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Short Communication

Scrutinize the Taxonomical Identity of Green Edible *Russula* from Sulawesi (Indonesia)

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ABSTRACT

Russula Pers. (Basidiomycota) is recognized as one of the most widely distributed macrofungi globally. This genus includes several edible species with distinct colour variations, such as *R. cyanoxantha*. However, in Indonesia, knowledge of this species remains limited as colourful mushrooms are often mistakenly considered poisonous. During a fungus expedition in Southeast Sulawesi (Indonesia), we collected a green edible wild mushroom consumed by locals. Our study aimed to verify the taxonomic classification of our specimens through morphological and molecular analysis. The fresh basidiomata were examined based on their macroscopic and microscopic characteristics. Molecular analysis using ITS 1/2 sequences was conducted to construct a phylogenetic tree. Our findings identified the green mushroom as *R. cyanoxantha* (Schaeff.) Fr. (Synonym: *R. lilacina*). Morphologically, our specimens can be distinguished from *R. virescens* by their smooth cap. They differ from *R. aeruginea* and *R. heterophylla* in that they have flexible and persistent gills. Additionally, *R. cyanoxantha* BO24636 can be separated from *R. dinghuensis* and *R. subpallidirosea* by their forked gills near the stalk. The BLAST comparison revealed a 96% similarity to *R. lilacina* from Thailand as the closest match. The phylogenetic tree (Maximum Likelihood) placed our specimens within the *R. lilacina* group (BS value 99%). Our research contributes to the taxonomic understanding of this edible wild green macrofungi in Indonesia, and future studies should explore its nutritional composition.

1. Introduction

The genus *Russula* was proposed by Persoon in 1796 and typified by *R. emetica*. Persoon and Fries initially placed *Russula* as a tribe of *Agaricus* but later elevated to the Genus level (Gray 1821). Species of *Russula* can be distinguished based on following features: mostly with colourful caps, brittle context due to abundant sphaerocytes, ornament on spore surface, having gloeocystidia, without latex on lamellae, and lacking clamp connections (Singer 1986). *Russula*, which is noted as ectomycorrhizal agaric genera, and at least 2000 species have been described worldwide (Adamčík *et al*. 2019). In addition, the GBIF (2023) listed that there are 1501 species of *Russula* with 986711 occurrences globally. In addition, Index Fungorum (2024) recorded a total of 3125 taxa of *Russula* across nations. This genus can be found in diverse habitats, including icy tundras and lush tropical rainforests (Looney *et al*. 2018). *Russula* displayed a high species diversity, and many are

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consumed, one of them is *R. cyanoxantha* (Schaeff.) Fr. (*R. lilacina*).

Index Fungorum (2024) recorded 3 taxa of *R. lilacina*, including *R. lilacina* Quél. ex Gillet (current name: *R. cyanoxantha* Schaeff.) Fr. has a range of pileus colours from purple to green. The second record is *R. lilacina* (A.H. Sm.) Trappe & T.F. Elliott, which combines the last remaining member of the genus *Macowanites* with *Russula* (Elliott and Trappe 2018). The last species is *R. lilacina* Sakolrak, Jangsantear, Sommai & Pinruan, which was recently reported from Thailand (Crouss *et al*. 2022). The name of this species is derived from the lilac hue of the cap, based on its etymology. *R. lilacina* can be determined by medium to large pileus, greyish lilac with yellowish white pileus, decurrent lamellae, globose to subglobose basidiospores with amyloid ornamentation, and minute warts (Crouss *et al.* 2022). To date, the distribution of *R. lilacina* Sakolrak, Jangsantear, Sommai & Pinruan was only known from the type locality in Chiang Mai (Thailand), in association with *Pinus merkusii* (Pinaceae).

Russula Pers. (Agaricales) is considered one of the top ectomycorrhizal mushrooms dispersed globally. However, many species of *Russula* in Indonesia are unexplored, both edible and poisonous. To date, no previous data indicated the occurrence and consumption of *R. lilacina* in Indonesia. In addition, no strain history nor herbarium collection number was noted in Indonesia. In 2023, we encountered the green basidiomata of wild *Russula*, which the local people consumed during our regular mushroom hunting held by the Indonesian mushroom hunter community in Southeast Sulawesi (Indonesia). The current study aimed to scrutinize the taxonomical identity of our green *Russula* specimens according to evidence from both morphology and molecular data.

2. Materials and Methods

2.1. Materials Collection

The samples were collected in December 2023 at Kolaka, Southeast Sulawesi, Indonesia (4° 7' 16.9932" S 122° 27' 40.4676" E), during a foraging event organized by the Indonesian mushroom hunter community (KPJI). The mushrooms were photographed in their natural habitat, and details such as coordinates, substrate, and surrounding vegetation were recorded. A portion of the samples were stored at the Herbarium Bogoriense Indonesia with the reference number BO24636.

2.2. Light Microscope Observations

The study analyzed the morphological characteristics of fresh basidiomata both in their natural habitat and in the Mycology Laboratory, Department of Biology, IPB University. Various macromorphological features such as colour, size, pileus, stipe ornamentation, margin, and lamellae were carefully observed. Microscopic details, including basidium, cystidia, spores (shape, size, colour, ornamentation), and clamp connection, were examined using a light microscope.

2.3. Scanning Electron Microscope Observations

Specimens were also examined using scanning electron microscopy (SEM) following established protocols (Goldstein *et al*. 1992) in the ILAB BRIN, Bogor, Indonesia. Lamellae samples were prepared by cutting them into small pieces, pre-fixing them in glutaraldehyde, tannic acid, and cacodylate buffer, dehydrating them, infiltrating them with t-butanol, freeze-drying them, mounting them on aluminum stubs, coating them with gold, and observing them using the JSM IT 200 SEM system. The specimens were identified based on relevant reference materials (Singer 1945; Kuo 2009; Crous *et al*. 2022).

2.4. Molecular Analyses

Fresh basidiomata were utilized for the isolation of DNA, which was then extracted following the protocol of a Qiagen Dneasy Plant Mini Kit. A Thermo Scientific Arktik Thermal Cycler from ThermoFisher-Scientific was employed for DNA amplification. The PCR amplification was done with ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers (White *et al*. 1990). The PCR amplification was performed in a 40 µL total reaction containing 12 µL ddH_2O, 2 µL of 10 pmol of each primer, 20 µL PCR mix from 2× Kappa Fast 2G, and 4 µL 100 ng template DNA. The PCR condition was set as follows: initial denaturation at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 45 seconds, and extension at 72 °C for 1 minute. The final extension was set at 72 °C for 10 minutes (Putra *et al.* 2024). The PCR product was then analyzed using agarose gel electrophoresis and visualized with FloroSafe DNA stain. Finally, the PCR products were sent to 1st Base Malaysia for sequencing.

The sequences were assembled using ChromasPro software and deposited in GenBank to receive an accession number. The sequence was then compared with previous data using the Basic Local Alignment Search Tool (BLAST) on NCBI. The results were analyzed for taxonomic matches based on the BLASTN result, which showed the highest sequence similarity. Selected sequences (Table 1) from the BLAST results of this study, as well as *Russula* sequences from a previous study (Song *et al*. 2018), were used to create a phylogenetic tree. Certain *Russula* species were used as the outgroup. The phylogenetic tree was constructed using the maximum likelihood (ML) method with MEGA X software (Kumar *et al*. 2018). Bootstrap 1000 replications were used to test the strength of the internal branches. Default parameters in MEGA X software were used for the ML analysis, and Bootstrap values of 50% or higher were displayed on the tree.

Table 1. Species, outgroup, herbarium voucher, and GenBank accession numbers used in this study

Spesies	Voucher/strain	Accesion no.	Reference	Location
Russula aeruginea	Nl1292	UDB000341	Song *et al*. 2018	Germany
R. albidogrisea	GDGM 48783	KY767807	Song *et al*. 2018	China
R. alboareolata	SUT-1	AF345247	Song *et al*. 2018	Thailand
R. atroaeruginea	HKAS53626	JX391967	Song *et al*. 2018	China
R. aureoviridis	GDGM 48786	KY767809	Song *et al*. 2018	China
R. crustosa	BB2004-214, PC	EU598193	Song *et al*. 2018	USA
R. cyanoxantha	HKAS78376	KF002766	Song *et al*. 2018	China
R. cyanoxantha	RUS24	AY061669	Song *et al*. 2018	Europe
R. dinghuensis	K15052704-3	KU863581	Song *et al*. 2018	China
R. grisea	Watling27098, E	AY061679	Song *et al*. 2018	Europe
R. heterophylla	Hue103 (TUB)	AF418609	Song *et al*. 2018	Germany
R. heterophylla	Buyck99.803, PC	AY061681	Song *et al*. 2018	Europe
R. ilicis	Sarnari10/18/99	AY061682	Song *et al*. 2018	Europe
R. indoalba	AG15-628	KX234820	Song *et al*. 2018	India
R. ionochlora	BB72-407	HM189875	Song *et al*. 2018	Europe
R. kanadii	CAL1162	KJ866933	Song *et al*. 2018	India
R. nigrovirens	HKAS55222	KP171173	Song *et al*. 2018	China
R. pallidirosea	UTC00274382	KR831283	Song *et al*. 2018	USA
R. shingbaensis	KD11-094	KM386692	Song *et al*. 2018	India
R. subpallidirosea	GDGM 45242	KU863582	Song *et al*. 2018	China
R. variata	JMP0078	EU819436	Song *et al*. 2018	USA
R. verrucospora	GDGM 71136 (holotype)	MG786052	Song *et al*. 2018	China
R. verrucospora	GDGM 71140	MG786053	Song *et al*. 2018	China
R. verrucospora	GDGM 71137	MG786054	Song *et al*. 2018	China
R. vesca	Buyck99.802, PC	AY061723	Song *et al*. 2018	Europe
R. vesca	AT2002091, UPS	DQ422018	Song *et al*. 2018	Europe
R. virescens	Buyck99.808, PC	AY061727	Song *et al*. 2018	Europe
R. werneri	IB1997/0786	DQ422021	Song *et al*. 2018	Europe
R. xanthovirens	GDGM 71145	MG786055	Song *et al*. 2018	China
R. granulata	BB2004-226, PC	EU598192	Song *et al*. 2018	USA
R. granulata	BB2004-228, PC	EU598189	Song *et al*. 2018	USA
R. pectinatoides	MICH52692	KF245518	Song *et al*. 2018	USA
R. archaea	IS79	AY061737	Song *et al*. 2018	Europe
R. camarophylla	IS68	AY061662	Song *et al*. 2018	Europe
R. densifolia	Ue116 (TUB)	AF418606.1	Song *et al*. 2018	Europe
R. nigricans	Fo46792 (TUB)	AF418607	Song *et al*. 2018	Europe
R. nigricans	MC01-511	AM113962	Song *et al*. 2018	Europe
R. lilacina	MMCR00191	MT940809	Song *et al*. 2018	Thailand
R. cyanoxantha	BO24636	ITSPP210105	This study	Indonesia

3. Results

3.1. Taxonomy

Russula cyanoxantha (Schaeff.) Fr., Monogr. Hymenomyc. Suec. (Upsaliae) 2(2): 194 (1863) Figs. 1-5

Synonym:

Agaricus cyanoxanthus Schaeff., Fung. bavar. palat. nasc. (Ratisbonae) 4: 40 (1774)
Agaricus pectinatus var. cyanoxanthus (Schaeff.) Duby, Bot. Gall., Edn 2 (Paris) 2: 843 (1830)
Russula cutefracta Cooke, Grevillea 10(no. 54): 46 (1881)
Russula cyanoxantha f. atroviolacea J.E. Lange, Dansk bot. Ark. 9(no. 6): 99 (1938)
Russula cyanoxantha f. cutefracta (Cooke) Sarnari, Boll. Assoc. Micol. Ecol. Romana 10(no. 28): 35 (1993)
Russula cyanoxantha f. pallida Singer, Z. Pilzk. 2(1): 4 (1923)
Russula cyanoxantha f. peltereaui Singer, Z. Pilzk. 5(1): 15 (1925)
Russula cyanoxantha var. cutefracta (Cooke) Sarnari, Boll. Assoc. Micol. Ecol. Romana 9(no. 27): 38 (1992)
Russula cyanoxantha var. flavoviridis (Romagn.) Sarnari, Boll. Assoc. Micol. Ecol. Romana 9(no. 27): 41 (1992)
Russula cyanoxantha var. subacerba Reumaux, in Reumaux, Bidaud & Moënne-Loccoz, Russules Rares ou Méconnues (Marlioz): 284 (1996)
Russula cyanoxantha var. variata (Banning) Singer, Feddes Repert. Spec. Nov. Regni veg. 33: 351 (1934)
Russula flavoviridis Romagn., Bull. mens. Soc. linn. Lyon 31(6): 175 (1962)
Russula lilacina Quél. Ex Gillet, Tabl. analyt. Hyménomyc. France (Alençon): 48 (1884)
Russula peltereaui (Singer) Moreau, Bull. trimest. Féd. Mycol. Dauphiné-Savoie 140: 7 (1996)
Russula variata Banning, Bot. Gaz. 6(1): 166 (1881)
Russula vesca var. cyanoxantha (Schaeff.) Fr., Summa veg. Scand., Sectio Post. (Stockholm): 311 (1849)

Fruiting bodies are solitary (Figure 1A). Pileus (Figure 1B) convex to applanate, medium to large, 5–6 cm diameter, smooth on the surface, slightly moist, somewhat depressed at center, green to olive green from the center to margin, shades mottled concolorous with pileus, darker near margin, with some shades of brown in irregular manners. Pileus is unchanging when bruised. Lamellae (Figure 1C) white to cream, dense, rarely forked, margin entire annexed to slightly decurrent. Stem 0.7-1.2 × 2.6-4.5 cm, center, cylindric to tapering downward, without ornamentation, dry, unchanging when injured. Basidiospores (Figure 3D) globose to subglobose, prominent minute warts on the surface and indentation on another side when observed with SEM (Figure 4), 3.5-6.7 × 2.7-5.4 µm, hyaline. Basidia (Figure 3A) clavate, 20-23 × 8-10 µm, sterigmata 2.5-5.4 µm in length, mostly 4-spored with some basidia showed 2-3 sterigmata. Trama is mainly composed of sphaerocysts (Figure 5B), 12-29 µm diam. Clamp connection absent. Hymenial cystidia (Figure 3B-C) present, clavate. Pileipellis (Figure 5A) is composed of thin-walled and septate interwoven hyphae, oleiferous hyphae can be observed. Habitat: solitary to gregarious on soil. Note: considered a delicacy by local people at the sampling site (Figure 2A-D).

Specimen examined: South Konawe, Southeast Sulawesi, Indonesia, 4° 7' 16.9932" S 122° 27' 40.4676" E, 56 m a.s.l, near *Castanopsis buruana* Miq., BO24636.

3.2. Molecular Analyses

The final aligned sequence was submitted to GenBank with the registration number ITS PP210105. The BLAST results displayed that specimen BO24636 posed a similarity to *R. cyanoxantha* and *R. lilacina* as the top hits. The ITS 1/2 gene-based phylogenetic tree grouped specimen BO24636 with *R. lilacina* in the same clade with a high bootstrap value of 99%. *R. lilacina* MMCR00191 (specimen from Thailand) was closely related to material BO24636 (Figure 6).

4. Discussion

Russula Pers. is known for its wide variety of taxa found across the globe (Kalichman *et al*. 2020), and many species remain to be re-examined in many countries. The comprehensive taxonomical data on the genus *Russula* in Indonesia is paid less. In addition, the knowledge of many species of edible *Russula* in Indonesia was scarce, and *R. cyanoxantha* is not an exception. *R cyanoxantha* posed a wide range of pileus colour (Kuo 2009). In Indonesia, this species was previously reported with violet pileus from the tropical rain forest of East Kalimantan (Retnowati 2007), primary and secondary forest at West Java, respectively (Putra and Nurhayat 2022; Haryadi *et al*. 2023). The current work reports for the first time *R. cyanoxantha* with greenish pileus for Indonesia. In addition, our

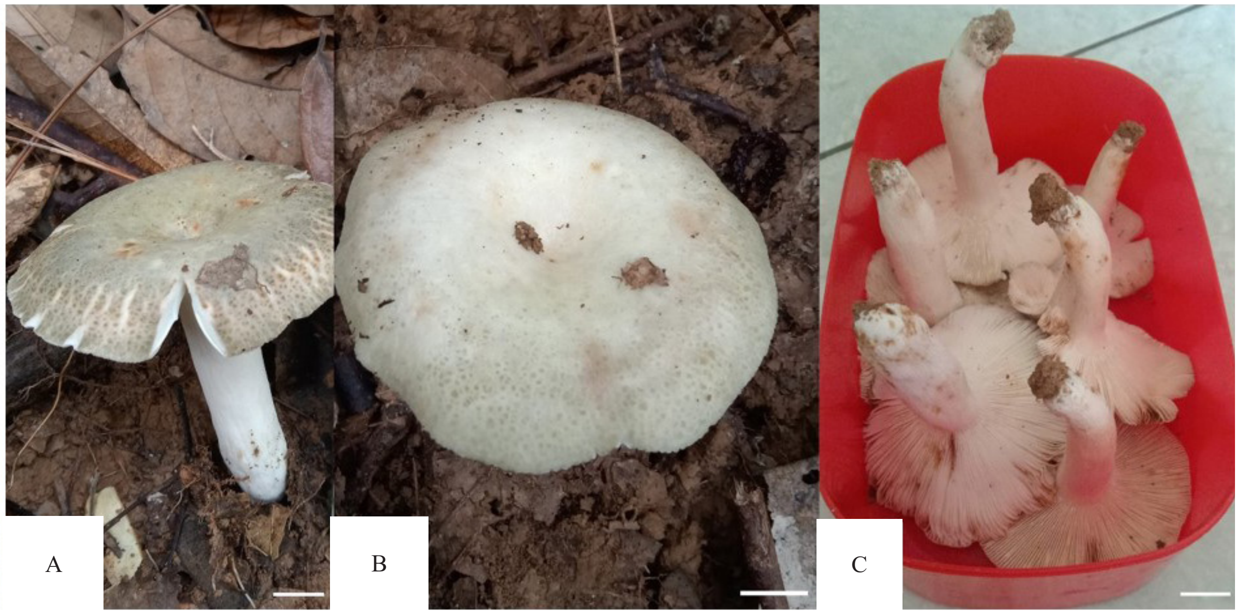

Figure 1. Macroscopic characters of *Russula cyanoxantha* BO24636. A-B: Upper side of pileus. B: Lamellae. Bars = 1 cm

Figure 2. The consumption of *Russula cyanoxantha* BO24636 by the local community. A: Fresh basidiomata obtained from the field. B: The fruiting bodies were boiled for several minutes. C-D: The mushrooms were then cooked

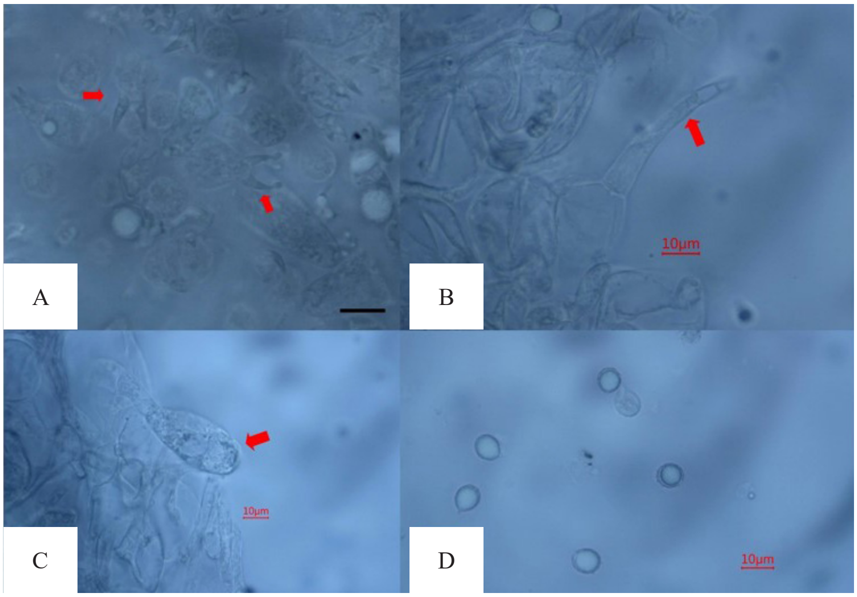

Figure 3. Microscopic characters of *Russula cyanoxantha* BO24636. A: Basidia. B: Cuticle cystidia. C: Cystidia. D: Basidiospores

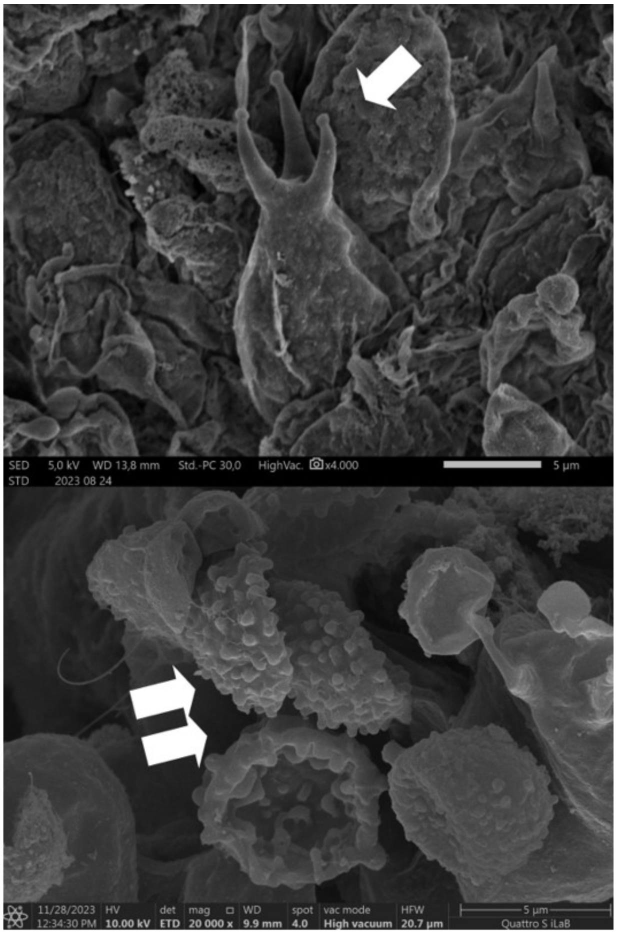

Figure 4. Scanning electron microscope image of *Russula cyanoxantha* BO24636. Basidium (arrow). Basidiospore with amyloid ornamentation (double arrow)

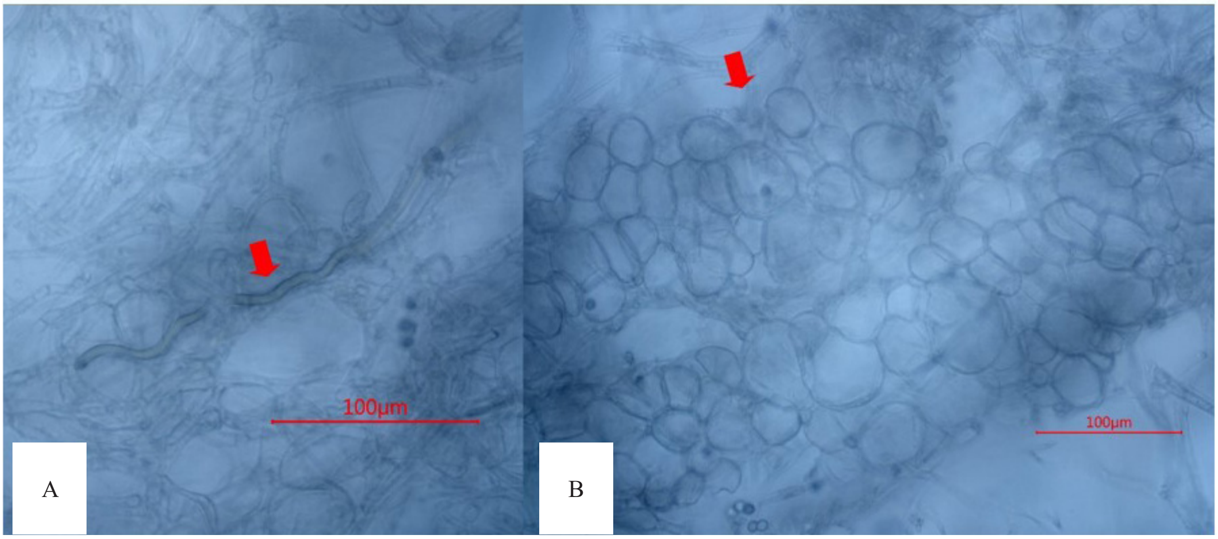

Figure 5. Microscopic characters of *Russula cyanoxantha* BO24636. A: Pileipellis with oleiforeous hyphae (arrow). B: Sphaerocycts on trama (arrow)

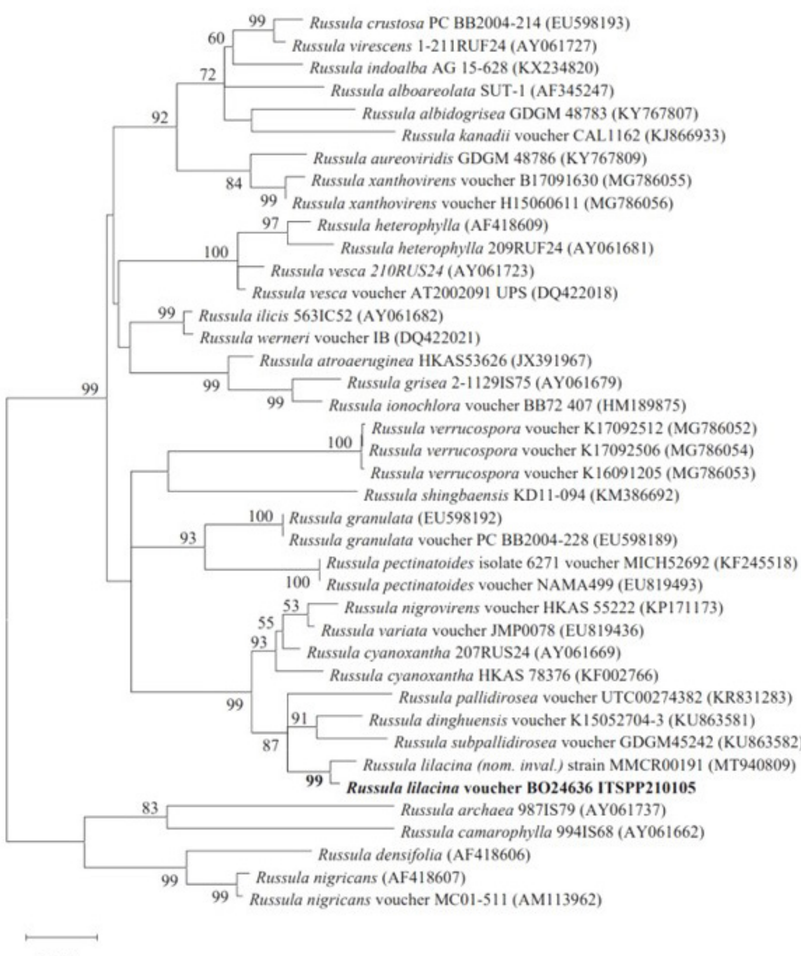

Figure 6. The phylogenetic tree of *Russula* BO24636 based on ITS1/2 region using maximum likelihood method and 1000 bootstrap analysis. Our specimen is bold on the phylogenetic tree

work expanded the distribution information of this species in another location (secondary forest, Southeast Sulawesi) in Indonesia. The genus *Russula* is noted as ectomycorrhizal mushroom-forming fungi. However, the host plant of *R. cyanoxantha* BO24636 remains unclear. The basidiomata can be found near *Castanopsis* at the sampling site. Many ectomycorrhizal are gourmet mushrooms, including *Russula* spp. (Wang *et al*. 2015).

Our initial field identification suggested that our specimens were either *R. virescens* or *R. cyanoxantha* as they posed a green pileus. The smooth cap of our specimens sets them apart from *R. virescens.* Furthermore, the cap of the former species had a green-coloured cuticle with patches of the same colour arranged in a radial pattern around the center in an areolate pattern (McKnight and McKnight 1998). *R. cyanoxantha* often has a multicolored pileus with twelve current recorded taxa (Index Fungorum 2024). Of those species, our specimens are morphologically similar to *R. cyanoxantha* var. peltereaui with green smooth cap. Other green caps morphologically resemble our specimens *R. aeruginea* and *R. heterophylla*. Our materials can be separated from those species that have flexible gills, which pertain to young to mature basidiomata (Cullington 2004). Moreover, *R. cyanoxantha* BO24636 delimited from *R. dinghuensis* and *R. subpallidirosea* by posing a forked of lamellae only near the stipe following the description of Zhang *et al*. (2017). The spore dimension of our specimens was smaller than those reported by Cullington (2004). The cap cuticle of *R. cyanoxantha* BO24636 was different in morphology compared to materials described by Cullington (2004). In the current study, we noted the oleiferous hyphae in the pileipellis cross-section.

Russula is considered one of the most diverse and complex groups of macrofungi (Buyck *et al*. 2018). Therefore, we combine the morphological and molecular analyses in this study. The comparison between our material sequence and database on GenBank via BLAST revealed that specimen BO24636 was similar to *R. cyanoxantha* and *R. lilacina* as the top hits. Interestingly, the analysis of ITS 1/2 genes revealed that our specimen was highly similar to *R. lilacina* MMCR00191 from Thailand, with a 99% bootstrap support value; however, it had a notably different length of the branch. Index Fungorum (2024) records three taxa of *R. lilacina*: *R.lilacina* Quél. ex Gillet 1884, *R. lilacina* (A.H. Sm.) Trappe & T.F. Elliott 2022, and *R. lilacina* Sakolrak, Jangsantear, Sommai & Pinruan 2022. Of those taxa, *R.lilacina* Quél. ex Gillet 1884 is now noted for *R. cyanoxantha* (Schaeff.) Fr. as the current name (Index Fungorum 2024). For the time being, we consider our specimens to be *R. cyanoxantha* var. peltereaui because only a single gene was used in this study.

Russula is one of the most popular wild edible mushrooms for food in ASEAN region (Manassila *et al*. 2005; Anh *et al*. 2023). In the sampling site, *R. cyanoxantha* BO24636 is considered a choice for self-consumption, but the information is limited to a few local people. They usually collected the fruiting bodies around October to December in the rainy season. The mushroom hunters report that the fruiting bodies usually appear in medium to high density on the soil of secondary forests. Previously, Retnowati (2007) reported the violet-brown *R. cyanoxantha* from East Kalimantan (local name: kulat deriyan), which is noted as a delicacy mushroom. The local people in East Kalimantan usually cook mushrooms by stir-frying them with soy sauce. In the current study, the local people usually boil the basidiomata first before stirring it with a lot of water to prepare a soupy dish. Another report of this species in Indonesia was provided by Haryadi *et al*. (2023). However, there is no information regarding the edibility of the mushroom. Considering the edibility of *R. cyanoxantha* BO24636, our future study will be focused on the analysis of the nutritional status and the cultivation efforts of this wild mushroom in Indonesia.

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