# Russula dinghuensis sp. nov. and R. subpallidirosea sp. nov., two new species from southern China supported by morphological and molecular evidence

Jianbin ZHANG, Jingwei LI, Fang Li & Lihong QIU\*

State Key Lab of Biocontrol, School of Life Science, Sun Yat-sen University, Guangzhou 510275, China

Abstract – Two new taxa of *Russula* from the Dinghu Mountain, Guangdong Province, China were described and illustrated based on both morphological data and phylogenetic analysis of the internal transcribed spacer sequences. *Russula dinghuensis* is characterized by the olive green pileus, acute and incurved margin, white and rarely forked lamellae, white spore print, globose to ellipsoid basidiospores with stocky and isolated warts, thick metachromatic pileipellis, and slender, furcated and septated terminal elements of pileipellis. *Russula subpallidirosea* is recognized by the pale pink to pale grayish-pink pileus, white and forked lamellae, white spore print, subglobose to ellipsoid basidiospores with the isolated, subcylindrical to conical warts, the metachromatic pileipellis, and the short, furcated and septate terminal elements of pileipellis. Both molecular and morphological analyses consistently confirm that these two new taxa are placed into *Russula* subg. *Heterophyllidia* subsection *Cyanoxanthinae*. The morphological differences among these two novel species and the closely related taxa are discussed.

Cyanoxanthinae / Dinghu Mountain / ITS / phylogeny / taxonomy

#### INTRODUCTION

Russula Pers. (Russulaceae, Basidiomycota), erected by Persoon in 1796, is considered one of the most abundant and widely distributed ectomycorrhizal agaric genera in the world (Buyck et al. 2008, 2015; Buyck & Horak 1999). Species of Russula are recognized by the combination of the following characteristics: mostly conspicuous fruit bodies with colorful pileus, amyloid spore ornamentation, presence of gloeocystidia, brittle context with abundant sphaerocytes, absence of latex, and hyphae lacking clamp connections (Romagnesi 1967; Sarnari 1998; Singer 1986). The genus Russula consists of more than 750 species and ca. 160 of these have been reported from China (Kirk et al. 2008; Song et al. 2007). Compared with the long and rich taxonomic history of Russula in Europe (Romagnesi 1967; Sarnari 1998), new Russula taxa from Asian countries are rarely reported. Up to now, only 22 species and 3 varieties were originally described from China (Bi & Li 1986; Chiu 1945; Li et al. 2012; Li et al. 2011; G.J. Li et al. 2015; Li et al. 2013; Li et al. 2013; Y.K. Li et al. 2015; Singer 1935; Song et al. 2007; Wang et al. 2009; Wen &

<sup>\*</sup> Corresponding author: qiulh@mail.sysu.edu.cn

Ying 2001; Ying 1983; 1989; Zang & Yuan 1999; Zhao *et al.* 2015). Because of the lack of systematic studies on *Russula* in China, it is believed that many Chinese *Russula* species are misplaced under European or American species names, which probably led to underestimates of the real diversity of *Russula* in China (Li *et al.* 2012; Li *et al.* 2011).

The Dinghu Mountain, located in Zhaoqing city, China, is considered a region highly diverse in macrofungi (Bi et al. 1994; Zheng et al. 1985). More than 51 species of Russula have been recorded from this subtropical region. During recent surveys of Russula in the area, several specimens representing two Russula taxa were collected. Morphological study together with molecular analyses (ITS) showed they represent two novel species of Russula subg. Heterophyllidia (Romagnesi) Sarnari 1998.

## MATERIALS AND METHODS

## Sampling and morphological studies

Random collection was conducted in September 2013 and May 2015 in Dinghushan Biosphere Reserve, Guangdong Province, China (112°33′ E, 23°10′ N). Specimens were dried at 50-60°C and deposited in the Herbarium of Guangdong Institute of Microbiology, China. Fresh basidiomes were photographed using a Canon IXUS 220 hs digital camera, and macroscopic characteristics of the intact fresh fruit bodies were recorded under daylight conditions in the field. HTML Color Codes (http://www.htmlcolorcode.org/) were used to describe the color of the specimens. For the microscopic characters, tissue sections were immersed in 5% KOH and then stained with 1% aqueous Congo red solution. All tissues were also examined in Cresyl blue to verify presence of ortho- or metachromatic reactions as explained in Buyck (1989). Sulfovanillin (SV) was used to test for reactions of cystidia. Micromorphological features including the pileipellis, basidia, basidiospores, cystidia, and stipitipellis, were observed and photographed using a NIKON E200 microscope equipped with an NIKON E4500 camera. Basidiospores were observed in Melzer's reagent and measured in side view, excluding ornamentation and apiculus which were observed by SEM. The abbreviation [x/y/z] indicates x basidiospores measured from y fruit bodies of z specimens. In the basidiospore dimension notation "(a-) b-m-c (-d)", b-c is the range including 95% of the measured values for length or width, with a and d corresponding to the extremes of all measurements, and "m" mean value. Q indicates length/width ratio of basidiospores, with Qm the average Q of all basidiospores  $\pm$  standard deviation.

## DNA extraction, PCR and sequencing

DNA was extracted from a fresh fruitbody using the method described by Xu et al. (2010). For PCR, sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA were amplified with the primer pair ITS1F/ITS4 (Gardes & Bruns 1993; White et al. 1990). The protocols for PCR amplification consisted of a 5 min activation at 94°C, followed by 32 cycles of 30 s at 94°C, 30 s at 52°C and 1 min at 72°C, and a final 12 min extension at 72°C. Direct sequencing of PCR

products was performed using the PCR primers as sequencing primers. Sanger dideoxy sequencing were performed with ABI 3730 DNA analyzer (IGE, Guangzhou, China). If direct sequencing of PCR products failed, it was subcloned with the PMD18-T vector (Takara Bio, Japan) before sequencing. The generated sequences were then submitted in GenBank.

### PHYLOGENETIC ANALYSES

BLAST query in GenBank indicated that sequences of the two novel species are close to *R. cyanoxantha* (Schaeff.) Fr., a species in *Russula* subgenus *Heterophyllidia* subsection *Cyanoxanthinae*. To establish their taxonomic position within subgenus *Heterophyllidia*, 28 sequences were added to the ITS dataset representing 19 *Russula* from five subsections of subg. *Heterophyllidia* (Table 1), according to the classification of Sarnari (1998). Based on the molecular phylogenetic results of Miller & Buyck (2002), *Albatrellus flettii* Morse ex Pouzar and *Gloeocystidiellum aculeatum* Sheng H. Wu were chosen as outgroups. Sequences were aligned with Clustal X and manually modulated when necessary. Some ambiguously aligned terminal regions were excluded. The final aligned result was submitted to TreeBASE (ID 18975).

For phylogenetic analyses, both Neighbor-joining (NJ) and Bayesian inference (BI) algorithms were employed. NJ analysis of the phylogenetic relationships among the taxa was performed using MEGA 5.05 with the Kimura-2-parameter model and gaps in alignment were treated as missing data. Bootstrap analysis was conducted with 1000 replicates. Bootstrap value (BV) exceeding 70% was considered as significantly supported. Bayesian inference analyses were performed with MrBayes v3.2.5 using the Markov chain Monte Carlo method under the GTR + I + G model. Analyses were run with 4 chains of 1,000,000 generations, and trees were sampled every  $100^{\rm th}$  generation. Bayesian posterior probabilities (PP) values were obtained from the 50% majority-rule consensus trees.

#### RESULTS

# Phylogenetic analysis

A total of 41 ITS sequences (average length 682 bp, see Table 1) representing 23 taxa including two outgroups, were analyzed by NJ and BI analysis. In the aligned ITS matrix, 263 characters were constant, 390 characters were variable, and 295 characters were parsimony-informative. Both the NJ and BI analyses produced similar tree topologies and only the tree inferred from NJ analysis is shown (Fig. 1). The molecular phylogenetic analysis showed that *Russula* subg. *Heterophyllidia* was a strongly supported (BV 87%, PP 0.68), monophyletic group. And five subsections of this subgenus also formed well-supported, distinct clades. The overall structure of tree was in line with the result of Dutta *et al.* (2015) and Zhao *et al.* (2015). The two new taxa formed a strongly supported clades (BV 100%, PP 1.00) that nested well within subsection *Cyanoxanthinae* and were distinct from each other

Table 1. The rDNA ITS sequences of Russula subg. Heterophyllidia used in this study

Taxon	Voucher	Locality	GenBank Accession No
Albatrellus flettii	S. MillerWF3	USA	AY061738
Gloeocystidiellum aculeatum	GB-2647	China	AY061739
Russula aeruginea	DG88	UK	JQ888195
R. aeruginea	NI1292	Germany	UDB000341
R. alboareolata	SUT-1	Thailand	AF345247
R. anatina	13216	Italy	JF908698
R. atroaeruginea	HKAS53626	China	JX391967
R. aurata	S. Miller 6001	Europe	AY061659
R. crustosa	BB2004-208, PC	Europe	EU598194
R. cyanoxantha	SM/BB 5, PC	Europe	AY061669
R. cyanoxantha	HKAS 78376	China	KF002766
R. cyanoxantha	HKAS 78385	China	KF002775
R. dinghuensis	GDGM 45244	China	KU863579
R. dinghuensis	GDGM45243	China	KU863580
R. dinghuensis	K15052704-3	China	KU863581
R. grisea	Watling 27098, E	Europe	AY061679
R. heterophylla	Buyck 99.803, PC	Europe	AY061681
2. ilicis	Sarnari 10/18/99	Europe	AY061682
R. ionochlora	BB28_302		HM189873
R. ionochlora	BB72_407		HM189875
R. kanadii	CUH AM086	India	KJ866936
R. kanadii	CUH AM087	India	KM275230
R. mustelina	Buyck 2422, PC	Europe	AY061693
R. nigrovirens	HKAS55042	China	KP171174
R. nigrovirens	HKAS69567	China	KP171176
R. paludosa	Buyck 6075, PC	Europe	AY061703
R. parazurea	Buyck 99.812, PC	Europe	AY061704
R. variata	JMP0078	USA	EU819436
R. variata	NEHU.MBSRJ37	India:	KP843880
R. vesca	Buyck99.802, PC	Europe	AY061723
R. vesca	AT2002091, UPS	Europe	DQ422018
R. virescens	Buyck99.808, PC	Europe	AY061727
R. virescens	HJB9989, UPS	Europe	DQ422014
R. werneri	IB1997/0786, IB	Europe	DQ422021
R. subpallidirosea	K15052627	China	KU863578
R. subpallidirosea	GDGM 45242	China	KU863582
R. subpallidirosea	K15052902	China	KU863583
R. subpallidirosea	K15060709	China	KU863584
R. subpallidirosea	K15070102	China	KU863585
R. subpallidirosea	MF03_2014/5	China	KU863586

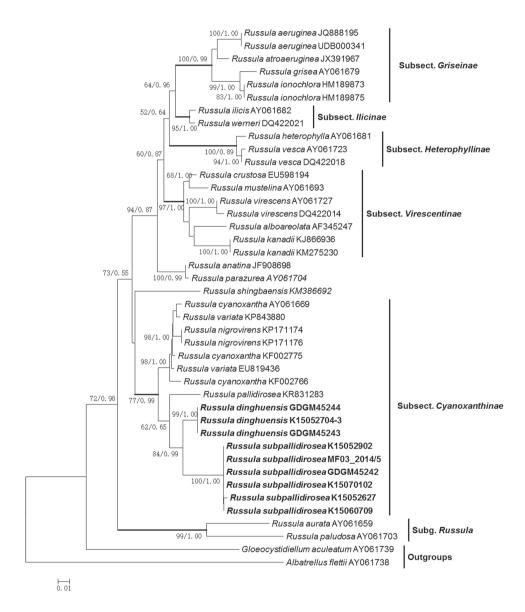


Fig. 1. Neighbor-joining tree of *Russula* subg. *Heterophyllidia* based on ITS dataset. Values to the left of the "/" are Bootstrap values (BV, significant when > 70%), and those to the right indicate Bayesian posterior probabilities (PP, significant when > 95%) of that clade. BV  $\ge 50\%$  and PP values > 0.5 are shown. *Russula dinghuensis* sp. nov. and *Russula subpallidirosea* sp. nov. are placed in bold font to highlight its phylogenetic position in the tree. Classification of *Russula* followed Sarnari (1998).

phylogenetically with an ITS sequence similarity ranging from 91.61% to 91.85%. They were also distinct from the other included species of the subsection *Cyanoxanthinae* with low ITS sequence similarities (The highest is 92.28%).

## **Taxonomy**

Russula dinghuensis J. B. Zhang and L. H. Qiu sp. nov.

Figs 2, 3

Mycobank: MB 815907

*Etymology*: Referring to the locality, Dinghu Mountain, Guangdong Province, China, where the type specimen was collected.

*Holotype*: CHINA. **Guangdong Province**, <u>Zhaoqing City</u>, Dinghu Mountain, 27 May 2015, J. B. Zhang and L. H. Qiu K15052704 (GDGM 45244)

**Basidiomata** middle-size. **Pileus** 4-8 cm in diam., hemispheric when young, plano-convex, expanding to applanate when mature, center depressed with age; margin even or incurved, slight striated with age, sometimes cracking; surface viscid when moist, cracking into small patches, sometimes becoming smooth at centre when mature, pale ochre (#A87235) when young, then becoming olive green (#9DC209) to dark green (#436800), mixed with rusty tone (#B95837). **Lamellae** adnate to subdecurrent, crowded, rarely forked, with scattered lamellulae, white, cream yellow when dried, unchanging when bruised, not brittle when touching. **Stipe** 3-6.5 × 0.8-1.2 cm, cylindrical, subglabrous, smooth, dry, white to whitish. **Context** 3-4 mm thick, white to cream when dry, without color changing when bruised. **Odour** indistinct. **Taste** unrecorded. **Spore print** whitish.

**Basidiospores** [60/3/3] (5.5)  $6-6.9-8.\overline{0}$  (8.5)  $\times$  5.0-6.3-7.0 µm [Q = 1.0-1.40, Qm =  $1.18 \pm 0.11$ ], globose to subglobose or broadly ellipsoid to ellipsoid: ornamentation amyloid; warts bluntly conical to subcylindrical, not exceeding 0.4 µm in height, isolated or connected with irregular lines or ridges, not forming a reticulum; suprahilar plage indistinct, not amyloid; hyaline in 5% KOH. Basidia 29-50 × 8-12 µm, 4-spored with some 2- and 3-spored basidia present, narrowly clavate to clavate, inflated at the apex; sterigmata < 5 μm in length. Lamellar trama mainly composed of nested spherocytes (17.5-30 × 15-30 µm) surrounded by connective hyphae. **Pleurocystidia** 44-67 × 6-10 μm, abundant, slender, clavate to subfusiform, apex obtuse, bluntly acuminate or mucronate, with abundant refractive subacerose contents, dark grey in SV. Cheilocystidia 45-52 × 4-6 µm, slender, clavate to subfusiform, apex obtuse, with abundant refractive subacerose contents. Marginal cells  $18-28 \times 3-5$  µm, cylindrical to narrowly clavate, hyaline. Pileipellis metachromatic in cresyl blue, 225-325 µm thick, composed of epipellis and subpellis; epipellis a trichoderm 125-150 µm thick, consisting of thin-walled, often ramifying, septate, interwoven, ascending to repent hyphae (2.5-5 µm diam.); terminal elements  $11-24 \times 2-4$  µm, apices obtuse or attenuate; pileocystidia  $32-53 \times 2.5-5$  µm cylindrical to clavate, apex mucronate or acuminate, always one-celled. Stipitipellis a cutis, composed of somewhat gelatinized, thin-walled, cylindrical hyphae 1.5-5 µm diameter; caulocystidia,  $43-76 \times 5-6.3 \mu m$ , subcylindrical to narrow clavate. Clamp connections absent.

*Habitat and distribution*: Gregarious in monsoon evergreen broadleaf forest and pine-broadleaf mixed forest.

Additional specimens examined: CHINA, Guangdong Province, Zhaoqing City, 27 May 2015, J. B. Zhang GDGM 45243; ibid., 27 May 2015, J. B. Zhang K15052704-3.



Fig. 2. **a-c.** Russula dinghuensis (GDGM 45244, Holotype); **d-f.** Russula subpallidirosea (GDGM 45242, Holotype). Photographs scale bar = 1 cm, Scanning Electronic Micrographs scale bar = 1 μm.

# Russula subpallidirosea J. B. Zhang and L. H. Qiu sp. nov.

Figs 2, 4

Mycobank: MB 816245

*Etymology*: Referring to the characteristics that its pileus color is similar to that of *Russula pallidirosea* Kropp.

*Holotype*: CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, 27 May 2015, J. B. Zhang and L. H. Qiu K15052818 (GDGM 45242)

**Pileus** 3-7 cm in diam., first hemispherical, becoming convex or planoconcave with a slightly depressed center when mature; surface pale pink (#FAC5AF)

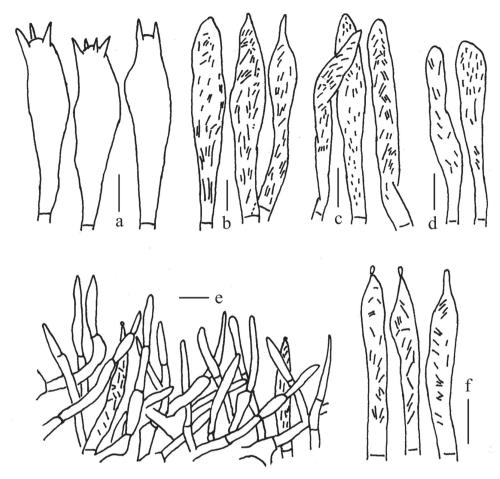


Fig. 3 *Russula dinghuensis* (Holotype GDGM 45244). **a.** Basidia; **b.** Pleurocystidia; **c.** Cheilocystidia; **d.** Caulocystidia; **e.** Pileipellis; **f.** Pileocystidia. Scale bars = 10 μm.

to pale grayish-pink (#F7E7CE), sometimes with yellowish brown spot (#F59B00), viscid when moist, cracked in dry condition; margin even or incurved, with slight striate; **Lamellae** adnate, 3-6 mm, crowded, often forking, with scattered lamellulae, white, sometime becoming yellowish brown when bruised, not brittle when touching. **Stipe** 3.1-6.5 × 0.8-1.3 cm, cylindrical, tortuous, slightly expanded towards the base, subglabrous, smooth, dry, white to whitish. **Context** 3-5 mm thick, white to cream when dry, without color changing when bruised. **Odour** indistinct. **Taste** unrecorded. **Spore print** whitish.

**Basidiospores** [80/4/4] (5.5) 6-6.7-8.0 (9.0)  $\times$  5.0-5.9-7.0 (8.0)  $\mu$ m [Q = 1.0-1.40 (1.43), Qm = 1.19  $\pm$  0.10], subglobose or broadly ellipsoid to ellipsoid, rarely globose; ornamentation amyloid; warts conical to subcylindrical, not exceeding 0.6  $\mu$ m in height, mostly isolated or some connected with irregular lines or ridges, not forming a reticulum; suprahilar plage indistinct, not amyloid; hyaline in 5% KOH. **Basidia** 31-43  $\times$  6-10  $\mu$ m, 4-spored with some 2- and 3-spored basidia present, narrowly clavate to clavate, inflated at the apex; sterigmata < 4  $\mu$ m in length. **Lamellar** 

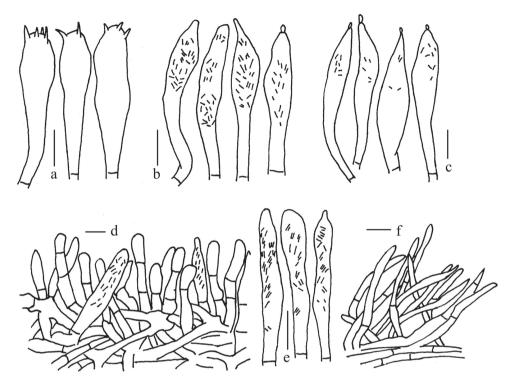


Fig. 4 Russula subpallidirosea (Holotype GDGM 45242). **a.** Basidia; **b.** Pleurocystidia; **c.** Cheilocystidia; **d.** Pileipellis; **e.** Pileocystidia; **f.** Terminal cells in stipitipellis. Scale bars = 10 μm.

trama mainly composed of nested spherocytes ( $20\text{-}45 \times 17.5\text{-}31.2 \,\mu\text{m}$ ) surrounded by connective hyphae. **Pleurocystidia**  $35\text{-}50 \times 5\text{-}8 \,\mu\text{m}$ , abundant, projecting 12-18  $\,\mu\text{m}$  beyond hymenium, slender, clavate to subfusiform, apex obtuse, bluntly acuminate or mucronate, with abundant refractive subacerose contents, grey in SV. Lamellae edge fertile; cheilocystidia  $55\text{-}63 \times 6\text{-}10 \,\mu\text{m}$ , narrowly clavate to clavate or fusiformis with a moniliform to papillate appendage, with some refractive subacerose contents. **Marginal cells**  $20\text{-}35 \times 5\text{-}7 \,\mu\text{m}$ , cylindrical to clavate, hyaline. **Pileipellis** metachromatic in cresyl blue,  $125\text{-}230 \,\mu\text{m}$  thick, composed of epipellis and subpellis; epipellis a trichoderm  $62\text{-}75 \,\mu\text{m}$ , consisted of thin-walled, often ramifying, septate, interwoven, ascending to repent hyphae ( $3\text{-}5 \,\mu\text{m}$  diam.); terminal elements  $9\text{-}18 \times 4\text{-}5 \,\mu\text{m}$ , apices obtuse, sometimes attenuate; **pileocystidia**  $27\text{-}38 \times 3\text{-}5 \,\mu\text{m}$ , one-celled, apex mucronate or subterminally constricted, grey in SV. **Stipitipellis** mostly a cutis, with some erect or oblique terminal elements ( $25\text{-}45 \times 2.5\text{-}3.8 \,\mu\text{m}$ ), composed of thin-walled, cylindrical hyphae  $1.5\text{-}5 \,\mu\text{m}$  in diam.; caulocystidia  $50\text{-}83 \times 4\text{-}6 \,\mu\text{m}$ , subcylindrical to narrow clavate. **Clamp connections** absent.

*Habitat and distribution*: Gregarious in monsoon evergreen broadleaf forest and pine-broadleaf mixed forest.

Additional specimens examined: CHINA. **Guangdong Province**, <u>Zhaoqing City</u>, 4 May 2014, J. B. Zhang MF03; 26 May 2015, J. B. Zhang K15052627; 29 May 2015, J. B. Zhang K15052902; 7 June 2015, J. B. Zhang K15060709; 1 July 2015, J. B. Zhang K15070102.

#### DISCUSSION

Russula dinghuensis sp. nov. is well characterized by the olive green pileus, acute and incurved margin, white and rarely forking lamellae, white spore print, the globose to ellipsoid basidiospores with the stocky and isolated warts, the thick metachromatic pileipellis, and the slender, furcated and moderately septate, terminal elements of pileipellis. Russula subpallidirosea sp. nov. is recognized by the pale pink to grayish pink pileus with yellowish brown spots, equally white but frequently forked lamellae, white spore print, subglobose to ellipsoid basidiospores with the isolated, subcylindrical to conical warts, the metachromatic pileipellis, and the short, furcated and strongly septate hyphal extremities of pileipellis. The combination of multicolored pileus, white lamellae, white spore print, basidiospores with isolated warts and non-amyloid suprahilar spots, and finally the metachromatic pileipellis with septate terminal cells assigns the two new taxa into Russula subg. Heterophyllidia subsection Cyanoxanthinae (Sarnari 1998), which is also supported by the molecular analysis based on ITS sequences (Fig. 1).

NJ and BI phylogenetic analysis showed R. dinghuensis sp. nov. and R. subpallidirosea sp. nov. formed a highly supported sister group to the other species of Cyanoxanthinae. R. dinghuensis morphologically resembles R. subpallidirosea in the size of basidiomata, color of lamellae and spore print, the shape and size of basidia and basidiospores and the hyphal extremities of pileipellis. However, the morphological differences between the two species are very obvious (Table 2). Macromorphologically, apart from the difference in cap color, the lamellae of R. dinghuensis rarely fork from the centre of pileus to the margin, but forked lamellae are common in R. subpallidirosea. Micromorphologically, the warts of basidiospores of R. dinghuensis seem generally stockier than those of R. subpallidirosea. As for the structure of cheilocystidia, those of R. subpallidirosea have often a moniliform to papillate appendage and are less slender (55-63 × 6-10 µm) than those of R. dinghuensis (45-52  $\times$  4-6  $\mu$ m). Terminal elements of the pileipellis of R. dinghuensis are narrower (11-24  $\times$  2-4  $\mu$ m) than in R. subpallidirosea (9-18  $\times$  4-5  $\mu$ m) and the pileipellis of R. dinghuensis is distinctly thicker (225-325 vs 125-230 µm) than that of R. subpallidirosea. So, both the molecular analysis and morphological differences strongly suggest that they are different taxa.

The ITS sequence analysis indicated that *R. dinghuensis* was different from published sequences of known *Russula* species of the subsection *Cyanoxanthinae*. It is closely related to the recently reported Chinese *Russula nigrovirens* Zhao *et al.* (2015), the European *Russula cyanoxantha* (Schaeff.) Fr. and the North American *Russula variata* Banning (1881).

The European *R. cyanoxantha* is often larger (cap 5-15 cm in diam.) and having a multicolored pileus. Two formae of this species, *R. cyanoxantha* f. *peltereaui* and *R. cyanoxantha* f. *cutefracta*, resemble *R. dinghuensis* in the green pileus, for the latter form also with cracking cuticle. The North American *R. variata*, growing in forests of oaks and other hardwoods, also has repeatedly forked lamellae, acrid context and an often strongly disrupting pileipellis. The recently described Chinese *R. nigrovirens* Zhao *et al.* (2015), differs from our species by its strongly cracked and darker pileus color, the larger basidia (45-75  $\times$  9-14  $\mu$ m), the basidiospore ornamentation with some irregular lines and the slender terminal elements of pileipellis (15-46  $\times$  2.5-5  $\mu$ m). An unnamed *Russula* sp. PUN 322 from India (Buyck & Atri 2011) placed in subsection *Cyanoxanthinae*, distinguished our species by its white to pale yellow pileus and the strongly reticulate and almost poroid configuration of the stipe hymenium. Considering the green, somewhat cracked pileus, some

Table 2 The comparision of morphological features between R. dinghuensis and R. subpallidirosea as well as some closely related species

Characters	R. dinghuensis	R. subpallidirosea	R. cyanoxantha (Li 2014)	R. variata (Li 2014)	R. pallidirosea (Kropp 2016)	R. nigrovirens (Zhao et al. 2015)
Pileus size (cm)	4-8	3-7	5-15	4-9	1.5-2.5	3-10
Pileus color	olive green with rusty tone	pale pink with yellowish brown spot	multicolor	multicolor	pinkish	green white to grayish green
Lamellae	rarely forked	often forked	Forked only near the stipe	often forked	occasionally forked	rarely forking near the stipe
Stipe (cm)	$3-6.5 \times 0.8-1.2$	$3.1-6.5 \times 0.8-1.3$	$4-10 \times 1-3$	$3.2-6.0 \times 0.6-1.8$	$1.2-2.2 \times 0.3-0.5$	$6-10 \times 1.0-2.5$
Taste	nnrecorded	unrecorded	a little sweet	acrid	mild	mild
Basidiospores (µm)	Basidiospores (μm) (5.5)6-6.9-8.0(8.5) × 5.0-6.3-7.0	6-6.7-8.0 (9.0) × 5.0-5.9-7.0	$6.3-9.3 \times 5.5-7.6$	$6.5-7.8 \times 6.0-7.0$	$6.0-8.5 \times 5.0-7.0$	$6.5-8.5 \times 6.0-8.0$
Basidia (µm)	$29-50 \times 8-12$	$31-43 \times 6-10$	$24-38 \times 7-11$	$35-57 \times 9-12$	$37-43 \times 9-10$	45-75 × 9-14
Pleurocystidia ( $\mu$ m) 44-67 × 6-10	$44-67 \times 6-10$	$35-50 \times 5-8$	$31-66 \times 4-12$	$57-74 \times 11-12$	Similar to Cheilocystidia	$47-72 \times 7-10$
Cheilocystidia ( $\mu$ m) 45-52 × 4-6	$45-52 \times 4-6$	$55\text{-}63\times6\text{-}10$	1	ı	$40-55 \times 5-7$	$46\text{-}55\times6.5\text{-}8.5$
Pileocystidia (μm)	$32-53 \times 2.5-5$	$27-38 \times 3-5$	ı		$17-50 \times 2-4$	$18-62 \times 3-6.5$
Caulocystidia ( $\mu$ m) 43-76 × 5-6.3	$43-76 \times 5-6.3$	$50-83 \times 4-6$	1	1	similar to pileocystidia	$29-96 \times 3.5-6.5$

\*Characters with bold font indicate that the feature differs significantly from that of R. dinghuensis; while those underlined indicate that it differs significantly from that of R. subpallidirosea.

species from other subsections of *R*. subg. *Heterophyllidia* can also be very similar to *R. dinghuensis*, *i.e. R. virescens* (Schaeff) Fr., *R. parvovirescens* Buyck, D. Mitch. & Parrent, *R. subgraminicolor* Murrill, *R. heterophylla* Fr., *R. aeruginea* Lindblad, *R. griseoviridis* McNabb, *R. sikkimensis* K. Das, Atri & Buyck and *R. viridella* var. *yunnanensis* Singer, but they all have an orthochromatic pileipellis in cresyl blue, pale cream spore print and either more prominent or different basidiospore ornamentation.

For *R. subpallidirosea*, the pale pink to pale grayish pink cap makes it similar to *R. vesca* in subsect. *Heterophyllinae*, but the latter species has needle shaped cells in the pileipellis and lacks abundantly forking gills.

**Acknowledgements.** We would like to thank Prof. B. Buyck from the Paris' Museum of Natural History in France and Dr. Tai-hui Li of the Guangdong Institute of Microbiology for their valuable comments and advice during this study, also the Administrative Bureau of Dinghushan Biosphere Reserve for the assistance with collection of samples. This research was partly supported by the National Natural Science Foundation of China (Project no. 31030015 and J1310025).

#### REFERENCES

- BANNING M.E., 1881 New species of fungi found in Maryland: *Agaricus (Tricholoma) cellaris*. *Botanical Gazette Crawfordsville* 6(1):165-166.
- BI Z.S. & LI T.H., 1986 A preliminary note on *Russula* species from Guangdong, with a new species and a new variety. *Guihaia* 6(3):193-199.
- BI Z.S., ZHENG G.Y. & LI T.H., 1994 Macrofungus flora of Guangdong Province. *Guangdong Science and Technology Press, Guangzhou*:1-879.
- BUYCK B., 1989 Revision du genre Russula Persoon en Afrique Centrale. Ghent Belgium Rijksuniversiteit: Introductory part 98.
- BUYCK B. & ATRI N.S., 2011 A *Russula* (Basidiomycota, Russulales) with an unprecedented hymenophore configuration from northwest Himalaya (India). *Cryptogamie, Mycologie* 32(2):185-190.
- BUYCK B., HOFSTETTER V., EBERHARDT U., VERBEKEN A. & KAUFF F., 2008 Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* subsect. *Ochricompactae*. Fungal Diversity 28:15-40.
- BUYCK B. & HORAK E., 1999 New species of *Russula* (Basidiomycotina) associated with *Anisoptera* (Dipterocarpaceae) in Papua New Guinea. *Australian Systematic Botany* 12(5):727-742.
- BUYCK B., JÁNČOVIČOVÁ S. & ADAMČÍK S., 2015 The study of Russula in the Western United States. Cryptogamie, Mycologie 36(2):193-211.
- CHIU W.F., 1945 The Russulaceae of Yunnan. Lioydia 8:31-59.
- DUTTA A.K., PALOI S., PRADHAN P. & ACHÁRYA K., 2015 A new species of *Russula* (Russulaceae) from India based on morphological and molecular (ITS sequence) data. *Turkish Journal of Botany* 39:850-856.
- GARDES M. & BRUNS T.D., 1993 ITS primers with enhanced specificity for basidiomycetesapplication to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2):113-118.
- KIRK P., CANNON P., MINTER D. & STALPERS J., 2008 Dictionary of the fungi. 10th Edition. KROPP B.R., 2016 Russulaceae in American Samoa: new species and further support for an
- Australasian origin for Samoan ectomycorrhizal fungi. *Mycologia*:15-171. LI G.J., 2014 Research on Taxonomy of *Russula* in China. *Institute of microbology Chinese academy of science*.
- LI G.J., LI S.F., LIU X.Z. & WEN H.A., 2012 Russula jilinensis sp. nov. (Russulaceae) from northeast China. Mycotaxon 120(1):49-58.
- LI G.J., LI S.F. & WEN H.A., 2011 Russula zhejiangensis sp. nov. from East China. Cryptogamie, Mycologie 32(2):127-133.
- LI G.J., ZHAO D., LI S.F. & WEN H.A., 2015 Russula chiui and R. pseudopectinatoides, two new species from southwestern China supported by morphological and molecular evidence. Mycological Progress 14(6):1-14.

- LI G.J., ZHAO D., LI S.F., YANG H.J., WEN H.A. & LIU X.Z., 2013 Russula changbaiensis sp. nov. from northeast China. Mycotaxon 124(1):269-278.
- LI G.J., ZHAO Q., ZHAO D., YUE S.F., LI S.F., WEN H.A. & LIU X.Z., 2013 Russula atroaeruginea and R. sichuanensis spp. nov. from southwest China. Mycotaxon 124(16):173-188.
- LI Y.K., ZHANG X., YUAN Y., CAO Z. & LIANG J.F., 2015 Morphological and molecular evidence for a new species of *Russula* (Russulaceae) from southern China. *Phytotaxa* 202(2):94-102.
- MILLER S.L. & BUYCK B., 2002 Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. *Mycological Research* 106(3):259-276.
- ROMAGNESI H., 1967 Les Russules d'Europe et d'Afrique du Nord. Bordas Paris.
- SARNARI M., 1998 Monografia illustrata del genre Russula in Europa. Associazione Micologica Bresadola, Trento.
- SINGER R., 1935 Supplemente zu meiner monographie der guttung Russula. Annales Mycologici 33:297-352.
- SINGER R., 1986 The Agaricales in modern taxonmy. 4th ed. *Koeltz Scientific Books, Koenigstein*. SONG B., LI T.H., WU X.L., LI J.J., SHEN Y.H. & LIN Q.Y., 2007 Known species of *Russula* from China and their distribution. *Journal of Fungal Research* 5(1):20-42.
- WANG X.H., YANG Z.L., LI Y.C., KNUDSEN H. & LIU P.G., 2009 Russula griseocarnosa sp. nov. (Russulaceae, Russulales), a commercially important edible mushroom in tropical China: mycorrhiza, phylogenetic position, and taxonomy. Nova Hedwigia 88(1-2):269-282.
- WEN H.A. & YING J.Z., 2001 Studies on the genus Russula from China II. Two new taxa from Yunnan and Guizhou. Mycosystema 20(2):153-155.
- WHITE T.J., BRUNS T.D., LEE S.B. & TAYLOR J.W., 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18:315-322.
- XU F., LIU Y. & WANG S., 2010 A Simple Method For Extraction of Genomic DNA of Mushroom. *Acta Edulis Fungi*:12-14.
- YING J.Z., 1983 A study on *Russula viridi-rubrolimbata* sp. nov. and its related species of Subsection *Virescentinae*. *Mycosystema* 2(1):34-37.
- YING J.Z., 1989 Studies on the genus *Russula* Pers. from China 1. New taxa of *Russula* from China. *Acta Mycology Sinica* 8(3):205-209.
- ZANG M. & YUAN M.S., 1999 Contribution to the knowledge of new basidiomycetous taxa from China. *Acta Botanica Yunnanica* 21(1):37-42.
- ZHAO Q., LI Y.K., ZHU X.T., ZHAO Y.C. & LIANG J.F., 2015 Russula nigrovirens sp. nov. (Russulaceae) from southwestern China. Phytotaxa 236(3):249-256.
- ZHENG G.Y., LOH T.C., LI C. & LI T.H., 1985 Some new species and varieties of Agaricales from Dinghu Mountain. *Microbiological Journal* (1):24-30.