Three New Species of Hypoxylon (Xylariales, Ascomycota) on a Multigene Phylogeny from Medog in Southwest China

Zi-Kun Song 1,2,†, An-Hong Zhu 3,†, Zhen-Dong Liu 4, Zhi Qu 1, Yu Li 2 and Hai-Xia Ma 1,5,6,*

1 Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China; michellesong2021@yeah.net (Z.-K.S.); quzhi@itbb.org.cn (Z.Q.)
2 College of Plant Protection, Jilin Agricultural University, Changchun 130118, China; liyu@itbb.org.cn
3 Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China; 18289679317@163.com
4 Food Science College, Tibet Agriculture & Animal Husbandry University, Nyingchi 860000, China; liuzhendong@xza.edu.cn
5 Hainan Institute for Tropical Agricultural Resources, Haikou 571101, China
6 Hainan Key Laboratory of Tropical Microbe Resources, Haikou 571101, China
* Correspondence: mahaixia@itbb.org.cn
† These authors contributed equally to this work.

Abstract: During a survey of hypoxylaceous fungi in Medog county (Tibet Autonomous Region, China), three new species, including Hypoxylon damuense, Hypoxylon medogense, and Hypoxylon zangii, were described and illustrated based on morphological and multi-gene phylogenetic analyses. Hypoxylon damuense is characterized by its yellow-brown stromatal granules, light-brown to brown ascospores, and frequently indehiscent perispore. Hypoxylon medogense is morphologically and phylogenetically related to H. erythrostroma but differs in having larger ascospores with straight spore-length germ slit and conspicuously coil-like perispore ornamentation. Hypoxylon zangii shows morphological similarities to H. texense but differs in having Amber (47), Fulvous (43) and Sienna (8) KOH-extractable pigments and larger ascospores with straight spore-length germ slit. The multi-gene phylogenetic analyses inferred from the datasets of ITS-RPB2-LSU-TUB2 supported the three new taxa as separate lineages within Hypoxylon. A key to all known Hypoxylon species from China and related species worldwide is provided.

Keywords: Ascomycota; Hypoxylon; multigene phylogeny; taxonomy; wood-decomposing fungi; Xylariales

1. Introduction

Polyphasic taxonomic studies based on phylogenetic, chemotaxonomic, and morphological data were extensively applied to identify species and reflect evolutionary relationships of hypoxylaceous fungi in recent years [1–3]. Since resurrected and emended by Wendt et al. [2], 15 genera were rearranged and recognized to Hypoxylaceae by having stromatal pigments and a nodulisporium-like anamorph. According to the arrangement of the families in Sordariomycetes by Hyde et al. [4], 19 genera were accepted in Hypoxylaceae as saprobes and endophytes. Interesting, Hypoxylon species in endophytic stages may play an important ecological role in protecting their host plants from pathogens [4], and some species are related to insect vectors [2,5–7]. As the main family of Xylariales, Hypoxylaceae exhibits high diversity in tropical and subtropical areas [8–11]. In the classification system of Ju and Rogers [12], the genus Hypoxylon Bull. contains two subclades, the Annulata and Hypoxylon sections. Then they were segregated and the Annulata section was accepted as a new genus, Annulohypoxylon, based on molecular phylogenetic data inferred from ACT and TUB2 sequences [13]. Hypoxylon species are mainly saprobic on dead and decaying wood of angiospermous plants [14]. In this genus, more than 200 species with 1189 epithets included in the Index Fungorum have been reported so far [4,15,16]. Despite species of
Hypoxylon being widely distributed throughout Asia, only 57 species were reported in China currently [17–21].

Medog county, Tibet Autonomous Region is located in southwest China, at the eastern end of the Himalayas and the lower reaches of the Yarlung Zangbo River, and belongs to a subtropical humid climate zone in the Himalayas, with abundant rainfall and an average annual temperature of 18.0 °C [22]. These unique climatic conditions contribute to the abundant resources of macro-fungi. In the current study, we surveyed hypoxylaceous taxa in Medog county, and three undescribed species of *Hypoxylon* were identified. The morphological characteristics of the three new species were described, and their nucleotide sequences were analyzed phylogenetically to confirm their status within *Hypoxylon*.

2. Materials and Methods

2.1. Collection of Specimens

The studied specimens were collected from Medog county (Tibet Autonomous Region), which is located in southwestern China. The explored sites are approximately at elevations from 800 to 1600 m above sea level (m.a.s.l.). The collected samples were dried with a portable drier (manufactured in Germany). Dried samples were labeled and then stored by ultrafreezing at −80 °C for a week to kill insects and their eggs before they were ready for studies. The Fungarium of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences (FCATAS) is responsible for the preservation of specimens.

2.2. Morphological Observations

Sexual structures of the collected specimens were used for morphological observations and identification. The stroma and perithecia were observed, photographed and measured with a VHX-600E 3D microscope from the Keyence Corporation (Osaka, Japan). Fresh material was respectively immersed in water, 10% KOH, and Melzer’s reagent to observe micromorphological structures as determined by Ma et al. and Song et al. [20,21]. The observations, micrographs, and measurements of asci and ascospores were performed by using an Olympus IX73 inverted fluorescence microscope (Olympus, Tokyo, Japan) and the CellSens Dimensions Software (Olympus, Tokyo, Japan). The observations and photgraphs of ornamentation of ascospores were examined by scanning electron microscope (SEM) (Phenom Corporation, The Netherlands) as given in Friebes and Wendelin [23]. The stromatal color and KOH-extractable pigments were assigned following the mycological color chart of Rayner [24]. The present paper contains the following abbreviations: KOH = 10% potassium hydroxide; n = number of measuring objects; M = arithmetical average of sizes of all measuring objects.

2.3. DNA Extraction, Amplification, and Sequencing

Fresh tissue of stroma was used for DNA extraction and sequence generation following the suggestions by Ma et al. and Song et al. [20,21]. Sequences of four DNA loci—ITS (internal transcribed spacer regions), nrLSU (nuclear large subunit ribosomal DNA), RPB2 (RNA polymerase II second largest subunit), and β-tubulin (beta-tubulin) were selected for multi-gene phylogenetic analyses [2,25]. The target sequences were amplified by the primers ITS4/ITS5, LR0R/LR5, fRPB2-7CR/fRPB2-5F, and T1/T22 [26–30]. In total, six ITS, six LSU, six RPB2, and six β-tubulin sequences of new *Hypoxylon* specimens collected from Medog were obtained and submitted to GenBank.

2.4. Molecular Phylogenetic Analyses

The listed Hypoxylaceae and Xylariaceae species in Table 1 originated from previously published studies. Besides *Hypoxylon* spp., the backbone tree contained species of related genera including *Annulohypoxylon*, *Daldinia*, *Hypomontagnella*, *Jackrogersella*, *Pyrenopolyporus*, *Rhopalostroma*, and *Thamnomycyes* with *Xylaria hypoxylon* (L.) Grev. and *Biscogniauxia nummularia* (Bull.) Kuntze chosen to be outgroups.
The alignment, trimming, and concatenation of sequences followed Song et al. [21]. The multi-gene phylogenetic analyses were performed by using two methods of maximum likelihood (ML) and Bayesian analyses (BA) based on ITS-LSU-RPB2-β-tubulin datasets and ITS-β-tubulin datasets. The latter was used for an added validation to the former. Maximum likelihood analyses used raxmlGUI 2.0 with 1000 bootstrap replicates and GTR+GAMMA+G as a substitution model [20,31,32]. Bayesian analyses used MrBayes 3.2.6 with jModelTest 2 conducting model discrimination and Markov chain Monte Carlo (MCMC) sampling. Every 100th generation was sampled as a tree with 1,000,000 generations running for six MCMC chains [20,33]. Phylogenetic trees were viewed and edited by FigTree version 1.4.3 and Photoshop CS6.

### Table 1

Bank accession numbers of sequences used in the multi-gene phylogenetic analyses. T and ET represent holotype and epitype specimens, respectively. Species in bold were derived from this study. N/A: not available.

| Species Name | Specimen No. | Locality | ITS | GenBank Accession No. | β-Tubulin | Status | References |
|--------------|--------------|----------|-----|-----------------------|-----------|--------|------------|
| *A. annulatum* | CBS 140775 | USA | KU604559 | KY610418 | KY624263 | KX76353 | ET | [2,11,25] |
| *A. moriforme* | CBS 123579 | Martinique | KX763621 | KY610425 | KY624289 | KX271261 | T | [25] |
| *A. truncatatum* | CBS 114741 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2,9,34] |
| *A. dieckmannii* | MUC 49214 | Austria | JX658512 | KY610439 | KY624248 | KX772761 | ET | [2,9,34] |
| *Hypomontagnella barbarensis* | STMA 14081 | Argentina | MK131720 | MK131718 | MK135891 | MK135893 | T | [35] |
| *H. annulatum* | MUC 54604 | China | KY610404 | KY610405 | KY624305 | KX727273 | ET | [2] |
| *H. macrocarpum* | CBS 115280 | France | KC966829 | KY610457 | KY624226 | KX767267 | ET | [2,9] |
| *H. submonticulosa* | MUC 52797 | Ethiopia | KC968931 | N/A | N/A | N/A | T | [9] |
| *H. hirsutum* | CBS 123578 | Australia | MG400190 | N/A | N/A | N/A | T | [36] |
| *H. barbarensis* | UCH 9545 | Panama | KC968938 | N/A | N/A | N/A | T | [9] |
| *H. scabriusculum* | CBS 115280 | France | KC968931 | N/A | N/A | N/A | T | [9] |
| *H. placentum* | MUC 51264 | USA | KM186294 | KM186295 | KM186296 | KM186297 | T | [9] |
| *H. submonticulosa* | CBS 119004 | France | KY648907 | KY610445 | KY624255 | KY72688 | T | [2] |
| *H. sp* | CBS 2714 | China | OL615106 | OL584225 | OL584229 | T | [20] |
| *H. cristatellum* | CBS 119004 | France | KY648907 | KY610445 | KY624255 | KY72688 | T | [2] |
| *H. cacophlamis* | CBS 123578 | Martinique | OL615106 | OL584225 | OL584229 | T | [20] |
| *H. pseudocentaureum* | MUC 51264 | Germany | KM186294 | KM186295 | KM186296 | KM186297 | ET | [20] |
| *H. pseudocentaureum* | CBS 118183 | Malaysia | KY610401 | KY610482 | KY624299 | KX727273 | T | [2] |
| *H. guianense* | MUC 51264 | Germany | KM186294 | KM186295 | KM186296 | KM186297 | ET | [2] |
| *H. leucostoma* | MUCL 3621 | Martinique | KM287533 | KM287545 | KM287558 | KM267571 | T | [25] |
| *H. hirsutum* | CBS 114880 | France | KC875331 | KM287535 | KM287549 | KX767262 | T | [25] |
| *H. leucostoma* | MUCL 3621 | Martinique | KM287533 | KM287545 | KM287558 | KM267571 | T | [25] |
| *H. leucostoma* | MUC 49214 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2,9] |
| *H. elegans* | MUC 49214 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2] |
| *H. elegans* | MUC 49214 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2] |
| *H. elegans* | MUC 49214 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2] |
| *H. elegans* | MUC 49214 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2] |
| *H. elegans* | MUC 49214 | Australia | JX658477 | KY610435 | KY624244 | KX767262 | T | [2] |
Table 1. Cont.

| Species Name          | Specimen No. | Locality | ITS   | GenBank Accession No. | β-Tubulin Status | Reference |
|-----------------------|--------------|----------|-------|-----------------------|-------------------|-----------|
| H. liviae             | CBS 11528    | Norway   | NR15514 | N/A                    | N/A              | KC97265  |
| H. lividicolor        | YM 70        | China    | JN97432 | N/A                    | N/A              | KY624306 |
| H. lividipigmentum    | YM 73        | Mexico   | JN97433 | N/A                    | N/A              | KY624306 |
| H. macrosporum        | YM 47        | Canada   | JN97434 | N/A                    | N/A              | KY624306 |
| H. medogense          | FCATAS4041   | China    | ON075425 | ON075431              | N/A              | ON093244 |
| H. medogense          | FCATAS4320   | China    | ON075426 | ON093230              | N/A              | ON093244 |
| H. museum             | MFLUCC 53765 | Guadeloupe | KY610488 | KY624306       | KY624306        | N/A      |
| H. notatum            | YM 250       | USA      | JQ009305 | N/A                    | N/A              | KY624306 |
| olivaceopigmentum     | DSM 10792    | USA      | MK287542 | MK287545              | MK287555         | MK287568 |
| H. papillatum         | ATCC 58729   | USA      | NR15513 | KY610454              | KY624223         | KY972568 |
| H. perforatum         | CBS 115281   | France   | KY610391 | KY610455              | KY624224         | KY972568 |
| H. petriniae          | CBS 114746   | France   | KY610518 | KY610494              | KY624279         | KY972568 |
| H. pilgerianum        | STA 13455    | Martinique | KY610412 | N/A                    | N/A              | KY972568 |
| H. porphyreum         | CBS 139022   | France   | KY610486 | KY624225             | KY972568         | N/A      |
| H. pseudofonderi      | MFLUCC 11-0639 | Thailand | KL940156 | N/A                    | N/A              | N/A      |
| H. pseudofuscum       | 18264        | Germany  | MW367857 | MW367848              | MW373858         | MW373867 |
| H. pulicidum          | CBS 122222   | Martinique | JX183075 | KY610492              | KY624280         | KY972568 |
| H. rubiginosum        | MFLUCC 52807 | Germany  | KY610469 | KY624266              | KY972568         | N/A      |
| H. rutilum            | YM 181       | France   | N/A     | KY610466              | KY624269         | KY972568 |
| H. samuelisi          | MFLUCC 51843 | Guadeloupe | KY610486 | KY624269             | KY972568         | N/A      |
| H. shearii            | YM 29        | Mexico   | EP002142 | N/A                    | N/A              | KY972568 |
| H. sporistratificatum  | STA 14082    | Argentina | KL604573 | N/A                    | N/A              | KY972568 |
| H. subgileum          | YM 8813007   | China    | JQ009315 | N/A                    | N/A              | KY972568 |
| H. subelairoides       | IF 13062     | Sri Lanka | KM610291 | N/A                    | N/A              | KM610291 |
| H. tenuifiloideum     | DSM 107935   | USA      | MK287536 | MK287548              | MK287561         | MK287574 |
| H. ticenese           | CBS 115271   | Germany  | KY610471 | KY610472              | KY624272         | KY972568 |
| H. trigolodes         | MFLUCC 54794 | Martinique | JX183075 | KY610471              | KY624272         | KY972568 |
| H. ulmophilum         | MFLUCC 52087 | Germany  | KY610469 | KY624266              | KY972568         | N/A      |
| H. Vogesicium        | CBS 115273   | France   | KY698920 | KY610417              | KY624283         | KY972568 |
| H. wuxiense           | MGBCM213     | China    | MT668854 | MT668853              | MT668852         | MT668851 |
| H. wuchishanense      | FCATAS2708   | China    | OL467292 | OL615104              | OL584220         | OL584227 |
| H. zangii             | FCATAS4029   | China    | ON075423 | ON093247              | N/A              | ON093247 |
| H. zangii             | FCATAS4319   | China    | ON075424 | ON093248              | N/A              | ON093248 |
| Jackrogersella coharenis | CBS 119126  | Germany  | KY610396 | KY610497              | KY624270         | KY972568 |
| j. multiflora         | CBS 119016   | Germany  | KY610473 | KY610497              | KY624290         | KY972568 |
| Pyrenoporus sphyrius   | MFLUCC 52673 | Ivory Coast | KY610421 | KY610472              | KY624309         | KY972568 |
| P. leucosporus        | CBS 117739   | Burkina Faso | AM574992 | KY610489              | KY624307         | KY972568 |
| P. multisporella      | CBS 126414   | Ivory Coast | KY610420 | KY610495              | KY624228         | KY972568 |
| Thamnomyces dudoviae   | CBS 123578   | Guinea   | FNA48283 | KY610467              | KY624232         | KY972568 |
| Xylaria hypoxylon      | CBS 122620   | Sweden   | KY610407 | KY610495              | KY624231         | KY972568 |
| Biscogniauxia nummularia | MFLUCC 51395 | France   | KY610382 | KY610427              | KY624236         | KY972568 |

This study selected 89 taxa from 10 genera to perform phylogenetic analysis, including 3 Annulohypoxylon spp., 2 Daldinia spp., 3 Hypomontagnella spp., 72 Hypoxylon spp., 2 Jackrogersella spp., 3 Pyrenoporus spp., 1 Rhopalostroma spp., and 1 Thamnomyces sp. with X. hypoxylon and B. nummularia added as the outgroups. The sequence datasets comprised 306 sequences with 91 ITS, 62 LSU, 62 RPB2, and 91 β-tubulin sequences. After being aligned and trimmed, the combined dataset contained 3530 characters including gaps with 587 characters for ITS, 867 characters for LSU, 729 characters for RPB2, and 1347 characters for β-tubulin alignment, of which 1537 characters were parsimony-informative.

3. Results

3.1. Phylogenetic Analysis

The best-scoring ML tree was built with a final ML optimization likelihood value of −77,579.19847. Bayesian posterior probabilities were calculated with a final average standard deviation of split frequencies of less than 0.01. Phylogenetic trees of BA and ML analyses were found to be highly similar in topology, and the ML tree is represented in Figure 1. ML bootstrap support (BS) ≥50% and Bayesian posterior probabilities (PP) ≥0.95 were labelled along the branches, while branches with BS ≥70% and PP ≥0.98 were considered to be significant.
Figure 1. Phylogram of the best ML trees of the Hypoxylon species from an analysis based on multi-gene alignment of ITS-LSU-RPB2-β-tubulin. ML bootstrap support (BS) ≥ 50% and Bayesian posterior probabilities (PP) ≥ 0.95 are labelled above or below the respective branches (BS/PP). Species in bold were sequenced in this study.

Multi-gene phylogeny shows that our new species are clustered within the clades H2 and H3. Hypoxylon damuense and H. zangii are phylogenetically well differentiated. Hypoxylon damuense clustered with H. hypomiltum Mont. and H. wujiangense Y.H. Pi, Q.R. Li in a full support subclade (BS = 100%, PP = 1) in clade H2. Hypoxylon zangii clustered together with H. guilanense Pourmogh., C. Lamb. and H. texense Kuhnert, Sir in a full
support subclade as a sister to *H. rubiginosum* (Pers.) Fr. *Hypoxylon medogense* formed a subclade with *H. erythrostroma* J.H. Mill. with full support in clade H3. The phylogenetic tree shows that *Hypoxylon* is a paraphyletic group with other genera embedded (e.g., *Annulohypoxylon*, *Daldinia*, and *Hypomontagnella*).

### 3.2. Taxonomy

**Hypoxylon damuense** Hai X. Ma, Z.K. Song and Y. Li, sp. nov., Figure 2.

*MycoBank:* MB 843581

**Diagnosis.** Differs from *H. rubiginosum* in its larger asci, light-brown to brown ascospores with conspicuous coil-like ornamentation and most of the perispore indehiscent. Differs from *H. hypomiltum* in its smaller perithecia, larger asci and apical apparatus. Differs from *H. wujiangense* in its larger stromata and stromatal KOH-extractable pigments.

**Etymology.** *Damuense* (Lat.): referring to the holotype locality of species in Damu Township.

**Holotype.** CHINA: Tibet Autonomous Region, Medog County, Damu Township, Kabu Village, 29°38′42″ N, 95°37′44″ E, alt. 1280 m, saprobic on the bark of dead wood, 2 October 2021, Haixia Ma, Col. XZ207 (FCATAS 4207).

**Teleomorph.** Stromata pulvinate to effused-pulvinate, 1–9 cm long × 0.4–2 cm broad × 0.6–0.9 mm thick; with inconspicuous to conspicuous perithecial mounds; surface Bay (6), Rust (39) and Livid Purple (81), exposing black subsurface layer when colored coating worn off; with yellow-brown granules immediately beneath the surface and between perithecia; yielding luteous (12) and ochreous (44) to fulvous (43) KOH-extractable pigments; tissue below the perithecial layer black, 0.1–0.46 mm thick. Perithecia ovoid, black, 0.16–0.3 mm broad × 0.3–0.45 mm high. Ostioles umbilicate, opening lower than the stromatal surface or at the same level as the stromatal surface. Asci cylindrical with eight obliquely uniseriate ascospores, long-stipitate, 102–242 µm total length, the spore-bearing portion 60–72 µm long × 6.2–8.6 µm broad, and stipes 41–174 µm long, with amyloid apical apparatus bluing in Melzer’s reagent, discoid, 0.8–1.5 µm high × 1.6–2.4 µm broad. Ascospores light-brown to brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 8.2–10.5 × 4.1–5.5 µm (n = 60, M = 9.2 × 4.8 µm), with straight spore-length germ slit on the convex side; most of the perispore indehiscent in 10% KOH, occasionally dehiscent, with conspicuous coil-like ornamentation in SEM; epispore smooth.

**Additional specimens examined.** CHINA: Tibet Autonomous Region, Medog County, Damu Township, Kabu Village, 29°38′48″ N, 95°37′46″ E, alt. 1310 m, saprobic on the bark of dead wood, 2 October 2021, Haixia Ma, Col. XZ321 (FCATAS 4321).

**Note.** *Hypoxylon damuense* was found in the subtropics, and characterized by large pulvinate stromata, long asci stipes, amyloid apical apparatus, light-brown to brown ascospores with straight germ slit, most of the perispore indehiscent in 10% KOH, occasionally dehiscent, with conspicuous coil-like ornamentation. The new species is quite similar to *H. rubiginosum* in ascospore dimensions and KOH-extractable pigments, but the latter has darker colored ascospores, smaller asci (100–170 µm total length), dehiscent perispores and smooth or with inconspicuous coil-like ornamentation. *Hypoxylon rubiginosum sensu stricto* was always discovered in the temperate northern hemisphere except for samples reported in Florida [12,15,48]. Moreover, the status of *H. damuense* as a new species is also supported in the phylogenetic trees, where it appears distant from *H. rubiginosum*.

Although phylogenetic analyses showed that *H. damuense* clustered with *H. hypomiltum* and *H. wujiangense* in a clade with strong supported values (100%/1), there are distinct morphological differences among them. *Hypoxylon hypomiltum* differs in having larger perithecia ((0.2–)0.3–0.5 mm broad × 0.5–0.7 mm high), smaller asci (90–132(–145) µm total length), smaller apical apparatus (0.3–0.6 µm high × 1.2–1.5 µm broad) and slightly oblique to sigmoid germ slit [12]. *Hypoxylon wujiangense* can be distinguished by its smaller stromata with white pruina surface, Sienna (8) KOH-extractable pigments and larger apical apparatus 1.5–2 µm high × 2.5–3 µm broad [19].
with inconspicuous coil-like ornamentation. *Hypoxylon rubiginosum* sensu stricto was always discovered in the temperate northern hemisphere except for samples reported in Florida [12,15,48]. Moreover, the status of *H. damuense* as a new species is also supported in the phylogenetic trees, where it appears distant from *H. rubiginosum*. Although phylogenetic analyses showed that *H. damuense* clustered with *H. hypomiltum* and *H. wujiangense* in a clade with strong supported values (100%/1), there are distinct morphological differences among them. *Hypoxylon hypomiltum* differs in having larger perithecia (0.2–0.3 mm broad × 0.5–0.7 mm high), smaller asci (90–132(–145) µm total length), smaller apical apparatus (0.3–0.6 µm high × 1.2–1.5 µm broad) and slightly oblique to sigmoid germ slit [12]. *Hypoxylon wujiangense* can be distinguished by its smaller stromata with white pruina surface, Sienna (8) KOH-extractable pigments and larger apical apparatus 1.5–2 µm high × 2.5–3 µm broad [19].

Figure 2. *Hypoxylon damuense* (holotype FCATAS 4207). (a,b) Stromata on the bark of dead wood. (c) Stromatal surface. (d,e) Stroma in vertical section showing perithecia and ostioles. (f) KOH-extractable pigments. (g) Asci in water. (h) Asci in Melzer’s reagent. (i) Ascospores in water. (j) Ascospore in 10% KOH showing germ slit. (k) Apical apparatus in Melzer’s reagent. (l) Ascospores in 10% KOH. (m,n) Ascospores under SEM. Scale bars: (a) = 1 cm; (b) = 1000 µm; (c) = 500 µm; (d,e) = 200 µm; (g–l) = 10 µm; (m,n) = 5 µm.

*Hypoxylon medogense* Hai X. Ma, Z.K. Song and Y. Li, sp. nov., Figure 3.
**Hypoxylon damuense** (holotype FCATAS 4207). (a,b) Stromata on the bark of dead wood. (c) Stromatal surface. (d,e) Stroma in vertical section showing perithecia and ostioles. (f) Asci in water. (g) Asci in Melzer’s reagent. (h) Apical apparatus in Melzer’s reagent. (i) KOH-extractable pigments. (j) Ascospore in 10% KOH. (k) Ascospore in water showing germ slit. (l) Ascospores in water. (m,n) Ascospore under SEM. Scale bars: (a) = 1 cm; (b) = 1000 µm; (c) = 500 µm; (d,e) = 200 µm; (f–h,j–l) = 10 µm; (m) = 5 µm; (n) = 8 µm.

**Hypoxylon medogense** Hai X. Ma, Z.K. Song and Y. Li, sp. nov., Figure 3.

MycoBank: MB 843582

**Diagnosis.** Differs from *H. erythrostroma* in its larger ascospores with straight spore-length germ slit and very conspicuous coil-like perispore ornamentation. Differs from *H. laschii* in ovoid to obovoid perithecia, shorter asci, and larger ascospores with very conspicuous coil-like perispore ornamentation.
**Etymology.** *Medogense* (Lat.): referring to the holotype locality of species in Medog county.

**Holotype.** CHINA: Tibet Autonomous Region, Medog County, Dexing Township, Deguo village, 29°24′58″ N, 95°23′6″ E, alt. 814 m, saprobic on the bark of dead wood, 25 September 2021, Haixia Ma, Col. XZ61 (FCATAS 4061).

**Teleomorph.** Stromata plane, pulvinate to effused-pulvinate, 3.9–16.5 cm long × 2.5–6.2 cm broad × 0.52–0.72 mm thick; with inconspicuous to conspicuous perithecial mounds; surface cinnamon (62), fulvous (43), ochreous (44) and bay (6); with orange or reddish-orange granules immediately beneath the surface and between perithecia; yielding amber (47), orange (7) or scarlet (5) KOH-extractable pigments; tissue below the perithecial layer inconspicuous, black. Perithecia ovoid to obovoid, black, 0.16–0.3 mm broad × 0.25–0.4 mm high. Ostioles with conical black papillae, opening higher than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, 91–142 μm total length, the spore-bearing portion 60–79 μm long × 6.9–9.4 μm broad, and stipes 25–85 μm long, with amyloid apical apparatus bluing in Melzer’s reagent, discoid, 0.9–1.4 μm high × 2.4–2.9 μm broad. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 9.9–12.8 × 4.6–7 μm (n = 60, M = 11.1 × 5.7 μm), with 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: Tibet Autonomous Region, Medog County, Dexing Township, Deguo village, 29°25′28″ N, 95°23′26″ E, alt. 808 m, saprobic on the bark of dead wood, 25 September 2021, Haixia Ma, Col. XZ320 (FCATAS 4320).

**Note.** *Hypoxylon medogense* is characterized by having a bright orange red waxy layer beneath the surface, orange (7) or scarlet (5) KOH-extractable pigments, ostioles higher than the stromatal surface, brown to dark brown ascospores with straight germ slit and dehiscent perispore with very conspicuous coil-like ornamentation. Although the phylogenetic trees (Figure 1 and Figure S1) show that *H. medogense* and *H. erythrostroma* are closely related, as well as similar to each other in stromatal morphology and KOH-extractable pigments, *H. erythrostroma* was originally described and illustrated by Miller (1933) from Florida, and can be distinguished from *H. medogense* by having smaller ascospores (6.5–9.5 × 3–4.5 μm) and a shorter spore-bearing portion of asci (40–50 μm). Ju and Rogers [12] reexamined the isotype of *H. erythrostroma* (GAM 2374) from the USA and other specimens from Brazil, French Guiana, Madagascar, Mexico, Papua New Guinea, and Puerto Rico, and found that the fungi has smaller ascospores ((7–)7.5–9.5 × 3–4.5 μm) with sigmoid germ slit spore-length and inconspicuous coil-like perispore ornamentation; the species was also reported in Guadeloupe (French West Indies) by Fournier et al. [10].

Notably, *Hypoxylon medogense* shows morphological similarities to *H. crocopeplum* Berk., M.A. Curtis and *H. laschii* Nitschke in stromatal morphology. *Hypoxylon crocopeplum* can be distinguished by obovoid to long tubular perithecia (0.1–0.3(–0.4) mm broad × 0.2–1.5 mm high), longer asci ((100–)120–205(–217) μm total length) and slightly larger ascospores ((9–)9.5–15(–17.5) × 4–7(–7.5) μm) with inconspicuous to conspicuous coil-like perispore ornamentation. *Hypoxylon laschii* has longer asci (165–190 μm total length) and smaller ascospores (8–10 × 3.5–4.5 μm) with no perspore ornamentation [12]. In the phylogenetic trees, *H. medogense* is distant from the two species.

**Hypoxylon zangii** Hai X. Ma, Z.K. Song and Y. Li, sp. nov., Figure 4.

- **MycoBank:** MB 843580

**Diagnosis.** Differs from *H. fendleri* and *H. retpela* in its smaller ascospores. Differs from *H. rubiginosum* in its stromatal granules and a subtropical distribution. Differs from *H. texense* in its stromatal KOH-extractable pigments and larger ascospores. Differs from *H. guilanense* in its stromatal morphology.

**Etymology.** *Zangii* (Lat.): referring in honor to Chinese mycologist Dr. Zang Mu, who is also the author of "Field Records in the Mountains and Valleys: Discovery Journey to the Third Pole—Notes and Drawings of Zang Mu Scientific Expeditions".
Diagnosis. Differs from H. fendleri and H. retpela in its smaller ascospores. Differs from H. rubigi nosum in its stromatal granules and a subtropical distribution. Differs from H. texense in its stromatal KOH-extractable pigments and larger ascospores. Differs from H. guilanense in its stromatal morphology.

Etymology. Zangii (Lat.): referring in honor to Chinese mycologist Dr. Zang Mu, who is also the author of "Field Records in the Mountains and Valleys: Discovery Journey to the Third Pole—Notes and Drawings of Zang Mu Scientific Expeditions".

Holotype. CHINA: Tibet Autonomous Region, Medog County, Yarlung Zangbo River, the large bend of Linduo, 29°27′52″ N, 95°26′39″ E, alt. 781 m, saprobic on the bark of dead wood, 24 September 2021, Haixia Ma, Col. XZ29 (FCATAS 4029).

Teleomorph. Stromata effused-pulvinate, 1.2–4.1 cm long × 0.8–1 cm broad × 0.25–0.45 mm thick; with conspicuous perithecial mounds; surface livid red (56) and vinaceous (57); with orange or reddish orange granules immediately beneath the surface and between perithecia; yielding amber (47), fulvous (43) and sienna (8) KOH-extractable pigments; tissue below the perithecial layer inconspicuous, brown. Perithecia spherical, ovoid to obovoid, black, 0.2–0.4 mm broad × 0.3–0.5 mm high. Ostioles umbilicate, sometimes overlain with conspicuous white substance, opening lower than the stromatal surface. Asci cylindrical, eight-spored, uniseriate, 85–145 μm total length, the spore-bearing portion 65–92 μm long × 7.1–10.9 μm broad, and stipes 12–66 μm long, with

Figure 4. Hypoxylon zangii (holotype FCATAS 4029). (a) Stroma on the bark of dead wood. (b,c) Stromatal surface. (d,e) Stroma in vertical section showing perithecia and ostioles. (f) KOH-extractable pigments. (g,h) Asci in water. (i) Ascospores in water showing germ slit. (j) Apical apparatus in Melzer’s reagent. (k) Ascospore in 10% KOH. (l,m) Ascospores in water. (n,o) Ascospores under SEM. Scale bars: (a) = 1 cm; (b) = 1 mm; (c–e) = 200 μm; (g,i–m) = 10 μm; (h) = 20 μm; (n) = 5 μm; (o) = 8 μm.
amyloid apical apparatus bluing in Melzer’s reagent, discoid, 0.8–1.3 µm high × 2–2.9 µm broad. Ascospores light-brown to brown, unicellular, ellipsoid-inequilateral, with slightly acute to narrowly rounded ends, 10.9–14.6 × 4.8–6.4 µm (n = 60, M = 12.2 × 5.5 µm), with straight spore-length germ slit on the convex side; perispore dehiscent in 10% KOH, with inconspicuous coil-like ornamentation in SEM; epispore smooth.

**Additional specimens examined.** CHINA: Tibet Autonomous Region, Medog County, Yarlung Zangbo River, the larger bend of Linduo, 29°27′35″ N, 95°26′32″ E, alt. 780 m, saprobic on the bark of dead wood, 24 September 2021, Haixia Ma, Col. XZ319 (FCATAS 4319).

**Note.** The stromatal morphology of *H. zangii* is similar to *H. fendleri* Berk. ex Cooke, *H. retpela* Van der Gucht, Van der Veken and *H. rubiginosum*. However, *H. fendleri* differs by having slightly thicker stromata at 0.5–0.8 mm, smaller ascospores ((8–)9–12 × 4–5.5 µm) with sigmoid germ slit spore-length, while *H. retpela* has thicker stromata at 0.5–0.8 mm, and smaller ascospores ((9–)9.5–12 × 4.5–5 µm) with very conspicuous coil-like ornamentation [12]. *Hypoxylon rubiginosum* can also be distinguished by its yellowish-brown or brown stromatal granules, thicker stromata (0.5–1.2–1.5 mm) and smaller ascospores ((8–)9–12 × 4–5.5 µm). In addition, *H. rubiginosum* prefers to distribute in the northern temperate region, while *H. zangii* was found in subtropical region [12,15,47]. These three species are distant from *H. zangii* in the phylogenetic trees (Figure 1).

*Hypoxylon zangii* clustered with *H. guilanense* and *H. texense* in a strong support clade in the phylogenetic trees. *Hypoxylon texense* shows morphological similarities to *H. zangii* with reddish-orange stromatal granules, but differs in having rust (39) to dark brick (86) instead of amber (47), fulvous (43) and sienna (8) KOH-extractable pigments, and smaller ascospores ((9–)9.5–12 × 4.5–5 µm) with very conspicuous coil-like ornamentation [12].

### Dichotomous key to Hypoxylon species from China and related species worldwide

1. Ascospores nearly equilateral ................................................................. 2
2. Ascospores inequilateral ........................................................................ 8
3. Ostioles lower than the stromatal surface ........................................... 8
4. Ostioles lower than the stromatal surface ........................................... 1
5. Perithecia spherical, (0.2–)0.3–0.4 mm broad ...................................... *H. cromem*
6. Perithecia spherical to tubular, 0.3–0.6 mm broad × 0.4–0.8 mm high. *H. parkosianum*
7. Perispore dehiscent in 10% KOH .......................................................... *H. hypomiltum*
8. Perispore indehiscent in 10% KOH ...................................................... 5
9. Perithecia tubular to long tubular ......................................................... 6
10. KOH-extractable pigments orange (7) ............................................. *H. cinnabarum*
11. KOH-extractable pigments greenish yellow (16), dull green (70), or dark green (21) ................................................................. *H. investiens*
12. Stromatal surface brown vinaceous (84), sepia (63), or chestnut (40); without apparent KOH-extractable pigments or with dilute grayish sepia (106) to blackish pigments ...................................................... *H. dieckmannii*
13. Stromatal surface fawn (87) or umber (9); KOH-extractable pigments hazel (88) .............................................................................. *H. gilbertstonii*
14. Ostioles lower than the stromatal surface ........................................... 9
15. Perithecia tubular .................................................................................. 15
16. Perithecia spherical, ovoid to obvoid ................................................ 10
17. Stromatal granules black .................................................................. *H. hainanense*
18. Stromatal granules colored ................................................................. 11
11. Stromata glomerate; KOH-extractable pigments hazel (88) ........................................ H. lenormandii
12. Stromata pulvinate; KOH-extractable pigments orange (7) ........................................ 12
13. Sigmoid germ slit ............................................................... H. erythrostroma
14. Perispore smooth or with conspicuous coil-like ornamentation .............................. 13
15. Straight germ slit ................................................................................................................. 13
16. Perispore with very conspicuous coil-like ornamentation ............................... H. medogense
17. Sigmoid germ slit slightly less than spore-length; stromata glomerate, with conspicuous perithecial mounds; KOH-extractable pigments pure yellow (14) with citrine (13) tone, greenish olivaceous (90), or orange (7) ......................... H. musceum
18. KOH-extractable pigments vinaceous purple (101) .................................................. 14
19. Perispore smooth or with inconspicuous coil-like ornamentation ....................... 15
20. Sigmoid germ slit much less than spore-length; stromata glomerate, with conspicuous perithecial mounds; KOH-extractable pigments pure yellow (14) with citrine (13) tone, greenish olivaceous (90), or orange (7) ......................... H. musceum
11. Stromata pulvinate to effused-pulvinate, sometimes hemispherical, plane; perithecia 0.1–0.2 mm diam ................................................................. H. rutilum
12. Perispore smooth or with inconspicuous coil-like ornamentation .............................. 16
13. Perispore with very conspicuous coil-like ornamentation ............................... H. cyclobalanopsidis
14. Perispore dehiscent in 10% KOH ................................................................. 17
15. Perispore dehiscent in 10% KOH .................................................................................. 17
16. Sigmoid germ slit ................................................................................................................. 18
17. Sigmoid germ slit spore-length; stromata pulvinate or effused-pulvinate, with inconspicuous to conspicuous perithecial mounds; KOH-extractable pigments with other colors ................................................................. 18
18. KOH-extractable pigments orange (7) ................................................................. H. fendleri
19. Perispore infrequently dehiscent in 10% KOH ......................................................... 19
19. Perispore dehiscent in 10% KOH .................................................................................. 20
20. Stromata saprobic on surface of dead bamboo .......................................................... 20
21. Stromata saprobic on the bark of dicot wood .............................................................. 21
22. Perispore smooth or with inconspicuous coil-like ornamentation ....................... 22
23. Stromata glomerate or hemispherical ............................................................................. 22
24. Stromata pulvinate to effused-pulvinate; stromatal granules scarlet (5) to orange (7) ................................................................. H. retpela
25. KOH-extractable pigments orange (7) ................................................................. H. baihualingense
26. Stromata glomerate to pulvinate; stromata granules dull yellow or rust ...................... H. guilanense
27. Stromatal granules pale brown to dull reddish-brown; KOH-extractable pigments pale luteous (11), honey (60) and ochreous (44); apical apparatus highly reduced or lacking, not bluing in Melzer’s reagent; ascospores light-brown to dark brown, with slightly broad rounded ends, 8–10.6(–11.1) µm × 4.1–6.3(–7.1) µm ........................... H. chrysalidosporum
27. Stromatal granules pale brown to dull reddish-brown; KOH-extractable pigments pale luteous (11), honey (60) and ochreous (44); apical apparatus highly reduced or lacking, not bluing in Melzer’s reagent; ascospores light-brown to dark brown, with slightly broad rounded ends, 8–10.6(–11.1) × 4.1–6.3(–7.1) µm ........................... H. chrysalidosporum
27. Stromatal granules dull reddish-brown to blackish; KOH-extractable pigments isabelline (65) or amber (47); apical apparatus bluing in Melzer’s reagent; ascospores brown to dark brown, with narrowly rounded ends, 9.5–13(–14.5) × 4.5–6.5 µm ........................................ H. dengii
28. KOH-extractable pigments greenish to olivaceous .............................................. 29
28. KOH-extractable pigments with other colors .................................................... 33
29. Stromata pulvinate to effused-pulvinate ............................................................. 30
29. Stromata glomerate or hemispherical ................................................................. 31
30. Ascospores brown to dark brown, 8.5–13.5 \( \times \) 4–6 \( \mu m \) ...................... *H. anthochromum*
30. Ascospores light brown to brown, 5.5–8 \( \times \) 2.5–3.5 \( \mu m \) ...................... *H. brevisporum*
31. Apical apparatus highly reduced or lacking, not bluing in Melzer’s reagent .......... *H. notatum*
31. Apical apparatus bluing in Melzer’s reagent .................................................. 32
32. Perithecia spherical to obovoid, 0.1–0.3(–0.4) mm broad \( \times \) 0.2–0.5 mm high; slightly sigmoid germ slit ...................................................................................... *H. fuscum*
32. Perithecia long tubular, 0.3–0.6 mm broad \( \times \) (0.6–)0.8–2 mm high; straight germ slit ..................................................... *H. placentiforme*
33. Stromata hemispherical .................................................................................. 34
33. Stromata pulvinate to effused-pulvinate .......................................................... 37
34. Perithecia long tubular ..................................................................................... *H. haematostruma*
34. Perithecia spherical to obovoid ....................................................................... 35
35. KOH-extractable pigments amber (47) with greenish yellow (16) tone, or greenish yellow (16) with citrine (13) tone ...................................................... *H. perforatum*
35. KOH-extractable pigments orange (7) .................................................................. 36
36. Apical apparatus bluing in Melzer’s reagent, 0.8–1.2 \( \mu m \) high \( \times \) 2.2–2.8 \( \mu m \) broad; ascospores (10.5–)11–15 \( \mu m \times \) 5–6.5(–7) \( \mu m \) ...................... *H. fragiforme*
36. Apical apparatus bluing in Melzer’s reagent, 0.4–0.8 \( \mu m \) high \( \times \) 1.2–2 \( \mu m \) broad; ascospores 7–9(–10) \( \mu m \) \times \) 3–4.5 \( \mu m \) ............................................. *H. howeanum*
37. Perithecia tubular ........................................................................................... 38
37. Perithecia spherical to obovoid ...................................................................... 42
38. Stromatal granules black; KOH-extractable pigments dark livid (80) .......... *H. lividicolour*
38. Stromatal granules colored; KOH-extractable pigments with other colors .......... 39
39. KOH-extractable pigments pure yellow (14) or amber (47) ......................... *H. trugodes*
39. KOH-extractable pigments orange (7) ............................................................... 40
40. Apical apparatus bluing in Melzer’s reagent, 0.2–0.5 \( \mu m \) high \( \times \) 1–1.5 \( \mu m \) broad ................................................................. *H. jecorinum*
40. Apical apparatus lightly bluing or bluing in Melzer’s reagent, more than 1.5 \( \mu m \) broad ................................................................. *H. crocopeplum*
41. Perithecia spherical, obovoid to long tubular, up to 1.5 mm high; ascospores (9–)9.5
15(–17.5) \( \times \) 4–7(–7.5) \( \mu m \); *Virgariella*-like conidiogenous structure .................................................. *H. crocopeplum*
41. Perithecia obovoid to tubular, up to 0.7 mm high; ascospores 7–11 \( \times \) 3.5–5 \( \mu m \); *Nodulisporium*-like conidiogenous structure .................................................. *H. subgilvum*
42. Stromata saprobid on dead bamboo ............................................................... *H. pilgerianum*
42. Stromata saprobid on dicot wood ................................................................. 43
43. Ascospores 15.5–22.9(–23.6) \( \times \) 7.3–10.6 \( \mu m \) .................................................. *H. larissae*
43. Ascospores length less than 15 \( \mu m \) .......................................................... 44
44. Perithecia subglobose, 0.5–0.7 mm broad; straight or slightly sigmoid germ slit nearly
spore-length ................................................................. *H. vuijiangense*
44. Perithecia less than 0.5 mm broad; straight germ slit spore-length ................ 45
45. Stromatal granules orange or reddish orange; ascospores light-brown .................. 46
45. Stromatal granules yellowish-brown or dull purplish-brown; ascospores dark
brown ................................................................. *H. zangii*
46. KOH-extractable pigments rust (39) to dark brick (86); ascospore (8.7–)9.1–10.8(=11.5)
(4.0–)4.5–5.4 \( \mu m \) ............................................. *H. texense*
46. KOH-extractable pigments amber (47), fulvous (43) and sienna (8); ascospore 10.9–14.6
\( \times \) 4.8–6.4 \( \mu m \) ............................................. *H. zangii*
mm high; smooth or with inconspicuous coil-like ornamentation perispore; \textit{Periconiella}-like conidiogenous structure .................................................. \textit{H. rubiginosum}

47. Stromatal granules dull purplish-brown; perithecia 0.1–0.2 mm broad × 0.2–0.3 mm high; smooth perispore; \textit{Nodulisporium}-like conidiogenous structure .............................................................................................. \textit{H. vinosopulvinatum}

4. Discussion

In the present study, three species of \textit{Hypoxylon} from Medog in China, \textit{H. damuense}, \textit{H. medogense}, and \textit{H. zangii}, are described as new species based on molecular analyses and morphological features. Phylogenetic analyses on the species of \textit{Hypoxylon} presented confirmed that \textit{Hypoxylon} is a polyphyletic genus. The species analyzed appeared mainly distributed in six separate clades (except \textit{H. papillatum} Ellis, Everh. and \textit{H. dieckmannii} Theiss.). \textit{Hypoxylon damuense} and \textit{H. zangii} were clearly separated from other sampled species of \textit{Hypoxylon} and from each other in the clade H2, and \textit{H. medogense} was included in clade H3 containing \textit{H. fragiforme} (Pers.) J. Kickx f., the type species of the genus. The phylogenetic tree shows that the classification of \textit{Hypoxylon} is confusing. It did not suggest any apparent correlation in morphological features with the distribution of species in the phylogenetic trees. Therefore, more collections, more gene sequences and new taxonomic features, as well as the application of polyphasic taxonomic approaches based on morphological (sexual and asexual), chemotaxonomic, and phylogenetic data of this genus are needed in the further studies. Previously numerous new species have been found in Southwest China \cite{49,50}, and present paper confirmed that more known fungal species in the area.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof8050500/s1, Figure S1: ML phylogram inferred from ITS-TUB2 sequences. ML bootstrap support (BS) ≥ 50% and Bayesian posterior probabilities (PP) ≥ 0.95 are labelled above or below the respective branches (BS/PP). Species in bold were sequenced in the this study.

Author Contributions: Z.-K.S., A.-H.Z., Z.-D.L., Z.Q. and H.-X.M. prepared the samples; Z.-K.S. made morphological examinations and performed molecular sequencing; A.-H.Z. performed phylogenetic analyses. Z.-K.S., A.-H.Z. and H.-X.M. wrote the manuscript; Y.L. revised the language of the text; H.-X.M. conceived and supervised the work. All authors have read and agreed to the published version of the manuscript.

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