MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF AN EDIBLE *RUSSULA* MUSHROOM IN NORTHEAST VIETNAM

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ABSTRACT

Wild mushrooms are extensively gathered by rural communities throughout the uplands of northern Vietnam and nearby countries for food and medicine. These non-wood forest products are important for the livelihood of numerous rural communities. Many of these fungi form symbiotic relationships with the roots of certain species of forest trees, and the fruiting bodies can not be produced in artificial media. In the northeast region of the country, an edible red *Russula* (Nấm chẹo or Nấm hồng cô) has been harvested from under wild *Castanopsis, Engelhardia and Lithocarpus* trees by local people for a long time. However, the identity of this *Russula* is unknown. We collected basidiomes of this edible *Russula* in Cao Bang, Bac Giang and Quang Ninh provinces and described its morphological features. We used ITS rDNA and LSU rDNA sequence analysis to compare with other similar taxa in GenBank. Morphological and molecular analysis identified the taxon as *Russula griseocarnosa*, previously described from southern Yunnan, China. There is strong interest by local governments to commercialize *R. griseocarnosa* in Vietnam. However, over-harvesting could have detrimental effects on the population of this edible species. Therefore, further studies are needed to quantify the ecological requirements and to develop sustainable harvesting protocols of this edible species in Vietnam.

Keywords: Fagaceae, rural livelihood, Russula griseocarnosa, wild mushroom.

1. INTRODUCTION

Russula is a large and important genus of ectomycorrhizal fungi and is widely distributed in forests and shrublands from the cool temperate to tropical climate zones [1, 2]. Many species have been reported in South Asia [3], Southeast Asia [4] and China [2, 5, 6] and new species continue to be collected and described [7, 8]. In SE Asia, basidiomes of *Russula* are most commonly observed under host trees in the Pinaceae, Fagaceae and Dipterocarpaceae [9].

Russula contains edible, medicinal and poisonous species [4, 10]. The fruiting bodies are all collected from the wild as attempts to cultivate them have been unsuccessful. Because of their flavour when cooked and nutritional

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value, rural communities in the uplands of Asia actively collect favoured species for domestic consumption and local trade. Also, *Russula* can be processed into commercial products [5].

In Vietnam, 16 species of *Russula* have been recognized [11-13]. Among these, *R. vinosa* [11], *R. albidula*, *R. rosea*, *R. variata*, *R. virescens* [12], *R. paludosa* and *R. cystidiosa* [13] are reported to be collected for food. In the northeast provinces of the country, an edible *Russula* mushroom with a red pileus, known locally as Nấm cheo or Nấm hồng cô, has been collected by local people for a long time. The taxonomic status of this mushroom is unknown. It is important to correctly identify this *Russula* sp. as some red-capped species contain poisons, some are edible and some are a source of medicines. Therefore, using morphological and molecular traits, this study was undertaken to establish the identity of the edible *Russula* mushroom being collected for food in Cao Bang, Bac Giang and Quang Ninh provinces.

2. RESEARCH METHODOLOGY

Sampling and morphological chracterization

In May and June 2022, field surveys were undertaken in Cao Bang, Bac Giang and Quang Ninh provinces (Figure 1) where edible red *Russula* mushrooms are harvested and consumed by local people. The forests in Dong Son and Ky Thuong awards, Ha Long city, Quang Ninh province are primary forests and form part of Dong Son-Ky Thuong Nature Reserve. The remaining forests in Quang Ninh, Bac Giang and Cao Bang provinces are secondary and community forests managed by local people. Ten sites were chosen with high density of edible Russula based on indigenous knowledge of the local people (Table 1). Basidiomes were collected around the base of five host tree species: Castanopsis tonkinensis, C. cerebrina, Engelhardia roxburghiana, Lithocarpus dealbatus and L. ducampii (Table 1). At each site, 30 basidiomes were selected for observation and measurement in situ. Then the basidiomes were harvested, wrapped in tissue paper, stored over ice in a cold container and transported to the laboratory of the Forest Protection Research Centre (FPRC), Vietnamese Academy of Forest Sciences in Hanoi, Vietnam.



Figure 1. Geographical locations where the edible red *Russula* were collected in northeast Vietnam (names of sampling sites are given in Table 1)

In the laboratory, basidiomes were dissected using a knife and photographed using a Canon G9X camera. Samples of basidiospores, basidia, hymenium, subhymenium and pleuromacrocystidia were mounted in Optika immersion oil and observed under an optical Olympus BX50 microscope. Tissue samples (10 g) were taken from inside the pileus for molecular work. Finally, 30 specimens were dried at 40°C using a food drier and curated into the FPRC fungal herbarium.

in natural forests in Northeast vietnam							
Sample	Host plant	Number in	Geographical	Location (ward, district,			
		figure 1	coordinates	province)			
BG1	Engelhardia	2	21.258953,	Nghia Phuong, Luc Nam,			
	roxburghiana Lindl.		106.461720	Bac Giang			
CB2	Engelhardia	1	22.766888,	Doai Duong, Trung Khanh,			
	roxburghiana Lindl.		106.499185	Cao Bang			
QN7	Engelhardia	4	21.420044,	Ha Lau, Tien Yen, Quang			
	roxburghiana Lindl		107.306033	Ninh			
QN8	Lithocarpus ducampii	4	21.425544,	Ha Lau, Tien Yen, Quang			
	(Hickel & A.Camus)		107.302358	Ninh			
	A.Camus						
QN11	Lithocarpus dealbatus	5	21.296574,	Thanh Son, Ba Che, Quang			
	(Hook.f. & Thomson ex		107.241280	Ninh			
	Miq) Rehder						
QN19	Engelhardia	5	21.301132,	Thanh Son, Ba Che, Quang			
	roxburghiana Lindl.		107.246516	Ninh			
QN20	Castanopsis tonkinensis	6	21.185119,	Ky Thuong, Ha Long,			
	Seemen		107.118468	Quang Ninh			
QN22	Engelhardia	6	21.180197,	Ky Thuong, Ha Long,			
	roxburghiana Lindl.		107.145569	Quang Ninh			
QN29	Castanopsis cerebrina	3	21.541572,	Vo Ngai, Binh Lieu, Quang			
	(Hickel & A.Camus)		107.343699	Ninh			
QN50	Engelhardia	3	21.510154,	Vo Ngai, Binh Lieu, Quang			
	roxburghiana Lindl.		107.347711	Ninh			

 Table 1. Information on host species and collection sites for the edible red Russula in natural forests in Northeast Vietnam

Sequencing and phylogenetic analysis

DNA was extracted from 10 basidiomes (BG1, CB2, QN7, QN8, QN11, QN19, QN20, QN22, QN29 and QN50) and processed for ITS rDNA and LSU rDNA gene amplification using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [14], and primers LROR (5'-ACCCGCTGAACTTAAGC-3') and LR7 (5'-TACTACCACCAAGATCT-3') [15]. Amplifications were carried out in 50-µlvolume reactions containing 20 µl Master Mix (Eppendorf, Germany), 1 µl of each forward and reverse primer, 1 µl of DNA template and 27 µl sterilized water. The PCRs were performed with a C1000 TouchTM thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 3 minutes at 94°C, followed by 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 1 minute. The amplification was completed at 72°C for 10 minutes and then the PCR product was stored at 10°C. The PCR amplicons were sequenced at 1st BASE (Seri Kembangan, Malaysia). The DNA sequences were compared to the GenBank database via the nucleotidenucleotide BLAST search interface located at National Center Biotechnology the for Information. Bethesda. USA (Table 2). Relevant sequences were transferred and then processed using BioEdit software [16]. The Russula species selected for comparison had basidiome traits (shape, colour, size) similar to the edible Russula in this study. Sequences belonging to two different vouchers of some Russula species were used to construct the consensus tree in order to cover the two sequences of interest.

с ·	Voucher	GenBank accession		Df
Species		ITS rDNA	LSU rDNA	Keierence
R. griseocarnosa	BG1	-	-	This study
R. griseocarnosa	CB2	-	-	This study
R. griseocarnosa	QN7	-	-	This study
R. griseocarnosa	QN8	-	-	This study
R. griseocarnosa	QN11	-	-	This study
R. griseocarnosa	QN19	-	-	This study
R. griseocarnosa	QN20	-	-	This study
R. griseocarnosa	QN22	-	-	This study
R. griseocarnosa	QN29	-	-	This study
R. griseocarnosa	QN50	-	-	This study
R. griseocarnosa	KUN F51713	EF627041	EF627044	[8]
R. griseocarnosa	E138	JAGKRU010000012	JAGKRU010000067	[17]
R. decolorans	FH12196	KT933992	-	[18]
R. decolorans	549/BB 07.322	-	KU237541	-
R. amethystina	929IF52	AY061653	-	[19]
R. amethystina	hue215, TUB	-	AY606971	[20]
R. vinosa	500RUF26	AY061724	-	[19]
R. vinosa	KUN F52026	-	EF627045	[8]
R. integra	FH12172	KT933984	-	[18]
R. integra	518/BB 07.198	-	KU237513	-
R. xerampelina	2-684RUS28	AY061734	-	[19]
R. xerampelina	OSA-MY-1762	-	AB154752	[21]
R. laeta	R70	MG679812	-	[22]
R. laeta	519/BB 07.267	-	KU237514	-
R. badia	R75	MG679813	-	[22]
R. badia	587/BB 07.324	-	KU237571	-
R. minutula	BPL574	KY509454	-	[23]
R. minutula	539/BB 08.636	-	KU237531	-
R. azurea	3515	JF908662	-	[24]
R. azurea	537/08.668	-	JN940591	-
R. lilacea	BPL645	KY509453	-	[23]
R. lilacea	435/07.213	-	JN940592	-
R. laricina	1112IS75	AY061685	-	[19]
R. laricina	575/08.681	-	JN940593	-
R. nauseosa	FH12173	KT933985	-	[18]
R. nauseosa	588/BB 07.285	-	KU237572	
R. zvarae	FH12175	KT933986	-	[18]
R. zvarae	538/08.639	-	JN940603	-
Albatrellus ovinus	Dai15171	MW534158	MW534173	[25]

Table 2. GenBank accession numbers of Russula voucher in this study

Phylogenetic analyses were performed using the ITS and LSU sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [26].

Consensus trees with the highest log likelihoods (1158.71) were created. Evolutionary analyses were conducted in MEGA7 [27]. *Albatrellus ovinus* was used as the outgroup taxon to root the tree.

3. RESULTS

Morphology

Pileus dark pink to red (Figure 2a, b), bellshaped when young (Figure 2a, c), later hemispherical (Figure 2d), and finally flat (Figure 2b) or slightly sunken in the centre when mature (Figure 2f). Pileus edge flat (Figure 2e, g), margin does not striate when young, shortly and very obscurely striate. Pileus surface smooth, glossy, and hairless; when the weather is humid, it can be slightly slimy/sticky, easy to separate from the basidiomata flesh. Mature pileus (8.0-) 10.6 (- 13.2) cm in diameter, (1.5) 1.8 (-2.1) cm in thickness. Gills and internal basidiomata pulp white (Figure 2e, g).



Figure 2. Appearance of the basidiomes of the edible red *Russula*: a. basidiome cluster; b. top of pileus; c, d. young basidiomes; e. dissected basidiome; f. pileus of mature basidiome; g. gill arrangement

Stipes white, slightly pinkish, cylindrical (Figure 2d), slightly bigger towards the base then tapering (Figure 2a, c), (5.1-) 5.6 (-6.2)

cm in length, (1.8-) 2.2 (2.5) cm in diameter, firm when young, but soft, spongy, dense, and interior grayish-white when old (Figure 2e).



Figure 3. Microscopic characteristics of the edible red *Russula*: a. pileipellis; b. hymenium and subhymenium with one pleuromacrocystidium (red arrow); c. basidia and attached basidiospores; d. basidiospores

Pileipellis an ixotrichoderm (155-) 180.1 (-245) µm thick, composed of (4.1-) 5.5 (-7.2) µm thick (Figure 3a). Terminal cells (1890-) 1950 (-2010) µm long, inflating up to (8.0-) 9.1 (-10.1) µm thick. Pleuromacrocystidia (90-) 116.3 (-142) × (12.2-) 15.3 (-18.3) μ m, abundant, emergent, projecting (29-) 40.5 (-51) µm beyond the hymenium (Figure 3b), fusiform, mostly with acute apex, some thick-walled in centre, wall (1.1-) 1.3 (-1.5) μ m thick. Basidia (35-) 43.5 (-52) × (9-) 10.5 (-12) µm, 4-spored, clavate (Figure 3c). Basidiospores (8.5-) 9.4 (-10.2) \times (7.5-) 7.7 (-8.1)subglobose to ellipsoid, μm, with conical ornamented large spines, sometimes enlarged and connected at the base.

Spines (1.3-) 3.7 (-2.2) μ m long, strongly but often partially amyloid (Figure 3d).

Sequence analysis

The ITS rDNA and LSU rDNA gene sequences of ten samples in this study were compared with reference sequences obtaned from the National Center for Biotechnology (NCBI) GenBank for *Russula amethystina*, *R. azurea*, *R. badia*, *R. decolorans*, *R. integra*, *R. laeta*, *R. badia*, *R. decolorans*, *R. integra*, *R. laeta*, *R. laricina*, *R. lilacea*, *R. minutula*, *R. nauseosa*, *R. griseocarnosa*, *R. vinosa*, *R. xerampelina*, *R. zvarae* (Table 2). The results indicated that all ten samples had 99.8% homology with *Russula griseocarnosa* (Figure 4). Bootstrap values were equal to or greater than 50% derived from 1,000 iterations.



Figure 4. Consensus tree of the concatenated sequences of ITS rDNA and LSU rDNA from different species of the genus *Russula*. EF627041 and EF627044 are the GenBank numbers of *R.* griseocarnosa holotype. The Maximum Likelihood method was used to construct the tree. The bar represents an expected sequence variation of 1.0%. *Albatrellus ovinus* was used as the outgroup taxon to root the tree.

4. DISCUSSION

Russula griseocarnosa X.H. Wang, Zhu L. Yang & Knudsen is identified as the main species of red *Russala* being collected for food in northeast Vietnam. Mushroom collectors report on high population densities of this species in natural and secondary forests. Now the authorities in these provinces, especially Quang Ninh, are formulating plans to comercialize speciality mushrooms including ectomycorrhizal fungi from the wild [28]. The true identity of the red *Russula* will allow scaling up of the marketing brand of *R. griseocarnosa* in Vietnam and give confidence of its edibility to consumers.

The morphological characteristics of *R*. griseocarnosa in this study (shape, colour, size

of basidiomes; and microscope examination of basidiospores, basidia, hymenium, subhymenium and pleuromacrocystidia) are highly similar to those described for the type collection of *R. griseocarnosa* collected in Xishuangbanna Nature Reserve in Yunnan Province [8]. There, *R. griseocarnosa* was associated with members of the Fagaceae. This kind of evergreen forest is contiguous with the upland forests of northern Vietnam. Therefore, it is likely that *R. griseocarnosa* has a wider geographical range across northern Vietnam than reported in this study.

The shape, colour and size of basidiomes of *R. griseocarnosa* are similar to *R. rosea* [11, 13], which is widely distributed in Quang Nam province [13]. However, the basidiospores,

basidia, hymenium, subhymenium and pleuromacrocystidia differ from R. rosea. Further taxonomic work is required to establish the status of R. rosea as this is a European species and is unlikely to occur in SE Asia.

Molecular methods are considered to be essential to identify cryptic species within the large *Russula* genus [7, 8, 29-32]. The ITS rDNA and LSU rDNA gene sequences were used to identify *R. griseocarnosa* sp. nov. in China [8]. Previously *R. vinosa*, a European species, had been used for this taxon. Based on these gene sequences, the basidiomes analysed in this study all showed high homology with the holotype of *R. griseocarnosa*.

Russula griseocarnosa is preferred widely by rural people in northeast Vietnam as a specialty food. Prices for fresh and dried basidiomes are estimated to reach 8-10 US\$/kg and 50-70 US\$/kg, respectively [28]. To support sustainable harvesting, further research should investigate options for forest management, and whether the edible red *Russula* can be cultivated in association with suitable host trees in home gardens.

5. CONCLUSION

Based on morphology and sequence analysis of ITS rDNA and LSU rDNA, the edible red *Russula* (Nấm chẹo) in Cao Bang, Bac Giang and Quang Ninh provinces is *Russula griseocarnosa*, which is previously described from Yunnan, China.

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ĐỊNH DANH LOÀI NẤM CHẠO THU THẬP TẠI VÙNG ĐÔNG BẮC VIỆT NAM

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TÓM TẮT

Các loài nấm hoang đã được người dân miền núi phía Bắc Việt Nam và các quốc gia lân cận thu hái làm thực phẩm và dược liệu. Những lâm sản ngoài gỗ này có vai trò quan trọng đối với sinh kế của người dân. Nhiều loài nấm không thể hình thành thể quả trên môi trường nhân tạo mà phải cộng sinh với hệ rễ của cây rừng. Một loài nấm ăn được thuộc chi *Russula* với tên gọi phổ thông là Nấm chẹo hoặc Nấm hồng cô thường được người dân ở vùng Đông Bắc sử dụng làm thực phẩm nhưng chưa được giám định loài. Nghiên cứu này đã thu thập Nấm chẹo ở tỉnh Cao Bằng, Bắc Giang và Quảng Ninh để mô tả đặc điểm hình thái. Đồng thời giải trình tự hai gen ITS rDNA & LSU rDNA và so sánh với các trình tự tham chiếu trong ngân hàng gen. Kết quả nghiên cứu đặc điểm hình thái và giải trình tự gen cho thấy loài Nấm chẹo có tên khoa học là *Russula griseocarnosa*. Đặc điểm hình thái của Nấm chẹo tương đồng với loài *R. griseocarnosa* ở Trung Quốc. Nhiều địa phương ở Việt Nam đang đẩy mạnh thương mại hóa loài Nấm chẹo. Tuy nhiên, việc khai thác quá mức đã làm suy giảm mật độ quần thể. Các nghiên cứu tiếp theo cần làm rõ đặc điểm sinh thái và kỹ thuật thu hái Nấm chẹo.

Từ khóa: nấm ăn, Nấm chẹo, nấm cộng sinh, Russula griseocarnosa.

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