Multi-Gene Phylogeny and Taxonomy of *Hypoxylon* (Hypoxylaceae, Ascomycota) from China

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1. Introduction

*Hypoxylon* Bull., described by Bulliard in 1791 [1], is a genus that contains primarily saprotrophs and endophytes of angiospermous plants [2,3]. The genus *Hypoxylon*, together with *Annullohypoxylon* Y.M. Ju, J.D. Rogers, H.M. Hsieh and *Daldinia* Ces., De Not., are all closely associated with both dicots and, infrequently, monocots in forest ecosystems [4]. Most hypoxylaceous fungi have a strong capacity to degrade cellulose and lignin and are important elements in forest ecosystems, playing a key ecological role in carbon circulation [5]. In addition, the endophytic stages of these fungi may even benefit their host plants by protecting them from pathogens [6,7].

The type genus *Hypoxylon* is the largest genus in the Hypoxylaceae, with more than 200 species [8] and 1173 epithets in the Index Fungorum (http://www.indexfungorum.org/names/names.asp, accessed on 1 November 2021). Members of the genus have a world-wide distribution, but they display a higher diversity in the tropics and subtropics [4,6,9,10].
In the 20th century, the generic concept of *Hypoxylon* was based only on morphological characteristics [1,4,11–15]. Currently, morphological, phylogenetic, and chemotaxonomic evidence, has also been used to infer species limits in inter- and intra-genera in Hypoxylaceae [3,6,10] and to segregate some new genera such as *Annulohypoxylon* [16], *Hypomontagnella* [17], *Jackrogersella*, and *Pyrenopolyporus* [18] from the genus *Hypoxylon*. The genus *Hypoxylon* is quite common in China; however, the occurrence of the species in China has not been confirmed by molecular phylogenetic analyses, and the species diversity and distribution of the genus in China are unclear [19–22].

Hainan Tropical Rainforest National Park is located in south-central Hainan province, between 18°33′16″–19°14′16″ N and 108°44′32″–111°04′43″ E and has a tropical monsoon climate. More than 3577 plant species, 1142 genera, and 220 families have been reported in the rainforest park (http://www.hntrnp.com, accessed on 15 November 2021), including abundant hypoxylaceous fungi. During investigations on Xylariales from Hainan Province, China, some specimens of Hypoxylaceae were collected. These collections were carefully studied using both morphological and phylogenetic methods, and four undescribed species of *Hypoxylon* were identified. The aims of this study were to confirm the taxonomic status of the new species, explore the species diversity of *Hypoxylon* in Hainan Tropical Rainforest National Park, and infer the evolutionary relationships of the genus *Hypoxylon*.

2. Materials and Methods

2.1. Sample Sources

The studied specimens were collected from Hainan Tropical Rainforest National Park, China, in 2020. These specimens were deposited at the Fungarium of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences (FCATAS).

2.2. Morphological Characterization

The micromorphological observations, micrographs, and measurements were obtained using an Olympus IX73 inverted fluorescence microscope (Tokyo, Japan) with the laser capture microdissection system of model MMI CellCut Plus (Zurich, Switzerland), while the same processes for observing the morphological characteristics of stromatal surfaces and perithecia were performed using a VHX-600E microscope from the Keyence Corporation. The photographs of ascospores were examined by scanning electron microscope (SEM) (Hitachi Corporation, Tokyo, Japan). Sexual structures were microscopically observed in water, 10% KOH, and Melzer’s reagent, as determined by Ju and Rogers [4]. The color codes appearing in this article refer to Rayner [23]. In the text, the following abbreviations are used: KOH = 10% potassium hydroxide, n = number of ascospores measured from a given number of specimens, M = arithmetical average of sizes of all ascospores.

2.3. DNA Extraction, PCR Amplification, and Sequencing

Following the instructions of the manufacturer, total genomic DNA of studied samples was extracted using an improved cetyltrimethylammonium bromide (CTAB) rapid extraction kit for plant genomes (Aidlab Biotechnologies, Beijing, China) and a Thermo Scientific Phire Plant Direct PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA). Four DNA loci of ITS (internal transcribed spacer regions), nLSU (nuclear large subunit ribosomal DNA), RPB2 (RNA polymerase II second largest subunit), and β-tubulin (beta-tubulin) were amplified by polymerase chain reaction (PCR) using HS Taq Mix (Dongsheng Biotech, Guangzhou, China). The 40 µL PCR mixtures contained 16 µL of ddH2O, 20 µL of 2 × HS™ Mix, 2 µL of DNA template, and 1 µL of each forward and reverse primer. The primer pairs ITS5/ITS4, LR0R/LR5, fRPB2-7CR/fRPB2-5F, and T1/T22 were used to amplify ITS, LSU, RPB2, and β-tubulin, respectively [24–28]. The PCR thermal cycling program for ITS was set as initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 55.8 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. For generation of LSU sequence data, the following program was
used: initial denaturation at 94 °C for 3 min, 36 cycles of 1 min at 94 °C, 50 s at 52 °C and 1 min at 72 °C, with a final extension period of 10 min at 72 °C. The PCR amplification of RPB2 and β-tubulin was set up as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 52 °C for 30 s, 72 °C for 1 min, with a final extension of 72 °C for 10 min [29]. PCR sample purification and DNA sequencing were carried out by BGI, Guangzhou, China. The generated sequences were submitted to GenBank to acquire accession numbers.

2.4. Phylogenetic Analysis

All newly generated sequences and relevant sequences of closely related species within the genus *Hypoxylon* and among genera of the family Hypoxylaceae and some related genera of the Xylariales based on ITS, LSU, RPB2, and β-tubulin were obtained in the phylogenetic analysis (Table 1). The other genera included *Annulohypoxylon*, *Daldinia*, *Hypomontagnella*, *Jackrogersella*, *Pyrenopolyporus*, *Rhopalostroma*, and *Thamnomyces*. The phylogenetic trees were rooted with *Xylaria hypoxylon* (L.) Grev. and *Biscogniauxia nummularia* (Bull.) Kuntze as outgroups.

Table 1. A list of species, specimen numbers, locality, GenBank accession numbers, and references used in this study. Holotype and epitype specimens are labelled as T and ET respectively. Species highlighted in bold were derived from this study. N/A: not available.

| Species Name | Specimen No. | Locality | GenBank Accession No. | Status | Reference |
|--------------|--------------|----------|-----------------------|--------|-----------|
| *Annulohypoxylon annulatum* | CBS 140775 | USA | IT5: KX605459, KY610418, KY624263, KX376335 | ET | [10,18,30] |
| | CBS 135779 | Martinique | LSU: KX376321, KY610425, KY624289, KX271261 | | [30] |
| | CBS 140778 | USA | RPB2: KX376329, KY610419, KY624277, KX376352 | ET | [18,30] |
| | Daldinia dennisii | CBS 114741 | Australia | IT5: JX658477, KY610435, KY624244, KC977262 | T | [6,18,31] |
| | Hypomontagnella barbarensis | STA 14081 | Argentina | LSU: MK131720, MK131718, MK135891, MK135893 | ET | [17] |
| | Hypoxylon anthochromum | MUC 54604 | France, Guiana | RPB2: KY610404, KY624305, KX271273 | ET | [18] |
| | Hypoxylon submonticulosa | CBS 113280 | France | IT5: KC968923, KY610457, KY624226, KX376357 | ET | [18] |
| | Hypoxylon anthrocheirum | YM 9'1 | Mexico | LSU: JK060819, N/A, N/A | | [16] |
| | H. begae | YM 215 | Portugal | IT5: JK060820, N/A, N/A | | [16] |
| | H. brevicornis | YM 36 | Puerto Rico | LSU: JK060821, N/A, N/A | | [16] |
| | H. carneum | MUC 54177 | France | LSU: KY610400, KY624297, KX271270 | ET | [18] |
| | H. ceradicola | CBS 119094 | France | LSU: KC968908, KY610444, KY624254, KX271270 | ET | [6,18] |
| | H. chrysosubalveus | FCATA82571 | China | LSU: OL462794, OL615106, OL848222, OL848229 | ET | [18] |
| | H. chrysosubalveus | FCATA82571 | China | RPB2: OL462795, OL615107, OL848223, OL848230 | ET | [18] |
| | H. croceopilem | CBS 119094 | France | LSU: KC968907, KY610445, KY624255, KX271270 | ET | [18] |
| | H. cyclalbalanopsideis | FCATA82714 | China | LSU: OL462799, OL615108, OL848223 | ET | [18] |
| | H. cyclalbalanopsideis | FCATA82715 | China | RPB2: OL462799, OL615109, OL848226 | ET | [18] |
| | H. dyeckmannii | YM 90104 | USA | LSU: JN979413, N/A, N/A | | [16] |
| | H. duranti | YM 85 | USA | LSU: JN979414, N/A, N/A | | [16] |
| | H. erythrostoma | YM 90602 | China | LSU: JN979416, N/A, N/A | | [16] |
| | H. eurasiatricum | MUC 57720 | Iran | LSU: MW376841, N/A, N/A | | [16] |
| | H. fendleri | DSM 10792 | USA | LSU: MK267533, MK287545, MK287558, MK287571 | ET | [34] |
| | H. ferrugineum | CBS 14129 | Austria | LSU: KX09079, N/A, N/A | | [35] |
| | H. fragiliforme | MUC 51264 | Germany | LSU: KM186294, KM186295, KM186296 | ET | [36] |
| | H. fraxinophilum | MUC 54176 | France | LSU: KC968938, N/A, N/A | | [37] |
| | H. fuscomarginatum | MFLUCC 13-0589 | Thailand | LSU: KP401576, N/A, N/A | | [30] |
| | H. fuscomarginatum | CBS 113049 | France | RPB2: KY610401, KY610482, KY624299, KX271271 | ET | [18] |
| | H. griseobrunneum | CBS 331 | India | LSU: KY610402, KY624296, KX271270 | ET | [6,18,38] |
| | H. guilameii | MUC 57726 | Iran | LSU: MT214997, MT214998, MT212235, MT212239 | T | [9] |
| | H. haematostroma | MUC 53301 | Martinique | LSU: KC968911, KY610403, KY624301, KX277291 | ET | [18] |
| | H. hainanense | FCATA82712 | China | LSU: OL462796, OL616132, OL848224 | ET | [18] |
| | H. hainanense | FCATA82713 | China | RPB2: OL462797, N/A, N/A | ET | [18] |
| | H. hinnuleum | MUC 3621 | USA | LSU: MK267537, MK287547, MK287562 | T | [34] |
| | H. hinnuleum | MUC 4799 | Germany | LSU: AM479928, KY610448, KY624258, KX271277 | ET | [6,18,39] |
| | H. hypomiltum | MUC 53147 | Guadeloupe | LSU: KY610403, KY610449, KY624302, KX271249 | T | [18] |
| | H. lividicolor | YM 70 | China | LSU: KT809193, MT830132, MT813037 | T | [40] |
| | H. lividicolor | YM 233 | Mexico | LSU: JN979433, N/A, N/A | | [16] |
| | H. macrosporum | YM 47 | Canada | LSU: JN979434, N/A, N/A | | [16] |
| | H. macrosporum | MUC 53765 | Guadeloupe | RPB2: KC968926, KY610488, KY624306 | | [16] |
The sequence alignment was conducted using fast Fourier transformation (MAFFT) online (http://mafft.cbrc.jp/alignment/server/), accessed on 22 November 2021) [48]. Further sequence processing was conducted using BioEdit 7.0.5, and the concatenation of four DNA loci of ITS, LSU, RPB2, and β-tubulin was completed using MEGA 6.0 [29,49,50].

A combined matrix of ITS–LSU–RPB2–β-tubulin was used to construct phylogenetic trees and analyzed by two methods: Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum Likelihood (ML) analysis was performed using raxmlGUI 2.0 with rapid bootstrap search executing 1000 replicates, setting the substitution model as GTRGAMMA+G [45,51]. Bayesian analysis was performed using MrBayes v.3.2.6, based on using jModelTest 2 to conduct model discrimination [52]. The selected applicable model implemented the Markov Chain Monte Carlo (MCMC) algorithm, which determined posterior probabilities (PP) [53]. Six simultaneous Markov chains were run for 1000000 generations, from which every 100th generation was sampled as a tree [54–58]. Phylogenetic trees were viewed in FigTree 1.4.2 [59]. The phylogenetic tree and multi-gene sequence alignment were deposited in TreeBASE (www.treebase.org/treebase-web/home.html, accessed on 20 December 2021) with accession number S29126.

### 3. Results

#### 3.1. Phylogenetic Analysis

The sequence datasets for the contributions of the molecular phylogenetic trees consisted of 82 ITS, 57 LSU, 58 RPB2, and 81 β-tubulin sequences. All of the 278 sequences came from 79 strains including 4 newly described Hypoxylon taxa, 58 known Hypoxylon taxa, 3 Annulohypoxylon taxa, 2 Daldinia taxa, 3 Hypomontagnella taxa, 2 Jackrogersella taxa, 3 Pyrenoporyphorax taxa, 1 Rhopalostroma taxon and 1 Thaumamycetes taxon, as well as the X. hypoxylon and B. nummularia as outgroups.

After aligned by MAFFT online, the sequence datasets contained 2046 character positions for ITS alignment, 3320 character positions for LSU alignment, 1285 character positions for RPB2 alignment, and 1285 character positions for β-tubulin alignment. The alignment positions for ITS, LSU, and RPB2 were 3320, 1285, and 1285, respectively, and the alignment positions for β-tubulin were 1285.

The sequence dataset was deposited in TreeBASE (www.treebase.org/treebase-web/home.html, accessed on 20 December 2021) with accession number S29126. The sequence alignment was conducted using fast Fourier transformation (MAFFT) online (http://mafft.cbrc.jp/alignment/server/), accessed on 22 November 2021) [48]. Further sequence processing was conducted using BioEdit 7.0.5, and the concatenation of four DNA loci of ITS, LSU, RPB2, and β-tubulin was completed using MEGA 6.0 [29,49,50].

A combined matrix of ITS–LSU–RPB2–β-tubulin was used to construct phylogenetic trees and analyzed by two methods: Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum Likelihood (ML) analysis was performed using raxmlGUI 2.0 with rapid bootstrap search executing 1000 replicates, setting the substitution model as GTRGAMMA+G [45,51]. Bayesian analysis was performed using MrBayes v.3.2.6, based on using jModelTest 2 to conduct model discrimination [52]. The selected applicable model implemented the Markov Chain Monte Carlo (MCMC) algorithm, which determined posterior probabilities (PP) [53]. Six simultaneous Markov chains were run for 1000000 generations, from which every 100th generation was sampled as a tree [54–58]. Phylogenetic trees were viewed in FigTree 1.4.2 [59]. The phylogenetic tree and multi-gene sequence alignment were deposited in TreeBASE (www.treebase.org/treebase-web/home.html, accessed on 20 December 2021) with accession number S29126.
positions for RPB2 alignment, and 2298 character positions for β-tubulin alignment. With less informative positions trimmed, and four DNA loci connected, the generated multi-gene alignment (MGA) had an aligned length of 3836 characters, of which 1977 characters were parsimony-informative. Phylogenetic trees generated from BI and ML analyses of the combined dataset of ITS–LSU–RPB2–β-tubulin were highly similar in topology. Only the ML tree is shown in Figure 1, with ML bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.95 labelled along the branches.

Figure 1. ML phylogram of the Hypoxylon species based on the multi-gene alignment of ITS–LSU–RPB2–β-tubulin. Support values of ML and BI analyses (bootstrap support ≥ 50%, posterior probabilities value ≥ 0.95) are labelled above or below the respective branches (ML/BI). New species are labelled in bold.
The phylogenies reveal a paraphyly of *Hypoxylon*, with the genera *Annulohypoxylon*, *Daldinia*, *Hypomontagnella*, *Jackrogersella*, *Pyrenoporypora*, and *Thamnomyces* embedded within the former. The phylogeny inferred from the ITS–LSU–RPB2–β-tubulin sequences demonstrated that the four new species, i.e., *H. wuzhishanense*, *H. hainanense*, *H. chrysalidosporum*, and *H. cyclobalanopsidis*, formed distinct well-supported lineages (Figure 1).

3.2. Taxonomy

*Hypoxylon chrysalidosporum* Hai X. Ma, Z.K. Song, sp. nov., Figure 2.

![Figure 2](image-url)

**Figure 2.** *Hypoxylon chrysalidosporum* (holotype FCATAS 2710). (a) Stromata on dead corticated branch. (b,c) Stromatal surface. (d–f) Stroma in vertical section showing the perithecia and tissue below the perithecial layer. (g,h) Ascus in Melzer’s reagent. (i) Ascus in water. (j,l) Ascospore in water. (k) KOH-extractable pigments. (m) Ascospores in Melzer’s reagent showing germ slit. (n) Ascospores in 10% KOH. (o) Ascospore under SEM. Scale bars: (a) = 1 cm; (b) = 1 mm; (c–f) = 200 µm; (g–j,l–n) = 10 µm; (o) = 5 µm.

MycoBank: MB 841956.

**Diagnosis.** Differs from *H. duranii* and *H. notatum* in its KOH-extractable pigments, highly reduced or absent in amyloid apical apparatus, and smaller ascospores with straight germ slit.

**Etymology.** *Chrysalidosporum* (Lat.): referring to the chrysalis-shaped ascospores.

**Holotype.** CHINA: Hainan Province, Ledong County, Hainan Tropical Rainforest National Park, Jianfengling National Natural Reserve, approximately 108°51′ E and 18°47′ N, elevation approximately 700 m, saprobic on surface of dead corticated branches, 24 October 2020, Haixia Ma, Col. J214 (FCATAS 2710).

**Teleomorph.** Stromata glomerate to effused–pulvinate, with conspicuous perithecial mounds, 0.1–0.7 cm long × 0.1–0.3 cm broad × 0.3–0.5 mm thick; surface Bay (6), Rust (39), Dark Brick (60) and Livid Purple (81); with pale brown to dull reddish brown granules immediately beneath the surface and between perithecia; yielding Pale Luteous (11), Honey (60) and Ochreous (44) pigments in 10% KOH; tissue below the perithecial layer black, inconspicuous, 0.1–0.4 mm thick. Perithecia spherical to obvoid, black, 0.2–0.4 mm broad × 0.3–0.4 mm high. Ostioles umbilicate, encircled with a paler area, opening lower than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, 75–139 µm total length.
× 6.6–11.9 µm broad; the spore-bearing portion 49–82 µm long, and stipes 19–71 µm long, with inamyloid apical apparatus highly reduced or absent, not bluing in Melzer’s reagent. Ascospores light-brown to brown, unicellular, ellipsoid-inequilateral, with slightly broad rounded ends, 8–10.6–(11.1) × 4.1–6.3–(7.1) µm (n = 60, M = 9.2 × 5.3 µm), with conspicuously straight spore-length germ slit on the convex side; perispore dehiscent in 10% KOH, with very conspicuous coil-like ornamentation in SEM; epispore smooth.

Additional specimens examined. CHINA: Hainan Province, Ledong County, Hainan Tropical Rainforest National Park, Jianfengling National Natural Reserve, approximately 108°49’ E and 18°50’ N, elevation approximately 750 m, saprobic on surface of dead corticated branches, 24 October 2020, Haixia Ma, Col. J1059 (FCATAS 2711).

Hypoxylon cyclobalanopsidis Hai X. Ma, Z.K. Song, sp. nov., Figure 3.

Figure 3. Hypoxylon cyclobalanopsidis (holotype FCATAS 2714). (a) Stromata on branches. (b) Stromatal surface and ostioles. (c,d) Stroma in vertical section showing the perithecia and tissue below the perithecial layer. (e) Mature ascus in water showing germ slit. (f) Ascospore under SEM. (g) Ascospore in 10% KOH. (h) KOH-extractable pigments. (i) Apical apparatus in Melzer’s reagent. (j) Ascospore in water showing germ slit. (k) Ascus in Melzer’s reagent. Scale bars: (a) = 1 cm; (b–d) = 200 µm; (e,g,i–k) = 10 µm; (f) = 5 µm.

MycoBank: MB 841957.

Diagnosis. Differs from H. porphyreum in its larger ascospores, KOH-extractable pigments, host plant and distribution. Differs from H. eurasiaticum, H. fuscum, and H. pseudofuscum in its smaller apical apparatus, host plant and tropical distribution.

Etymology. Cyclobalanopsidis (Lat.): referring to the host genus Cyclobalanopsis which the fungus inhabits.

Holotype. CHINA: Hainan Province, Ledong County, Hainan Tropical Rainforest National Park, Jianfengling National Natural Reserve, approximately 108°53’ E and 18°43’ N, elevation approximately 720 m, saprobic on dead corticated branches of Cyclobalanopsis, 23 October 2020, Haixia Ma, Col. J217 (FCATAS 2714).

Teleomorph. Stromata pulvinate to effused–pulvinate, 0.1–2 cm long × 0.1–0.6 cm broad × 0.25–0.45 mm thick; with inconspicuous perithecial mounds; surface Livid Purple (81), Livid Vinaceous (83) and Violet (32), with colored coating worn off exposing Dark Purple (36) areas, sometimes with tiny cracks appearing; with pale-brown to orange-brown granules immediately beneath the surface and between perithecia; yielding Amber (47) and Ochreous (44) to Fulvous (43) pigments in 10% KOH; tissue below the perithecial layer
inconspicuous and pale-brown to black. Perithecia ovoid to obovoid, black, 0.1–0.3 mm broad × 0.1–0.4 mm high. Ostioles umbilicate, encircled with a white area, opening lower than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, short-stipitate, 76–117 µm total length × 6.9–12.9 µm broad, the spore-bearing portion 65–93 µm long, and stipes 6–28 µm long, with apical apparatus highly reduced and minute, faintly bluing in Melzer’s reagent. Ascospores brown to dark-brown, unicellular, ellipsoid-inequilateral, with narrowly to broadly rounded ends, 11–15.2 × 5.1–7 µm (n = 60, M = 13 × 6.3 µm), with more sigmoid to less straight spore-length germ slit on the convex side; perispore dehiscent in 10% KOH, with conspicuous coil-like ornamentation in SEM; epispore smooth.

**Additional specimens examined.** CHINA: Hainan Province, Ledong County, Hainan Tropical Rainforest National Park, Jianfengling National Natural Reserve, Mingfeng Valley, approximately 108°51′ E and 18°45′ N, elevation approximately 700 m, saprobic on dead corticated branches of *Cyclobalanopsis*, 23 October 2020, Haixia Ma, Col. J200 (FCATAS 2715).

*Hypoxylon hainanense* Hai X. Ma, Z.K. Song, sp. nov., Figure 4.

Figure 4. *Hypoxylon hainanense* (holotype FCATAS 2712). (a) Stromata on wood. (b) Stromatal surface. (c,d) Stroma in vertical section showing the perithecia and tissue below the perithecial layer. (e) Ascus in Melzer’s reagent. (f) Ascus in water. (g) Ascospore in 10% KOH. (h) Apical apparatus in Melzer’s reagent. (i) Ascospore in water. (j) Ascospore in 10% KOH showing germ slit. (k) Ascospore under SEM. (l) KOH-extractable pigments. Scale bars: (a) = 1cm; (b–d) = 200 µm; (e–j) = 10 µm; (k) = 5 µm.

MycoBank: MB 841955.

**Diagnosis.** Differs from *H. brevisporum* in having larger and wider ascospores, spherical to obovoid perithecia, and slightly larger apical apparatus. Differs from *H. lividicolor* in its thinner stromata, spherical to obovoid perithecia, and smaller ascospores.

**Etymology.** *Hainanense* (Lat.): referring to the holotype locality of species in Hainan Province.

**Holotype.** CHINA: Hainan Province, Ledong County, Hainan Tropical Rainforest National Park, Jianfengling National Natural Reserve, approximately 108°48′ E and 18°45′ N, elevation approximately 650 m, saprobic on surface of dead decorticated wood, 29 December 2020, Haixia Ma, Col. J233 (FCATAS 2712).
**Teleomorph.** Stromata effused–pulvinate, 0.8–7.2 cm long × 0.6–3.3 cm broad × 0.7–1.5 mm thick; with inconspicuous to conspicuous perithecial mounds; surface Violet (32), Livid Purple (81) and Dark Violet (33); highly carbonaceous black granules immediately beneath surface and between perithecia; yielding Pale Vinaceous (85) to Livid Vinaceous (83) and Vinaceous Purple (101) pigments in 10% KOH; tissue below the perithecial layer black, conspicuous, 0.3–0.8 mm thick. Perithecia spherical to obovoid, black, 0.2–0.5 mm broad × 0.3–0.6 mm high, occasionally with pale-brown perithecial contents. Ostioles opening at the same level or slightly higher than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, 70–139 µm total length × 4.6–6.8 µm broad, the spore-bearing portion 47–61 µm long, and stipes 19–83 µm long, with amyloid apical apparatus bluing in Melzer’s reagent, discoid, 0.6–1.1 µm high × 1.3–1.8 µm broad. Ascospores light-brown to brown, unicellular, ellipsoid-inequilateral, with slightly broad rounded ends, 6.1–9.6 × 3.2–5 µm (n = 60, M = 7.7 × 4 µm), with conspicuously straight germ slit less than spore-length on the convex side; perispore dehiscent in 10% KOH, with inconspicuous coil-like ornamentation in SEM; epispore smooth.

**Additional specimens examined.** CHINA: Hainan Province, Ledong County, Hainan Tropical Rainforest National Park, Jianfengling National Natural Reserve, approximately 108°50’ E and 18°46’ N, elevation approximately 600 m, saprobic on surface of dead decorticated wood, 29 December 2020, Haixia Ma, Col. J1058 (FCATAS 2713).

*Hypoxylon wuzhishanense* Hai X. Ma, Z.K. Song, sp. nov., Figure 5.

**Figure 5.** *Hypoxylon wuzhishanense* (holotype FCATAS 2708). (a) Stromata on dead Bamboo sp. (b) Stroma in vertical section showing the perithecia and tissue below the perithecial layer. (c) Stromatal surface and ostioles. (d) Ascus in 10% KOH. (e) Ascospores in Melzer’s reagent showing germ slit. (f) Ascus in water. (g) Ascus in Melzer’s reagent. (h) KOH-extractable pigments. (i,j) Apical apparatus in Melzer’s reagent. (k) Ascospore in water. (l,m) Ascospore in 10% KOH. (n) Ascospore under SEM. Scale bars: (a) = 1 mm; (b,c) = 200 µm; (d–g,i–m) = 10 µm; (g) = 5 µm.

MycoBank: MB 841954.

**Diagnosis.** Differs from *H. pseudofendleri* by having smaller prithecia, larger ascospores and lower ostioles. Differs from *H. pilgerianum* in having larger apical apparatus, larger and wider ascospores, and most of perispore indehiscent in 10% KOH.
**Key to Hypoxylon species from China and related species around the world**

1. Ostioles barely to slightly higher than the stromatal surface ........................................ 2
2. Ostioles lower than the stromatal surface ................................................................. 8
3. Ascospores nearly equilateral ................................................................................. 3
4. Ascospores inequilateral ......................................................................................... 4
5. Stromata glomerate to pulvinate; ascospores 8.5–12(–13.5) × 4–5 μm ........... H. croceum
6. Stromata pulvinate to effused–pulvinate; ascospores 11–14.5 × (4.5–)5–6.5 μm........ H. parksonianum
7. Perithecia 0.3–0.5(–0.6) mm broad; ascospores 9.5–15(–16) × 4–6.5(–7) μm ........... H. lenormandii
8. Ascospores nearly equilateral ................................................................................ 9
9. Ascospores inequilateral ....................................................................................... 14
10. KOH-extractable pigment Orange (7) ................................................................. H. cinnabarinum
11. Without apparent KOH-extractable pigments or with dilute Grayish Sepia (106) to
    blackish pigments ...................................................................................... H. dieckmannii
12. Perithecia tubular to long tubular, 0.3–0.4 mm broad × 0.5–1 mm high ........ H. investiens
13. Stromatal surface Vinaceous Gray (116), Purplish Gray (128), Livid Vinaceous (83),
    Dark Vinaceous (82), or Brown Vinaceous (83), becoming blackish when aged; dull reddish-brown

**Etymology.** *Wuzhishanense* (Lat.): referring to the holotype locality of species in Wuzhishan National Natural Reserve.

**Holotype.** CHINA: Hainan Province, Wuzhishan City, Hainan Tropical Rainforest National Park, Wuzhishan National Natural Reserve, approximately 109°38′ E and 18°55′ N, elevation approx. 600 m, saprobic on surface of dead Bamboo sp., 30 December 2020, Haixia Ma, Col. W2 (FCATAS 2708).

**Teleomorph.** Stromata pulvinate, 0.8–14 cm long × 0.4–3.2 cm broad × 0.2–0.3 mm thick; with inconspicuous-to-conspicuous perithecial mounds; surface Rust (39), Livid Purple (81) to Dark Brick (60), with colored coating worn off exposing Dark Purple (36) areas, with yellowish-brown granules immediately beneath the surface and between perithecia; yielding Amber (47) and Ochreous (44) to Fulvous (43) pigments in 10% KOH; tissue below the perithecial layer inconspicuous. Perithecia spherical, black, 0.2–0.3 mm broad × 0.1–0.2 mm high. Ostioles umbilicate, opening slightly lower than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, 71–106 μm total length × 6.4–9 μm broad, the spore-bearing portion 58–91 μm long, and stipes 9–28 μm long, with amyloid apical apparatus bluing in Melzer’s reagent, discoid, 1–1.9 μm high × 2.2–3.4 μm broad. Ascospores light-brown to brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, (9.5–)10–14 × 5.4–6.7 μm (n = 60, M = 11.4 × 6 μm), with straight to less frequently sigmoid germ slit spore-length on the convex side; most of perispore indehiscent in 10% KOH, occasionally dehiscent, with inconspicuous coil-like ornamentation in SEM; epispore smooth.

**Additional specimens examined.** CHINA: Hainan Province, Wuzhishan City, Hainan Tropical Rainforest National Park, Wuzhishan National Natural Reserve, approximately 109°38′ E and 18°55′ N, elevation approximately 600 m, saprobic on surface of dead Bamboo sp., 30 December 2020, Haixia Ma, Col. X469 (FCATAS 2709).

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**Wuzhishan National Natural Reserve.**

**Etymology.** Wuzhishanense (Lat.): referring to the holotype locality of species in Wuzhishan National Natural Reserve.

**Holotype.** CHINA: Hainan Province, Wuzhishan City, Hainan Tropical Rainforest National Park, Wuzhishan National Natural Reserve, approximately 109°38′ E and 18°55′ N, elevation approx. 600 m, saprobic on surface of dead Bamboo sp., 30 December 2020, Haixia Ma, Col. W2 (FCATAS 2708).

**Teleomorph.** Stromata pulvinate, 0.8–14 cm long × 0.4–3.2 cm broad × 0.2–0.3 mm thick; with inconspicuous-to-conspicuous perithecial mounds; surface Rust (39), Livid Purple (81) to Dark Brick (60), with colored coating worn off exposing Dark Purple (36) areas, with yellowish-brown granules immediately beneath the surface and between perithecia; yielding Amber (47) and Ochreous (44) to Fulvous (43) pigments in 10% KOH; tissue below the perithecial layer inconspicuous. Perithecia spherical, black, 0.2–0.3 mm broad × 0.1–0.2 mm high. Ostioles umbilicate, opening slightly lower than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, 71–106 μm total length × 6.4–9 μm broad, the spore-bearing portion 58–91 μm long, and stipes 9–28 μm long, with amyloid apical apparatus bluing in Melzer’s reagent, discoid, 1–1.9 μm high × 2.2–3.4 μm broad. Ascospores light-brown to brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, (9.5–)10–14 × 5.4–6.7 μm (n = 60, M = 11.4 × 6 μm), with straight to less frequently sigmoid germ slit spore-length on the convex side; most of perispore indehiscent in 10% KOH, occasionally dehiscent, with inconspicuous coil-like ornamentation in SEM; epispore smooth.

**Additional specimens examined.** CHINA: Hainan Province, Wuzhishan City, Hainan Tropical Rainforest National Park, Wuzhishan National Natural Reserve, approximately 109°38′ E and 18°55′ N, elevation approximately 600 m, saprobic on surface of dead Bamboo sp., 30 December 2020, Haixia Ma, Col. X469 (FCATAS 2709).
granules immediately beneath surface and between perithecia; asci with apical apparatus bluing in Melzer’s reagent; ascospores dark-brown to blackish-brown, pyriform to obovoid, (11.5–)12–15–(16) × 5.5–7 µm .............................................................. H. fuscopurpureum
13. Stromatal surface Fawn (87) or Umber (9); blackish granules immediately beneath surface and between perithecia; asci with apical apparatus lightly bluing in Melzer’s reagent; ascospores brown, ellipsoid, 7–8.5 × 4–4.5 µm ........................................ H. gilbertsonii
14. Sigmoid germ slit ................................................................. 15
15. Straight spore-length germ slit; perispore dehiscent in 10% KOH .............................................................. H. dengii
15. Sigmoid germ slit slightly less than spore length; perispore infrequently dehiscent in 10% KOH .............................................................. H. dengii
16. Asci with apical apparatus bluing in Melzer’s reagent, 0.5–1.2 µm high × 1.8–2.5 µm broad; KOH-extractable pigment Orange (7) .............................................................. H. fendleri
16. Asci with apical apparatus bluing in Melzer’s reagent, 0.5–0.8 µm high × 3–3.4 µm broad; KOH-extractable pigment Vinaceous Purple (101) .............................................................. H. fuscosides
17. Straight germ slit ................................................................. 18
17. Straight or slightly sigmoid germ slit .............................................................. 26
18. Straight germ slit slightly less than spore length; perispore infrequently dehiscent in 10% KOH .............................................................. H. dengii
18. Straight spore-length germ slit; perispore dehiscent in 10% KOH .............................................................. H. dengii
19. Perithecia long tubular .............................................................. 20
19. Perithecia spherical to obovoid .............................................................. 21
20. Stromatal surface Fulvous (43), Sienna (8), or Rust (39); stromata containing orange-red granules, with KOH-extractable pigments Orange (7) or Scarlet (5); asci with apical apparatus bluing in Melzer’s reagent, 1–2(–2.5) µm high × 3–4 µm broad ................................... H. haematostroma
20. Stromatal surface Brown Vinaceous (84), Dark Brick (60), Sepia (63), or Chestnut (40); stromata containing dark-reddish-brown or blackish granules, with KOH-extractable pigments Olivaceous (48), Greenish Olivaceous (90), Isabelline (65), or Dull Green (70), or infrequently without apparent pigments; asci with apical apparatus bluing in Melzer’s reagent, 0.5–1 µm high × 2.5–3 µm broad .............................................................. H. placentiforme
21. KOH-extractable pigments Olivaceous Gray (12), Greenish Olivaceous (90), or Gray Olivaceous (107) .............................................................. H. brevisporum
21. KOH-extractable pigments with other colors .............................................................. 22
22. Conspicuous coil-like ornamentation of perispore; asci with apical apparatus not bluing in Melzer’s reagent .............................................................. 23
22. Smooth or with inconspicuous coil-like ornamentation of perispore; asci with apical apparatus bluing to lightly bluing in Melzer’s reagent .............................................................. 24
23. KOH-extractable pigments Orange (7), Sienna (8), and Amber (47). H. baihualingense
23. KOH-extractable pigments Pale Luteous (11), Citrine (13) and Honey (64) .............................................................. H. chrysalidosporum
24. Stromata on bamboo ........................................................................ H. pilgerianum
24. Stromata on dicot wood ........................................................................ 25
25. Stromata effused–pulvinate, plane, or with inconspicuous to conspicuous perithecial mounds; perithecia 0.2–0.5 mm broad × 0.3–0.6 mm high; smooth or with inconspicuous coil-like ornamentation of perispore ...................................................... H. rubiginosum
25. Stromata pulvinate with conspicuous perithecial mounds; perithecia 0.1–0.2 mm broad × 0.2–0.3 mm high; smooth perispore ...................................................... H. vinosopulvinatum
26. Perithecia 0.5–0.7 mm broad .............................................................. H. wujiangensis
26. Perithecia less than 0.3 mm broad .............................................................. 27
27. Perispore indehiscent in 10% KOH .............................................................. H. wuzhishanense
27. Perispore dehiscent in 10% KOH .............................................................. 28
28. Conspicuous coil-like ornamentation of perispore .............................................................. 29
28. Smooth or with inconspicuous coil-like ornamentation of perispore ........................ 32
29. Stromata glomerate to hemispherical ................................................................. 30
29. Stromata pulvinate to effused–pulvinate ......................................................... 31
30. Stromata glomerate, restricted-pulvinate to effused–pulvinate, 0.1–6 cm long × 0.1–1.5 cm broad; asci with apparatus bluing in Melzer’s reagent; ascospores with straight or slightly sigmoid spore-length germ slit .............................. H. duranii
30. Stromata hemispherical, pulvinate to effused–pulvinate, up to 12 cm long × 0.2–2 cm broad; asci with apical apparatus bluing in Melzer’s reagent; ascospores with more sigmoid or less straight spore-length germ slit ......................................................... H. eurasiaticum
31. Ascii with apical apparatus highly reduced, minute, faintly bluing in Melzer’s reagent; ascospores 11–15.2 × 5.1–7 µm, with more sigmoid or less straight spore-length germ slit ............................. H. cyclobalanopsidis
31. Ascii with apical apparatus highly reduced or lacking, not bluing in Melzer’s reagent; ascospores (9–)9.5–12 × 4.5–5 µm, with straight or slightly sigmoid spore-length germ slit ......................................................... H. retpeula
32. Stromata hemispherical to spherical ............................................................... 33
32. Stromata pulvinate to effused–pulvinate .......................................................... 36
33. KOH-extractable pigments Orange (7) or Rust (39) ........................................... H. howeanum
33. KOH-extractable pigments with other colors ................................................... 34
34. Ascii with apical apparatus highly reduced or lacking, not bluing in Melzer’s reagent; ascospores (11–)12–16 × (5.5–)6–7.5 µm ................................................... H. notatum
34. Ascii with apical apparatus bluing in Melzer’s reagent ....................................... 35
35. Perithecia spherical to obvoid, 0.1–0.3 mm broad × 0.2–0.5 mm high; ascospores 8–20 × 4–8 µm, with slightly sigmoid germ slit ......................................................... H. fuscom
35. Perithecia spherical, 0.1–0.3 mm diameter; ascospores (8–)9–12 × 4–6 µm, with straight or slightly sigmoid germ slit ......................................................... H. perforatum
36. Ascospore length less than 11 µm .................................................................. 37
36. Ascospore length more than 11 µm ............................................................... 39
37. KOH-extractable pigments Pure Yellow (14) or Amber (47) ............... H. trugodes
37. KOH-extractable pigment Orange (7) ............................................................. 38
38. Stromata surface Fulvous (43), Ochreous (44), or Apricot (42); ascii with apical apparatus 0.2–0.5 µm high × 1–1.5 µm broad; ascospores 8–9.5(–11) × 4–5 µm; Periconiella-like conidiogenus structure ......................................................... H. jecorinum
38. Stromata surface Umber (9), Sepia (63), Rust (39), Sienna (8), Dark Brick (60), or Bay (6); ascii with apical apparatus 0.3–1 µm high × 1.5–2.2 µm broad; ascospores 7–11 × 3.5–5 µm; Nodulisporium-like conidiogenus structure ......................................................... H. subgilvum
39. KOH-extractable pigment Orange (7) ............................................................. H. crocopeplum
39. KOH-extractable pigments with other colors .................................................. 40
40. KOH-extractable pigment Dark Livid (80) ....................................................... 41
40. KOH-extractable pigment Dark Livid (80) ....................................................... 41
41. Stromata 2.5 mm thick; ascospores 11–12.5 × 4.5–5 µm ................................ H. lividicolor
41. Stromata 0.8–1 mm thick; ascospores 10–13.5(–15) × 4.5–6 µm ................ H. lividipigmentum
42. Perithecia obvoid to tubular ................................................................. H. anthonochrom
42. Perithecia spherical to obvoid ................................................................. 43
43. Perithecia 0.18–0.35 mm broad × 0.28–0.42 mm high; ascospores (9–)10–13.5 × 4–5 µm ......................................................... H. porphyreum
44. Perithecia 0.12–0.28 mm broad × 0.19–0.36 mm high; ascospores 11–16 × 4.5–7.3 µm ................................................................. H. pseudofuscum

4. Discussion

Hainan Tropical Rainforest National Park is primarily tropical lowland and tropical mountain rainforest, enjoying a tropical island monsoon climate moderated by a hot and moist climate with annual rainfall often over 2200 mm (http://www.hntrnp.com, accessed on 15 November 2021). The pattern raises the high diversity and the high number
of endemic species of vegetation and fungi in the region. *Hypoxylon* is a cosmopolitan genus, but in tropical and subtropical regions it displays a higher diversity [4]. In the present study, four new species of *Hypoxylon* from Hainan Tropical Rainforest National Park are described, based on morphological characteristics and phylogenetic analyses of the ITS, LSU, RPB2, and β-tubulin sequences. The secondary metabolite profiles generated from chemotaxonomic studies provide strong support for identifying species. However, chemotaxonomic data were not generated in this study [3].

Phylogenetically, *H. chrysalidosporum* is closely related to *H. duranii* J. D. Rogers, based on a combined ITS–LSU–RPB2–β-tubulin dataset. *Hypoxylon duranii* was originally described from Mexico, but the holotype lacked phylogenetic data. Sequence data for *H. duranii* collected from China were referenced in this study [4,16]. Morphologically, *H. duranii* is similar to *H. chrysalidosporum*, sharing glomerate and effused–pulvinate perithecia, spherical, to obvoid perithecia, and dehiscent perispore with conspicuous coil-like ornamentation. However, *H. duranii* can be distinguished from *H. chrysalidosporum* by its KOH-extractable pigments Isabelline or Amber, amyloid apical apparatus, bluing in Melzer’s reagent, and slightly larger ascospores [9.5–13(–14.5) × 4.5–6.5 μm] with a straight or slightly sigmoid germ slit [4]. *Hypoxylon chrysalidosporum* resembles *H. notatum* Berk., M. A. Curtis apud Berk. and H. shearii Y.-M. Ju, J. D. Rogers in having a similar stromatal morphology, apical apparatus being highly reduced or absent, not bluing in Melzer’s reagent, and having dehiscent perispore [4]. However, the type of *H. notatum* was selected by Miller (1961) from the southern United States, and differs in having KOH-extractable pigments Pure Yellow with Greenish Yellow tone and Dark Brown, and larger ascospores [(11–)12–16 × (5.5–)6–7.5 μm] which are strongly curved [1,4]. *H. shearii* has a buff or fawn stromatal surface, with Luteous KOH-extractable pigments, larger perithecia (0.4–0.7 mm diameter), and brown-dark, larger ascospores [12–14 × 5.5–6.5(–7) μm] [4].

*Hypoxylon cyclobalanopsidis* is closely related to *H. porphyreum* Granmo, *H. eurasiaticum* Pourmoghaddam, Krisai-Greilhuber, Khodap., *H. fuscum* (Pers.: Fr.) Fr., and *H. pseudofuscum* Pourmoghaddam, Krisai-Greilhuber in the phylogenetic analyses (Figure 1). *Hypoxylon porphyreum* differs from *H. cyclobalanopsidis* in its smaller ascospores [(9–)10–13.5 × 4–5 μm, M = 11.4 × 4.8 μm], KOH-extractable pigments Brown with a Greenish tone, and growing on *Quercus* from southeastern Norway, Sweden, France and the USA [60,61]. *Hypoxylon eurasiaticum* can be distinguished from *H. cyclobalanopsidis* by its larger discoid apical apparatus (0.5–1.5 μm high × 2.5–3.5 μm wide), smaller ascospores (9–12.5 × 4–6 μm), and by growing on *Quercus castaneifolia* from Iran [33]. *Hypoxylon fuscum* is primarily distinguished from *H. cyclobalanopsidis* by its hemispherical to pulvinate perithecia with dull orange, dull orange-brown, or dull reddish-brown granules immediately beneath the surface and between perithecia, larger discoid apical apparatus (0.5–2 μm high × 1.2–3.5 μm wide), slightly larger ascospores (12.5–15.5 × 5–7 μm), and it frequently occurs on *Cornus avellana* in Europe [4,62,63]. *Hypoxylon pseudofuscum* has larger discoid apical apparatus (0.5–1.5 μm high × 2–3.5 μm wide), KOH-extractable pigments Isabelline, or Hazel, slightly larger ascospores (11–16 × 4.5–7.3 μm), and it grows on *Alnus* and *Salix* from Germany and Iran [33].

*Hypoxylon hainanense* is closely related to *H. brevisporum* Y.M. Ju, J.D. Rogers, *H. lividipigmentum* F. San Martin, Y.M. Ju, J.D. Rogers, and *H. lividicolor* Y.-M. Ju, J. D. Rogers, with a weak bootstrap value according to ML phylogenetic analyses (Figure 1). *Hypoxylon brevisporum* differs from *H. hainanense* in having KOH-extractable pigment Olivaceous Gray, obvoid to tubular perithecia, smaller and thiner ascospores (5.5–8 × 2.5–3.5 μm), and slightly smaller apical apparatus (0.2–0.4 μm high × 1.2–1.5 μm broad) [4]. *Hypoxylon lividicolor* is distinguished by its thicker, chestnut stromata with KOH-extractable pigment Dark Livid, tubular to long tubular perithecia, and larger dark-brown ascospores (11–12.5 × 4.5–5 μm) with straight or slightly sigmoid germ slit [4]. *Hypoxylon lividipigmentum* differs in having tubular to long tubular perithecia and larger, dark-brown ascospores [10–13.5(–15) × 4.5–6 μm] [4].
Hypoxylon wuzhishanense is closely related to H. pseudofendleri D.Q. Dai, K.D. Hyde in our phylogenetic analyses (Figure 1). Unfortunately, RPB2 and β-tubulin sequences of H. pseudofendleri are not available for phylogenetic analysis in GenBank. Morphologically, H. pseudofendleri is similar to H. wuzhishanense in having large, purplish-brown stromata, yellowish-brown granules beneath the surface and between perithecia, similar asci and apical apparatus. However, H. pseudofendleri differs from H. wuzhishanense in having larger perithecia (500–850 µm × 350–500 µm high), with ostioles slightly higher than the stromatal surface, and slightly smaller ascospores (9–11.5 × 4.5–6.5 µm, M = 10.2 × 5.7 µm) with slightly pointed at the ends and smooth wall [42]. There are no descriptions of stromatal pigments in 10% KOH, germination site of ascospores, or perispore in 10% KOH for H. pseudofendleri, so we cannot compare these characteristics between the two species. The two species group together with H. pilgerianum Henn. Hypoxylon pilgerianum was originally described from Brazil on Chusquea sp., with ascospores 10–12 × 4–5 µm (M = 11 × 4.5 µm), and reinstated by Ju and Rogers on dead culms of bamboo, with ascospores 8.5–12(–13.5) × 4–5(–5.5) µm (M = 10.3 × 4.5 µm) [4,9,64]. Fournier et al. described two collections from Martinique as H. cf. pilgerianum sp. 1 and H. cf. pilgerianum sp. 2, with ascospores (7.6–)7.9–9.1(–10) × (3.4–)3.7–4.3(–4.4) µm (M = 8.5 × 4 µm) and (10.3–)10.9–12.5(–12.8) × (4.9–)5.2–6.1(–6.7) µm (M = 11.6 × 5.7 µm), respectively [9]. Hypoxylon pilgerianum s. Ju, Rogers resembles H. wuzhishanense in stromatal morphology, but the former has slightly smaller apical apparatus (0.5–1 µm high × 2.5 µm broad), and smaller and thinner ascospores [8.5–12(–13.5) × 4–5(–5.5) µm], with perispore dehiscent in 10% KOH [4]. Hypoxylon fuscopurpureum (Schwein.) M. A. Curtis somewhat resembles H. wuzhishanense, sharing its stromatal morphology, but differs in having greenish KOH-extractable pigments and larger asci (115–150 × 8–10 µm) [4].

Most species of Hypoxylon play an important ecological role in tropical rainforests as wood-decomposers [3], and some might have beneficial effects on their hosts during their endophytic life stage [65]. In addition, many species have been found to produce highly bioactive secondary metabolites [41,43,66–70]. Although approximately 33 species of Hypoxylon have been recorded in China [4,19–21,29,71], species diversity, evolution, population dynamics, and the host–fungus interactions of this genus are still obscure. Therefore, comprehensive studies on the diversity, phylogeny, evolution, host–fungus interactions, and secondary metabolites of the genus Hypoxylon are needed in the future.

5. Conclusions

The current study revealed four new taxa of Hypoxylon from Hainan Tropical Rainforest National Park based on morphological characteristics, ecological distributions, and a combined ITS–LSU–RPB2–β-tubulin phylogeny.

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