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Two New and One First Recorded Species of *Xylaria* Isolated from Fallen Leaves in Hainan Tropical Rainforest National Park in China

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Abstract: *Xylaria* is a widely distributed genus in the Ascomycota phylum that can decompose wood. It is an essential decomposer in ecosystems and a source of bioactive secondary metabolites. Based on morphological characteristics and molecular evidence, this article thoroughly describes two new species discovered on the fallen leaves in Hainan Tropical Rainforest National Park, along with illustrations and comparisons with similar species. *Xylaria diaoluoshanensis* is characterized by filamentous stromata with long infertile apexes, ascospores sometimes with non-cellular appendages. *Xylaria fulvotomentosa* differentiates itself from other *Xylaria* species that grow on fallen leaves by its stroma surface, being yellow tomentose. These two new species of the genus *Xylaria* were found by phylogenetic analysis using the ITS-β-tubulin-RPB2 sequence dataset. Furthermore, a species first discovered in China, *X. petchii*, is described. Finally, a search table for 44 species related to fallen leaves and petioles in the world is established.

Keywords: Xylariaceae; *Xylaria*; molecular phylogenetics; taxonomy; Ascomycota

Citation: Pan, X.; Chen, Z.; Lei, J.; Chen, X.; Wu, T.; Li, Y.; Chen, Y. Two New and One First Recorded Species of *Xylaria* Isolated from Fallen Leaves in Hainan Tropical Rainforest National Park in China. *Diversity* **2024**, *16*, 179. https://doi.org/10.3390/d16030179

Academic Editor: Ipek Kurtboke

Received: 28 December 2023
Revised: 4 March 2024
Accepted: 11 March 2024
Published: 14 March 2024

1. Introduction

Xylaria Hill ex Schrank is the largest genus in Xylariaceae [1,2]. Over 300 *Xylaria* species have been reported worldwide [3], and there are 879 records related to *Xylaria* in Index Fungorum (http://www.indexfungorum.org/, accessed on 21 December 2023). Many species in this genus exhibit significant changes in their stromata morphology at different development stages [4]. They are generally characterized by cylindrical or filamentous upright stromata with a pale interior, asci with amyloid apical rings that turn blue in an iodine solution, eight brown unicellular ascospores with germ slit, and geniculosporium-like conidiophores [2,5–7]. To date, most reported *Xylaria* species grow on wood and branches, few grow on fallen fruits and seeds, termite nests and soil, and fallen leaves and petioles [7–13]. Fallen leaves and petioles are one of the growth substrates of *Xylaria* species. However, species growing on fallen leaves and petioles are usually overlooked due to their fragile and tiny stromata [14]. Generally, they have a small number of stromata, and different species may grow on the same leaf [14]. This challenges identification and makes the research on this group more difficult than that on other substrate groups. Ju and Hsieh [14] systematically combed global *Xylaria* species related to fallen leaves and petioles and profoundly promoted research on this group of substrates. However, the specimens collected of most of the species in that article were insufficient, making it difficult to determine the existence of substrate-specific species. For instance, there is only one specimen for *X. allima* Y.M. Ju & H.M. Hsieh, *X. hispidipes* Y.-M. Ju & H.-M. Hsieh, *X. neblinensis* Y.-M. Ju & H.-M. Hsieh, and *X. noduliformis* Y.-M. Ju & H.-M. Hsieh [14]. However, some species are not restricted to fallen leaves and petioles, they can also grow on woods, such as *X. meliacearum* Læssøe and *X. petchii* Lloyd [14]. *Xylaria clusiae* K.F. Rodrigues, J.D. Rogers & Samuels, *X. duranii* F. San Martín & Vanoye, and *X. heloidea* Penz. & Sacc. can be found on fallen leaves and fallen fruits or seeds [7]. Therefore, continuing to explore *Xylaria* species that grow on fallen leaves and petioles is of great ecological significance.

Xylaria species related to fallen leaves and petioles are mainly distributed in tropical and subtropical regions. The Hainan Tropical Rainforest National Park is located in the middle-south of Hainan Island, China, at latitude 18°33′16″–19°14′16″ N and longitude 108°44′32″–110°04′43″ E (https://www.hntrnp.com/, accessed on 21 December 2023). It belongs to the tropical island monsoon climate, with sufficient hydrothermal resources and abundant plant and fungal resources, and contains numerous endemic species. [4,15]. In this article, through morphological comparison and molecular investigation, three *Xylaria* species that grow on fallen leaves found in China are identified for the first time, including two new species and one first record in China. Finally, this paper establishes a key to *Xylaria* species related to fallen leaves and petioles worldwide.

2. Materials and Methods

2.1. Sample Source

The samples were collected at the Diaoluoshan Management Bureau of Hainan Tropical Rainforest National Park in February and June 2023 and stored in the Forest Resource Institute of Hainan Academy of Forestry.

2.2. Morphological Investigation

The habitat photos of the specimens were taken using a Canon D3 (Canon Corporation, Tokyo, Japan) and a Huawei Mate 50 (Huawei Corporation, Shenzhen, China). Fresh specimens were dried with a portable dryer (made in China). The dried specimens were marked and stored at minus 80 °C for morphological and molecular examination. The macroscopic morphology of the specimens was observed with a VHX-5000 digital microscope (Keyence Corporation, Osaka, Japan), focusing on the surfaces of the stromata, ostioles, and perithecia. Microscopic characteristics were observed and measured using three aqueous solution agents, water, Melzer's reagent, and 1% Sodium Dodecyl Sulfate (SDS), under a full-automatic optical microscope DM6B (Leica Corporation, Wetzlar, Germany) [13]. In this study, N represented the observed and measured number of ascospores, and M denoted the average size of ascospores.

2.3. Molecular Research

The total DNA of the specimens was extracted using cetyltrimethylammonium bromide (CTAB) plant genome rapid extraction kits (Aidlab Biotechnology, Beijing, China). The gene sequences at three sites, ITS, RPB2, and β-tubulin, were amplified. The 40 μL system was employed in all PCR reactions (ddH_2O 16 μL, 2 × HS™ Mix 20 μL, forward primer 1 μL, reverse primer 1 μL, and DNA template 2 μL). ITS was amplified using the primers ITS4/ITS5 [16]. The PCR program was as follows: initial denaturation at 95 °C for 3 min; 30 cycles at 94 °C for 40 s, 55.8 °C for 45 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min [4]. The amplification primers for RPB2 and β-tubulin were 7CR/5F [17] and T1/T22 [18], respectively. The PCR programs for these two sites were: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min [19]. The PCR products were sent to the Tianyi Huiyuan Gene Technology Co., Ltd. (Wuhan, China) for sequencing. Serial numbers were obtained after the sequences were submitted to GenBank.

2.4. Phylogenetic Analyses

The newly obtained sequences and the Xylariaceae and Graphromataceae sequences collected from the National Center for Biotechnology Information (NCBI) were used to construct phylogenetic trees based on the sequence dataset ITS-β-tubulin-RPB2 (Table 1). *Hypoxylon fragiforme* (Pers.) J. Kickx f. and *Camillea obularia* (Fr.) Læssøe, J.D. Rogers & Lodge were selected as outgroups. Sequences were verified and adjusted in MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/, accessed on 7 November 2023). BioEdit v.7.0.5.2 was adopted for manual cropping and optimization [20]. MEGA v.6.0 was used in site splicing [21,22]. Phylogenetic analyses were performed based on the Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The ML analysis was conducted in RAxML v.8.2.10 [23] using the GTRGAMMA substitution model and 1000 bootstrap inferences. The BI analysis was carried out with MrBayes v.3.2.6 [24]. The applicable model was automatically selected according to the Bayesian information criterion (BIC) with initial generations set to be 1,000,000 [24,25]. The phylogenetic trees were inspected and adjusted in FigTree v.1.4.3, using Adobe Photoshop CS6 to add background colors.

Table 1. Sequences and species used in the phylogenetic analysis, including growth substrate, origin, specimen number, GenBank access numbers, and references. Type specimens are labeled with HT. The new sequences from this study are in bold. NA: not available.

Taxon	Substrate/Origin	Specimen No.	GenBank No.			Reference
			ITS	β-Tubulin	RPB2	
Xylaria acuminatilongissima	Termite nests/China	HAST 623	EU178738	GQ502711	GQ853028	[5]
X. aethiopica	Pods of *Millettia ferruginea*/Ethiopia	YMJ 1136	MH790445	MH785221	MH785222	[26]
X. adscendens	Wood/Guadeloupe	HAST 570	GU300101	GQ487708	GQ844817	[5]
X. allantoidea	Trunk/China	HAST 94042903	GU324743	GQ502692	GQ848356	[5]
X. amphithele	Dead leaves/Guadeloupe	HAST 529	GU300083	GQ478218	GQ844796	[5]
X. apoda	Bark/China	HAST 90080804	GU322437	GQ495930	GQ844823	[5]
X. arbuscula	Bark/China	HAST 89041211	GU300090	GQ478226	GQ844805	[5]
X. arbuscula var. *plenofissura*	Wood/China	HAST 93082814	GU339495	GQ478225	GQ844804	[5]
X. aristata	Petioles/Indonesia	YMJ 1823 (HAST 145971)	OQ883719	NA	NA	[14]
X. atrodivaricata	Termite nests/China	HAST 95052001	EU178739	GQ502713	GQ853030	[5]
X. badia	Bamboo culm/China	HAST 95070101	GU322446	GQ495939	GQ844833	[5]
X. bambusicola	Bamboo culm/Thailand	JDR 162	GU300088	GQ478223	GQ844801	[5]
X. berteri	Bark/USA	JDR 256	GU324750	GQ502698	GQ848363	[5]
X. berteri	Bark/China	HAST 90112623	GU324749	AY951763	GQ848362	[5]
X. betulicola	Leaves of *Betula*/China	FCATAS 750	MF774332	NA	NA	[15]
X. brunneovinosa	Termite nests/China	HAST 720	EU179862	GQ502706	GQ853023	[5]
X. castorea	Wood/New Zealand	PDD 600	GU324751	GQ502703	GQ853018	[5]
X. cirrata	Termite nests/China	HAST 664	EU179863	GQ502707	GQ853024	[5]
X. coccophora	Wood/French	HAST 786	GU300093	GQ487701	GQ844809	[5]
X. crinalis	Wood/China	FCATAS 751	MF774330	NA	NA	[15]
X. crozonensis	Bark/France	HAST 398	GU324748	GQ502697	GQ848361	[5]
X. cubensis	Log/Russian Far East	HAST 477	NA	GQ502699	GQ848364	[5]
X. culleniae	Pod/Thailand	JDR 189	GU322442	GQ495935	GQ844829	[5]
X. delicatula	On decaying leaves/French Guiana	GS 2775 (HAST 145973)	OQ883720	NA	NA	[14]

Table 1. *Cont.*

Taxon	Substrate/Origin	Specimen No.	GenBank No.			Reference
			ITS	β-Tubulin	RPB2	
X. diaoluoshanensis	Fallen leaves/China	HAFFR 115	OR702611	OR726655	NA	This study
X. diaoluoshanensis	Fallen leaves/China	HAFFR 117 (HT)	OR702612	OR726656	OR757125	This study
X. diaoluoshanensis	Fallen leaves/China	HAFFR 127	OR702613	OR726657	NA	This study
X. escharoidea	Termite nests/China	HAST 658	EU179864	GQ502709	GQ853026	[5]
X. fabacearum	Seed pods of Fabaceae/Thailand	MFLU 16-1061	NR171104	MT212220	MT212202	[27]
X. fabaceicola	Seed pods of Fabaceae/Thailand	MFLU 16-1072	NR171103	MT212219	MT212201	[27]
X. feejeensis	Bark/China	HAST 92092013	GU322454	GQ495947	GQ848336	[5]
X. ficicola	Fallen leaves and petioles of *Ficus auriculata*/China	HMJAU 22818	MZ351258	NA	NA	[13]
X. filiformis	Herbaceous stem/Iran	GUM 1052	KP218907	NA	NA	[28]
X. fimbriata	Termite nests/French West Indies	HAST 491	GU324753	GQ502705	GQ853022	[5]
X. fulvotomentosa	Fallen leaves/China	HAFFR 124 (HT)	OR702619	OR726658	OR757121	This study
X. fulvotomentosa	Fallen leaves/China	HAFFR 129	OR702620	OR726659	OR757122	This study
X. cf. *glebulosa*	Fruit/French West Indies	HAST 431	GU322462	GQ495956	GQ848345	[5]
X. grammica	Wood/China	HAST 479	GU300097	GQ487704	GQ844813	[5]
X. griseosepiacea	Termite nests/China	HAST 641	EU179865	GQ502714	GQ853031	[5]
X. hedyosmicola	Fallen leaves of *Hedyosmum orientale*/China	FCATAS 856	MZ227121	MZ221183	MZ683407	[13]
X. hedyosmicola	Fallen leaves of *Hedyosmum orientale*/China	FCATAS 857	MZ227023	MZ221184	MZ851780	[13]
X. hypoxylon	Wood/Belgium	HAST 152	GU300096	GQ260187	GQ844812	[5]
X. hypoxylon	Wood/China	HAST 95082001	GU300095	GQ487703	GQ844811	[5]
X. ianthinovelutina	Fruit of *Swietenia*/Martinique	HAST 553	GU322441	GQ495934	GQ844828	[5]
X. intraflava	Termite nests/China	HAST 725	EU179866	GQ502718	GQ853035	[5]
X. juruensis	*Arenga engleri*/China	HAST 92042501	GU322439	GQ495932	GQ844825	[5]
X. laevis	Wood/Martinique	HAST 419	GU324746	GQ502695	GQ848359	[5]
X. leavis	Bark/China	HAST 95072910	GU324747	GQ502696	GQ848360	[5]
X. lindericola	Fallen leaves of *Lindera robusta*/China	FCATAS 852	MZ005635	MZ031978	MZ031982	[13]
X. lindericola	Fallen leaves of *Lindera robusta*/China	FCATAS 853	MZ005636	MZ031979	MZ048749	[13]
X. liquidambaris	Fruits of *Liquidambar formosana*/China	HAST 93090701	GU300094	GQ487702	GQ844810	[5]
X. longissima	Wood/China	FCATAS 749	MF774331	NA	NA	[15]
X. longissima	Wood/Iran	IRAN 16582 F	KP218906	NA	NA	[28]
X. meliacearum	Petioles and infructescence of *Guarea guidonia*/Puerto Rico	JDR 148	GU300084	GQ478219	GQ844797	[5]
X. minuscula	Fallen leaves of *Castanopsis carlesii* var. *Sessilis*/China	YMJ 90102701 (HAST 145978 HOLOTYPE)	OQ883721	NA	NA	[14]

Table 1. *Cont.*

Taxon	Substrate/Origin	Specimen No.	GenBank No.			Reference
			ITS	β-Tubulin	RPB2	
X. multiplex	Wood/USA	JDR 259	GU300099	GQ487706	GQ844815	[5]
X. muscula	Dead branch/French West	HAST 520	GU300087	GQ478222	GQ844800	[5]
X. nigripes	Termite nests/China	HAST 653	GU324755	GQ502710	GQ853027	[5]
X. oxyacanthae	Fallen seeds/USA	JDR 859	GU322434	GQ495927	GQ844820	[5]
X. oxyacanthae	Fruits/Germany	LZ 2010-502	HQ414587	NA	NA	[29]
X. palmicola	Fruits/New Zealand	PDD 604	GU322436	GQ495929	GQ844822	[5]
X. petchii	Fallen branches/China	HAFFR 60	OR702616	OR735171	NA	This study
X. petchii	Fallen leaves of *Daphniphyllum paxianum*/China	HAFFR 118	OR702617	OR735172	OR757123	This study
X. petchii	Fallen leaves of *Daphniphyllum paxianum*/China	HAFFR 126	OR702618	OR735173	OR757124	This study
X. phyllocharis	Dead leaves/French West	HAST 528	GU322445	GQ495938	GQ844832	[5]
X. plebeja	Trunk/China	HAST 91122401	GU324740	GQ502689	GQ848353	[5]
X. polymorpha	Wood/USA	JDR 1012	GU322460	GQ495954	GQ848343	[5]
X. polymorpha	Stump/Germany	M:M-0125909	FM164944	NA	NA	[30]
X. polysporicola	Fallen leaves of *Polyspora hainanensis*/China	FCATAS 848	MZ005592	MZ031976	MZ031980	[13]
X. polysporicola	Fallen leaves of *Polyspora hainanensis*/China	FCATAS 849	MZ005591	MZ031977	MZ031981	[13]
X. reevesiae	Fruits of *Reevesia formosana*/China	HAST 90071609	GU322435	GQ495928	GQ844821	[5]
X. regalis	Log of *Ficus racemose*/India	HAST 920	GU324745	GQ502694	GQ848358	[5]
X. rogersii	Fruits of *Magnolia* sp./China	FCATAS 915	MZ648827	NA	MZ707121	[4]
X. schimicola	Fruits of *Schima noronhae*/China	FCATAS 896	MZ648850	MZ695787	MZ707114	[4]
X. schweinitzii	Bark/China	HAST 92092023	GU322463	GQ495957	GQ848346	[5]
X. sicula f. *major*	Fallen leaves/China	HAST 90071613	GU300081	GQ478216	GQ844794	[5]
X. simplicissima	On herbaceous stems/Finland	MP 111004 (HAST 145982 EPITYPE)	OQ883722	NA	NA	[14]
X. striata	Branch/China	HAST 304	GU300089	GQ478224	GQ844803	[5]
X. theaceicola	Fruits of *Schima villosa*/	FCATAS 903	MZ648848	MZ695788	MZ707115	[4]
X. venosula	Twigs/USA	HAST 94080508	EF026149	EF025617	GQ844806	[5]
X. venustula	Bark/China	HAST 88113002	GU300091	GQ487699	GQ844807	[5]
X. vittatipiliformis	Dead leave/Guadeloupe	CLLGUAD 029 (HAST 145985 ISOTYPE)	OQ883723	NA	NA	[14]
X. vivantii	Fruits of *Magnolia* sp./Martinique	HAST 519	GU322438	GQ495931	GQ844824	[5]
X. wallichii	Fruits of *Schima wallichii*/China	FCATAS 923	MZ648861	MZ695793	MZ707118	[4]
X. xylarioides	Wood/Iran	GUM 1151	KP218909	NA	NA	[28]
Hypoxylon fragiforme	Bark/France	HAST 383	JN979420	AY951720	NA	[19]
Camillea obularia	–/Puerto Rico	ATCC 28093	KY610384	KX271243	NA	[31]

3. Results

3.1. Molecular Phylogeny

This paper used 86 ITS sequences, 72 β-tubulin sequences, and 68 RPB2 sequences for the phylogenetic analyses. Among them, 205 came from the NCBI database and 21 were obtained for this article, including 224 *Xylaria* species, one *Hypoxylon*, and one *Camillea* species. The sequence length of ITS was 796 character positions, β-tubulin 2241, and RPB2 1240. After cropping, the remaining character positions of ITS, β-tubulin, and RPB2 were 530, 1444, and 907, respectively. The complete dataset had a length of 2881 characters, containing 1217 parsimony-informative. The results of phylogenetic analyses showed no significant differences between the ML and BI trees. RAxML bootstrap values (≥70%) and Bayesian posterior probability (≥0.95) were labeled on the phylogenetic trees, respectively (Figure 1). The phylogenetic tree revealed that *X. diaoluoshanensis* sp. nov. clustered with *X. minuscula* Y.M. Ju & H.M. Hsieh. *Xylaria fulvotomentosa* sp. nov. and *X. hedyosmicola* Hai X. Ma & X.Y. Pan clustered together. *Xylaria petchii* is closely related to *X. amphithele* F. San Martín & J.D. Rogers and *X. ficicola* Hai X. Ma, Lar.N. Vassiljeva & Yu Li.

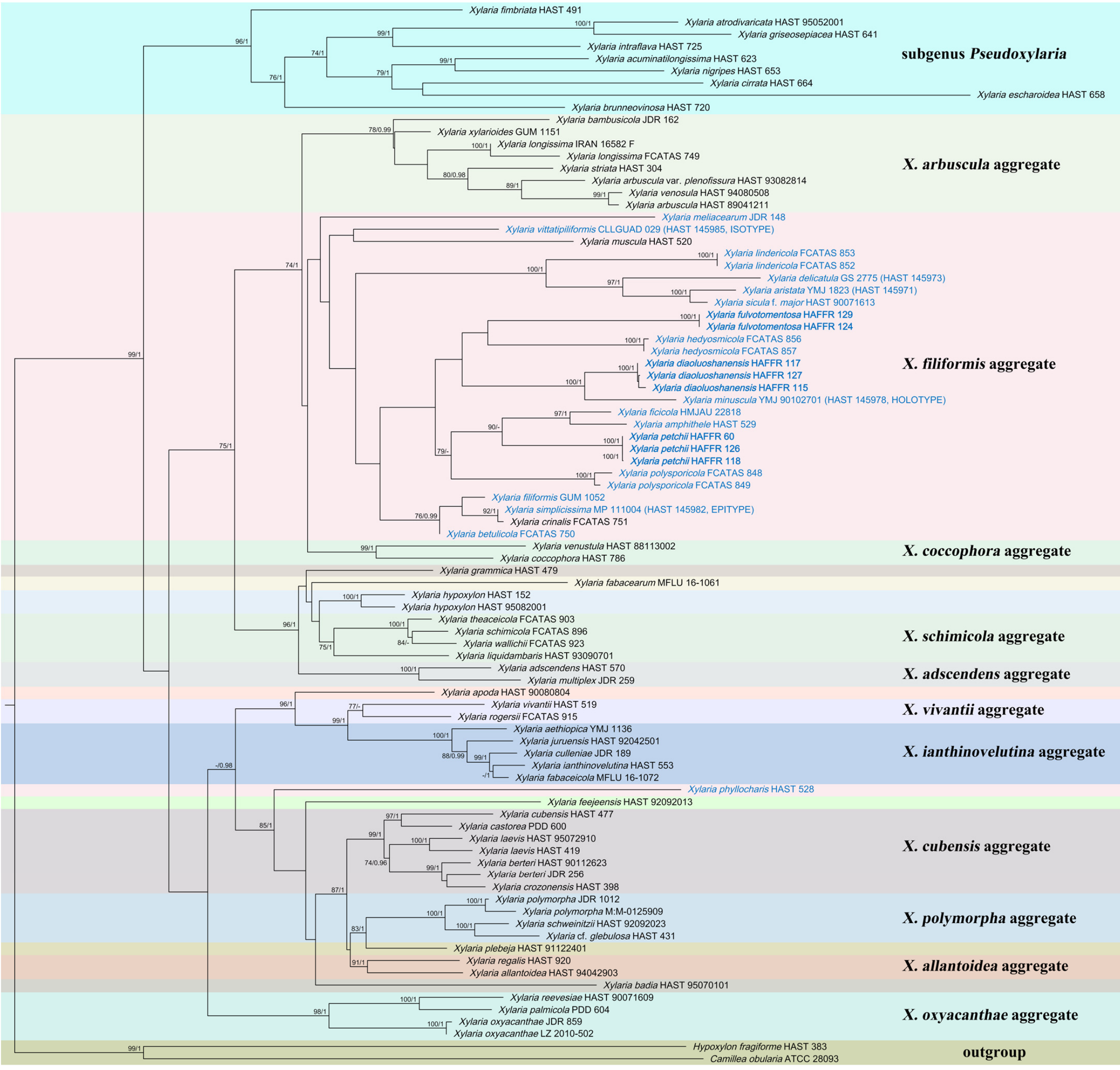

Figure 1. The ML phylogenetic tree of *Xylaria* constructed with ITS-β-tubulin-RPB2 sequences. Support values of ML and BI analyses (bootstrap supports ≥ 70% and posterior probability values ≥ 0.95) are indicated above or below the branches. Species related to fallen leaves and petioles are in blue, and those described in this article are in bold.

3.2. Taxonomy

Xylaria diaoluoshanensis Xiao Y. Pan, sp. nov. Figure 2.

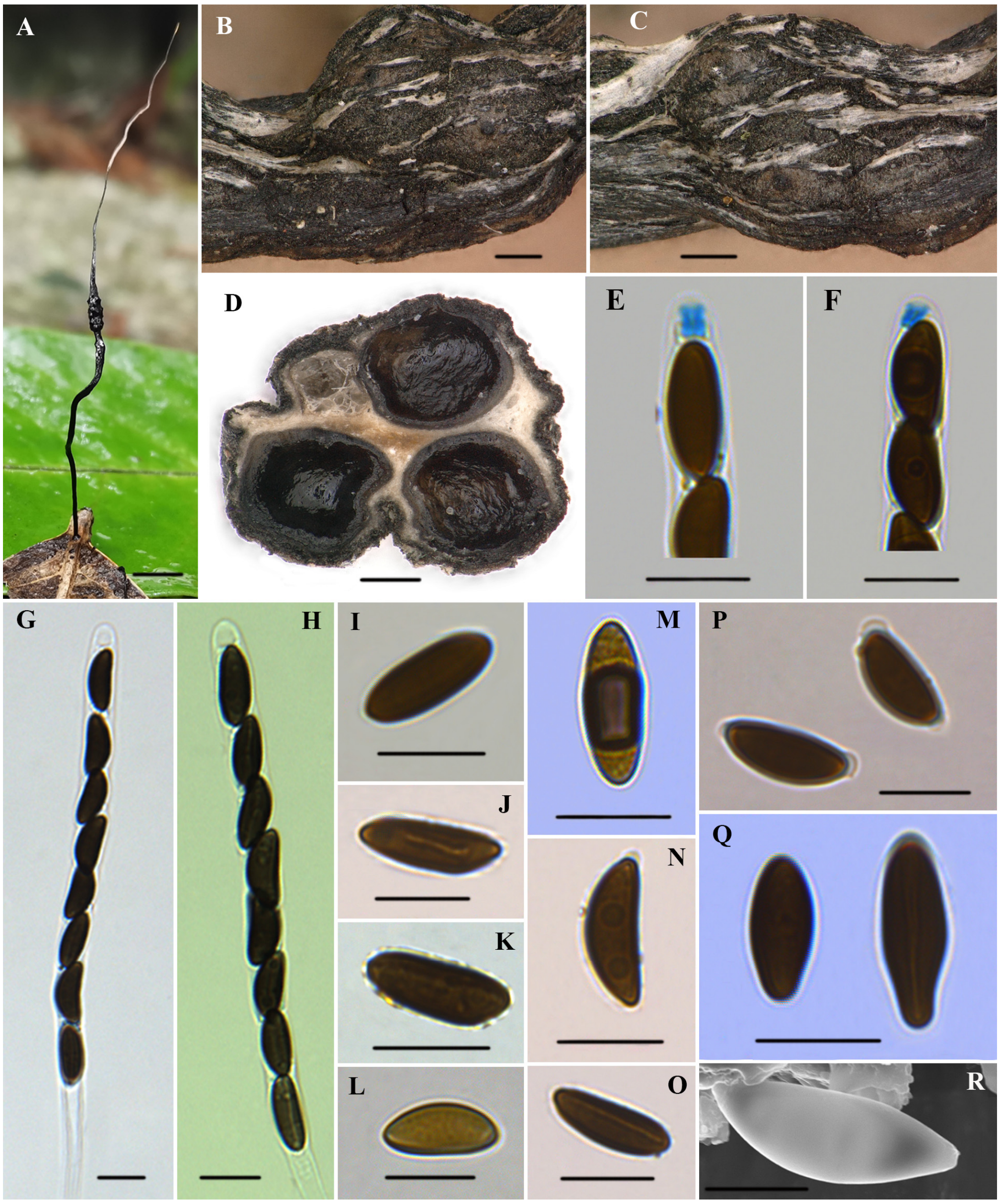

Figure 2. *Xylaria diaoluoshanensis* (HAFFR 117). (**A**) Stromata on leaves; (**B**,**C**) stromatal surface and ostioles; (**D**) section through stroma, showing perithecia; (**E**,**F**) ascal apical apparatus in Melzer's reagent; (**G**,**H**) asci in 1% SDS; (**I**,**L**,**M**,**Q**) ascospores in Melzer's reagent; (**J**) ascospore in 1% SDS, with a slightly curved germ slit along almost half of the spore-length; (**K**) ascospore in water; (**N**) ascospore in 1% SDS; (**O**) ascospore in 1% SDS, with nearly spore-length germ slit; (**P**) ascospores in 1% SDS, presenting non-cellular appendages; (**R**) ascospore under SEM; scale bars: (**A**) = 0.5 cm; (**B**–**D**) = 200 µm; (**E**–**Q**) = 10 µm; (**R**) = 5 µm.

MycoBank: 851069

Diagnosis. It is differentiated from *X. minuscula* by its stromata lacking peeling layers and smaller ascospores. Differences from *X. vittatipiliformis* by its stromata lacking peeling layers and longer and thinner ascospores. Differences from *X. vermiformis* by its stromata surfaces with flatter perithecial contours and ostioles and larger ascospores.

Etymology. Dedicated to the place where the type specimen was collected, the Diaoluoshan Management Bureau.

Holotype. CHINA: Hainan Province, Hainan Tropical Rainforest National Park, Diaoluoshan Management Bureau, on fallen leaves, 18 June 2023, Xiaoyan Pan (HAFFR 117).

Teleomorph. Stromata were solitary, upright, filiform, unbranched, 30–65 mm total height; sterile filiform apexes were 10–35 mm long; fertile parts were 2–10 × 1–3 mm, cylindrical, consisting of closely packed perithecia; stipes were glabrous, 15–35 × 0.2–1 mm, with longitudinal wrinkles, slightly enlarged base; surface of sterile apex, fertile part (with slightly to half-exposed perithecial contours) and stipe were all roughened, white to cream-colored at the young stage and black at the mature stage; interior was white; consistency was soft. Perithecia were subglobose to depressed-spherical, 250–600 μm in diameter. Ostioles were slightly papillate. Asci were cylindrical, with eight uniseriate ascospores, were 110–165 μm long in total, spore-bearing parts were 75–105 × 6.5–7.7(–8.8) μm, stipes were 25–75 μm long, with apical rings turning blue in Melzer's reagent, which were tubular to slightly urn-shaped, and (2.1–)2.5–3.5(–4.5) × 1.5–3 μm. Ascospores were brown to dark brown, unicellular, ellipsoid to fusiform, inequilateral, with slightly narrowly to broadly rounded ends, one end occasionally squeezed, smooth, (10.3–)11.5–14(–16.5) × (4.1–)4.6–5.7(–6.8) μm (M = 12.7 × 5.2 μm, N = 60), germ slit was mostly straight in nearly the spore-length, few slightly curved germ slit with nearly half of the spore-length, some with a hyaline sheath slightly swelling at both ends to form non-cellular appendages in 1% SDS.

Additional specimens examined. CHINA: Hainan Province, Hainan Tropical Rainforest National Park, Diaoluoshan Management Bureau, on fallen leaves, 18 June 2023, Xiaoyan Pan (HAFFR 115 and 127).

Xylaria fulvotomentosa Xiao Y. Pan, sp. nov. Figure 3.

MycoBank: 851070

Diagnosis. It can be distinguished from most *Xylaria* species by its yellow tomentose stromatal surface. It differs from *X. appendiculata* in that *X. fulvotomentosa* has evident perithecial mounds on its stromata and smaller ascospores.

Etymology. *fulvotomentosa* (lat.) denotes the primary features of yellow tomentose stromatal surfaces.

Holotype. CHINA: Hainan Province, Hainan Tropical Rainforest National Park, Diaoluoshan Management Bureau, on fallen leaves, 18 June 2023, Xiaoyan Pan (HAFFR 124).

Teleomorph. Stromata were solitary to scattered, upright, cylindrical, unbranched, 3–13 mm total height; fertile parts were 1.5–8 × 1–1.5 mm, cylindrical, composed of tightly arranged perithecia, apices attenuated or broadly rounded; stipes were 1.5–5 × 0.2–0.6 mm, with longitudinally fine stripes slightly expanded at bases; surface of fertile part (with slightly to half-exposed perithecial contours) and stipe were all roughened, except for the black upper apexes of protuberant perithecium, other parts were densely covered by yellow tomentum that gradually faded away with age and turned greyish brown, especially in fertile parts; interior was dark brown; consistency was hard. Perithecia were subglobose, 160–400 μm in diameter. Ostioles were papillate, up to 18 μm long. Asci were cylindrical, with eight uniseriate ascospores, were 80–115 μm long in total, spore-bearing parts were 60–80 × 6–7.8 μm, stipes were 15–38 μm long, with apical apparatuses turning blue in Melzer's reagent, were tubular to urn-shaped, and 2.3–3.4 × 1.4–2.5(–3) μm. Ascospores brown, unicellular, elliptical, inequilateral, with narrowly rounded ends, smooth, 9.2–10.8(–11.2) × 3.7–4.9(–5.3) μm (M = 10.1 × 4.3 μm, N = 60), straight germ slit nearly spore-length, with a hyaline sheath swelling at both ends to form non-cellular appendages in 1% SDS.

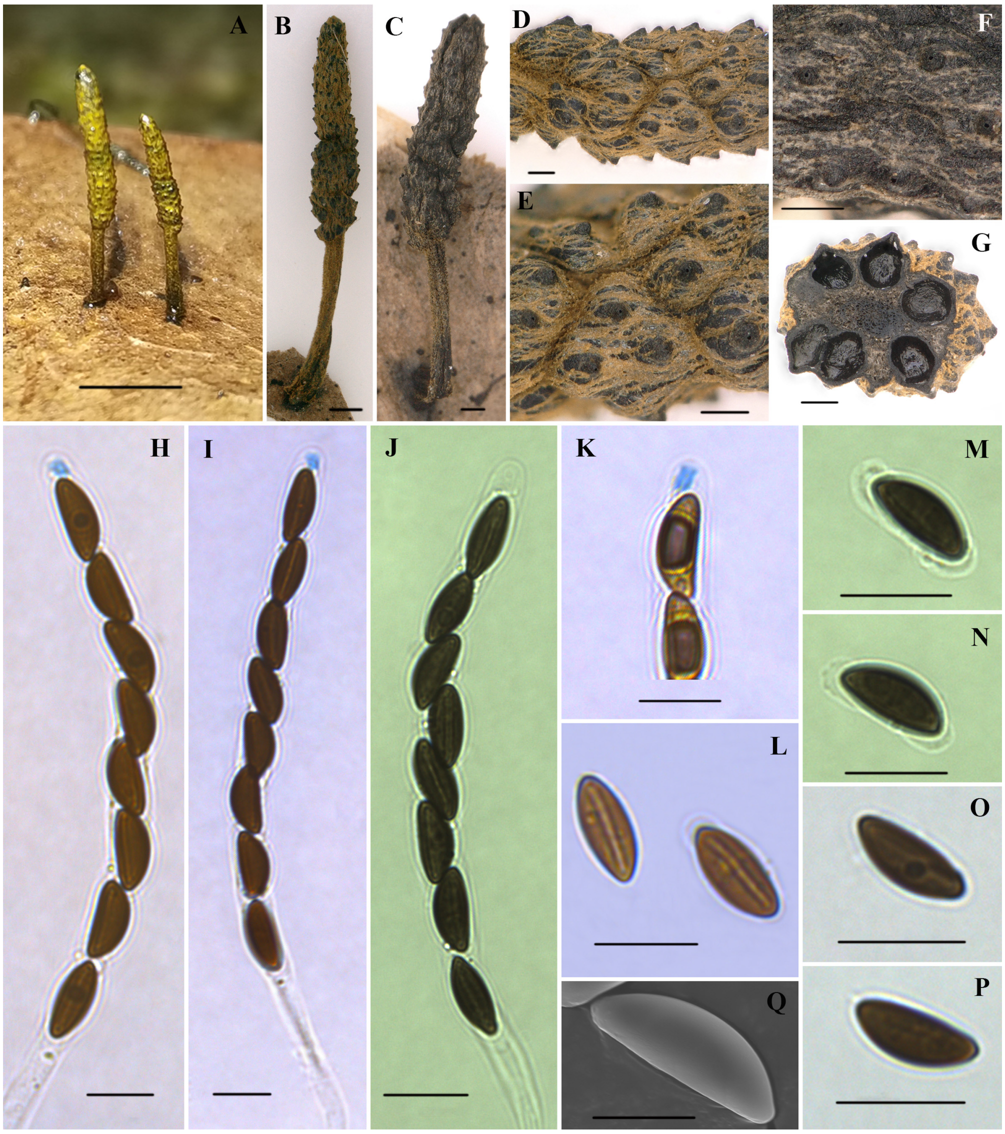

Figure 3. *Xylaria fulvotomentosa* (holotype HAFFR 124). (**A**–**C**) Stromata on leaves ((**C**), HAFFR 129); (**D**–**F**) stromatal tomentose surface and ostioles ((**F**), HAFFR 129); (**G**) section through stroma, showing perithecia; (**H**,**I**) asci in Melzer's reagent; (**J**) ascus in 1% SDS; (**K**) ascal apical ring in Melzer's reagent; (**L**) ascospores in 1% SDS, showing germ slit; (**M**,**N**) ascospores in 1% SDS, with non-cellular appendages; (**O**,**P**) ascospores in water; (**Q**) ascospore under SEM; scale bars: (**A**) = 0.5 cm; (**B**–**G**,**I**) = 200 µm; (**H**,**J**–**P**) = 10 µm; (**Q**) = 5 µm.

Additional specimen examined. CHINA: Hainan Province, Hainan Tropical Rainforest National Park, Diaoluoshan Management Bureau, on fallen leaves, 18 June 2023, Xiaoyan Pan (HAFFR 129).

Xylaria petchii C. -G. Lloyd, Mycol. Writings 7: 1310. 1924. Figure 4.

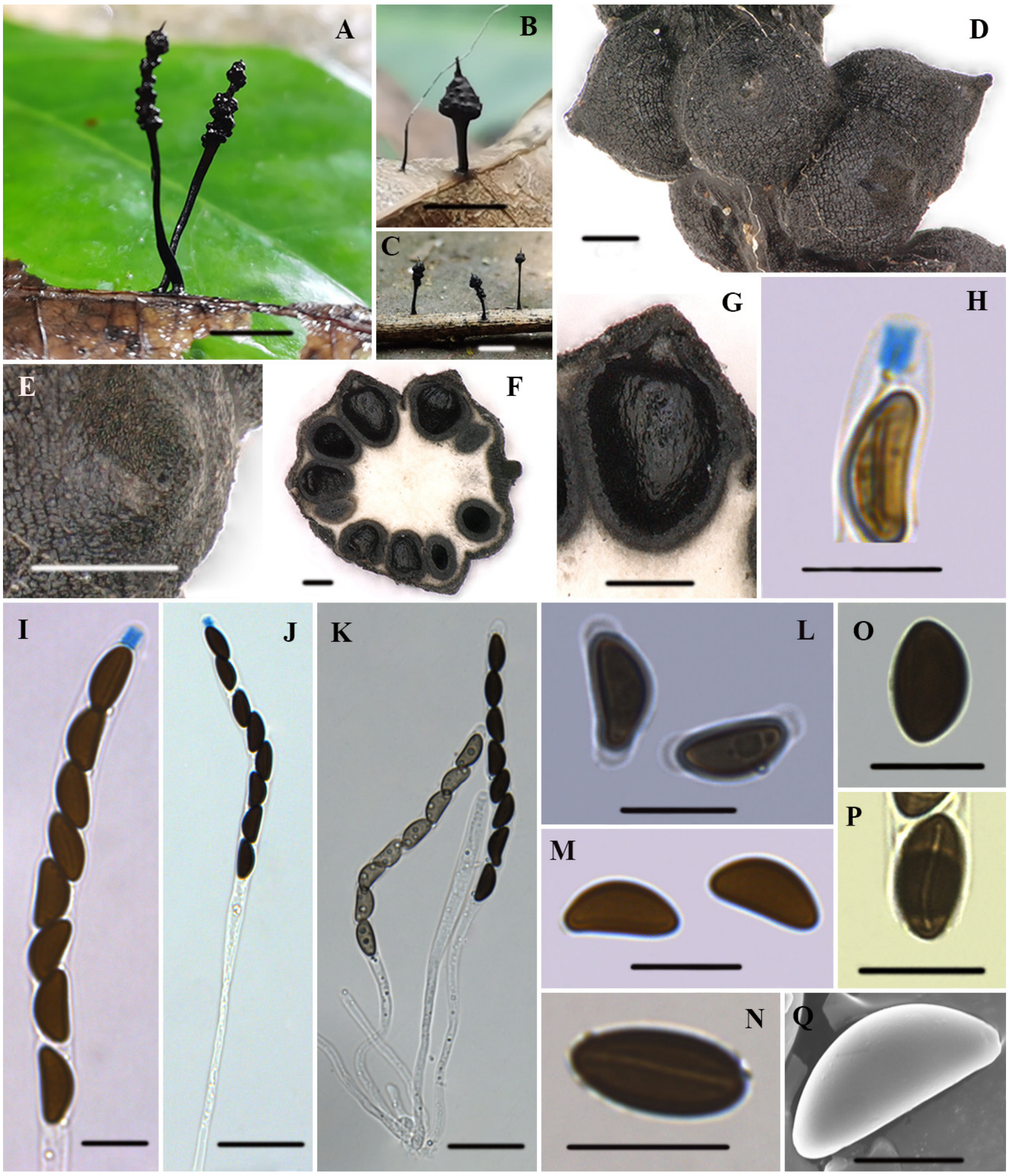

Figure 4. *Xylaria petchii* (HAFFR 118). (**A**,**B**) Stromata on leaves ((**B**), HAFFR 57); (**C**) stromata on branches (HAFFR 60); (**D**) stromatal surface; (**E**) ostioles; (**F**,**G**) section through stroma, showing perithecia; (**H**) ascal apical apparatus in Melzer's reagent; (**I**,**J**) asci with apical apparatus in Melzer's reagent; (**K**) asci in water; (**L**) ascospores in 1% SDS, presenting non-cellular appendages; (**M**) ascospores in Melzer's reagent; (**N**) ascospore with a spore-length germ slit in water; (**O**) ascospore in water; (**P**) ascospore in Melzer's reagent, showing a slightly sigmoid germ slit; (**Q**) ascospore under SEM; scale bars: (**A**–**C**) = 0.5 cm; (**D**–**G**) = 200 µm; (**H**,**I**,**L**–**P**) = 10 µm; (**J**,**K**) = 25 µm; (**O**) = 5 µm.

Teleomorph. Stromata were solitary to scattered, upright, cylindrical, and unbranched or occasionally branched once at stipe, 5–21 mm long in total; acute sterile apexes were 0.1–1 mm long; fertile parts were 1–10 × 0.5–2 mm, cylindrical or conical to subglobose, usually composed of clusters of perithecium near the top of the stromata, with a few occasionally scattered below; stipes were glabrous, 3–12 × 0.1–1.5 mm, with longitudinally fine stripes slightly swollen at bases; surface of sterile apex, fertile part (with conspicuous to fully exposed perithecial contours), and stipe were all roughened, black, without outer layer; interior was white; consistency was soft. Perithecia were subglobose, 250–550 µm in diameter. Ostioles were papillate. Asci were cylindrical, with eight uniseriate ascospores, a total length of 95–160 µm, spore-bearing parts of 60–110 × 7.5–11 µm, stipes that were 30–80 µm long, with apical apparatus turning blue in Melzer's reagent, which were tubular to slightly urn-shaped, 2.5–5 × 2–4 µm. Ascospores were brown to blackish brown, unicellular, elliptical, inequilateral, with narrowly to broadly rounded ends, smooth, (8.5–)10–12.5(–15) × (4.5–) 5–6.5(–7) µm (M = 11.3 × 5.7 µm, N = 60), with a straight to slightly sigmoid germ slit that was nearly spore-length, with a hyaline sheath swelling at both ends to form non-cellular appendages in 1% SDS.

Specimens examined. CHINA: Hainan Province, Hainan Tropical Rainforest National Park, Diaoluoshan Management Bureau, on fallen branches, 26 February 2023, Xiaoyan Pan (HAFFR 60); on fallen leaves of *Daphniphyllum paxianum*, 18 June 2023, Xiaoyan Pan (HAFFR 118 and 126).

4. Discussion

Nine new species have been described in the Hainan Tropical Rainforest National Park [4,32,33], indicating the abundant species diversity in this region. This article combines morphological features and molecular evidence to continue species description in this region, with two new species (*X. diaoluoshanensis* and *X. fulvotomentosa*) and one species first recorded in China (*X. petchii*).

In the phylogenetic tree, *X. diaoluoshanensis* and *X. minuscula* cluster together, sharing somewhat similar stromal morphology. By comparison, *X. minuscula* has smaller stromata (3–14 mm total length), dull grayish brown, with peeling layers that split into narrow or thread-like stripes, and larger ascospores [(13.5–)14–15(–17) × (4.5–)5–6(–7) µm (M = 14.5 × 5.7 µm)] [14]. *Xylaria vittatipiliformis* Y.-M. Ju, H.-M. Hsieh & Fournier and *X. vermiformis* Y.-M. Ju & H.-M. Hsieh are also similar to *X. diaoluoshanensis* in stroma morphology. *Xylaria vittatipiliformis* has the stromata outer layer peeling and splitting into band-like stripes, and the ascospores are shorter and wider, (10–)11–12 (–12.5) × (5.5–) 6–7 (–7.5) µm (M = 11.4 × 6.5 µm) [14]. *Xylaria vermiformis* is distinguished from *X. diaoluoshanensis* by its more prominent perithecial mounds (half-exposed) and its sharper ostioles (conical-papillate) on the surface of the stromata and smaller ascospores [(9–)9.5–10.5(–11) × (3.5–)4–4.5(–5) µm (M = 10.1 × 4.4 µm)], without non-cellular appendages [14]. *Xylaria appendiculatoides* Y.M. Ju & H.M. Hsieh is somewhat similar to *X. diaoluoshanensis* in stroma morphology. *Xylaria appendiculatoides* is separated from *X. diaoluoshanensis* by its stromata surface color (blackish brown to black), sharper ostioles (conical-papillate), and larger ascospores [(14–)15–16(–17) × (6.5–)7.0–7.5(–8) µm] [14]. *Xylaria betulicola* Hai X. Ma, Lar.N. Vassiljeva & Yu Li, *X. crinalis* Hai X. Ma, Lar.N. Vassiljeva & Yu Li, *X. eugeniae* F. San Martín, Vanoye & P. Lavín, *X. filiformis* (Alb. & Schwein.) Fr., and *X. hedyosmicola* all have filamentous stromata [13–15,34], slightly similar to *X. diaoluoshanensis*. However, *X. eugeniae* has smaller stromata (15–20 mm total length) with more conspicuous perithecial mounds (half to fully exposed) and smaller ascospores (12–13.5 × 4–5) [34]. *Xylaria betulicola*, *X. crinalis*, *X. filiformis*, and *X. hedyosmicola* can be clearly separated from *X. diaoluoshanensis* in the phylogenetic analyses.

Xylaria fulvotomentosa and *X. hedyosmicola* cluster together in the system analysis (Figure 1). However, a significant difference in morphology between the two is the long sterile filiform apexes and hairless stipes of the filamentous stromata of *X. hedyosmicola* [13]. Morphologically, *X. appendiculata* Ferd. & Winge and *X. fulvotomentosa* share cylindrical fertile parts growing on hairy stipes and the colors of their stromata are somewhat similar. However, *X. appendiculata* has smoother stromata without obvious perithecial mounds and larger ascospores, (11.5–)12.5–14(–15) × (6–)6.5–7.5(–9) µm (M = 13.1 × 7.2 µm) [14]. *Xylaria friabilis* J. Fourn. & Lechat is similar to *X. fulvotomentosa* in the color of stromata. However, *X. friabilis* has stromata with hairless stalks and ascospores lacking appendages [10].

Xylaria petchii was originally collected by Petch on fallen leaves in Sri Lanka and described by Lloyd in 1924 [14,35]. Hladki and Romero (2010) published *X. filiformoidea* Hladki & A.I. Romero collected in Argentina [36]. Ju and Hsieh (2023) believed that *X. petchii* and *X. filiformoidea* belonged to the same species [14]. The major characteristic of *X. petchii* is the diverse morphology of the fertile parts of stromata, with most perithecium clustered near the top of the stromata and a few scattered below. Ju and Hsieh (2023) observed *X. petchii* collected from Sri Lanka. Compared with specimens gathered in China, except for their larger ascus [(95–160 × 7.5–11 μm vs. 95–125 × 6–7 μm)] and ascospores [(8.5–)10–12.5(–15) × (4.5–)5–6.5(–7) μm vs. (7.5–)8.5–9.5(–10) × (3.5–)4–4.5(–5) μm)], other features are basically in accordance with the observation results of Ju and Hsieh (2023) [14]. Therefore, here they were identified as the same species. Unfortunately, since no gene sequences related to *X. petchii* were found in the NCBI, the Chinese sequences could not be compared with them. On the other hand, our sequences did not group with others included in this analysis. This study is the first to provide the gene sequence of *X. petchii* and determine the phylogenetic relationship between it and other *Xylaria* species. The results of the phylogenetic analyses showed that *X. amphithele* and *X. ficicola* are closely related to *X. petchii*. Moreover, they have some similarities in morphology. *Xylaria amphithele* differs from *X. petchii* mainly in its smaller stromata (≤50 mm) with conspicuous to half-exposed perithecial mounds and larger ascospores [(12–)12.5–15.5(–17) × (5–)6–7.5(–8) μm (M = 14.0 × 6.7 μm)] [14]. *Xylaria ficicola* is distinguished from *X. petchi* by larger ascospores [(16–)17.5–21(–22.7) × 6.5–8.5 μm] and larger apical apparatuses [5–6.5(–7.5) × 3–3.5 μm] [37]. *Xylaria filiformis*, placed in another clade (Figure 1), has similar stromata to *X. petchii*. However, there is an apparent difference in the ascospores of the two. In the former, the ascospores are short fusoid and light brown [14], while the latter has elliptical brown to blackish brown ascospores.

After carefully examining all *Xylaria* species that were lacking molecular data, these species were separated from the two new species described in this article primarily based on stromata morphology, lack of yellow tomentum on the stromal surface, perithecia, ostioles, ascospore size, germ slit, and appendage. The research results show that *X. diaoluoshanensis* has filamentous stromata with long sterile apexes, *X. fulvotomentosa* possesses cylindrical stromata with yellow tomentum, and *X. petchii* has cylindrical stromata with variable fertile parts, implying diversity in their stroma morphology. Meanwhile, the three species exhibit a degree of unity, such as having a few tiny and fragile stromata, papillate ostioles, and brown ascospores. According to the observations of Ju and Hsieh (2023), this kind of uniformity generally appears in *Xylaria* species related to fallen leaves and petioles [14]. This study collected gene sequences of 18 *Xylaria* species related to fallen leaves and petioles, of which 17 species clustered in the same branch of the phylogenetic tree. *Xylaria phyllocharis* Mont. is separated from other species related to fallen leaves and petioles and clusters with wood-inhabiting *Xylaria* species (Figure 1). It is not notably different from the findings of Hsieh et al. (2010) and Pan et al. (2022) [5,13], verifying that the systematic analysis results of this paper are not paradoxical. *Xylaria diaoluoshanensis*, *X. fulvotomentosa*, and *X. petchii* cluster in the different sub-branches of *X. filiformis* aggregate (Figure 1). In contrast with the *Xylaria* species on other substrates, they are highly correlated but also clearly separated. In *X. filiformis* aggregate, 19 species are gathered. Excluding two wood-inhabiting species (*X. muscula* Lloyd and *X. crinalis*), the remaining 17 are related to fallen leaves and petioles, demonstrating that genes of the species growing on this substance are more similar and may be evolving into a distinctive taxon in *Xylaria*. Moreover, *X. clusiae*, *X. hedyosmicola*, *X. pisoniae* D. Scott, J.D. Rogers & Y.M. Ju, and *X. polysporicola* Hai X. Ma & X.Y. Pan were all named after their hosts [13,38,39]. However, it is currently unclear whether these four species and most other *Xylaria* species that grow on fallen leaves and petioles have host specificity. Therefore, in-depth research is critical to unravel the above mystery.

Ju et al. (2018) argued that *X. ficicola* and *X. heloidea* were the same species [7]. Ju and Hsieh (2023) summarized *Xylaria* species related to fallen leaves and petioles in the world and reclassified many species [14]. For example, they believed that *X. crinalis* was *X. simplicissima* (Pers.) Y.M. Ju & H.M. Hsieh, *X. hainanensis* Y.F. Zhu & L. Guo was *X. aristata* var. *aristata* Mont. Based on the above studies, 42 *Xylaria* species growing on fallen leaves and petioles have been officially published worldwide. This paper describes two new species. A search table for 44 *Xylaria* species related to fallen leaves and petioles is established [7,13,34,38,40–42], as shown below.

The key to the species of *Xylaria* related to fallen leaves and petioles worldwide

1 Stromata branched, long stipes that bear one to three clavae on each terminal branch ..... *X. luxurians*
1 Stromata unbranched to occasionally branched 2
2 Stipes tomentose or glabrous to tomentose 3
2 Stipes glabrous 15
3 Fertile parts filiform ..... *X. duranii*
3 Fertile parts not filiform 4
4 Fertile parts cylindrical 5
4 Fertile parts capitate 11
5 Fertile parts overlain by dark long spikes ..... *X. asperata*
5 Fertile parts without dark long spikes 6
6 Fertile parts glabrous ..... *X. appendiculata*
6 Fertile parts overlain by tomentum 7
7 Fertile parts densely covered by yellow tomentum ..... *X. fulvotomentosa*
7 Fertile parts with non-yellow tomentum 8
8 Ascospores with non-cellular appendages 9
8 Ascospores without non-cellular appendages 10
9 Ascospores (14.5–)15–16.5(–17) × (8–)8.5–9.5(–10) µm ..... *X. allima*
9 Ascospores (10–)10.5–12(–14) × (5–)6–7(–7.5) µm ..... *X. lima*
10 Surface of fertile parts with half-exposed to fully exposed perithecial contours ..... *X. castilloi*
10 Surface of fertile parts lacking perithecial mounds or with slight perithecial mounds ..... *X. maitlandii*
11 Stromata with an acute apex 12
11 Stromata with a rounded apex 14
12 Ascospores with non-cellular appendages ..... *X. axifera*
12 Ascospores without non-cellular appendages 13
13 Consistency fragile, ascospores (10–)10.5–12.5(–14) × (5.5–)6–7(–7.5) µm ..... *X. aristata* var. *aristata*
13 Consistency soft, ascospores (15–)15.5–17(–18) × (6.5–)7.5–9(–9.5) µm ..... *X. hispidipes*
14 Ascospores (13.5–)14–16(–17) × (5.5–)6–7(–7.5) µm ..... *X. aristata* var. *hirsuta*
14 Ascospores 8–9(–9.5) × 4–4.5(–6.6) µm ..... *X. imminuta*
15 Fertile parts cylindrical or conical to subglobose, most perithecia gather near the top of the stromata, with several occasionally scattered below ..... *X. petchii*
15 Fertile parts with uniform morphology, perithecia lacking the above cluster patterns 16
16 Fertile parts cylindrical 17
16 Fertile parts not cylindrical 30
17 Ascospores with non-cellular appendages 18
17 Ascospores without non-cellular appendages 25
18 Stromata with an outer peeling layer split into band-like stripes, perithecia 150–200 µm ..... *X. vittiformis*
18 Stromata without an outer peeling layer or the outer peeling layer without band-like stripes, perithecia greater than 250 µm 19
19 Surface of fertile parts lacking perithecial mounds, ascospores (22–)23.5–27(–28) × (8.5–)9–10.5(–11) µm ..... *X. spiculaticlavata*
19 Surface of fertile parts with conspicuous perithecial mounds, ascospores length less than 19 µm and width nearly less than 9 µm 20
20 Stromata with an outer peeling layer split into narrow or thread-like stripes, ascospores (13.5–)14–15(–17) × (4.5–)5–6(–7) µm ..... *X. minuscula*
20 Stromata without an outer peeling layer or the outer peeling layer without narrow or thread-like stripes 21
21 Stromata with a blunt apex, ascospores 8–10 × 4–6 µm ..... *X. kamatii*
21 Stromata with an acute apex, ascospores length greater than 10.3 µm 22

22 Ostioles conic-papillate, tilting upwards, 120–150 μm broad at base............................ ....*X. appendiculatoides*
22 Ostioles papillate or slightly papillate, less than 80 μm broad at base............................23
23 Stromata surface blackish brown, ascospores (15–)16.5–18(–19) × (7.5–)8–9(–9.5) μm ....*X. phyllophila*
23 Stromata surface black, ascospores length less than 16.5 μm and width nearly less than 7.5 μm...24
24 Stromata filiform, with long sterile filiform apexes up to 10–35 mm, ascospores ellipsoid to fusiform, (10.3–)11.5–14(–16.5) × (4.1–)4.6–5.7(–6.8) μm........*X. diaoluoshanensis*
24 Stromata cylindrical, with a mucronate apex 2 mm, ascospores ellipsoid, (11.5–)12.5–14.5(–15) × 5.5–8 μm...*X. polysporicola*
25 Stromata surface dark vinaceous brown, ascospores strongly inequilateral.............. ....*X. phyllocharis*
25 Stromata surface not dark vinaceous brown, ascospores inequilateral...........................26
26 Ascospores light brown to brown, (5.5–)6–7 × 3–3.5(–4) μm...........................*X. diminuta*
26 Ascospores brown to blackish brown, length greater than 8.5 μm and width larger than 4 μm...27
27 Stromata without an outer layer...*X. neblinensis*
27 Stromata with an outer layer..28
28 Stromata with an acuminate or mucronate apex, ascospores (9–)9.5–10.5(–11) × (5.5–)6–6.5(–7) μm..*X. noduliformis*
28 Stromata with a long acicular apex...29
29 Stromata 23–35 mm total length, overlain by an outer peeling layer split into narrow or thread-like stripes, ascospores (8.5–)9–11 × 4–6 μm..*X. foliicola*
29 Stromata 61–78 mm total length, overlain by an outer peeling layer split into band-like stripes, ascospores (10–)11–12(–12.5) × (5.5–)6–7(–7.5) μm........*X. vittatipiliformis*
30 Fertile parts filiform..31
30 Fertile parts not filiform..36
31 Ascospores with spiral germ slit..*X. meliacearum*
31 Ascospores with straight germ slit...32
32 Consistency fragile, ascospores (15–)16.5–19(–21.5) × (5–)5.5–6.5(–7.5) μm.................... ....*X. simplicissima*
32 Consistency soft, ascospores length less than 14.5 μm...33
33 Ascospores with non-cellular appendages...34
33 Ascospores without non-cellular appendages...35
34 Ascospores light brown, short fusoid...*X. fliformis*
34 Ascospores brown to dark brown, ellipsoid...*X. vagans*
35 Stromata 15–20 mm total length, ascospores ellipsoid, 12–13.5 × 4–5 μm.......*X. eugeniae*
35 Stromata 35–83 mm total length, ascospores ellipsoid to shortly fusoid, (9–)9.5–10.5(–11) × (3.5–)4–4.5(–5) μm...*X. vermiformis*
36 Ascospores with non-cellular appendages...37
36 Ascospores without non-cellular appendages...43
37 Surface of fertile parts with conspicuous perithecial mounds..38
37 Surface of fertile parts lacking perithecial mounds..42
38 Ascospores with cellular appendage on one end..39
38 Ascospores without cellular appendage...40
39 Stromata 50 mm total length, without a long apex, ostioles slightly papillate, ascospores (12–)12.5–15.5(–17) × (5–)6–7.5(–8) μm...*X. amphithele*
39 Stromata 91–147 mm total length, with a long apex, ostioles coarsely papillate, ascospores (10.5–)11.5–13.5(–15) × (5–)5.5–6.5(–7.5) μm..................................*X. nainitalensis*
40 Stromata without an acute apex, ascospores (14.5–)15.5–18(–19) × (5–)5.5–6.5(–7) μm ....*X. heloidea*
40 Stromata with an acute apex..41
41 Stromata with a mucronate apex, ascospores (10–)10.5–12(–12.5) × (5–)5.5–6(–6.5) μm

....X. *pisoniae*
41 Stromata with a long acicular apex, much longer than the fertile part, ascospores (8.5–)9.5–11(–12) × (4–)4.5–6(–6.5) µm..X. *sicula*
42 Stromata surface dull grayish brown, overlain with a thin pellicle cracked reticulately into plaques 100–200 µm broad, fertile parts capitate...X. *hypsipoda*
42 Stromata surface dark brown to blackish, without the above plaques, fertile parts peltate ....X. *memecyli*
43 Ascospores dark brown, nearly semicircular to broadly ellipsoid, (10–)10.5–12.5(–14) × (5.5–)6–7(–7.5) µm..X. *delicatula*
43 Ascospores dark brown to blackish brown, ellipsoid, (12.5–)13–15(–16) × (7.5–)8–9(–10) µm ....X. *clusiae*

Author Contributions: Conceptualization, Y.C.; Data curation, X.P. and J.L.; Formal analysis, J.L. and X.C.; Funding acquisition, Z.C. and Y.C.; Investigation, X.P., T.W. and Y.L.; Methodology, X.P. and Z.C.; Project administration, Z.C. and Y.C.; Visualization, X.P. and Z.C.; Writing—original draft, X.P.; Writing—review and editing, X.P. and Y.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study is supported by the Technical Innovation Project of Hainan Provincial Research Institute (SQKY2022-0036) and the Forestry and Grassland Ecological Protection and Restoration Fund (National Park Subsidy) Project (HDZB-2023-071).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Datasets analyzed in this study are publicly available. All the gene sequences obtained in this article can be searched in GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Table 1). All new taxa are saved in MycoBank (https://www.mycobank.org/).

Acknowledgments: We would like to thank the Hainan Tropical Rainforest National Park Service for their assistance during the specimen collection process.

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

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