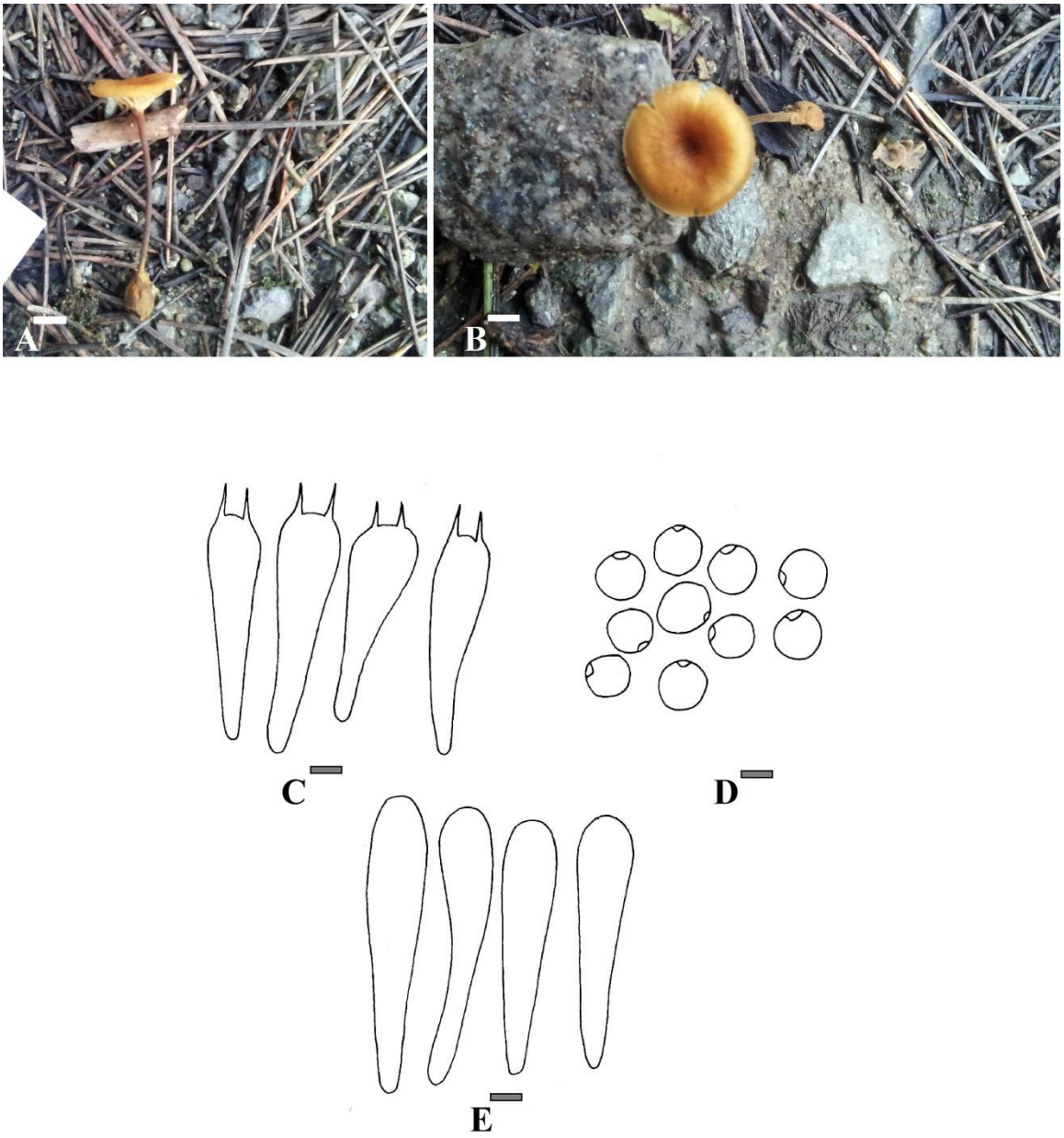


Figure (A-E) Morpho-anatomical shapes of ***Xeromphalina flavens*** nom. prov. (A-B) Basidiomata (C) Basidia (D) Basidiospores (E) Cheilocystidia. Scale bars: A = 0.5 cm, B = 1 cm, C = 3 µm, D = 1 µm, E = 2.4 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 643 base pairs. In initial BLAST search of GenBank database, closest hit was *Xeromphalina setulipes* (GenBank NR_121458, Query coverage - 100%, expect value - 0.0 and Identity index - 97%) from USA followed by *Xeromphalina setulipes* (GenBank GQ890701 Query coverage - 100%, expect value - 0.0 and Identity index - 97%) from Spain. Except these two sequences of *Xeromphalina setulipes,* all other matches were below the cut off value.

The newly generated ITS sequence of the *Xeromphalina flavens* nom. prov. along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 476/698 were conserved characters, 200 were variable, 144 were parsimony informative and 49 were singletons. The analysis was carried out for 13 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 387 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.29).

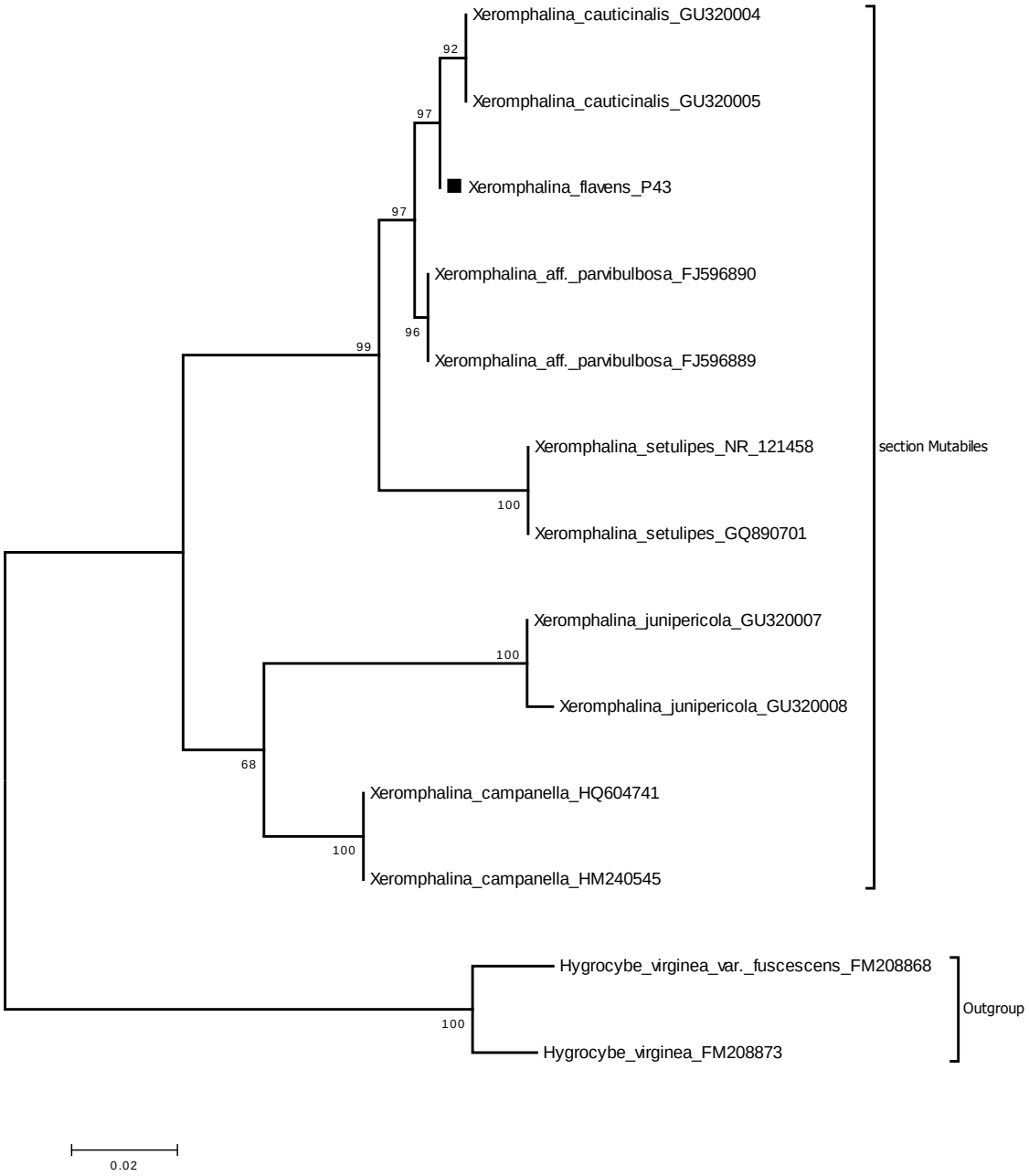


Figure 3.29 Molecular phylogenetic analysis of ***Xeromphalina flavens*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1188.0486) is shown. Collection of Swat is represented by black cube (■).

Pleurotaceae

Pleurotus

30 *Pleurotus ostreatus*

30. *Pleurotus ostreatus* (Jacq.) P. Kumm., *Führ. Pilzk.* (Zerbst): 104 (1871)

Plate 35 – Figs. A-H & 3.30

Morpho-Anatomical characterization

PILEUS: 5 - 8. cm diam, infundiliform to depressed, umbillicate central disc, pale orang yellow (10YR 8/4) with smooth glabrous velvety surface; curvature recurved; context color white; LAMELLAE: decurrent, crowdy, thick, white; breadth 0.5 - 1 cm margins ragged eroded; STIPE: 1.3 – 6.6 × 0.6 – 2.6 cm, laterally attached, soft, equal, cylindrical to flattened; white; surface smooth.

BASIDIOSPORES: (3.6) 3.9 – 4.9 (5.0) × 9.6 (9.9) – (11.0) 11.6 µm [X = 9.5 × 4.4 µm, Q = 2.159], ellipsoid to oblong, apiculus present, thin-walled, hyaline in KOH, anamyloid. BASIDIA: 6.7 – 9.0 × 15.6 – 24.4 µm, clavate, thin-walled,monosporic to quadrisporic, red in phloxin, hyaline in KOH. TRAMA HYPHAL, cylindrical, uneven 5.0 – 13.0 µm, thin walled, hyaline in KOH, red in phloxin. CHEILOCYSTIDIA: 5.0 – 8.1 × 21.7 – 31.4 µm clavate to cylindrical, thin-walled, hyaline in KOH, red in phloxin PLEUROCYSTIDIA: 5.7 – 8.1 × 19.4 – 29.5 µm clavate to cylindrical, thin-walled, hyaline in KOH, red in phloxin a cutis, hyphae cylindrical, cutis, septae present 5.0 – 9.0 µm, thin-walled, hyaline in KOH. STIPTIPELLIS: cylindrical, cutis, septae present 5.0 – 13.0 µm, hyaline to pale yellow in KOH. CLAMP CONNECTIONS: present in all tissues.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Matta, Under *Populus euphritica* (Oliv), 14 September 201; ISP # 282 (LAH).

Plate 35

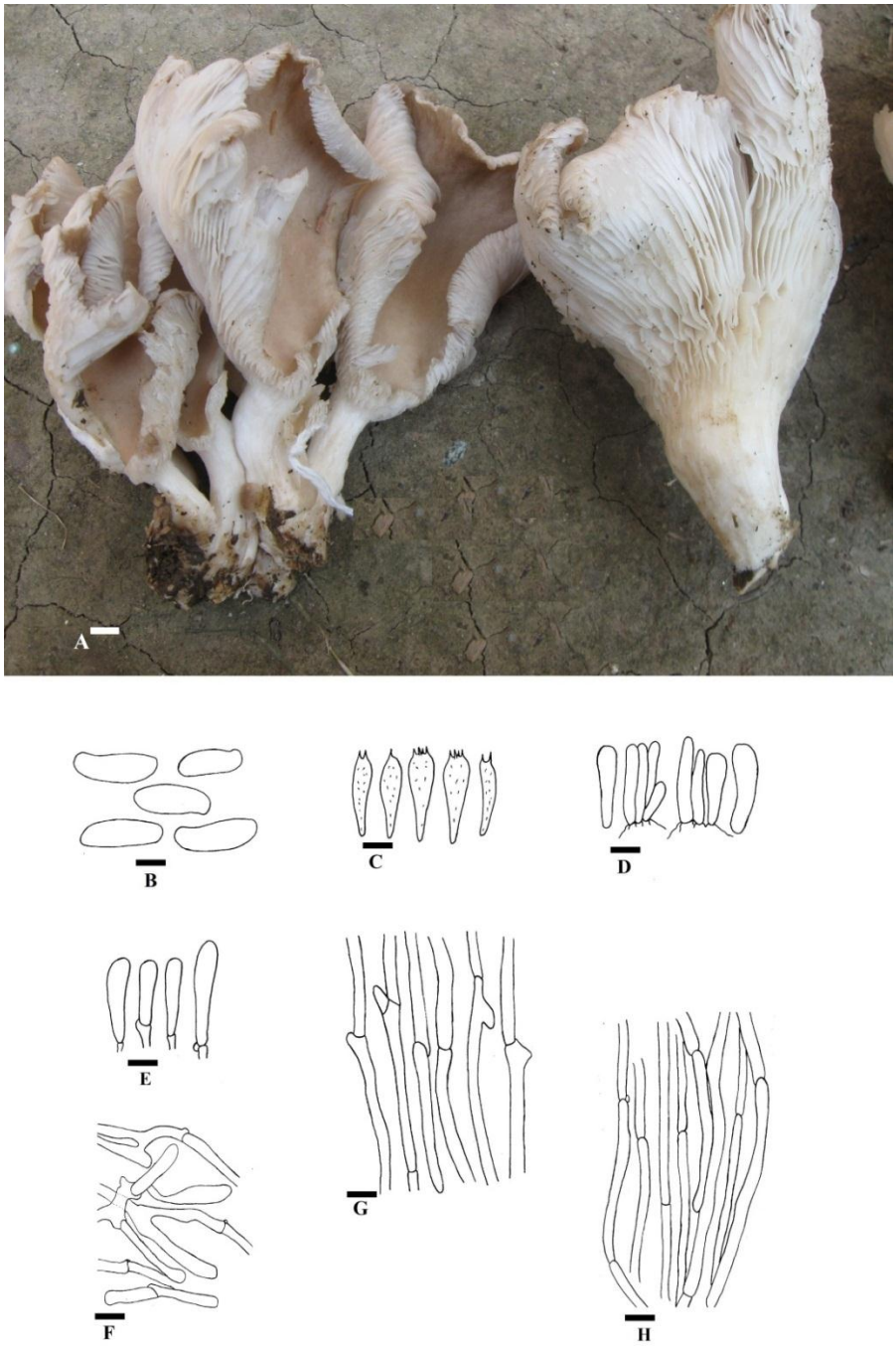


Figure (A-H) Morpho-anatomical shapes of ***Pleurotus ostreatus*** (A) Basidiomata (B) Basidiospore (C) Basidia (D) Cheilocystidia (E) Pleurocystidia (F) Tramal hyphae (G) Pileal hyphae (H) Stiptipellis. Scale bar: A = 0.8 cm, B = μm, C-E = 8 μm G-H = 9 μm,

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 643 base pairs. In initial BLAST search of GenBank database, closest hit was *Pleurotus pulmonarius* sensu auct. = *Pleurotus ostreatus* (GenBank KJ670292, Query coverage - 100%, expect value - 0.0 and Identity index - 100%) followed by 9 other sequences of *Pleurotus pulmonarius* = *Pleurotus ostreatus* 6 of *Pleurotus cornucopiae* (Paulet) Rolland and 3 of *Pleurotus eryngii* (DC.) Quél. having the same query coverage score, expect value and identity.

The newly generated ITS sequence of the this collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 502/662 were conserved characters, 170 were variable, 167 were parsimony informative and 03 were singletons. The analysis was carried out for 29 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 446 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.30).

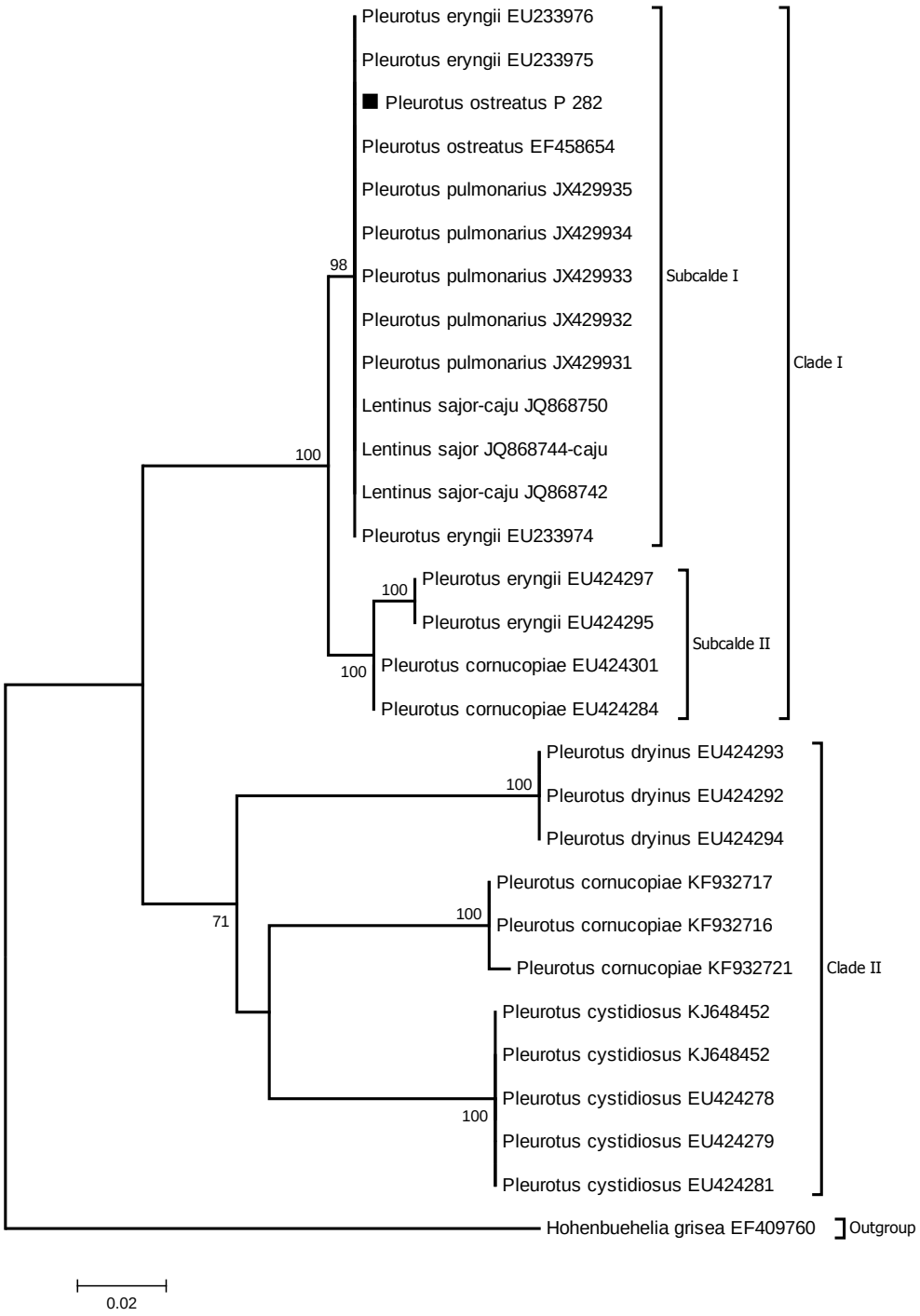


Figure 3.30 Molecular phylogenetic analysis of ***Pleurotus ostreatus*** by Maximum Likelihood method. Maximum log likelihood value = (-1224.6158) is shown. Collection of Swat is represented by black cube (■).

Russulaceae

Russula

31 *Russula kalamica* nom. prov.

32 *Russula deodara* nom. prov.

33 *Russula umbra* nom. prov.

34 *Russula puellaris*

35 *Russula mankialica* nom. prov.

36 *Russula sichuanensis*

31. ***Russula kalamica*** nom. prov. Plate 36 – Figs. A-G & 3.31

Etymology: The specific epithet may be referred to the type locality of this species.

Morpho-Anatomical characterization

PLEUS: 2.5 – 3.5 cm across, depressed, obtuse, dark brown to light brown at margins (7.5YR 6/4 – 5YR 1/4) smooth, appressed fibrillose, moist and slightly sticky surface; curvature incurved; margins cottony, white; LAMELLAE: adnate, close to slightly crowded,white ragged eroded. STIPE: 2.6 – 3.5 × 1.0 – 1.6 cm, centrally attached, white, equal, cylindrical, surface smooth.

BASIDIOSPORES: (6.8) 7.0 – 7.4 (8.0) × (6.8) 7.4 – 8.7 (9.7) µm [x = 7.3 × 9.7 µm, Q = 1.328], aculeate, globose, apiculus present, spiny, thin-walled, amyloid, hyaline in KOH. BASIDIA: 23.2 – 45.6 × 10.9 – 13.9 µm, clavate, four spored, thin-walled, hyaline in KOH, sterigmata, pointed, four spored, 3.1 – 7.8 µm. CHEILOCYSTIDIA: 49.1 – 64.4 × 8.3 – 12.6 µm, fusoid. PLEUROOCYSTIDIA: 49.8 – 69.2 × 19.9 – 9.7 µm. STIPTIPELLIS: cylindrical and parallel, cutis, 1.9 – 4.5 µm, thin walled, hyaline in KOH).

MATERIAL EXAMINED: PAKISTAN, KHYBER PUKHTUNKHWA, kalam Swat, Solitary Under *Cedrus dedara*., 03 September, 2013; ISP # 032 (LAH).

Plate 36

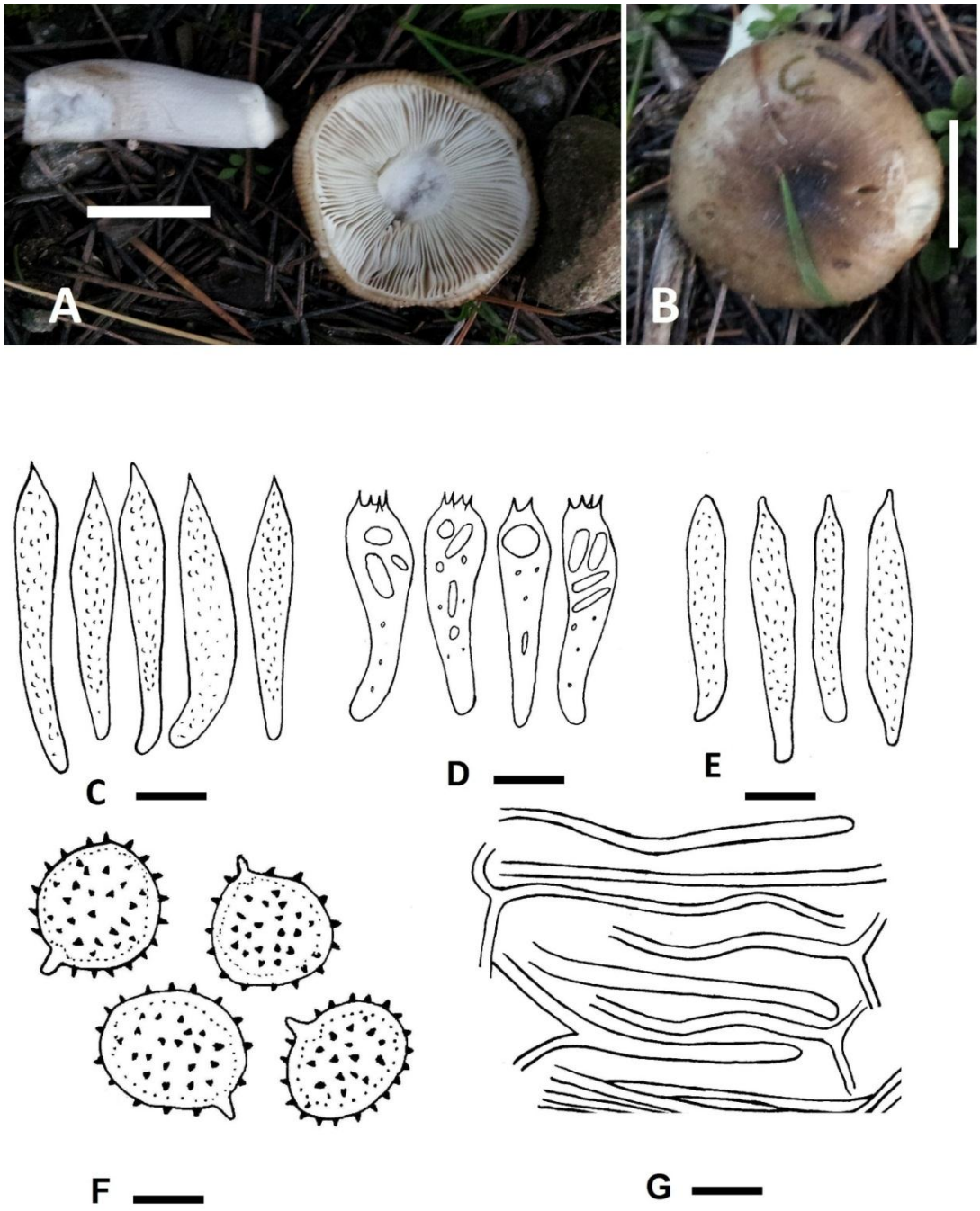


Figure (A-G) Morpho-anatomical shapes of ***Russula kalamica*** nom. prov. (A-B) Basidiomata (C) Pleurocystidia (D) Basidia (E) Cheilocystidia (F) Basidiospore (G) Stiptipellis. Scale bar: A-B= .5 cm, C = 15 µm D = 11 µm, E = 15 µm, F = 5 µm G = 12 µm.

32. ***Russula deodara*** nom. prov. Plate 37 – Figs. A-E – 3.19.5.2 & 3.31

Etymology: The specific epithet may be referred to the occurrence of this species in *Cedrus deodara* forest.

Morpho-Anatomical characterization

PLEUS: 3 – 4 cm diam, hemispheric obtuse, pale orangish yellow with darkish shades to golden (7.5YR 8/4); surface appressed fibrillose, moist; curvature incurved; margins straight to cottony; LAMELLAE: adnate and ascending, close, thick, pale yellowish pink (7.5YR 9/2); margins smooth, convex, entire. STIPE: 4 – 4.5 × 1 – 1.5 cm, centrally attached; cartilage like; white; soft; clavate, surface smooth.

BASIDIOSPORES: 6.8 – 12.5 × 5.1 – 9.2 µm [X = 9.4 × 8.0 µm, Q = 1.181], globose, apiculus very distinct 2.5 – 4.2 µm, warty, thin-walled, hyaline in KOH. BASIDIA: 42.1 – 50.1 × 12.1 – 15.6 µm, clavate, bissterigmate to tetrasterigmate, thin-walled, hyaline in KOH; Sterigmata, 4.2 – 8.1 µm. CHEILOCYSTIDIA: 26.7 – 59 × 9.0 – 11.8 µm clavate to fusiform, thin-walled, hyaline in KOH. PLEUROCYSTIDIA: 53.3 – 60.6 × 9.9 – 11.8 µm clavate to fusiform, thin-walled, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Miandam, Solitary on ground in *Cedrus deodara* forest, 08 September, 2013; ISP # 078 (LAH).

Plate 37

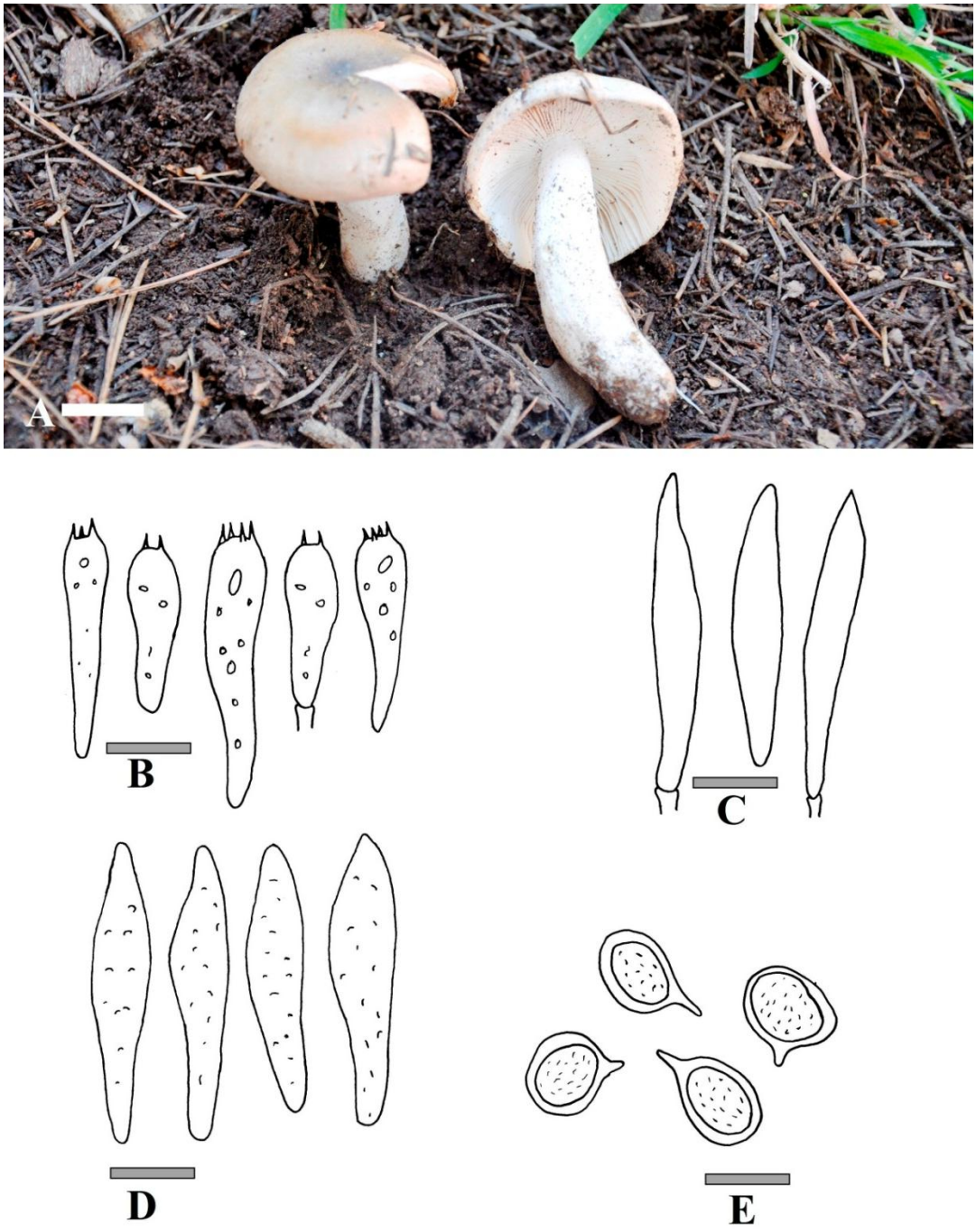


Figure (A-E) Morpho-anatomical shapes of ***Russula deodara*** nom. prov. (A) Basidiomata (B) Basidia (C) Cheilocystidia (D) Pleurocystidia (E) Basidiospore. Scale bar: A = 1.5 cm B-D =15 μm, E = 8 μm.

33. ***Russula umbra*** nom. prov. Plates 38 & 39 – Figs. A-D & 3.31

Etymology: The specific epithet may be referred to the shady habitate of this species.

PLEUS: 7 – 8 cm diam, depressed umbillicate. White with very sparse yellowish pink shades (5YR 7/4); surface appressed fibrillose, moist; curvature straight and flaring; margins straight to slightly plicate; LAMELLAE: adnexed, close, thick, off white; margins smooth, convex, entire. STIPE: 5.3 – 5.9 × 2.3 – 2.8 cm, centrally attached; white; soft; cylindrical, surface smooth.

SPORES 8.5 – 11.4 × 5.1 – 7.6 µm [X = 9.5 × 6.4 µm, Q = 1.095], globose, apiculus present, spiny, thin-walled, amyloid, hyaline in KOH. BASIDIA: 24.0 – 57.1 × 9.2 – 14.6 µm, clavate, bisporic to tetrasporic, thin-walled, hyaline in KOH. CYSTIDIA: absent. PILEIPELLIS: lxolattice, hyphae cylindrical, 1.5 – 3.0 µm, thin-walled, hyaline in KOH.

HABITAT AND DISTRIBUTION: PAKISTAN, Khyber Pakhtunkhwa, Swat, Miandam, Solitary under *Pinus wallichiana* A. B. Jacks., 09 September, 2013; ISP # 113(LAH).

Plate 38

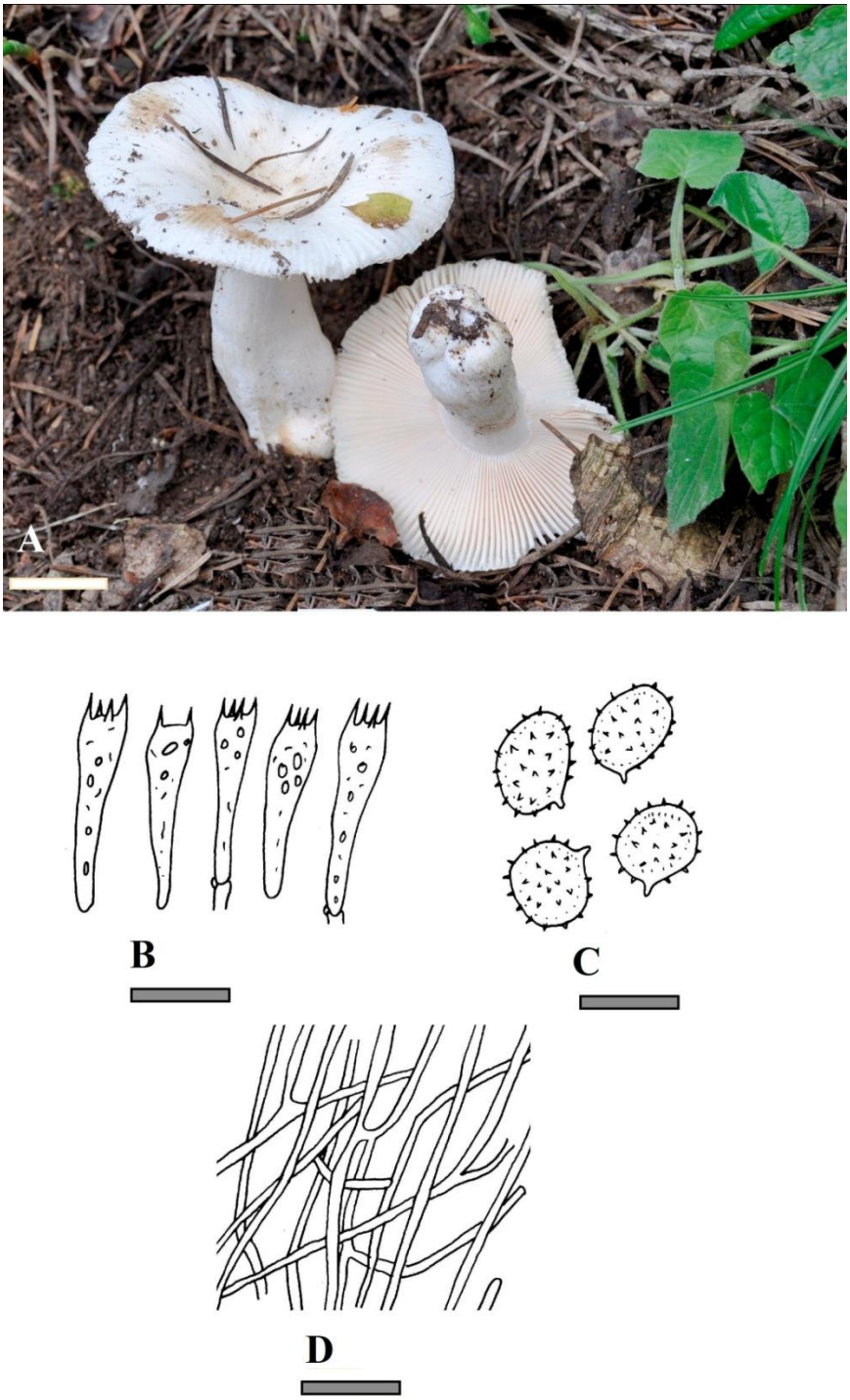
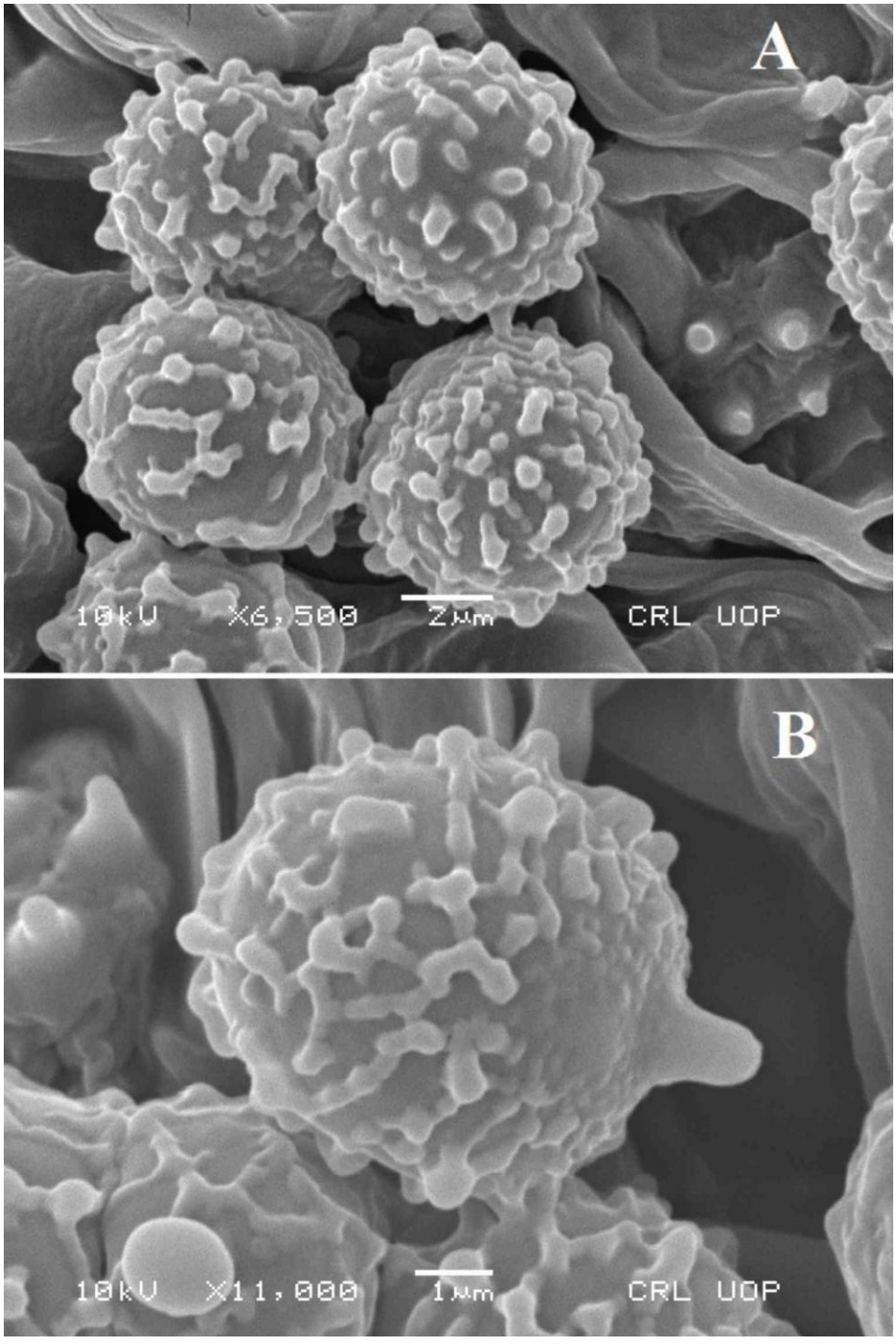


Figure (A-D) Morpho-anatomical shapes of ***Russula umbra*** nom. prov**.** (A) Basidiomata (B) Basidia (C) Basidiospore (D) Pillipillis. Scale bar: A = 2 cm, B = 25 µm, C = 0.8 µm & D = 8 µm.

Plate 39



(A-B) Scanning electron micrographs of basidiospores of ***Russula umbra*** nom. prov.

34. *Russula puellaris* Fr., *Epicr. Syst. Mycol.* (Upsaliae): 362 (1838)

Plate 40 – Figs. A-F & 3.31

Morpho-Anatomical characterization

PLEUS: 2.5 - 3.5 cm across, convex to pulvinate, vivid red to very deep red in center (7.5R2/14 – 735R1/10) becoming moderate red from center in margins (5R5/8); sticky and glabrous; central disc obtuse; margin cottony; flesh thick and white. LAMELLAE: adnexed, soft, whitish to pale 1.2-1.4 cm in breadth. STIPE: 3 - 4 × 0.5 – 1.4 cm, equal or slightly clavate, smooth and cylindrical, milky white.

BASIDIOSPORES: 9.0 - 11.1 × 9.7 – 10.1 µm [X = 9.5 × 10.2 µm, Q = 1.073], globose to sub-globose, apiculus present, warty, hyaline in KOH, non-amyloid. BASIDIA: 42.5 - 49.3 × µm, clavate, two to three spored basidia, thin-walled, hyaline in KOH; Sterigmata 4.2–5.2 13.4 - 19.9 µm. CHEILOCYSTIDIA: 29.0 – 47.9 × 9.4 – 15.6 µm, clavate, thin walled, hyaline in KOH. PLEUROCYSTIDIA: 31.9 – 43.7 × 10.1 - 16.4µm, clavate, thin-walled, hyaline in KOH. Pileipellis: a, hyphae cylindrical, 6.6 – 9.7 µm, thin-walled, hyaline in KOH. STIPTIPELLIS : cylindrical, 6.6 – 7.3 µm, hyaline in KOH. CLAMP CONNECTIONS: not observed.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, Solitary on ground in *Cedrus deodara* forest, 03 September, 2013; ISP # 034 (LAH).

COMMENTS: The species is new record for Pakistan.

Plate 40

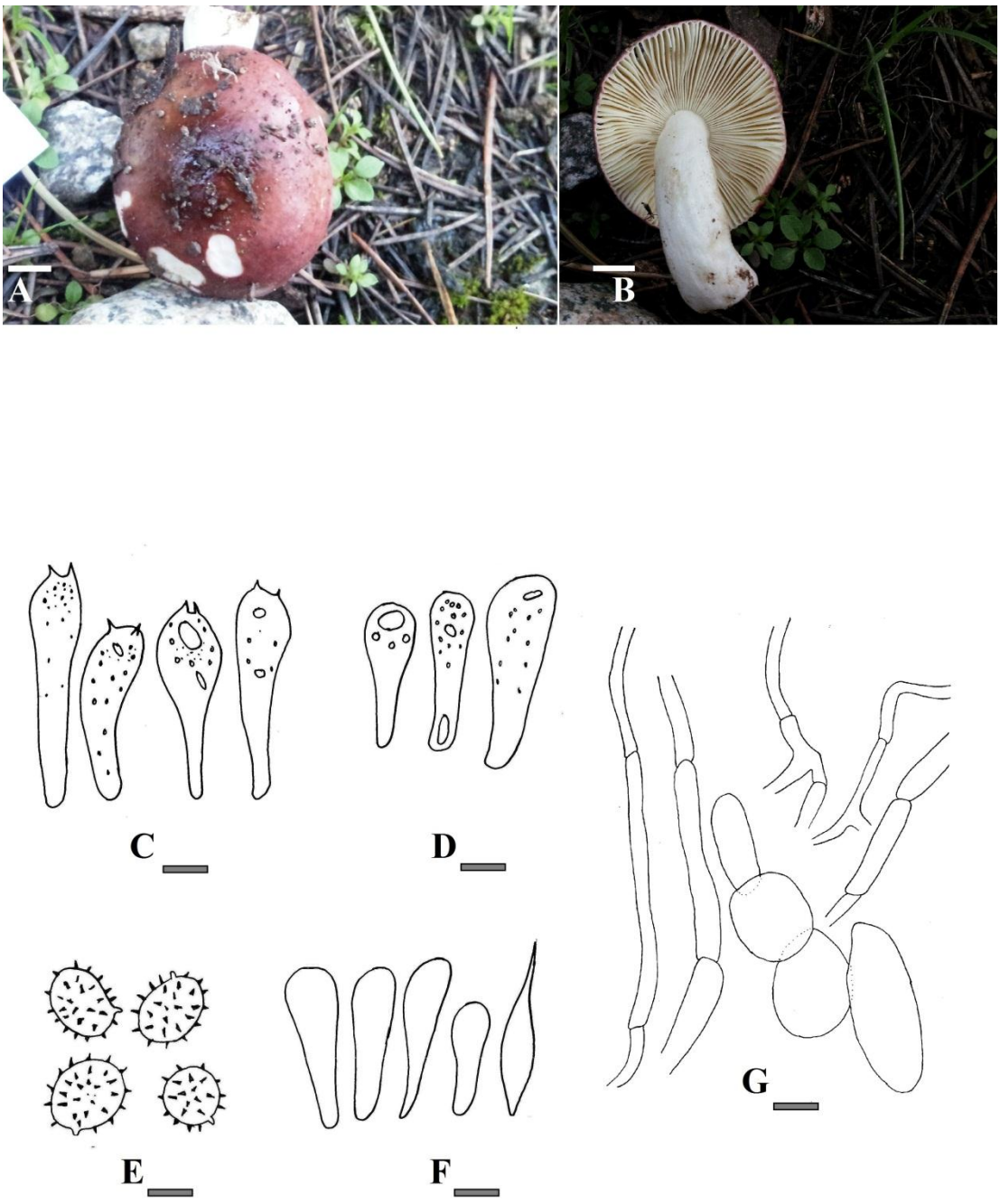


Figure 3.19.2.1 (A-F) *Russula puellaris* (A-B) Basidiomata (C) Basidia (D) Cheilocystidia (E) Basidiospore (F) Pleurocystidia (G) pillipillis. Scale bar: A-B= .5 cm, C-D = 9 µm, E = 7 µm, F-G = 11 µm.

35. ***Russula mankialica*** nom. prov. Plates 41 & 42 – Figs. A-D & 3.31

Etymology: mankialica refers to the type locality.

Morpho-Anatomical characterization

PLEUS: 4.5 – 8 cm diam, hemispheric, greyish reddish to dark greyish red (7.5YR 4/4 – 2.5R 3/2); surface glandular when young glabrous on maturity, moist; central disc obtuse; curvature incurrved; margins cottony; flesh white. LAMELLAE: adnate and ascending, close, thin, white entire. STIPE: 5.6 – 9.3 × 0.9 – 2.8 cm, white, central, soft; solid, cylindrical, surface smooth.

BASIDIOSPORES: 7.9 – 9.7 × 6.9 – 9.2 µm [X = 8.0 × 8.8 µm, Q = 1.088], aculeate globose, apiculate, thin-walled, olive green in KOH, reddish olive green in phloxin. BASIDIA: 36.5 – 53.8 × 11.8 – 20.8 µm, clavate, bisporic to quadrispored, guttiulate, thin-walled, hyaline in KOH. CHEILOCYSTIDIA: 55 – 63.5 × 8.7 – 13.7 µm, cylindrical with obtue apex, somewhat clavate content golden brown. PLEUROOCYSTIDIA: same to cheilocystidia.

HABITAT AND DISTRIBUTION: PAKISTAN, KHYBER PAKHTUNKHWA, SWAT, MASH KOON, MANKIAL, solitary to gregarious on ground in dry temprate coniferous forest under *Cedrus deodara*, and *Abies pidrow*. 04 September, 2013, ISP # 070 (LAH).

Plate 41

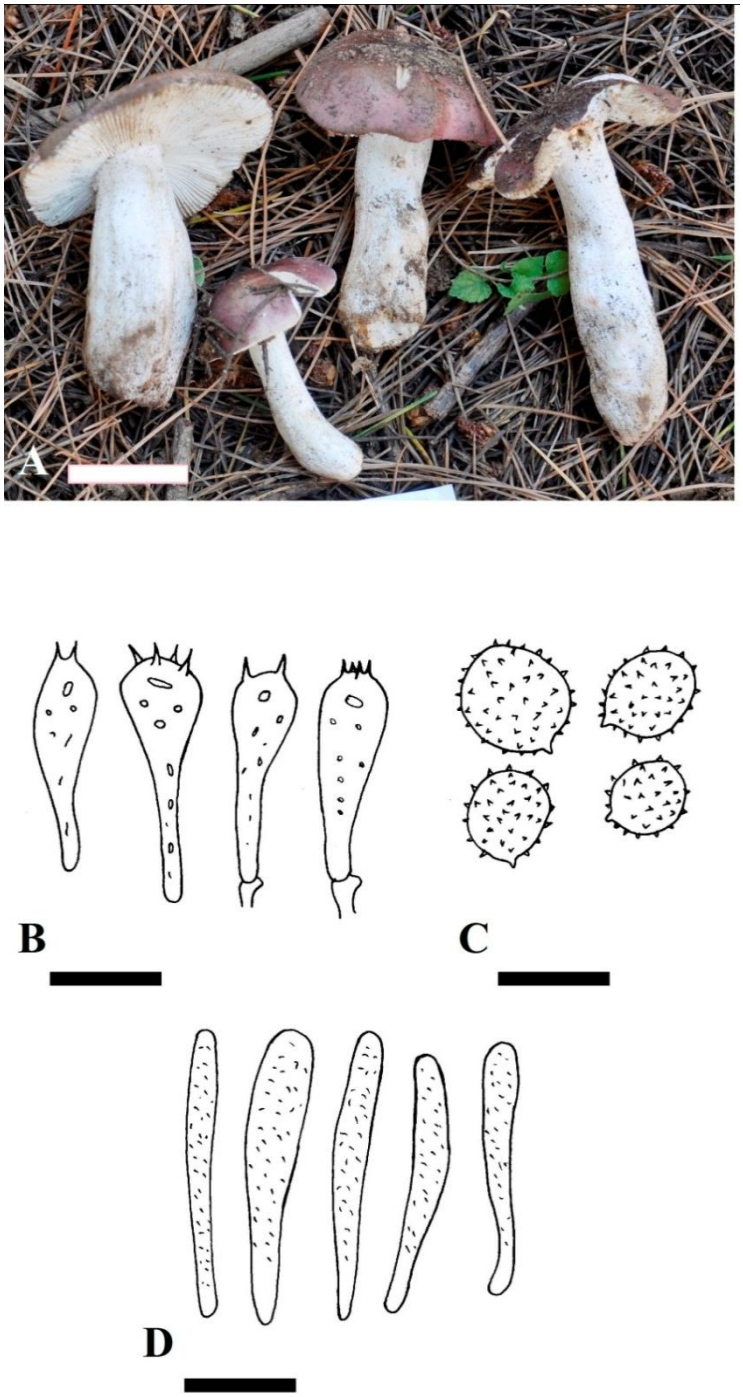
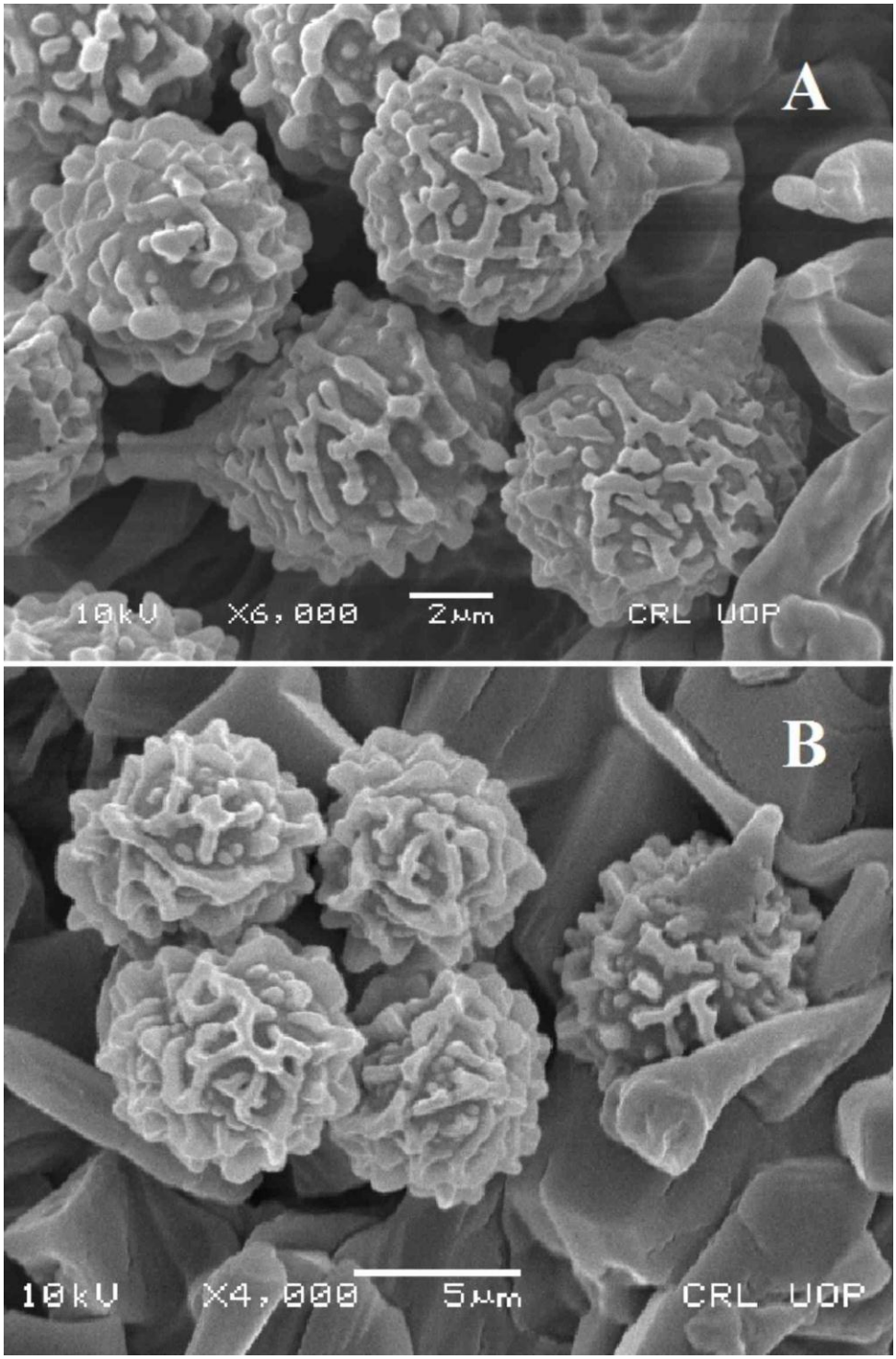


Figure (A-D) Morpho-anatomical shapes of ***Russula mankialica*** nom. prov. (A) Basidiomata (B) Basidia (C) Basidiospore (D) Cheilocystidia. Scale bar: A = 3 cm, B = 23 µm, C = 8 µm & D = 22 µm.

Plate 42



Scanning electron micrographs of ***Russula mankialica*** nom. prov. (A-C)

36. ***Russula sichuanensis*** G.J. Li & H.A. Wen, *Mycotaxon* 124: 179 (2013)

Plate 43 – Figs. A-H & 3.31

Morpho-Anatomical characterization

PLEUS: 3.5 – 4.0 cm diam, depressed to infunduliform with umbillicate depressed central disc. Deep reddish purple in center becoming light redish purlple toward the edges (5RP1/6-5RP5/8) with smooth glabrous velvety and slightly sticky surface; curvature uplifted; margins plicate; LAMELLAE: free and approximate, close, thick, pale to reddish orange to brownish orange light pale to yellow red (5YR8/6) margins smooth, convex, entire. STIPE: 4.0 – 5.0 × 0.5 – 0.7 cm, centrally attached, white, soft hollow, cylindrical, surface smooth.

SPORES: 10.6 – 11.3 × 10.3 – 11 µm [x = 10.8 × 10.9 µm, Q = 1.009], aculeate globose, apiculus present, spiny, thin-walled, amyloid, hyaline in KOH. BASIDIA: 30.4 – 38.5 × 14.9 – 17.2 µm, clavate, four spored, thin-walled, hyaline in KOH. CHEILOCYSTIDIA: 29.5 – 49.6 × 10.3 – 17.7 µm. PLEUROOCYSTIDIA: 27.8 – 40.12 × 11.8 – 17.9 µm. PILEIPELLIS: a cutis, hyphae cylindrical, 4.9 – 6.8 µm, thin-walled, hyaline in KOH. STIPE HYPHAE: cylindrical and parallel, 4.5 – 8.2 µm, hyaline in KOH.

MATERIAL EXAMINED: PAKISTAN, KHYBER PUKHTUNKHWA, Yakh Tangay Shangla, Solitary Under *Pinus wallichiana* A. B. Jacks., September 02, 2013; ISP # 03(LAH).

COMMENTS: Repoted from Pakistan

Plate 43

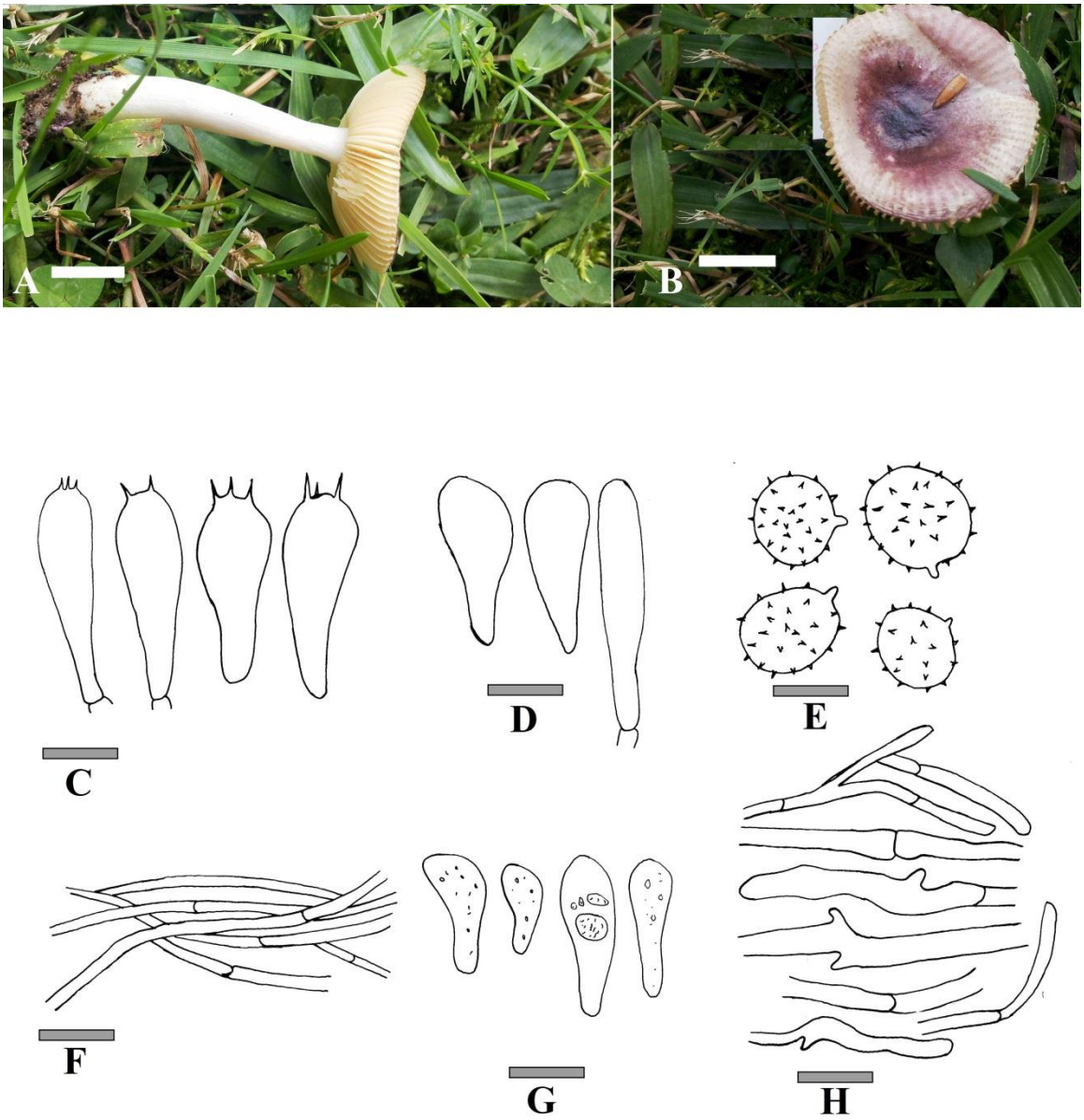


Figure 3.19.6.1 (A-H) ***Russula sichuanensis*** (A-B) Basidiomata (C) Basidia (D) Cheilocystidia (E) Basidiospores (F) Stipe hyphae (G) Pleurocystidia (H) Pileipellis. Scale bar: A-B = 1.5 cm.

Molecular Phylogenetic Analysis

ITS region of *Russula mankialica* nom. prov., *Russula umbra* nom. prov., *Russula puellaris, Russula sichuanens, Russula deodara* nom. prov. and *Russula kalamica nom. prov.* generated sequences of 616, 506, 393, 609, 609 and 632 base pairs respectively. In initial BLAST search of GenBank database, closest hit for *Russula mankialica* nom. prov. was *Gymnomyces abietis* Trappe & Castellano (AY239348) with 93% Identity index, followed by *Gymnomyces monticola* (Harkn.) Trappe (AY239315) with 93% Identity index from USA. The genus name *Gymnomyces* used for sequestrate taxa, is now recognized as synonym of *Russula* "MycoBank: *Russula*". (MycoBank. Retrieved 2014-11-29). In initial BLAST search of GenBank database, closest hit for *Russula umbra* nom. prov. was *Russula* aff. *mustelina* (JF834362) and *Russula mustelina* Fr. (AY061693) with 93% Identity index from USA. In initial BLAST search of GenBank database, closest hit for *Russula puellaris* was *Russula nauseosa* (KJ748441) with 97% Identity index from USA, followed by *Russula abietina* (GenBank EU248590, Query coverage - 100%, expect value - 0.0 and Identity index - 97%) from Canada.In initial BLAST search of GenBank database, closest hit for *Russula sichuanens* was *Russula sichuanens* (KM596859) with 99% Identity index reported by M.Saba and A.N Khalid from the Pakistan. In initial BLAST search of GenBank database, closest hit for *Russula deodara nom. prov.* was an ectomycorrhizal sequence of *Russula sp*. (HG796943) with 99% identity index from Pakistan by Ilayas *et al*. In initial BLAST search of GenBank database, closest hit for *Russula kalamica nom. prov.* was ectomycorrhizal sequence *Russula sp*. (HG796943) with 87% identity index from Galyat Pakistan.

The newly generated ITS sequence of *Russula mankialica* nom. prov., *Russula umbra* nom. prov., *Russula puellaris, Russula sichuanens, Russula deodara* nom. prov. and *Russula kalamica nom. prov.* along with those retrieved from GenBank based on the result from BLAST search and published in research articles were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The analysis was carried out for 30 nucleotide sequences. Dataset included 1750 characters among which only 711 (325 conserved, 363 variable, parsimony informative 265 and 94 singleton) were used after timmining both sides of the alighnment file with conserved motifs. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 377 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.31).

Phylogenetic tree is shown with two major clades, clade I and clade II. Clade I is furher devided into eight sublades with best bootstrap values. Clade I depicts nesting of *Russula sichuanens* in subclade I, *Russula puellaris* and *Russula mankialica* nom. prov. in subclade II and *Russula umbra* nom. prov. in subclade VIII. Clade II depicts clustering of *Russula kalamica* nom. prov. and *Russula deodara* nom. prov. in subclade II.

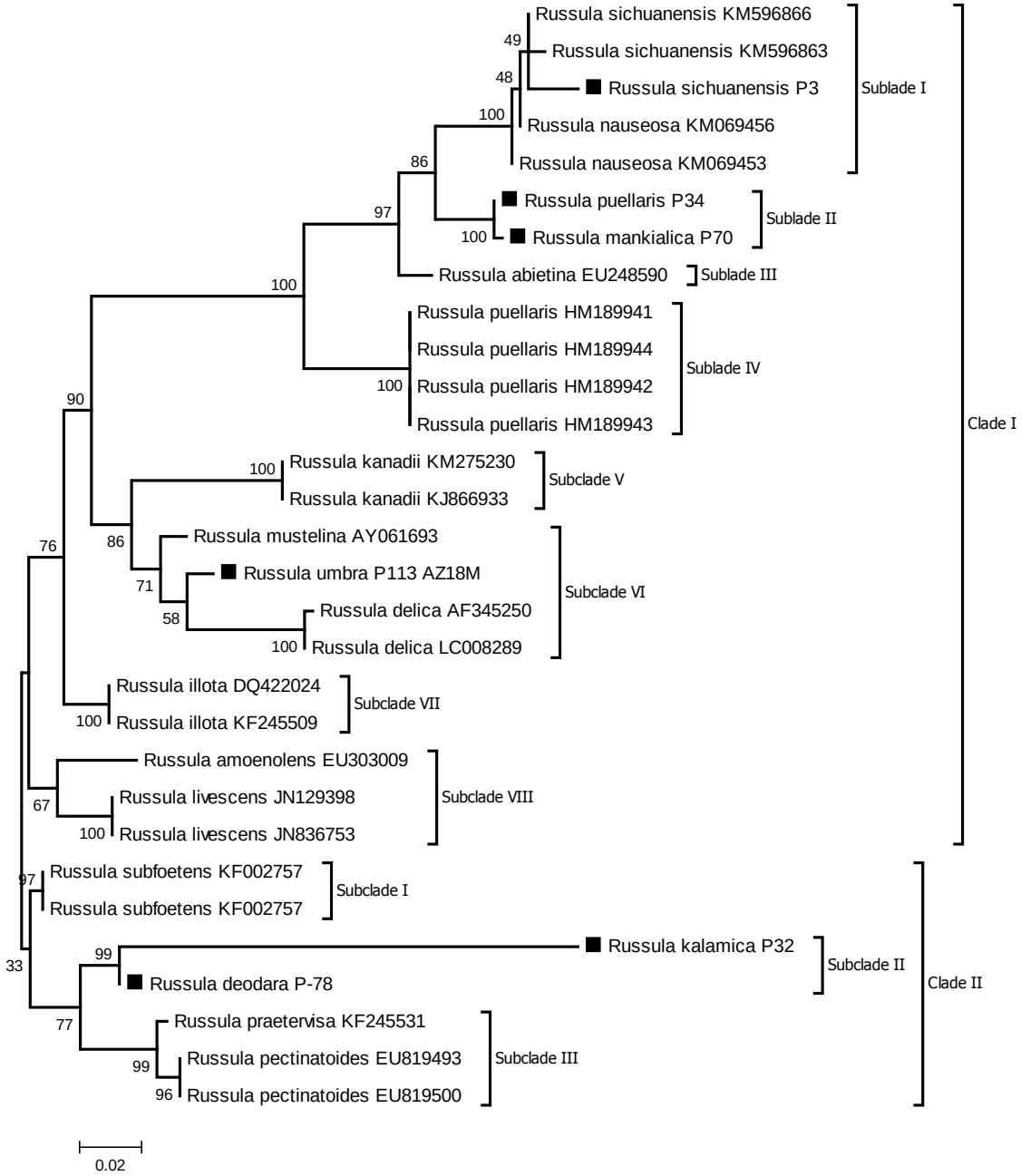


Figure 3.31 Molecular phylogenetic analysis of of ***Russula*** species by Maximum Likelihood method. Tree with highest log value (-1796.5811) is presented. Collection of Swat is represented by black cube (■). The percentage of trees in which the associated taxa clustered together is given next to the branches.

Strophariaceae

Cyclocybe

37 *Cyclocybe erebia*

Pachylepyrium

38 *Pachylepyrium kalamicum* nom. prov.

Pholiota

39 *Pholiota gummosa*

40 *Pholiota lanceolta* nom. prov.

41 *Pholiota manga* nom. prov.

42 *Pholiota spumosa*

37. ***Cyclocybe erebia*** (Fr.) Vizzini & Matheny, in Vizzini, *Index Fungorum* 154: 1 (2014) Plate 44 – Figs. A-G & 3.32

Morpho-Anatomical characterization

PLEUS: 2 – 3.5 cm diam, convex to plano convex, light reddish brown to dark reddish brown (10R4/6 – 10R5/4); umbonate to broadly umbonate central disc; with aerolate to appressed fibrillose moist surface; curvature incurved deflexed; margins plicate to wavy plicate cottony. LAMELLAE: sinuate, close to crowdy, grayish orange to reddish orange (2.5YR6/6); margins ragged eroded; breadth 1-1.5 cm. STIPE: 4.5 – 6 cm, centrally attached, soft, cylindrical light brown to light reddish brown (7.5YR6/4 – 2.5YR6/4); smooth and equal.

BASIDIOSPORES: 14.16 – 16 × 8.7 – 10.6 µm [X = 9.7 × 15.5 µm, Q = 1.59], ellipsoid or globoid, apiculus present, smooth, thin-walled, yellow brown in KOH. BASIDIA: 7.5–10.6 × 18.9–26.0 µm, club shaped to clavate, two spored basidia, thin-walled, hyaline in KOH; Sterigmata 4.1–4.4 µm. CHEILOCYSTIDIA: 8.5 – 11.3 × 25.4 – 34.2 µm, clavate, guttiulate thin walled, hyaline in KOH, PLEURICYSTIDIA 7.5 – 10.4 × 21.2 –28.5 µm, clavate, thin walled, hyaline in KOH, guttiulate. PILEIPELLIS: cellular, thin-walled, hyaline in KOH. STIPE HYPHAE: cylindrical, 11.8– 15.3 µm, hyaline in KOH.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Kalam, solitary on ground in dry temprate mixed forest of *Cedrus deodara* and *Quercus semicopifolia*. 04 September, 2013, ISP # 021

COMMENTS: This is new record for Swat. Previously it is reported by Murakami (1993) from AJK.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 576 base pairs. In initial BLAST search of GenBank database, closest hit was *Agrocybe erebia* (Fr.) Kühner ex Singer = *Cyclocybe erebia* (Fr.) Vizzini & Matheny (GenBank JN020964, Query coverage - 100%, expect value - 0.0 and Identity index - 96%).

The newly generated ITS sequence of the current *Agrocybe erebia* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. The analysis was carried out for 35 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 469 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.32).

Plate 44

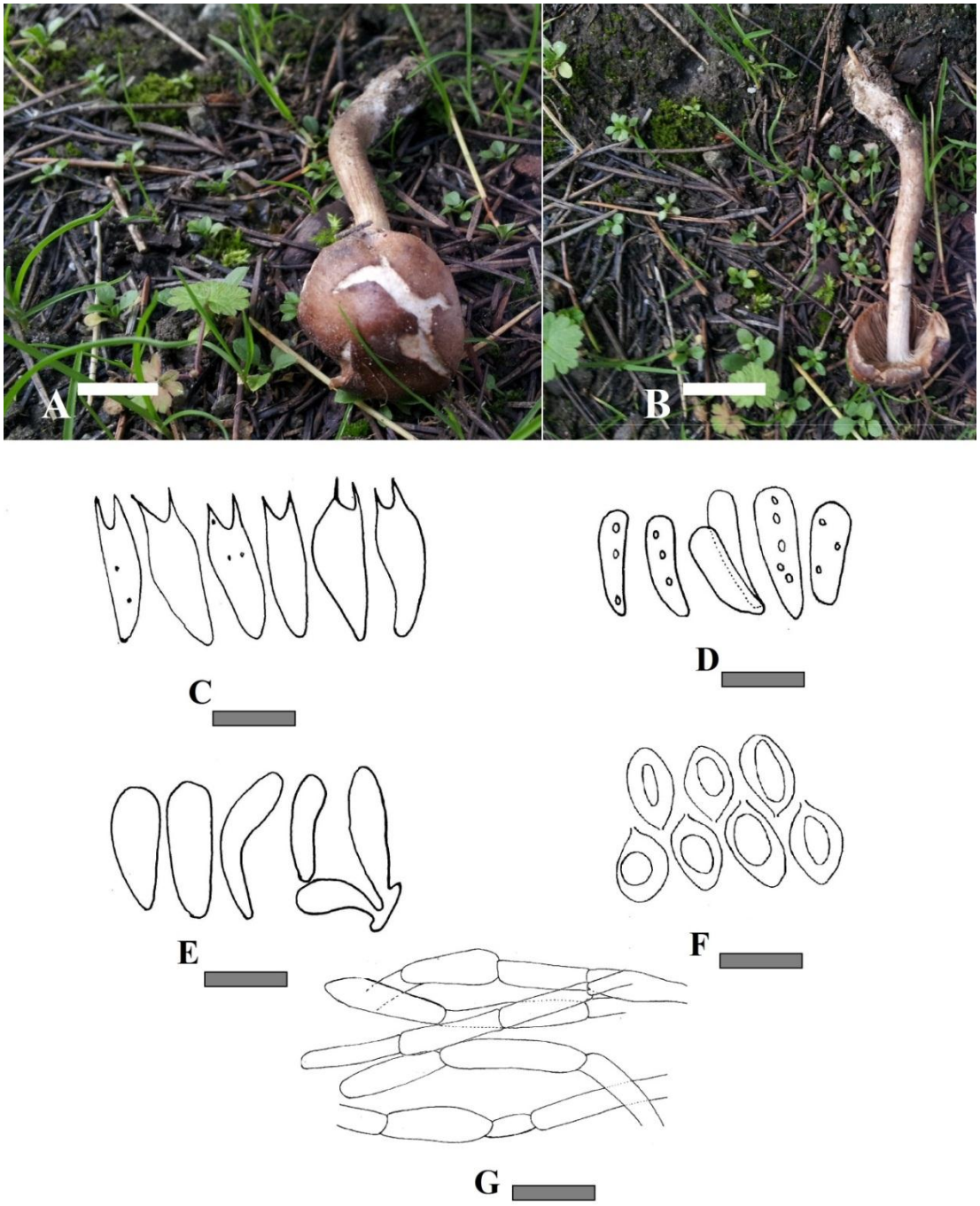


Figure (A-G) Morpho-anatomical shapes of ***Cyclocybe erebia*** A-B Basidiomata. C. Basidia. D. Cheilocystidia. E. Pleurocystidia. F. Basidiospores. G. Stipe hyphae. Scale bar: A: 1.5 cm B: 1.3 cm C: 13 µm, D: 18 µm, E: 12 µm, F: 12 µm, G: 30 µm.

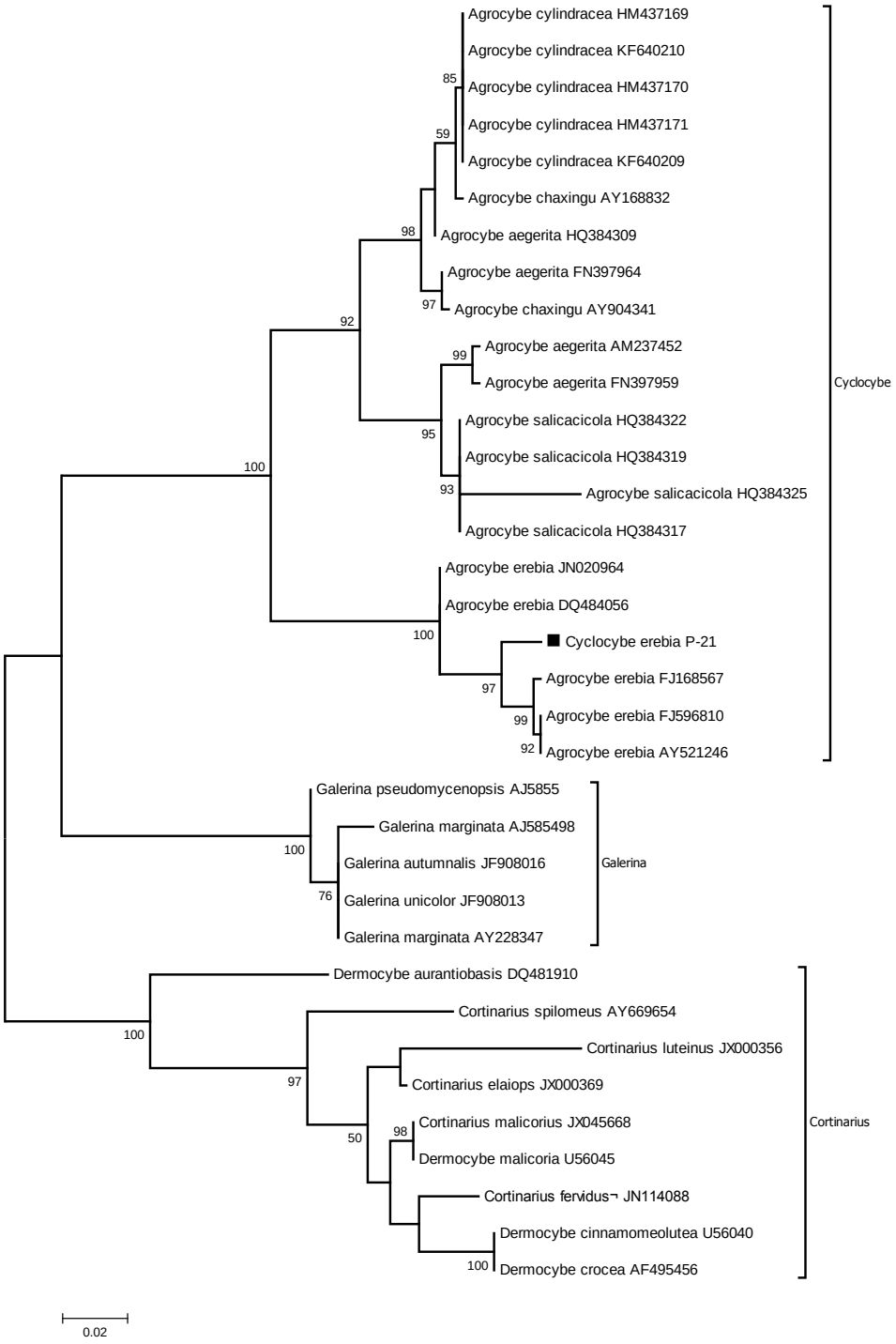


Figure 3.32 Molecular phylogenetic analysis of ***Cyclocybe erebia*** by Maximum Likelihood method.

Collection of Swat is represented by black cube (■).

38. ***Pachylepyrium kalamicum*** nom. prov. Plate 45 – Figs. A-D – 3.33

Etymology: The specific epithet may be referred to the type locality.

Morpho-Anatomical characterization

PLEUS: 1 – 2 cm diam, hemispheric, deep brown (2.5YR 1/8); obtuse central disc; appressed fibrillose velvety surface; curvature incurved; margins cottony. LAMELLAE: straight and flaring, sub distant, entire, strong brownish orange (2.5YR 5/8); margins. STIPE: 4.0 – 4.5 × 0.2 – 0.4 cm, centrally attached, soft, cylindrical deep brown (2.5YR 5/8); smooth and equal.

BASIDIOSPORES: 7.7 – 9.9 × 8.7 – 12.0 µm [X = 8.3 × 10.6 µm, Q = 1.277], globose to sub-globose to angular ovate to kite shaped, thick-walled, dark brown in KOH. BASIDIA: 32.3 – 39.6 × 10.4 – 12.3 µm, clavate, moderately-walled, hyaline in KOH. CYSTIDIA not observed. STIPE HYPHAE cylindrical, 2.5 – 12.9 µm, hyaline in KOH.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Kalam, solitary to on ground in dry temprate mixed forest of *Cedrus deodara* and *Quercus semicopifolia*. 03 September, 2013, ISP # 027 (LAH)

Plate 45

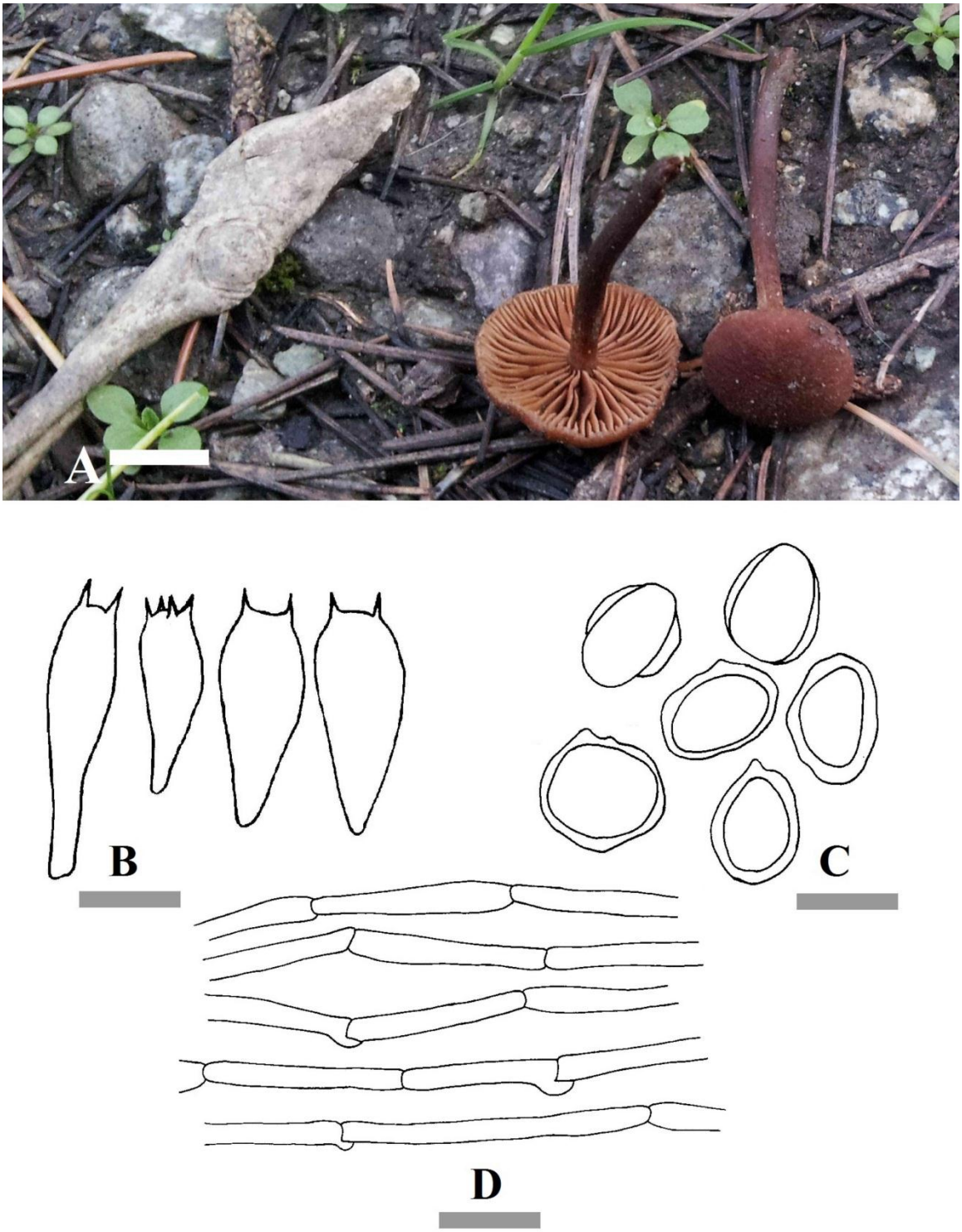


Figure (A-D) Morpho-anatomical shapes of ***Pachylepyrium kalamicum*** nom. prov. (A) Basidiomata (B) Basidia (C) Basidiospores (D) Stipe Hyphae Scale bar: A = 1 cm, B = 15 µm, C = 10 µm, D = 15 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 629 base pairs. In initial BLAST search of GenBank database, closest hit was *Pachylepyrium carbonicola* (A.H. Sm.) Singer (GenBank JF506863, Query coverage - 100%, expect value - 0.0 and Identity index - 87%).

The newly generated ITS sequence of the current *Pachylepyrium* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 847/652 were conserved characters, 158 were variable, 107 were parsimony informative and 50 were singletons. The analysis was carried out for 24 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 575 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.33).

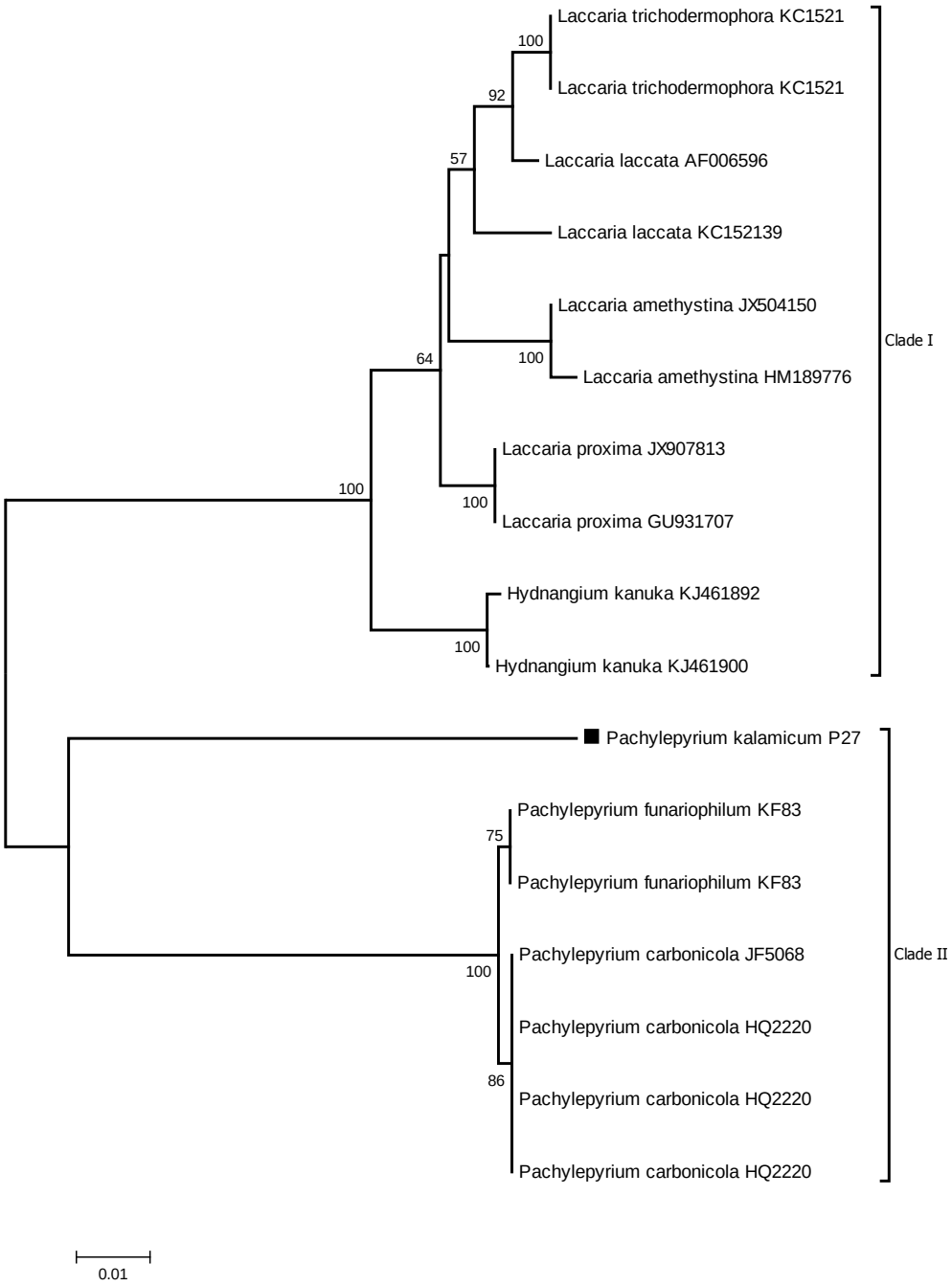


Figure 3.33 Molecular phylogenetic analysis of ***Pachylepyrium kalamicum*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1945.2111) is shown. Collection of Swat is represented by black cube (■).

39. *Pholiota gummosa* (Lash) Singer *Lilloa* 22: 517 (1951)

Plate 46 – Figs. A-H & 3.34

Morpho-Anatomical characterization

PLEUS: 3.5 – 4.0 cm diam, depressed, central disc umbillicate; brownish orange to strong brown (5YR5/8 – 5YR4/10); surface apressed fibrillose with tiny squamules spreading all over the surface; curvature inrolled; margins inrolled to cottony. LAMELLAE: adnate and slightly acsending, close, thin, light orange yellow to vivid orange yellow (10YR7/8 – 7.5YR8/16); marginate; STIPE: 2.3 – 2.9 × 1.1 cm, centrally attached, soft, hollow, cylindrical, ventricose milky white in the upper half to strong yellowish brown in the lower half (10YR5/6); surface squamolose.

Basidiospores: 5.4 – 8.2 × 3.9 – 5.4 µm [X = 7.0 × 4.7 µm, Q = 1.489], ellipsoid to subglobose, smooth, thin-walled, hyaline in KOH, non-amyloid. Basidia: 15.8 – 23.0 × 4.7 – 7.9 µm, clavate, quadrisporic , thin-walled, hyaline in KOH; CHEILOCYSTIDIA: 28.0 – 40.4 × 7.4 – 9.4 µm, fusiform, thin walled. PILEIPELLIS: cutis to trichoderm, hyphae cylindrical, (2.4–) 3.4 – 7.8 (–9.0) µm, thin-walled, hyaline in KOH. STIPTIPELLIS: cylindrical, (2.9–) 3.4 – 11.3 (–12.1) µm, hyaline to pale yellow in KOH.

HABITAT AND DISTRIBUTION: Pakistan, Khyber Pakhtunkhwa, Swat, Kalam, solitary to on ground in forest of *Cedrus deodara*, 04 September, 2013, ISP # 017 (LAH)

COMMENTS: This is new record for Pakistan.

Plate 46

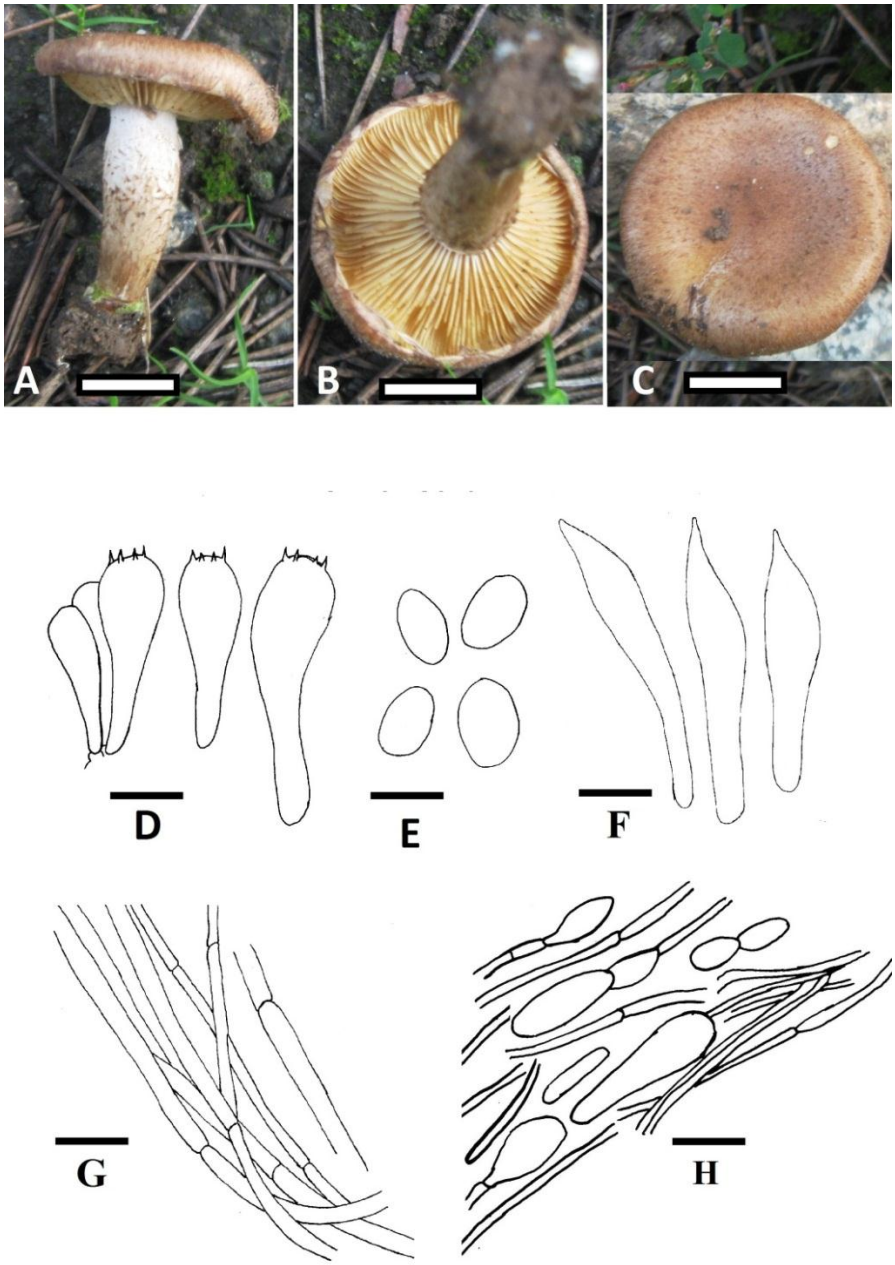


Figure (A-H) Morpho-anatomical shapes of ***Pholiota gummosa*** (A-C) Basidiomata; (D) Basidia (E) Basidiospores (F) Cheilocystidia (G) Stiptipellis (H) Pilipellus. Scale bars: A-B = 1.2 cm, D = 6 µm E = 7.5 µm, F = 10 µm, G = 33 µm, H = 40 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 558 base pairs. In initial BLAST search of GenBank database, closest hit was *Pholiota gummosa* (GenBank JF908580, Query coverage - 74%, expect value - 0.0 and Identity index - 99%).

The newly generated ITS sequence of the current *Pholiota* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 358/592 were conserved characters, 219 were variable, 153 were parsimony informative and 65 were singletons. The analysis was carried out for 33 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 142 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.34).

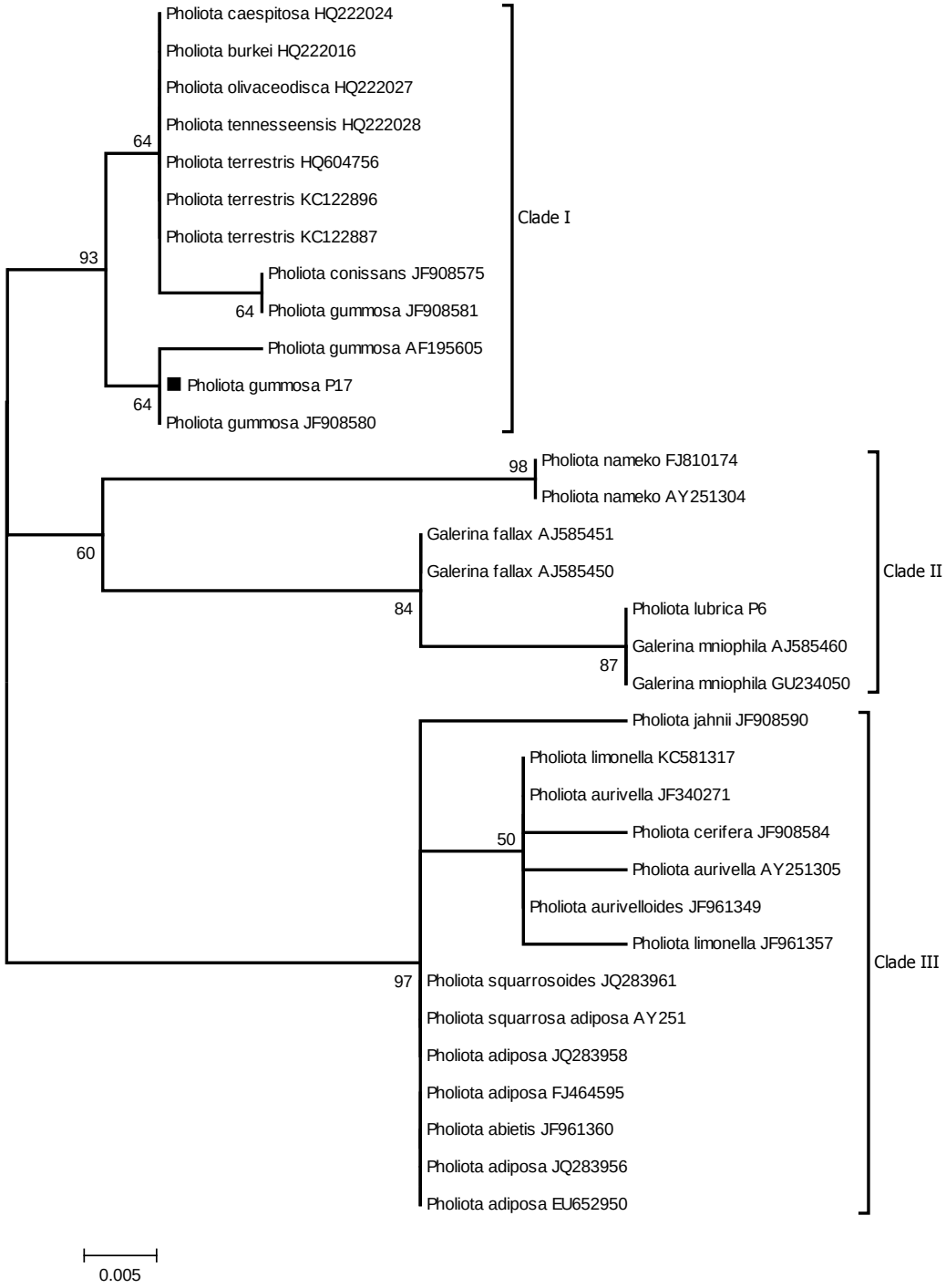


Figure 3.34 Molecular phylogenetic analysis of ***Pholiota gummosa*** by Maximum Likelihood method. Maximum log likelihood value = (-336.93) is shown. Collection of Swat is represented by black cube (■).

40. ***Pholiota lanceolata*** nom. prov. Plate 47– Figs. A-G & 3.35

Etymology: The specific epithet may be referred to the lance (elliptic) shaped cystidia of this species.

Morpho-Anatomical characterization

PLEUS: 2.5 – 3.1 cm diam, depressed with umbillicate central disc. Color; strong yellowish brown to pale yellow orange (10YR 5/6– 10YR 9/4) at margins; surface, fibrillose, velvety moist; curvature, straight; margins, inrolled, pubescent; LAMELLAE: areuate, close, thin, light yellow (2.5YR 9/6); margins curved. STIPE: 1.9 – 2.3 × 0.4 – 0.6 cm, centrally attached; whitish at apex, strong yellowish brown at lower half (10YR 5/6); soft; solid, cylindrical, equal slightly reticulated.

BASIDIOSPORES: 7.6 – 9.7 × 4.4 – 6.5 µm [X = 8.4 × 5.3 µm, Q = 1.573], elliptical, smooth, thin-walled, olive green in KOH. BASIDIA: 18.4 – 26.2 × 9.0 – 10.6 µm, clavate, two to four spored, thin-walled, hyaline in KOH. Sterigmata, 4.2 – 7.0 µm, pointed CYSTIDIA 22.1 – 41.3 × 6.6 – 11.8 µm, fusoid thin walled, hyaline in KOH. STIPTIPELLIS cylindrical and parallel, 5.7 – 10.4 µm, hyaline in KOH. CLAMP CONNECTIONS: present.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, on soil in mixed *Pinus wallichiana* A. B. Jacks., and *Cedrus deodara* forest. 04 September, 2013, ISP # 022(LAH).

Plate 47

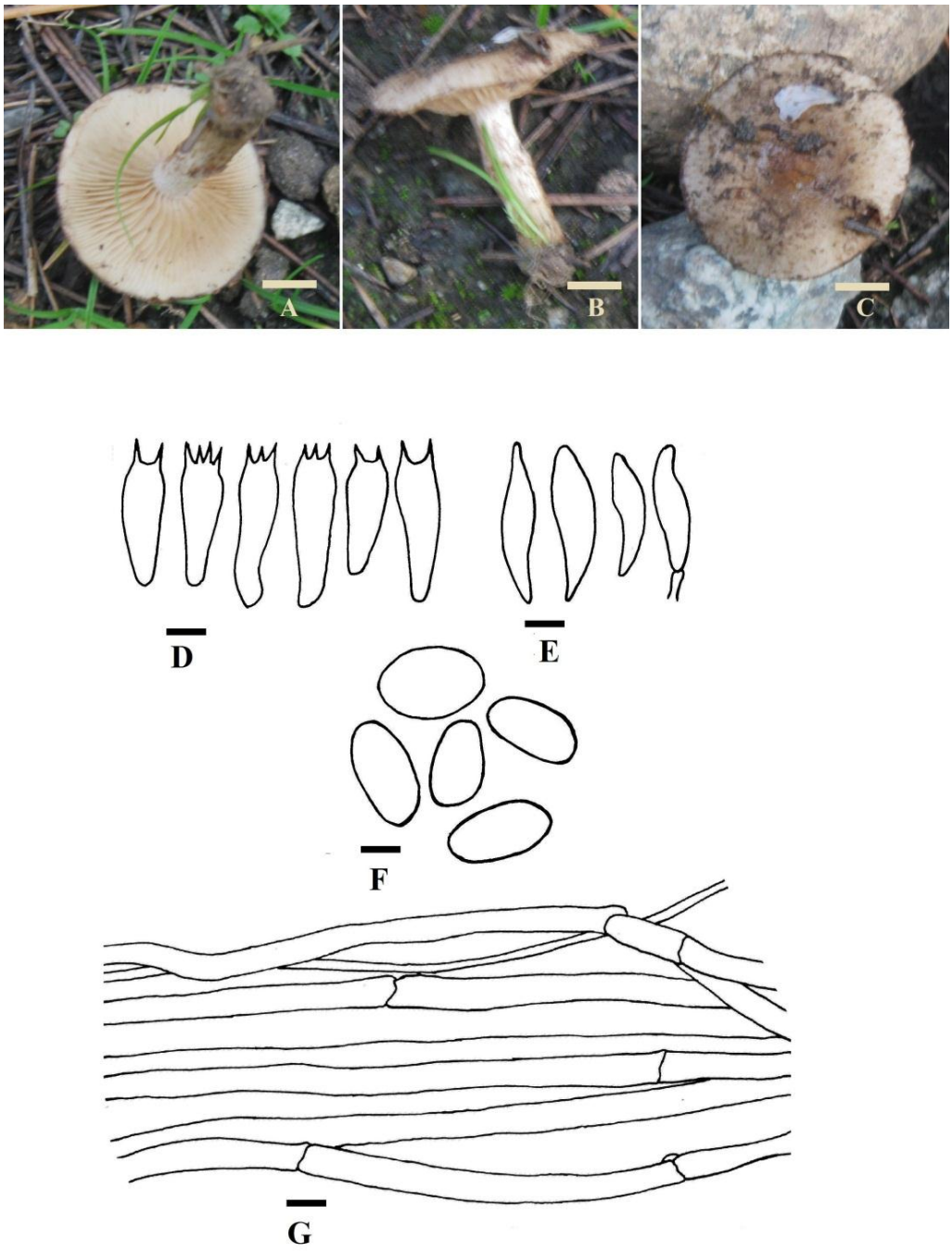


Figure (A-G) Morphological structures of ***Pholiota lanceolata*** nom. prov. (A-C) Basidiomata; (D) Basidia (E) Cheilocystidia; (F) Basidiospores (G) Stiptipellis. Scale bars: A-C = 0.8 cm, D = 6.5 µm E = 8 µm, F = 2.8 µm, G = 33 µm.

Molecular Phylogenetic Analysis

ITS region of *P. lanceolata* generated sequence of 613 base pairs. In a megablast search of the GenBank nucleotide database using the ITS sequence, the closest hit was *Pholiota adiposa* (Batsch) P. Kumm., Führ. Pilzk. (Zerbst) 1871 (GenBank AB470888; maximum identity 94%) from China, followed by *Pholiota gummosa* (Lasch) Singer 1951 (GenBank JF908580; maximum identity 94%) from Italy.

The newly generated ITS sequence of the newly discovered *Pholiota lanceolata* nom. prov. along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). The analysis was carried out for 30 reprentative sequences. Both coding and non-coding positions were included in the analysis. Among these 525/621 were conserved characters, 91/621 were variable, 62/621 were parsimony informative and 28/621 singletons. Those positions in which data was missing were not taken into account. Final dataset had 569 positions exclusively. Maximum likelihood (ML) analysis was conducted using MEGA version 6, with 1000 heuristic bootstrap (BS) replicates (Fig. 3.35).

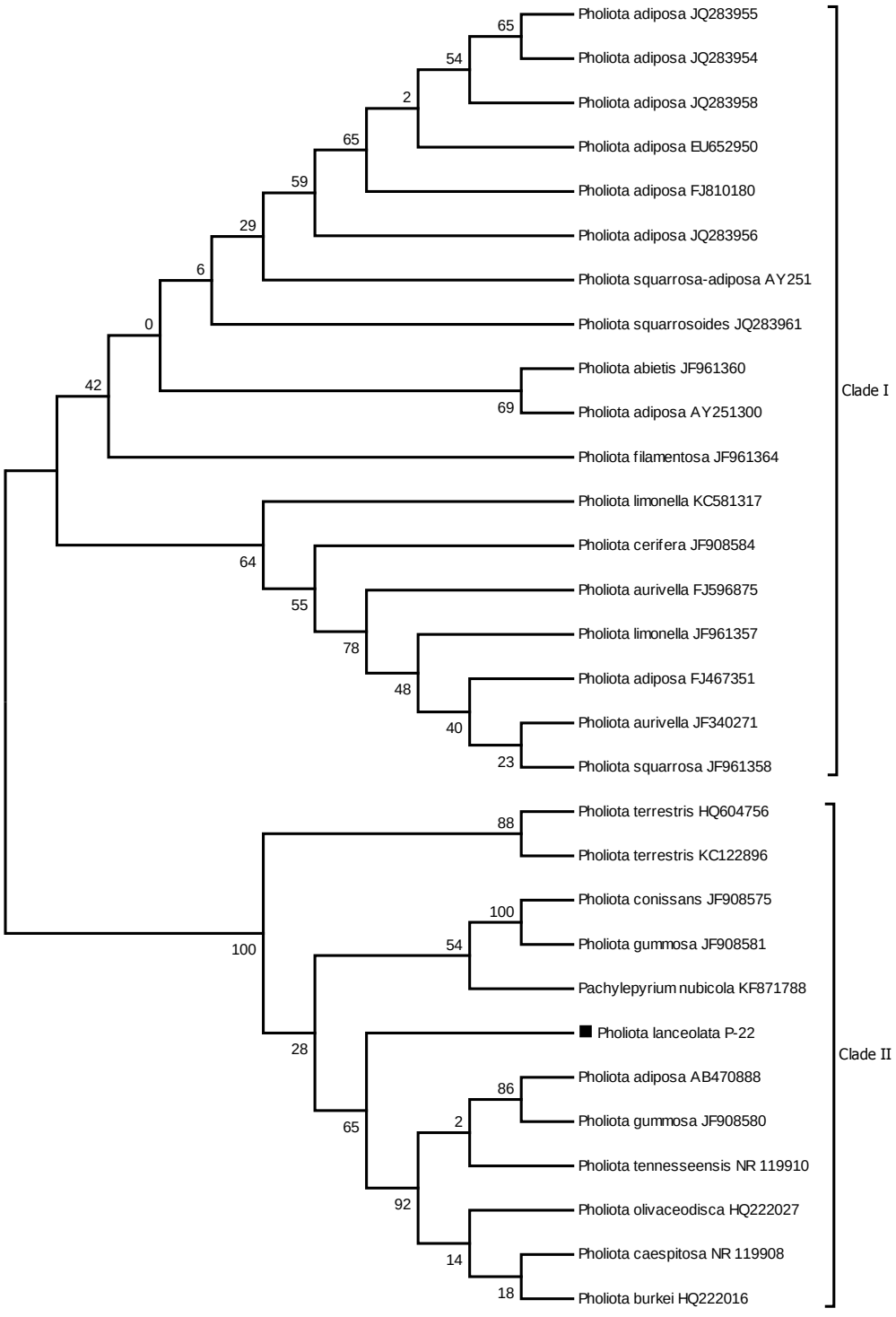


Figure 3.35 - Molecular phylogenetic analysis of of ***Pholiota lanceolata*** nom. prov. by Maximum Likelihood method. Highest log value = -1581.4. Black box (■) represents the new species.

41. ***Pholiota manga*** nom. prov. Plate 48 – Figs. A-G & 3.36

Etymology: The specific epithet may be referred to the larger pileus of this species.

Morpho-Anatomical characterization

PLEUS: 5.7 – 7.0 cm diam, plane with umbonate central disc; strong brown to strong orange (7.5YR 3/8 – 2.5YR 6/12); Surface, smooth, glabrous, velvety and slightly sticky; curvature, uplifted; margins, plicate; LAMELLAE: adnate and horizental, close, thin, strong brown to deep brown (5YR 4/8 – 5YR 2/6); margins smooth, convex, entire. STIPE: 3.0 – 4.0 × 0.1 – 0.7 cm, centrally attached; brownish orange to strong brown (5YR 5/8 – 5YR 4/8); soft, solid, cylindrical, smooth.

SPORES 5.4 – 7.1 × 7.5 – 10.7µm [x = 6.1 × 8.7 µm, Q = 1.421], globose to hemiglobose to oblong, smooth, thin-walled. BASIDIA: 20.3 – 25.7 × 7.5 – 10.4 µm, clavate, three spored, thin-walled, hyaline in KOH. Sterigmata, 4.2 – 7.8 µm, pointed CHEILOCYSTIDIA: 41.3 – 60.4 × 11.0 – 19.8 µm, thick walled, hyaline in KOH. PLEUROOCYSTIDIA: 56.1 – 70.3 × 15.3 – 18.4 µm, thick walled, hyaline in KOH. PILEIPELLIS: a cutis, hyphae cylindrical, 3.8 – 9.0 µm, thin-walled, hyaline in KOH. STIPTIPELLIS: cylindrical and parallel, 4.7 – 11.8 µm, hyaline in KOH.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Malam Jabba valley, on soil in mixed *Pinus wallichiana* A. B. Jacks., and *Cedrus deodara* forest. 02 September, 2013, ISP # 006(LAH).

Plate 48

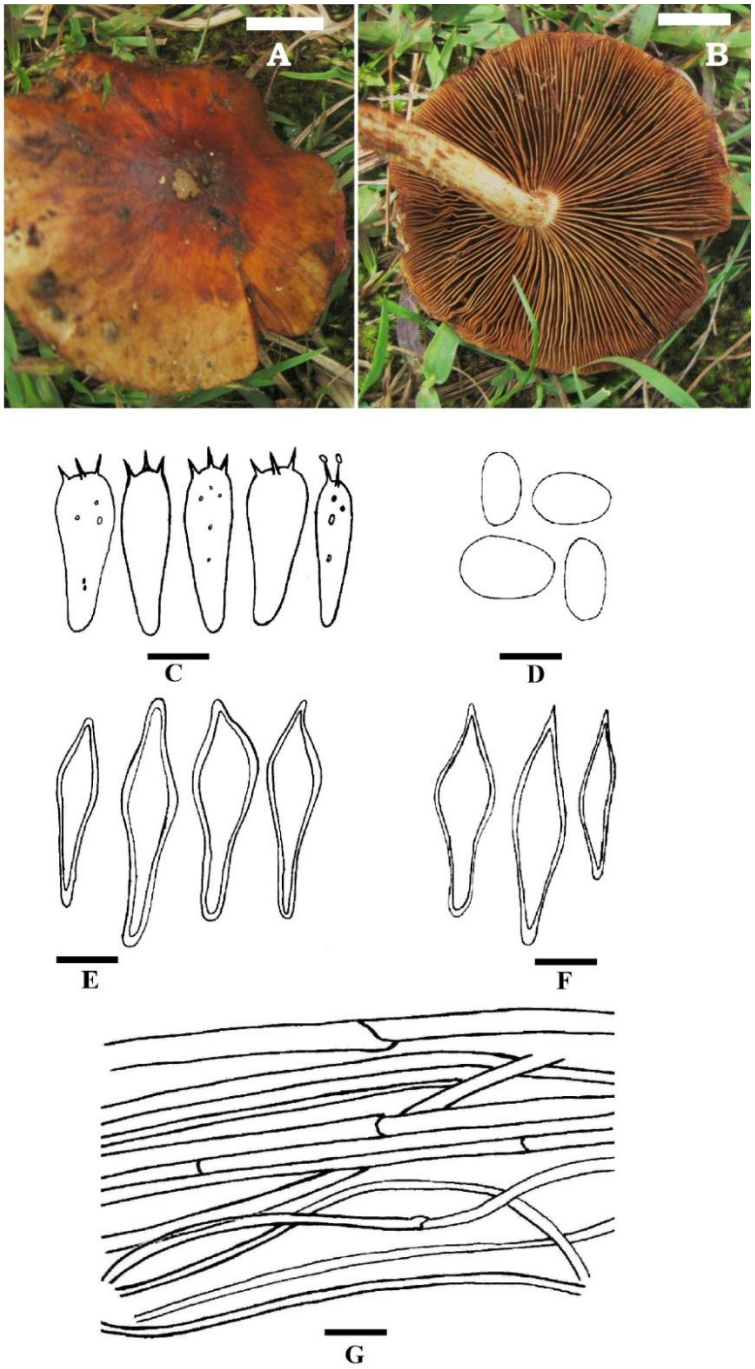


Figure 3.22.3.1 (A-G) Morphological structures of ***Pholiota manga*** nom. prov. (A-B) Basidiomata; (C) Basidia (D) Basidiospores (E) Cheilocystidia; (F) Pleurocystidia (G) Stiptipellis. Scale bars: A-B = 1.3 cm, C = 8 µm D = 6 µm E = 13 µm, F = 19 µm, G = 25 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 658 base pairs. In initial BLAST search of GenBank database, closest hit was *Pholiota lubrica* (Pers.) Singer 1951 (GenBank HG007986, Query coverage - 99%, expect value - 0.0 and Identity index - 92%) from Czech Republic.

The newly generated ITS sequence of *Pholiota manga* nom. prov. along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 447/655 were conserved characters, 186 were variable, 110 were parsimony informative and 76 were singletons. The analysis was carried out for 29 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 524 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.36).

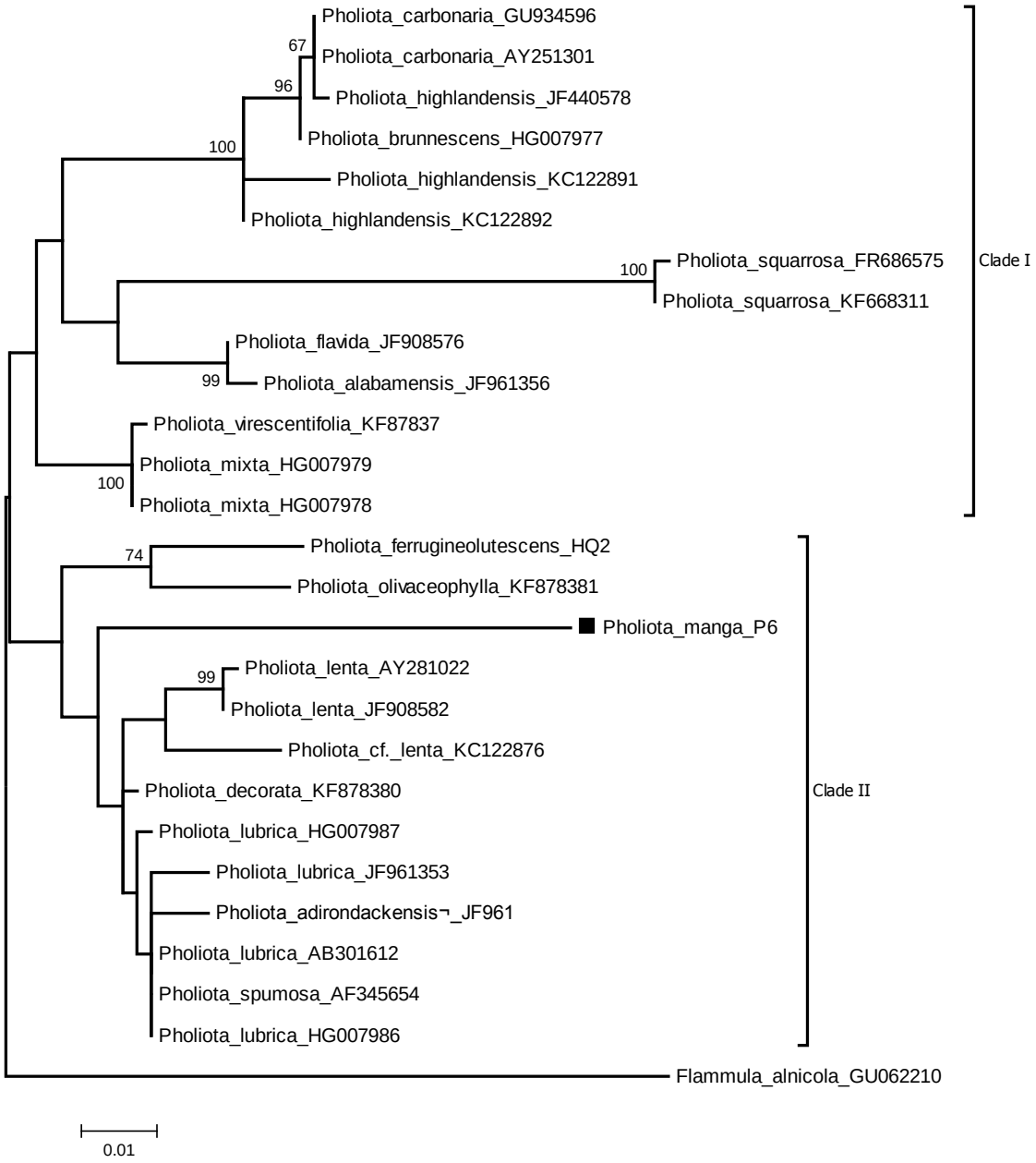


Figure 3.36 Molecular phylogenetic analysis of ***Pholiota manga*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1962.0963) is shown. Collection of Swat is represented by black cube (■).

42. *Pholiota spumosa* (Fr.) Singer, *Lilloa* 22: 517 (195

Plate 49 – Figs. A-F & 3.37

Morpho-Anatomical characterization

PLEUS: 2.0 – 3.5 cm diam, convex obtuse to umbonate, deep orange to orange yellow (2.5YR – 7.5YR 8/16 – 10YR 9/8), surface squamolose, dry, velvety; curvature deflexed; margins plicate to straight; LAMELLAE: sinnuate, close to sub distant, thin, light orange yellow (10YR 9/8); STIPE: 2.1 – 5.0 × 0.3 – 0.5 cm, soft, centrally attached, equal, cylindrical, light orange yellow (10YR 9/8).

BASIDIOSPORES: 6.0 – 9.1 × 4.5 – 5.5 µm [X = 7.9 × 4.8 µm, Q = 1.645], broadly ellipsoid, smooth, thick-walled, olive green to yellow in KOH, BASIDIA: 20.7 – 25.5 × 6.5 – 8.4 µm, clavate, tetrasporic , thin-walled, hyaline in KOH. CHEILOCYSTIDIA: 31.6 – 44.5 × 9.1 – 15.5 µm, fusoid venticose to utriform, thin walled. PLEUROCYSTIDIA: 42.0 – 59 × 11.1 – 15.1 µm, fusoid venticose to ninepin-shaped, thin walled.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Malam Jabba valley, on rotton wood trunk in mixed *Pinus wallichiana* A. B. Jacks., and *Cedrus deodara* forest. 02 September, 2013, ISP # 001(LAH).

COMMENTS *Pholiota spumosa* is reported by Iqbal and Khalid (1996) previously from Pakistan. This species is also reported from Swat earlier.

Plate 49

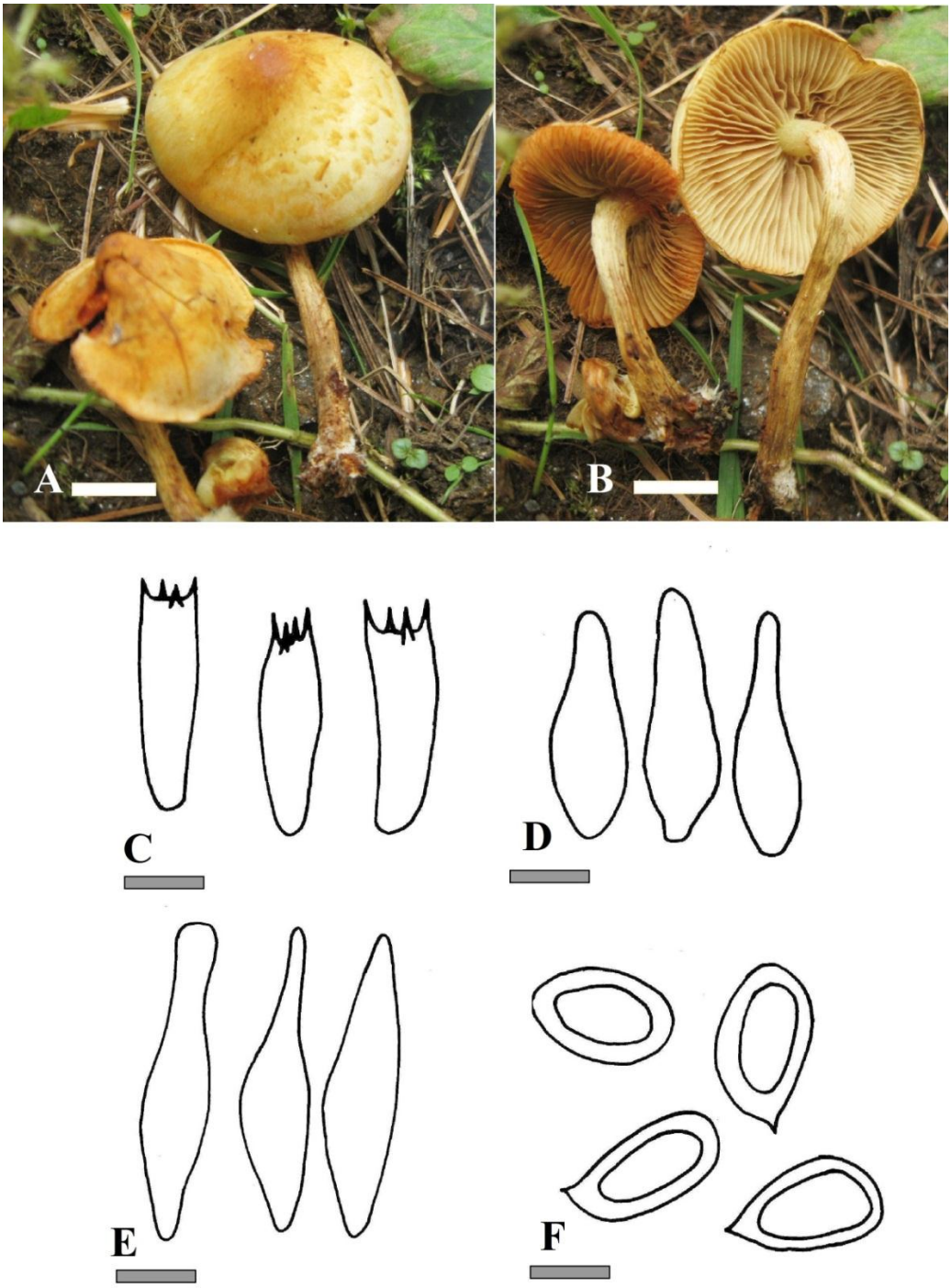


Figure (A-F) Morpho-anatomical shapes of ***Pholiota spumosa*** (A-B) Basidiomata (C) Basidia (D) Cheilocystidia (E) Pleurocystidia (F) Basidiospore. Scale bar: A-B = 0.5 µm, C = 8 µm, D = 12 µm, E = 14 µm, F = 3.5 µm

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 621 base pairs. In initial BLAST search of GenBank database, closest hit was *Pholiota spumosa* (GenBank KC122890, Query coverage - 100%, expect value - 0.0 and Identity index - 99%).

The newly generated ITS sequence of *Pholiota spumosa* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 503/645 were conserved characters, 128 were variable, 97 were parsimony informative and 30 were singletons. The analysis was carried out for 24 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 555 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.37).

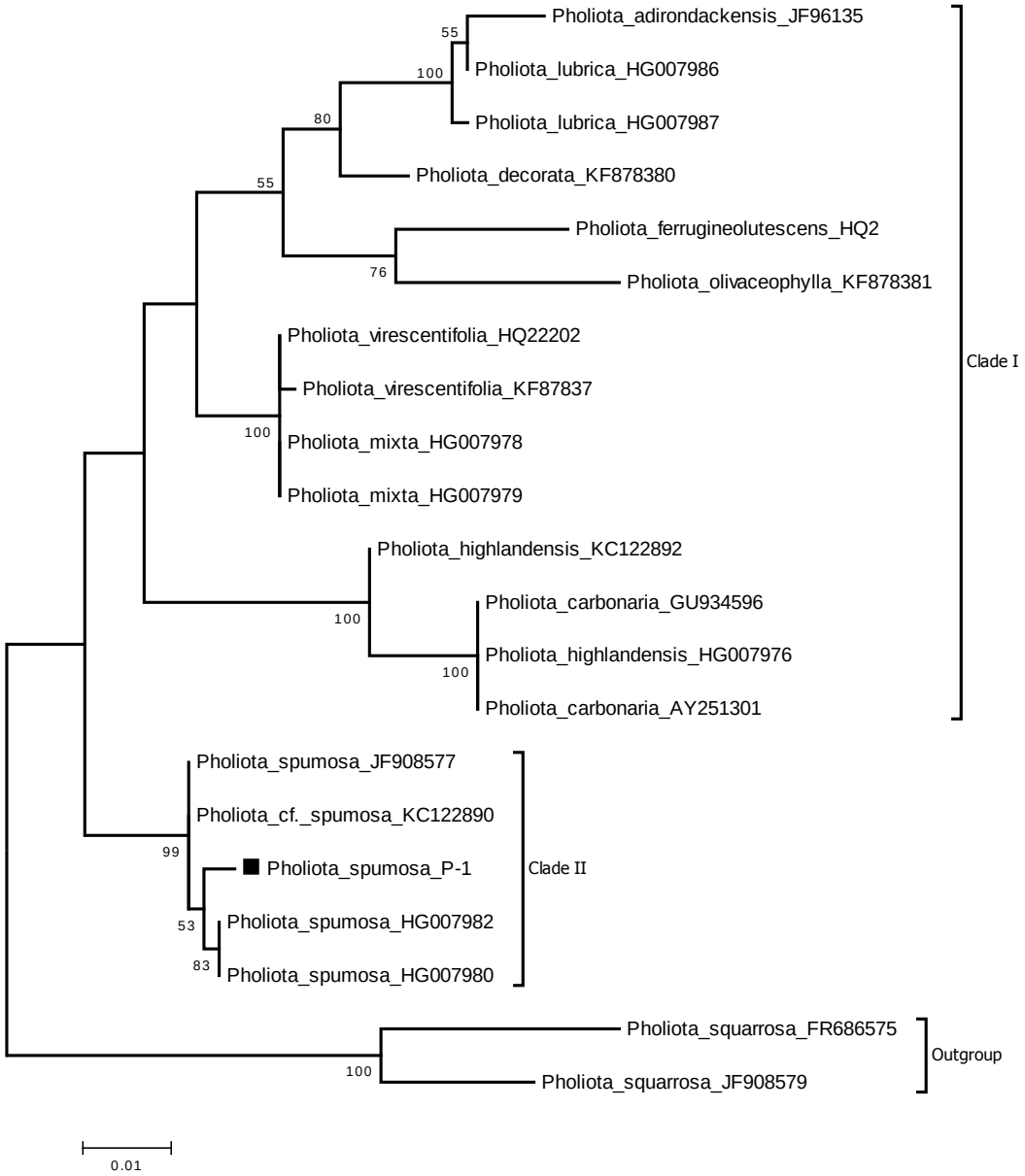


Figure 3.37 Molecular phylogenetic analysis of ***Pholiota spumosa*** by Maximum Likelihood method. Maximum log likelihood value = (-1757.7345) is shown. Collection of Swat is represented by black cube (■).

Tricholomataceae

Clitocybe

43 *Clitocybe gibba*

Lepista

44 *Lepista irina*

Pseudoomphalina

45 *Pseudoomphalina conifera* nom. prov.

Leucocalocybe

46 *Leucocalocybe mongolica*

43. *Clitocybe gibba* (Pers.) P. Kumm., *Führ. Pilzk*. (Zerbst): 123 (1871)

Plate 50 – Figs. A-F & 3.38

Morpho-Anatomical characterization

PLEUS: 4.5 – 6.5 cm diam, depressed umbillicate, moderate orang yellow to dark orange yellow (2.5YR7/8 – 10YR7/8 – 7.5YR6/8), appressed fibrillose, velvety, dry; curvature recurrved; margins uplifted pubescent; LAMELLAE: decurrent, close, thin, off white; margins ragged eroded. STIPE: 4.0 × 0.8 cm, centrally attached, soft, equal and cylindrical slightly tapered at the apex off white in the upper region to yellowish brown in the lower region (2.5Y6/6).

BASIDIOSPORES: (5 – 8.5) × 8.5 – 6.7 µm [x = 7.0 × 8.0 µm, Q = 1.14], hemispheric to ellipsoid, apiculus no present, smooth, hyaline in KOH, thin-walled, guttiulate. BASIDIA: 22 – 32 × 8.5 – 12.5 µm, clavate, thin-walled, monosporic to quadrisporic, hyaline in KOH; TRAMAL HYPHAE: cylindrical, hyaline in KOH, thin walled. PLEUROCYSTIDIA: 15 – 28.3 × 5.7 – 8.3 µm clavate, thin-walled, hyaline in KOH, guttiulate. CHIELOCYSTIDIA: 16.5 – 33 × 6 – 10.6 µm clavate, hyaline in KOH, thin-walled, guttiulate. STIPE HYPHAE: thin walled. cylindrical, 4.7 – 15 µm, hyaline in KOH.

MATERIAL EXAMINED: PAKISTAN, KHYBER PUKHTUNKHWA, Kalam Swat, on soil in mixed *Pinus wallichiana* A. B. Jacks., and *Cedrus deodara* forest. 02 September, 2013, ISP # 013(LAH)

COMMENTS: Previously reported from Balakot (Mansehra) Pakistan by Murakami (1993).

Plate 50

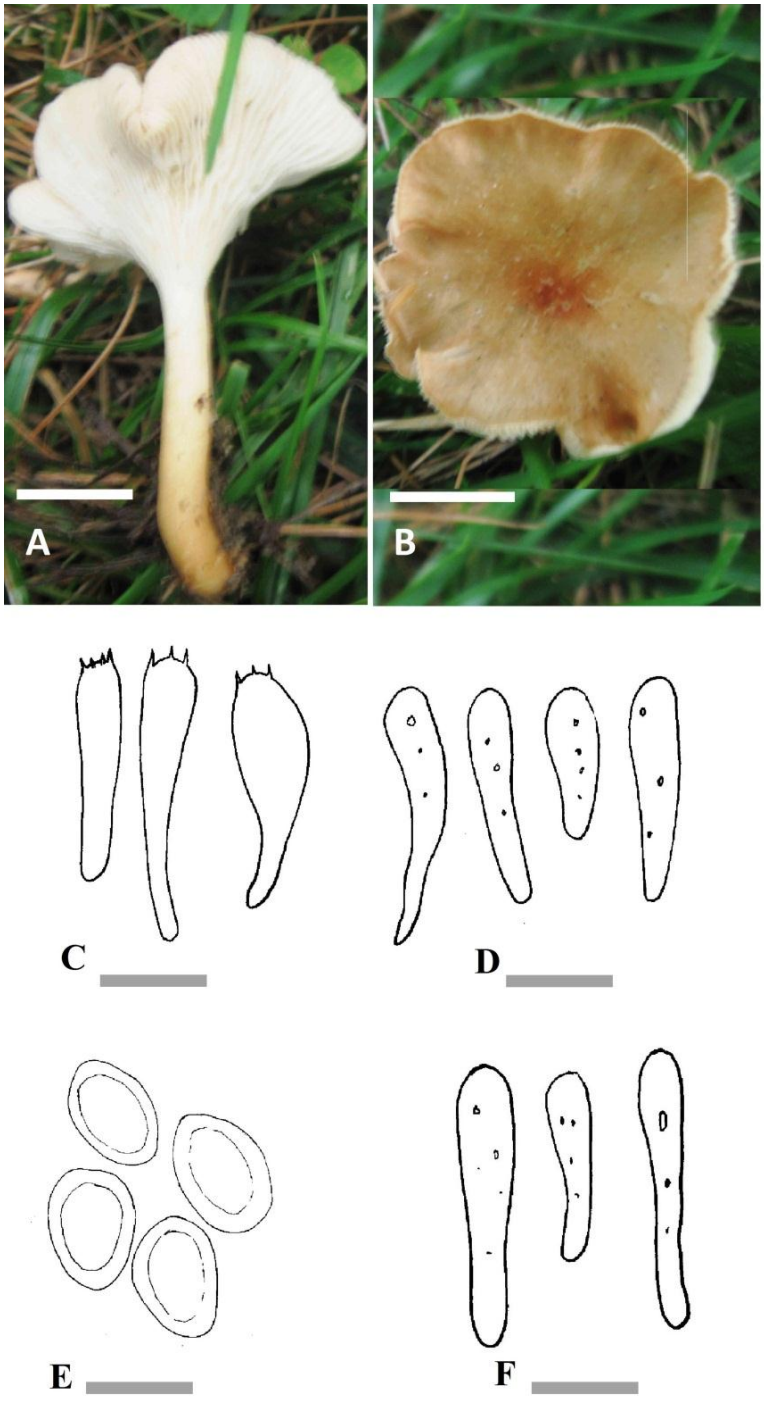


Figure A-F Morpho-anatomical shapes of ***Clitocybe gibba*** (A-B) Basidiomata (C) Basidia (D) Chielocystida (F) Pleurocystidia (E) Basidiospores. Scale bars: A = 1.5 cm, B = 2 cm C = 4.5 µm, D = 4, µm, E = 6.5 µm, F = 5 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 609 base pairs. In initial BLAST search of GenBank database, closest hit was *Infundibulicybe gibba* Pers.) Harmaja 2003 = *Clitocybe gibba* (GenBank GU188436, Query coverage - 100%, expect value - 0.0 and Identity index - 97%), followed by *Infundibulicybe gibba* (GenBank FJ596815, Query coverage - 100%, expect value - 0.0 and Identity index - 96%) from USA.

The newly generated ITS sequence of the current *Clitocybe* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 415/695 were conserved characters, 247 were variable, 132 were parsimony informative and 103 were singletons. The analysis was carried out for 22 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 536 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.38).

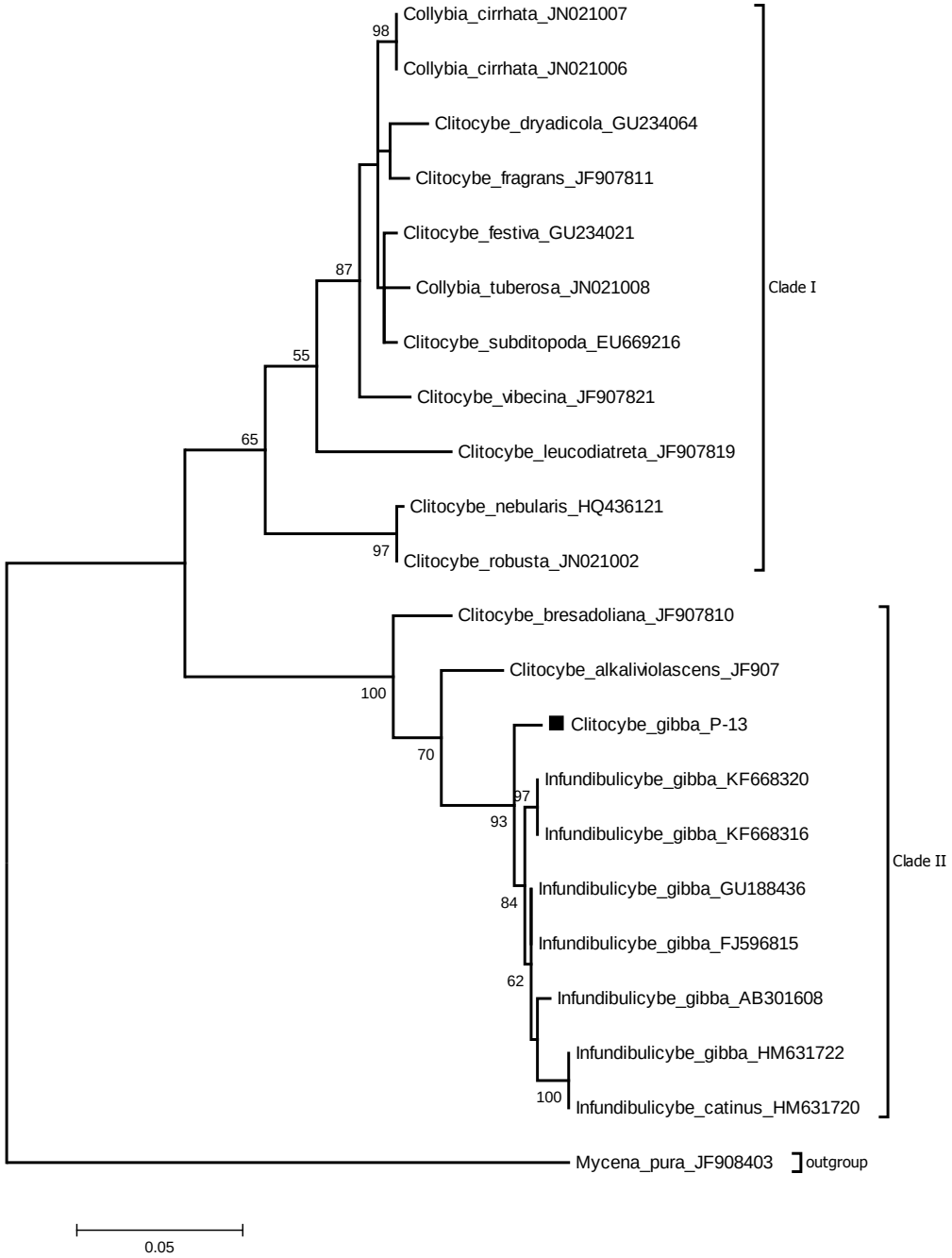


Figure 3.38 Molecular phylogenetic analysis of ***Clitocybe gibba*** by Maximum Likelihood method. Maximum log likelihood value = (2158.5866) is shown. Collection of Swat is represented by black cube (■).

44. *Lepista irina* (Fr.) H. E. Bigelow. *Can. J. Bot.* 37: 775 (1959)

Plate 51 – Figs. A-G & 3.39

Morpho-Anatomical characterization

PLEUS: 2.5 – 8.9 cm in diameter, convex, central disc slightly depressed; whitish when young moderate orang to deep brownish orange when mature (5YR5/8 – 5YR6/8) with surface smooth glabrous velvety, margins rolled to cottony become eroded at maturity, curvature deflexed to inrolled. LAMELLAE: adnate, close, thin, margins ragged eroded, light reddish orange to light brownish orange (10R4/12 – 5YR5/8). STIPE: 1.6 – 8.6 × 0.6 – 3 cm, cylindrical, hollow, centrally attached, soft, white to light brownish to moderate brownish orange (5YR7/8 – 5YR5/8).

BASIDIOSPORES: 3.2 – 4.2 × 5.6 – 9.7 µm [X = 4.0 × 6.5 µm, Q = 1.625], ellipsoid to oblong, spiny, apiculate, hyaline in KOH, thin-walled, non-amyloid. BASIDIA: 22.5 – 28.1 × 6.2 – 8.8 µm, clavate, thin-walled, one to four spored basidia, hyaline in KOH. TRAMAL HYPHAE: regular, cylindrical, 6.1–8.9 µm, hyaline in KOH, thin walled. CYSTIDIA: absent. PILEIPELLIS: a cutis, hyphae thin-walled, cylindrical, 4.0 –8.6 µm, hyaline in KOH. STIPE HYPHAE: cylindrical, 3.7 – 8.6 µm, hyaline to pale yellow in KOH. CLAMP CONNECTIONS: present in all tissues.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Shangla, Yakh Tangai, on soil in mixed *Pinus wallichiana* A. B. Jacks., and *Cedrus deodara* forest. 02 September, 2013, ISP # 005, 010, 011 & 012 (LAH)

COMMENTS: *Lepista irina* is new record for Pakistan.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 700 base pairs. In initial BLAST search of GenBank database, closest hit was *Lepista irina* (GenBank HM237136, Query coverage - 100%, expect value - 0.0 and Identity index - 99%).

The newly generated ITS sequence of the current *Lipista* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among studied characters 484 out of 659 were conserved, 145 were variable, 72 were parsimony informative and 65 were singletons. The analysis was carried out for 15 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 506 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.39).

Plate 51

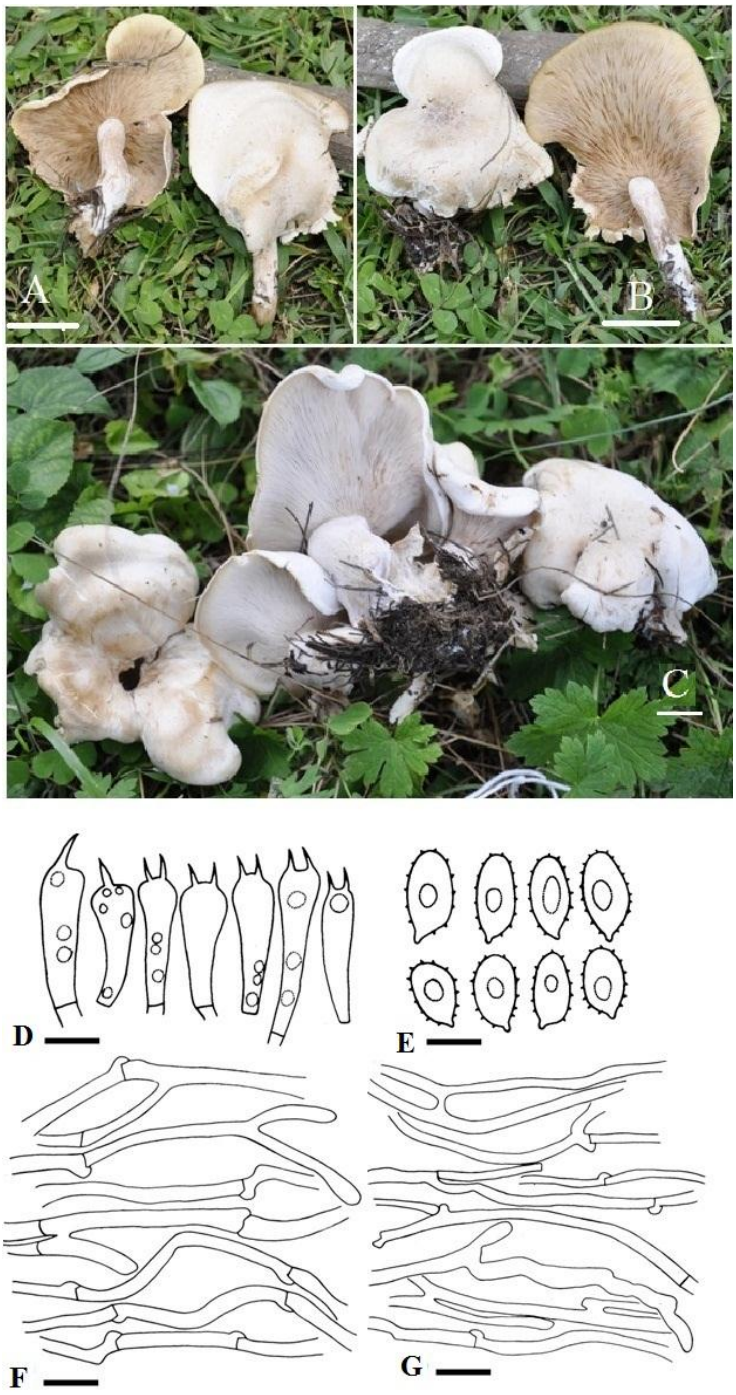


Figure Morpho-anatomical shapes of ***Lepista irina*** (A–G) Basidiomes (D) Basidia (E) Basidiospores (F) Pilepellis (G) Stipe elements. Scale bars: A-C= 1 cm, D = 7µm, E = 4.5µm, & F-G =21.5µm.

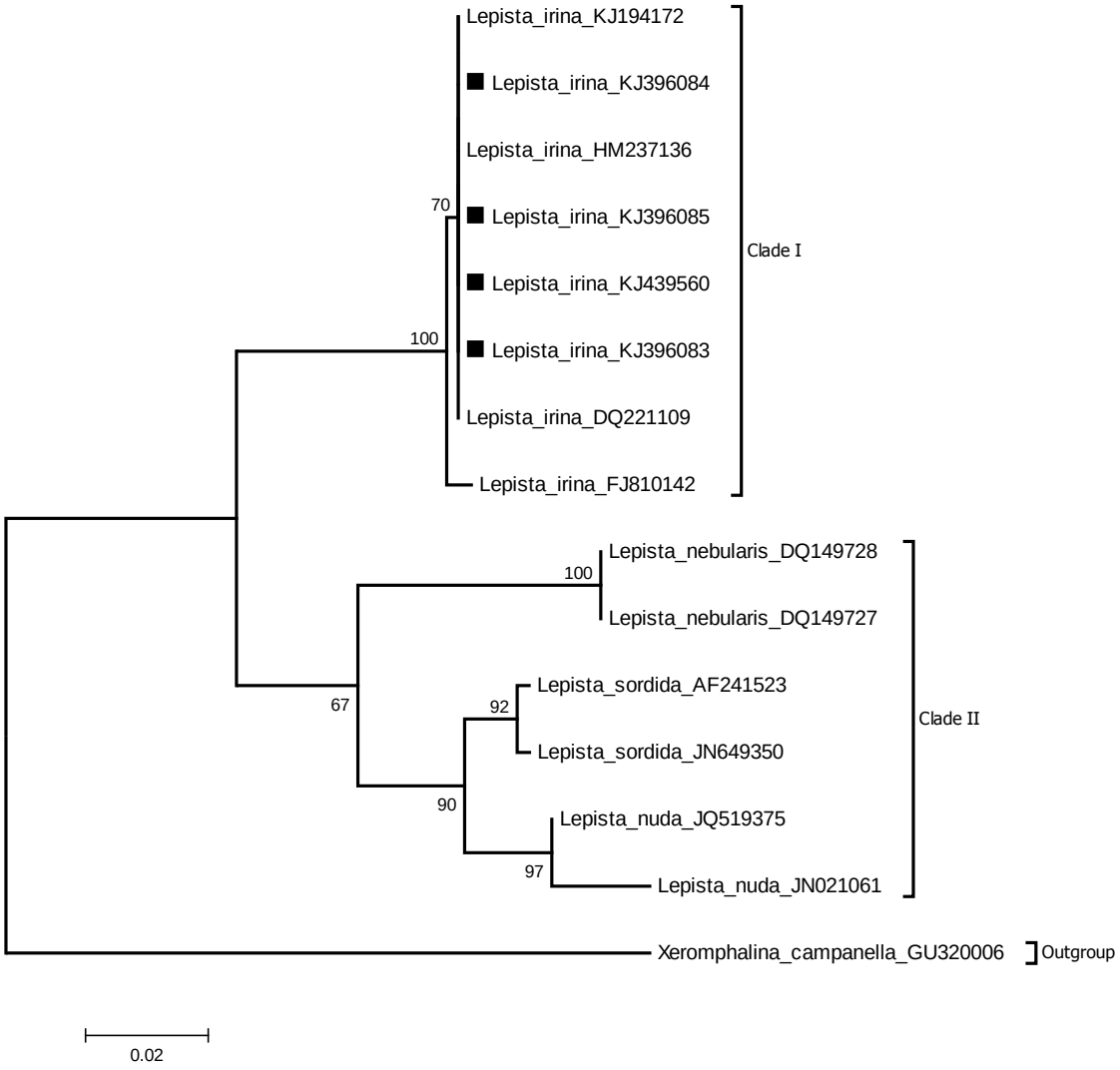


Figure 3.39 Molecular Phylogenetic affiliation of ***Lepista irina*** with associated members of *Lipista* based on Maximum Likelihood method through nrITS sequences. Highest log likelihood value is (-1374.8033). Collection entertained in current study is represented by (■).

45. ***Pseudoomphalina conifera*** nom. prov. Plate 52 – Figs. A-F & 3.40

Etymology: The specific epithet may be referred to the habitat of taxa in coniferus forests.

Morpho-Anatomical characterization

PLEUS: 105 – 2.5 cm in diameter, depressed, umbillicate central disc; pale orange yellow to deep orange brown in center (2.5Y 9/4 – 2.5YR 5/8) with smooth apressed fibillose most surface; curvature straight; margins rolled to cottony; LAMELLAE: decurrent, subdistant, thin, snow white; margins ragged curved. STIPE: 3.0 – 3.3 × 0.2 – 0.3 cm, centrally attached, soft, , cylindrical and equal, pale yellow (2.5R 9/4); surface smooth, pseudorhizzae present.

BASIDIOSPORES: (4) 4.2 – 6.5 (7.4) × (7.4) 7.8 – 16.2 (16.7) µm [X = 5.4 × 12.1 µm, Q = 2.24], narrowly ellipsoid to oblong, apiculate, smooth, thin-walled, olive green in KOH and phloxin. BASIDIA: 18.3 – 32.1 × 5.5 – 7.9 µm, narrowly clavate, bisterigmate, guttiulate, thin-walled, hyaline in KOH; Sterigmata 3 – 4 µm. CHEILOCYSTIDIA: 24.0 – 31.2 × 5.0 – 7.1 µm, narrowly clavate, , guttiulate, thin-walled, hyaline in KOH; STIPTIIPILLIS a laxolattice, hyphae cylindrical, 4.0 – 7.8 µm, thin-walled, hyaline in KOH.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Kalam, on soil in mixed *Pinus wallichiana* A. B. Jacks., and *Cedrus deodara* forest. 02 September, 2013, ISP # 030(LAH)

Plate 52

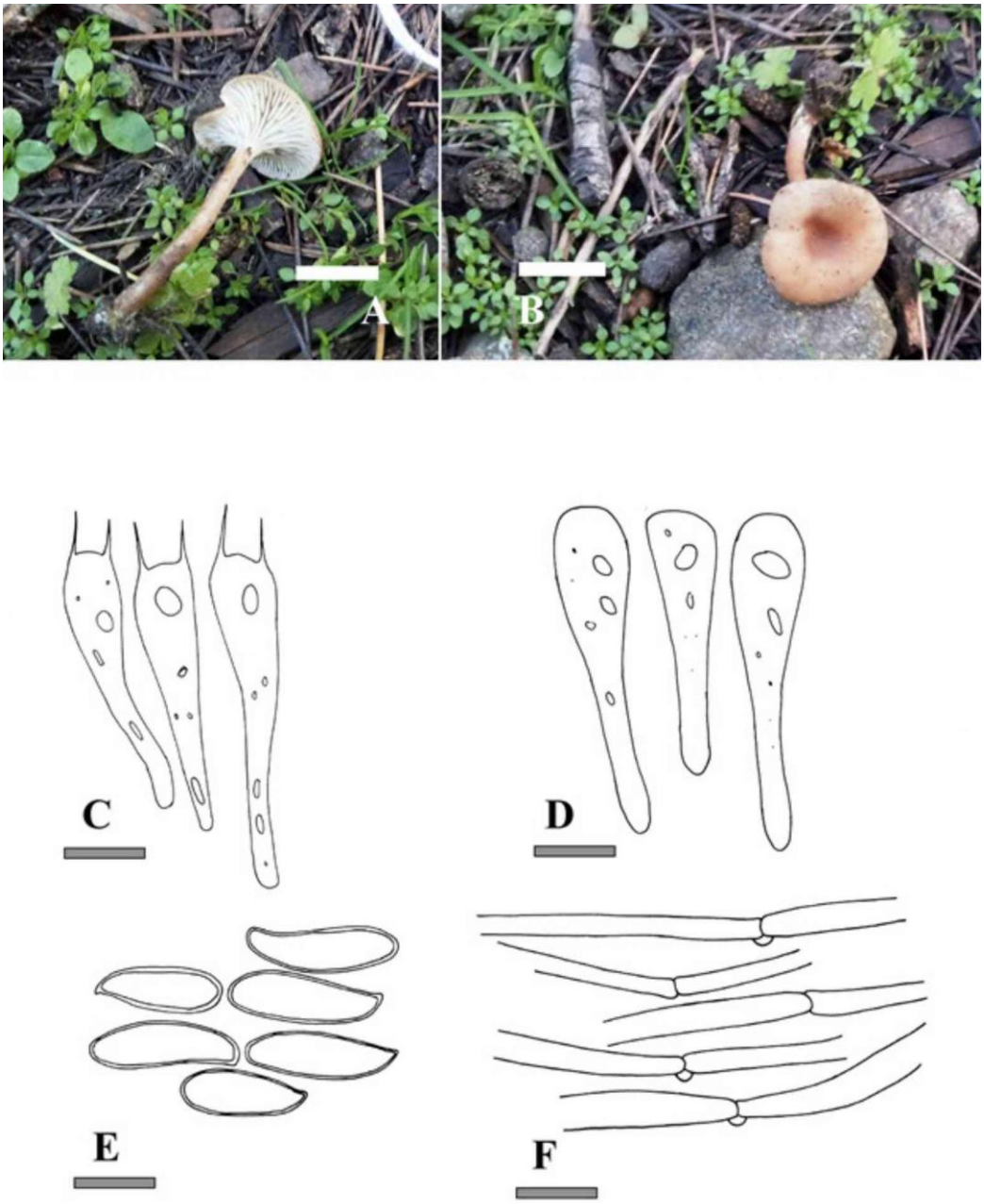


Figure A-F Morpho-anatomical shapes of ***Pseudoomphalina conifera*** nom. prov. (A-B) Basidiomata (C) Basidia (D) Chielocystida (E) Basidiospores (F) Stitipellis. Scale bars: A = 1.5 cm, B = 2 cm C = 4.5 µm, D = 4, µm, E = 6.5 µm, F = 5 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 642 base pairs. In initial BLAST search of GenBank database, the closest hit was *Pseudoomphalina kalchbrenneri* (Bres.) Singer 1956 (GenBank KF381526; maximum identity - 100%, E-value – 0.0 Query coverage - 90%) from Denmark. Comparison of ITS sequence data obtained for the present *Pseudoomphalina* species with the identical nucleotide sequences in GenBank depicted the identity of our collection as *species novem*.

The generated ITS sequence of *Pseudoomphalina conifer* nom. prov. along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Among studied characters 462 out of 699 were conserved, 299 were variable, 215 were parsimony informative and 14 were singletons. The analysis was carried out for 21 nucleotide sequences. Those positions in which data was missing were not taken into account. Final dataset had 510 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.40).

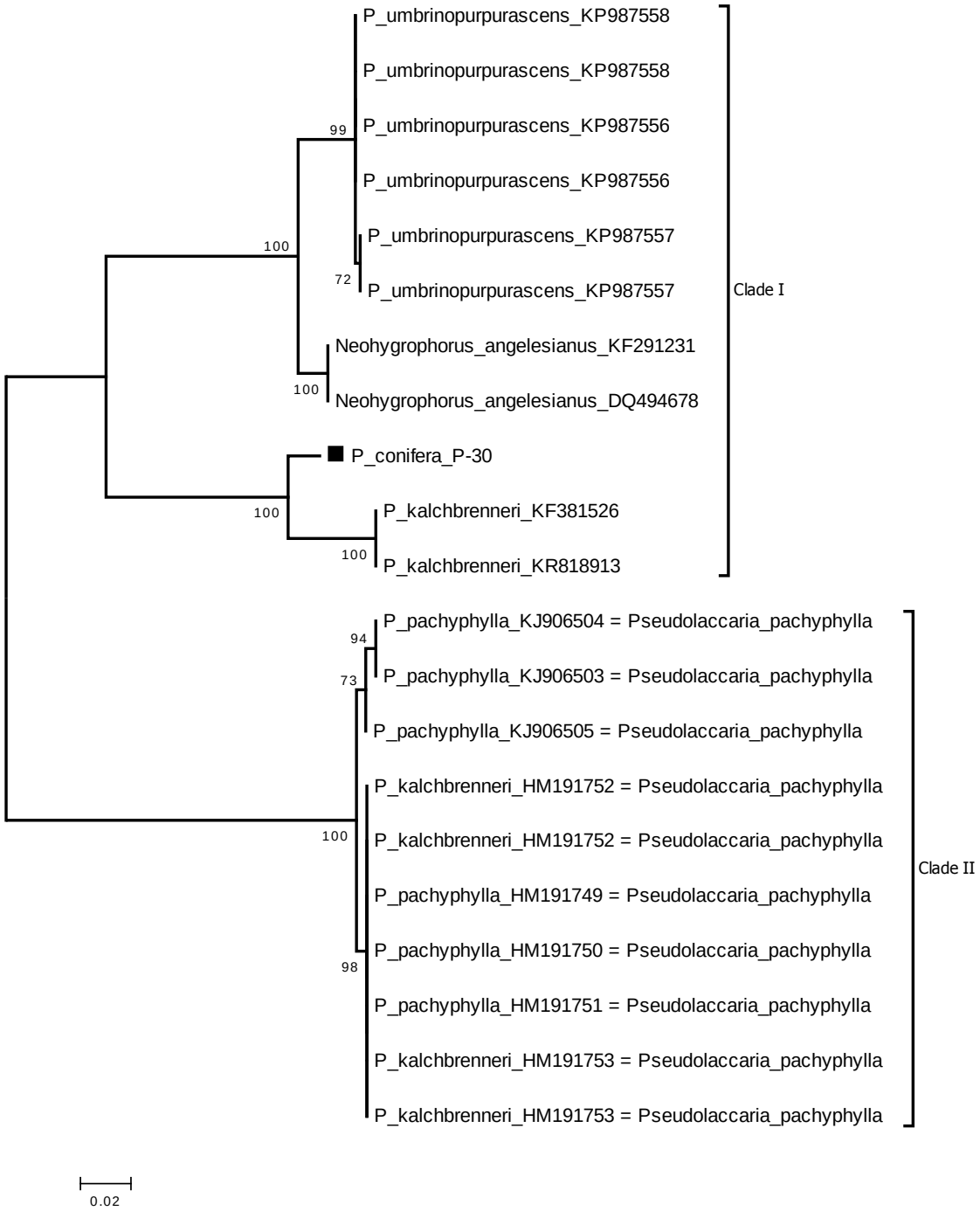


Figure 3.40 Molecular phylogenetic analysis of ***Pseudoomphalina conifer*** nom. prov. by Maximum Likelihood method. Maximum log likelihood value = (-1545.1774) is shown. Collection of Swat is represented by black cube (■).

46. *Leucocalocybe mongolica* (S. Imai) X.D. Yu & Y.J. Yao [as *'mongolicum'*], *Afr. J. Microbiol. Res.* 5(31): 5751 (2011) **var. *purpura*** var. nov.

Plate 53 – Figs. A-E & 3.41

Morpho-Anatomical characterization

PLEUS: 6 – 7 cm across, plano convex, slightly obtuse, very pale blue to light yellowish brown at places (10B 8/2 – 10YR 6/4), surface, appressed fibrillose, velvety; curvature, deflexed; margin, cottony. LAMELLAE: sinuate, crowded, thick, color same to pileus; marginate; STIPE: 2 – 2.5 × 1.3 – 1.7 cm, centrally attached, cartilage like, equal, cylindrical.

BASIDIOSPORES: (4.0) 4.3 – 5.6 (6.0) × 7.3 (7.5) – (8.7) 9.0 µm [x = 8.1 × 4.9 µm, Q = 1.653], pulvinate to oblong, thin-walled, hyaline in KOH. BASIDIA: (7.8) 8.4 – 11.4 (11.8) × (23.4) 25.9 – 36.4 (37.1) µm, clavate, quadrisporic, thin-walled, hyaline in KOH, red in phloxin; CYSTIDIA: absent. STIPE HYPHAE: cylindrical, cutis, septae present 3.5 – 6.6 µm, hyaline KOH.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Swat, Miandam, on soil in mixed *Pinus, Cedrus,Taxus* and *Abies* forest. 02 September, 2013, ISP # 083(LAH)

COMMENTS: *Leucocalocybe mongolica* is new record for Pakistan.

Plate 53

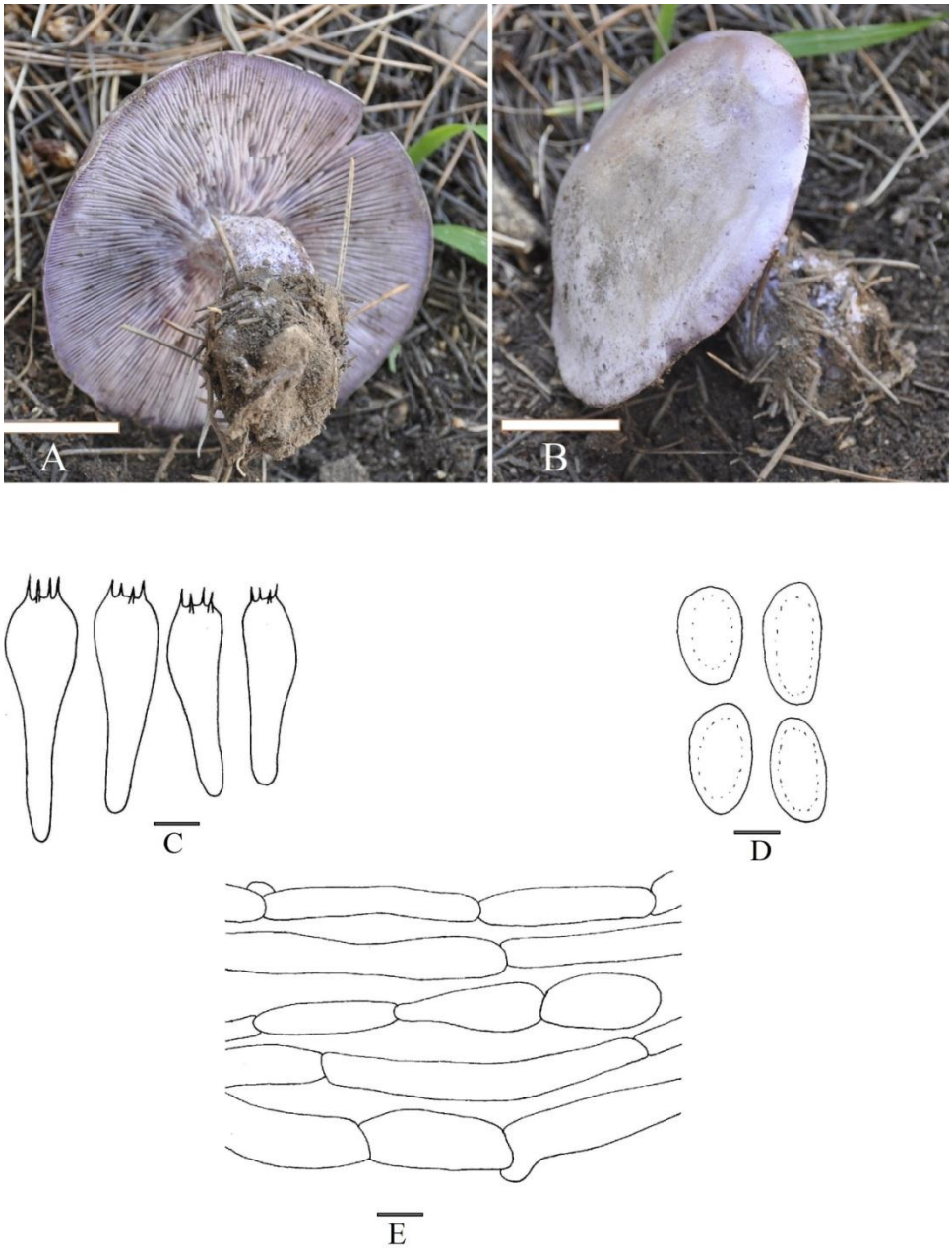


Figure A-E Morpho-anatomical shapes of ***Leucocalocybe mongolica*** **var.** ***purpura*** var. nov. (A-B) Basidiomata (C) Basidia (D) Chielocystida (F) Pleurocystidia (E) Basidiospores. Scale bars: A-B = 1.7 C = 6 µm, D = 3, µm, E = 4 µm.

Molecular Phylogenetic Analysis

ITS region of this collection generated a sequence of 621 base pairs. In initial BLAST search of GenBank database, closest hit was *Tricholoma mongolicum* S. Imai = *Leucocalocybe mongolica* (S. Imai) X.D. Yu & Y.J. Yao (GenBank KC413949, Query coverage - 100%, expect value - 0.0 and Identity index - 97%) from China.

The newly generated ITS sequence of the current *Leucocalocybe* collection along with those retrieved from GenBank based on the result from BLAST search were aligned using MUSCLE version 3.8 (Edgar 2004). Bio N.J. & Neighbor-Join data algorithms were used to acquire preliminary tree(s) for the experiential search automatically to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Evolutionary affiliations of the taxa analyzed were represented by selecting the bootstrap consensus tree inferred from 1000 replicatesis. Among these 537/640 were conserved characters, 97 were variable, 70 were parsimony informative and 27 were singletons. The analysis was carried out for 30 reprentative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were not taken into account. Final dataset had 560 positions exclusively. Evolutionary affiliations of the closest taxa used herein were sorted out in MEGA6 (Fig. 3.41).

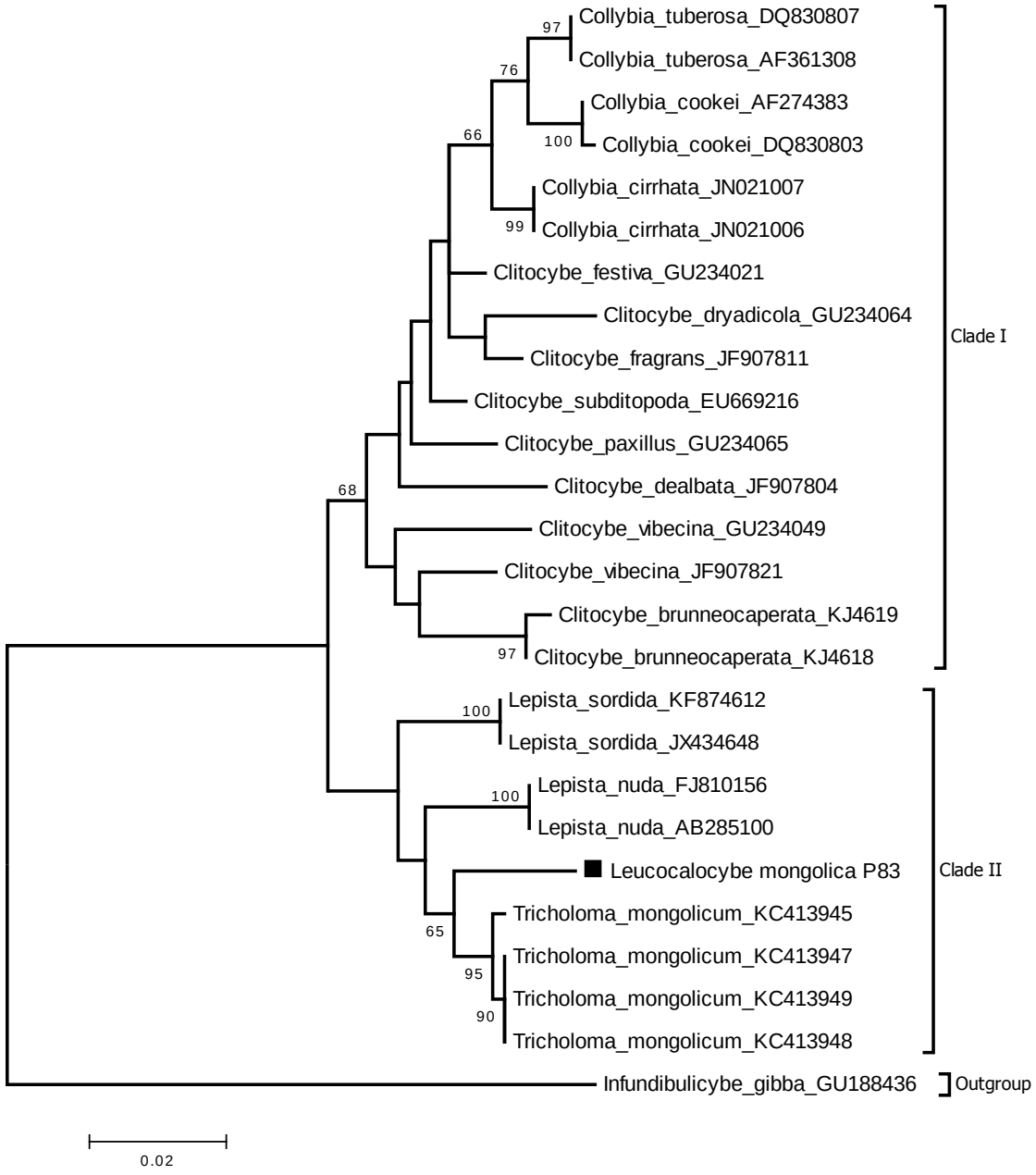


Figure 3.41 Molecular phylogenetic analysis of ***Leucocalocybe mongolica*** **var.** ***purpura*** var. nov. by Maximum Likelihood method. Maximum log likelihood value = (-1822.7797) is shown. Collection of Swat is represented by black cube (■).

CHAPTER 4 DISCUSSION

ITS barcoding of the nrDNA is a novel system of accurate and rapid fungal species identification. It is accepted as universal barcode of fungi (Peay, 2008). In this study morphological descriptions have been coupled with ITS barcoding for authentic identification and phylogenetic inference of mushroom forming fungi of Swat.

Agaricaceae

Agaricaceae is monophyletic group of saprotrophic fungi that exhibits huge diversity. Agaricoid, as well as secotioid and gasteroid taxa are included in this family (Singer, 1986; Redhead *et al*. 2001; Kruger *et al*. 2001; Moncalvo *et al*. 2002; Vellinga 2004a and Kirk *et al*. 2008). In present study this family is represented by 3 species of 3 genera viz *Echinoderma asperum, Lepiota granulata* and *Leucocoprinus cretacious*.

The genus *Lepiota* having more than 400 species inhabits mostly the temperate regions of the world (Vellinga 2004b; Kirk *et al*. 2008). Fries (1821) included squamose to annulate white-spored mushrooms in genus *Lepiota*. However, *Leucocoprinus* Pat., *Cystoderma* Fayod, *Limacella* Earle, and all the more recent split-offs from the genus, like *Leucoagaricus* Locq. ex Singer, *Macrolepiota* Singer, and *Cystolepiota* Singer are established by a much more restricted description of the genus *Lepiota* (Singer 1986; Bon 1993; Candusso & Lanzoni 1990; Vellinga 2001a). The present-day definition of this genus is much narrower and is mainly based on microscopic characters (Vellinga, 2003).

Among the studied lepiotoid agarics, the Freckled Dapperling- ***Echinoderma asperum*** is new report for Swat. Previously considered as member of *Lepiota*, features of

Echinoderma asperum,like arrangement of acute squamules on the pileus which are made up of round to ellipsoidal cells in agglutinoid chains and anamoyloidity of basidiospores placed it within section- *Echinatae* Fayod (Zelený, 2006). However, section *Echinatae* is treated as a separate genus, *Echinoderma* (Locq. ex Bon) Bon, by Bon (1993) in his narrowest genus concept. Shibata (1992) and Razzaq *et al*. (2013) reported *Echinoderma asperum* from Pakistan as *Lepiota acutesquamosa* (Weinm.) P. Kumm. Razzaq *et al.* (2013) provided a comprehensive phylogenetic treatment for this species. His collection of *Echinoderma asperum* as *Lepiota acutesquamosa* is from a geographically different moist temperate region of Pakistan, Khanspur, which is floristically less diversified than Swat. Present molecular phylogenetic analysis is in close agreement to that of Razzaq *et al.* (2013) however, the bi-sporic to tetra-sporic variance in structure of basidia of present collections and the one described by Razzaq *et al.* (2013) demonstrates that they could be two different varieties of the same species. In the current phylogenetic inference of *Echinoderma asperum* based on related nrITS sequences, *Echinoderma asperum* depicts a well delineated position with *Echinoderma perplexum* (Knudsen) Bon (*Lepiota perplexa*) as sister taxa with strong bootstrap support (Figs. 3.4).

Lepiota granulata nom. prov. is featured by the presence of very prominent golden color granules throughout its pileus and stipe. *Lepiota granulata* makes monophyletic sister clade with members of sect. *Cristatae* which are characterized by spurred spores and a hymenidermal pileus covering (Vellinga and Huijser 1999) and with members of sect. *Stenosporae*, characterized by trichoderm having long cylindrical elements and spurred spores (Vellinga 2003). Among phylogeneticaly close relatives of *Lepiota granulata* viz *Lepiota pilodes* Vellinga & Huijser and *Lepiota castaneidisca* Murrill, *Lepiota granulata*

differ from *Lepiota pilodes* in having broader and very slightly spurred basidiospores (Vellinga & Huijser 1993) whereas *Lepiota castaneidisca* differs from our collection in having ellipsoid spores and round red brown pileus as described by Vellinga (2010) (Fig. 3.4).

The genus *Leucocoprinus*, having *Leucocoprinus cepistipes* (Sowerby) Pat. as type of the genus, is widespread in distribution and contains about 40 species (Kirk *et al*. 2008). Our collection ***Leucocoprinus cretaceus***, easily identifiable by its bright white squamulose sporocarps (Niveiro *et al*. 2013), is chacterized by hemispherical to companulate pileus becoming plano-convex with distinct umbo. Its thin to moderately thick context is of bright white appearance. In North America and Europe *L. cretaceus* dwells in tropical regions and neighboring temperate regions (Vellinga 2001, Birkebak 2010). Our current collection from a moist temperate region of Swat Pakistan, dominated by trees of evergreen pines and deciduous oaks and prevailing monsoon rains lasting for whole summer (Ahmad *et al*. 2015), contains about 8.5 × 11.24 µm sub globose to ellipsoid, smooth, and dextrinoid basidiospores with distict apiculus. Phylogenetically our collection of *L. cretaceus* clusters with other accessions of *L. cretaceus* of the genebank supported by 100% bootstrap value (Fig. 3.5, Clade-2).

Amanitaceae

The ectomycorrhizal genus *Amanita* Pers. is the type and largest genus of Amanitaceae. Members of this family constitute the most conspicuous edible and poisonous mushrooms. It is suggested that *Amanita* contains more than 500 species worldwide (Deng *et al*. 2014). Phylogenetic inference of the nLSU gene of *Amanita* species supports

the dissimilarity of *Amanita* Pers. and *Lepidella a* (E.-J.Gilbert) Veselý *emend*. Corner & Bas at subgeneric level and the nine sections previously circumscribed by Singer (1986) viz sections *Amanita* Pers., *Amidellae, Caesareae, Lepidella, Mappae, Phalloideae, Ovigerae, Vaginata* and *Validae* (Drehmel *et al.* 1999). Subg. *Amanita* is characterized by inamyloid spores whilst subg. *Lepidella* is characterized by amyloid spores (McGurk *et al*. 2016). Acorrding to recent taxonomic treatments *Lepidella* is further subdivided into four sections: Sect. *Amidella* (J.-E Gilbert) Veselý, sect. *Lepidella* sensu Bas, sect. *Phalloideae* (Fr.) Quél., and sect. *Validae* (Fr.) Quél whereas sect. *Amanita* is subdivided into be three sections: sect. *Amanita,* sect. *Caesareae* Singer ex Singer, and sect. *Vaginatae* (Fr.) Quél. s.s. (Yang 1997).

Amanita flavipes, a member of *Amanita* subgenus *Lepidella* section *Validae* (Fr.) Quél., is easily recognized in the field by moderate to small sized basidiocarps with bright yellow coloration. Most morphological characters of our collection are in closer agreement with Sanmee *et al*. (2008), however the splendid yellow pileus color of Pakistani collection suggests very slight contrast with that reported from Thailand. *A. flavipes* is considered to occure in the most extended ranges known for any Asian mushroom taxa (Kim *et al.* 1993; Yang, 1997; Tulloss, 2005). However, as considerable difference based on genetical and morphological chararacters is reported between *A. flavipes* collections of different geographic locations within Asia, range description of this widely occurring taxa need to be revised (Zhang *et al*. 2004). Varius taxonomic works on Asian *Amanita* including, Pakistan, India, and China have confirmed that previous cited names were misidentified (Yang, 1997, 2002; Yang and Doi, 1999; Bhatt *et al*. 2003). ITS sequence data obtained for the present *Amanita* species supports its

identity as *Amanita flavipes* through initial BLAST and phylogenetic analysis with identical sequences of Genbank. In the phylogenetic cladogram of Sect. *validae* (Fig. 3.7), present collection clusters with accessions of *A. flavipes* and *Amanita flavoconia* (G. F. Atk.) E.-J.Gilbert. However, morphologically our collection differs from *A. flavoconia* by ovate to pulvinate and bright yellow appearance (Kuo, 2013a).

Amanita aurumana nom. prov., *Amanita longiformis* nom. prov. and *Amanita superiora* nom. prov. seem undescribed previously. Comparison of ITS sequences of the present *Amanita* accession with the closely related identical sequences in GenBank confirms the identity of our collection as *species novem*.

***Amanita aurumana* nom. prov.** is depicted by a sister lineage with other monophyletic and polyphyletic clades in phylogenetic cladogram of *Amanita* section *Amanita* (Fig. 3.6). Phylogenetic tree validates *A. pantherina* accessions in *Pantherina* clade and *Amanita parvipantherina* Yang, *Amanita multisquamosa* Peck and *Amanita subglobosa* Yang accessions in *Parvipantherina* clade, respectively. Morphologically *A. aurumana* is similar to *A. pantherina* in pileus color and shape of warts but differs from the later in having larger and broader basidiospores with a tendency to form globose shape (Yang, 1997). *Amanita pervipantherina* differs our collection in cape color (pale brown) and smaller size of warts and brownish color (Yang *et al*. 2004).

***Amanita longiformis* nom. prov.** forms an out group with related sequences retrieved from genbank (Figs. 3.8). *Amanita longiformis* is characterized by a long stipe with prominent bulbous base. It also lacks partial veil. Intial blast revealed its maximum identity with *Amanita griseofolia* described by Yang (2004). *A. longiformis* differs from

A. griseofolia in being having a prominent basal bulb and no volval leftovers. *A. longiformis* is placed in the subgenus *Amanita* because of having inamyloid basidiospores and rough cap margin (Drehmel *et al.* 1999).

***Amanita superiora* nom. prov.** Initial BLAST search revealed *Amanita franchetii* (Boud.) Fayod as gentically the closest match of our newly proposed species. In the molecular phylogenetic analysis *Amanita superiora* nom. prov. maintains its monophyletic placement along with other members of *Amanita* subgen. *Lepedilla* sect. *Validae* like *Amanita franchetii, Amanita rubescens* Pers. and *Amanita excelsa* (Fr.) Bertill. as *Amanita spissa* (Fr.) Kumm. (Fig. 3.9).

Auriscalpiaceae

Lentinellus P. Karst, s a genus of white rot, wood decay, lamellate agaric in the family Auriscalpiaceae. This is common genus of the temperate ecosystems. *Lentinellus* is characterized by possessing mostly pleurotoid basidiomata with serrate lamellae, nodulose and amyloid basidiospores. It was originally classified in Tricholomataceae; tribe Leucopaxilleae, but was shown later by Maas Geesteranus (1963) to have affinities with *Auriscalpiurn* Gray (Aphyllophorales). He proposed a new family for both genera, the Auriscalpiaceae Maas Geest. Jtilich (1981) incorporated this family in his newly erected order, Hericiales, in which he brought together fungi characterized by smooth to warted, amyloid basidiospores, mono- or dimitic hyphal systems, and gloeoplerous hyphae.

***Lentinellus horridus* nom. prov.** is described herein as distinct from the closely related species both in terms of morphological and phylogenetic features. *L. horridus* is distingueshed by umbillicate pinkish cap, omphialoid basidioma and amoyloid spores. This new species is different from the closely related taxa, *L. cystidiosus* Petersen by having larger basidiome and pinkish cap contrary to deep tan or deep brown color of *L. cystidiosus* (Hughes and Petersen 2004). Based on available shared genetic characters analyzed in present study, *L. cystidiosus* is closest to *L. cystidiosus, L. montanus* Mill. and *L. flabelliformis* (Bolton) Ito having less than 97% identity index. However, none of them is morphologically similar to *L. horridus*. Phylogenetic cladogram also depicts monophyletic status of *L. horridus* among closely related taxa as shown in subclade II (Fig. 3.10). All this proves *L. horridus* as sp. nov.

Bolbitiaceae

Conocybe semiglobata* var. *campanulata, a member Bolbitiaceae is illustrated in this work. The genus *Conocybe* with poorly known spescies from Pakistan is a wide spread genus of the semi desert and semi savannah of Middle Asia (Hausknecht, 2009). Only 7 species of *Conocybe* have been reported from Pakistan including *C. semiglobata* var. campanulata from Ladhar, Sheikhupura. (Ahmad 1980). This species is reported hereby for the first time from Swat. The semiglobate type of pileus of our conocyboid collection is in close agreement with the one described by Ahmad (1980). Its smaller fruit body makes it member of the section *Conocybe* (Hausknecht *et al*. 2004) within the genus *Conocybe*. Phylogenetic analysis infers the confirmation of our collection to be *C. semiglobata* var. campanulata as it groups within the Conocyboid clade among *C.*

semiglobata var. campanulata accessions (Genbank; JX968169 and JX968168) from Hungary (Figs. 3.6.1.2). Macro and micro morphological description supplemented with molecular analysis approves our collection as *C. semiglobata* var, companulata by having longer stipe, larger spores and larger basidia than the close species *C. subpubescens* reported from Pakistan by Ahamd (1980) and Iqbal & Khalid (1996).

Boletaceae

Boletaceae was first erected by François Fulgis Chevallier in 1826 as distinct from Agaricaceae and included five genera in his circumscription: *Boletus*, *Laetiporus*, *Perenniporia*, *Polyporus*, and *Fistulina* (Chevallier, 1826). Later on Singer (1986) incorporated 26 genera and 415 species in the family. Binder and Hibbett (2006) included 38 genera in this family. Though recent classifications moved many members out of Boletaceae, still it remains a diverse family with many genera. About 800 species in 50 genera have been recognized in Boletaceae (Kirk *et al*. 2008). Wu *et al*. (2014) illustrated seven major clades at subfamily level and 59 generic groups, with four new subfamilies (1. Austroboletoideae, 2. Chalciporoideae, 3. Leccinoideae, and 4. Zangioideae) and 22 new potential genera. Ecologically, most members of Boletaceae are significant ectomycorrhizal (ECM) fungi in the ecosystem and can form ECM relationships with members of more than plant 10 families (Thoen and Bâ 1989; den Bakker *et al*. 2004; Sato *et al*. 2007; Smith and Pfister 2009; Becerra and Zak 2011; Henkel *et al*. 2012; Husbands *et al*. 2013).

Among collected mushroom froming fungi of Swat, more than 20 species were Boletes. All of these gave positive results for DNA extaraction by modified CTAB method of

Bruns (1995), whereas, only 4 amongst them showed amplified bands for ITS1F and ITS4 and editable sequences were obtained for only 2 viz *Boletus reticuloceps* and *Xerocomellus fulvus* nom. prov.

Boletus reticuloceps was collected from two locations of district Swat, viz, Mankial and Kalam (dry temperate forests). *B. reticuloceps* is characterized by orang red hemispheric basidiomata which does not change color upon bruising and hence becomes member of the sub family Bolitoidae (Wu *et al*. 2014). Current phylogenetic treatment depicts clustering of *B. reticuloceps* with other accessions of the same taxa from different parts of the world in a monophyletic clade with strong bootstrap support, viz, *B. reticuloceps* (GenBank; JN563882, JN563885 and JN563884) from China and *B. reticuloceps* (GenBank; EU231968) from USA. Seven clades showing ingroup taxa of the same genus are evident in the phylogentic tree (Fig. 3.12) whereas out member of the other genus, *Austroboletus amazonicus* Vasco-Palac. & López-Quint. forms the out group taxa. Very slight difference is evident between Pakistani collection and the one originally described by Wang and Yao (2005). Basidiospores size of our collection ranges from 14.0 – 19.5 × 5.0 – 9.0 µm whereas that of the type ranges from 15.0-17.5 × 5.0-6.5 µm.

Xerocomellus is a newly described genus of Boletaceae. It is well separated from *Xerocomus* based on phylogenetic analysis (Sutara, 2008). *Xerocomellus* is shown as heterogenous taxon based on morphological and molecular evidences (Ladurner & Simonini 2003; Binder & Hibbett 2004, 2007; Sutara 2008). This genus is represented by 8 species in Pakistan (Ahmad 1980; Iqbal & Khalid 1996; Razaq 2007; Sarwar 2013;

Sarwar *et al.* 2014; Sultana *et al.* 2011). Current study reports one new species of *Xerocomellus* from Swat.

Xerocomellus fulvus has been described morpho–anatomically as well as molecularly. In the phylogenetic analyses, this species makes sister clade with *B. dryophilus* Thiers (Fig. 3.13). Morphologically *X. fulvus* is characterized by deep pink to strong yellowish brown pileus color and smooth spores. This species can be easily differentiated from those having striated spores. It shows very close resemblance with *B. dryophilus* but can be distinguished by smaller pileus, dark reddish brown stipe base and slightly larger spores as compared to reddish pileus, yellowish stipe base and smaller spores in *B. dryophilus*. After careful observation and comparison of present taxon with closely related species, it was concluded that *X. fulvus* is a new species and morphologically resembles with *B. dryophilus* group.

Cortinariaceae

Cortinarius (Pers.) Gray is a largest genus of Basidiomycetes in the family Cortinariaceae. The number of species estimated for *Cortinarius* are ca. 2000 (Kirk *et al*. 2008) with a worldwide occurrence. This genus is characterized by a cobweb-like veil attached between the stipe and pileus and rust brown or brown, verrucose basidiospores. Species of *Cortinarius* are important ectomycorrhiza formers (Kårén *et al*. 1996). Probability of occurrence of new species is much more in *Cortinarius* because of the systematic revisions of this genus which have verified that diversity of *Cortinarius* is much more than previously thought (Garnica *et al*. 2002; Peintner *et al*. 2003; Frøslev *et al*. 2006, Ortega *et al*. 2008, 2009; Niskanen *et al*. 2008, 2009, 2012a, b, 2013; Vila *et al*.

2008; Bojantchev 2011; Bojantchev and Davis 2011). But the large number of taxa, together with the lack of reliable microscopic characters and the plasticity of the widely used macro-morphological characters make species delimitation within the genus difficult. The genus *Cortinarius* is one of the largest genera of mushrooms. The CABI Bioscience and CBS Database of Fungal Names list 5170 records of *Cortinarius* (http:// www.indexfungorum.org/Names/NAMES.ASP).

Species of *Cortinarius* with fleshy basidiomes, vivid colors, a viscid to glutinous pileus surface and a dry stipe are circumscribed in subgenus *Phlegmacium* Fr. *Phlegmacium* species are widely distributed globally and occur in ectomycorrhizal association with coniferous and deciduous trees (Moser *et al*. 1994, 1995). *Phlegmacium* has been classificated through various classification systems Various in Europe. Moser (1960) documented these sections *Caerulescentes, Amarescentes, Calochroi, Laeticolores, Fulvi, Triumphantes* and *Phlegmacium*. Name of the section *Laeticolores* is changed to *Scauri* and a new section *Tenues* added by Moser (1983).

Cortinarius cistoglaucopus is new record for Pakistan. *C. cistoglaucopus* is characterized by white cap and light gray gills and stipe. *C. cistoglaucopus* is recently erected new taxon using molecular and morphological data (Fernandez-Brime, *et al*. 2014). Initial BLAST search for this species resulted into a number of different species with the same percent identity. These included accessions of *Cortinarius van-campiae* Consiglio and *Cortinarius misermontii* Chevassut & Rob. Henry. *Cortinarius cistoglaucopus* share numerous morphological features with *Cortinarius van-campiae* (= *C. misermontii* sensu Ballara` *et al*. 2005) i.e. attenuated to radicant stipe base, pale basidiomes, fibrillose

pileus surface and spores. These similarities are sufficient to consider *Cortinarius cistoglaucopus* as a variety of *C. van-campiae*. However, the phylogenetic results recover both taxa in two separate, well supported, monophyletic groups, and their sister relationship is weakly supported (Fernandez-Brime, *et al*. 2014). An in-depth morphological study revealed that, although they are closely related species, they can be distinguished by these characters: (i) the bulb in *C. van-campiae* is conical and has an evident margin, while in *C. cistoglaucopus* it is not conical and is hardly marginate; (ii) the universal veil is red or reddish in *C. vancampiae* but white or whitish in *C. cistoglaucopus*, which gives the basidiomes a grayish white, grayish cream, ochraceous or brownish ocher color but never with pink-reddish, blood-reddish or red wine hues. In general the differences among species in section *Glaucopodes* are small and the separation of species usually is based on a few subtle traits (Fernandez-Brime, *et al*. 2014). Phylogenetic cladogram of current work depicts monophyletic status of *C. cistoglaucopus* while treated with closest species of the same percent identity in BLAST search. Our phylogenetic cladogram is represented by three monophyletic clades well supported by statistical values. Clade I includes accessions *C. van-campiae, C. misermontii* and C. alticaudus amongst which *C. cistoglaucopus* clusters with *C. cistoglaucopus* (KF738089) forming a separate lineage (Fig. 3.14).

***Cortinarius elegantior* (Fr.) Fr.**, a member of *Phlegmacium* sect. *Calochroi* is a new record for Pakistan. This species is characterized by hemispheric yellowish pileus, yellow gills and white stipe. Recently seven major monophyletic groups were established by Garnica *et al*. (2009) within sect. *Calochroi* based on a multigene phylogeny. These include *Calochroi, Caroviolacei, Dibaphi, Elegantiores, Napi, Pseudoglaucopodes* and

Splendentes. Subsection *Elegantiores*, which comprises of as principal taxa *C. elegantior* shares similar colouration of pileus, gills and stipe coloration. Only a slight difference in basidiospores size is observable between Pakistani and European collections; 13 – 15.5 × 7.5 – 12.0 µm vs 13.5-16 × 8-9.5. The yellow-greenish colours of the basidiome of *C. elegantior* correspond to flavomannin-di- and trimethyl ether pigments (Brandrud 1998). Phylogenetically *C. elegantior* clusters with *C. elegantior* (GU363462, JX219779, GU363463, GU363464, JX219778 and JX219777) by strong bootstape support (Figs. 3.15).

Cortinarius kohistanicus **nom. prov.**, belonging to subgenus *Phlegmacium* section *Calochroi*, is encountered from dry temperate coniferous forest of Swat in current work. Section *Calochroi* contains species making visible fruiting bodies with a globose to adruptly bulbous stem base, a cap with a well-developed sticky layer, and the existence of pigments which often bruise with KOH. *C. kohistanicus* nom. prov. is characterized by yellow orang pink basidiome and purple gills. Our phylogenetic execution for this new species is in close agreement with Garnica *et al*. (2009). *C. kohistanicus* nom. prov. is represented by a well delineated position in clade I (Figs. 3.16). Clade I include taxa belonging to Pseudoglaucopodes clade of section *Calochroi* (Garnica *et al*. 2009). *C. kohistanicus* nom. prov. clusters with *C. flavaurora* Moser & McKnigh EU056946, *C. corrugatus* Peck EU056949, *C. praetermissus* ergeron ex Reumaux EU684535, *C. elotus* Fr. EU056953 and *C. olivellus* Rob. Henry EU056955 from Germany, *C. elotoides* Moser & McKnigh KF732300 from Finland, *C. pseudoglaucopus* (Jul. Schäff. ex M.M. Moser) Quadr. JF273633 from USA, *C. xanthodryophilus* Bojantchev & R.M. Davis JF273637 from USA, and *C. humolens* Brandrud DQ663321 from Denmark in the ingroup taxa. *C.*

kohistanicus nom. prov. differ the closely matched *C. elotus* by smaller pilius and larger spores (11.4 – 15.4 × 6.8 – 8.6 vs 10.5-12.5 × 6-7.5) (Ballarà 1999).

Hygrophoraceae

Mushrooms with widely-spaced, thick and waxy gills producing white spores, and the stipe without veil remnants are classified in the genus *Hygrocybe* (Boertmann 1995). Genus *Hygrocybe* belong to Hygrophoraceae family. Fries (1821) used the term *Hygrocybe* for the group as a member of the 'tribus' *Clitocybe* ('subtribus' *Hygrocybe*) at first and subsequently transferred them as a 'tribus' to the genus *Hygrophorus* in 1838. Kummer (1871) raised it to the rank of a separate genus. Multiple separate genera are recognized in genus *Hygrocybe* by some taxonomists however the division of this genus into three subgenera is widely accepted, these include subgenus *Hygrocybe* s.str. Bon 1976, subgenus *Pseudohygrocybe* Bon 1976 and subgenus *Cuphophyllus* Donk 1962 (Boertmann 1995, Candusso 1997, Krieglsteiner 2001).

Hygrocybe decoriae **nom. prov.** is collected from moist temperate forest. This blackening wax cap is characterized by conical to papillate yellow red and slimy cap surface with deflexed, inrolled to cottony margins and centrally attached, pliable, yellow green stipe about 5.6 - 6.4 cm tall. Phylogenetically it differs from identical species by forming a distinctive lineage in Nigrescentes clade of the Phylogram (Fig. 3.17). This clade includes *Hygrocybe conica* (Schaeff.) Kumm. (JN021030, JN021031, JN021032 and FM208880), *Hygrocybe pseudoconica* Lange (JF908060) and *Hygrocybe singeri* (Sm. & Hesler) Singer (KC581351) of Sect. *Nigrescentes* (Bat.) Candusso. The well bootstrap supported phylogram (Figs. 3.17) established in this study for comparison of

Hygrocybe decoriae nom. prov. is in accorandance with the phyogeney of Babos *et al*. (2011). Morphologically *Hygrocybe decoriae* nom. prov. differs from the genetically closest relative, *Hygrocybe conica*, by having larger basidiospores (5.7 – 19.0 × 8.3 – 11.6 µm vs 7 – 11.5 × 4.5 – 6.5 µm) and shorter basidia (34.5 – 42.7 µm vs 40 – 60 µm).

Hygrophorus chrysodon is another species of Hygrophoraceae encountered in this work. This species is characterized by 1.5 – 3.5 cm depressed, white pileus with slightly strigose margin. The gills and stipe of this species are white. *Hygrophorus chrysodon* is reported by Razzaq (2013) from the same floristic and climatic region of Abbotabad, KP Pakistan. Molecular Phylogentic analysis conducted for this species resulted in a well boostrap supported cladogram of equal topology (Figs. 3.11.1.2). Five clades are obvious in the tree in which *Hygrophorus chrysodon* clusters with other accessions of the same species in clade II (Fig 3.18).

Hymenogastraceae

The ectomycorrhizal basidiomycetus genus, *Hebeloma* (Fr.) P. Kumm., has its main distribution in the temperate zones of the northern hemisphere. Fries (1821) recognised *Hebeloma* as a tribe of Agaricus. *Hebeloma* was raised to the level of genus by Kummer (1871), (Kuyper & Vesterholt 1990; Vesterholt 2004). Three subgenera within *Hebeloma*: *Myxocybe*, *Porphyrospora*, and *Hebeloma* and two sections within the subgenus *Hebeloma*: *Hebeloma* (species with a persisting cortina) and *Denudata* (cortina only present in the primordia) were recognized by Singer (1986). Provision of useful information about natural affinities within *Hebeloma* has been shown in recent phylogenetic studies of (Aanen *et al*. 2000) using sequences of the rDNA ITS

The phylogentic tree established for ***Hebeloma theobrominum*** in this study shows major clades in accordance to Boyle *et al*. (2006) phylogenetic classification of *Hebeloma* of Europe (Figs. 3.19). These include *Hebeloma, Amygdalina, Velutipes, Denudata, Scabrispora, Theobromina*, and *Sacchariolentia*. Outgroup taxa used herein as *Naucoria, Hymenogaster, Galerina* and *Alnicola* depict differing degrees of resolution as monophyletic clades similarly shown by Peintner *et al*. (2001). Pakistani collection of *Hebeloma theobrominum* lies in the *Theobromina* clade which consists of two other members of the same species as *Hebeloma truncatum = Hebeloma theobrominum* (FJ168588 from USA) and *Rhodocybe truncate = Hebeloma theobrominum* (FJ770393 from UK). Section *Theobromina* was introduced in 2005 (Vesterholt 2005) when it became apparent, based on molecular studies, that *Hebeloma theobrominum* was phylogenetically distant from *Hebeloma sinapizans* (Paulet) Gillet, with which it had been placed in sect. *Sinapizantia* (Quadraccia 1987; Aanen *et al*. 2000; Vesterholt 2004). Morphologically our collection shares similar outlines with *Hebeloma theobrominum* described from other parts of the world. However, the spores dimensions of Pakistani collection (10 – 8.2 × 8.2 – 13.4) µm are larger and basidia (20 – 25 × 7.5 – 10.0 µm) are smaller in contrast to the compared ones (7 – 11 × 4.5 – 5.5 µm) and (24 – 30 × 6.5 – 8.0 µm) respectively.

Inocybaceae

Inocybaceae is a family of the mushroom forming fungi in order Agaricales. Type genus of the family Inocybaceae, *Inocybe*, is noteworthy for its ectomycorrhizal ecology and poisonousness. Members of *Inocybe* are characterized by dull to brown spore print,

brownish basidiomata, sometimes with a lilac or purplish shade, pileus being fibrillose or squamulose in texture, brownish lamellae and existence on soil. Several species have mutioloid cystidia and smooth or nodulose basidiospores while others lack mutioloid cystidia and nodulose basidiospores (Matheny 2009). Kuyper (1986) placed *Inocybe* in Cortinariaceae, however, recent taxonomic works support Jülich's (1982) placement of *Inocybe* in its own family, the Inocybaceae (Matheny 2005, 2009). In current study 5 species of *Inocybe* were encountered which proved to be the most successful members for molecular treatment.

***Inocybe kohistanensis* Jabeen, Ahmad and Khalid** collected in the dry and moist temperate pine forests of Swat is described as new to science. Reddish brown pileus, metuloid cystidia, nodulose basidiospores, and marginate bulb makes *Inocybe kohistanensis* one belonging to sect. *Marginatae* of *Inocybe*. Its identical to *I. dunensis* and *I. oblectabilis* and clusters with these species in subsection *Oblectabiles*. Key feature of sect. *Marginatae* is identified very easily in field by presence of bulb at the base of the stipe. Molecular phylogenetic assessment separates two subgroups in sect. *Marginatae*, supported by morphological characters. One of this is characrteized by white, or yellowish stipe when young, buff, lacking any pinkish or reddish coloration designated as subcect. *Praetervisae* and the other being characterized by more or less pinkish to reddish tinge on the stipe or at least on the upper surface of the stipe is named as subcect. *Oblectabiles* (Bon, 1988). Subsect. *Oblectabiles* is shown into two clades in molecular phylogenetic cladogram. *I. kohistanensis* sp. nov. forms monophyletic clade with *I. dunensis* and *I. oblectabilis.* It is because of the difference in spore morphology of all other related species. The spore shapes of these species vary between between nodular

and smooth. *I. kohistanensis* sp. nov is disjointed from *I. dunensis* (Genbank accsssions: JF908092, JF908093, JF908262), *I. oblectabilis* (Genbank accsssions: AM882832, AM882833), and *I. pallida* (Genbank No. JF908198 by 93% staistical value) making a sister clade in tree (Figs. 3.20). *I. kohistanensis* also differs from these taxa morphologically.

Inocybe mimica is found associated with *Cedrus deodara* and *Pinus wallichiana* in cold temperate forest of Swat Kohistan. The region is dominated by trees of *Prunus cornuta, Aesculus indica,* and *Taxus wallichiana* (Ahmad *et al*. 2015). *I. mimica* is characterized by orang yellow conical pileus with papillate ubmo and white stipe. Massee (1904) described *I. mimica* from Yorkshire, UK. Saba *et al*. (2015) reported, for the first time, *I. mimica* from Asia and its first record of its association with *Pinus wallichiana*. *I. mimica* belongs to clade *inosperma* (Larsson *et al*. 2009). It is confirmed by molecular phylogentic analysis that bearing phaseoliform spores while having rubescent context by most species in this clade is its key feature. The current molecular phylogenetic treatment of this species depicts its clustering with other accessions of *I. mimica* from different parts of the world in a monophyletic clade within section *Inosperma* (Fig. 3.21).

Inocybe pallida is characterized by plano umbonate and reddish orange cap, free and remote, orange yellow gills, brilliant orange to light orange and even stipe, nodulose basidiospores and metuloid cheilosystidia. Current molecular phylogentics analysis and morphological characters supports this species conspecific with *Inocybe pallida* (JF908198) from USA in sect. *Inocybe*. Our phylogenetic analysis (Fig. 3.22) is in close

agreement with Jabeen *et al*. (2016) and Matheny (2009). *I. pallida* was found growing gregariously in coniferous forest.

Inocybe rimosa is collected in the moist temperate region of Swat. The region is dominated by *Cedrus deodara*, *Pinus wallichiana* and *Viburnum grandiflorum* Wall. ex DC. *Cedrus deodara* and *Pinus wallichiana* are potential ectomycorrhizal hosts of *Inocybe* (Watling and Abraham, 1992; Saba *et al*. 2015). This species portrays a broad ecological range covering all biomes from deciduous forests to the arctic-alpine zone (Larsson *et al*. 2009). *Inocybe rimosa* is characterized by plano-umbillicate orange brown pileus, strong yellowish brown to light yellowish brown fee and approximate gills and white stipe, slightly swollen at the base. Current molecular phylogenetic treatment places *Inocybe rimosa* in the subclade *Pseudosperma* of super-clade *Rimosae* (Fig. 3.23). Section *Rimosae* includes species characterized by fibrillose to rimose pileus, ellipsoid to phaseoliform spores, and absence of metuloid pleurocystidia but with compact, cylindrical, clavate to pyriform hymenial cheilocystidia that make the gill edge look particularly white in mature members.

Inocybe swatica **nom. prov.** is another member of section *Inocybe*. Distinctive features of *I. Swatica* includes; convex to slightly conical when young, becoming sub campanulate, moderate orange yellow to yellowish brown pileus, sinuate and white gills and cylindrical stipe with marginate base. Very clear morphological dissimilarity is obvious between our collections and the ones shown as identical in the initial blast. In the molecular phylogenetic analysis *Inocybe swatica* forms a monophyletic clade within *Inocybe* clade (Fig. 3. 24). All of our collections from different parts of Swat cluster

together with 100% bootstrap support. The monophyletic clade of *I. swatica* is further clustered with *I. praetervisa* by 48% bootstrap support which is morphologically dissimilar from our species by having broader spores and smaller fruit body along with great geographical distinction (Bresadola 1883; Kaufman, 1924; Kobayashi 2002).

Marasmiaceae

Marasmiaceae is a family of white spore's mushroom forming fungi. Its members have tough stipes and the ability of withering up during a dry period and later recovering. The important edible fungus, the shiitake mushroom, *Lentinula edodes* (Berk.) Pegler is a member of this family. This family contains 54 genera and 1590 species (Kirk *et al*. 2008). *Gymnopus* (Pers.) Roussel is a large and cosmopolitan genus of Marasmiaceae with about 350 taxa (Coimbra *et al*. 2015). It is characterized by collybioid or tricholomatoid basidiocarps, free lamellae and central and cylindrical stipe (Noordeloos, 1997).

Gymnopus barbipes characterized by depressed, umbillicate and orange to strong brown pileus with appressed fibrillose surface. Free and approximate, pale yellow gills with white shades in the base to pale yellow cylindrical stipe. In phylogenetic tree *Gymnopus barbipes* of Swat clusters with other identical accessions of *Gymnopus barbipes* with strong bootstrap support. It lies in a monophyletic clade containing members of section *Impudicae* Antonín & Noordel. The molecular phylogenetic tree depicts 2 major clades, *Impudicae and Levipedes* (Fig. 3.25). Section *Levipedes* is characterized by lobed or coralloid or inflated cells of pileipellis, (a Dryophilatype cutis), well-developed cheilocystidia, smooth stipe, and hyphae only rarely becoming green in alkali or not

(Antonín & Noordeloos 2010). Current molecular phylogenetic analysis is consistent with Coimbra *et al*. (2015) and Petersen and Hughes (2014).

***Gymnopus subcampanulatus* nom. prov.** is characterized by conical to convex reddish orange pileus, slightly conical in youth, becoming sub campanulate to more or less umbonate upon maturity with plicate, strigose and incurved margin, fusiform to sharply ovoid basidiospores and cylindric to ventricose basidia It falls in *Gymnopus* section *Vestipedes,* subsection *Vestipedes* (Antonín & Noordeloos 1997). *G. subcampanulatus* forms a distinct lineage in Clade I of the molecular phylogenetic phylogram produced in this study (Fig. 3.26). *G. subcampanulatus* differs from the closest species *Gymnopus confluens* (Pers.) Antonín, Halling & Noordel in smaller pileus and stipe (.5 – 2 vs. 1 – 6 cm) and (6 – 7.5 vs. 2.5 – 13 cm) respectively. Furthermore *G. subcampanulatus* is distinctive from *G. confluens* by the presence of pleurocystidia (Kuo, 2013b).

***Macrocystidia obscurum* nom. prov.** belongs to a small genus *Macrocystidia* Joss. In family Marasmiaceae. This genus is represented by only 15 records of 8 species in Index Fungorum (http:// www.indexfungorum.org/Names/NAMES.ASP). The Genbank contains only 4 sequences of 2 species. The molecular phylogenetic tree (Fig. 3.27) obtained for *M. obscurum* nom. prov., using identical sequences of Genbank, contains 4 monophyletic clades with strong bootstrap support, Viz *Tricholoma* clade; including species of the famly Tricholomataceae Lotsy, *Omphalina* clade; including members of the genus *Omphalina* Quél., *Panella* clade; including accessions of *Sarcomyxa serotina* (Pers.) P. Karst. = *Panellus serotinus* (Pers.) Kühner and *Macrocystidia* clade; including members of *Macrocystidia*. *M. obscurum* clusters with *M. reducta* (JQ694109) from New

Zealand and forms a sister group with accessions of the type species of the genus, *Macrocystidia cucumis* (Pers.) Joss (HQ604758) from Canada and (DQ490640) from USA (Fig. 3.27). Morphologically *M. obscurum* differs from *M. reducta* by longer stipe, smaller pileus, slightly smaller basidiospores and smaller basidia (28 – 35 × 6 – 8 μm) vs. (20 – 29 × 6.0 – 9.0 μm).

Mycenaceae

Mycenaceae Roze is a family of the saprobic mushrooms forming fungi in Agaricales containing 10 genera and 705 species (Kirk *et al*. 2008). A large-scale phylogenetic analyses study of the Agaricales published by a consortium of mycologists in 2002 adopted the name Mycenaceae for a strongly supported clade consisting of *Dictyopanus* Pat., *Favolaschia* (Pat.) Pat., *Mycena* (Pers.) Roussel, *Panellus* P. Karst., and *Resinomycena* Redhead & Singer. Taxa in the Mycenaceae have a cosmopolitan distribution, and are found in almost all ecological zones (Cannon and Kirk 2007).

***Mycena bractea* nom. prov.** is featured by plano-umbonate white pileus with appressed fibrillose surface and plicate margin, free and approximate and sub distant white lamellae, broadly ellipsoid, appiculate and mono guttiuate basidiospores and broadly fusoid to fusoid-venticose lamellar cystidia. In the molecular phylogram (Fig. 3.28) *Mycena bractea* nests with a number of varieties of *Mycena pura,* it is distinct by having long branch length and lies close to *Mycena rosella* in the sister subclade belonging to *Subsect. Violacellae* of *Mycena* sect. *Calodontes*. Most varieties of *M. pura* (e.g. *multicolor, ianthina, violacea*) were earlier found to have no phylogenetic basis (Harder *et al*. 2010). However, the addition the 3-gene phylogenetic analysis using TEF and

RPB1 with ITS recognized 11 varieties of *M. pura* including *M. pura var. lutea* as valid phylospecies (Harder, *et al*. 2013). Morpholgically *Mycena bractea* differs from *Mycena pura* var. violacea Gillet = *Mycena pura*, (FN394578) from Denmark, a phylogenitically closed species by smaller basidiomata, white color of lamellae and broadly ellipsoid basidiospores.

***Mycena lacerata* nom. prov.** is characterized by convex, obtuse and yellowish pink pileus with dry and glabrous surface approximate, free and approximate, distant, thick and marginate lamellae, ellipsoid and apiculate basidiospores with germ pore and clavate basidia and pleurocystidia. The molecular phylogenetic tree (Fig. 3.28) depicts this species as a sister phyla of *Mycena diosma* Krieglst. & Schwöbel (FN394619 and FN394618) from Denmark. *Mycena lacerata* nom. prov. nests in subclade belonging to s*ubsect. Violacellae* of *Mycena* sect. *Calodontes*. Morphologically *Mycena lacerata* nom. prov. differs from *Mycena diosma* by small size and yellowish pink color of basidiomata in contrast to dark brownish violet to reddish violet coloration.

Mycena pura collected in the cool temperate area of Swat is featured by hemispheric and umbonate light violet to pale violet pileus with smooth velvety surface and translucent margin, free and crowded, thin and light violet lamellae with curved margins, ellipsoid or oblong, apiculate, smooth and thin-walled basidiospores, clavate and bisporic to quadrisporic basidia and fusoid to fusoid-venticose, cheilocystidia. Harder, *et al*. (2013) reported discrepancy in morphological species recognition with the ITS data within *M. pura* complex. Phylogentic tree of this work (Fig. 3.28) depicts closeness of Pakistani *M. Pura* with *M. Pura* (JF908451) from Italy and *M. Pura* (FN394592) from Denmark and

paraphyletic nature of *Mycena pearsoniana* Dennis ex Singer as shown by Harder *et al*. (2010).

***Xeromphalina flavens* nom. prov.** is characterized by depressed, umbillicate brilliant yellow to dark orange yellow pileus with appressed fibrillose and velvety surface, light yellow, decurrent, distant to sub distant and thin lamellae with ragged margins, centrally attached, pliable, hollow, and cylindrical yellowish white stipe with equal and smooth surface and globose, smooth, thin-walled, golden yellow in KOH and amyloid basidiospores with evident germpore. *X*. *flavens* belongs to section *Mutabiles* Smith. In the phylogenetic tree (Fig. 3.29), *X*. *flavens* forms sister lineage with *Xeromphalina cauticinalis* (Fr.) Kühner & Maire (GU320005) and (GU320004) from Spain. Morphologically *X*. *flavens* is distinct from *X*. *cauticinalis* by shorter stipe (2 – 2.5 × 0.1 – 0.3 cm) vs. (3.5 – 8 × 1– 2.5 cm) and smaller basidiospores (2.4 – 3.4 × 2.5 – 3.8 µm) vs. (4.2 – 6.8 × 2.5 – 3.5 µm), basidiospores being globose in *X*. *flavens* and ellipsoid in *X*. *cauticinalis*.

Pleurotaceae

The family Pleurotaceae possesses small and white spored mushrooms. It contains 94 species in 4 genera (Kirk *et al*. 2008). Morphologically some members of Pleurotaceae look like members of Marasmiaceae. Oyster mushroom, *Pleurotus ostreatus* is best known and widly consumed member of this family.

Pleurotus ostreatus, collected in warmer subtropical region of Swat in hot summer season, is characterized by depressed, umbillicate and semicircular pale orang yellow

pileus with smooth glabrous and velvety surface, recurved curvature, white context, decurrent, crowdy, thick and white lamellae with ragged eroded margins and a soft, laterally attached, equal and cylindrical to flattened white stipe with smooth surface. Molecular phylogenetic analysis of current work (Fig. 3.30) represents clustering of *P. ostreatus* with *P. eryngii* (EU233975 and EU233976) and *P. ostreatus* (JX429935). *P. ostreatus* clearly differs from *P. eryngii* by its association with a *Populus* host contrary to the root parasitic nature of *P. eryngii* with plants of the Umbelliferae. Morphologically *P. ostreatus* differs from *P. eryngii* by shorter and evenly cylindrical to flate stipe (Estrada *et al*. 2008). The phylogentic tree shows clustering of *P. eryngii* at different positions i.e in both subclades of Calde I. This clearly means that sequences like EU233975 and EU233976 have been misnamed as *P. eryngii*.

Russulaceae

Russulaceae Lotsy is a big fungal family of the order Russulales. It holds about 1,900 known species of cosmopolitan occurrence. Russulaceae include the beautiful brittle-gills and the milkcap mushrooms and include some edible mushroom-forming fungi. This family also includes pleurotoid, secotioid or gasteroid and corticioid members. Molecular phylogenetics analyses confirms close affinities of russulaceous fungi with very dissimilar basidiome types. Main genera of this family includes *Multifurca* Buyck & V. Hofst., *Lactifluus (*Pers.) Roussel, *Lactarius* Pers., *Russula* Pers., and the crust-forming genera *Gloeopeniophorella* Rick *Boidinia* Stalpers & Hjortstam and *Pseudoxenasma* K.H. Larss. & Hjortstam.

***Russula mankialica* nom. prov.** a dweller of the dry temperate pine forest of Swat is characterized by hemispheric dark red pileus with surface which is glandular when young, fetching glabrous on maturity, adnate to ascending, close, thin and white lamellae, white, central, brittle, solid and cylindrical stipe with smooth surface, aculeate globose and apiculate basidiospores olive green in KOH and reddish olive green in phloxin. In the molecular phylogram (Fig. 3.31) *Russula mankialica* nom. prov. clusters with *R. puellaris* in subclade II of calde I. Subclade II belongs to section *Puellarinae*. (Shimono, *et al*. 2004) However, a great variation in morphological characters is apparent between *R. mankialica* nom. prov. and *R. puellaris* species. *R. mankialica* nom. prov. differs from *R. puellaris* by larger size and dark reddish color of basidiomata in contrast to of purple coloration and basidiospores size (8 – 10 × 7 – 9 µm) vs. (6.5 – 9 × 5.5 – 7).

***Russula umbra* nom. prov.** is found in the moist temperate mixed forest of needle to broad leaved trees under *Pinus wallichiana*. *R. umbra* nom. prov. is distinctive for depressed umbillicate white pileus with very sparse yellowish pink shades and appressed fibrillose surface, adnexed, close, thick and off white lamellae with smooth, convex and entire margins, globose, apiculate, spiny, thin-walled and amyloid spores and absence of cystidia. Phylogenetic analys show monophyletic nature of *R. umbra* nom. prov. in subclade VI of Clade I (Fig. 3.31). *R. umbra* nom. prov. forms close association with *Russula delica* Fr. (AF345250, LC008289) and *Russula mustelina* (AY061693) through strong bootstrap values. *R. umbra* nom. prov. is distinctive from *Russula delica* in being smaller in overall size, basidiospores size and shape (8.5 – 11.5 × 5 – 7.5 µm) vs. (8 – 12 × 7 – 9 µm) and gills attachment (adnexed vs. deccurent). *R. umbra* nom. prov. differs from the other phylogenetically close species, *Russula mustelina* by smaller basidiomata,

pileus 7 – 8 cm vs. 12 – 15 cm, stipe 5 – 6 vs. 3 – 11 cm, white color of basidiomata in the former and brown in the later.

Russula puellaris found in the cool dry temperate region of Swat is characterized by convex to pulvinate pileus, sticky, vivid red to very deep red becoming moderate red at margins with white and thick flesh, adnexed, soft, whitish to pale lamellae and equal to slightly clavate, smooth and cylindrical, milky white stipe. This is identified as new record for Pakistan herein. In the phylogenetic tree (Fig. 3.31) *R. puellaris* clusters with *Russula mankialica* nom. prov. by strong bootstrap percentage and does not significantly differ morphologically from the species description in the literature.

Russula sichuanensis originally described by Li *et al*. (2013) from Sichuan province, China is recently reported by Saba and Khalid (2015) from different regions of Pakistan. Morphologically quite variable from the type specimen, identification of Saba and Khalid (2015) collections mostly rely on initial BLAST similarity and clustering in the same lineage during phylogenetic reconstruction (Nilsson *et al*. 2008). *R. sichuanensis* described in this work is characterized by depressed to infunduliform to umbillicate depressed pileus, deep reddish purple in center becoming lighter toward the edges with smooth glabrous velvety and slightly sticky surface, uplifted curvature, plicate margins, free and approximate, close and thick lamellae with pale to reddish orange to brownish orange to yellow red in color and milky white stipe. Morphological description of this work is in close agreement with the description provided by Li *et al*. (2013) for type specimen; however, slight variation is evident in the sizes of microscopic structures.

***Russula deodara* nom. prov.** and ***Russula kalamica* nom. prov.** are collected in moist temperate dry temperate pine forest of Swat respectively. *Russula deodara* nom. prov. is distinctive for hemispheric and obtuse pileus of pale orangish yellow to golden coloration which becomes darkish in center, adnate and ascending, close, thick, pale yellowish pink lamellae with smooth, convex, and entire margins, cartilage like, centrally attached, clavate, and white stipe with smooth surface. *Russula deodara* nom. prov. clusters with another proposed new *Russula* species, *Russula kalamica* nom. prov. of this work in a distict lineage (Fig. 3.31). This lineage is shown as subclade II of clade II. Sister group of subclade II i.e subclade III represents *Russula praetervisa* Sarnari (KF245531) and *Russula pectinatoides* Peck (EU819493, EU819500). *Russula kalamica* nom. prov., characterized by depressed, obtuse, dark brown to light brown pileus, smooth, appressed fibrillose, moist and slightly sticky surface, incurved curvature, white cottony margins, adnate, close to slightly crowded,white lamellae with ragged eroded margins differs from *Russula deodara* nom. prov. by broader basidiospores with very distict appiculus and slightly warty (6.8 – 12.5 × 5.1 – 9.2 µm) surface in comparison to spiny basidiospores (6.8 – 8.0 × 6.8 –9.7 µm) of the later. *Russula deodara* nom. prov. and *Russula kalamica* nom. prov. are clearly differentiated from the closer species shown in phylogenetic tree by their spores size and shape (5.5 – 9 × 4.5 – 6 µm, broadly elliptical and warty) (Kuo 2005).

Strophariaceae

Cyclocybe erebia is featured by a ring on stipe and dark brown basidiome. It is characterized by occurrence in the moist temperate pine forest, convex to plano convex,

light reddish brown to dark reddish brown pileus, sinuate, close to crowdy, grayish orange to reddish orange lamellae and centrally attached, soft, cylindrical light brown to light reddish brown ringed stipe. The molecular phylogentic tree (Fig. 3.32) obtained for this species is based on closely matched sequences of the intial BLAST of Genbank. Tree is featured by three strongly supported clades viz *Cyclocybe* clade featured by species of *Agrocybe* Fayod = *Cyclocybe* Velen., *Galerina* clade including species of the genus *Galerina* Earle and *Cortinarius* clade with species of *Cortinarius* (Pers.) Gray and *Dermocybe* (Fr.) Wünsche = *Cortinarius* (Pers.) Gray genera. *Cyclocybe erebia* nests within other accessions of the same species; (FJ168567, FJ596810) and (DQ484056) from USA, (AY521246) from Korea, and (JN020964) from UK. Morphologically Pakistani collection show minor variability with the type in cap size and shape and basidiospores size.

Pachylepyrium kalamicum **nom. prov.** collected in dry temperate region is featured by hemispheric, obtuse and deep brown pileus with appressed fibrillose velvety surface, straight and flaring strong brownish orange lamellae, a deep brown cylindrical stipe and globose to sub-globose, angular ovate to kite shaped, thick-walled, dark brown basidiospores. Molecular phylogenetic analysis did for *P. kalamicum* nom. prov. reveals two clades with strong bootrape support (Fig. 3.33). It depicts a monophyletic stature of *P. kalamicum* nom. prov. in clade I. The sister clade being of *Pachylepyrium funariophilum* (M.M. Moser) Singer = *Crassisporium funariophilum* (M.M. Moser) Matheny and *Pachylepyrium carbonicola*. Our molecular phylogenetic analysis is consistent with results of Matheny *et al*. (2015) who showed *Pachylepyrium* Singer as polyphyletic genus using multi gene phylogeny.

Genus *Pholiota* of Strophariaceae species are widespread and noticeable wood parasites, decomposers and soil saprotrophs. Kirk *et al*. (2008) quotes about 150 species of *Pholiota* in the world, while Neda (2008) estimates about 362 species of *Pholiota* to prevail in the world. In current study four species of this genus were encountered mostly linked to pine forests.

Pholiota gummosa found in the moist pine temperate forest of Swat is characterized by brown depressed and umbillicate pileus with apressed fibrillose surface imprinted with tiny squamules thoroughly, adnate and slightly ascending, close, thin, light and orange yellow to vivid orange yellow lamellae, centrally attached, soft, hollow, cylindrical, ventricose and milky white in the upper half to strong yellowish brown in the lower half stipe with squamolose surface and ellipsoid to subglobose, smooth, thin-walled, and anamyloid basidiospores. *Pholiota gummosa* belongs to section *Sordidae* of subgenus *Hemipholiota* (Smith and Hesler 1968). The molecular phylogentic tree reveals three distinct clades with strong bootstrap values in which our collection clusters with *P. gummosa* (JF908580) from USA (Fig. 3.34).

Pholiota lanceolata **nom. prov.** making a unique lineage with the polyphyletic clade of *Pholiota adiposa*, *Pholiota gummosa*, *Pholiota tennesseensis* A.H. Sm. & Hesler, *Pholiota olivaceodisca* A.H. Sm. & Hesler, *Pholiota caespitosa* A.H. Sm. & Hesler and *Pholiota burkei* A.H. Sm. & Hesler in clade II of the molecular phylogram (Fig. 3.35), *Pholiota lanceolata* nom. prov. is distinguished by smaller pileus (2.5 – 3.1 cm) and stipe (1.9 – 2.3 × 0.4 – 0.6 cm), attachment of gills (areuate) and larger basidiospores (7.6 – 9.7 × 4.4 – 6.5 µm) (Smith and Hesler 1968). Comparison of ITS sequence data obtained

from the *Pholiota lanceolata* nom. prov. with the identical nucleotide sequences in GenBank also support the identity of this species as a new species by showing 94% maximum identity with *Pholiota adiposa*.

***Pholiota manga* nom. prov.** is another find of the moist temperate pine forests of Swat. Sequence match of *Pholiota manga* nom. prov. show maximum identity (92%) with *Pholiota lubrica* (HG007986) and phylogenetic acquaintance with *Pholiota lenta* sensu Wakefield & Dennis = *Pholiota mixta* (Fr.) Kuyper & Tjall.-Beuk. (AY281022), closest accession in the sister clade. Phylogenetic analysis reveals 2 clades in the cladogram in which *Pholiota manga* nom. prov. is depicted by a distinct lineage (Fig. 3.36). *Pholiota manga* nom. prov. is characterized by plano umbonate strong brown to strong orange pileus with smooth, glabrous, velvety and slightly sticky surface, adnate and horizental, close, thin, strong brown to deep brown lamellae with smooth, convex and entire margins, centrally attached, concolorus with pileus, soft, solid, cylindrical and smooth stipe, globose to hemiglobose to oblong, smooth and thin-walled spores, and clavate and three spored basidia with 4.2 – 7.8 µm pointed sterigmata.

Pholiota spumosa is a conspicuous member of *Pholiota*. *Pholiota spumosa* is a widespread species, commonly recorded in Europe, North America, and occasionally in Africa (Cortez and Coelho 2006). This the first report of this species from Swat Pakistan. It was collected in moist temperate forest. The molecular phylogenetic analysis executed for this species resulted into a phylogenetic tree (Fig. 3.37) with best topology and strong bootstrap support. The tree contains two clades in which *Pholiota spumosa* of this study

clusters with other accessions of the same species in clade II phylogenetic analysis of this study is in close agreement with Holec *et al*. (2014).

Tricholomataceae

Clitocybe gibba, a common and widespread mushroom species of Swat is easily recognized in field by depressed-umbillicate pileus with moderate orang yellow to dark orange yellow coloration, appressed fibrillose, velvety and dry surface, recurrved curvature, uplifted and pubescent margins, decurrent, close, thin and off white lamellae with ragged eroded margins and centrally attached, soft, equal and cylindrical stipe which is slightly tapered at the apex with off white coloration in the upper region to yellowish brown in the lower region. Molecular phylogenetic analysis conducted for this species with closely related species of *Clitocybe* (Fr.) Staude include 19 sequences of 12 different species, two species of *Collybia* (Fr.) Staude, each with one sequence and *Mycena pura* (JF908403) as outgroup species belonging to the nearest family, resulted in a phylogram with best topology and strongly supported 2 clades. Collection of Swat clusters with other accessions of *Infundibulicybe gibba* = *Clitocybe gibba* with very strong bootstrap support in clade II (Fig. 3.38).

Lepista irina is another widespread common species of the moist temperate forests of Swat. *L. irina* is an edible species of Tricholomataceae. It can easily be deciphered by broadly white pileus when younger becoming brownish orange upon maturity, convex cap with slightly depressed central disc, surface being smooth glabrous and velvety, centrally attached, hollow, soft, cylindrical whitish to light brownish orange stem with striated to reticulated surface and ellipsoid or oblong, appiculate, spiny, thin-walled and

anamyloid basidiospores. Molecular phylogenetic analysis of this species produced a tree with best topology and strong statistical values. All of the four collections of *Lepista irina* from Swat clusters in the monophyletic clade I. Clade I also includes other accessions of *Lepista irina* as KJ194172 from Pakistan, HM237136 from New Zealand, DQ221109 from USA and FJ810142 fom China (Fig. 3.39).

Pseudoomphalina conifera **nom. prov.** belongs to the dry temperate forest of Swat. It is characterized by depressed, umbillicate pileus; orange yellow at margns to orange brown in center with smooth apressed fibillose and moist surface, snow white, decurrent, subdistant and thin lamellae with ragged and curved margins, pale yellow, centrally attached, soft, cylindrical and equal stipe with smooth surface and apparent pseudorhizzae, narrowly ellipsoid to oblong, apiculate, smooth and thin-walled olive green basidiospores and narrowly clavate, bisterigmate, guttiulate and thin-walled basidia. Molecular phylogenetic analysis reveals placement of all of the identical sequences in two strongly supported clades viz Clade I and Clade II of the phylogenetic tree. Our phylogenetic analysis is in close agreement with that of Lavorato *et al*. (2015). *Pseudoomphalina conifera* nom. prov. makes sister lineage with accessions of with strong bootstrap value (Fig. 3.40). Another strongly supported calde near to *P.kalchbrenneri* nom. prov. is of *Neohygrophorus angelesianus* = *Pseudoomphalina angelesianus* (A.H. Sm. & Hesler) Vizzini, Contu & Z.W. Ge, comb. nov. accessions (Lavorato *et al*. 2015). Matheny *et al*. (2006) showed uncertain position of *Neohygrophorus* in the tricholomatoid clade whearas phylogenetic analysis of Lodge *et al*. (2006 and 2014) depicted *Neohygrophorus angelesianus* to be sister to sister to the type of the genus *Pseudoomphalina*, i.e., *P*. *kalchbrenneri*). All species of Clade II have

been segregated to a new genus *Pseudolaccaria* Vizzini, Contu & Z.W. Ge, gen. nov. based on the molecular analysis showing polyphyletic nature of the members (Lavorato *et al*. 2015).

***Leucocalocybe mongolica* var. *purpura* var. nov.** is a saprotrophic member of *Leucocalocybe*. *Leucocalocybe mongolica* principally known from North China and Mongolia (Imai, 1937), is consumed as edible worldwide (Imai, 1937; Mao, 2000). *Leucocalocybe mongolica* var. *purpura* var. nov. of the present work, collected from moist temperate pine forest of Swat, exhibits appealing difference in coloration with the type species i.e pale yellowish to white. *Leucocalocybe mongolica* var. *purpura* var. nov. is notable by plano convex, slightly obtuse, very pale blue to light yellowish brown pileus with appressed fibrillose and velvety surface, sinuate, crowded and thick lamellae concolorus to pileus and a centrally attached, cartilage like, equal and cylindrical stipe. In the molecular phylogenetic analysis it forms a distict lineage sister with accessions of *Tricholoma mongolicum* with strong statistical values (Fig. 3.41).

CONCLUSION

In this dissertation we explored different parts of Swat district for making further additions to the mushrooms of the region. Sum 46 species of 26 genera (15 families) have been described and identified on the basis of morphological and molecular phylogenetic studies. Among these 50% seems new to science and 17% are new reports for Pakistan. Whereas 6% and 25% are already reported from Swat District and other regions of the country, respectively. As a result of present contribution the number of known mushrooms from this area has increased to 84.

From these results it can be concluded that mycotbiota of Swar District is very rich and is mostly unexplored. Great potential has been found in the discovery of new mushrooms (with 50% undescribed species). Among the previously known mushrooms reported in this study most belong to ectomycorrhizal groups which indicates that fungal species, particularly mushrooms play important role in keeping biodiversity of Swat District enriched.

Current study elucidates the effectiveness of nrITS sequences in phylogenetic studies of mushrooms and resolution of species delineation.

RECOMMENDATIONS

- ✓ Most recent estimates based on high-throughput sequencing methods suggest that as many as 5.1 – 13.5 million fungal species exist, which indicates that still there occur a great diversity of fungi, including mushrooms, for which more detailed surveys are required to explore and document.
- ✓ As Swat district is floristically more diverse, the presence of ectomycorrhizal mushrooms and its host trees is more likely to occur, it is therefore ought to yield many new and interesting forms.
- ✓ Growth performance of nursery plantations for afforestration could be inhanced by inoculating seedlings with most frequently associated ectomycorrhizal fungi of forest trees.
- ✓ In biosystematics studies of mushrooms gross morphology should be coupled with molecular phylogenetics based on nrITS sequences for obtaining more reliable classification.
- ✓ High-throughput sequencing methods may be used to assess geographical distribution and host-substrate specificity of the mushrooms of the region.
- ✓ Mushroom biodiversity is rich in Swat District but poorly explored. It is proposed that collaborative research projects with Agriculture and Forest Departments are required to make more efficient use of mushrooms which are famous for their food value and symbiotic association with forest trees, research should also focus on other aspects including taxonomy, ecology, and ethnomycology.

- ✓ As the folks mostly rely on natural food sources including mushrooms and very less is known about the mycobiota of the region so there is a great chance of introducing new edible mushrooms to the mushroom cultivation industry.

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Appendix

(Published Papers)