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Rhizomaticola guizhouensis gen. et sp. nov. and Five Rosellinia Like Species Isolated from Decaying Wood

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Abstract: During the investigation of xylarialean taxa in China and Thailand, six rosellinia like taxa were collected. *Rhizomaticola* gen. nov. with type species of *Rh. guizhouensis* is established based on its morphology and multi-gene molecular data. *Rhizomaticola* owns no carbonaceous stromata and has black ascospores without a germ slit which are distinguished from those of *Rosellinia*, *Dematophora*, *Stilbohypoxylon* and *Xylaria*. Five rosellinia like species are introduced based on their morphology, inducing three new species (*Dematophora populi*, *Rosellinia thailandica*, *Ro. vitis*), one new record for China (*Ro. cainii*) and one known species (*D. necatrix*). Their descriptions and illustrations are detailed.

Keywords: 1 new genus; 3 new species; β-tubulin; ITS; phylogeny; *rpb*2; taxonomy; *Xylariaceae*

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1. Introduction

Xylariaceae is one of the largest families of *Xylariomycetidae*, distributed worldwide, with high biodiversity [1–5]. Most species of the family were reported in tropical and subtropical regions [4–7]. Many xylariaceous species are saprophytes, including wood, dung, litter, termite nests and other substrates [8–10]. There were also a large number of endophytes and pathogenic fungi of plants were reported in *Xylariaceae* [8,11,12]. Many novel compounds with biological activity were found in the species of *Xylariaceae* [13,14]. A total of 32 genera were included in *Xylariaceae* by Wijayawardene et al. [15]. *Xylaria* Hill ex Schrank, *Rosellinia* De Not., *Nemania* Gray and *Stilbohypoxylon* Henn. are the most common genera in this family.

Rosellinia is characterized by carbonized, hard, uniperithecioid stromata usually growing from a subiculum, asci with a J+ apical apparatus bluing in Melzer's reagent, ascospores with or without a germ slit [16]. According to morphological characteristics, 142 species were accepted by Petrini [16]. Only 48 species of *Rosellinia* have been reported from China [16–31]. Based on chemotaxonomic markers and a multi-locus phylogeny, the genus *Dematophora* R. Hartig was resurrected to include *D. arcuata* (Petch) C. Lamb., Wittstein and M. Stadler, *D. buxi* (Fabre) C. Lamb., Wittstein and M. Stadler, *D. bunodes* (Berk. and Broome) C. Lamb., Wittstein and M. Stadler, *D. necatrix* R. Hartig and *D. pepo* (Pat.) C. Lamb., Wittstein and M. Stadler and other allied species [32]. *Dematophora* differs from *Rosellinia* by its dematophora like asexual morph and forming a well-defined clade in phylogeny. With regard to the morphology of sexual morph, Wittstein et al. [32] did not point out the differences between *Rosellinia* and *Dematophora*.

In the process of investigating xylarialean taxa in China and Thailand, six rosellinia like species were collected from the forests, which were regard as an undescribed xylariaceous

genus, *Rhizomaticola* to accommodate *Rh. guizhouensis*, as well as three species of *Rosellinia* and two species of *Dematophora*. Their descriptions and illustrations are provided.

2. Materials and Methods

2.1. Collection and Isolation

Samples with black dots were collected from forests in China and Thailand in the rainy season. Samples were put into paper bags with some silica gel desiccant. Macroscopic characteristics of stromata were observed and photographed under an Olympus SZ61 stereomicroscope. Materials were mounted in water and Melzer's reagent for anatomical examination [31]. Macroscopic photographs of asci and ascospores were taken under a Nikon digital camera (700 D) fitted to a light microscope (Nikon Ni, Nikon Corporation, Tokyo, Japan). At least 30 ascospores and 30 asci were measured using the Tarosoft ® image framework (v. 0.9.0.7). Macroscopic photographs were made with the necessary changes and arranged for a plate. Single-spore isolation was used to obtain pure cultures [33]. Herbarium materials were deposited in the Herbarium of Guizhou Agricultural College (GACP); the Herbarium of the Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University (GZUH); the Herbarium of Mae Fah Luang University (MFLU); and the living cultures are deposited in Guizhou University Culture Collection (GZUHC) or Mae Fah Luang University Culture Collection (MFLUCC).

2.2. DNA Extraction, Polymerase Chain Reaction (PCR) Amplification and Sequencing

Culture was grown on potato dextrose agar (PDA) and the hyphae were scraped off with a scalpel to extract DNA. Some stromata were cut and the contents were picked for DNA extraction directly. Total DNA was extracted by BIOMIGA Fungus Genomic DNA Extraction Kit (BW-GD2416-02, Biomiga, USA), following the manufacturer's instructions. Regions of internal transcribed spacers (ITS), segments of large-subunit ribosomal RNA (LSU), partial β-tubulin gene (*tub2*), the second largest subunit of the RNA polymerase II (*rpb2*) were amplified with primer pairs ITS1/ITS4, LROR/LR5, Bt2a/Bt2b, RPB2-5F/RPB2-7Cr, respectively [34–37]. The components of a 25 µL volume PCR mixture were used as follows: double distilled water 9.5 µL, PCR master mix 12.5 µL, 1 µL of each primer, 1 µL template DNA [38]. PCR reaction systems were as follows: Long et al. [38] and Pi et al. [39]. Qualified PCR products checked with 1.5% agarose gel electrophoresis stained with GoldenView were sent to Sangon Co., China, for sequencing.

2.3. Sequence Alignment and Phylogenetic Analyses

All sequences for phylogenetic tree construction were chosen following published literature [40] and top hits of ITS blasted in the GenBank database (Table 1). ITS, LSU, *tub2*, *rpb2* sequence data including all introns and exons were aligned separately using the MAFFT v.7.110 online programme (http://mafft.cbrc.jp/alignment/server/ (accessed on 15 August 2022) [41]) with the default settings. Multiple sequence alignments were generated and adjusted using BioEdit v.7.0.5.3 [42]. The MrModeltest 2.2 was used to perform the model of evolution [43]. The final ML search was conducted using the GTRGAMMA + I model. The phylogenetic analyses were carried out for maximum likelihood in CIPRES web portal [44] using RAxML 7.4.2 Black Box [45].

Bayesian analyses were performed in CIPRES web portal by using MrBayes on XSEDE [46]. The model of evolution was calculated by using MrModeltest v. 2.2 [43]. Markov Chain Monte Carlo sampling (MCMC) was used to determine posterior probabilities (PP) [47] in MrBayes on XSEDE. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 1000th generation. The first 25% of trees were discarded during the burn-in phase of each analysis [48]. Phylogenetic trees were visualized and arranged using FigTree v1.4.0. and were edited with Adobe Photoshop CS6 [38]. The alignments were uploaded in TreeBASE (www.treebase.org/treebase-web/home.html (accessed on 15 August 2022) under ID 24609 for ITS-LSU-*rpb2*-*tub2* alignment.

Table 1. List of taxa used for phylogenetic reconstruction.

Species	Strains	GenBank Accession Number				References
		ITS	LSU	*rpb2*	*tub2*	
Amphirosellinia fushanensis	HAST 91111209^{HT}	NR_153514	N/A	GQ848339	GQ495950	[3]
A. nigrospora	HAST 91092308^{HT}	NR_153513	N/A	GQ848340	GQ495951	[3]
Annulohypoxylon annulatum	CBS 140775^{ET}	NR_153579	KY610418	KY624263	N/A	[49]
A. truncatum	CBS 140778^{ET}	NR_153580	KY610419	KY624277	N/A	[49]
Anthostomella thailandica	MFLUCC 15-0017^{HT}	NR_153556	KX533448	KX599538	KX600496	[50]
Astrocystis bambusae	HAST 89021904	GU322449	N/A	GQ844836	GQ495942	[3]
A. mirabilis	HAST 94070803	GU322448	N/A	GQ844835	GQ495941	[3]
Barrmaelia rappazii	Cr2 = CBS 142771^{HT}	MF488989	MF488989	MF488998	MF489017	[51]
B. rhamnicola	BR = CBS 142772^{ET}	MF488990	MF488990	MF488999	MF489018	[51]
Biscogniauxia arima	122 WSP^{IT}	EF026150	N/A	GQ304736	AY951672	[3]
Brunneiperidium gracilentum	MFLUCC:14-0011^{HT}	KP297400	KP340549	KP340529	KP406611	[52]
Cainia anthoxanthis	MFLUCC 15-0539^{HT}	NR_138407	NG_070382	N/A	N/A	[53]
C. globosa	MFLUCC 13-0663^{HT}	NR_171724	KX822123	N/A	N/A	[50]
Camillea tinctor	YMJ 363	JX507806	N/A	JX507790	JX507795	[54]
Clypeosphaeria mamillana	WU 33598^{ET}	NR_153909	NG_067338	MF489001	MH704637	[55]
Collodiscula japonica	CJ = CBS:124266	JF440974	MH874889	KY624273	KY624316	[56]
C. leigongshanensis	GZ70 = GZUH0107^{HT}	KP054281	KP054282	KR002588	KR002587	[29]
Coniocessia cruciformis	IRAN 1475C^{HT}	NR_145220	GU553347	N/A	N/A	[57]
C. nodulisporioides	CBS 125778	MH863756	MH875224	N/A	N/A	[57]
Coniolariella gamsii	IRAN 2506C	KY052004	KY052005	N/A	N/A	(submitted directly)
C. hispanica	CBS 124506^{T}	MH863381	MH874902	N/A	N/A	[57]
Creosphaeria sassafras	CBS 127876	MH864737	MH876173	N/A	N/A	[57]
Dematophora pepo	CBS:123592	MN984620	N/A	N/A	MN987246	[32]
Diabolocovidia claustri	CPC37593^{HT}	NR_170827	NG_074445	N/A	N/A	[58]
Diatrype lijiangensis	MFLU 19-0717^{HT}	NR_165229	MK810546	N/A	MK852583	[59]
Diatrypella heveae	MFLU:17-1216^{HT}	MF959501	NG_069531	N/A	MG334557	[60]
D. vulgaris	CBS 128327^{T}	NR_159873	NG_069986	N/A	N/A	[57]
Durotheca comedens	YMJ 90071615	EF026128	N/A	JX507793	EF025613	[38]
D. guizhouensis	GMBC0065^{HT}	MH645423	MH645421	MH645422	MH645420	[38]
Emarcea eucalyptigena	CBS 139908^{HT}	MK762711	NG_066346	MK791286	N/A	[61]
Engleromyces sinensis	BJTC 200803	MZ622705	MZ622702	N/A	N/A	(submitted directly)
Entalbostroma erumpens	ICMP:21152^{HT}	NR_154013	N/A	KX258204	KX258205	[62]
Entoleuca mammata	JDR 100	GU300072	N/A	GQ844782	GQ470230	[3]
Eutypa lata	CBS 208.87^{NT}	MH862066	MH873755	KF453595	DQ006969	[57]
Eutypella citricola	CBS 128332	MH864883	MH876331	N/A	N/A	[57]
Furfurella nigrescens	CBS:143622^{HT}	MK527844	MK527844	MK523275	MK523333	[51]
F. stromatica	CBS 144409^{HT}	MK527846	MK527846	MK523277	MK523334	[51]
Graphostroma platystomum	CPC:37153	MT223799	MT223894	MT223680	MT223734	[63]
Halorosellinia oceanica	BCC < THA > :60405	MK606079	MK629003	N/A	N/A	(submitted directly)
H. xylocarpi	MFLU 18-0545^{HT}	NR_166290	NG_068301	N/A	MN077076	[40]
Hansfordia pulvinata	CBS 194.56	KU683763	MH869122	KU684307	N/A	[12]
Hansfordia pulvinata	CBS:144422	MK442587	MK442527	N/A	N/A	[64]
Helicogermslita clypeata	MFLU 18-0852^{HT}	NR_175685	NG_081506	MW658647	MW775614	[65]
Hypocopra rostrata	NRRL 66178	KM067909	KM067909	N/A	N/A	(submitted directly)
Hypocreodendron sanguineum	169 (JDR)	GU322433	N/A	GQ844819	GQ487710	[3]
Hypoxylon rickii	MUCL 53309^{ET}	NR_137115	KY610416	KY624281	KC977288	[49]
Idriella lunata	CBS:204.56^{T}	MH857584	MH869129	N/A	N/A	[57]
Induratia thailandica	MFLU 18-0784^{HT}	MK762707	MK762714	MK791283	N/A	[61]
Jackrogersella multiformis	CBS 119016^{ET}	NR_154784	KY610473	KY624290	KX271262	[49]
Kretzschmaria clavus	JDR 114	EF026126	N/A	GQ844789	EF025611	[3]
Kretzschmaria lucidula	JDR 112	EF026125	N/A	GQ844790	EF025610	[3]

Table 1. *Cont.*

Species	Strains	GenBank Accession Number				References
		ITS	LSU	*rpb*2	*tub*2	
Kretzschmariella culmorum	JDR 88	KX430043	N/A	KX430045	KX430046	(submitted directly)
Lopadostoma turgidum	CBS 133207^{ET}	NR_132036	KC774618	KC774563	MF489024	[66]
Lunatiannulus irregularis	MFLUCC:14-0014HT	KP297398	KP340540	KP340526	KP406609	[52]
Microdochium phragmitis	CBS:285.71^{ET}	MH860125	KP858949	KP859122	MH704636	[67]
Nemania bipapillata	HAST 90080610	GU292818	N/A	GQ844771	GQ470221	[3]
N. serpens	HAST 235	GU292820	N/A	GQ844773	GQ470223	[3]
N. sphaeriostoma	JDR 261	GU292821	N/A	GQ844774	GQ470224	[3]
Neoxylaria arengae	MFLUCC 15-0292^{HT}	NR_171264	N/A	MT502418	N/A	[68]
Nigropunctata nigrocircularis	MFLU 19-2130^{HT}	NR_175683	NG_081504	N/A	MW775612	[65]
N. bambusicola	MFLU 19-2145^{HT}	NR_175684	NG_081505	MW658646	N/A	[65]
Podosordaria mexicana	176 WSP	GU324762	N/A	GQ853039	GQ844840	[3]
Podosordaria muli	167 WSP^{HT}	GU324761	N/A	GQ853038	GQ844839	[3]
Poronia pileiformis	WSP 88113001^{ET}	NR_158882	N/A	GQ853037	GQ502720	[3]
Requienella fraxini	CBS 140475^{HT}	NR_138415	MH878686	N/A	N/A	[57]
R. seminuda	CBS 140502^{ET}	NR_154630	MH878683	MK523300	N/A	[57]
Rhizomaticola guizhouensis	**FJS12 = GZUH0101^{HT}**	**ON815473**	**0N815474**	**ON897692**	**ON924997**	**This study**
Rh. guizhouensis	**2022FJS24 = GZUH0335**	**OP177724**	**OP177725**	**OP184058**	**OP184057**	**This study**
Rosellinia aquila	MUCL 51703	KY610392	KY610460	KY624285	KX271253	[49]
Ro. corticium	STMA 13324	MN984621	MN984627	MN987237	MN987241	[32]
Sarcoxylon compunctum	CBS:359.61	KT281903	KY610462	KY624230	KX271255	[49]
Stilbohypoxylon elaeicola	94082615 (HAST)	GU322440	N/A	GQ844827	GQ495933	[68]
Stilbohypoxylon elaeidis	MFLUCC 15-0295a^{HT}	MT496745	NG_074460	MT502416	MT502420	[68]
Stilbohypoxylon quisquiliarum	JDR 172	EF026119	N/A	GQ853020	EF025605	[3]
Vamsapriya bambusicola	MFLUCC11-0477 ^{HT}	KM462835	NG_067527	KM462834	KM462833	[58]
V. indica	MFLUCC 12-0544	KM462839	KM462840	KM462841	KM462838	[58]
Wawelia regia	CBS:110.10	MH854595	MH866123	N/A	N/A	[57]
Xylaria bambusicola	WSP 205^{HT}	EF026123	N/A	GQ844801	AY951762	[3]
X. feejeensis	HAST 92092013	GU322454	N/A	GQ848336	GQ495947	[3]
X. hypoxylon	HAST 95082001	GU300095	N/A	GQ844811	GQ487703	[3]
Zygosporium pseudomasonii	CBS 146059^{HT}	MN562147	MN567654	MN556815	N/A	(submitted directly)

Notes: Type specimens are labeled with HT (holotype), ET (epitype), IT (isotype), NT (neotype), T (type). N/A: sequence not available. New sequences are marked as bold. HAST: Herbarium, research Center for Biodiversity, Academia Sinica, Taipei; CBS: Westerdijk Fungal Biodiversity Institute (CBS-KNAW Fungal Biodiversity Centre), Utrecht; MFLUCC: Mae Fah Luang University Culture Collection, Thailand; WSP: Washington State University, U.S.A.; YMJ: Herbarium of Yu-Min Ju; Wu: Herbarium of the Institute of Botany, University of Vienna, Austria; GZUH: Herbarium of Guizhou University; IRAN: Iranian Research Institute of Plant Protection, Tehran, Iran; ICMP: International Collection of Microorganisms from Plants; JDR: Herbarium of Jack D. Rogers; CPC: Culture collection of Pedro Crous, housed at CBS; BCC: Universitat de Barcelona; MFLU: Mae Fah Luang University Herbarium, Chiang Rai, Thailand; NRRI: Natural Resources Research Institute, University of Minnesota Duluth, Duluth, Minnesota; MUCL: University Catholique de Louvain.

3. Results

3.1. Phylogenetic Analyses

Multiple sequence alignment for constructing the phylogenetic tree (Figure 1) included 81 taxa, 2988 positions including gaps (ITS: 1–538, LSU: 539–1372, *rpb*2: 1373–2435, *tub*2: 2436–2988). All characters have equal weight. Of these characters, 1424 characters are constant, 315 variable characters are parsimony-uninformative. Number of parsimony-informative characters is 1249. Gaps were treated as "missing". *Rhizomaticola guizhouensis* showed a distinct clade on the base of *Xylariaceae* (Figure 1), but bootstrap support values were not high (48, 0.91).

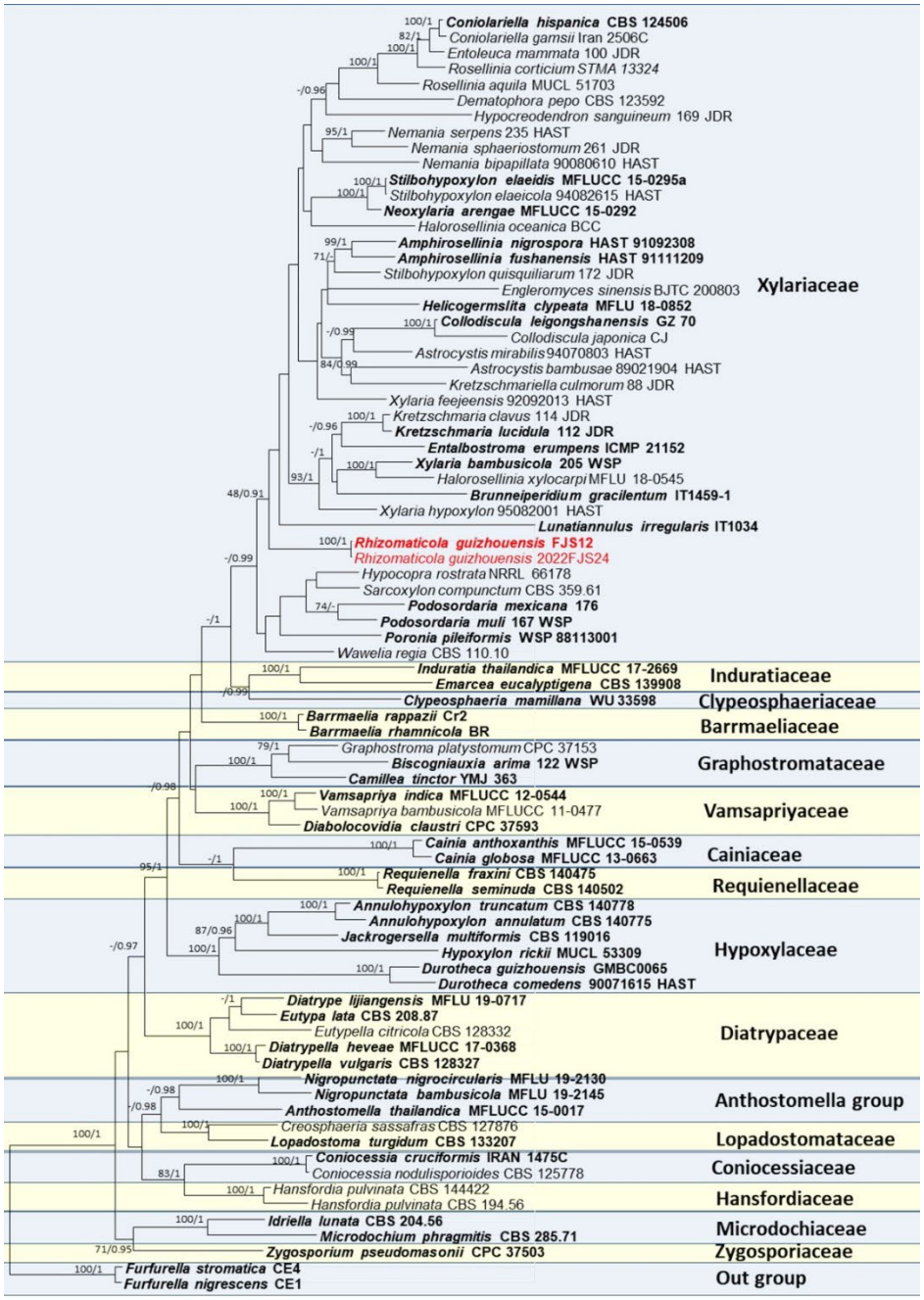

Figure 1. Phylogeny of *Xylariales* obtained from a Maximum Likelihood analysis of the combined ITS, LSU, *rpb*2 and *tub*2 using RAxML-HPC BlackBox software online. *Furfurella nigrescens* (CE1) and *F. stromatica* (CE4) were taken as outgroup taxa. Strains or specimen numbers were followed by their names. Type and authority strains are marked in bold. Bayesian posterior probabilities ≥0.95 and bootstrap support values for maximum likelihood (ML) higher than ≥70% are marked above the nodes; an en-dash ("-") indicates a value < 0.95 (PP) or <70% (BS).

3.2. Taxonomy

Rhizomaticola Q.R. Li and J.C. Kang gen. nov.
MycoBank no.: 844445
Etymology: In reference to rhizome where the fungus is inhabited.
Holotype: GZUH0101
Type species: *Rhizomaticola guizhouensis* Q.R. Li and J.C. Kang, sp. nov.

Saprobic on dead rhizoma of *Phragmites australis* (Cav.) Trin. ex Steud., **Sexual morph**: No subiculum observed. *Stromata* scattered or gregarious, solitary, superficial, orbicular in outline, black, no carbonaceous, containing a single perithecium; Surface convex, black alternating with white, crack. The tissue between surface and perithecia white. *Ostioles* papillate on the central, black. *Peridium* black. *Paraphyses* hyaline, unbranched, septate, longer than asci. *Asci* 8-spored, unitunicate, long-cylindrical, long-stipitate, apically rounded with a J+, barrel-shaped apical apparatus. *Ascospores* overlapping uniseriate, dark brown to black, unicellular, long ellipsoidal to fusiform, prominent at ends, smooth-walled, lacking germ slits, appendages and clear sheaths. **Asexual morph**: Undetermined.

Notes—Morphologically, this genus is similar to *Dematophora*, *Rosellinia*, *Stilbohypoxylon* and *Xylaria*, all of which have large stromata visible to the naked eye and unitunicate asci with a J+ apical ring bluing in Melzer's reagent, ascospores with germ slits [3,16,69,70]. However, *Rhizomaticola* has no subiculum, non-carbonaceous stromata cracking on its surface, white external stromata and ascospores lacking germ slits which are different from those close genera. *Rhizomaticola* differs from *Collodiscula* and *Astrocystis* by its non-carbonaceous stromata. Moreover, *Collodiscula* has ascospores with one too many septa, most species of *Astrocystis* have the ascospores with germ slits [19,29]. Molecular phylogenetic studies based on ITS, LSU, *tub2* and *rpb2* sequences in this study showed *Rhizomaticola* formed a distinct branch in *Xylariaceae*. Although the support values (48/0.91) are not high, its morphological characteristics are consistent with those of *Xylariaceae*. We would like to propose to temporarily place it in the *Xylariaceae*.

Rhizomaticola guizhouensis Q.R. Li and J.C. Kang, sp. nov. Figure 2.

Mycobank No.: 844446

Etymology: In reference to its collection location, Guizhou province, China.

Holotype—CHINA, Guizhou Province, Tongren city, The Fanjing Mountain Nature Reserve, on dead rhizome of *P. australis*, March, 2015, Q.R. Li and Lili Liu, FJS12 (GZUH0101, **holotype**, DNA was extracted directly from specimen; GACP QR0159). CHINA, Guizhou Province, Tongren city, The Fanjing Mountain Nature Reserve, on dead rhizome of *P. australis* (Cav.) Trin. ex Steud., July, 2022, Q.R. Li, 2022FJS24 (GZUH0335).

Description—Saprobic on dead rhizoma of *P. australis*. **Sexual morph**: No subiculum observed. *Stromata* 600–1000 μm diam., 500–800 μm high, scattered or gregarious, solitary, superficial, orbicular in outline, black, non carbonaceous, containing a single perithecium; Surface convex, black alternating with white, cracked. The tissue between surface and perithecia white. *Ostioles* papillate on the central, black. *Peridium* 55–65 μm thick, black. *Paraphyses* 2.5–4 μm wide, hyaline, unbranched, septate, longer than asci. *Asci* 221.5–320.5 × 12–18.5 μm (av. = 271.5 × 15.5 μm, n = 30), 8-spored, unitunicate, long-cylindrical, long-stipitate, the spore bearing part up to 155 μm long, apically rounded with a J+, barrel-shaped apical ring, 5–6.5 μm high, 4–5 μm broad. *Ascospores* 29.5–34.5 × 9–11 μm (av. = 32.5 × 9.5 μm, n = 30), overlapping uniseriate, dark brown to black, unicellular, long ellipsoidal to fusiform, prominent at both ends, smooth-walled, lacking germ slits, without appendages and sheaths. **Asexual morph**: Undetermined.

Culture characteristics—no culture was obtained; DNA was extracted directly from asci and ascospores in stromata.

Notes—*Rhizomaticola guizhouensis* is designated as the type species of *Rhizomaticola*. *Rh. guizhouensis* was found from Guizhou, China. *Rhizomaticola guizhouensis* differs from the uniperithecial species of *Xylaria* by it non-carbonaceous stromata, the ascospores lacking germ slits and observation of no multiple perithecia on a stroma [3–5]. Unfortunately, we could not obtain the pure culture of this species after many attempts. Ascospores did not germinate on PDA, OA (oatmeal agar) and MEA (malt extract agar) media.

Dematophora necatrix R. Hartig, Untersuch. Forstbot. Inst. München 3: 126 (1883). Figure 3.

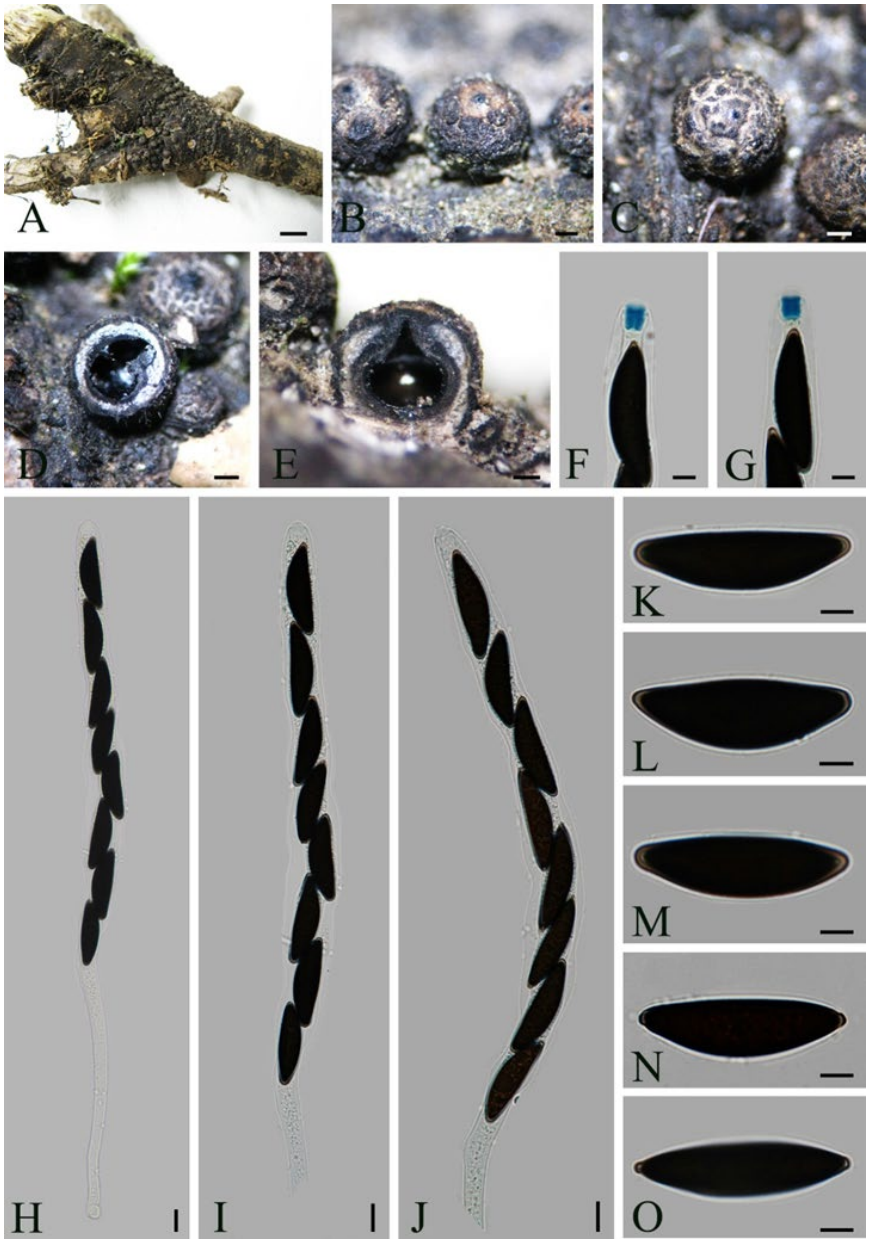

Figure 2. *Rhizomaticola guizhouensis* (GZUH0101,holotype) (**A**) Material; (**B**,**C**) Stromata on the surface of host; (**D**,**E**) Sections of stromata; (**F**,**G**) Ascus apex with a J+, apical ring (stained in Melzer's reagent); (**H**–**J**) Asci with ascospores; (**K**–**O**) Ascospores. Scale bars: (**A**) = 0.5 cm, (**B**–**E**) = 200 μm, (**F**,**G**) = 5 μm, (**H**–**J**) = 10 μm, (**K**–**O**) = 5 μm.

Synonymy:
Rosellinia necatrix Berl. ex Prill., Bull. Soc. mycol. Fr. 20: 34 (1904)
Hypoxylon necatrix (Berl. ex Prill.) P.M.D. Martin, Jl S. Afr. Bot. 34: 187 (1968)
Hypoxylon necatrix (Berl. ex Prill.) P.M.D. Martin, Jl S. Afr. Bot. 42(1): 73 (1976)
Rosellinia radiciperda sensu auct. NZ; fide NZfungi (2008)
Pleurographium necator (R. Hartig) Goid., Ann. Bot., Roma 21(1): 48 (1935)
Rhizomorpha necatrix R. Hartig, Untersuch. Forstbot. Inst. München 3: 125 (1883)
Mycobank no.: 216282

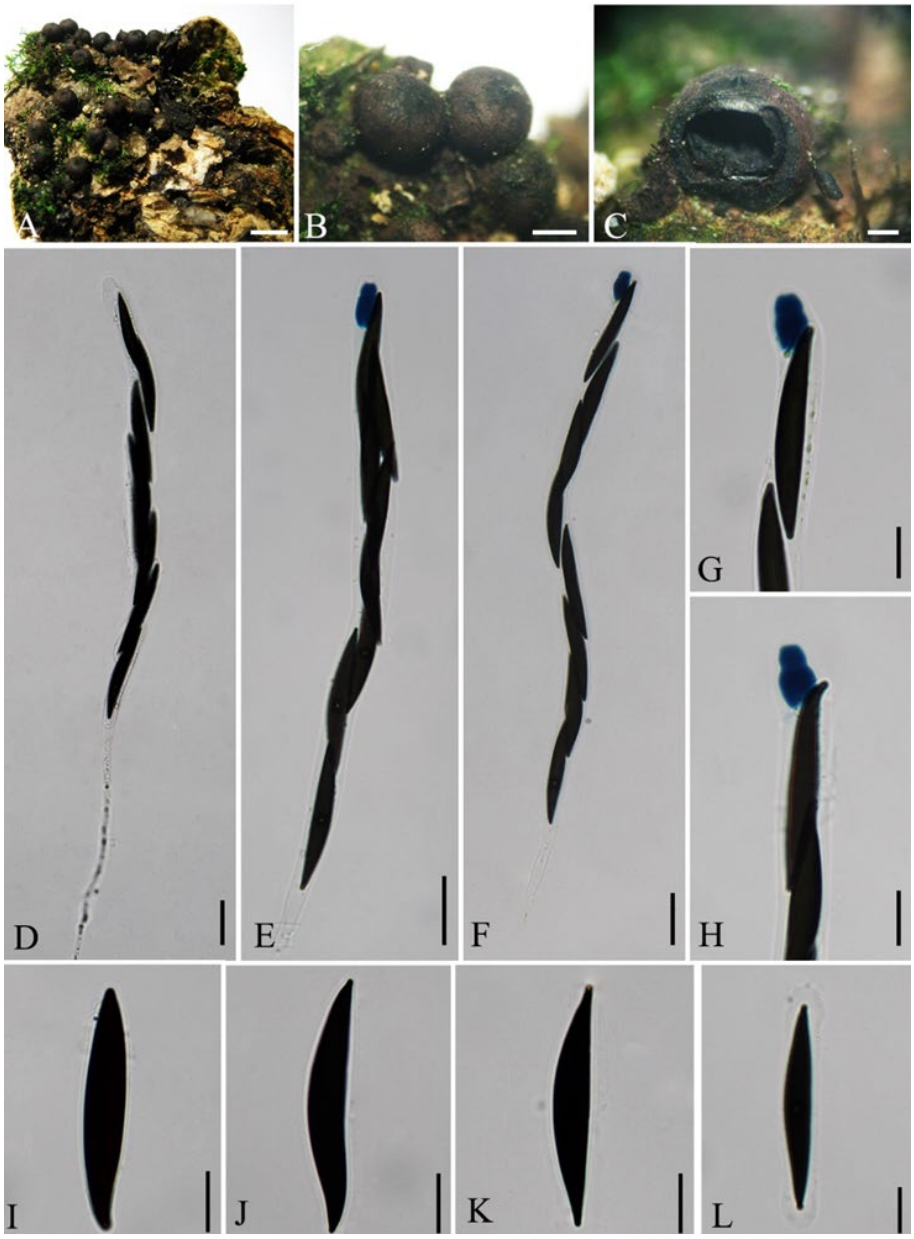

Figure 3. *Dematophora necatrix* (GZUH0139) (**A**,**B**) Stromata on the host; (**C**) Vertical section of a stroma; (**D**–**F**) Asci; (**G**,**H**) Urn–shaped J+ apical rings (stained in Melzer's reagent); (**I**–**L**) Ascospores. Bars: (**A**) = 2 mm, (**B**) = 500 µm, (**C**) = 200 µm, (**D**) = 20 µm, (**E**–**H**) = 20 µm, (**I**–**L**) = 10 µm.

Descrption—see Petrini (2013).

Distribution—China, France, Italy, Portugal, Spain

Specimen examined—CHINA, Guizhou Province, Guiyang city, Huaxi District, on unidentified plant stem, June 2014, Qirui Li, GZ28 (GZUH0139, GACP QR0198).

Notes—*Dematophora necatrix* is distributed worldwide. Morphologically, *D. necatrix* resembles *D. bothrina* (Berk. and Broome) C. Lamb., Wittstein and M. Stadler, *D. compacta* (Takemoto) C. Lamb., Wittstein and M. Stadler, *D. paraguayensis* (Starbäck) C. Lamb., Wittstein and M. Stadler, *D. grantii* (L.E. Petrini) C. Lamb., Wittstein and M. Stadler, *D. siggersii* (L.E. Petrini) C. Lamb., Wittstein and M. Stadler and *D. acutispora* (Theiss.) C. Lamb., Wittstein and M. Stadler [16,71]. Petrini [16] pointed out their differences. Morphological characteristics of new collection (GZUH0139) are consistent with those of *D. necatrix*.

Dematophora populi Q.R. Li and J.C. Kang sp. nov. Figure 4.

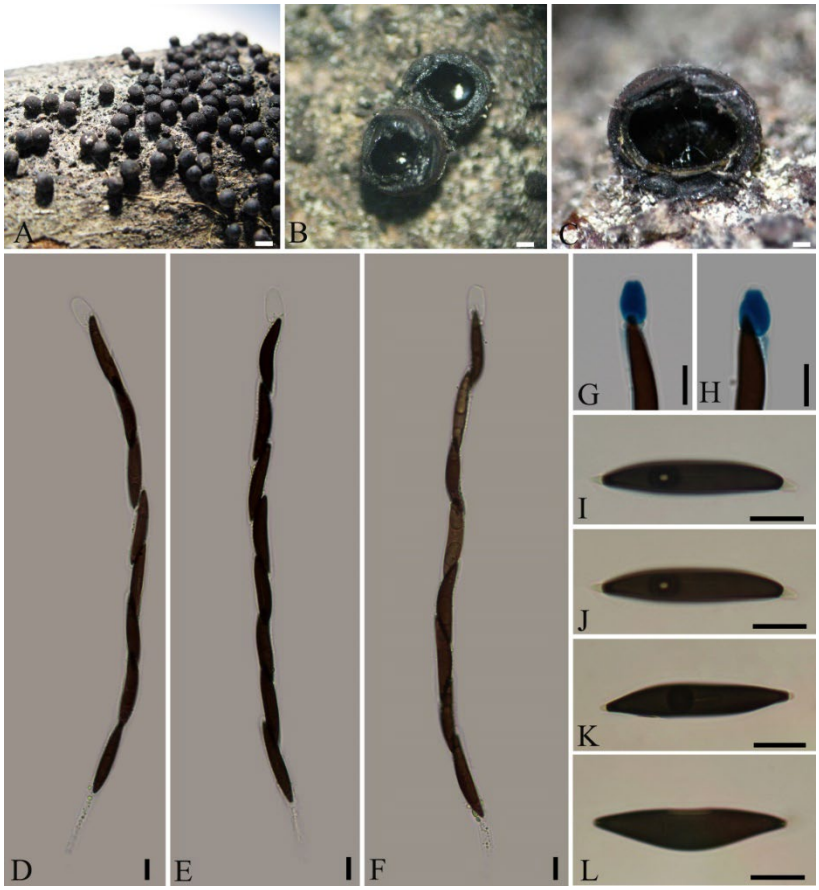

Figure 4. *Dematophora populi* (GZUH0116) (**A**–**C**) Stromata on the host; (**D**–**F**) Asci.; (**G**,**H**) Urn-shaped J+ apical rings (stained in Melzer's reagent); (**I**–**L**) Ascospores with short cell appendage on the ends. Bars: (**A**) = 1000 µm, (**B**) = 500 µm, (**C**) = 200 µm, (**D**–**L**) = 10 µm.

Mycobank no.: 844442
Etymology: The name refers the name of host, *Populus* sp.

Holotype—CHINA: Guizhou Province, Guiyang city, Baiyun park, on dead branch of *Populus* sp., May 2014, Qirui Li, GZ7 (GZUH0116, **holotype**; GACP QR0214); CHINA: Guizhou Province, Guiyang city, Guiyang Forest Park, on dead branch of an unknown plant, June 2014, Qirui Li, GYSLGY09 (GZUH0117; GACP QR0215).

Description—Saprobic on dead branch of *Populus* sp., **Sexual morph**: *Subiculum* evanescent, black, felty. *Stromata* 1.5–2 mm diam., 1.0–2.0 mm high, carbonaceous, globose to subglose, with fine papillate. *Ectostroma* up to 150 µm thick, black. *Entostroma* disappearing at matutity. *Perithecia* 1000–1500 µm diam., 800–1425 µm high, black. *Asci* 240–315 × 11–13 µm (av. = 287 × 12 µm, *n* = 15), 8-spores, unitunicate, long-cylindrical, apically rounded, with an urn-shaped apical ring, 10.5–12.5 µm high (av. = 11.6 µm, *n* = 20), upper width 3.5–4.5 µm (av. = 4.2 µm, *n* = 20), lower width 6–7 µm (av. = 6.7 µm, *n* = 20). *Ascospores* 34–44 × 6–8 µm (av. = 38.5 × 7.1 µm, *n* = 30), extremely narrowly ellipsoidal to almost fusiform, brown to dark brown, smooth-walled, with short cell appendages on the ends; germ slit short, central, straight, far less than half of spore length, lacking sheaths. **Asexual morph**: Undetermined.

Notes—In term of ascospores dimension, *D. populi* belongs to *Rosellinia necatrix* group, and which is close to *D. bothrina* (≡ *Ro. arcuata* Petch), *D. necatrix*, *D. paraguayensis* Starbäck in this group [16]. However, possessing cell appendages on both ends of ascospores, *D. populi* are clearly different from them. *Dematophora populi* morphologically shows similarities to *Ro. desmazieri* (Berk. and Br.) Sacc. but differs by its longer ascospores (34–44 × 6–8 µm vs. 25–30 × 6.6–8.1 µm) and shorter germ slit of ascospores [16]. Although

its anamorph was not observed, we put it in *Dematophora* here, since the *Dematophora populi* belongs to *Rosellinia necatrix* group, and the species of this group were transferred to *Dematophora* [32].

Rosellinia cainii L.E. Petrini, Index Fungorum 25: 1 (2013). Figure 5.

Mycobank no.: 550201

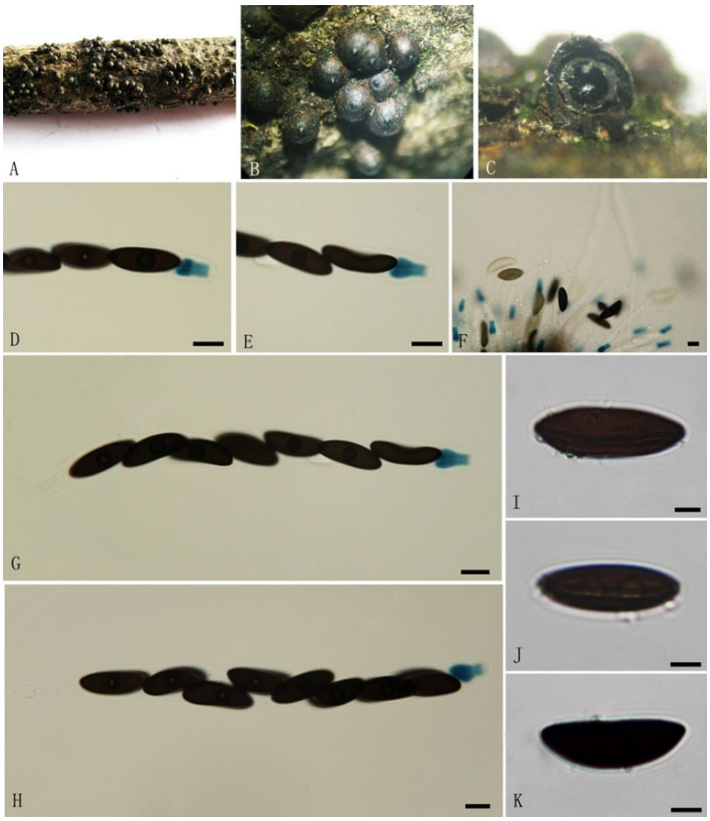

Figure 5. *Rosellinia cainii* (GZUH0119). (**A**,**B**) Stromata on the host; (**C**) Longitudinal section of stroma; (**D**,**E**) Urn–shaped J+ apical rings (stained in Melzer's reagent); (**F**) Paraphyses; (**G**,**H**) Asci. (**I**–**K**) Ascospores. Bars: (**A**) = 5 mm, (**B**) = 1 mm, (**C**) = 200 µm, (**D**–**H**) = 10 µm, (**I**–**K**) = 5 µm.

Description—Saprobic on dead branches of an unknown plant. **Sexual morph**: *Subiculum* woolly, wiry, felted brown to black, persistent or not. *Stromata* 600–800 µm diam., 500–900 µm high, solitary or densely, smooth, superficial, spherical with a papillate ostiole, containing single perithecia in one stroma. *Ostioles* finely papillate. *Ectostroma* up to 30 µm thick, black. *Entostroma* black, easily separated from ectostroma at matutity. *Paraphyses* 3–5 µm, hyaline, unbranched, septate. *Asci* 170–206 × 10.5–16 µm (av. = 187.8 × 13.5 µm, *n* = 30), 8-spores, unitunicate, cylindrical, apically rounded, with a blue, urn-shaped, J+, apical rings stained in Melzer's reagent, 10.5–12.5 µm high (av. = 11 µm, *n* = 30), upper width 3.5–4.5 µm (av. = 4 µm, *n* = 30), lower width 5–7.5 µm (av. = 6.5 µm, *n* = 30). *Ascospores* 22.5–30.5 × 7.5–9.5 µm (av. = 25.7 × 8.4 µm, *n* = 30), overlapping uniseriate, ellipsoidal to asymmetrically ellipsoidal with broadly rounded ends, dark brown to black, smooth-walled, with a straight germ slit nearly spore length, lacking sheaths and appendages. **Asexual morph**: undetermined.

Distribution—Canada, China

Specimen examined—CHINA, Yunnan Province, Pu'er city, Wuliangshan National Nature Reserve, on unknown plant, June 2014, Qirui Li, WLS1 (GZUH0119, GACP QR0217).

Notes—*Rosellinia cainii* was introduced by Petrini [16] as a new species, since its broadly rounded ascospores without slimy sheaths and caps. No available description for asci in the original literature. The first collection was found on *Corylus rostrata* hort. ex Dippel from Canada. This is the first report for *Ro. cainii* collected in China.

Rosellinia thailandica Q.R. Li and J.C. Kang sp. nov. Figure 6.
Mycobank no.: 844443
Etymology: in reference to the collection country, Thailand.

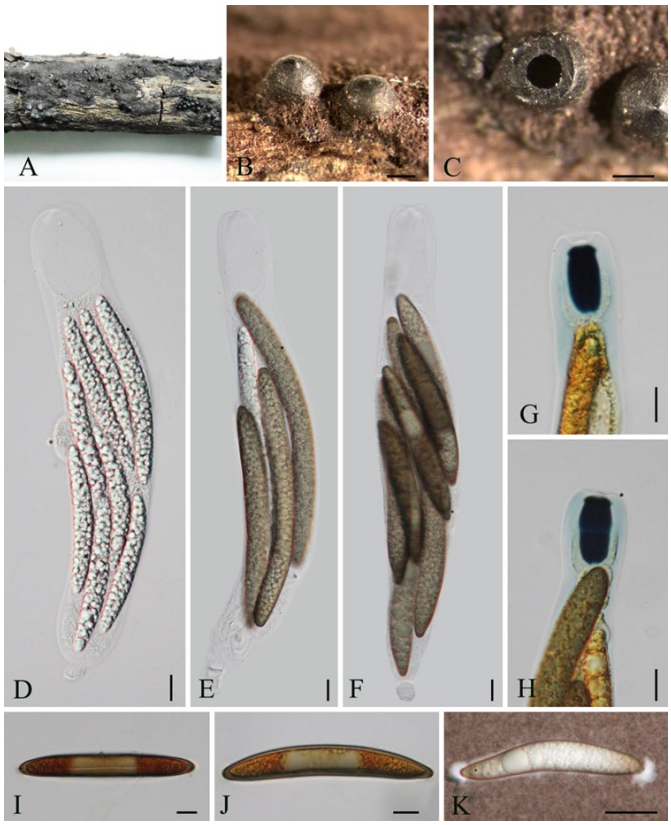

Figure 6. *Rosellinia thailandica* (MFLU12-2136, holotype). (**A**,**B**) Stromata of on the host; (**C**) Cross-section of a stroma; (**D**–**F**) Asci; (**G**,**H**) Asci apical rings (stained in Melzer's reagent); (**I**–**K**) Ascospore with a germ slit and end-sheaths; (**I**,**J** stained in Melzer's reagent; **K**, stained in ink). Bar: (**B**,**C**) = 500 μm, (**D**–**K**) = 10 μm.

Holotype—THAILAND, Chiang Mai, Campus of Mae Fah Luang University, on dead-wood, November 2012, Q.R. Li, T24 (MFLU12-2136 **holotype**, ex-type culture MFLUCC 13-0166; GZUH0058); THAILAND, Chiang Mai, on unknown plant dead branches, December 2012, Qirui Li, T35 (MFLU 12-2146, GZUH0065)

Description—Saprobic on dead branches of unknown angiosperm plant. **Sexual morph**: *Subiculum* woolly, brown to black, persistent, gathering at the bottom of stromata. *Stromata* 950–1200 μm wide, 600–800 μm high, carbonaceous, subglobose to globose, solitary or gregarious, embedded up to the middle the subiculum, smooth, with metallic luster. *Ostioles* finely papillate, well-developed. *Ectostroma* 120 μm, black. *Entostroma* black. *Asci* 170–235 × 26–39 μm (av. = 203 × 33 μm, *n* = 30) 8-spored, unitunicate, cylindrical to clavate, short pedicellate, apically rounded, with a J+, urn–shaped apical ring, bluing in Melzer's reagent, 19–24 μm (av. = 22 μm, *n* = 30) high, 9–11 μm (av. = 10 μm, *n* = 30) wide. *Ascospores* 72.5–144.5 × 10.0–15.0 μm (av. = 109.1 × 12.7 μm, *n* = 30), overlapping, fusiform, with round ends, asymmetrically equilateral, dark brown at maturity, unicellular, smooth, with a germ slit in the center of the ascospores, nearly half of spore-length, possessing thin, slimy sheaths covering ends of ascospores, lacking appendages. **Asexual morph**: Undetermined.

Notes—In term of stromata and ascospores dimension, *Rosellinia thailandica* belongs to *Ro. emergens* group [16]. Species with similar ascorspore dimension are *Ro. macrosperma* Speg., *Ro. markhamiae* Sivan., *Ro. megalosperma* Syd. and P. S.yd. [16,72]. However, *Ro. thailandica* possess the higher apical rings than those of them. Moreover, *Ro. megalosperma* and *Ro. markhamiae* have spore-length germ slit. Sheaths were not observed on the ascospores

of *Ro. megalosperma* [16,72]. *Rosellinia macrosperma* owns narrower ascospores (10 ± 1.7 μm vs. 10–14 μm) without germ slits [16].

Rosellinia vitis Q.R. Li and J.C. Kang, sp. nov. Figure 7.

Mycobank no.: 844444

Etymology: The name refers the host of vine.

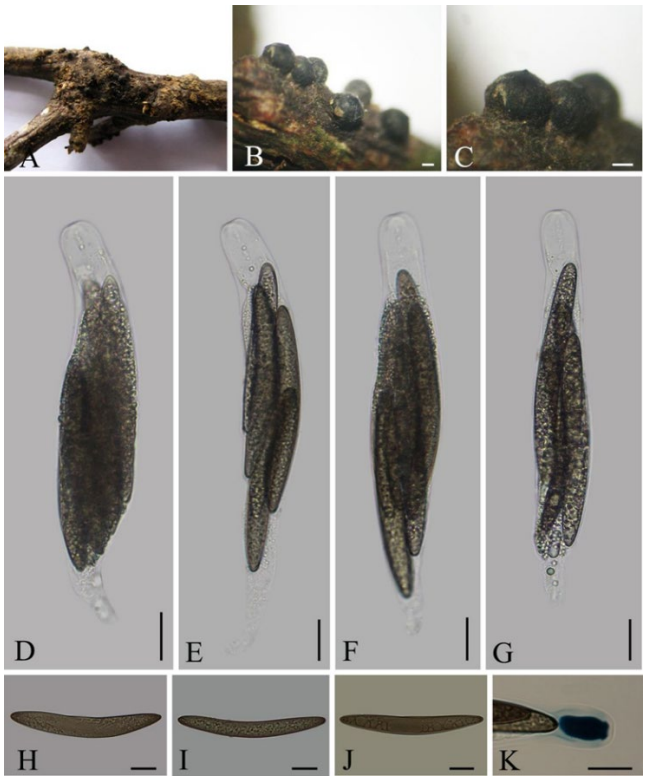

Figure 7. *Rosellinia vitis* (GZUH0123). (**A**–**C**) Stromata on the host; (**D**–**G**) Asci (**H**–**J**) Ascospores; (**K**) Urn-shaped J+ apical ring (stained in Melzer's reagent). Bars: (**B**,**C**) = 200 μm, (**D**–**J**) = 20 μm, (**K**) = 10 μm.

Holotype—CHINA, Yunnan Province, Pu'er city, Xishuangbanna Nature Reserve, on the dead vines of unknown plants, June 2014, Qirui Li, XSBN25 (GZUH0123 **holotype**, GACP QR0222)

Description—Saprobic on the dead vines of unknown plants. **Sexual morph**: *Subiculum* woolly, brown to black, persistent, gathering at the bottom of stromata. *Stromata* 550–1100 μm wide, 420–790 μm high, carbonaceous, subglobose to globose, solitary or gregarious, embedded up to the bottom the subiculum, smooth. *Ostioles* finely papillate, well-developed. *Ectostroma* 80 μm, black. *Entostroma* black. *Asci* 165–270 × 27–35 μm (av. = 210.5 × 31.4 μm, *n* = 30), 8-spored, unitunicate, short cylindrical to clavate, short pedicellate, apically rounded, with a J+, urn-shaped apical rings, bluing in Melzer's reagent, 21.5–26.5 μm (av. = 24 μm, *n* = 30) high, 7–15 μm (av. = 12 μm, *n* = 30) wide. *Ascospores* 92–116.5 × 12.5–18.5 μm (av. = 109.9 × 13.7 μm, *n* = 30), overlapping, fusiform, with round ends, asymmetrically equilateral, dark brown at maturity, unicellular, smooth, lacking germ slits, sheathes and appendages. **Asexual morph**: Undetermined.

Notes—In term of stromata and ascospores dimension, *Rosellinia vitis* belongs to *Ro. emergens* group [16]. *Rosellinia vitis* is most close to *Ro. capetribulensis*, *Ro. markhamiae*, and *Ro. macrosperma* [16,72,73]. *Rosellinia vitis*, however, differs from *Ro. capetribulensis* and *Ro. markhamiae* by lacking germ slit on ascospores. Entostroma of *Ro. macrosperma* is white and its ascospores (103.3 ± 8.5 × 10 ± 1.7 μm) are narrower than those of *Ro. vitis*.

4. Discussion

Xylariaceae is a worldwide distributed group which includes common characteristics such as ascomata visible to the naked eye, unitunicate asci with or without a J+, apical apparatus, brown to black, rarely hyaline, 1–2-celled ascospores mostly with a germ slit, geniculosporium-like or nodulisporium-like asexual morph [40]. Here, we introduce a new genus, *Rhizomaticola* to accommodate the type species of *Rh. guizhouensis* isolated from China. *Rhizomaticola* have black ascospores without a germ slit and no hard carbonaceous stromata which can be distinguished from its similar genera.

Rosellinia and *Dematophora* are widely distributed in tropical and subtropical regions and mainly saprophytes on plant branches [27,32,74]. In this paper, we introduced five species of *Rosellinia* and *Dematophora* which were collected from China and Thailand and identified them based on their morphology. We have attempted to isolate the pure cultures of these specimens, but only a part of the isolations has been obtained. We found that the larger the ascospores, the less likely it is to germinate in *Rosellinia* and *Dematophora*. Moreover, the culture is likely to die after being stored for a while at 4 °C.

Many taxonomic features are used for the identification of species within *Rosellinia* and *Dematophora*. The commonly used morphological characteristics mainly include: the size and shape of the stromata; the size and shape of the apical ring of ascus; the size and shape of the ascospores; the length of germ slits; the type of appendages; and the presence and type of sheaths covering the ascospores [16,75–77]. Anamorph is used for species identification as well, although only a few species of asexual stage have been observed [32,49,74]. DNA sequences have also been carried out for the identification of species within those genera [30,73]. However, there are only a few DNA sequences of *Rosellinia* available on Genbank. Secondary metabolites were attempted to be taken as a taxonomic feature to identify species of *Rosellinia* and *Dematophora* [32]. *Dematophora* was resurrected as inferred from polythetic taxonomy, while the results of utility of secondary metabolites as genus-specific chemotaxonomic markers were inconclusive [32].

Author Contributions: Investigation and collected specimens, Q.L., L.L.; checked specimens and DNA extract, S.L.; Microphotography, Q.L.; Validation, J.K.; Visualization, Y.L.; writing, original draft preparation Q.L., L.L.; writing, review and editing, S.L., Y.L., X.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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