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The molecular phylogeny and morphology revealed a new wood-rotting fungus *Vararia yunnanensis* (Peniophoraceae, Russulales) in Yunnan Province, China

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Abstract

Vararia yunnanensis sp. nov. is proposed based on a combination of morphological features and molecular evidence. *Vararia yunnanensis* is characterized by the resupinate basidiomata with a cracking hymenial surface, a dimitic hyphal system with generative hyphae bearing simple-septa, and colorless, slightly thick-walled, smooth, amyloid basidiospores measuring $5-12 \times 3.5-9 \mu m$. The phylogenetic analyses of ITS+nLSU in Russulales showed that *V. yunnanensis* nested into the family Peniophoraceae, in which *V. yunnanensis* grouped within the genus *Vararia*. Furthermore, the ITS sequence data indicated that the new species is sister to *Vararia ellipsospora*.

Keywords: 1 new species, Basidiomycetes, Molecular phylogeny, Taxonomy, Peniophoraceae, Wood-inhabiting fungi

Introduction

Vararia P. Karst. (1888: 2), typified by *V. investiens* (Schwein.) P. Karst. (1888: 2), is a corticioid wood-inhabiting fungal genus with a wide distribution (Bernicchia & Gorjón 2010). The genus is characterized by the resupinate basidiomata, a dimitic hyphal structure with simple-septate or clamped generative hyphae and often dextrinoid dichohyphae in Melzer's reagent, the presence of gloeocystidia, and variously shaped smooth basidiospores with or without an amyloid reaction (Karsten 1898, Boidin & Lanquetin 1975, Boidin 1980, Bernicchia & Gorjón 2010). Index Fungorum (http://www.indexfungorum.org, accessed on 11 Sep 2022) registers 96 specific and infraspecific names in *Vararia* (Boidin & Lanquetin 1987, Stalpers 1996, Larsson & Larsson 2003, Bernicchia & Gorjón 2010, Liu & He 2016).

Molecular studies involving *Vararia* based on single-gene or multi-gene datasets have been carried out by Larsson & Larsson (2003). Inferred from a combined dataset of ITS and nLSU sequence data of Peniophoraceae revealed that *Vararia* formed a monophyletic lineage and then grouped with related species of *Scytinostroma* Donk (1956: 19) (Liu *et al.* 2017). Reclassification of *Parapterulicium* Corner (1952: 285) (Pterulaceae, Agaricales), contributions to Lachnocladiaceae and Peniophoraceae (Russulales) indicated that the taxa of *Vararia* clustered into Varariaceae, in which they grouped with related genera, *Dichostereum* Pilát (1926: 223) and *Parapterulicium* (Leal-Dutra *et al.* 2018). The phylogenetic analyses based on ITS and nLSU sequences within the Peniophoraceae clade in the Russulales revealed that the genera *Vararia* and *Scytinostroma* are of polyphyletic origin, while the genus *Dichostereum* and *Asterostroma* Massee (1889: 154) are of monophyletic origin (Liu *et al.* 2017).

We collected basidiomata of an undescribed taxon from Yunnan Province, P. R. China. We present morphological and molecular phylogenetic evidence based on the internal transcribed spacer (ITS) region and the large subunit nuclear ribosomal RNA gene (nLSU) sequences that support the recognition of the new species, *Vararia yunnanensis*.

Materials and methods

Morphological studies

The specimens are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological descriptions were based on field notes. Color terms followed Petersen (1996). All the materials were examined under a Nikon 80i microscope. The measurements and drawings were made from slide preparations stained with cotton blue (0.1 mg aniline blue dissolved in 60 g pure lactic acid), Melzer's reagent (1.5 g potassium iodide, 0.5 g crystalline iodine, 22 g chloral hydrate, aq. dest. 20 mL) and 5% potassium hydroxide. Spores were measured from sections cut from the hymenial layer, and in presenting spore size data, 5% of the measurements excluded from each end of the range are shown in parentheses. The following abbreviations were used: KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

DNA extraction, PCR amplification, sequencing and phylogenetic analyses

A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens according to the manufacturer's instructions that were slightly modified by grinding a small piece of the dried fungal specimen (30 mg) to powder with liquid nitrogen. The powder was transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 mL phenol-chloroform (24:1) was added to each tube, and the suspension was shaken vigorously. After centrifugation at 13 thousand rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer. The mixture was then transferred to an Adsorbing Column (AC) for centrifugation at 13, 000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added to the AC for centrifugation at 12 thousand rpm for 0.5 min. After washing twice with 0.5 mL washing buffer, the AC was transferred to a sterility centrifuge tube, and 100 mL elution buffer was added to the middle of the adsorbed film to elute the genome DNA. The internal transcribed spacer region (ITS) was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). The nuclear large subunit region (nLSU) was amplified with primer pairs LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna primers for fungi/). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min; and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 48 °C 1 min, and 72 °C for 1.5 min; and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at the Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, People's Republic of China. All newly generated sequences were deposited in GenBank (Table 1).

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Species name	Sample no.	GenBank ac	cession no.	References
		ITS	nLSU	
Asterostroma bambusicola	He 4132	KY263865	KY263871	Unpublished
A. cervicolor	TMI: 21362	AB439560	AB439560	Suhara et al. 2010
A. cervicolor	KHL9239	AF506408	AF506408	Unpublished
A. laxum	EL 33-99	AF506410	AF506410	Larsson & Larsson 2003
A. macrosporum	TMI: 25696	AB439544	AB439544	Suhara et al. 2010
A. macrosporum	TMI: 25697	AB439545	AB439545	Suhara et al. 2010
A. muscicola	TMI: 25860	AB439551	AB439551	Suhara et al. 2010
Baltazaria galactina	CBS 752.86	MH862034	MH862034	Liu 2019
B. galactina	CBS: 753.86	MH862035	MH862035	Vu et al. 2019
B. neogalactina	CBS 755.86	MH862037	MH862037	Liu 2019
B. neogalactina	CBS: 758.86	MH862040	MH862040	Vu et al. 2019
Confertobasidium olivaceoalbum	FP90196	AF511648	AF511648	Larsson & Larsson 2003

TABLE 1. List of species, specimens, and GenBank accession numbers of sequences used in this study.

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TABLE 1 (Continued)

Species name	C1	GenBank accession no.		ЪС
	Sample no.	ITS	nLSU	KUEIEIIUES
Dichostereum boidinii	He 4410	MH538315	MH538331	Liu & He 2018
D. boidinii	He 5026	MH538324	MH538330	Liu & He 2018
D. pallescens	CBS: 719.81	MH861457	MH861457	Vu et al. 2019
D. pallescens	CBS: 718.81	MH861456	MH861456	Liu & He 2018
Lachnocladium schweinfurthianum	KM 49740	MH260033	MH260051	Liu 2019
Metulodontia nivea	NH13108	AF506423	AF506423	Larsson & Larsson 2003
Michenera artocreas	GHL-2016-Oct	MH204688	MH204692	Liu et al. 2019
Peniophora cinerea	Не 3725	MK588769	MK588769	Unpublished
P. cinerea	CBS: 261.37	MH855905	MH855905	Vu et al. 2019
P. incarnata	CBS 430.72	MH860518	MH860518	Vu et al. 2019
P. incarnata	NH 10271	AF506425	AF506425	Larsson & Larsson 2003
P. quercina	CBS 407.50	MH856687	MH856687	Liu 2019
P. quercina	CBS: 410.50	MH856690	MH856690	Vu et al. 2019
P. nuda	LZ 15-07	MT859929	MT859929	Hibbett et al. 2000
Scytinostroma alutum	CBS 763.81	MH861483	MH861483	Liu 2019
S. alutum	CBS: 766.81	MH861486	MH861486	Vu et al. 2019
S. duriusculum	CBS 757.81	MH861477	MH861477	Liu 2019
S. duriusculum	CBS: 758.81	MH861478	MH861478	Vu et al. 2019
S. portentosum	EL 11-99	AF506470	AF506470	Larsson & Larsson 2003
Vararia abortiphysa	CBS 632.81	MH861387	-	Vu et al. 2019
V. ambigua	CBS 634.81	MH861388	MH861388	Liu 2019
V. amphithallica	CBS 687.81	MH861431	-	Vu et al. 2019
V. aurantiaca	CBS 641.81	MH861393	-	Vu et al. 2019
V. aurantiaca	CBS 642.81	MH861394	-	Liu 2019
V. breviphysa	CBS 644.81	MH861396	-	Vu et al. 2019
V. calami	CBS 646.81	MH861398	-	Vu et al. 2019
V. calami	CBS 648.81	MH861399	-	Vu et al. 2019
V. callichroa	CBS 744.91	MH874000	-	Liu 2019
V. cinnamomea	CBS 641.84	MH861794	-	Vu et al. 2019
V. cinnamomea	CBS 642.84	MH873488	-	Liu 2019
V. cremea	CBS 651.81	MH873147	-	Vu et al. 2019
V. dussii	CBS 652.81	MH873148	-	Liu 2019
V. dussii	CBS 655.81	MH861405	-	Vu et al. 2019
V. ellipsospora	HHB-19503	MW740328	MW740328	Unpublished
V. fusispora	PDD: 119539	OL709443	-	Unpublished
V. gallica	CBS 656.81	MH861406	MH861406	Vu et al. 2019
V. gallica	CBS 234.91	MH862250	MH873932	Vu et al. 2019
V. gillesii	CBS 660.81	MH873153	-	Liu 2019
V. gomezii	CBS 661.81	MH873154	-	Liu 2019
V. gracilispora	CBS 663.81	MH861411	-	Vu et al. 2019
V. gracilispora	CBS 664.81	MH861412	-	Vu et al. 2019
V. insolita	CBS 668.81	MH861413	-	Vu et al. 2019
V. intricata	CBS 673.81	MH861418	-	Vu et al. 2019
V. investiens	FP-151122	MH971976	-	Liu 2019

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TABLE 1 (Continued)

Species name	Sample no.	GenBank accession no.		Defenences
		ITS	nLSU	Kelerences
V. malaysiana	CBS 644.84	MH873490	-	Liu 2019
V. minispora	CBS 682.81	MH861426	-	Vu et al. 2019
V. ochroleuca	CBS 465.61	MH858109	-	Liu 2019
V. ochroleuca	JS24400	AF506485	-	Liu 2019
V. parmastoi	CBS 879.84	MH861852	-	Vu et al. 2019
V. pectinata	CBS 685.81	MH861429	-	Liu 2019
V. perplexa	CBS 695.81	MH861438	-	Vu et al. 2019
V. pirispora	CBS 720.86	MH862016	-	Liu 2019
V. rhombospora	CBS 743.81	MH861470	-	Vu et al. 2019
V. rosulenta	CBS 743.86	MH862028	-	Liu 2019
V. rugosispora	CBS 697.81	MH861440	-	Liu 2019
V. sigmatospora	CBS 748.91	MH874001	-	Liu 2019
V. sphaericospora	CBS 700.81	MH873185	-	Liu 2019
V. sphaericospora	CBS 703.81	MH861446	-	Vu et al. 2019
V. trinidadensis	CBS 650.84	MH873495	-	Liu 2019
V. trinidadensis	CBS 651.84	MH861803	-	Vu et al. 2019
V. tropica	CBS 704.81	MH861447	MH873189	Liu 2019
V. vassilievae	UC2022892	KP814203	-	Unpublished
V. verrucosa	CBS 706.81	MH861449	-	Vu et al. 2019
V. yunnanensis	CLZhao 18283	ON454116	ON502653	Present study
V. yunnanensis	CLZhao 17725	ON454115	ON502654	Present study

The sequences were aligned in MAFFT version 7 (Katoh *et al.* 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). Sequences of *Confertobasidium olivaceoalbum* (Bourdot & Galzin) Jülich (1975: 167) and *Metulodontia nive* (P. Karst.) Parmasto (1968: 118) retrieved from GenBank were used as outgroups in the ITS+nLSU (Fig. 1) analysis following Leal-Dutra *et al.* (2018). Sequences of *Peniophora incarnata* (Pers.) P. Karst. (1889: 27) and *Peniophora nuda* (Fr.) Bres. (1897: 114) retrieved from GenBank were used as outgroups in the ITS+nLSU (Fig. 2) analysis following Leal-Dutra *et al.* (2018).



FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of two new species of *Vararia* and related species in Peniophorales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values equal to or higher than 70%, parsimony bootstrap values equal to or higher than 50% and Bayesian posterior probabilities equal to or higher than 0.95.



FIGURE 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of two new species and related species in *Vararia* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap values equal to or higher than 70%, parsimony bootstrap values equal to or higher than 50% and Bayesian posterior probabilities equal to or higher than 0.95. The new species are in bold.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analyses followed Zhao & Wu (2017), and the tree construction was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian Inference (BI). BI was calculated with MrBayes 3.2.7a with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees, for 500,000 generations (Fig. 1) and 1,900,000 generations (Fig. 2) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received a maximum likelihood bootstrap (BS) \geq 70%, maximum parsimony bootstrap (BT) \geq 50%, or Bayesian posterior probabilities (BPP) \geq 0.95.

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 37 fungal specimens representing 24 species among *Vararia* and related genera. The dataset had an aligned length of 2225 characters, of which 1346 characters were constant, 190 were variable and parsimony-uninformative, and 689 were parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious trees (TL = 2769, CI = 0.5432, HI = 0.4568, RI = 0.6933, RC = 0.3766). The best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.004514 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 156. The Bayesian tree is shown here.

The ITS dataset included sequences from 48 fungal specimens representing 38 taxa. The dataset had an aligned length of 879 characters, of which 295 characters are constant, 123 are variable and parsimony-uninformative, and 461 are parsimony-informative. Maximum parsimony analysis yielded 5 equally parsimonious tree (TL = 3826, CI = 0.3116, HI = 0.6884, RI = 0.3984, RC = 0.1241). The best model for ITS estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The bayesian analysis resulted in a similar topology with an average standard deviation of split frequencies = 0.009769 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 696. The Bayesian tree is shown here (Fig. 2). The phylogeny (Fig. 2) based on the ITS sequences demonstrated that *Vararia yunnanensis* was sister to *V. ellipsospora* (Bourdot & Galzin) Liberta (1966: 319).

The phylogenetic tree (Figs. 1, 2) inferred from ITS+nLSU sequences revealed that the new taxa are nested in *Vararia* and grouped with *V. ellipsospora*.

Taxonomy

Vararia yunnanensis Y.L. Deng & C.L. Zhao, sp. nov. Figs. 3, 4 MycoBank no.: MB 844043

Holotype:—CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, E 103°30'10", N 23°42'07", elev. 1500 m, on fallen branch of angiosperm, 1 August 2019, CLZhao 17725 (SWFC!), GenBank No. (ITS ON454115; nLSU ON502654).

Etymology:--yunnanensis refers to the location "Yunnan Province" where the type specimen was collected.

Diagnosis:—This species is characterized by the resupinate basidiomata with a cracking hymenial surface, a dimitic hyphal system with generative hyphae bearing simple-septa, and colorless, slightly thick-walled, smooth, amyloid basidiospores measuring $5-12 \times 3.5-9 \mu m$.

Basidiomata:—Basidiomata annual, adnate, resupinate, without odor or taste when fresh, up to 8 cm long, 4 cm wide, $80-100 \mu$ m thick. Hymenial surface smooth, cream (4A2/3), cracking with age. Sterile margin thin, cream (4A2/3).

Hyphal structure:—Hyphal system dimitic, *generative hyphae* bearing simple-septa, dichohyphae cream (4A2/3), capillary, thick-walled, dichotomously to irregularly branched with main branches and acute tips, weakly to moderately dextrinoid in Melzer's reagent, CB–, tissues unchanged in KOH. Subiculum composed of colorless, generative hyphae rarely branched, slightly thick-walled, 1.5–2.5 μ m in diam., dichohyphae predominate, frequently branched, 1.5 μ m diameter. Subhymenium composed of colorless, generative hyphae rarely branched, slightly thick-walled, 1–2 μ m diameter; dichohyphae predominate, frequently branched, 0.5–1.5 μ m diameter;

Hymenium:—Gloeocystidia various with three types, (i) subcylindrical cystidia, colorless, thin- to slightly thick-walled, smooth, tapered or gradually elongated apex, $16.5-58.5 \times 4-10 \mu m$; (ii) tapering cystidia, usually with a constriction at the tip, colorless, thin- to slightly thick-walled, smooth, $27.5-42 \times 5.5-9 \mu m$; (iii) fusiform cystidia, colorless, thin- to slightly thick-walled, smooth, $18.5-43.5 \times 7-9 \mu m$. Basidia cylindrical, thin-walled, with four sterigmatas and a basal simple septum, $17.5-32 \times 5-9.5 \mu m$; basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores:—Ellipsoid, colorless, slightly thick-walled, smooth, with oil drops, amyloid, CB–, $(5.1-)5.9-11.5(-11.8) \times (4.3-)4.7-8.6(-9) \mu m$, L = 9.14 μm , W = 6.35 μm , Q = 1.4 (n = 60/2).



FIGURE 3. Basidiomata of Vararia yunnanensis (CLZhao 17725). Bars: A = 0.5 cm; B = 0.5 mm. Photos by: Ying-Lian Deng.

Additional specimen (paratype) examined:—CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, E 103°30'10", N 23°42'07", elev. 1500 m, on fallen angiosperm branch, 2 August 2019, CLZhao 18283 (SWFC!), GenBank No. (ITS ON454116, nLSU ON502653).

Discussion

Corticioid fungi are an extensively studied group of Basidiomycota (Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Dai *et al.* 2015, Fan *et al.* 2021, Jiang *et al.* 2021, Wu *et al.* 2022, Wu *et al.* 2022a) and *Vararia* is a group of 'corticioid fungi' (Liu & He 2016). The family Peniophoraceae includes several closely related genera such as *Asterostroma, Peniophora* Cooke (1879: 20), *Scytinostroma* and *Vararia* in which all the genera found in Europe and America (Bernicchia & Gorjón 2010). The genus *Dichostereum* was once treated as a subgenus of *Vararia* since the two genera are very similar in morphology, except that the species of *Vararia* has smooth basidiospores (Boidin 1967, Parmasto 1970, Lanquetin 1973). However, recent molecular and morphological studies showed that two species of *Parapterulicium* Corner with coralloid basidiomata belonging to Peniophoraceae in the Russulales rather than Pterulaceae of the Agaricales (Leal-Dutra *et al.* 2018).



FIGURE 4. Microscopic structures of *Vararia yunnanensis* (drawn from the holotype). A: Basidiospores. B: Basidia and basidioles. C: Subcylindrical cystidia. D: Tapering cystidia. E: Fusiform cystidia. F: Skeletal mycelium. G: A section of hymenium. Bars: $A-G = 10 \mu m$. Drawings by: Ying-Lian Deng.

Many recently described wood-inhabiting fungi taxa have been reported in the subtropics and tropics, including those of the genus *Vararia* (Liu & He 2016). In the present study, a new species, *Vararia yunnanensis* is described based on phylogenetic analyses and morphological characteristics.

A phylogeny of corticioid species in the Russulales revealed that eight genera clustered in the Peniophoraceae (Leal-Dutra *et al.* 2018). In this study, the new species clustered in the genus *Vararia* (Fig. 1) within the Russulales and three species of *Vararia viz., V. ambigua* Boidin, Lanq. & Gilles (1980: 286), *V. Gallica* (Bourdot & Galzin) Boidin (1951: 78) and *V. tropica* A.L. Welden (1965: 516) grouped together. Furthermore, phylogeny within the *Vararia* showed that *V. yunnanensis* is sister to *V. ellipsospora* and is closely grouped with *V. ambigua* and *V. gallica* based on the ITS sequence data (Fig. 2). However, morphologically, *V. ellipsospora* differs by the fimbriate basidiomata, clamped generative hyphae, and flexuous-cylindrical gloeocystidia (Cunningham 1955).

Morphologically, Vararia amphithallica Boidin, Lang. & Gilles (1980: 288), V. rugosispora Boidin, Lang. & Gilles (1980: 328) and V. yunnanensis have similar, slightly thick-walled generative hyphae and dichohyphae. However, V. amphithallica can be easily delimited from V. yunnanensis by its soft basidiomata, pinkish buff to cinnamon-buff hymenophore, generative hyphae with clamp connections, and two sterigmatic basidia (Liu & He 2016). Vararia *rugosispora* can be distinguished from V. *yunnanensis* by its larger basidia $(17.5-32 \times 5-9.5 \,\mu\text{m vs} \, 50-55 \times 9-11 \,\mu\text{m})$, and cinnamon brown, larger basidiospores ($(5.1-)5.9-11.5(-11.8) \times (4.3-)4.7-8.6(-9) \mu m vs 12-16 \times 7-8 \mu m$) (Boidin et al. 1980). Vararia yunnanensis is similar to nine species based on simple septa genitive hyphae viz. V. ambigua, V. breviphysa Boidin & Lanq. (1975: 462), V. cinnamomea Boidin, Lanq. & Gilles (1984: 250), V. cremea Boidin, Lang. & Gilles (1980: 301), V. fusispora G. Cunn. (1955: 977), V. gallica (Bourdot & Galzin) Boidin (1951: 78), V. sigmatospora Boidin, Gilles & Lang. (1987: 132) and V. trinidadensis A.L. Welden. (1965: 515). However, V. ambigua differs in its cream to buff hymenophore, and smaller basidiospores ($(5.1-)5.9-11.5(-11.8) \times (4.3-)4.7-8.6(-9) \mu m vs$ $4.5-5.2 \times 2.5-3 \mu m$) (Boidin et al. 1980, Liu & He 2016). Vararia breviphysa is distinguished from V. yunnanensis by having light yellow to light brown basidiomata, and larger basidiospores ($16-20 \times 4-5 \mu m$, Liu *et al.* 2019). Vararia cinnamomea differs in its tan basidiomata, yellowish-brown dichohyphae, and oval to cylindrical basidiospores (11-13 \times 5–6 µm, Boidin & Languetin 1984). Vararia cremea is separated from V. yunnanensis by the larger gloeocystidia $(40-90 \times 7-15 \ \mu\text{m})$, and larger basidiospores ($(5.1-)5.9-11.5(-11.8) \times (4.3-)4.7-8.6(-9) \ \mu\text{m} \text{ vs} 15-20 \times 2.7-3.5 \ \mu\text{m}$) (Boidin *et al.* 1980). Vararia fusispora differs in having the larger basidiospores $((5.1-)5.9-11.5(-11.8) \times (4.3-)4.7 8.6(-9) \ \mu m \ vs \ 14-17 \times 4-6 \ \mu m)$ (Cunningham 1955). Vararia gallica differs in its gradually tapered dichohyphae, swollen basidial base, and fusiform gloeocystidia (Grosse-Brauckmann & Kummer 2004). Vararia sigmatospora differs from V. yunnanensis by having narrowly spindle-shaped pink basidiospores $(13-22 \times 2.5-3.5 \ \mu m)$ (Boidin et al. 1987). Vararia trinidadensis differs in its powdery hymenophore, and longer basidiospores ((5.1-)5.9-11.5(-11.8) × (4.3-)4.7-8.6(-9) µm vs $13-17 \times 2.5-3.2$ µm) (Welden 1965).

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