New Records of three Russula species from the Temperate-Forests of KP, Pakistan; validation based on anatomical and morphological assessments combined with molecular phylogenetic analysis

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Abstract

In this paper, three new records of the genus *Russula* from the temperate forests of Pakistan are described and illustrated based on phylogenetic analysis of the Internal Transcribed Spacer (ITS) ribosomal DNA sequences and morpho-anatomical investigation. These taxa are *Russula byssina* (from subsect. Lactarioideae) *R. Foetentula* (from subsect. Foetentinae) and *R. pseudoamoenicolor* (from subsect. Amoeninae). Their taxonomic position is supported by the molecular phylogenetic and morphological analyses. A comparison of the described taxa and other similar species is also addressed in this study.

Keywords –: Basidiomata – morphology – phylogeny – Russulaceae – Russulales – taxonomy

Introduction

The genus *Russula* Pers. is one of the most conspicuous gill mushroom groups among Basidiomycetes. Up to now, over 2500 names and 750 species have been recorded according to Index Fungorum since the establishment of *Russula* in 1796 (Kirk *et al.*, 2008). It is characterized by a heteromerous context and amyloid spore ornamentation (Romagnesi 1967 & 1985; Sarnari 1998; 2005 and Li 2014). This genus contains hymenoid and sequestrate species, and the latter claim has been widely proved by molecular phylogenetic analyses (Lebel & Tonkin 2007 and Buyck *et al.*, 2010). Basidiocarps of *Russula* are common in various boreal forests (Romagnesi 1967 & 1985; Sarnari 1998 and Li 2014) and form an ectomycorrhizal system in association with diverse host plants (Trappe 1962 and Roberts *et al.*, 2004). The basidiocarps of many *Russula* species are collected for food and medicine (Buyck 2008; Dai & Yang 2008; Dai *et al.*, 2010 and Li *et al.*, 2010), however, some species are poisonous (Li *et al.*, 2010 and Li 2014). Phylogenetic analyses of *Russula* and Russulaceae were carried out to investigate the relationship between infrageneric groups and monophyly of the genus (Eberhardt 2002; Miller & Buyck 2002; Park *et al.*, 2013; Adamčík *et al.*, 2016a and 2016b). Although *Russula* was shown to be paraphyletic in some early phylogenetic analyses (Miller *et al.*, 2001 & 2006 and Shimono *et al.*, 2004), additional analyses suggested that *Russula* is a monophyletic genus (Eberhardt 2002; Larsson & Larsson 2003; Buyck 2008; Buyck *et al.*, 2010 and Zhao *et al.*, 2017). To establish new *Russula* taxa, internal transcribed spacer (ITS) phylogeny is commonly used as evidence. In recent years, Asia has become a hot spot for the exploration of unknown *Russula* species (Li 2014 and Li *et al.*, 2015).

The related topography and climatic change, specifically rainfall and the flora are different from place to place in the studied valley. The mountains are mostly covered with coniferous forests, including chir-pine (*Pinus roxburghii* Sarg), blue-pine (*Pinus wallichiana* A. B. Jackson), the Himalayan yew (*Taxus wallichiana* Zucc), the pindrow fir (*Abies pindrow* Royle), the Himalayan spruce (*Picea* *smithiana* (Wall.) Boiss) and cedar (*Cedrus deodara* (Roxb. ex D. Don), G. Don) (Ibrar *et al.*, 2007; Sher *et al.*, 2013 and Razzaq *et al.*, 2013). District Shangla which is a moist temperate region covered by 90% of the coniferous forest has enormous potential for fungal diversity (Ullah, 2018). During a mushroom survey in Shangla Top KP, Pakistan (2021), a wide diversity of this genus was observed and collections were made. Out of these collections, *Russula byssina*, *R. Foetentula*, and *R. pseudoamoenicolor* are taxonomically described and are the first-time reports from Pakistan.

Description of sampling site

Collections were taken mainly from moist temperate woodlands of the Shangla Top forests of Shangla District, Khyber Pakhtunkhwa province, Pakistan. (34–31◦ to 33–08◦ North latitude and 72–33◦ to 73–01◦ East longitude).

Sampling and morphological analysis

Basidiomata were collected, described, and photographed in the field and deposited in the Herbarium of Islamia College Peshawar (ICP), KP, Pakistan. Color designations are from Munsell system Universal color language (UCL) level 3 color names. Collections were dried using a fan, heater, and sunlight. Microscopic characters were observed in the laboratory using hand-cut sections of basidiomata mounted in a 5% aqueous solution of potassium hydroxide (KOH) and Congo red. Spores ornamentation was observed under oil immersion (100X) objective lens and the other structures on (40X) objective lens. Twenty basidiospores and 10 every basidium, cheilocystidia, pleurocystidia, pileal, and stipe components were assessed and measured from one basidiom by an ocular micrometer (Zeiss Eyepiece Screw Micrometer). The number of measured basidiospores was given as n/m/p, where n, m, and p represented the total number of basidiospores, basidiomata, and collections measured, respectively. Dimensions of basidiospores were given as (a–) b–c (–d), where the range b–c represented a minimum of 90% of the measured values (5th to 95th percentiles) and extreme values (a and d) whenever present ($a <$ 5th percentile, $d >$ 95th percentile), were given in parentheses. Q referred to the length/width ratio of basidiospores while the other measured component values were given as length/width and from small to large ranges.

Molecular procedures

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from a small section of gills by using the CTAB method (Bruns, 1995). Amplification of Internal Transcribed Spacer ITS1 and 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 Pakistan. Amplified PCR products were purified and were bidirectionally sequenced at commercial sequencing services (china).

Sequence alignment and phylogenetic analysis

Generated ITS sequences were trimmed with the conserved motifs 5'–CATTA– and –GACCT–3' (Dentinger *et al*., 2011), and newly obtained raw sequences were spliced and deposited in GenBank (accession numbers are shown in Fig 04), and the alignment portion between these motifs was included in subsequent analyses. The nrDNA ITS sequences were matched using the Basic Local Alignment Search Tool (BLAST), National Center for Biotechnology Information (NCBI) for comparison and confirmation of identifications. BioEdit (Version 7.2.5.0) was used for cleaning and editing the sequences where required. Newly generated sequences were aligned with other sequences of the GenBank, using the muscle alignment tool (www.ebi.ac.uklTools/msalmuscle). In the aligned dataset, all characters were equally pondered, while treating gap positions as missed data. A phylogenetic tree was made for each species sequence. Maximum Likelihood (ML) analysis was done using Molecular Evolutionary Genetic Analysis (MEGA ver-6.0) with default settings of the program i.e. Jukes-Cantor Model and for ML Heuristic Nearest Neighbor-Interchange (NNI) method was used (Tamura *et al.*, 2013). About 1000 bootstrapping was implemented for analysis replicates.

Results

Taxonomy

Russula byssina G.J.Li & C.Y.Deng (2020) Figures. 01 & 04

Morpho-anatomy

BASIDIOCARPS is small to medium-sized. PILEUS: 4 – 5 cm in diameter. Shape depressed to deeply indented, pale yellow-green (10Y 9/2) to light yellow (2.5Y 9/6) in color, velvety and sticky surface feel, and smooth to rimose surface appearance. CURVATURE; deflexed to inflexed. MARGIN; even, the context color is pale and creamy. GILLS: insertion adnate and horizontal, thin, close to crowded in spacing, milky white to creamy color, and margins are smooth. STIPE: length 3 cm and width 1 – 1.5 cm. Location central, cylindrical to sub-clavate in shape, smooth to fibrillose surface, color is white to milky white and pliable to cartilage-like consistency. ANNULUS and VOLOVA: absent. VEIL and its patches are also absent.

BASIDIOSPORES: [20/2/1] 9 – 14 (15) × (7) 8 – 11 (12) µm, Q = 1.21. Color red in congo-red, globose in shape, smooth surface, thick-walled, absent of guttiule and present of apiculus, CHEILOCYSTIDIA: 60 – 80 × 7.5 – 15 µm. The color is red in congo-red, aciculate to lamproeystidum, thin-walled, and absent of guttiule. PLEUROCYSTIDIA: 80 – 100 × 7.5 – 12.5 µm. Color red in congo-red, aciculate to lamproeystidum in shape, thin-walled and absent of guttiule. BASIDIA: 40 – 70 × 10 – 15 µm. The color red to pink in congo-red, clavate in shape, 4-spored with pointed sterigmata, thick-walled, and present of guttiule. PILLEPELLIS: 5 – 10 µm in width. Color light red in congo-red, thick-walled, septated, and irregular trichoderm in the arrangement. STIPETIPELLUS: 2.5 – 7.5 µm in width. The color is red to orange in congo-red, thin-walled, septated, and irregular trichoderm to transition between hymeniderm and epithelium.

Habitat and distribution. Pakistan, Khyber Pakhtunkhwa, Shangla Top forest, 1700m a.s.l, gregarious on the ground in the mixed forest of *pinus wallichina* and *Quercus*, 21 August 2021, IS # 064, Imtiaz khan.

Comments. This species belongs to a subsect. Lactarioideae is a new record for Pakistan, based on the nrITS phylogenetic analysis coupled with the morpho-anatomical characterization.

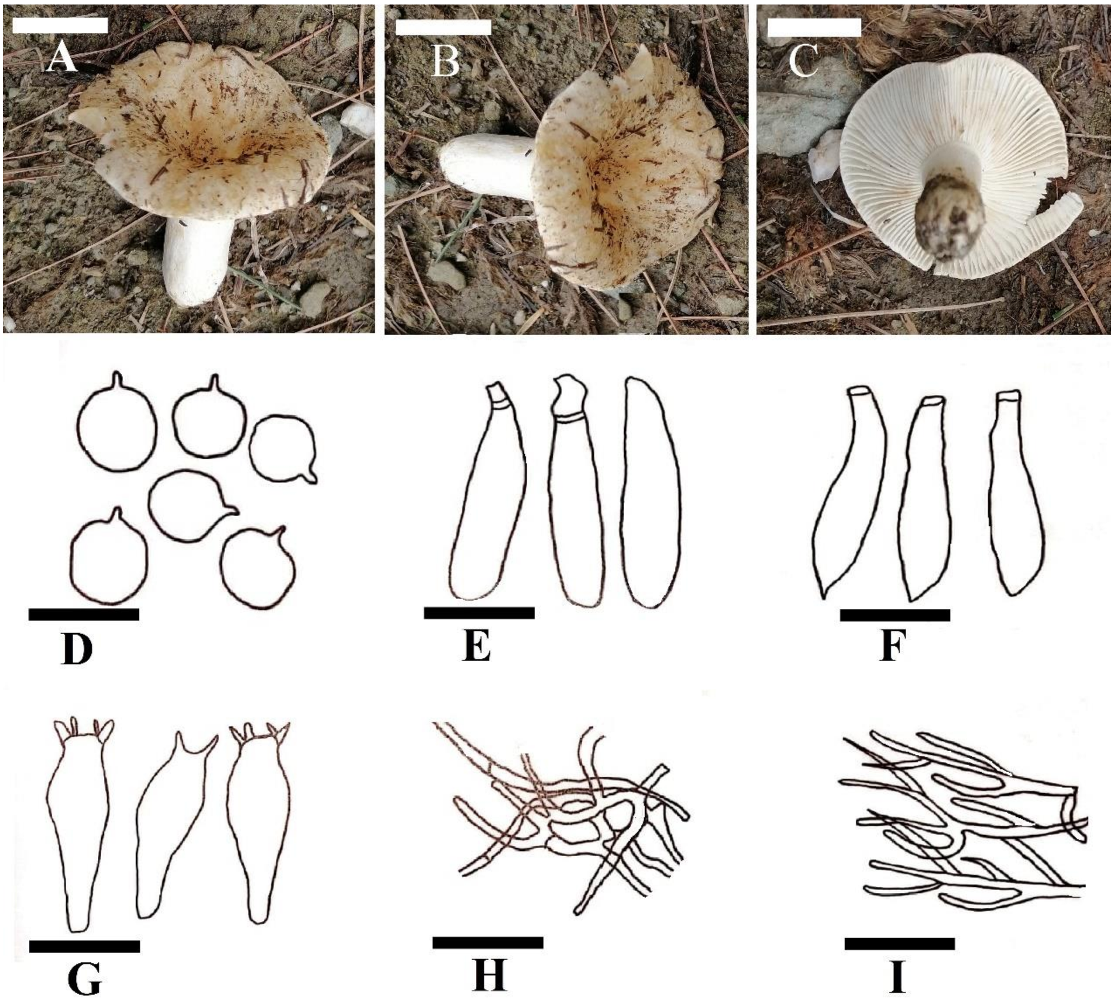

Figure – 01: A-C: Basidiomata of *Russula byssina*: A- Pileus, B- Stipe, C- Gills, D-I: Anatomical structures of *Russula byssina*: D- Basidiospores, E- Cheilocystidia, F- Pleurocystidia, G- Basidia, H- Pillepellis, I- Stipetipellus. Scale bars: A-C = 02 cm. D = 18 µm, E = 38 µm, F = 50 µm, G = 40 µm, H = 12 µm, I = 9 µm.

Russula Foetentula peck (1907) Figures. 02 & 04

Morpho-anatomy

BASIDIOCARPS is small to medium-sized. PILEUS: 5 – 5.5 cm in diameter. Shape broadly convex to hemispheric/pulvinate and the color is light yellow (5Y 9/6) to deep orange-yellow (7.5YR 6/12). Velvety and sticky surface feel, smooth to fibrillose surface appearance, and the context color is light orange-yellow (7.5YR 9/8). CURVATURE; inflexed to appendiculate. MARGIN; crenulate to eroded. GILLS: adnate and horizontal insertion, thick, spacing close to crowded, creamy in color, and the margins are smooth. STIPE: length 4.5 and width 1 – 1.5 cm. Central in location, the shape is cylindrical and smooth to the fibrillose surface. White to pale creamy and the consistency is soft to cartilage-like. VOLOVA: absent. ANNULUS: absent. Patches of the universal veil are also absent.

BASIDIOSPORES: [20/1/1] (6) 7 – 9 (10) × (5) 6 – 7 (8) µm. Q = 1.32. Color light red to brown in congo-red, globose in shape, echinate surface, thick-walled, present of guttiule and apiculus. CHEILOCYSTIDIA: 50 – 70 × 7.5 – 10 µm. The color is red to brown in congo-red, narrowly cylindrical to moniliform in shape, thin-walled, and absent of guttiule. PLEUROCYSTIDIA: 50 – 80 × 10 – 15 µm. The color is red to brown in congo-red, narrowly cylindrical to narrowly clavate in shape, thin-walled, and absent of guttiule. BASIDIA: 40 – 52.5 × 10 – 15 µm. Color light red in congo-red, clavate in shape, 2-spored with pointed sterigmata, thin-walled, and absent of guttiule. PILLEPELLIS: 2.5 – 5 µm. Color light red to orange in congo-red, thin-walled, unseptated, and irregular trichoderm in the arrangement. STIPETIPELLUS: 2.5 – 7.5 µm. Color light red in congo-red, thin-walled, unseptated, and ixotrichoderm in the arrangement.

Habitat and distribution. Pakistan, Khyber Pakhtunkhwa, Shangla Top forest, 1700m a.s.l, solitary on the ground in the mixed forest of *pinus wallichina* and *Quercus*, 21 August 2021, IS # 057, Imtiaz khan.

Comments. This species belongs to a subsect. Foetentinae is a new record for Pakistan, based on the nrITS phylogenetic analysis coupled with the morpho-anatomical characterization.

Russula pseudoamoenicolor Ghosh (2016) Figures. 03 & 04

Morpho-anatomy

BASIDIOCARPS is small to medium-sized. PILEUS: 9.5 – 10 cm in diameter. Shape slightly depressed to depressed, pale purplish pink (5RP 9/4) to dark purplish-red (7.5RP 3/8) in color, dry and velvety surface feel, smooth to shaggy surface appearance, and the context color is pale creamy. CURVATURE; straight to deflexed. MARGIN; undate to erodead. GILLS; adnate and horizontal, thin, close to crowded in spacing, color pale yellow (5Y 9/4) to light yellow (2.5Y 9/6), and smooth margins. STIPE: length 5 cm, width 2 – 2.5 cm. Location central, clavate to ventricose shape, glabrous to the pruinose surface, and pale purplish pink (5RP 9/4) to moderate purplish pink (5RP 7/6) in color. The nature of the pith is hollow and its consistency is soft to pliable. ANNULUS: absent. VOLOVA: absent. VEIL and its patches are also absent.

BASIDIOSPORES: [20/1/1] (6) 7 – 9 (11) × (5) 6 – 7 (8) µm, Q = 1.25. Color light red to brown in congo-red, globose to subglobose in shape, echinate surface, thick-walled, present of guttiule and apiculus. CHEILOCYSTIDIA: 82.5 – 147.5 × 17.5 – 25 µm. The color is red to pink in congo-red, narrowly lageniform to fusoid-ventricose in shape, thick-walled, and absent of guttiule. PLEUROCYSTIDIA: 125 – 145 × 20 – 27.5 µm. Color red in congo-red, fusoid to ventricose in shape, thick-walled and absent of gutiule. BASIDIA: 35 – 60 × 10 – 17.5 µm. Color light red to brown in congo-red, clavate in shape, 4-spored with pointed sterigmata, thin-walled, and absent of guttiule. PILLEPELLIS: 2.5 – 10 µm in width. Color red to orange in congo-red, thin-walled, septated, and intricate trichoderm to irregular trichoderm in the arrangement. STIPETIPELLUS: 2.5 – 7.5 µm in width. Color light red in congo-red, thick-walled, septated, and ixotrichoderm in the arrangement.

Habitat and distribution. Pakistan, Khyber Pakhtunkhwa, Shangla Top forest, 1700m a.s.l, solitary on the ground in the mixed forest of *pinus wallichina* and *Quercus*, 21 August 2021, IS # 060, Imtiaz khan.

Comments. This species belongs to a subsect. Amoeninae is a new record for Pakistan, based on the nrITS phylogenetic analysis coupled with the morpho-anatomical characterization.

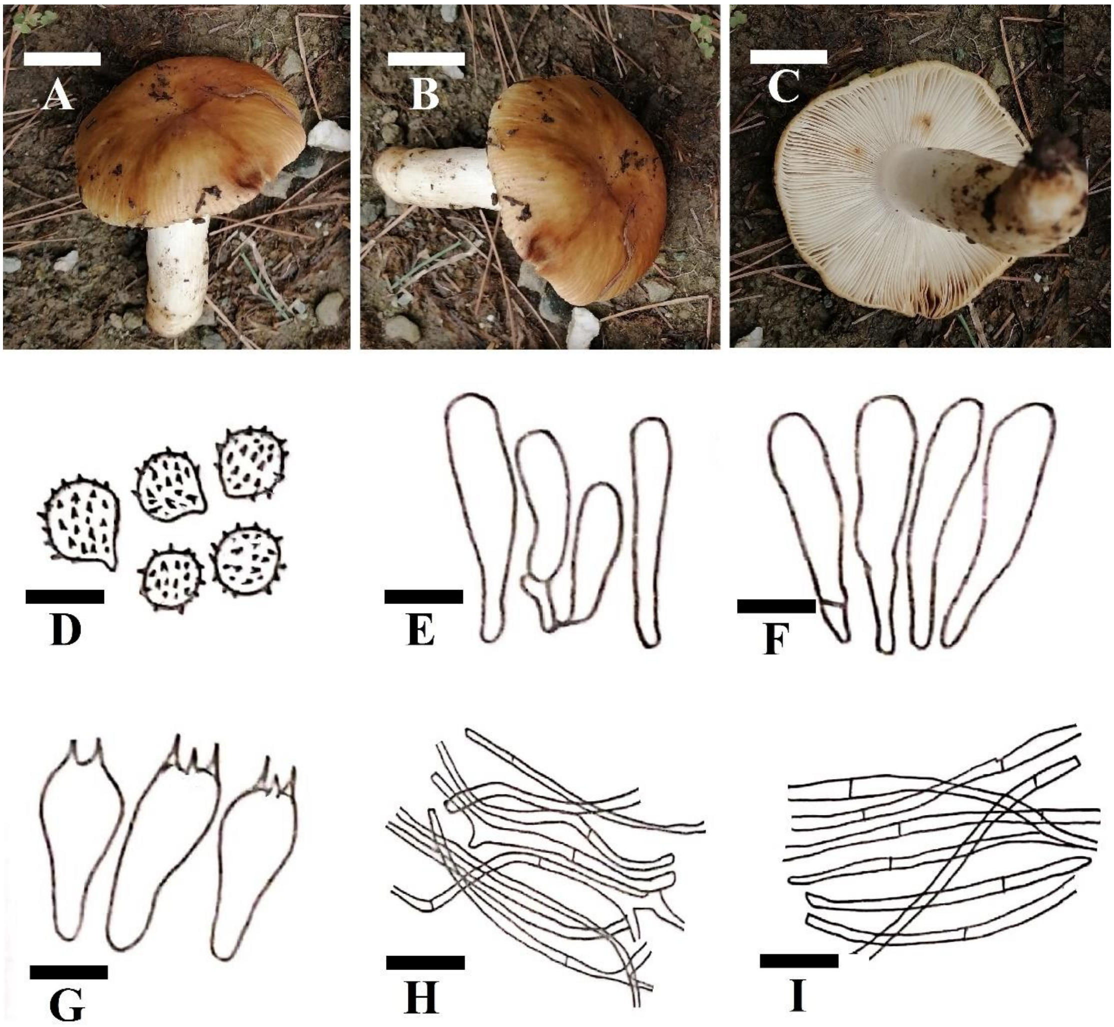

Figure – 02: A-C: Basidiomata of *Russula Foetentula*: A- Pileus, B- Stipe, C- Gills, D-I: Anatomical structures of *Russula Foetentula*: D- Basidiospores, E- Cheilocystidia, F- Pleurocystidia, G- Basidia, H- Pillepellis, I- Stipetipellus. Scale bars: A-C = 02 cm. D = 08 μm, E = 18 μm, F = 20 μm, G = 16 μm, H = 04 μm, I = 06 μm.

Nucleotide alignment datasets and phylogenetic inferences

The consensus sequences for the ITS region of *Russula byssina* were 623 bp. Initial BLAST analysis revealed that *Russula byssina* has maximum similarity with *Russula byssina* (GenBank MN648950), Query coverage – 100%, expect value 0.0, and Identity index – 99.04% collected from China. Secondly, *Russula foetentula* consensus sequences for the ITS region were 615 bp. BLAST results revealed that *Russula foetentula* has maximum similarity with *Russula foetentula* (GenBank MK5645966.1), Query coverage – 100%, expect value 0.0, and Identity index – 97.24% collected from the USA. Whereas consensus sequences for the ITS region of *Russula pseudoamoenicolor* were 622 bp. BLAST analysis revealed that *Russula pseudoamoenicolor* has maximum similarity with *Russula pseudoamoenicolor* (GenBank KX234819.2), Query coverage – 100%, expect value 0.0, and Identity index – 98.07% collected from India.

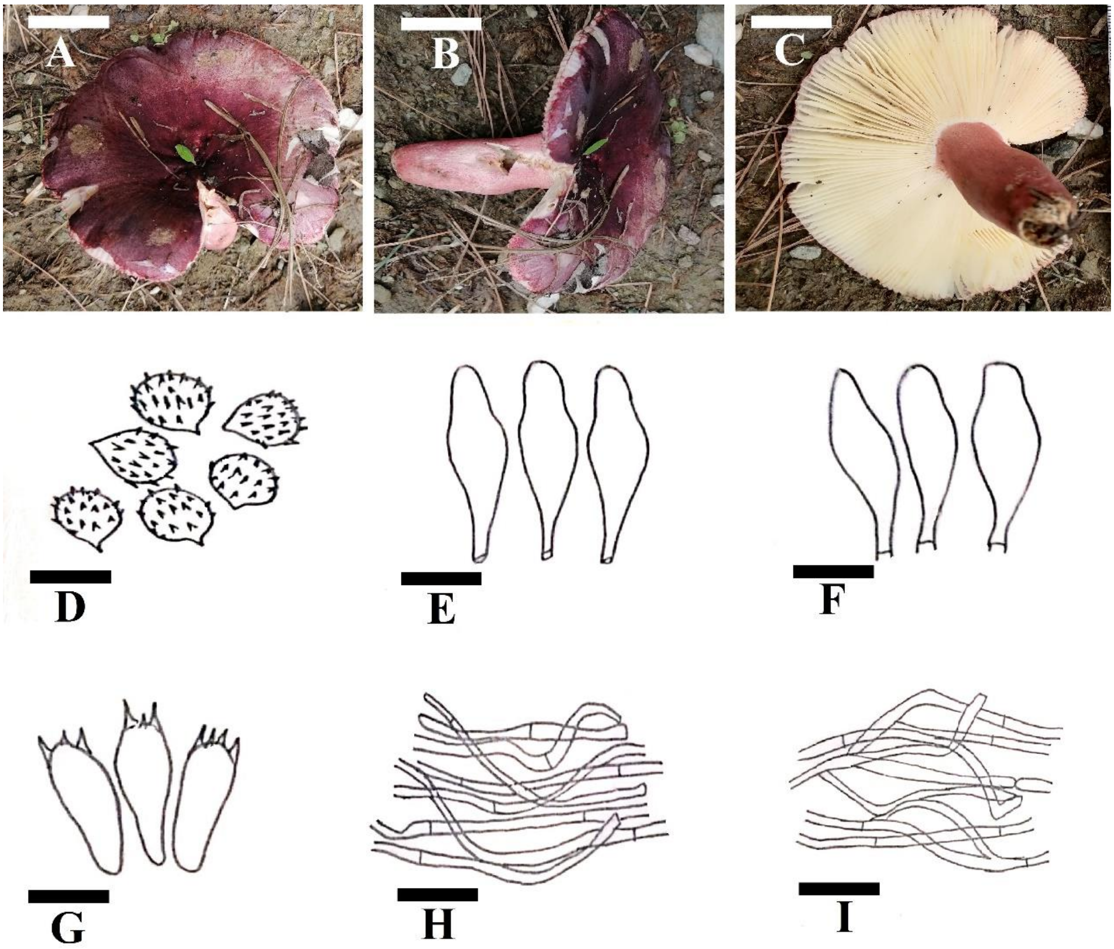

Figure – 03: A-C: Basidiomata of *Russula pseudoamoenicolor*: A- Pileus, B- Stipe, C- Gills, D-I: Anatomical strucrures of *Russula pseudoamoenicolor*: D- Basidiospores, E- Cheilocystidia, F- Pleurocystidia, G- Basidia, H- Pillepellis, I- Stipetipellus. Scale bars: A-C = 2.5 cm. D = 10 µm, E = 57 µm, F = 56 µm, G = 28 µm, H = 09 µm, I = 07 µm.

The phylogenetic tree of the studied collections accessions was done by involving 66 closest sequences from Genbank using the Maximum Likelihood method. *Albatrellus ovinus* (GenBank AY198202) was selected as an outgroup taxon (Saba & Khalid, 2015). The data set contained 711 characters out of which 392 were conserved, 317 were variable, 297 were parsimony informative and 20 were singletons. Positions with gaps and unavailable data were not taken into account. The final dataset maintained 383 positions exclusively. Initial tree(s) for the heuristic search were acquired automatically by application of Neighbor-Join and Bio NJ data algorithms to a set of pairwise distances. Estimations were done by employing the MCL (Maximum Composite Likelihood) criteria. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Subsequently, the topology with the uppermost log-likelihood value was selected. The tree is figured out by 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. 04). These three new record for Pakistan were confirmed by each forming strongly supported clade with the related taxa.

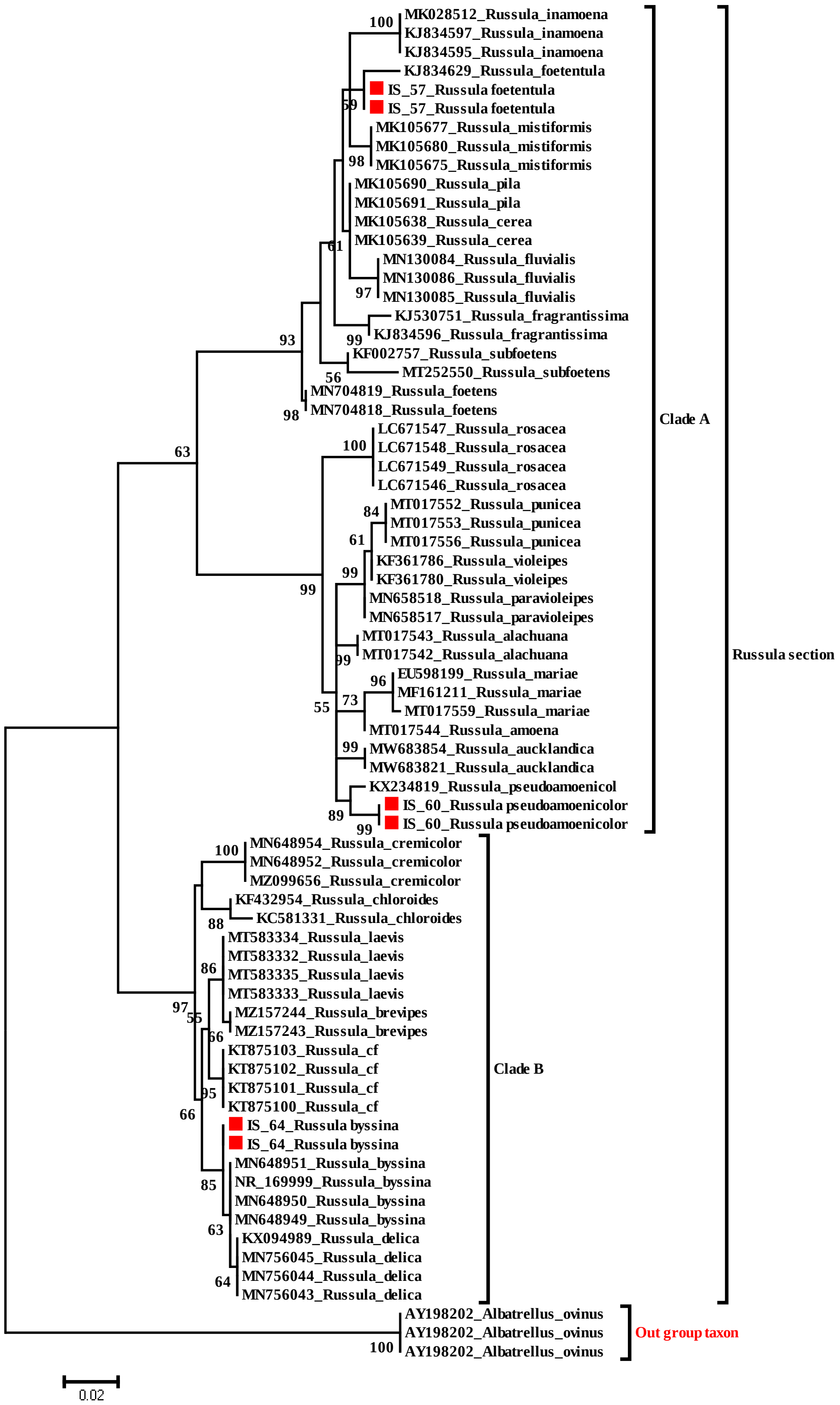

Figure – 04: Molecular phylogenetic relationship of the studied collections with related species based on the Maximum Likelihood method. The tree with the highest log likelihood (-1755.0335) is shown. The collections of Shangla are represented by a red cube (🟥).

TABLE 01. Specimens and GenBank accession numbers of ITS sequences used in molecular analysis. New sequences are indicated in bold.

Taxon	Accession No.	Origin	Voucher
Russula byssina	MN648950	China	HGAS-MF009913
Russula byssina	MN648949	China	HGAS-MF009921
Russula byssina	NR169999	China	HGAS:MF009907
Russula byssina	MN648951	China	HGAS-MF009907
Russula delica	KX094989	China	HMJAU 32182
Russula delica	MN756045	China	1069
Russula delica	MN756044	China	0785
Russula delica	MN756043	China	1604
Russula chloroides	KF432954	Ireland	RUS-12091401
Russula chloroides	KC581331	Canada	UBCF20353
Russula cremicolor	MZ099656	China	GH593 (HGASMF01-4448)
Russula cremicolor	MN648954	China	HGAS-MF009919
Russula cremicolor	MN648952	China	HGAS-MF009908
Russula cf.	KT875103	Mexico	HC-PNNT-093
Russula cf.	KT875102	Mexico	AR09597
Russula cf.	KT875101	Mexico	HC-PNNT-026
Russula cf.	KT875100	Mexico	HC-PNNT-176
Russula brevipes	MZ157244	China	MG720473
Russula brevipes	MZ157243	China	MG720473
Russula laevis	MT583333	USA	CLC_1642
Russula laevis	MT583335	USA	CLC_2271
Russula laevis	MT583334	USA	CLC_1740
Russula laevis	MT583332	USA	CLC_1690
Russula cerea	MK105639	Poland	F-2012-28 (KRA), JMV800667 (BCN)
Russula cerea	MK105638	Germany	GH20090620, JMV800660 (BCN)
Russula pila	MK105690	Spain	JMV970816-8 (BCN)
Russula pila	MK105691	Spain	JMV800654 (BCN)
Russula fluvialis	MN130084	Finland	KUO (JR8666)
Russula fluvialis	MN130086	Finland	KUO (JR8196)
Russula fluvialis	MN130085	Finland	KUO (JR8313)
Russula foetens	MN704819	China	fruit body176
Russula foetens	MN704818	China	fruit body175
Russula fragrantissima	KJ530751	Italy	98
Russula fragrantissima	KJ834596	Italy	108
Russula subfoetens	KF002757	China	HKAS 78367
Russula subfoetens	MT252550	Laos	WA0000072202

Russula foetentula	KJ834629	Canada	165
Russula inamoena	KJ834595	Italy	109
Russula inamoena	MK028512	Switzerland	Montri-91
Russula inamoena	KJ834597	Italy	107
Russula mistiformis	MK105677	Spain	JC170305NR, JMV800661 (BCN)
Russula mistiformis	MK105680	Spain	AMC H-69, JMV971127 (BCN)
Russula mistiformis	MK105675	Greece	JMV800652 (BCN)
Russula foetens	MN704818	China	fruit body175
Russula alachuana	MT017543	USA	SAV_F-20108
Russula alachuana	MT017542	USA	SAV_F-20113
Russula pseudoamoenicolor	KX234819	India	AG 15-739
Russula amoena	MT017544	Slovakia	SAV_F-3147
Russula mariae	MF161211	USA	FH:BHI-F229
Russula mariae	MT017559	USA	SAV_F-4564
Russula mariae	EU598199	USA	PC BB2004-213
Russula aucklandica	MW683854	New Zealand	PDD:106909
Russula aucklandica	MW683821	New Zealand	PDD:104168
Russula rosacea	LC671546	Thailand	SNA001
Russula rosacea	LC671549	Thailand	DNP064-3
Russula rosacea	LC671547	Thailand	PSD039
Russula rosacea	LC671548	Thailand	DNP064-1
Russula paravioleipes	MN658518	China	HBAU15002
Russula paravioleipes	MN658517	China	HBAU15001
Russula violeipes	KF361786	South Korea	HCCN21655
Russula violeipes	KF361780	South Korea	HCCN11773
Russula punicea	MT017556	South Korea	SFC20170731-02

Discussion

ITS barcoding of the nrDNA is a novel system of accurate and rapid fungal species identification. It is accepted as a universal barcode of fungi (Schoch *et al*., 2012). Among Basidiomycotina, Russulaceae Lotsy is a big fungal family of the order Russulales. It holds about 1,900 known species of cosmopolitan occurrence (Buyck *et al*., 2022). So in this paper also, three taxa of the *Russula* genus were studied morphologically and sequences of the ITS region of nrDNA were analyzed for each species.

The studied ***Russula byssina*** Clustered with several specimens that are currently identified as *R. brevipes*, *R. chloroides*, and *R. delica* receiving low bootstrap values. But most morphological characters of our collection are in closer agreement with Li *et al.*, (2020). However, our sample slightly differs by pileus shape, color, and margins. In blasting and the phylogenetic tree, our collection also clustered with *Russula byssina* of accessions (MN648950, (MN648949.1) from China. ***Russula foetentula*** is a typical member of the subsect. Foetentinae (Melzer & Zvára) Singer of subgenus

Ingratula Romagn. This is rarely reported from North America, perhaps because Singer (1943) synonymized it with *R. foetens* var., minor Singer, whereas Shaffer (1972) synonymized it with the European *R. subfoetens* W.G. Sm. Both these taxa, however, have a very unpleasant smell and different spores. Peck (1907) situated his species near *R. foetens* Pers., but the distinct almond odor mentioned in the protologue, as well as its spore ornamentation, suggests otherwise. Indeed, the presence of an almond smell is a good and reliable character for a *Russula* species, but the fact that *R. foetentula* possesses this odor seems largely ignored when considering it a synonym of *R. subfoetens*. *Russula foetentula* in this work is morpho-anatomically closely related to the Carteret & Buyck, (2013) *foetentula*. However show slight variation in pileus shape, surface appearance, margins, and context color. Phylogenetically this species also clustered with the same species. ***Russula pseudoamoenicolor*** **t**he Etymology is due to the lookalike of *Russula amoenicolor,* a European species. The combination of characters in *Russula pseudoamoenicolor* comprising a purplish-red to violet-red or lilac sub-velvety pileus with a darker center, reddish-violet to pink-rose stipe, the occasional occurrence of typically subulate terminal cells of pileipellis, absence of dermatocystidia and inamyloid supra hilar spot place it in R. subgenus. Amoenula Sarnari (Sarnari 1998). The presently described Specimen is much closer to the *Russula pseudoamoenicolor* of (Hyde *et al.*, 2016*)*. But morphologically differ in pileus margin, stipe shape mainly their surface appearance, and also show slight variation in all part's color. The present study provides evidence that further research is needed to collect and identify the fungal diversity of Shangla Top forests Pakistan, which appears to be a global hotspot of fungal diversity.

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