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Article

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A new genus, *Rubroboletus*, to accommodate *Boletus sinicus* and its allies

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Abstract

Rubroboletus is erected as a new genus to accommodate *Boletus sinicus* and its allies based on morphological and molecular evidence. Morphologically, *Rubroboletus* differs from the remaining genera in Boletaceae by the combination of a reddish pileal surface, an orange-red to blood red surface of the hymenophore, yellow tubes, pink to red reticula or spots on the yellow background of the stipe, a bluish color-change when injured, a non-amyloid context, smooth spores which are olive-brown in deposit, and an interwoven trichodermal pileipellis. Our phylogenetic analyses based on five gene markers (ITS, nrLSU, *tef1-α, rpb1* and *rpb2*) recognized eight species in the genus, including one new species and seven new combinations. A key to the eight species is provided.

Keywords: Boletes, New taxa, *Rubroboletus*, Phylogeny, Taxonomy

Introduction

The genus *Boletus* L. (1753: 1176) has been widely studied by mycologists from all over the world (Fries 1838; Murrill 1909; Singer 1947, 1986; Dick 1960; Hongo 1960; Smith & Thiers 1971; Corner 1972; Nilson & Persson 1977; Pegler & Young 1981; Zang 1983, 2006; Høiland 1987; Both 1993, 1998; Watling & Li 1999; Li & Song 2000; Binder & Bresinsky 2002; Horak 2005, 2011; Binder & Hibbett 2006; Ortiz-Santana *et al*. 2007; Drehmel *et al*. 2008; Dentinger *et al*. 2010) since it was erected. Singer (1986) divided it into seven sections mainly based on morphological characters such as the color of the hymenophore, the color-change of the context when exposed to air and the taste of the basidioma. *Boletus* sect. *Luridi* Fr. (1838: 417) sensu Singer (1986: 778), typified by *B. luridus* Schaeff. (1774: 107), is the largest section in *Boletus* s. l. and harbors more than 40 species. It is characterized by small and discolorous pores, a pileus either viscid or with coverings, a context often containing poisonous substances and sometimes a finely reticulated stipe (Singer 1986).

Molecular techniques have accelerated the developments of the fungal taxonomy (Taylor *et al*. 2000; Weiss 2010; Hibbett *et al*. 2011; Yang 2011). Combined with morphological characters and molecular evidence, a batch of new genera of boletes were erected recently (Halling *et al*. 2007, 2012a, b; Desjardin *et al*. 2008, 2009; Li *et al*. 2011, 2014; Zeng *et al*. 2012, 2014; Hosen *et al*. 2013; Arora & Frank 2014; Gelardi *et al*. 2014). With these techniques, it was found that sect. *Luridi* was not monophyletic (Marques *et al*. 2010; Vizzini 2014a; Wu *et al*. 2014) and species of this section were split into at least six lineages (Clades 37, 39, 40, 41, 44 and 46) in Wu *et al*. 2014. The genus *Suillellus* Murrill (1909: 16) (Clade 44) was reconfirmed to accommodate *B. luridus* and its allies. Additionally, *B. magnificus* W.F. Chiu (1948: 221) in Clade 37, *B. firmus* Frost (1874: 103) in Clade 39, *B. rufo-aureus* Massee (1909: 204) in Clade 41, and *B. floridanus* (Singer 1945: 799) Murrill (1948: 23) and *B. frostii* J.L. Russell (1874: 102) in Clade 46 were transferred to *Neoboletus* Vizzini (2014d: 1), *Caloboletus* Vizzini (2014b: 1), and *Crocinoboletus* N.K. Zeng *et al.* (2014: 134), and *Exsudoporus* Vizzini (2014c: 1), respectively. However, taxonomically, it was not well clarified yet for the species in the Clade 40, a statistically well supported clade (BS=100%, PP=1.0) consisting *Boletus sinicus* W.F. Chiu (1948: 220) and its allies.

This study is to compare the morphological features between this lineage and related taxa, and to erect a new genus to accommodate *B. sinicus* and its allies.

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Materials and Methods

Morphological studies

Macroscopic descriptions were made based on field notes and images of basidiomata. Color codes follow Kornerup & Wanscher (1981). For microscopic observation, dried materials were sectioned and mounted in 5% KOH solution. Sections of the pileipellis and the surface of the stipe were made halfway across the pileus radius and at the stipe midsection, respectively. Pileipellis, basidia, basidiospores, pleuro- and cheilocystidia, and stipitipellis were studied using an Axioskop 40 microscope following the standard method described in previous studies (Li *et al*. 2009, 2011; Zeng *et al*. 2012, 2013; Hosen *et al*. 2013). All microscopic features were drawn by hand. The notation "basidiospores (n/m/p)" indicates that the measurements were made on n basidiospores from m basidiomata of p collections. Dimensions of basidiospores are given using the notation (a)b–c(d), where the range b–c represents a minimum of 90% of the measured values, and extreme values (a and d), whenever present, are given in parentheses. Q refers to the length/breadth ratio of basidiospores; Q_m refers to the average Q of basidiospores ± sample standard deviation. Examined specimens are deposited in the herbaria of the National Botanical Garden of Belgium (BR), University of Tennessee (TENN) and Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). The descriptions of species appear in alphabetical order by species epithet. The generic name *Boletus* is abbreviated as "*B*.", *Butyriboletus* as "*Bu*.", *Caloboletus* as "*C*.", *Neoboletus as* "*N*." and *Suillellus* as "*S*.", while "*Rubroboletus*" as "*R*."

DNA extraction, PCR and sequencing

Total DNA was extracted from basidioma dried by silica gel or from herbarium specimens using the CTAB method (Doyle & Doyle 1987). ITS5/ITS4 and LROR/LR5 were used for the amplifications of ITS and nrLSU region, respectively (Vilgalys & Hester 1990; White *et al*.1990). Primers designed by Wu *et al*. 2014 and Zhao *et al*. 2014 were used to amplify *tef1-α*, *rpb1* and *rpb2.* PCR reactions were conducted on an ABI 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) or an Eppendorf Master Cycler (Eppendorf, Netheler-Hinz, Hamburg, Germany). The PCR programs were as follows: pre-denaturation at 94°C for 3 min, then followed by 35 cycles of denaturation at 94°C for 40s, annealing at 50°C (for ITS), 53 °C (for nrLSU), 55 °C (for *tef1-α*, *rpb1* and *rpb2*) for 50s, elongation at 72°C for 90s, a final elongation at 72°C for 8 min was included after the cycles. PCR products were purified with a Gel Extraction & PCR Purification Combo Kit (Spin-column) (Bioteke, Beijing, China) and then sequenced on an ABI-3730-XL sequence analyzer (Applied Biosystems, USA) using the same primers as in the original PCR amplifications.

Phylogenetic analyses

In this study, 264 sequences, including 17 newly generated in this study and 247 retrieved from the GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/index.php?e0true) databases, were used in the phylogenetic analyses. Two datasets were analyzed, one combining four genes (nrLSU, *tef1-α*, *rpb1* and *rpb2*) and the other containing only ITS sequences. DNA sequences of five loci were independently aligned with MAFFT v6.8 (Katoh *et al.* 2005) and manually optimized in BioEdit v7.0.9 (Hall 1999). The datasets were then analyzed using RAxML v7.2.6 (Stamatakis 2006) and MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) for Maximum Likelihood (ML) and Bayesian Inference (BI), respectively.

To assess incongruence among individual genes, single-gene analyses were conducted using the ML method to detect the topologies of the four genes (BS>70%, Nuhn *et al*. 2013). Due to no significant incongruence detected, the alignments of nrLSU, *tef1*-α, *rpb1* and *rpb2* were then concatenated using Phyutility (Smith & Dunn 2008). Unavailable sequences of the loci of a few species were treated as missing data in the phylogenetic analyses. The final concatenated alignments, deposited in TreeBASE (http://purl.org/phylo/treebase; submission ID 16552), were analyzed using RAxML v7.2.6 and MrBayes v3.1.2 for ML and BI methods, respectively.

For both BI and ML analyses of the dataset, the substitution model was determined using the Akaike Information Criterion (AIC) complemented in MrModeltest v2.3 (Nylander 2004). GTR+I+G was chosen as the best model for the dataset. For ML analysis, all parameters were kept default (Stamatakis 2006), and the supports were calculated using nonparametric bootstrapping with 1000 replicates. Bayesian analyses were conducted by setting generations to one million for the dataset and runs were terminated once the average standard deviation of split frequencies went below 0.01 (Ronquist & Huelsenbec 2003). Other parameters were kept at their default settings. The chain convergence was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure sufficiently large ESS values. Trees were summarized and posterior probabilities (PPs) were calculated after discarding the first 25% generations as burn-ins.

For the combined analyses on the phylogeny of Boletaceae, species are mainly chosen from Nuhn *et al*. 2013 and Wu *et al*. 2014. *Boletinellus merulioides* (Schwein. 1832: 160) Murrill (1909: 7), *Gyrodon lividus* (Bull. 1791: 327) Sacc. (1888: 52), *Paragyrodon sphaerosporus* (Peck 1885: 33) Singer (1942: 25), *Paxillus vernalis* Watling (1969: 60) and *Phlebopus portentosus* (Berk. & Broome 1874: 46) Boedijn (1951: 218) were selected as outgroups for the combined dataset. *Caloboletus calopus* (Pers. 1821: 390) Vizzini (2014b: 1) and *Boletus erythropus* Pers. (1796: 23), which are closely related to the sect. *Luridi* (Vizzini *et al*. 2014; Wu *et al*. 2014), were selected as outgroups for the ITS dataset. The scientific names, collection information and accession numbers for the sequences used in the analyses are presented in Tables 1 and 2.

TABLE 1. Specimens used in multigene phylogenetic study and their GenBank accession numbers

Taxon	Voucher ID	Location	**GenBank accession numbers**			
			nrLSU	***tef1-α***	***rpb1***	***rpb2***
Aureoboletus gentilis	MG372a	Brancciano, Lazio, Italy	KF112344	KF134014	KF112557	KF112741
Austroboletus fusisporus	HKAS 75207	China	JX889720	JX889718	JX889721	-
Boletellus projectellus	AFTOL-713	MA, U.S.A.	AY684158	AY879116	AY662660	AY787218
Boletinellus merulioides	AFTOL-ID 575	MA, U.S.A.	AY684153	DQ056287	DQ435803	DQ366281
Boletus aokii	HKAS 59812	Wannling, Hainan, China	KF112378	KF112266	KF112597	-
Boletus bicolor	MB 07-001	Chestnut Ridge Park, NY, USA	KF030370	KF030405	KF030370	-
Boletus carminipes	MB 06-061	Erie Co., NY, USA	JQ327001	JQ327022	KF030363	-
Boletus edulis	Be3	Bavaria, Germany	KF030282	GU187682	GU187444	-
Boletus pallidus	179/97	Bavaria, Germany	AF457409	KF030424	KF030396	-
Boletus pulverulentus	9606	West Newton, MA, USA	KF030313	KF030418	KF030364	-
Boletus sp.	HKAS 55440	Deqin, Yunnan, China	KF112315	KF112145	KF112499	KF112652
Boletus sp.	HKAS 59660	Baoshan, Yunnan, China	KF112358	KF112153	KF112503	KF112664
Boletus sp.	HKAS 76661	Nanyang, Henan, China	KF112342	KF112205	-	KF112801
Borofutus dhakanus	HKAS 73789	Gazipur, Bangladesh	JQ928616	JQ928576	JQ928586	JQ928597
Bothia castanella	MB 03-053	Massachussetts	DQ867117	KF030421	KF030382	-
Buchwaldoboletus lignicola	Pul1	Maindreieck, Germany	JQ326997	JQ327040	-	-
Butyriboletus appendiculatus	Bap1	Bavaria, Germany	AF456837	JQ327025	KF030359	-
Caloboletus calopus	BR50201590638-05	Montenau, Belgium	KJ184554	KJ184566	KJ184560	**KP055030**
Caloboletus firmus	MB 06-060	Chestnut Ridge Park, NY, USA	KF030368	KF030408	KF030368	-
Chalciporus piperatus	MB04-001	Massachusetts, USA	DQ534648	GU187690	GU187453	-
Crocinoboletus laetissimus	HKAS 59701	Chuxiong, Yunnan, China	KF112436	-	-	KF112711
Crocinoboletus rufo-aureus	HKAS 53424	Chenzhou, Hunan, China	KF112435	KF112206	KF112533	KF112710
Crocinoboletus rufo-aureus	HKAS 59820	Baisha, Hainan, China	KF112434	-	KF112532	KF112709
Exsudoporus frostii	SAT1221511	Tennessee, USA	**KP055021**	**KP055018**	**KP055024**	**KP055027**
Exsudoporus frostii	NY815462	Dota, San José, Costa Rica	JQ924342	KF112164	-	KF112675
Fistulinella prunicolor	REH9502	Fraser Island, Qld, Australia	JX889648	JX889690	-	-
Gymnogaster boletoides	REH9455	SE Qld, Australia	JX889673	JX889683	-	-
Gyrodon lividus	REG Gl1	Bavaria, Germany	AF098378	GU187701	GU187461	GU187786
Harrya chromapes	ND4	North Carolina, USA	JX889664	JX889704	-	-
Heimioporus japonicus	HKAS 52237	Chuxiong, Yunnan, China	KF112347	KF112228	KF112618	KF112806
Hemileccinum impolitum	Bim1	Bavaria, Germany	AF139715	JQ327034	-	-
Leccinellum corsicum	Buf 4507	-	KF030347	KF030435	KF030389	-
Leccinum extremiorientale	HKAS 63635	Chuxiong, Yunnan, China	KF112403	KF112198	KF112535	KF112720
Leccinum variicolor	HKAS 57758	Lijiang, Yunnan, China	KF112445	KF112251	KF112591	KF112725
Mucilopilus castaneiceps	HKAS 75045	Nujiang, Yunnan, China	KF112382	KF112211	-	KF112735
Neoboletus erythropus	Ber1	-	AF139683	-	-	-

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TABLE 1. (Continued)

Taxon	Voucher ID	Location	GenBank accession numbers			
			nrLSU	***tef1-α***	***rpb1***	***rpb2***
Neoboletus luridiformis	AT2001087	Berkshire, England, UK	JQ326995	JQ327023	-	-
Neoboletus magnificus	HKAS 54096	Kunming, Yunnan, China	KF112324	KF112149	KF112495	KF112654
Neoboletus magnifrcus	HKAS 74939	Baoshan, Yunnan, China	KF112320	KF112148	KF112494	KF112653
Octaviania tasmanica	OSC132097	Tasmania, Australia	JN378494	JN378435	-	-
Paragyrodon sphaerosporus	MB06-066	Iowa, USA	GU187593	GU187737	-	GU187803
Paxillus vernalis	AFTOL-ID 715	China	AY645059	DQ457629	-	-
Phlebopus portentosus	Ph1	Africa	AF336260	FJ536679	FJ536646	FJ536606
Phylloporus pelletieri	Pp1	Bavaria, Germany	AF456818	JQ327036	KF030390	-
Porphyrellus brunneus	REH9508	Fraser Island, Qld, Australia	JX889646	JX889688	-	-
Porphyrellus holophaeus	HKAS 50508	Ninger, Yunnan, China	KF112465	KF112244	KF112553	-
Porphyrellus porphyrosporus	AFTOL-1779	Walhalla, Bavaria, Germany	DQ534643	GU187734	GU187475	GU187800
Pseudoboletus parasiticus	Xpa1	Bavaria, Germany	AF050646	KF030443	KF030394	-
Pulveroboletus aff. *ravenelii*	HKAS53351	Sanming, Fujian, China	KF112406	KF112261	KF112542	KF112712
Retiboletus griseus	HKAS63590	Dali, Yunnan, China	KF112417	KF112178	KF112537	KF112691
Royoungia boletoides	REH8774	Atherton, Qld, Australia	JX889660	JX889701	-	-
Rubroboletus dupainii	JAM 0607	Butner, NY, USA	KF030413	KF030413	KF030361	-
Rubroboletus latisporus	HKAS 63517	Qujing, Yuannan, China	**KP055022**	**KP055019**	**KP055025**	**KP055028**
Rubroboletus latisporus	HKAS 80358 (holotype)	Chongqing, China	**KP055023**	**KP055020**	**KP055026**	**KP055029**
Rubroboletus rhodosanguineus	4252	Chestnut Ridge Park, NY, USA	KF030252	KF030412	-	-
Rubroboletus satanas	MBinder-BS2	-	AF336242	-	-	AY218473
Rubroboletus sinicus	HKAS 56304	Deqin, Yunnan, China	KJ605673	KJ619483	KJ619482	**KP055031**
Rubroboletus sinicus	HKAS 68620	Nujiang, Yunnan, China	KF112319	KF112146	KF112504	KF112661
Solioccasus polychromus	REH9417	Australia	JQ287642	JQ287644	-	-
Spongiforma thailandica	DED 7873	Thailand	EU685108	KF030436	KF030387	-
Strobilomyces floccopus	AFTOL-716	MA, USA	AY684155	AY883428	-	AY786065
Suillellus aff. *amygdalinus*	HKAS57262	Qamdo, Tibet, China	KF112316	KF112174	KF112501	KF112660
Suillellus amygdalinus	112605ba	Mendocino Co., CA, USA	JQ326996	JQ327024	KF030360	-
Suillellus luridus	Bl2	-	AF139686	-	-	-
Sutorius eximius	9400	Ulster County, NY	JQ327004	JQ327029	-	-
Tylopilus felleus	HKAS 54926	Marburg, Germany	KF112411	HQ326866	KF112575	KF112737
Tylopilus sp.	HKAS 46334	Deqin, Yunnan, China	KF112471	KF112271	KF112581	KF112795
Tylopilus sp.	HKAS 50229	Xishuangbanna, Yunnan, China	KF112423	KF112216	KF112574	KF112769
Tylopilus sp.	HKAS 50281	Xishuangbanna, Yunnan, China	KF112451	KF112284	-	KF112730
Tylopilus virens	HKAS 76678	Liangshan, Sichuan, China	KF112438	KF112272	KF112582	KF112793
Veloporphyrellus aff. *subalpinus*	HKAS 57490	Lijiang, Yunnan, China	KF112380	KF112209	KF112555	KF112733
Xanthoconium stramineum	3518	Lake Mize, Gainsville, FL, USA	KF030353	KF030428	KF030386	-
Xerocomellus aff. *rubellus*	MB 03-033	Worcester, MA, USA	KF030294	KF030419	KF030371	-
Xerocomellus chrysenteron	Xch1	Bavaria, Germany	AF050647	KF030415	KF030365	-
Xerocomus aff. *macrobii*	HKAS 56280	Chuxiong, Yunnan, China	KF112418	KF112265	KF112541	KF112708
Xerocomus badius	MB 03-098a	Rutland, MA, USA	KF030355	KF030423	KF030393	-
Xerocomus subtomentosus	Xs1	Bavaria, Germany	AF139716	JQ327035	KF030391	-
Zangia roseola	HKAS 51137	Kunming, Yunnan, China	HQ326949	HQ326877	-	-

Accessions numbers in boldface indicate newly generated sequences.

TABLE 2. Specimens used in the ITS phylogenetic study and their accession numbers

Species	Voucher/Isolate/strain	Location	ITS Accession No.	References
Boletus amygdalinus	SOC1147	Southern Oregon, USA	FJ235148	Frank unpublished
Boletus erythropus	UF269	Portugal	HM347665	Marques *et al.* unpublished
Butyriboletus appendiculatus	BR 50200892955-50	Zoniënwoud, Belgium	KJ605668	Zhao *et al.* 2014
Caloboletus calopus	BR 50201590638-05	Montenau, Belgium	KJ605655	Zhao *et al.* 2014
Rubroboletus latisporus	HKAS 80358 (Holotype)	Chongqing, China	**KJ951990**	This study
Rubroboletus latisporus	HKAS 63517	Qujing, Yunnan, China	**KJ951989**	This study
Rubroboletus pulchrotinctus	GS0339	Capadno, RE, Italy	UDB000405	unpublished
Rubroboletus pulchrotinctus	GS0364	Capadno, RE, Italy	UDB000406	unpublished
Rubroboletus pulchrotinctus	GS 0860	Traversetolo, PR, Italy	UDB000407	unpublished
Rubroboletus rhodoxanthus	AT 2000182	Sardinia, Italy	UDB001116	unpublished
Rubroboletus rhodoxanthus	EDM13	Italy	EU444539	Di Marino *et al*. unpublished
Rubroboletus rhodoxanthus	MA-Fungi 47703	Portugal	AJ419189	Martin & Raidl 2002
Rubroboletus rubrosanguineus	GS1917	Valle di Calone, Italy	UDB000409	unpublished
Rubroboletus rubrosanguineus	GS1918	Valle di Calone, Italy	UDB000411	unpublished
Rubroboletus rubrosanguineus	GS 1971	Provincia di trieste, Italy	UDB000412	unpublished
Rubroboletus satanas	Bs 2	Zellingen, Germany	DQ534567	Binder & Hibbett 2006
Rubroboletus satanas	UF 1032	France	HM347649	Marques *et al.* unpublished
Rubroboletus satanas	Bsat-X-10	Zijevo Massif, Montenegro	JQ685717	Lazarevic *et al.* unpublished
Rubroboletus sinicus	HKAS 68620	Lanping, Yunnan, China	**KJ951991**	This study
Rubroboletus sinicus	HKAS 56304	Deqin, Yunnan, China	KJ605666	Zhao *et al.* 2014

Results

Molecular phylogenetic data

In this study, 17 sequences were newly generated, including three sequences of ITS, three sequences of nrLSU, three sequences of *tef1-α*, three sequences of *rpb1* and five sequences of *rpb2*. As no well-supported conflict (BS>70%, Nuhn *et al*. 2013) was detected among the topologies of the four genes, their sequences were then concatenated together for further multi-gene analyses. The combined dataset included 73 species and the alignment contained 3188 nucleotide sites (including gaps), consisted of 954, 652, 886 and 710 sites (including gaps) for nrLSU, *tef1-α, rpb1* and *rpb2*, respectively. Phylogenetic tree generated from the combined dataset showed that members of *Rubroboletus* formed an independent clade in the family Boletaceae. This clade is sister to *Caloboletus*, but with low support values (BS = 19%; PP = 0.52).

The ITS dataset included 20 sequences generated from 10 species and the alignment contained 610 nucleotide sites (including gaps). In this alignment, 418 characters were constant, while 192 characters were variable, of which 76 characters were parsimony informative. Sequences generated from 16 collections of *Rubroboletus* formed an independent clade with high support value (BS = 85%, PP = 1.0). This clade was further grouped into two subclades, one including *R. pulchrotinctus*, *R. rubrosanguineus, R. satanas* and *R. sinicus,* and the other including *R. latisporus* and *R. rhodoxanthus*.

Phylogenetic trees generated from ML and BI analyses were nearly identical with minimal variation in statistical support values, and, thus, only the trees inferred from the ML analysis are shown (Figs. 1 and 2).

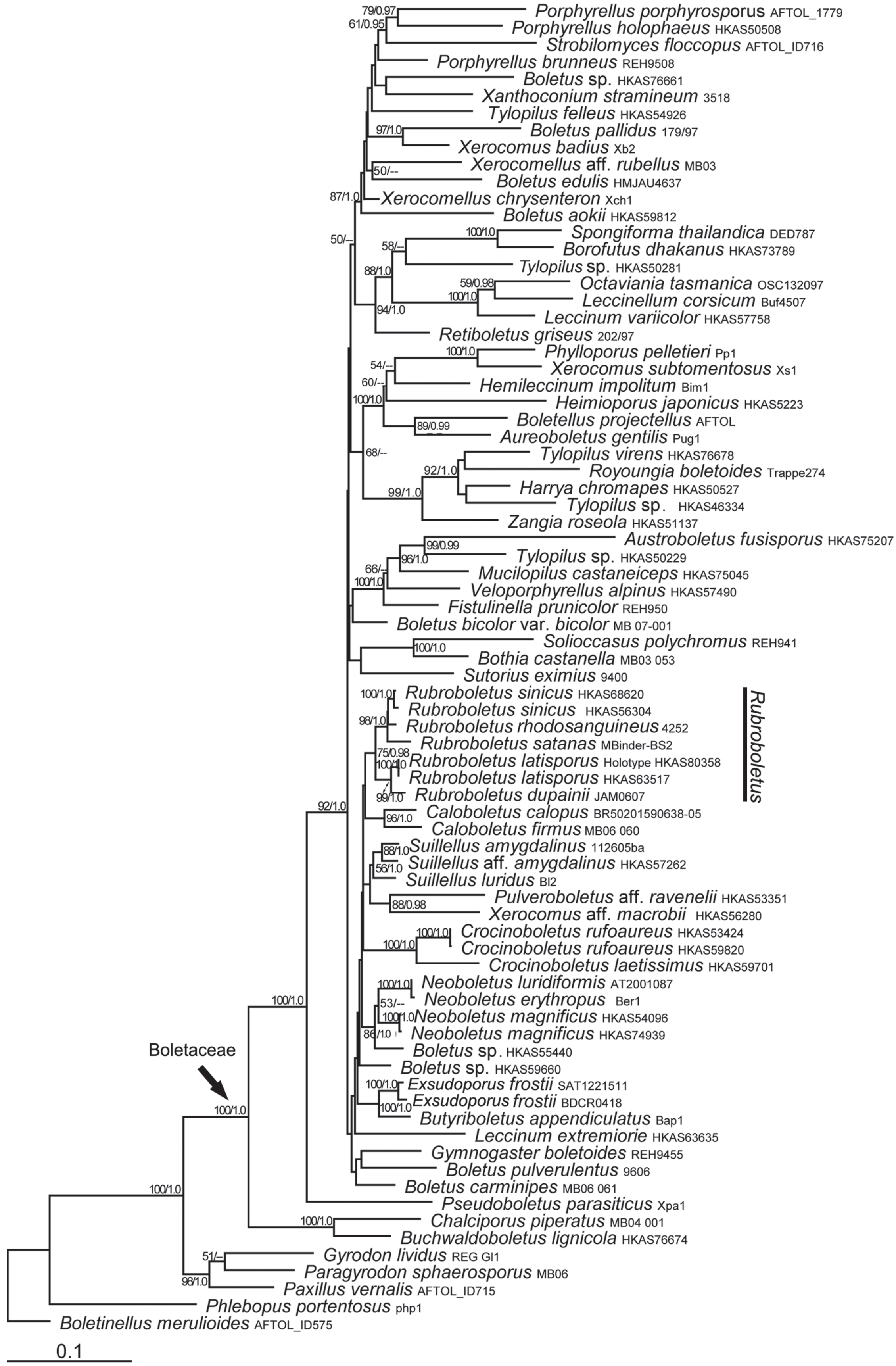

FIGURE 1. Maximum-Likelihood phylogenetic tree generated from the combined dataset (nrLSU, *tef1-α, rpb1* and *rpb2*). BS support values >50% for ML and PPs >0.95 for BI are indicated along branches (BS/PP).

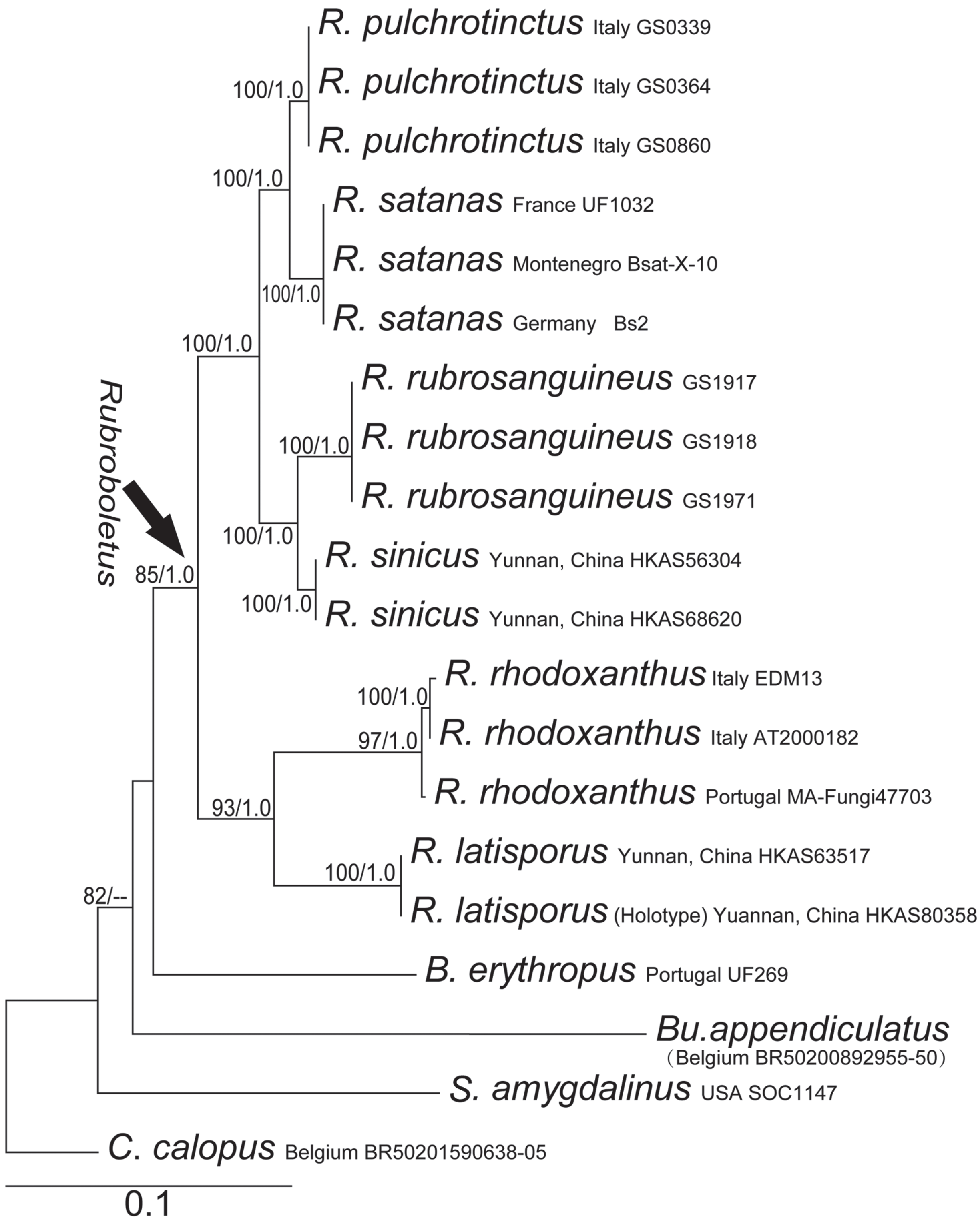

FIGURE 2. Maximum-Likelihood phylogenetic tree generated from ITS sequences. BS support values >50% for ML and PPs >0.95 for BI are indicated along branches (BS/PP).

Taxonomy

Rubroboletus Kuan Zhao et Zhu L.Yang, **gen. nov.**
Mycobank: MB 809235

Etymology: "*Rubro-"* refers to the red color of the pileus, the surface of the hymenophore and the reticulum (or spots) on the stipe.
Generic Type: *Rubroboletus sinicus* (W.F. Chiu) Kuan Zhao et Zhu L. Yang

Basidioma stipitate-pileate with tubular hymenophore. *Pileus* hemispherical, convex or applanate, grayish, pinkish to red; context white, yellowish to lemon-yellow, bluing quickly when exposed. *Hymenophore* surface orange red to blood red, sometimes orange-yellow when mature, rapidly bluing when bruised; tubes yellow to olivaceous green, turning blue promptly when injured, then back to the original color slowly. *Stipe* central, covered with pinkish, red to brownish red reticula or spots. *Pileipellis* an interwoven trichoderm composed of more or less vertically arranged, sometimes gelatinized filamentous hyphae. *Hymenophoral trama* boletoid. *Basidiospores* smooth, subfusiform to ovoid-ellipsoid, slightly thick-walled. *Pleuro-* and *cheilocystidia* lageniform, thin-walled. *Clamp connections* absent. *Amyloid reaction* not observed.

Rubroboletus latisporus Kuan Zhao et Zhu L. Yang, **sp. nov.** (Figs. 3–4)
MycoBank: MB 809242

Etymology: *latisporus* refers to the broad spores.
Holotype:—CHINA. Chongqing Municipality: Wu County, on the ground of a mixed forest dominated by *Pinus massoniana*, 950 m, 5 Jul 2013, *Lihong Han* 128 (HKAS 80358, holotype!).

Pileus 7–10 cm in diameter, hemispherical to convex; surface blood red (10D7–8), strongly viscid when wet and shiny when dry, becoming dark blue when bruised; *context* 1–1.5 cm thick, whitish to white (1A1) to cream-colored (4A2), becoming blue promptly when injured, then back to the original color slowly. *Hymenophore* depressed around stipe, surface orange-red (5B6–8) to yellow (2A6–8) when mature, rapidly bluing when bruised; *pores* angular, 2–3/mm; *tubes* up to 1 cm in depth, yellow (3B7–8) to olivaceous green (30C5–7), becoming blue very quickly when injured, then back to the original color slowly. *Stipe* 8–10 × 2–2.5 cm, sub-cylindrical, robust, tapering upwards, background yellow (4A7–8), reticulum confined to the upper part and concolorous with the stipe; spots dark red (11D7–8) to brown-red (12E6–8), irregularly distributed over nearly the entire stipe; mycelia at the base of the stipe white; context yellowish (3A2–3), turning blue quickly when injured, then back to the original color slowly. *Odor* and *taste* indistinct.

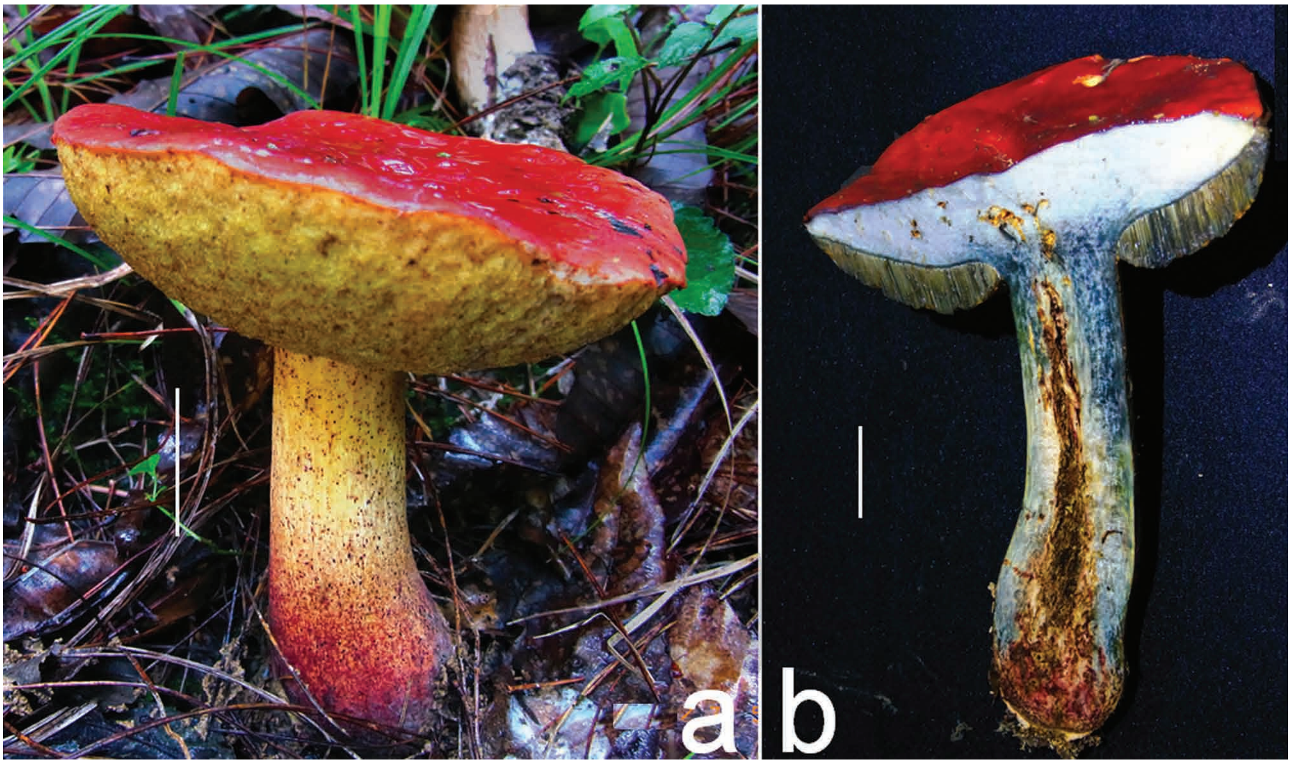

FIGURE. 3. Basidiomata of *Rubroboletus latisporus* (holotype). **a.** Mature basidioma. **b**. Bluish color change after injury (image taken immediately after sectioning). Bars: a–b=2 cm.

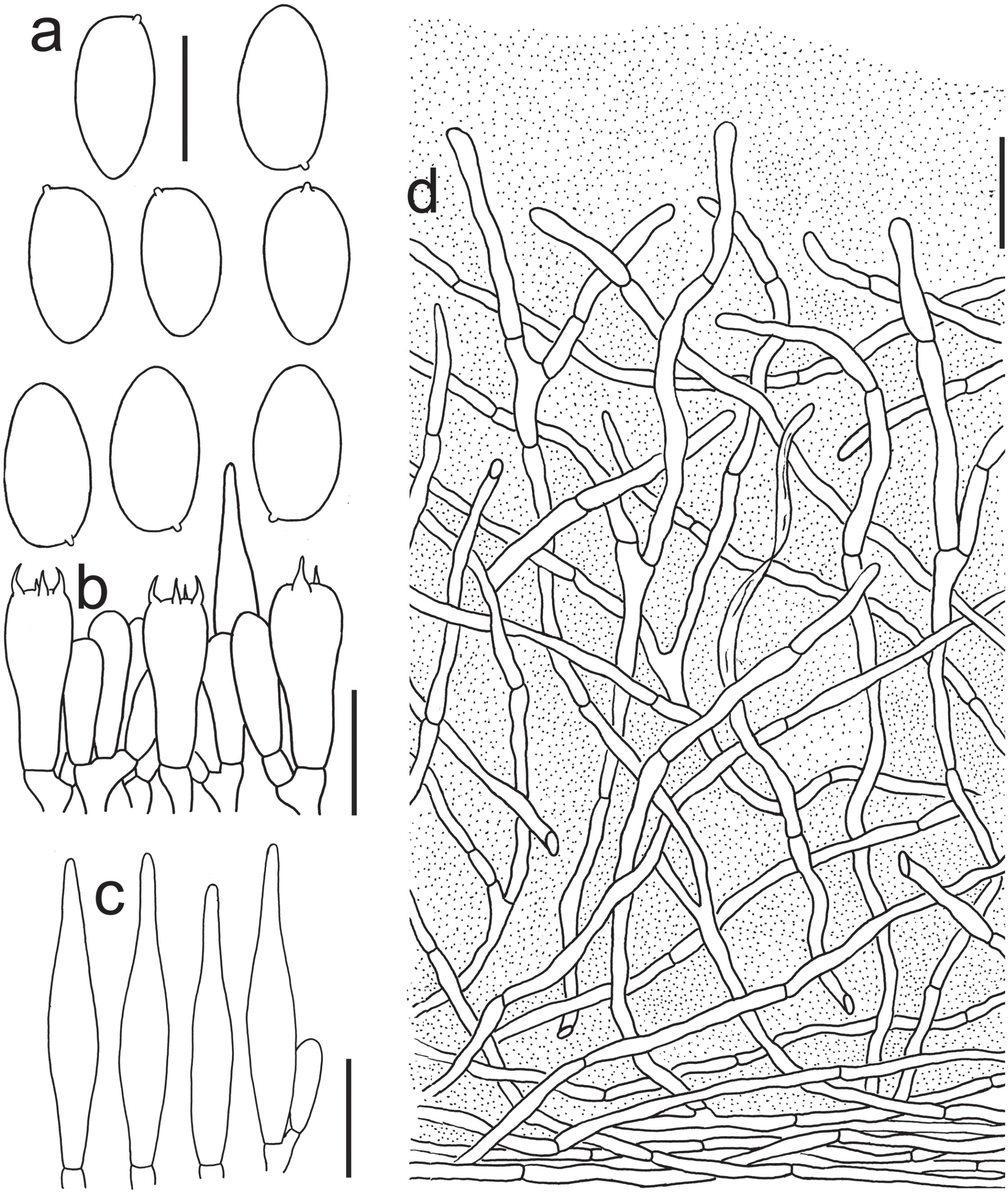

FIGURE. 4. Microscopic features of *Rubroboletus latisporus* (holotype). **a**. Basidiospores. **b**. Basidia and pleurocystidium. **c**. Cheilocystidia. **d**. Pileipellis in a gelatinized matrix. Bars: a=10 µm; b–d=20 µm.

Basidiospores [80/2/2] (9.5) 11–13 (14) × 6–6.5 (7) µm, [Q = (1.83) 1.91–2.15 (2.17), Q_m =2.02 ± 0.06], ovoid-ellipsoid, nearly colorless in KOH and yellowish brown in Melzer's reagent. *Basidia* 24–39 × 8–12 µm, clavate, 4-spored, sometimes 2-spored. *Cheilocystidia* 39–62 × 6–10 µm, narrowly lageniform to lageniform, thin-walled, colorless in KOH. *Pleurocystidia* similar with cheilocystidia in shape and size. *Pileipellis* an interwoven trichoderm composed of more or less vertically arranged thin-walled, filamentous hyphae 3.5–5 µm in diameter, embedded in a gelatinized matrix. *Stipe trama* composed of vertically arranged hyphae. *Clamp connections* absent in all tissues. *Amyloid reaction* none.

Habitat and distribution: Solitary or in groups under *Pinus massoniana* or in mixed forests of *Pinus* spp. and *Quercus* spp. Currently only known from southwestern China.

Paratype: CHINA, Yunnan Province: Shilin County, Gui Mountain, in a mixed forest dominated by *Pinus* and *Quercus*, alt. 2200 m, 8 Aug 2010, *Gang Wu* 286 (HKAS 63517).

Notes: *Rubroboletus latisporus* is characterized by its strongly viscid pileus when wet, brown-red spots on the stipe and broad spores. This species is closely related and similar to *R*. *dupainii*, originally described from France, as they share the vivid red and gelatinized pileus. However, the surface of the hymenophore of *R*. *latisporus* is orange-red to yellow when mature, while that of *R*. *dupainii* is blood red to dark red. Furthermore, the context of the former is whitish to cream-colored, while that of the latter is pale yellow (3A3).

New combinations in Rubroboletus

Based on molecular phylogeny and morphological evidence (see discussion below), the following new combinations were made.

Rubroboletus dupainii (Boud.) Kuan Zhao et Zhu L. Yang, **comb. nov.**
Mycobank: MB 809237

Basionym: *Boletus dupainii* Boud., Bulletin de la Société Mycologique de France 18: 139, 1902.

Rubroboletus pulchrotinctus (Alessio) Kuan Zhao et Zhu L. Yang, **comb. nov.**
Mycobank: MB 809238

Basionym: *Boletus pulchrotinctus* Alessio, *Boletus* Dill. ex L. 1: 231, 1985.

Rubroboletus rhodosanguineus (Both) Kuan Zhao et Zhu L. Yang, **comb. nov.**
Mycobank: MB 809239

Basionym: *Boletus rhodosanguineus* Both, Bulletin of the Buffalo Society of Natural Sciences 36: 219, 1998.

Rubroboletus rhodoxanthus (Krombh.) Kuan Zhao et Zhu L. Yang, **comb. nov.**
Mycobank: MB 809243

Basionym: *Boletus sanguineus* var. *rhodoxanthus* Krombh., Naturgetreue Abbildungen und Beschreibungen der Schwämme 5: 12, 1836.

Rubroboletus rubrosanguineus (Cheype) Kuan Zhao et Zhu L. Yang, **comb. nov.**
Mycobank: MB 809240

Basionym: *Boletus rubrosanguineus* Cheype, Documents Mycologiques 13(52): 53, 1983.

Rubroboletus satanas (Lenz) Kuan Zhao et Zhu L. Yang, **comb. nov.**
Mycobank: MB 809241

Basionym: *Boletus satanas* Lenz, Schwämme Mitteldeutschl.: 67, 1831.

Rubroboletus sinicus (W.F. Chiu) Kuan Zhao et Zhu L. Yang, **comb. nov.** (Figs. 5–6)
Mycobank: MB 809236

Basionym: *Boletus sinicus* W.F. Chiu, Mycologia 40: 220, 1948
Materials examined: Yunnan Province, Deqin County, Haba Snow Mountain, alt. 3600 m, 13 Aug 2008, Y. C. Li 1464 (HKAS 56304);

Kunming City, Ciba Town, in a wild mushroom market, G. Wu 255 (HKAS 63486); Lanping County, Tongdian Town, 15 Aug 2010, B. Feng 839 (HKAS 68620).

Notes: *Rubroboletus sinicus*, the type of *Rubroboletus*, is characterized by its rose-red to dark red pileal surface covered with brownish scales, ovoid-ellipsoid spores with an inconspicuous suprahilar depression and a bluish color change when bruised (Chiu 1948, 1957).

FIGURE. 5. Basidiomata of *Rubroboletus sinicus* (a, c from HKAS 68620; b from HKAS 63486).**a**. Mature basidioma. **b.** Blood red surface of the hymenophore. **c**. Bluish color change after injury (image taken immediately after sectioning). Bars: a–c=1 cm.

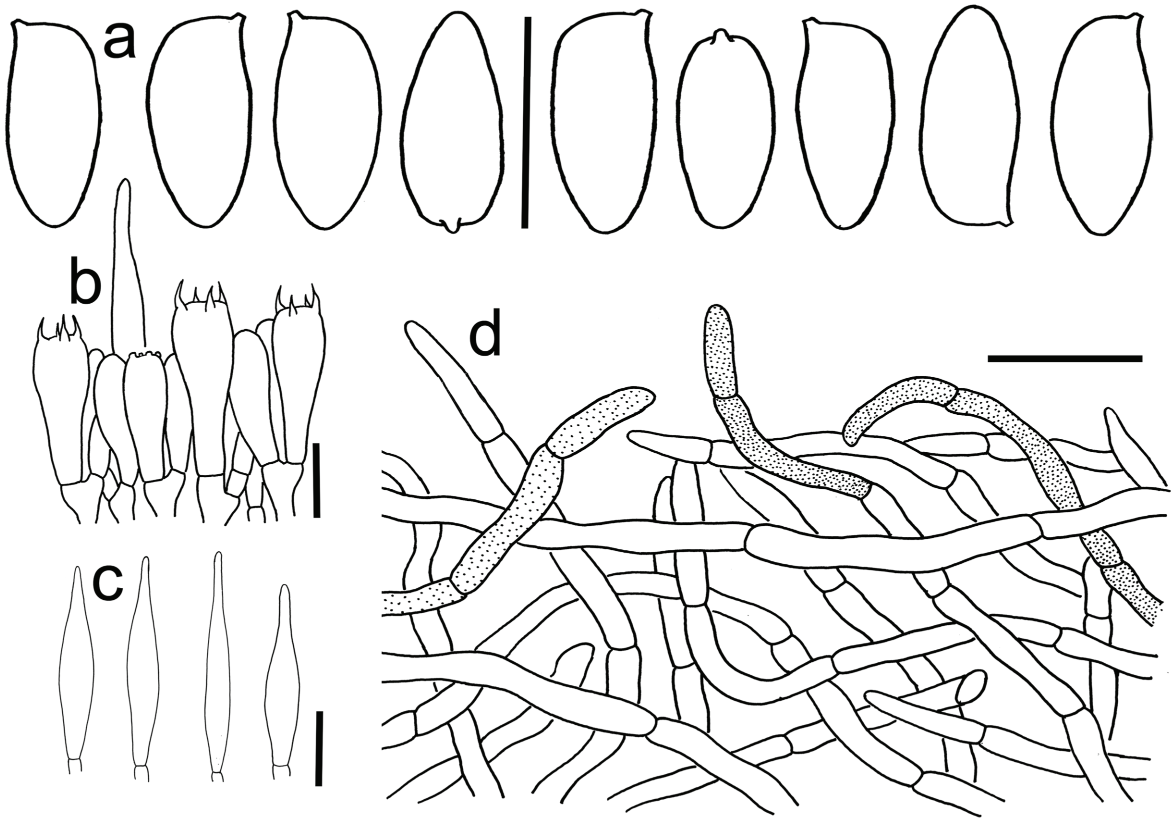

FIGURE. 6. Microscopic features of *Rubroboletus sinicus* (HKAS 63486). **a**. Basidiospores. **b**. Basidia and pleurocystidium. **c**. Cheilocystidia. **d**. Pileipellis. Bars: a=10 µm; b–d=20 µm.

Key to the species of Rubroboletus

1	Pileus shiny when dry and strongly viscid when wet; pileal surface turning dark blue when bruised	2
1	Pileus not shiny when dry, only slightly viscid when wet; pileal surface turning dark red or unchanging when bruised	3
2	Pileal surface scarlet to tinged ochraceous when mature; surface of the hymenophore blood red to dark red when mature; context pale yellow; in Europe, also reported from North and Central America	*R. dupainii*
2	Pileal surface blood red to dark red when mature; surface of the hymenophore orange red when mature; context whitish to cream-colored; in East Asia	*R. latisporus*
3	Pileal surface glabrous; spores subfusiform, with a conspicuous suprahilar depression; in North and Central America and Europe	4
3	Pileal surface felty-tomentose; spores ovoid-ellipsoid, with an inconspicuous suprahilar depression; in East Asia	*R. sinicus*
4	Context yellowish to pale yellow when mature; stipe club-shaped or sub-cylindrical	5
4	Context whitish to white when mature; stipe usually strongly bulbously swollen at the base	*R. satanas*
5	Both the context of the cap and the stipe becoming blue when injured	6
5	Context of the cap becoming blue but that of the stipe unchanging when injured	*R. rhodoxanthus*
6	Pileal surface unchanging when bruised; odor of hey or not distinct; taste slightly acid	7
6	Pileal surface turning dark red when bruised; odor of overripe fruit; taste sweet	*R. rhodosanguineus*
7	Stipe covered with red to dark red reticula; odor of hey; in coniferous forests, such as *Picea* spp. and *Abies* spp.	*R. rubrosanguineus*
7	Stipe covered with pink reticula; odor not distinct; in broad-leaved forests, such as *Quercus* spp.	*R. pulchrotinctus*

Discussion

Boletus sect. *Luridi* is the largest section in *Boletus* s. l. (Singer 1986: 778) and was proved to be not monophyletic. Members of this section were scattered in several independent clades (Vizzini 2014a; Wu *et al*. 2014). Our *Rubroboletus* corresponds to the "sect. *Luridi* 4" in Vizzini *et al*. (2014) and the "clade 40" of *Pulveroboletus* Group in Wu *et al*. (2014). In the last few years, some species of sect. *Luridi* have already been transferred to the following five genera, namely *Caloboletus*, *Crocinoboletus, Exsudoporus*, *Neoboletus* and *Suillellus*. However, they can be distinguished from *Rubroboletus* by their morphological characters.

Rubroboletus shares some characters with *Suillellus*, like the blood red surface of the hymenophore and the blusish color change. However, *Suillellus* is different from *Rubroboletus* by its yellowish brown to dark brown pileus, yellow to brown reticula on the stipe and strongly amyloid hyphae of the context (Muñoz 2005; Klofac 2007; Knudsen & Taylor 2012), while members of *Rubroboletus* have a grayish red to vivid red or dark red pileus, rose to red reticula and non-amyloid hyphae.

Caloboletus is somewhat related to *Rubroboletus* in our analyses (Fig. 1). Although both genera have a reticulated stipe and a bluish color change, *Caloboletus* is distinct by its unique bitter taste and yellow surface of the hymenophore (with an exception: orange red in *C. firmus*) (Zhao *et al*. 2014).

Crocinoboletus can be distinguished from *Rubroboletus* by its brilliant orange color of basidiomata contributed by the unusual boletocrocin polyene pigments and bluish olivaceous staining overall the entire basidiomata when bruised (Zeng *et al.* 2014).

Exsudoporus also has a reddish pileus, a blood red to orange red surface of the hymenophore and a bluish color change. However, it can be recognized by its conspicuous, strongly raised reticula on the stipe and unique pores forming exudate droplets when young (Vizzini 2014c).

A few species of *Neoboletus*, such as *N. magnificus* and *N. luridiformis* Rostk. (1844: 105) (=*B. erythropus* sensu Auct.) look like members of *Rubroboletus*. However, *N. magnificus* has a rarely reticulated stipe which always covered with red-dotted elements or streaked with red fibrils (Chiu 1948, 1957); *N. luridiformis* has a brown to dark brown pileus and a club-shaped stipe covered with dense orange-red floccules (Lannoy & Estades 2001; Vizzini *et al.* 2014a).

In this study, eight species were recognized in *Rubroboletus*. Our phylogenetic analysis based on the combined dataset covered five species of *Rubroboletus* (Fig. 1). The remaining three species, namely *R. pulchrotinctus*, *R. rhodoxanthus* and *R. rubrosanguineus*, were recognized by the phylogeny analyses based on ITS dataset as they clustered into the same clade with *R. sinicus* and its allies (Fig. 2). Morphologically, they also have yellow tubes, an orange red to blood red surface of the hymenophore, dark red to brown spots on the stem and a non-amyloid context (Singer & Kuthan 1976; Alessio 1985; Estadès & Lannoy 2004; Muñoz 2005), which are well consistency with the characters of *Rubroboletus*. Thus, these three species were transferred to this genus.

Some species, such as *B. fagicola* A.H. Sm. & Thiers (1971: 338), *B. fragrans* Vittad. (1835: 153), *B. impolitus* Fr. (1838: 421), *B. lupinus* Fr. (1838: 418), *B. rhodopurpureus* Smotl (1952: 31), *B. rubricitrinus* Murrill (1940: 66) and *B. sullivantii* Berk. & Mont. (1856: 152) were placed in sect. *Luridi* (Singer 1986). However, their morphological characters, like the color of the pileus and the surface of the hymenophore, and the color change of the context, are not in accordance with *Rubroboletus.* Their systematic positions can only be settled in the near future.

Several species of *Boletus* sect. *Luridi*, such as *R. satanas* and *R. rhodoxanthus*, were reported as poisonous (Ammirati *et al.* 1985; Ellis & Ellis 1990; Kretz *et al.* 1991; Rumack & Spoerke 1994; Benjamin & Denis 1995; Ennamany *et al*. 1998; Flammer 2008). Although *R. sinicus* was sporadically sold in wild mushroom markets in Yunnan, China (Chiu 1948, 1957; Wang *et al*. 2004), it has long been suspected that this taxon is probably poisonous and its edibility needs further chemical studies.

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