

Article

Morphological and Molecular Evidence for Two New Species within *Russula* Subgenus *Brevipes* from China

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Abstract: Two new *Russula* species, *R. subbrevipes* and *R. callainomarginis*, from China are described based on morphological and molecular characteristics. *Russula subbrevipes* has thus far only been found in southwestern China at altitudes of higher than 3400 m and is characterized by a yellow ochre pileal surface, glabrous or tomentose stipe, fruity odor, subglobose to ellipsoid basidiospores, isolated or partially connected warts and pleurocystidia with a cap appendage. *Russula callainomarginis* is characterized by a cream to white pileus, light turquoise lamellae margin, spongy stipe, light turquoise zone on the top of the stipe, pungent odor, globose to ellipsoid basidiospores and dominant isolated warts. The phylogenetic tree of *Russula* was constructed with multi-gene sequences, including the internal transcribed spacer regions (ITS), the ribosomal large subunit (nrLSU), the small subunit of the mitochondrial rRNA gene (mtSSU) and the second largest subunit of RNA polymerase II (*RPB2*). The results show that both *R. subbrevipes* and *R. callainomarginis* represent new lineages in *Russula* subg. *Brevipes*. Description and illustration of the two new species are presented.

Keywords: Russulales; Russulaceae; ectomycorrhizal fungi; phylogeny; taxonomy



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1. Introduction

Russula Pers. was erected by Persoon [1] and typified by *R. emetica* (Schaeff.) Pers. It is an important ectomycorrhizal genus worldwide, comprising more than 2000 species [2,3]. Species in the genus play a significant role in forest ecosystems, and many species are harvested for human consumption, especially in China [4,5]. The genus *Russula* has had a rich taxonomic history during the last two hundred years, and numerous infrageneric classification systems have been proposed [6–9]. Recent molecular phylogenetic studies based on a worldwide representative sampling have indicated eight subgenera within the genus: *Russula* subg. *Glutinosae* Buyck and X.H. Wang; *R.* subg. *Archaeae* Buyck and Hofst.; *R.* subg. *Compactae* (Fr.) Bon; *R.* subg. *Crassotunicatae* Buyck and Hofst.; *R.* subg. *Heterophyllidiae* Romagnesi; *R.* subg. *Malodorae* Buyck and Hofst.; *R.* subg. *Brevipes* Buyck and Hofst.; and *R.* subg. *Russula* [10,11]. *Russula* subg. *Brevipes* Buyck and Hofst was initially recognized as a lineage in the section *Plorantinae* belonging to *R.* subg. *Compactae* (Fr.) Bon.

Russula subg. *Compactae* is characterized by its large-to-small and hard fruiting bodies; dull-colored, white, brown, grey to black pilus; numerous lamellulae alternating with the lamellae; a poorly differentiated pileipellis that does not separate easily from the context; reddening, greying, blackening or browning context; white spore-print [8,12]. It was split into two subtaxa by Bataille in 1908: *Lactarioieae* (later *Plorantinae*), which do not blacken,

and *Nigricantinae*, which do. Romagnesi [6] followed this system with a minor modification to establish a new classification system, in which the sections *Archaeinae* Heim: Romagn., *Nigricantinae* Bataille and *Plorantinae* Bataille were included in *R. subg. Compactae*.

Recently, phylogenetic analyses were conducted to gain an insight into the genus *Russula*, and the results showed that sections *Archaeinae*, *Nigricantinae* and *Plorantinae* were well-supported, although they may not be as closely related as previously believed [4,13,14]. Buyck et al. [10] firstly studied the morpho-anatomical features of both fruiting bodies, as well as below-ground structures of Russulaceae, and presented a more comprehensive phylogeny based on nrLSU, mtSSU, RPB1, RPB2 and TEF1 sequences. A new classification system was proposed, and the sections *Archaeinae*, *Nigricantinae* and *Plorantinae* were elevated to four different subgenera with significant support: *R. subg. Archaea*, *R. subg. Compactae*, *R. subg. Malodora* and *R. subg. Brevipipes* [10,12].

The members in *R. subg. Brevipipes* are mostly medium to very large and thick-fleshed, only exceptionally small and thin-fleshed. Cap whitish, often rapidly developing yellowish brown to reddish brown stains. Well-developed annulus never present. Gills regularly unequal. Context turning yellowish to rusty brown, mostly with distinct smell, acrid to strongly acrid. Spore-print whitish to yellow. Spores with inamyloid or amyloid suprahilar spot. Primordial hyphae absent. Gloecystidia mucronate to obtuserounded in all parts of the fruiting body. Hyphal extremities of cap surface inflated or not [10].

This subgenus has a cosmopolitan distribution. In Europe, the group is represented by the well-known *R. chloroides* (Krombh.) Bres. and *R. delica* Fr. In Asia, *R. subg. Brevipipes* is frequently reported from the Himalayas and Kashmir, and Li et al. reported three new species (*R. leucocarpa* G.J. Li and C.Y. Deng; *R. byssina* G.J. Li and C.Y. Deng; *R. cremicolor* G.J. Li and C.Y. Deng) from Guizhou Province of China in 2020, based on ITS sequences [15]. However, the taxonomy of this group is exceptionally challenging due to nomenclatoric mess and dubious taxa within *R. delica* and *R. chloroides* [3,10].

This paper is part of an ongoing project in which taxonomic and phylogenetic studies focusing on *Russula* in China are being carried out to clarify the species diversity. Some new species have been described in recent years [16–19]. As a continuation of these surveys, two *Russula* species were found to be undescribed. Phylogenetic analyses based on ITS, nrLSU, mtSSU and RPB2 regions were carried out to verify their affinity within the *R. subg. Brevipipes*.

2. Materials and Methods

2.1. Morphological Studies

Collections were obtained from southwestern China during 2012–2015. Photographs and macro-morphological descriptions were based on fresh, mature fruitbodies, and specimens were then dried in an oven at 40 °C until completely desiccated. The studied specimens were deposited in the herbarium of Research Institute of Tropical Forestry, Chinese Academy of Forestry (RITF), the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS) and the personal herbarium of Haijiao Li (LI). Terminology for descriptive terms follows Vellinga [20]. The description templates and terminology of the micromorphological characters were taken from Adamčík, et al. [3]. Color names and codes follow Kornerup and Wanscher [21].

Microscopic examinations followed Adamčík, et al. [3]. Tissues of specimens were first immersed in 5% potassium hydroxide (KOH) and then stained with 1% aqueous Congo red solution for microscopic observation with an Axio Imager 2 upright microscope (Carl Zeiss Microscopy, GmbH, Germany) under a 100× oil immersion objective lens. Observations and measurements of the basidiospores and ornamentation were made in Melzer's reagent. All tissues were also examined in Cresyl blue to verify presence of ortho- or metachromatic reactions as explained in Buyck [22]. Sulphovanillin (SV) solution was used to test for reactions of cystidia. Scanning electron microscope (SEM) photos were captured with a JEOL JSM-6510 microscope (Tokyo, Japan). The abbreviation (n/m/p) indicates n basidiospores measured from m fruit bodies of p specimens. Basidiospore measurements are presented as (Min–)AV–SD–AV–AV+SD(–Max), where Min is the minimum value, Max is the maxi-

mum value, AV is the average value, SD is the standard deviation, and Q represents the length/width ratio of the basidiospores.

2.2. Molecular Study and Phylogenetic Analysis

Genomic DNA was extracted from dried mushrooms with the CTAB protocol [23]. The DNA was amplified with the primers: ITS4 and ITS5 for ITS [24]; LR0R and LR7 for nrLSU [25]; *RPB2* using the primers bRPB2-6f and fRPB2-7cr [26,27]; the mitochondrial small subunit (mtSSU) with primers MS1 and MS2 [24].

Amplifications were performed in a 50 μ L reaction volume containing 5 μ L of 10 \times PCR reaction buffer, 5 μ L dNTP mix (0.2 mmol), 2 μ L each of primers (5 μ mol) and 1.5 U of Taq DNA polymerase. The final volume was adjusted to 50 μ L with sterile distilled H₂O [28]. The PCR procedure for ITS, mtSSU and *RPB2* was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 48 °C for 45 s, 72 °C for 1 min and a final extension of 72 °C for 10 min. The PCR procedure for nrLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1.5 min and a final extension of 72 °C for 10 min. The PCR products were visualized by agarose gel electrophoresis and stored at –20 °C after visualization. The PCR products were purified by using TaKaRa MiniBEST Agarose Gel DNA Extraction Kit according to the operation manual. DNA sequencing was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology and Services Co. Ltd., Shanghai, China). The basic authenticity and reliability of newly generated sequences were established based on Nilsson et al. [29]. All newly generated sequences were submitted to GenBank and are listed in Table 1.

Table 1. A list of species, specimens, and GenBank accession number of sequences used in this study.

Species	Collection No.	Location	GenBank Accession No.			
			ITS	LSU	<i>RPB2</i>	mtSSU
<i>Lactifluus piperatus</i>	M. Lecomte:2001 08 19 55	France	KF220121	KF220214	KF220287	NC_038056
<i>Russula acrifolia</i>	r-05065	USA	JF834363	JF834510	JF834460	-
<i>R. acrifolia</i>	BB 08.662	Italy	-	KU237535	KU237821	KU237381
<i>R. acrifolia</i>	RITF3122	China	MH911600 ^a	MH911611 ^a	MH911626 ^a	-
<i>R. adusta</i>	5226	Italy	JF908669	-	-	-
<i>R. adusta</i>	BB 06.562	Canada	-	KU237476	KU237762	KU237320
<i>R. albonigra</i>	r-04105	USA	JF834355	JF834503	JF834452	-
<i>R. albonigra</i>	BB 07.291	Slovakia	-	KU237536	KU237822	KU237382
<i>R. amara</i>	FH12213	Germany	KT933998	KT933859	KT933930	-
<i>R. amoenolens</i>	BPL232	USA	KT933954	KT933813	KT933884	-
<i>R. archaeosuberis</i>	BB 12.085	Italy	KY800355	KU237593	KU237878	KU237441
<i>R. aff. areolata</i>	BB 06.090	Madagascar	-	KU237471	KU237757	KU237315
<i>R. australis</i>	JAC10732	New Zealand	MW683746	MW683616	-	-
<i>R. azurea</i>	BB 08.668	Italy	JN944002	KU237529	KU237815	KU237375
<i>R. blennia</i> sp. ined.	BB 08.066	Madagascar	-	KU237556	KU237842	KU237404
<i>R. brevipes</i>	BB 06.508	Mexico	-	KU237479	KU237765	KU237323
<i>R. cf. brevipes</i>	BB 06.441	Mexico	-	KU237483	KU237769	KU237327
<i>R. brevipes</i> var. <i>acrior</i>	JMP0058	USA	EU819422	-	-	-
<i>R. callainomarginis</i>	RITF2639	China	MH286463 ^a	MH286468 ^a	MH911624 ^a	MH911616 ^a
<i>R. callainomarginis</i>	Li160714-03	China	MH911604 ^a	-	-	-
<i>R. callainomarginis</i>	Li150731-09	China	MH911605 ^a	-	-	-
<i>R. callainomarginis</i>	Li160910-20	China	MH911606 ^a	-	-	-
<i>R. callainomarginis</i>	Li160714-04	China	MH911607 ^a	-	-	-

Table 1. Cont.

Species	Collection No.	Location	GenBank Accession No.			
			ITS	LSU	RPB2	mtSSU
<i>R. camarophylla</i>	PAM01081108	China	DQ421982	DQ421982	DQ421938	-
<i>R. cf. camarophylla</i>	MPG11-7-09	Spain	-	KU237579	KU237865	KU237427
<i>R. capillaris</i> sp. ined.	BB 08.172	Madagascar	-	KU237553	KU237839	KU237399
<i>R. aff. cellulata</i>	BB 06.045	Madagascar	-	KU237454	KU237740	KU237298
<i>R. chloroides</i>	UBCF20353	Canada	KC581331	KC581331	-	-
<i>R. aff. chloroides</i>	FH12273	Belgium	KT934015	KT933876	KT933947	-
<i>R. compacta</i>	BPL227	USA	KT933952	KT933810	KT933881	-
<i>R. compacta</i>	BB 06.295	USA	-	KU237480	KU237766	KU237324
<i>R. cortinarioides</i>	BB 07.103	USA	KP033480	KP033491	KP033502	KU237402
<i>R. cuprea</i>	FH12250	Slovakia	KT934010	KT933871	KT933942	-
<i>R. decipiens</i>	SAV F-1022	Slovakia	KY582683	-	KY616679	KY471572
<i>R. decolorans</i>	FH12196	Slovakia	KT933992	KT933853	KT933924	-
<i>R. delica</i>	FH12272	Belgium	KF432955	KR364224	KR364340	-
<i>R. aff. delica</i>	BB 12.086	Italy	-	KU237594	KU237879	KU237442
<i>R. cf. delica</i>	SA07.210	Slovakia	-	KU237600	KU237885	KU237449
<i>R. delicinae</i>	BB 06.476	Mexico	-	KU237484	KU237770	KU237328
<i>R. densifolia</i>	BB 07.344	Slovakia	-	KU237502	KU237788	KU237347
<i>R. dissimulans</i>	OSA-MY-1727	Japan	AB291731	AB154717	-	-
<i>R. earlei</i>	BPL245	USA	KT933961	KT933820	KT933891	-
<i>R. aff. earlei</i>	MT s.n.	Costa Rica	-	KU237598	KU237883	KU237446
<i>R. eccentrica</i>	BB 07.044	USA	KP033479	KP033490	KP033501	KU237353
<i>R. cf. eccentrica</i>	BB 07.132	USA	KP033478	KP033489	KP033500	KU237341
<i>R. edulis</i>	BB 08.167	Madagascar	-	KU237564	KU237850	KU237412
<i>R. fellea</i>	FH12185	Slovakia	KT933989	KT933850	KT933921	-
<i>R. fistulosinae</i> sp. ined.	BB 08.105	Madagascar	-	KU237527	KU237813	KU237373
<i>R. fragilis</i>	FH12197	France	KT933993	KT933854	KT933925	-
<i>R. globispora</i>	GENT:FH-2007-BT111	Germany	KU928144	-	KY616671	KY471564
<i>R. gossypina</i>	BB 06.002	Madagascar	-	KU237450	KU237736	KU237293
<i>R. griseobrunnea</i>	PDD:81525	New Zealand	GU222265	-	-	-
<i>R. aff. griseobrunnea</i>	BB 09.344	New Caledonia	-	KU237592	KU237877	KU237440
<i>R. hatsikiana</i> sp. ined.	BB 08.178	Madagascar	-	KU237557	KU237843	KU237405
<i>R. herrerae</i>	BB 06.532	Mexico	-	KU237486	KU237772	KU237330
<i>R. inornata</i> sp. ined.	BB 08.194	Madagascar	-	KU237558	KU237844	KU237406
<i>R. integra</i>	FH12172	Slovakia	KT933984	KT933845	KT933916	-
<i>R. laeta</i>	SAV F-3949	Slovakia	KY582708	-	KY616709	KY471600
<i>R. laevis</i>	JR4016	Finland	MN130091	MN130128	MN380529	MN161180
<i>R. laricina</i>	575/08.681	Italy	JN944008	JN940593	KU237846	-
<i>R. lateriticola</i>	BB 06.031	Madagascar	KP033476	KP033487	KP033498	KU237297
<i>R. lepida</i>	HJB9990	Belgium	DQ422013	DQ422013	DQ421954	KY471624
<i>R. cf. liberiensis</i>	BB 06.184	Madagascar	-	KU237474	KU237760	KU237318
<i>R. lilacea</i>	BB 07.213	Slovakia	JN944005	KU237498	KU237784	KU237343
<i>R. luteotacta</i>	FH12187	Slovakia	KT933991	KT933852	KT933923	-
<i>R. marangania</i>	MEL2293694	Australia	EU019930	EU019930	-	-
<i>R. mariae</i>	SFC20120922-08	South Korea	KF361778	KF361828	KF361728	-
<i>R. melliolens</i>	SAV F-4201	Slovakia	KY582719	-	KY616712	KY471611
<i>R. minutula</i>	BB 08.636	Italy	-	KU237531	KU237817	KU237377
<i>R. mustelina</i>	FH12226	Germany	KT934005	KT933866	KT933937	-
<i>R. nauseosa</i>	FH12173	Germany	KT933985	KT933846	KT933917	-
<i>R. nigricans</i>	UE20.09.2004-07	Sweden	DQ422010	DQ422010	-	-
<i>R. nigricans</i>	BB 07.342	Slovakia	-	KU237495	KU237781	KU237339
<i>R. ochroleuca</i>	FH12211	Germany	KT933996	KT933857	KT933928	-

Table 1. Cont.

Species	Collection No.	Location	GenBank Accession No.			
			ITS	LSU	RPB2	mtSSU
<i>R. odorata</i>	BB 07.186	Slovakia	JN944010	KU237518	KU237804	KU237364
<i>R. pallidospora</i>	JV02-218	Sweden	DQ422032	DQ422032	-	-
<i>R. aff. pallidospora</i>	MPG13-6-08	Spain	-	KU237580	KU237866	KU237428
<i>R. pectinatoides</i>	BPL276	USA	KT933975	KT933836	KT933907	-
<i>R. pelargonica</i>	r-04023	USA	JF834348	JF834496	JF834445	-
<i>R. persicina</i>	UE21.09.2003-01	Sweden	DQ422019	DQ422019	DQ421960	-
<i>R. polyphylla</i>	BB 07.134	USA	KP033486	KP033497	KP033508	KU237448
<i>R. polyphylla</i>	BB 07.023	USA	KP033481	KP033492	KP033503	KU237403
<i>R. polyphyllinae</i> sp. ined.	BB 09.215	New Caledonia	-	KU237590	KU237875	KU237438
<i>R. pseudoaurantiophylla</i>	BB 09.219	New Caledonia	-	KU237591	KU237876	KU237439
<i>R. pumicoidea</i>	Trappe14771	Australia	EU019931	EU019931	-	-
<i>R. raoultii</i>	FH12222	Germany	KT934002	KT933863	KT933934	-
<i>R. romellii</i>	FH12177	Germany	KT933987	KT933848	KT933919	-
<i>R. sesenagula</i>	BB 08.117	Madagascar	-	KU237526	KU237812	KU237372
<i>R. sinuata</i>	H4755	Australia	EU019943	-	-	-
<i>R. solaris</i>	BB 07.282	Slovakia	JN944007	JN940606	KU237835	KU237395
<i>R. subbrevipipes</i>	RITF3136	China	MH286460 ^a	MH286465 ^a	MH911625 ^a	MH911617 ^a
<i>R. subbrevipipes</i>	RITF2946	China	MH286462 ^a	MH286467 ^a	-	MH911618 ^a
<i>R. subbrevipipes</i>	RITF3002	China	MH286461 ^a	MH286466 ^a	-	MH911619 ^a
<i>R. cf. subfistulosa</i>	BB 08.176	Madagascar	-	KU237542	KU237828	KU237388
<i>R. subnigricans</i>	RITF2657	China	MH911602 ^a	MH911612 ^a	-	MH911620 ^a
<i>R. subnigricans</i>	Li160821-05	China	MH911603 ^a	-	-	-
<i>R. subnigricans</i>	YM-64	China	MH911601 ^a	-	-	-
<i>R. vesca</i>	BPL284	USA	KT933978	KT933839	KT933910	-
<i>R. vesicatoria</i>	BB 07.034	USA	-	KU237599	KU237884	-
<i>R. violeipes</i>	SFC20121010-06	South Korea	KF361808	KF361858	KF361758	-
<i>R. zvarae</i>	FH12175	Germany	KT933986	KT933847	KT933918	-

^a New sequences for this study.

Sequences were aligned in MAFFT 7 ([30]; <http://mafft.cbrc.jp/alignment/server/>, accessed on 22 January 2022) using the “G-INS-I” strategy and manually adjusted in BioEdit [31]. One thousand partition homogeneity test (PHT) replicates of ITS, nrLSU, mtSSU and RPB2 sequences were tested by PAUP* v4.0b10 [32] to determine whether the partitions were homogeneous. The PHT results indicated all the DNA sequences display a congruent phylogenetic signal (p -value = 0.03). This means that the genes’ sequence dataset did not show any conflicts in tree topology for the reciprocal bootstrap trees, which allowed us to combine them. Sequences of species and outgroup *Lactifluus piperatus* (L.) Kuntze were retrieved from GenBank (NCBI) and combined with the new sequences to construct a concatenated ITS+ nrLSU+mtSSU+RPB2 dataset. Sequence alignment was deposited at TreeBase (submission ID 23830).

The best-fit model of nucleotide evolution to the datasets was selected with AIC (Akaike Information Criterion) using MrModeltest 2.3 [33,34]. Best model for the combined ITS+nrLSU+mtSSU+RPB2 sequence dataset estimated was GTR. Bayesian inference (BI) and maximum likelihood (ML) analyses were applied to the dataset with four partitions (ITS, nrLSU, mtSSU and RPB2).

BI analysis was performed using MrBayes on XSEDE (3.2.6) on Abe through the Cipres Science Gateway (www.phylo.org, accessed on 27 January 2022) with 2 independent runs, each one beginning from random trees with 4 simultaneous independent chains, performing 2 million replicates, sampling one tree every 1000th generation. The first 25% of the sampled trees were discarded as burn-in, and the remaining ones were used to reconstruct a majority rule consensus and calculate Bayesian posterior probabilities (BPP) of the clades.

ML analysis searches were conducted with RAxML-HPC2 on XSEDE (8.2.10) on Abe through the Cipres Science Gateway (www.phylo.org, accessed on 27 January 2022) involving 100 ML searches under the GTRGAMMA model, with all model parameters estimated by the program. Only the maximum-likelihood best tree from all searches was kept. In addition, 100 rapid bootstrap replicates were run with the GTRCAT model to assess the reliability of the nodes.

Branches that received bootstrap support for maximum likelihood (BS) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (BS) and 0.95 (BPP), respectively, were considered as significantly supported.

3. Results

3.1. Molecular Phylogeny

The combined dataset included sequences from 101 fungal specimens or isolates representing 82 taxa. The dataset had an aligned length of 3108 characters including gaps, of which 2065 characters were constant, 247 were variable and parsimony-uninformative and 796 were parsimony-informative. Bayesian analysis and ML analysis resulted in a similar topology, with an average standard deviation of split frequencies of 0.008966 (BI). The ML topology is shown in Figure 1.

The phylogenetic analyses revealed that the subgenera proposed by Buyck et al. (2018) were well-supported with significant BI and ML values: *Russula* subg. *Malodora* (BS = 97%, BPP = 1.00); *Russula* subg. *Brevipes* (BS = 97%, BPP = 1.00); *Russula* subg. *Compactae* (BS = 98%, BPP = 1.00) and *Russula* subg. *Archaea* (BS = 100%, BPP = 1.00). Our collections from Southern China formed two new lineages (bold names in Figure 1) with significant support (BS = 100%, BPP = 1.00; BS = 100%, BPP = 1.00, respectively) and fell into the *Russula* subg. *Brevipes*. They were considered as distinct phylogenetic species.

3.2. Taxonomy

Russula subbrevipes J.F. Liang and J. Song, sp. nov. (Figures 2–4)

MycoBank: MB 829447 (<https://www.mycobank.org/page/Name%20details%20page/571620>).

Diagnosis—Differs from other *Russula* species in this group by yellow ochre pileus, tomentose stipe, fruity odor, cylindrical or clavate pleurocystidia with a cap appendage, subglobose to ellipsoid basidiospores ((7.0–)7.8–8.4–9.0(–9.7) × (6.4–)6.9–7.4–7.9(–8.4) μm), amyloid ornamentation with isolated or partial connected warts.

Etymology—Subbrevipes (Lat.): refers to the morphological similarity to *R. brevipes* Peck.

Holotype—CHINA. Yunnan Province, Diqing Tibetan Autonomous Prefecture, Shangri-la, Pudacuo National Park, 27°51′17″ N, 99°57′8″ E, elev. 3400 m, on ground of *Quercus*, *Betula* and *Pinus*, 25 August 2014, Zhao 2265 (RITF3136).

Description—basidiomata medium-sized to large. Pileus 90–140 mm in diam., first hemispherical, expanding to plano-concave with a depressed center, then broadly infundibuliform when mature; margin incurved when juvenile, not becoming straight, smooth, without striate, sometimes dehiscent with age; surface yellow ochre (5C7) when fresh, becoming pale yellow (1A3) to cinnamon (6D6) when dry, slightly viscid when moist, glabrous, smooth, without striate; suprapellis unpeelable; context hard, up to 5 mm thick towards center, white (1A1) when fresh, unchanging when bruising. Lamellae subdecurrent, close to crowded with 1–3 series lamellulae, no forking near the stipe, white when fresh, becoming brownish-orange (5C3) to brown (5E5) when dry, unchanging when bruising. Stipe 40–50 × 10–25 mm, cylindrical, solid, cream (4A3) to white (1A1), becoming pale yellow (1A3) when dry, unchanging when bruising, glabrous or tomentose on the upper half part. Odor fruity. Taste mild. Spore-print whitish.

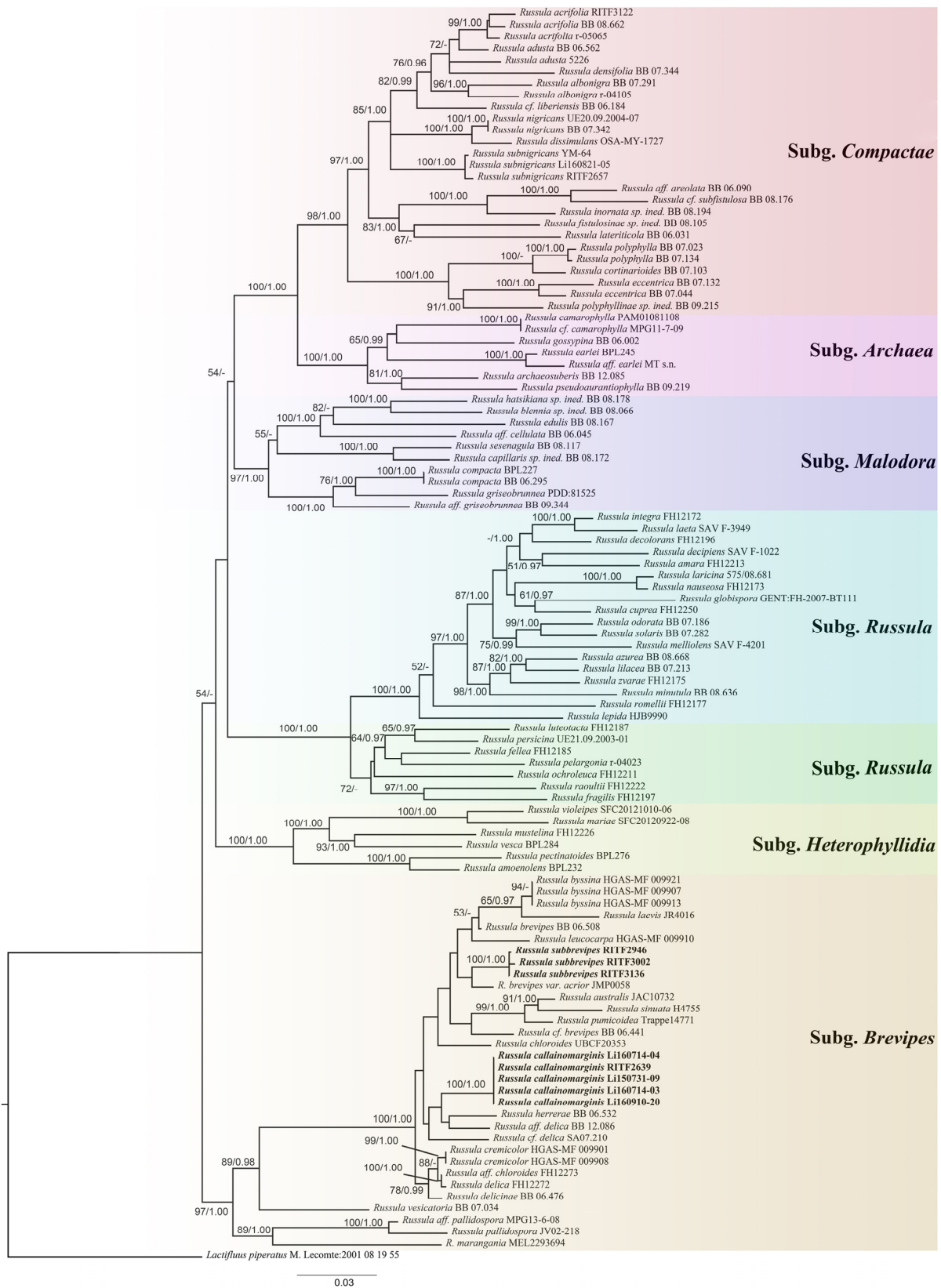


Figure 1. Phylogenetic consensus tree inferred from the maximum likelihood (ML) analysis based on ITS+nrLSU+mtSSU+RPB2 sequence data. Branches are labelled with maximum-likelihood bootstrap proportions (BS) higher than 50% and Bayesian posterior probabilities (BPP) more than 0.95; bold names = new species.

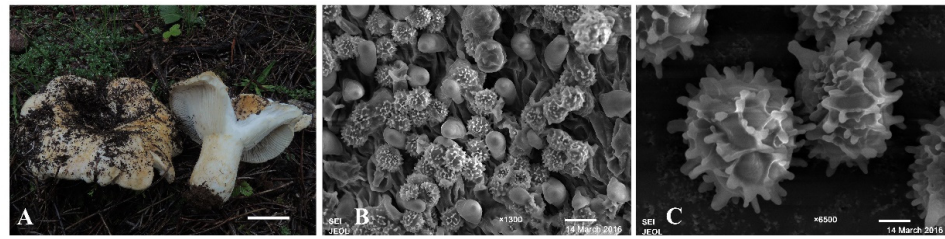


Figure 2. Basidiomata and microscopic structures of *Russula subbrevipis* (drawn from the holotype). (A) Basidiomata. (B,C) Basidiospores (SEM). Scale bars: (A) = 3 cm; (B) = 10 μm; (C) = 2 μm.

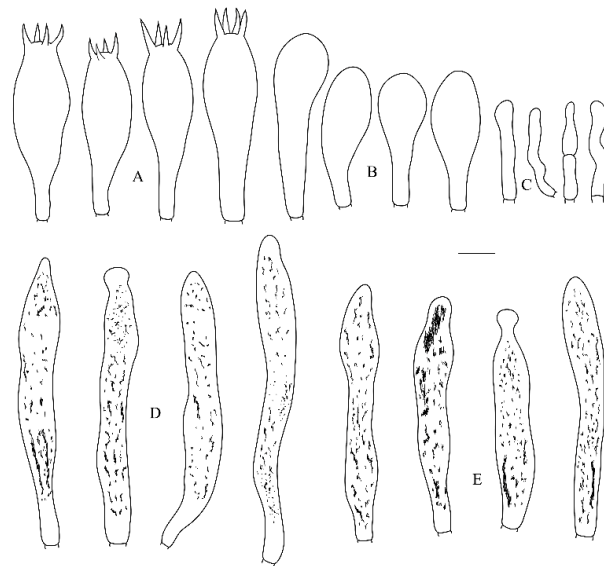


Figure 3. *Russula subbrevipis* (RITF3136). (A) Basidia. (B) Basidiola. (C) Marginal cells. (D) Hymenial cystidia on lamellae sides. (E) Hymenial cystidia on lamellae edges. Scale bar: (A–E) = 10 μm.

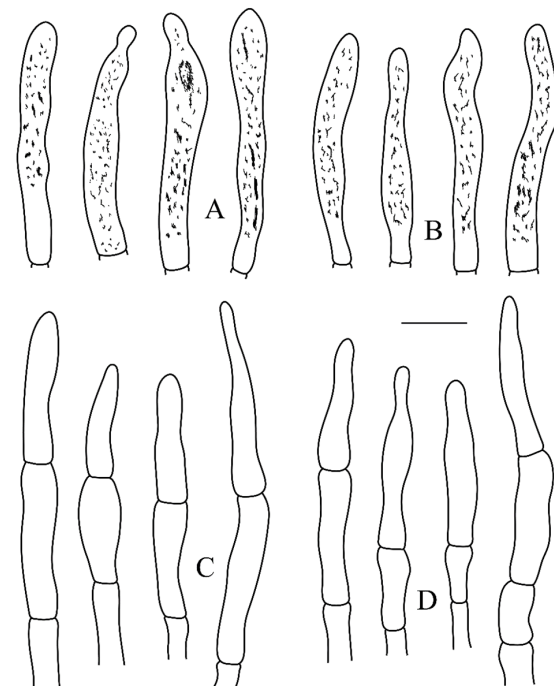


Figure 4. *Russula subbrevipis* (RITF3136). (A) Pileocystidia near the pileus margin. (B) Pileocystidia near the pileus center. (C) Hyphal terminations near the pileus margin. (D) Hyphal terminations near the pileus center. Scale bar: (A–D) = 10 μm.

Basidiospores (7.0–)7.8–8.4–9.0(–9.7) × (6.4–)6.9–7.4–7.9(–8.4) μm, Q = (1.01–)1.08–1.14–1.21(–1.31), subglobose to ellipsoid; ornamentation amyloid; warts bluntly conical to subcylindrical, up to 1.4 μm high, isolated or more commonly with light to heavy connectives forming a partial reticulum; suprahilar plage distinct, weakly amyloid. Basidia (37.0–)40.0–44.5–49.1(–52.3) × (11.2–)6.9–7.4–7.9(–15.9) μm, mostly 4-spored, mainly clavate; basidiola clavate or ellipsoid, ca. 9–14 μm wide. Hymenial cystidia on lamellae sides moderately numerous, (59.5–)64.0–72.5–81.0(–91.0) × (8.5–)9.0–9.7–10.3(–10.5) μm, mainly clavate or fusiform, apically often obtuse, sometimes with a cap appendage, thin-walled; contents heteromorphous or granulose, turning dark grey in SV. Hymenial cystidia on lamellae edges similar to on lamellae sides in shape and contents, but often shorter, (50.6–)54.0–61.1–68.3(–74.0) × (7.0–)7.9–9.3–10.6(–12.6) μm. Marginal cells (15.0–)17.9–20.8–23.8(–25.8) × (4.0–)4.4–5.1–5.8(–6.7) μm, subcylindrical, fusiform or lageniform, often flexuous. Pileipellis orthochromatic in Cresyl blue, sharply delimited from the underlying spherocytes of the context, ca. 150–285 μm deep; two layered with subpellis ca. 60–85 μm deep, horizontally oriented, intricate, less gelatinized, dense hyphae, 3–5.5 μm wide, and ca. 90–200 μm deep suprapellis of strongly gelatinized, repent, loose arranged hyphae, 3–5.5 μm wide. Hyphal terminations near the pileus margin rarely branched, sometimes flexuous, thin-walled, terminal cells 19.0–23.8–29.0(–33.2) × (3.6–)4.0–4.9–5.7(–6.3) μm, mainly attenuated or narrowly lageniform, occasionally subcylindrical, apically constricted or obtuse; subterminal cells frequently wider, ca. 4–7 μm wide, typically unbranched. Hyphal terminations near the pileus center similar to those near the pileus margin, (20.3–)21.8–25.8–29.8(–33.2) × (3.7–)4.2–4.8–5.4(–5.7) μm, mainly lageniform, occasionally subcylindrical; subterminal cells often shorter, ca. 3–6 μm wide, typically unbranched. Pileocystidia near the pileus margin always one-celled, scattered, (31.3–)33.9–39.5–45.1(–47.5) × (4.5–)4.9–5.7–6.6(–7.0) μm, mainly clavate, occasionally fusiform, apically typically obtuse, sometimes with a globose appendage, thin-walled; contents heteromorphous, blackish in SV. Pileocystidia near the pileus center similar to pileus margin in shape, size and contents, always one-celled, scattered, 32.0–37.6–43.0(–46.5) × 4.6–5.7–6.9(–8.0) μm. Clamp connections absent in all tissues.

Habitat and distribution—single or scattered in forests dominated by *Quercus* sp., *Betula* sp. and *Pinus* sp. at altitudes greater than 3400 m in south-western China.

Additional specimens (paratypes) examined—CHINA. Yunnan Province: Diqing Tibetan Autonomous Prefecture, Shangri-la, Pudacuo National Park, 25 August 2014, T25446 (RITF2946) and T25575 (RITF3002).

Russula callainomarginis J.F. Liang and J. Song, sp. nov. (Figures 5–7)

Mycobank: MB 829448 (<https://www.mycobank.org/page/Name%20details%20page/571632>).

Diagnosis—differs from other *Russula* species in this group by its cream to white pileus, adnate and folded lamellae with 1–2 series lamellulae, light turquoise lamellae margin, spongy stipe, pungent odor, globose to ellipsoid basidiospores ((6.4–)6.8–7.4–8.0(–8.5) × (5.2–)6.0–6.5–7.1(–7.5) μm), amyloid ornamentation and isolated warts.

Etymology—*Callainomarginis* (Lat.): referring to the light turquoise lamellae margin.

Holotype—CHINA. Hubei Province, Shennongjia Forestry District, Longjiangping, 31°26′36″ N, 110°29′18″ E, elev. 1850 m, 10 August 2015, LYK 91 (RITF2639).

Description—basidiomata medium-sized. Pileus 60–80 mm in diam., first hemispherical, then developing convex to umbilicate, centrally depressed; margin usually remaining somewhat decurved and inrolled even when dry; surface cream to white (1A1) when fresh, becoming wax yellow (3A5) to greyish-orange (5B6) upon drying, nonviscid when wet, glabrous, smooth, not striated, never cracked; suprapellis unpeelable; context hard, up to 7 mm thick towards center, white (1A1) when fresh, color unchanged when bruised. Lamellae adnate, usually folded, close with 1–2 series lamellulae, no forking seen near the stipe, white when fresh, becoming light brown spots and stains when bruised, becoming silver-white (2B2) to sienna (6D7) when dry, lamellae margin light turquoise (24A5) when young, becoming cream (4A3). Stipe up to 40 mm long, 16 mm wide, cylindrical with

slightly tapered base, white (1A1) when fresh with light turquoise (24A5) zone on the apex, becoming pale yellow (1A3) when dry. Spongy inside. Odor pungent. Taste slightly acrid. Spore-print whitish.

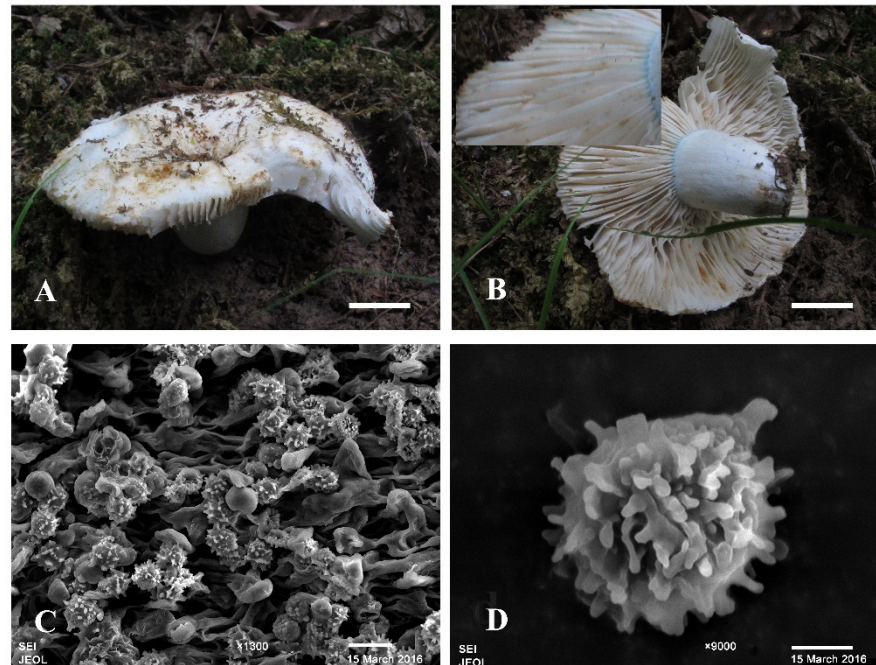


Figure 5. Basidiomata and microscopic structures of *Russula callainomarginis* (drawn from the holotype). (A,B) Basidiomata. (C,D) Basidiospores (SEM). Scale bars: (A,B) = 1.3 cm, (C,D) = 10 μ m.

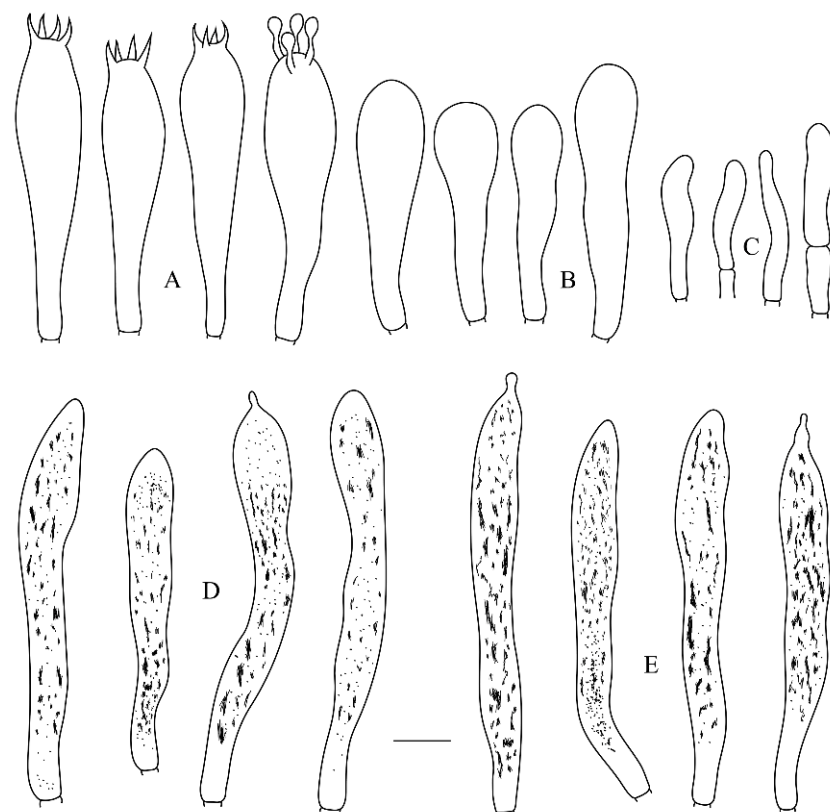


Figure 6. *Russula callainomarginis* (RITF2639). (A) Basidia. (B) Basidiola. (C) Marginal cells. (D) Hymenial cystidia on lamellae sides. (E) Hymenial cystidia on lamellae edges. Scale bar: (A–E) = 10 μ m.

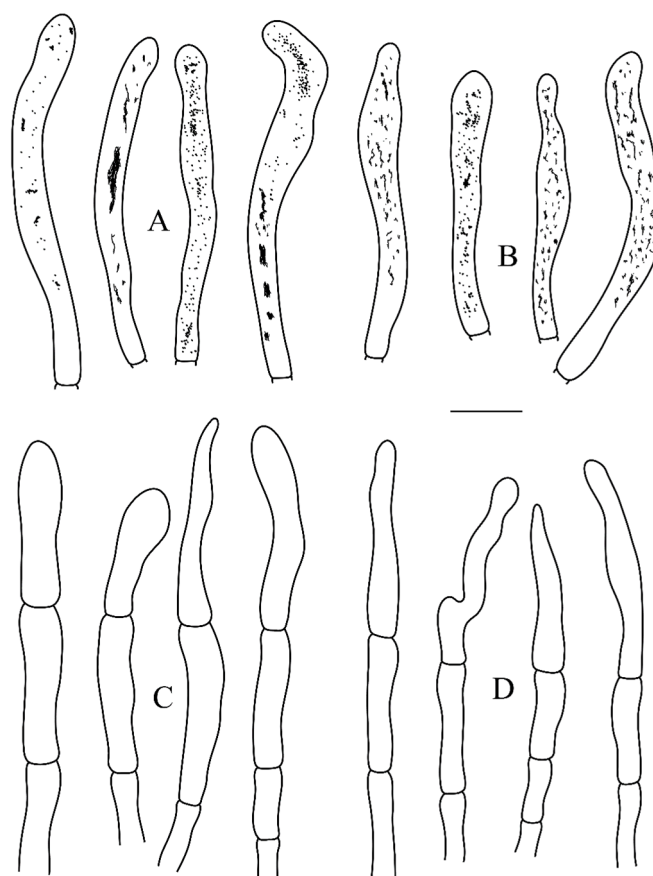


Figure 7. *Russula callainomarginis* (RITF2639). (A) Pileocystidia near the pileus margin. (B) Pileocystidia near the pileus center. (C) Hyphal terminations near the pileus margin. (D) Hyphal terminations near the pileus center. Scale bar: (A–D) = 10 μm .

Basidiospores (6.4–)6.8–7.4–8.0(–8.5) \times (5.2–)6.0–6.5–7.1(–7.5) μm , Q = (1.0–)1.07–1.13–1.20(–1.31), globose to ellipsoid, ornamentation amyloid; warts bluntly conical to subcylindrical, up to 1 μm high, isolated or connected with lines or ridges; suprahilar plage distinct, weakly amyloid. Basidia (40.0–)42.4–46.4–50.5(–52.0) \times (10.2–)11.1–12.0–12.9(–13.6) μm , mostly 4-spored, clavate or narrowly clavate; basidiola mainly clavate, ca. 8–13 μm wide. Hymenial cystidia on lamellae sides moderately numerous, (57.7–)59.6–70.0–80.4(–93.0) \times (7.6–)8.0–8.8–9.5(–10.6) μm , clavate, fusiform or subcylindrical, apically often obtuse, occasionally with round or ellipsoid appendage, thin-walled; contents heteromorphous or granulose, turning dark grey in SV. Hymenial cystidia on lamellae edges similar to on lamellae sides in contents, often smaller, (55.3–)56.4–65.6–75.0(–88.0) \times (6.8–)7.1–7.8–8.6(–9.3) μm , fusiform or clavate, apically often obtuse, sometimes with round or ellipsoid appendage, thin-walled. Marginal cells (17.0–)18.0–20.6–23.2(–24.8) \times (3.5–)3.9–4.5–5.0(–6.7) μm , clavate, fusiform or subcylindrical, sometimes flexuous. Pileipellis orthochromatic in Cresyl blue, not sharply delimited from the underlying spherocytes of the context, ca. 130–150 μm deep; vaguely divided in 80–100 μm deep subpellis, horizontally oriented, intricate, less gelatinized, dense hyphae, and ca. 50–70 μm deep suprapellis of strongly gelatinized, repent, loosely arranged hyphae forming a cutis, 3–7 μm wide. Hyphal terminations near the pileus margin rarely branched, sometimes flexuous, thin-walled, terminal cells (20.8–)22.5–28.0–33.6(–38.4) \times (4.0–)4.6–5.7–6.8(–7.8) μm , often clavate or narrowly lageniform, occasionally subcylindrical, apically obtuse or constricted; subterminal cells often longer, ca. 4–7 μm wide, typically unbranched. Hyphal terminations near the pileus center similar to those near the pileus margin, 19.7–23.5–27.2(–30.0) \times (3.7–)3.9–4.8–5.8(–6.2) μm , mainly subcylindrical and narrowly lageniform, occasionally clavate; subterminal cells often shorter, ca. 3–6 μm wide, typically unbranched. Pileocystidia near the pileus

always one-celled, scattered, $(29.3\text{--}33.0\text{--}42.8\text{--}52.6\text{--}58.3) \times 4.4\text{--}5.6\text{--}6.7\text{--}7.5$ μm , mainly clavate or subcylindrical, apically typically obtuse, thin-walled; contents granulose or crystalline, blackish in SV. Pileocystidia near the pileus center often shorter, always one-celled, scattered, $(30.0\text{--}33.1\text{--}38.6\text{--}44.0\text{--}49.0) \times 4.0\text{--}4.8\text{--}5.5\text{--}6.0$ μm , mainly clavate or fusiform, apically typically obtuse, thin-walled; contents granulose or heteromorphous, turning dark grey in SV. Clamp connections absent in all tissues.

Habitat and distribution—single or scattered on ground in forest dominated by Fagaceae during July to September. Presently known only from central and south China.

Additional specimens (paratypes) examined—CHINA. Yunnan Province, Kunming, Heilongtan Forest Park, elev. 1950 m, 1 September 2012, Zhao 117 (HKAS 77470); Chuxiong, Lufeng County, Guangtong town, Xibaoqiao Village, on ground of Fagaceae, elev. 1883 m, 31 July 2015, Li150731-09 (LI); Shandong Province, Taian, Taohuayu, Caojiazhuang Village, elev. 297 m, 14 July 2016, Li160714-03 and Li160714-04 (LI); Guizhou Province, Guizhou, Qianlingshan Park, on ground of Fagaceae, elev. 1145 m, 10 September 2016, Li160910-20 (LI).

4. Discussion

The genus *Russula* has a cosmopolitan distribution from Arctic tundra to tropical forests and forms ectomycorrhizae with a diverse range of plants in deciduous, evergreen, broadleaf and coniferous forests, scrubland, and even meadows [5,35]. In China, 158 species had been confirmed by Li et al. 2014, with especially high species richness in Yunnan Province and the Greater and Lesser Khinggan Mountains [36], and new species are still being identified.

Our phylogeny generated a tree which is consistent with formerly published studies [3,10,13]. Both the macro- and micro-morphological characters (large basidiocarps, white or white to yellow ochre pileal surface when fresh, hard and rigid context, close to crowded lamellae with numerous lamellulae, short stipe, whitish spore-print and weakly amyloid plages), as well as the significant support for the phylogenetic placement of *R. subbrevipipes* and *R. callainomarginis* (BS = 100%, BPP = 1.00 and BS = 100%, BPP = 1.00; Figure 1) together with *R. delica*, confirm that our new species belong to the subg. *Brevipes*.

Russula subbrevipipes and *R. callainomarginis* share similar characters: unpeelable suprapellis, hard context, white spore-print, subglobose to ellipsoid basidiospores and amyloid ornamentation; however, *R. subbrevipipes* differs from *R. callainomarginis* by producing a broadly infundibuliform pileus, yellow ochre pileal surface, tomentose stipe, fruity odor, partial connected warts, larger basidiospores, pleurocystidia with a cap appendage and never generating light turquoise lamellae margin.

Our phylogeny showed that *R. brevipes* was close to *R. subbrevipipes* and *R. callainomarginis* (Figure 1). Morphologically, *R. brevipes* Peck resembles *R. subbrevipipes* and *R. callainomarginis* by producing broadly medium-sized basidiomata, short stipe, close to crowded lamellae, whitish spore-print and amyloid ornamentation; however, *R. brevipes* differs from *R. subbrevipipes* and *R. callainomarginis* in having a longer stipe (30–80 \times 9–40 mm) and warts (0.7–2 μm), larger basidiospores (8–11.3 \times 7.8–9.4 μm), subreticulate warts, indistinctive or mushroomy odor [37]. Moreover, *R. brevipes* has only been reported in coniferous forest to date and never generate a generating light turquoise lamellae margin and pleurocystidia with cap appendage [37–39].

Considering the broadly infundibuliform basidiomata, white spore-print, hard context and blue green zone on top of the stipe, *R. delica* and *R. chloroides* are similar to *R. callainomarginis*. However, *R. delica* differs from *R. callainomarginis* by the peppery or bitter taste, forking lamellae, longer basidiospores (8–11.5 \times 6.5–8.7 μm); *R. chloroides* is distinguishable by yellowish to reddish pileal surface, larger basidiospores (7–11 \times 6–8.7 μm), forking lamellae and peppery taste [6]. In addition, *R. delica* do not produce a light turquoise lamellae margin [6].

Russula leucocarpa, *R. byssina* and *R. cremicolor* fall into the *R.* subg. *Brevipes* and are also newly reported from China. However, these species were only found in coniferous forest,

which is the remarkable difference. Morphologically, *R. leucocarpa* differs from *R. subbrevipipes* by producing a smaller basidiocarp, forked lamellae and smaller basidiospores; *R. byssina* can be distinguished from *R. subbrevipipes* by its smaller basidiocarp, peelable margin, shorter stipe and larger basidiospores; *R. cremicolor* differs from *R. callainomarginis* in its small basidomata, thick context and larger basidiospores [15].

Russula laevis Kälviäinen, Ruotsalainen and Taipale also resemble *R. subbrevipipes* and *R. callainomarginis* by producing ochraceous cream to pale brownish pileus, short stipe, abundant lamellae, cream spore-print and amyloid ornamentation; However, the basidiospores are larger ((9.2–)9.5–10–10.5(–11.3) × (7.6–)8–8.5–8.9(–9.6) μm), longer hymenial cystidia on lamellae ((72–)79.5–86.8–94(–98) × (7–)7.5–8–8.5(–9.5)), acrid taste, pileocystidia often with one or two central knobs and never generating a light turquoise lamellae margin [3].

For the time being, taxonomy and phylogeny of the *Russula* species in China has been relatively well-studied. However, many specimens are still unidentified in China, and comprehensive phylogeny of *Russula* at the genus level is lacking. Further studies based on broader sampling and more data are needed to clarify the fungi diversity and species affinities.

Author Contributions: Conceptualization, J.S.; data curation, J.S.; methodology, J.S. and B.C.; software, J.S. and B.C.; validation, J.S. and Q.C.; formal analysis, J.S. and H.L.; investigation, J.S. and S.W.; resources, J.S.; writing—original draft preparation, J.S. and B.C.; writing—review and editing, J.Z. and G.Y.; visualization, B.C.; supervision, J.L.; project administration, J.S.; funding acquisition, J.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable for studies not involving humans.

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Conflicts of Interest: The authors declare no conflict of interest.

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