

Research Article



Integrating Mitochondrial Genomics into the Taxonomy of Hypoxylaceae

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Received: 03 August 2025 / Accepted: 19 June 2026 / Published: 12 July 2026

Abstract

Hypoxylaceae is a species-rich family of ascomycetes, predominantly found in tropical forests, known for their ecological roles and rich production of secondary metabolites, yet their taxonomy and species delimitation remain challenging. In this study, we collected 50 specimens of *Hypoxylon* and its related genera from various regions in China and identified them based on morphology and comprehensive phylogenetic analyses performed on two datasets, viz. mitochondrial genome protein-coding genes (PCGs) and a multi-locus alignment of ITS, LSU, *tub2*, and *rpb2*. The analyses revealed sixteen novel species, which we herein introduce as *Annulohypoxylon baishaense*, *A. linjiangense*, *A. xishuiense*, *Hypomontagnella vinacea*, *H. yinggelingensis*, *Hypoxylon betulae*, *H. guiense*, *H. rongjiangense*, *H. huangjingense*, *H. lancangense*, *H. populi*, *H. pseudorubiginosum*, *H. qiongense*, *H. sichuanense*, *H. subjaklitschii*, and *Jackrogersella maolanensis*. We sequenced and annotated the complete mitochondrial genomes for all 50 specimens, representing the first mitogenomic data for the genera *Jackrogersella*, *Hypomontagnella* and *Hypoxylon*. Comparative analyses revealed significant variations in genome size (ranging from 26,809 bp in *Hypoxylon lancangense* to 251,279 bp in *Annulohypoxylon viridipigmentum*), GC content, and structure, while all genomes contained 14 core protein-coding genes (PCGs), tRNAs, and rRNAs. The *Annulohypoxylon* species exhibited higher and homogeneous GC skew values compared to the other genera. Collinearity analyses and sequence similarity comparisons of core genes among closely related lineages provided supportive evidence for species delimitation. Evolutionary rate analyses indicated that most mitochondrial genes are subject to purifying selection, whereas *atp8*, *cox1*, *nad2*, *nad3*, and *nad5* showed signatures of positive selection in certain lineages, suggesting lineage-specific adaptive evolution. Genes exhibiting higher variability, including *nad3*, *nad5*, *cox1*, and *cob*, may serve as informative supplementary markers when used in combination with nuclear loci and morphology. A significant gene rearrangement was detected in the *cox2* gene of *Jackrogersella maolanensis*, which may be associated with the evolutionary divergence of *Jackrogersella*. This study represents the first application of mitochondrial genome data as a supplementary tool for species delineation and genus-level classification within Hypoxylaceae, providing a foundation for future work to assess its value in systematic and evolutionary studies.

Key words: 16 new species, Karst ecosystem, Mitogenome analysis, Next generation sequencing

Citation: Zeng, W.-Y., Zhou, X., Habib, K., Pi, Y.-H., Hu, H.-M., Ren, Y.-L., Liu, L.-L., Kang, Y.-Q., Hyde, K.D., Shen, X.-C., Wang, X.-Y., Meng, Z.-B., Li, Q.-R., & Kang, J.-C. (2026) Integrating Mitochondrial Genomics into the Taxonomy of Hypoxylaceae. Fungal Diversity 136: 136011. <https://doi.org/10.65390/fdiv.2026.136011>

Introduction

Hypoxylaceae (Xylariales, Sordariomycetes) is a diverse family of mainly saprotrophic ascomycetous fungi, commonly found in angiosperm-dominated forests. Members of Hypoxylaceae have a worldwide distribution and are characterized by diverse lifestyles, including saprotrophic,

endophytic, and phytopathogenic modes of life, colonizing wood, leaves, and fruits, while some species are symbiotically associated with insect vectors in both terrestrial and aquatic habitats (Ju & Rogers 1996; Pažoutová et al. 2013; Wibberg et al. 2021; Cedeño-Sánchez et al. 2024a, b). They are not only associated with woody substrates but have also been recovered from seed plants, lichens, and

algae and have even been found in insect nests and sponges (Pažoutová et al. 2013; Medina et al. 2016; U'Ren et al. 2016; Leman-Loubière et al. 2017; Sir et al. 2019). The family shows remarkable diversity in morphology and secondary metabolite production, characterized by the presence of stromatal pigments and a nodulisporium-like anamorph (Wendt et al. 2018; Wibberg et al. 2021).

Historically, taxonomic delineation within Hypoxylaceae relied heavily on morphological characteristics (Ju & Rogers 1996). However, this approach proved insufficient due to phenotypic plasticity and convergent evolution, leading to misclassifications and unresolved species complexes, such as the *Hypoxylon fuscum* and *H. rubiginosum* groups (Lambert et al. 2021). With the advent of molecular phylogenetic studies based on ITS, LSU, *rpb2*, and *tub2* dataset, together with chemotaxonomy as an additional tool have significantly improved the resolution of taxa within the family. This has enabled the segregation of new genera such as *Hypomontagnella*, *Jackrogersella*, and *Parahypoxylon* (Lambert et al. 2019; Cedeño-Sánchez et al. 2023). Polyphasic taxonomy, which integrates morphological, chemotaxonomic, and molecular data, has become the standard for Hypoxylaceae classification. Despite these advances, challenges persist. Phylogenetically, certain genera remain polyphyletic, and intragenomic polymorphisms in ribosomal DNA (rDNA) loci, particularly the ITS region, show high intragenomic polymorphism and limited resolution at the species level (Stadler et al. 2020; Cedeño-Sánchez et al. 2023). Additionally, the cryptic diversity observed in stromatal pigments and secondary metabolite profiles underscores the limitations of relying solely on these markers. For example, many specimens classified as *H. rubiginosum* (often reported as *H. cf. rubiginosum*) show molecular differences but lack diagnostic morphological and chemical differences (Pourmoghaddam et al. 2020). These limitations highlight the need for alternative genetic markers. Recent research demonstrates that mitogenomes can provide robust, lineage-specific markers. For instance, in *Ganoderma*, the *atp9* and *nad4L* genes have proven to be reliable molecular markers and potential DNA barcodes, offering valuable insights into the phylogeny of the genus and the Ganodermataceae family (Chen et al. 2024).

Despite their potential, mitogenomic data remain scarce, with fewer than 0.02% of described fungal species sequenced (Fonseca et al. 2021), representing a critical gap that hinders the resolution of taxonomically complex groups such as Hypoxylaceae. The primary value of this study lies in contributing complete mitogenome sequences, which provide a foundational resource for future comparative genomics. Expanding mitogenomic datasets is essential for future research aiming to elucidate fungal evolution, diversity, and classification in greater detail. Given that only about 0.02% of available data currently contribute to resolving these aspects, broader and more representative taxonomic sampling is urgently needed. Increasing both taxonomic breadth and depth of mitogenomic sampling will allow researchers to evaluate mitogenome utility across hierarchical levels and to integrate mitochondrial markers with existing polyphasic frameworks for more robust species delimitation and phylogenetic inference. This will enable a thorough assessment of the usefulness of mitogenomic data for studies on fungal classification, diversity, and phylogeny, as this field remains in its preliminary stages.

This study presents an extensive analysis of the mitochondrial genomes of 50 *Hypoxylaceae* specimens, offering a comprehensive dataset that will serve as a foundation for future evolutionary and taxonomic research. By combining traditional methods (morphology and phylogeny) with mitochondrial genome data, 16 novel *Hypoxylaceae* species are introduced, and insights into their mitochondrial genome structure are provided. Further in-depth analysis and comparison of these mitochondrial genome data will be presented in subsequent articles.

Materials and methods

Sample collection

The fungal specimens were collected during surveys conducted in the tropical and subtropical regions of China from 2019 to 2021. All relevant habitat information was recorded in detail. Specimens were photographed using a Canon G15 camera (Canon Inc., Tokyo, Japan), placed in paper bags, and transported to the laboratory for further examination. To remove excess humidity, they were dried using a portable fan dryer. The dried specimens were carefully labelled and stored in an ultra-low freezer at -80 °C for one week after isolation to eliminate any insects and their eggs. Afterwards, the specimens were subjected to morphological analysis. All specimens were deposited at the herbarium of Guizhou Medical University (GMB) and the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Living cultures were deposited at the Guizhou Medical University Culture Collection (GMBC). All scientific names of fungi follow the entries in MycoBank and Index Fungorum, hence no authorities and years of publications are given in the text apart from the taxonomic entries. Colors and codes were determined using the color chart of Rayner (1970).

Isolation and morphological characterization

Macroscopic characteristics were examined under an Olympus SZ61 stereomicroscope (Japan) and photographed with a Canon 700D digital camera (Canon Inc., Tokyo, Japan). The samples were mounted in water for microscopic examination, and Melzer's reagent was added to assess the amyloid reaction of the ascus apical apparatus. The Tarosoft[®] Image Framework (v 0.9.7) program and Adobe Photoshop CS6 software (Adobe Systems, USA) were used for measuring and processing images. Axenic cultures were obtained from single spores or tissues as described by Senanayake et al. (2020). Germinating spores were observed with a Stereo Zoom microscope and transferred to potato dextrose agar (PDA; 39 g/l distilled water, Difco potato dextrose). The cultures were incubated at 25 °C for 4–6 weeks, with weekly observations of growth and development.

Mycelial fermentation and DNA extraction

Pure colonies were transferred onto PDA medium and incubated at 25°C for 7 days. Under sterile conditions, a sterile cork borer was used to create holes (0.3 cm in diameter) in the colonies, and 10 mycelial plugs were transferred into Erlenmeyer flasks containing 100 mL of PD liquid medium (Potato Dextrose Liquid Medium). The flasks were then incubated in a rotary shaking incubator at 25°C and 120 rpm for 10 days. After fermentation, the mycelium was filtered out. The filtered mycelium was subjected to DNA

extraction using the commercially available BIOMIGA Fungal Genomic DNA Extraction Kit (Biomiga, San Diego, USA). Extracted DNA was used for Sanger sequencing of target loci (ITS, LSU, *tub2*, *rpb2*) and whole-genome sequencing (WGS).

Sanger Sequencing for Multi-Locus Gene Regions

Internal transcribed spacers (ITS), large subunit (LSU), *tub2* (β -tubulin), and *rpb2* (RNA polymerase II subunit) regions were PCR-amplified using primer pairs ITS1/ITS4 (White et al. 1990; Gardes & Bruns 1993), LR0R/LR5 (Vilgalys & Hester 1990), Bt2a/Bt2b or T1/T22 (Glass & Donaldson 1995; O'Donnell & Cigelnik 1997), and *rpb2*-5F/*rpb2*-7cR (Liu et al. 1999), respectively. The 25 μ L PCR mix included 9.5 μ L ddH₂O, 12.5 μ L PCR Master Mix, 1 μ L of each primer, and 1 μ L template DNA. Amplification followed the thermal cycling conditions outlined in Samarakoon et al. (2022). PCR products were verified via 1.5% agarose gel electrophoresis with GoldenView staining. Successful amplicons were sent to Sangon Biotech (Sangon Co., China) for sequencing.

Raw sequence chromatograms were manually inspected and quality-trimmed using Geneious Prime V2023.2.1. Forward and reverse reads for each locus were assembled into consensus sequences. The final sequences for each locus (ITS, LSU, *tub2*, *rpb2*) were deposited in the National Center for Biotechnology Information (NCBI) GenBank database (Supplementary Table 9).

Whole-genome sequencing (WGS), assembly and annotation

Genomic DNA was sent to Sangon Biotech Co., Ltd. (Shanghai, China) for next-generation whole-genome sequencing and library preparation. The sequences obtained were proofread and then assembled using the software Geneious Prime V2023.2.1. The raw sequencing data underwent initial quality control using FastQC v0.11.9. Adapters and low-quality bases were trimmed using Trimmomatic v0.39. The following thresholds were applied: LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:50. The mitochondrial genome was assembled from the cleaned reads using SOAPdenovo2 v2.04 with a range of k-mer size and other parameters set to default. Open reading frames (ORFs) were identified using Geneious tools (Kearse et al. 2012). Annotation of the genomes was performed with MFannot (<https://megasun.bch.umontreal.ca/apps/mfannot/>) (Valach et al. 2014) and MITOS web server (<http://mitos.bioinf.uni-leipzig.de/>) (Bernt et al. 2013), adhering to the mitochondrial genetic code 4. Manual adjustments were made to the annotations, with reference to the mitochondria of closely related species within the same family. Finally, graphical maps of the Hypoxylaceae mitogenomes were drawn using OGDRAW (<https://proksee.ca/projects/new>) (Grant et al. 2023). The tRNA genes in the 50 Hypoxylaceae mitogenomes were also predicted using tRNAscan-SE v1.3.1 (Lowe & Chan 2016). Assemblies have been deposited in GenBase and GenBank accessions mentioned in Supplementary Table 9.

Mitogenome sequence analysis

The base composition of the 50 Hypoxylaceae mitogenomes was analyzed using the DNASTAR Lasergene v7.1 (<http://www.dnastar.com/>). Strand asymmetry of the 50 mitogenomes was assessed according to the following formulas: AT skew = $(A - T) / (A + T)$, and GC skew = $(G - C) / (G + C)$ (Wang et al. 2017). Genetic distances between each pair of the 14 core PCGs (*atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*), and *rps3* was calculated with MEGA 11 (Caspermeyer 2016), using the Kimura-2-parameter (K2P) model. The DnaSP v6 (Rozas et al. 2017) was used to calculate the nonsynonymous substitution rate (Ka) and the synonymous substitution rate (Ks) for all the 14 core PCGs from the 50 Hypoxylaceae specimens mitogenomes. Codon usage analysis was conducted using the Sequence Manipulation Suite (Stothard 2000), based on the genetic code 4. Genome synteny of the 50 Hypoxylaceae specimens mitogenomes and representative species from other genera was analyzed using the Mauve v2.4.0 (Darling et al. 2010).

Nuclear marker sequence alignment and phylogenetic analysis

The obtained ITS, LSU, *tub2*, *rpb2* sequences were compared with each other and all the known sequences in GenBank using the BLAST algorithm for precise identification (<https://www.ncbi.nlm.nih.gov>) (Altschul et al. 1990). The molecular phylogeny was inferred from a combined dataset of ITS, *tub2* and *rpb2* sequences. Reference sequences were obtained from open databases and published literature, encompassing all available representative strains across the target clades, together with the closest BLASTn matches to the newly generated sequences (Supplementary Table 8).

Phylogenies were inferred under both Maximum Likelihood (ML) and Bayesian Inference (BI) frameworks for each dataset. Sequences were aligned using the MAFFT v.7.110 online program (Katoh et al. 2019) with the default settings. The alignment was manually refined using BioEdit v7.0.5.3 (Hall 1999) to correct misalignments and trim regions with excessive gaps or ambiguities. The maximum likelihood (ML) analysis was implemented in RAxML v.8.2.12 using the GTRGAMMA substitution model with 1,000 bootstrap replicates (Stamatakis 2015). Bayesian inference was performed using MrBayes v. 3.2.2 (Ronquist et al. 2012). The Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.2.2 (Ronquist et al. 2012) was used to determine the posterior probabilities (PP). Six simultaneous Markov chains were run for 1,000,000 generations, and trees were sampled every 1,000th generation. The phylogenetic tree was visualized in FIGTREE v.1.4.3 (Rambaut 2012). All analyses were run on the CIPRES Science Gateway v 3.3 web portal (Miller et al. 2010). All the obtained sequences were deposited in the GenBank (Supplementary Table 9). The alignment file is attached to this paper as supplemental material.

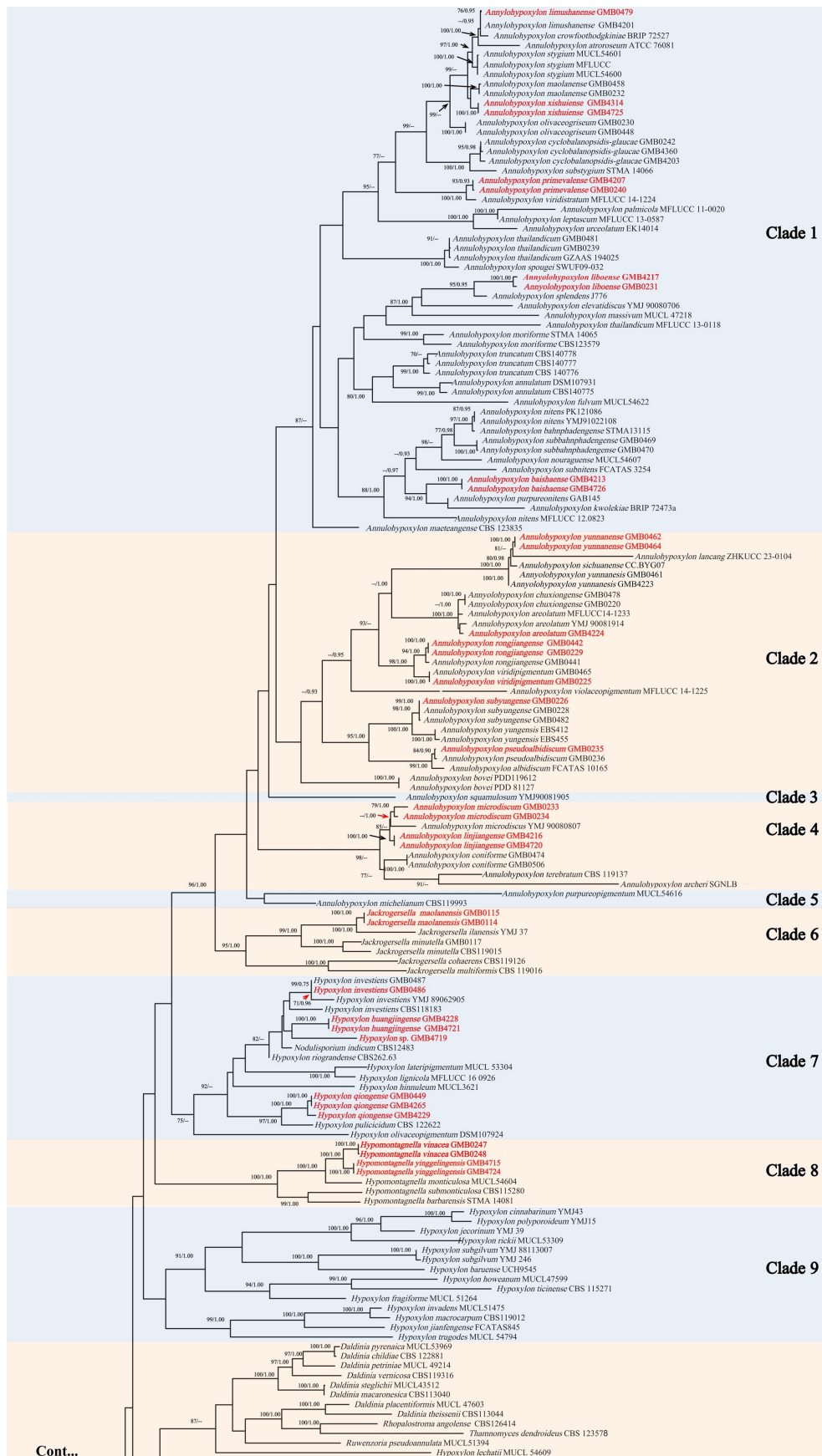


Fig. 1 RAxML tree based on a combined ITS, LSU, *rpb2* and *tub2* sequences dataset. Bootstrap support values for maximum likelihood (ML) greater than 70% and Bayesian posterior probabilities (BPP) greater than 0.90 are displayed above the respective branches (ML/BP). The sequences generated during this study are highlighted in red.



Fig. 1 (continued)

Mitogenome sequence alignment and mitogenome-based phylogenetic analysis

Mitogenome-based phylogenetic analyses were performed following the methods of (Wu et al. 2021). Mitochondrial genome sequences of representative related taxa were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov>) (Altschul et al. 1990).

The mitogenomic phylogenetic relationships of the 50 Hypoxylaceae specimens and other ascomycetes were investigated based on combined mitochondrial gene datasets (14 core PCGs). Five distinct datasets for phylogenetic analysis including concatenated nucleotide sequences of 14 conserved PCGs; PCG12 dataset comprising only the first and second codon positions of the 14 conserved genes; PCGt concatenated sequences of the 14 conserved PCGs with associated tRNAs; PCGA combining the amino acid sequences of the 14 PCGs and ITS-LSU-PCG a combined dataset of mitochondrial and nuclear markers, concatenating the sequences of the 14 conserved PCGs with two nuclear genes ITS and LSU were used (Li et al. 2020). Individual mitochondrial genes were aligned using MAFFT v7.037 software (Katoh et al. 2019), and then concatenated them into a combined mitochondrial gene dataset using SequenceMatrix v1.7.8 (Vaidya et al. 2011). A preliminary partition homogeneity test was carried out to detect potential phylogenetic conflicts between different genes. Best-fit models (GTRGAMMA substitution model) of evolution and partitioning schemes for the gene set was determined according to Partition Finder 2.1.1 (Lanfear et al. 2017). MrBayes v3.2.6 (Ronquist et al. 2012) was used to construct the phylogenetic tree using Bayesian inference (BI) method based on the combined gene sets. Two independent runs with four chains (three heated and one cold) were conducted simultaneously for 2×10^6 generations. Each run was sampled every 1000 generations. Assumption of stationarity to reach estimated sample size (ESS) greater than 100, and the potential scale reduction factor (PSRF) approached 1.0 was checked. The first 25% samples were discarded as burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (BPP) in a 50% majority-rule consensus tree. Maximum likelihood (ML) phylogenetic trees were constructed using RAxML v8.0.0 (Stamatakis 2015). The phylogenetic tree was visualized in FIGTREE v1.4.3 (Rambaut 2012). All analyses were run on the CIPRES Science Gateway v 3.3 web portal (Miller et al. 2010).

Comparative mitogenomic analysis and intron analysis

The genome sizes, gene numbers, intron numbers, base compositions, and gene arrangements of the 50 Hypoxylaceae specimens were analyzed to assess variations and conservation between different Hypoxylaceae species following the methods of Wu et al. (2021). The introns of *cox1* genes in published Ascomycota mitogenomes were classified into different position classes (Pcls) according to the method described by Férandon et al. (2010). The *cox1* genes of 50 Hypoxylaceae mitogenomes were aligned with the reference *cox1* gene of the medicinal fungus *Ganoderma calidophilum* (Li et al. 2019), using ClustalW (Thompson et al. 1994). Introns were named according to the insertion sites (nt) in the corresponding reference gene. Each Pcl was constituted by introns inserted at the same position of the

corresponding reference *cox1* gene (Cheng et al. 2021). Pcls with the same insert sites were considered as orthologous introns and had high sequence similarities (Férandon et al. 2010).

Evolutionary rates of the mitochondrial genes

The synonymous substitution rate (Ks), nonsynonymous substitution rate (Ka), and their ratios (Ka/Ks) are often employed to measure evolutionary rates (Kryazhimskiy & Plotkin 2008). Therefore, we selected 14 PCGs (*atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*) and *rps3* gene of 50 Hypoxylaceae specimens to calculate the values of Ka, Ks, and Ka/Ks. These genes were aligned using MEGA 11 (Tamura et al. 2021) according to the codons (parameters: Gap opening penalty: 10.00; Gap extension penalty: 0.10; and delay divergent cutoff: 30%). The Ka/Ks ratio was calculated using DnaSP v6 (Rozas et al. 2017).

Results

Phylogenetic analyses

Phylogenetic tree analysis based on combined loci of ITS, LSU, *rpb2* and *tub2*

The final dataset included 317 sequences representing 225 taxa, comprising both newly generated sequences and those retrieved from GenBank. Following the exclusion of ambiguously aligned regions and gaps, the final data matrix comprised 2,894 characters (ITS: 1-275 bp; LSU: 276-1043 bp; *rpb2*: 1044-1923 bp; *tub2*: 1924-2894 bp). *Biscogniauxia nummularia* (MUCL 51395), *Graphostroma platystoma* (CBS 270.87), *Xylaria arbuscula* (CBS 126415), and *X. hypoxylon* (CBS 122620) were added as out-groups. The RAxML analysis of the combined ITS-LSU-*rpb2*-*tub2* dataset yielded the best-scoring tree (Fig. 1). The tree topology (branching pattern and clade composition) from the Maximum Likelihood (ML) analysis closely resembled that of the Bayesian Inference (BI) analysis.

The phylogram is represented with 15 clades (Fig. 1). The genera *Annulohypoxylon* (clade 1-5), *Hypomontagnella* (clade 8), *Jackrogersella* (clade 6) and *Parahypoxylon* each form distinct, monophyletic clade. *Hypoxylon* species are distributed across multiple clades, from clade 7 to clade 15. The interposition of the genera *Daldinia*, *Hypomontagnella*, *Pyrenopolyporus*, *Rhopalostroma*, *Ruwenzoria*, and *Thamnomycetes* among these clades renders the status of *Hypoxylon* polyphyletic. The support values for several of these clades were generally low, which further complicated the reliable resolution and clustering of *Hypoxylon* species.

The sequences of our collected specimens clustered within clades that align with their respective genera, supporting their morphological identifications. The phylogenetic placement of each strain is discussed in the "Notes" section for the corresponding taxon.

Phylogenetic tree based on mitochondrial genome data

The dataset comprised 50 newly generated mitochondrial genomes plus five additional sequences retrieved from GenBank, which were used as outgroups (Supplementary Tables 8–9). We constructed maximum likelihood (ML) and

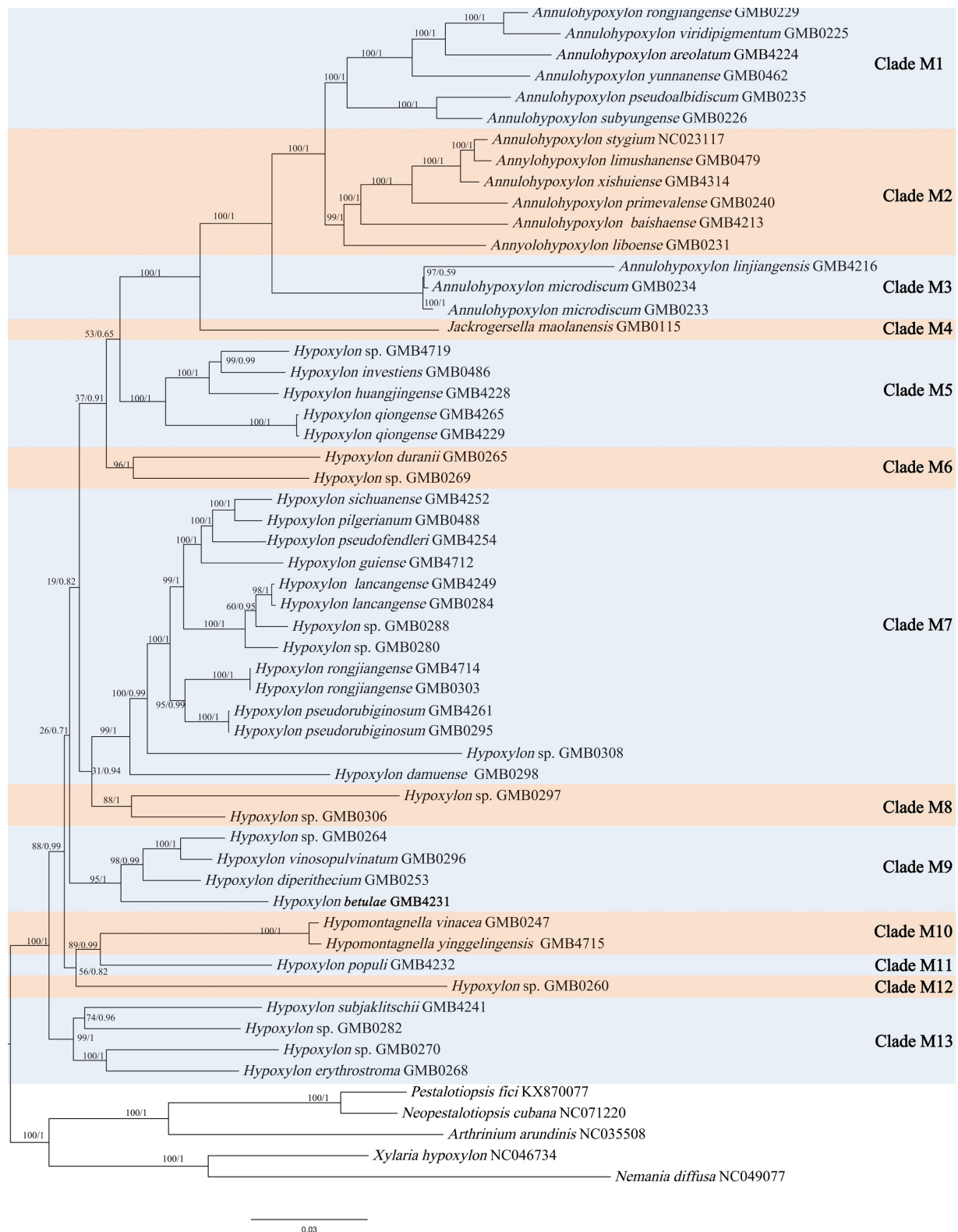


Fig. 2 Maximum likelihood tree of the combined 14 PCGs, ITS and LSU sequences dataset. Bootstrap support (BS) values for maximum likelihood (ML) greater than 70% and Bayesian posterior probabilities (BPP) greater than 0.90 are displayed above the respective branches (ML/BP).

bayesian Inference (BI) phylogenetic trees based on the following datasets: (1) a combined matrix of nuclear ribosomal loci (ITS, LSU) and the 14 mitochondrial protein-coding genes (PCGs); (2) the amino acid sequences of the 14 mitochondrial PCGs; (3) the nucleotide sequences of the 14 mitochondrial PCGs using only first and second codon

positions; (4) the full nucleotide sequences of the 14 mitochondrial PCGs; and (5) the nucleotide sequences of the 14 mitochondrial PCGs including tRNA genes. No significant differences were observed in branching patterns or clade composition among the phylogenetic trees inferred from mitochondrial genome sequences. The phylogenetic tree

based on the combined ITS–LSU–PCG dataset is presented in Fig. 2. The other phylogenetic trees are included in the supplementary documents (Supplementary Figs. 1–4).

The mitogenome phylogenetic analyses revealed strongly supported backbone nodes within Hypoxylaceae, particularly among *Annulohypoxylon* species, which formed monophyletic, well-resolved clades with high bootstrap support ($\geq 90\%$). *Hypoxylon* certain nodes and clades showed lower statistical support (bootstrap values $< 70\%$), though their topological placement remained consistent across repeated analyses. The phylogenetic results supported the monophyly of *Annulohypoxylon* and *Jackrogersella*. *Hypomontagnella* forms a distinct monophyletic clade in phylogenies based on ITS, LSU, *rpb2*, and *tub2* sequences (Fig. 2), but did not in the mitogenomic phylogeny, instead forming a well-supported sister branch to *Hypoxylon populi*. This highlights the need for broader sampling of mitogenomes to conclusively resolve the full phylogenetic classification within Hypoxylaceae. The absence of mitogenome data for many type species of key genera limits definitive conclusions, underscoring the necessity of including these taxa to resolve taxonomic relationships robustly.

Taxonomy

Annulohypoxylon baishaense H.M. Hu, K. Habib & Q. R. Li *sp. nov.*

Mycobank No.: MB858997; Fig. 3

Etymology: The epithet refers to the Baisha County, where the holotype was collected.

Material examined: China, Hainan Province, Baisha County, Yinggeling National Nature Reserve (19°33'45.80"N, 108°10'06.18"E), altitude 998 m, on dead wood, 18 July 2022, H.M. Hu, 2022YGL76 (GMB4213, holotype; GMBC4213, ex-type living culture; KUN-HKAS 148611, isotype).

Teleomorph: *Stromata* on the surface of dead wood, 0.4–3.5 cm long $\times$ 0.4–2 cm wide $\times$ 0.6–0.9 mm high ($\bar{x}$ = 2 cm $\times$ 1.2 cm $\times$ 0.75 mm, n = 10), effused-pulvinate, hemispherical, slightly constricted at base, surface black, shiny, carbonaceous; with perithecial mounds up to 1/3 exposed, tissue below the perithecial layer black, conspicuous, with 10% KOH extractable pigments Dark Brick (66). *Perithecia* 1.16–1.45 mm wide $\times$ 1.39–1.66 mm high ($\bar{x}$ = 1.26 $\times$ 1.49 mm, n = 20), spherical. *Ostioles* conical papillate, encircled with a bovei-type disc, 0.5–0.75 mm diam. *Asci* 160–195 $\times$ 4–6.5 μ m ($\bar{x}$ = 169.3 $\times$ 5.1 μ m, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 70.8–80 μ m ($\bar{x}$ = 77 μ m, n = 30) long, the stipes 90–115 μ m ($\bar{x}$ = 101 μ m, n = 30) long, with apical apparatus bluing in Melzer's iodine reagent, discoid, 2.0–2.4 $\times$ 0.9–1.5 μ m ($\bar{x}$ = 2.2 $\times$ 1.2 μ m, n = 20). *Ascospores* 7.5–9 $\times$ 2.8–4.2 μ m ($\bar{x}$ = 8 $\times$ 3.5 μ m, n = 30), uniseriate, unicellular, inequilateral ellipsoid, with rounded ends, brown, with an inconspicuous germ slit, covering the full spore length; perispore dehiscent in 10 % KOH, smooth with a thickening on the convex side, epispore smooth. Asexual morph: not formed.

Culture characteristics: Colonies grown on OA at 25 °C for 3 weeks reached a diameter of 5 cm. Colonies surface white, felty to cottony, thin; reverse light red. Not sporulating on OA nor on PDA.

Additional material examined: China, Hainan Province, Baisha County, Yinggeling National Nature Reserve (19°33'46.84"N, 108°10'04.63"E), altitude 1037 m, on dead wood. 20 July 2022, H.M. Hu, 2022YGL347 (GMB4726).

Notes: Morphologically and phylogenetically (Fig. 1), *Annulohypoxylon baishaense* is closely related to *A. purpureonitens*, sharing similarly sized ostiolar discs and ascospores. However, *A. purpureonitens* differs in having red-brown to black granules immediately beneath the surface (vs. black in *A. baishaense*), smaller ascomata (0.6–1 mm diam. vs. 1.16–1.45 mm diam.), Vinaceous Purple (101) KOH-extractable pigments [vs. Dark Brick (66)], and shorter asci (94–102.5 μ m in length vs. 160–195 μ m in length) (Sir et al. 2018; Cruz et al. 2021).

Phylogenetically, *Annulohypoxylon baishaense* is also closely related to *A. kwolekia*, a species proposed based on molecular sequence data without morphological descriptions. Pairwise sequence identity between *A. baishaense* and *A. kwolekia* (BRIP 72473a) was 92% for ITS, 100% for LSU, and 83% for *rpb2*. However, we do not accept *A. kwolekia* as a validly published species due to the absence of a proper description, photographs, and failure to meet the standard requirements for species introduction (Tan & Shivas 2022). Further research including the acquisition of morphological data for *A. kwolekia* is necessary to properly resolve its taxonomic status.

The KOH-extractable pigments of *Annulohypoxylon baishaense* is comparable to that of *A. bahnpadengense*, which yields Isabeline (65) to Grayish Sepia (106) pigmentation. However, *A. bahnpadengense* differs in having shorter asci (100–130 μ m in total length) with a shorter stipe (37–60 μ m) and is also phylogenetically distant (Fournier et al. 2010).

Other species in the clade that are morphologically similar include *Annulohypoxylon nouraguense* which can be distinguished by its dark vinaceous (82) to blackish brown surface, smaller ascospores (6–7 $\times$ 2.9–3.7 μ m vs. 7.5–9 $\times$ 2.8–4.2 μ m in *A. baishaense*), and Vinaceous Grey (116) to Vinaceous Purple (101) KOH-extractable pigments (Fournier & Lechat 2016).

Annulohypoxylon linjiangense H.M. Hu, K. Habib & Q.R. Li *sp. nov.*

Mycobank No.: MB858998; Fig. 4

Etymology: The epithet refers to the collection location, Linjiang Village.

Material examined: China, Guizhou Province, Xishui National Nature Reserve, Linjiang Village (28°13'35.9"N, 106°52'22.2"E), altitude 500 m, on dead wood of an unidentified plant, 5 Nov. 2022, H.M. Hu, 2022LJ5 (GMB4216, holotype; GMBC4216, ex-type; KUN-HKAS 148612, isotype).

Teleomorph: *Stromata* on the surface of dead wood, 0.1–1.5 cm long, 0.3–0.8 cm wide, and 0.5–1 cm thick, pulvinate, adnate, with perithecial mounds 1/2–3/4 exposed, carbonaceous, surface black, tissue between the perithecia black, tissue beneath perithecial layer inconspicuous, black, with 10% KOH extractable pigments Isabeline (65) to Grayish Sepia (106). *Perithecia* 0.84–1 mm wide $\times$ 0.76–1 mm high ($\bar{x}$ = 0.87 $\times$ 0.91 mm, n = 20), spherical. *Ostiole* papillate, slightly lower than the level of stromatal surface,

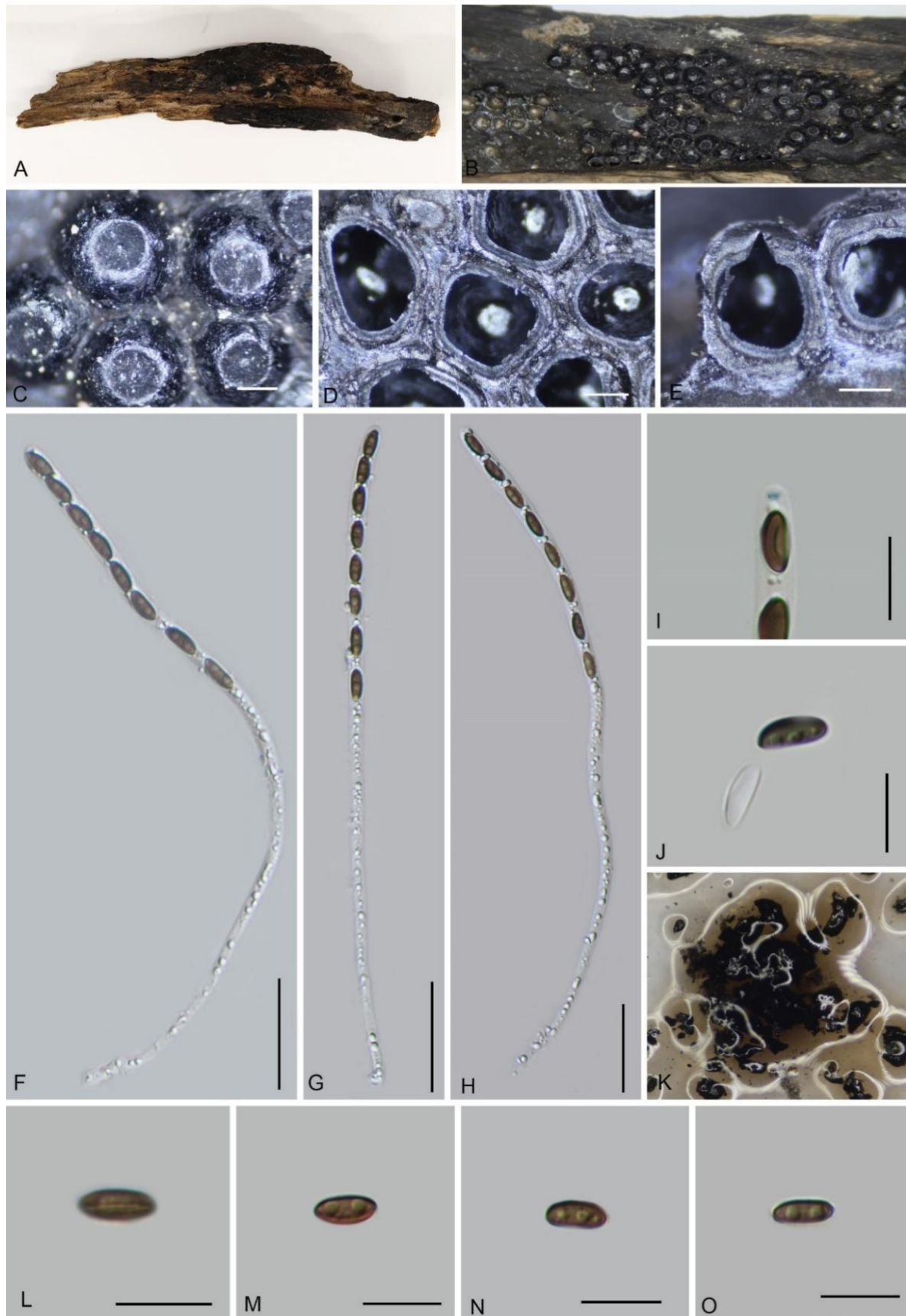


Fig. 3 *Annulohypoxylon baishaense* (GMB4213, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Transverse section of stromata. E Longitudinal section of stromata. F–H Asci in distilled water. I Ascical apical apparatus (stained in Melzer's reagent). J Perispore dehiscence in 10% KOH. K Stromatal KOH-extractable pigment. L–O Ascospores. Scale bars: C–E = 0.5 mm; F–J, L–O = 10 µm.



Fig. 4 *Annulohypoxyton linjiangense* (GMB4216). A Stromatal habit on wood. B Stromatal surface. C Ostiole; D Cross-section of stroma. E Longitudinal section of stroma. F–H Asci in distilled water. I Ascinal apical apparatus (non-bluing in Melzer's reagent). J Perispore dehiscent in 10% KOH. K Stromatal KOH-extractable pigment. L–O Ascospores. Scale bars: C–E = 0.5 mm; F–J = 20 μ m; L–O = 10 μ m.

surrounded by a black disc, 0.29–0.38 mm diam. Asci 140–170 × 5.6–7 µm ($\bar{x}$ = 150 × 6.6 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 70–90 µm ($\bar{x}$ = 84 µm, n = 30), apical apparatus not bluing in Melzer's reagent. Ascospores 8–12 × 4–5.6 µm ($\bar{x}$ = 10 × 4.8 µm, n = 60), uniseriate, unicellular, inequilateral ellipsoid, with rounded ends, brown, with a straight germ slit covering the full spore length, perispore dehiscent in 10 % KOH, smooth, with a thickening on the convex side, episporium smooth. Asexual morph: Not found.

Additional material examined: China, Guizhou Province, Xishui National Nature Reserve, Linjiang Village (28°13'34.1"N, 106°52'23.7"E), altitude 391 m, on dead wood of an unidentified plant, 1 Nov. 2022, H.M. Hu, 2022LJ307 (GMB4720).

Culture characteristics: Colonies grown on OA at 25 °C for 3 weeks reached a diameter of 4 cm. Colonies surface at first white become pale white, felty to cottony, thin; reverse light yellow. Not sporulating on OA nor on PDA.

Notes: Morphologically and phylogenetically, *Annulohypoxylon linjiangense* is closely related to *A. microdiscum*, sharing similar pigmentation, ascospore size, and non-amyloid apical apparatus. However, *A. microdiscum* can be distinguished by the presence of orange and blackish granules immediately beneath the surface and between the perithecia, much smaller asci (94–110 µm in length vs. 140–170 µm in *A. linjiangense*), and an indehiscent perispore (vs. dehiscent in *A. linjiangense*). Pairwise sequence identity between *A. linjiangense* and *A. microdiscum* was 98.87% for ITS, 94.59% for *tub2* (β -tubulin), and 97.91% for *rpb2*.

Other phylogenetically close species, *Annulohypoxylon coniformis* and *A. terebratum*, can be easily distinguished by the absence of an ostiolar disc. Moreover, *A. coniformis* produces Mouse Grey (119) pigments, while *A. terebratum* yields Greenish Olivaceous (90) pigments in KOH (Fournier et al. 2010; Li et al. 2025).

Annulohypoxylon xishuiense H.M. Hu, K. Habib & Q.R. Li *sp. nov.*

Mycobank No.: MB858999; Fig. 5

Etymology: The epithet refers to the Xishui County, where the holotype was collected.

Material examined: China, Guizhou Province, Xishui County, changqiangou village (28°40'63.23"N, 106°18'80.46"E), altitude 868 m, on dead branch of an unidentified plant, 4 Nov. H. M. Hu 2022CQG9 (GMB4314, holotype; GMB4314, ex-type; KUN-HKAS 148613, isotype).

Teleomorph: Stromata on dead branch of an unidentified plant, 3–10 cm long × 1.5–5 cm wide × 0.7–1 mm high, effused to effused-pulvinate, with inconspicuous perithecial mounds, carbonaceous, surface Cinnamon (62) or Brown Vinaceous (84), gradually fading to dull Isabelline (65), black below and between perithecia, carbonaceous; tissue below the perithecial layer inconspicuous; with 10% KOH extractable pigments Greenish Olivaceous (90). *Perithecia* 0.36–0.83 mm wide × 0.40–0.85 mm high ($\bar{x}$ = 0.55 × 0.71 mm, n = 20), spherical. *Ostioles* papillate, slightly higher than the level of stromatal surface, disc truncatum-type, 0.23–0.37 mm diam. Asci 85–110 × 4–5.5 µm ($\bar{x}$ = 96 × 4.6 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 47–60 µm ($\bar{x}$ = 54 µm, n = 30), the stipes 40–50 µm long, apical

apparatus not bluing in Melzer's reagent. Ascospores 5.8–7 × 2.5–3.5 µm ($\bar{x}$ = 6.2 × 2.8 µm, n = 30), uniseriate, unicellular, ellipsoid, almost equilateral, with rounded ends, brown, with a straight spore-length germ slit, perispore dehiscent in 10 % KOH, smooth, with a thickening on the convex side, episporium smooth. Asexual morph: not formed.

Culture characteristics: Colonies grown on OA at 25 °C for 4 weeks reached a diameter of 5 cm. Colonies surface at first white, felty to cottony, thin; reverse greyish white. Not sporulating on OA nor on PDA.

Additional material examined: China, Guizhou Province, Xishui County, changqiangou village (28°40'61.58"N, 106°18'79.28"E), altitude 902 m, on dead branch of an unidentified plant, 4 Nov. H.M. Hu 2022CQG313 (GMB4725).

Notes: Morphologically, *Annulohypoxylon xishuiense* closely resembles *A. maolanense* in stromatal surface coloration, KOH-extractable pigmentation, and the size of asci and ascospores. Phylogenetically, the two species also appear as sister taxa, albeit with low statistical support. A key phenotypic difference is the larger ostiolar disc in *A. xishuiense* (0.23–0.37 mm) compared to *A. maolanense* (0.15–0.2 mm). Moreover, *A. maolanense* has smaller perithecia (0.25–0.36 mm wide) than those of *A. xishuiense* (0.36–0.83 mm wide) (Li et al. 2025).

The stromata of *A. xishuiense* could be confused with *A. stygium*. However, *A. xishuiense* can be easily distinguished by its much larger ostiolar discs (0.23–0.37 mm vs. 0.1–0.2 mm in *A. stygium*). Additionally, Ju & Rogers (1996) and Fournier & Lechat (2016) reported the ascospores of *A. stygium* as ellipsoid-inequilateral, whereas the ascospores of the new species are almost equilateral. Based on these morphological differences and phylogeny, we propose *A. xishuiense* as a new species.

Hypomontagnella vinacea K. Habib, Y.H. Pi & Q.R. Li *sp. nov.*

Mycobank No.: MB859000; Fig. 6

Etymology: The epithet refers to the vinaceous color of the stromatal surface.

Material examined: China, Guizhou Province, Rongjiang County, Leigongshan Nature Reserve (26°22'54.31"N, 108°21'51.63"E), altitude 923 m, on dead branches of an unidentified plant, 29 Aug. 2020, Yinhuai Pi, 2020LGS48 (GMB0247, holotype; GMB0247, ex-type; KUN-HKAS 148614, isotype).

Teleomorph: Stromata on the surface of dead wood, 1.7–2.8 cm long × 0.8–1.5 cm wide × 0.5–0.65 mm high ($\bar{x}$ = 2.0 cm × 0.94 cm × 0.62 mm, n = 10), effused to effused-pulvinate, with inconspicuous perithecial mounds, surface dark Vinaceous Grey (116) or blackish, carbonaceous, tissue between perithecia black, tissue below the perithecial layer inconspicuous, without pigments in 10 % KOH. *Perithecia* 0.34–0.58 mm wide × 0.51–0.81 mm high ($\bar{x}$ = 0.44 × 0.69 mm, n = 20), spherical to obovate. *Ostioles* papillate, higher than the level of the stromatal surface, without a disc. Asci 80–145 × 4.5–7 µm ($\bar{x}$ = 111 × 6 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, spore-bearing part 45–55 µm ($\bar{x}$ = 50 µm, n = 30) long, stipes 55–75 µm ($\bar{x}$ = 57 µm, n = 30) long, apical apparatus amyloid, discoid, 1.3–2.3 µm wide × 0.9–1.8 µm high ($\bar{x}$ = 1.8 × 1.2 µm, n = 20). Ascospores 7.5–8.5 × 3–4.5 µm ($\bar{x}$ = 7.8 × 3.6 µm, n = 30), uniseriate, unicellular, ellipsoidal, inequilateral, with narrowly rounded ends, brown,

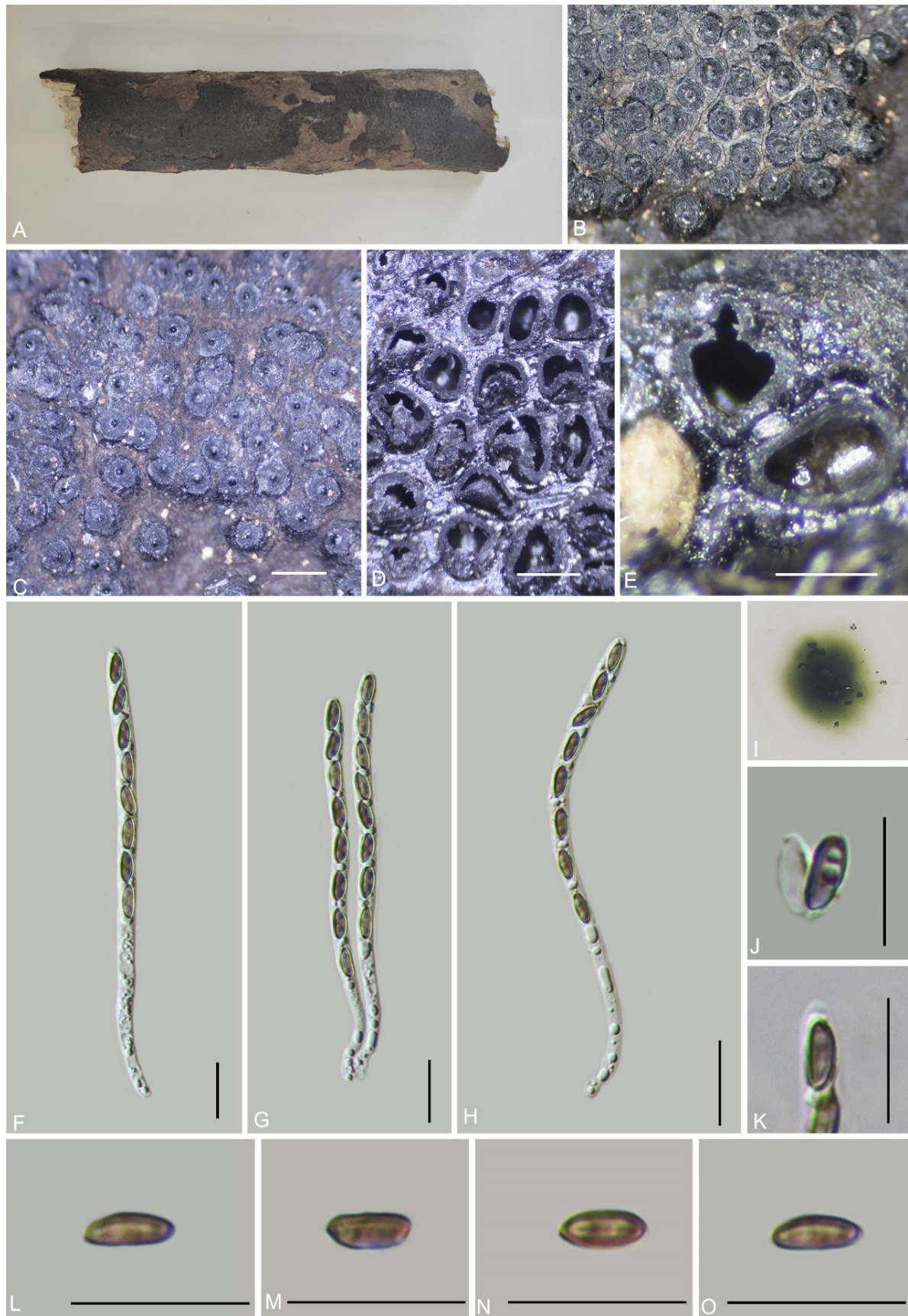


Fig. 5 *Annulohypoxylon xishuiense* (GMB4314, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Transverse section of stromata. E Longitudinal section of stromata. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascus apical apparatus (not bluing in Melzer's reagent). L–O Ascospores. Scale bars: C–E = 0.5 mm; F–H, J–O = 10 µm.

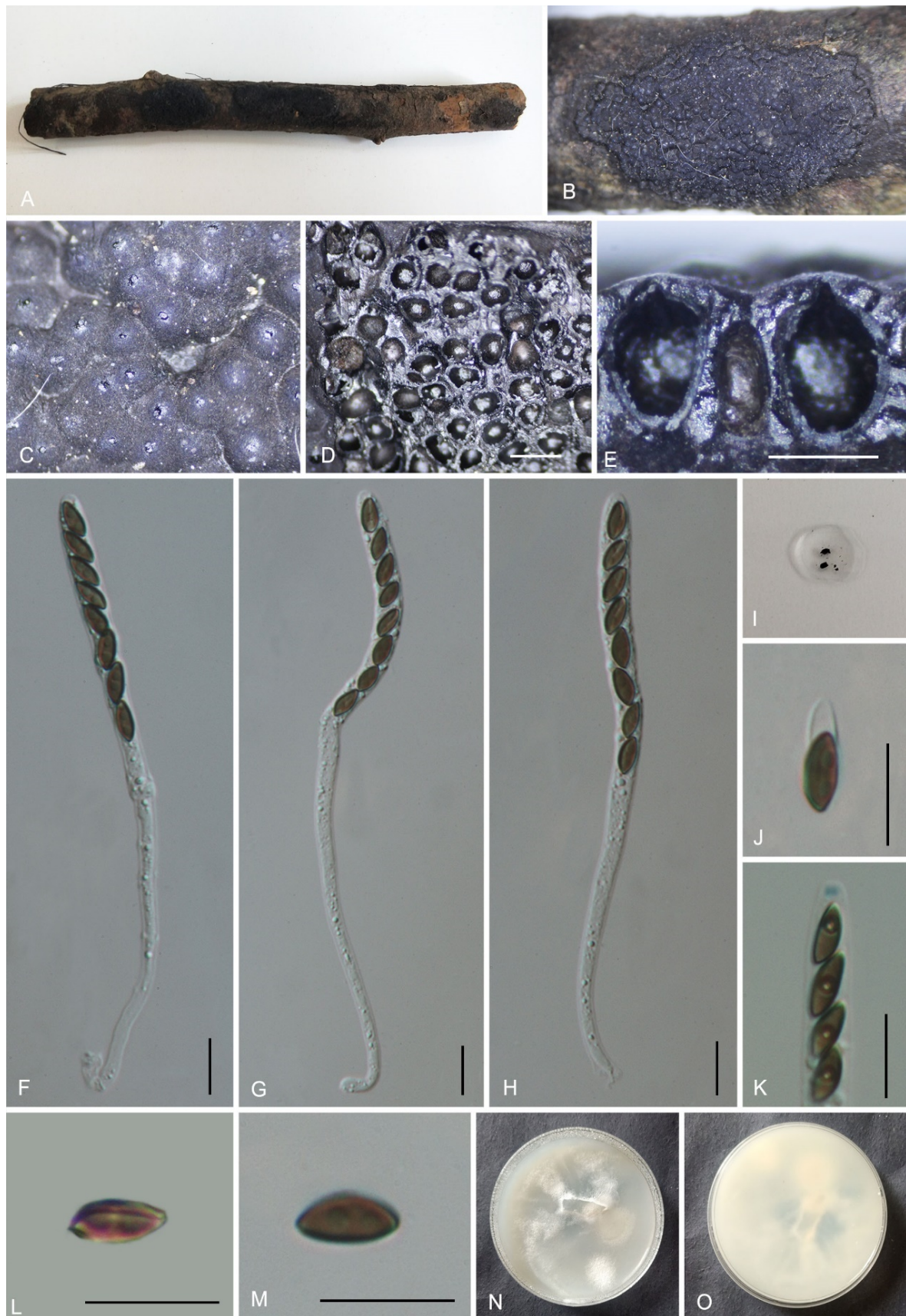


Fig. 6 *Hypomontagnella vinacea* (GMB0247, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross section of stromata. E Vertical section of stromata. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascical apical apparatus (bluing in Melzer's reagent). L, M Ascospores; N, O Culture on OA (N-above, O-lower). Scale bars: D, E = 0.5 mm; F–H, J–M = 10 μ m.

with an inconspicuous straight spore-length germ slit; perispore always dehiscent in 10% KOH, smooth, epispore smooth. Asexual morph: not formed.

Culture characteristics: Colonies on OA covering 4–5 cm diam. after 2 weeks at 25 °C, Colonies surface white, cottony, azonate with entire margins; after 4 weeks covering a 9 cm petri dish, becoming white grey, appressed with entire margins; reverse white. No conidia were observed.

Additional material examined: China, Guizhou Province, Libo County, Qiannan Buyi and Miao Autonomous Prefecture, Maolan National Nature Reserve (25°18'2.76"N, 108°4'29.48"E), altitude 545 m, on dead branches of an unidentified plant, 9 July 2021, Y.H. Pi, 2021MLB37 (GMB0248), living culture GMBC0248.

Notes: Morphologically, *Hypomontagnella vinacea* resembles *H. monticulosa*, sharing similar stromatal coloration. However, *H. monticulosa* is distinguished by a conspicuous disc surrounding the ostioles, larger ascospores ($\bar{x}$ = 10.4 × 4.8 µm) with a sigmoid germ slit, and young stromata produce Vinaceous Purple (101) pigments in KOH (Ju & Rogers 1996; Lambert et al. 2019). Pairwise sequence identity between *H. vinacea* (GMB0247) and *H. monticulosa* (MUCL 54604) was 96.4% for ITS, 99.6% for LSU, 93.7% for *rpb2*, and 96.9% for *tub2*. *Hypomontagnella barbarensis* also shares a vinaceous stromatal surface but differs in having much larger ascospores (13–19.3 × 6.9–9.4 µm vs. 7.5–8.5 × 3–4.5 µm) and ostiole surrounded by a disc. *Hypomontagnella vinacea* is distinguished from all known species in the genus by its smaller ascospores (7.5–8.5 × 3–4.5 µm) and the absence of a disc surrounding ostiole, whereas other species in the genus typically have ascospores longer than 9 µm and broader than 4.5 µm (Lambert et al. 2019).

Hypomontagnella yinggelingensis H.M. Hu, K. Habib & Q.R. Li sp. nov.

MycoBank No.: MB859001; Fig. 7

Etymology: The epithet refers to Yinggeling National Nature Reserve, where the holotype was collected.

Material examined: China, Hainan Province, Baisha County, Yinggeling National Nature Reserve (18°28'59.1"N, 109°20'26.2"E), altitude 1088 m, on dead twig of an unknown plant, 18 July 2022, H.M. Hu, 2022YGL39 (GMB4715, holotype; GMBC4715, ex-type; KUN-HKAS 148615, isotype)

Teleomorph: Stromata on the surface of dead wood, 0.3–6 cm long × 0.3–0.8 cm wide × 0.85–1 mm thick, cushion-shaped, surface black, carbonaceous, the tissue between perithecia black, tissue below the perithecial layer inconspicuous, with 10% KOH extractable pigments Vinaceous Purple (101). *Perithecia* 0.37–0.60 mm wide × 0.62–0.77 mm high ($\bar{x}$ = 0.49 × 0.68 mm, n = 20), obovate. *Ostioles* conical papillate, slightly higher than stromatal surface, without a disc. *Asci* 120–140 × 5–7 µm ($\bar{x}$ = 131 × 6 µm, n = 30), 8-spored, unitunicate, cylindrical, spore-bearing part 50–65 µm long ($\bar{x}$ = 56 µm, n = 30), apical apparatus bluing in Melzer's reagent, discoid, 1.5–1.8 × 0.6–1 µm ($\bar{x}$ = 1.6 × 0.8 µm, n = 20). *Ascospores* 7–8.4 × 3–4.3 µm ($\bar{x}$ = 7.8 × 3.8 µm, n = 30), uniseriate, unicellular, ellipsoid, inequilateral, brown, with narrowly rounded ends, with a sigmoid germ slit of spore-length; perispore dehiscent in 10 % KOH, smooth, epispore smooth. Asexual morph: Not observed.

Culture characteristics: Colonies on OA covering 5 cm diam. after 3 weeks at 25 °C, Colonies surface white, cottony,

azonate, margin thin; after 5 weeks covering a 9 cm petri dish, becoming yellowish, dense; reverse brownish grey. No conidia were observed.

Additional material examined: China, Hainan Province, Baisha County, Yinggeling National Nature Reserve (18°28'58.9"N, 109°20'26.3"E), altitude 1315 m, on dead twig of an unknown plant, 18 July 2022, H.M. Hu, 2022YGL327 (GMB4724).

Notes: Phylogenetically and morphologically, *Hypomontagnella yinggelingensis* is closely related to the above described *Hy. vinacea*. However, *Hy. vinacea* differs from *Hy. yinggelingensis* by its Vinaceous Grey (116) to blackish surface coloration (vs. black), without KOH extractable pigment (vs. Vinaceous Purple) and ascospores with a straight germ slit (vs. sigmoid germ slit) (this study).

Hypomontagnella yinggelingensis shares the presence of a sigmoid germ slit with *Hy. monticulosa* and *Hy. submonticulosa*. However, both species can be readily distinguished by their larger ascospores (*H. monticulosa* 7–11 × 3.5–4.5 µm; *Hy. submonticulosa* 9–13.5 × 4–5 µm). *Hypomontagnella monticulosa* further differs by having a vinaceous surface in young stromata, becoming brown-vinaceous to blackish in mature stromata, whereas *Hy. yinggelingensis* has a black stromatal surface in both immature and mature stages. *Hypomontagnella submonticulosa* further differs by having a straight to slightly oblique germ slit shorter than the spore length, while *H. yinggelingensis* has a spore-length sigmoid germ slit (Lambert et al. 2019).

Hypoxylon betulae K. Habib, Y.H. Pi & Q.R. Li sp. nov.

MycoBank No.: MB859002; Fig. 8

Etymology: The epithet refers to the host genus *Betula* sp.

Material examined: China, Guizhou Province, Kuankuoshui National Nature Reserve (28°31'54.18"N, 107°10'36.72"E), altitude 1556 m, on dead branches of *Betula* sp., 2 Nov. 2022, H.M. Hu, 2022KKS123 (GMB4231, holotype; GMBC4231, ex-type living culture; KUN-HKAS 148616, isotype).

Teleomorph: Stromata on dead branches of *Betula* sp., 0.9–3.5 cm long × 0.5–1.4 cm wide × 0.7–1 mm high, flat, surface Vinaceous Purple (101) or purplish grey (128), tissue black between perithecia; tissue below the perithecia dark brown to black, with 10% KOH extractable pigments Sienna (8) to Dark Brick (86). *Perithecia* 0.22–0.42 mm wide × 0.35–0.55 mm high ($\bar{x}$ = 0.33 × 0.46 mm, n = 20), spherical. *Ostiole* recessed below the surface, not prominent. *Asci* 110–140 × 6.5–8.5 µm ($\bar{x}$ = 127 × 7.3 µm, n = 30), 8-spored, unitunicate, long-cylindrical, long-stipitate, the spore-bearing part 40–50 µm long, the stipes 70–90 µm long, with apical apparatus bluing in Melzer's iodine reagent, discoid, 1.7–3 × 1–1.5 µm ($\bar{x}$ = 2.4 × 1.2 µm, n = 20). *Ascospores* 6–8.2 × 2.6–4.7 µm ($\bar{x}$ = 7.2 × 3.6 µm, n = 30), uniseriate, unicellular, ellipsoid-inequilateral, with rounded ends, light brown to brown, with an inconspicuous germ slit; perispore dehiscent in 10% KOH. epispore smooth. Asexual morph: not formed.

Culture characteristics: Colonies growing on OA at 25 °C for 3 weeks reached a diam. of 5 cm. Colony surface white, felty, flocculent, circular, dense; reverse white, center cyan. Not sporulating on OA nor on PDA.

Additional material examined: China, Guizhou Province, Kuankuoshui National Nature Reserve (28°31'53.58"N, 107°10'34.69"E), altitude 1494 m, on dead branches of *Betula* sp., 5 Nov. 2022, H.M. Hu, 2022KKS307 (GMB0445).



Fig. 7 *Hypomontagnella yinggelingensis* (GMB4715, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross section of stroma. E Vertical section of stroma. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascilar apical apparatus (stained in Melzer's Reagent). L–O Ascospores. Scale bars: D, E = 0.5 mm; F–H, J–O = 10 μ m.

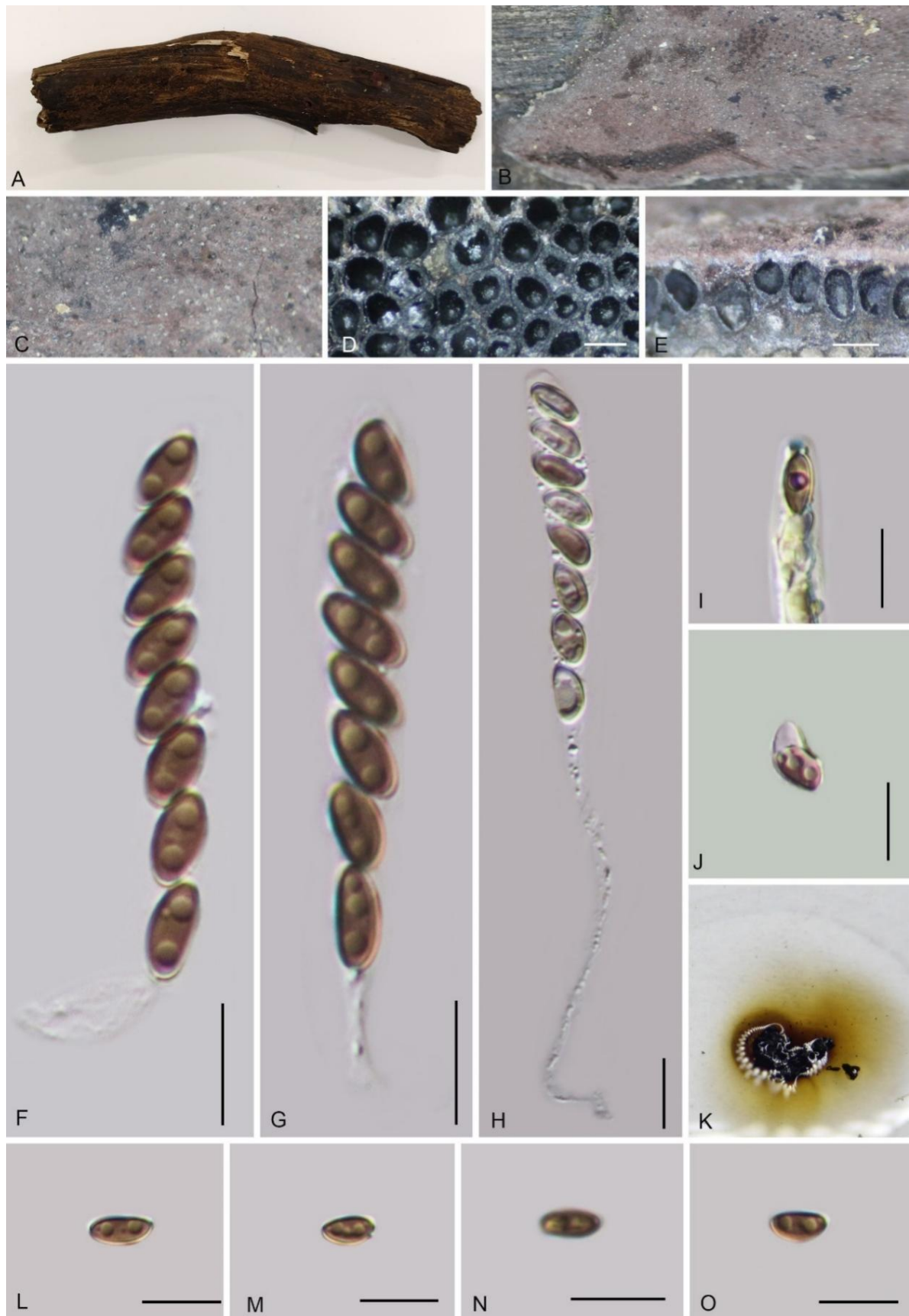


Fig. 8 *Hypoxylon betulae* (GMB4231, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross-section of stroma. E Longitudinal section of stroma. F–H Asci in distilled water. I Ascilar apical apparatus (bluing in Melzer's reagent). J Perispore dehiscent in 10% KOH. K Stromatal KOH-extractable pigment. L–O Ascospores. Scale bars: D, E = 0.5 mm; F–J, L–O = 10 μ m.

Notes: Phylogenetically (Fig. 1), *Hypoxylon betulae* is closely related to *H. fuscopurpureum*, and morphologically also resembles it, with both species sharing a purplish stromatal surface. However, *H. fuscopurpureum* can be differentiated by olivaceous KOH-extractable pigments, larger perithecia (0.3–0.6 × 0.22–0.41 mm), and larger ascospores (12–15 × 5.5–7 µm vs. 6–8.2 × 2.6–4.7 µm), and have an indehiscent perispore (Ju & Rogers 1996; Mühlbauer et al. 2002).

Its stromata could be confused with *H. fuscum*, as both species have a purplish stromatal surface. However, *H. fuscum* is distinguished by Amber (47), Isabelline (65), Olivaceous (48), Gray Olivaceous (107), or Greenish Olivaceous (90) KOH-extractable pigments, and larger ascospores (8–20 × 4–8 µm) (Ju & Rogers 1996; Mühlbauer et al. 2002).

Hypoxylon betulae can be distinguished from other *Hypoxylon* species that produce Sienna (8) stromatal KOH-extractable pigments, such as *H. erythrostroma* (asci 90–115 µm in total length, ascospores with acute ends and a sigmoid germ slit), *H. fendleri* (asci 86–156 µm in total length, ascospores 9–12 × 4–5.5 µm, with a sigmoid germ slit), *H. lateripigmentum* (asci 85–155 µm in total length, with olivaceous-yellow granules beneath the surface and between the perithecia), *H. musceum* (asci 83–110 µm in total length, ascospores 9–13 × 4.5–6 µm), *H. sepiaceum* (subperithecial tissue olivaceous-brown), and *H. arawakianum* (asci 100–110 µm in total length, ascospores 9.7–11.2 µm) (Rogers 1981; Kuhnert et al. 2014; Fournier & Lechat 2015).

Hypoxylon guinense W.Y. Zeng, K. Habib & Q.R. Li *sp. nov.*

Mycobank No.: MB859003; Fig. 9

Etymology: The epithet refers to "Gui" a local name for Guangxi Zhuang Autonomous Region, where the holotype was collected.

Material examined: China, Guangxi Zhuang Autonomous Region, Qinzhou City, Shiwandashan National Nature Reserve, (21°25'45.5"N, 107°11'29.8"E), altitude 650 m, on dead culm of bamboo, 14 Aug. 2023, W.Y. Zeng, 2023SWDS30 (GMB4712, holotype; ex-type GMBC4712; KUN-HKAS 148617, isotype)

Teleomorph: Stromata on the surface of dead culm of bamboo, 0.4–1.2 cm long × 0.2–0.8 cm wide × 0.2–1.2 mm thick, effused-pulvinate, surface Dark Brick (66) to Brown Vinaceous (84), tissue between the perithecia black, tissue below the perithecial layer blackish brown, with KOH-extractable pigments Dark Olivaceous Grey (121), Umber (9) to Sepia (63) after a while. *Perithecia* 0.37–0.46 mm wide × 0.38–0.44 mm high ($\bar{x}$ = 0.42 × 0.42 mm, *n* = 20), subglobose. *Ostiole* lower than the surface, inconspicuous. *Asci* 121–160 × 9–11 µm ($\bar{x}$ = 141 × 10.1 µm, *n* = 30), 8-spored, long cylindrical, spore-bearing part 97–126 µm long ($\bar{x}$ = 106 µm, *n* = 30), with apical apparatus bluing in Melzer's reagent, discoid, 3.3–3.8 × 0.7–1.1 µm ($\bar{x}$ = 3.5 × 0.8 µm, *n* = 20). *Ascospores* 15–20 × 7–10 µm ($\bar{x}$ = 17.2 × 8.1 µm, *n* = 30), brown to dark brown, ellipsoid, equilateral, with rounded ends, with inconspicuous straight germ slit, 1/3–1/2 of spore-length, perispore indehiscent in 10% KOH; episporium smooth. Asexual morph: not formed.

Culture characteristics: Colonies grown on OA at 25 °C for 3 weeks reached a diameter of 5 cm. Colonies surface white, felty, cottony, thin; reverse white. Not sporulating on OA nor

on PDA.

Additional material examined: China, Guangxi Zhuang Autonomous Region, Qinzhou city, Shiwandashan National Nature Reserve (21°25'44.4"N, 107°11'29.4"E), altitude 739 m, on dead culm of bamboo, 16 Aug. 2023, W.Y. Zeng, 2023SWDS308 (GMB4713).

Notes: Morphologically, *Hypoxylon guinense* closely resembles *H. wuzhishanense*, which has also been reported on bamboo and has similarly sized ascumata. However, *H. wuzhishanense* can be distinguished by its Pale Vinaceous (85) to Livid Vinaceous (83) and Vinaceous Purple (101) KOH-extractable pigments, shorter asci (70–139 µm total length vs. 121–160 µm), and smaller dehiscent ascospores (6.1–9.6 × 3.2–5 µm vs. indehiscent 15–20 × 7–10 µm) (Ma et al. 2022).

Morphologically, *H. guinense* also resembles *H. pilgerianum*, which has been reported on bamboo and shares similar surface coloration. Phylogenetically, the two species are also closely related. However, *H. pilgerianum* can be differentiated by the presence of dull orange-brown or dull reddish-brown granules immediately beneath the surface and between the perithecia, Ochreous (44), Honey (64), or Amber (47), KOH-extractable pigments and much shorter asci (68–97 µm total length) and ascospores (8–9.7 × 4–4.8 µm) (Ju & Rogers 1996).

Hypoxylon huangjingense H.M. Hu, K. Habib & Q.R. Li *sp. nov.*

Mycobank No.: MB859005; Fig. 10

Etymology: The epithet refers to Huangjing Old Forest Nature Reserve, where the holotype was collected.

Material examined: China, Sichuan Province, Huangjing Old Forest Nature Reserve, (28°8'6.9"N, 105°26'38.9" E), altitude 1100 m, on dead wood, 16 Aug. 2022, H.M. Hu, 2022HJLL34 (GMB4228, holotype; GMBC4228, ex-type; KUN-HKAS 148618, isotype)

Teleomorph: Stromata on the surface of dead wood, 1.5–6 cm long × 0.5–1 cm wide × 0.25–0.28 mm high, effused to effused-pulvinate, with inconspicuous perithecial mounds, slightly carbonaceous or cortical, surface Mouse Grey (118), tissue between the perithecia black, tissue below the perithecial layer conspicuous, black; with 10% KOH extractable pigments Grey Olivaceous (107) to Olivaceous Grey (121). *Perithecia* 0.42–0.63 mm wide × 0.55–0.81 mm high ($\bar{x}$ = 0.52 × 0.70 mm, *n* = 20), spherical. *Ostioles* umbilicate, slightly higher than the surface, inconspicuous. *Asci* 120–140 × 5–7.5 µm ($\bar{x}$ = 131.8 × 6.2 µm, *n* = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 60–68 µm ($\bar{x}$ = 63.7 µm, *n* = 30), with apical apparatus bluing in Melzer's reagent, discoid, 2–2.3 × 1.3–1.6 µm ($\bar{x}$ = 2.2 × 1.4 µm, *n* = 20). *Ascospores* 6.5–9 × 3.5–5 µm ($\bar{x}$ = 7.8 × 4.3 µm, *n* = 30), uniseriate, unicellular, brown, ellipsoidal, equilateral, with a faint straight germ slit, slightly less than spore-length, perispore dehiscent in 10 % KOH, smooth; episporium smooth. Asexual morph: Not observed.

Culture characteristics: Colonies on OA covering 3 cm diam. after 2 weeks at 25 °C, Colonies surface white, cottony, azonate, margin entire; after 6 weeks covering a 9 cm petri dish, white, dense; reverse yellow. No conidia were observed.

Additional material examined: China, Sichuan Province, Huangjing Old Forest Nature Reserve, (28°8'6.5"N, 105°26'38.3"E), altitude 1271 m, on dead wood, 19 Aug. 2022, H.M. Hu, 2022HJLL309 (GMB4721).

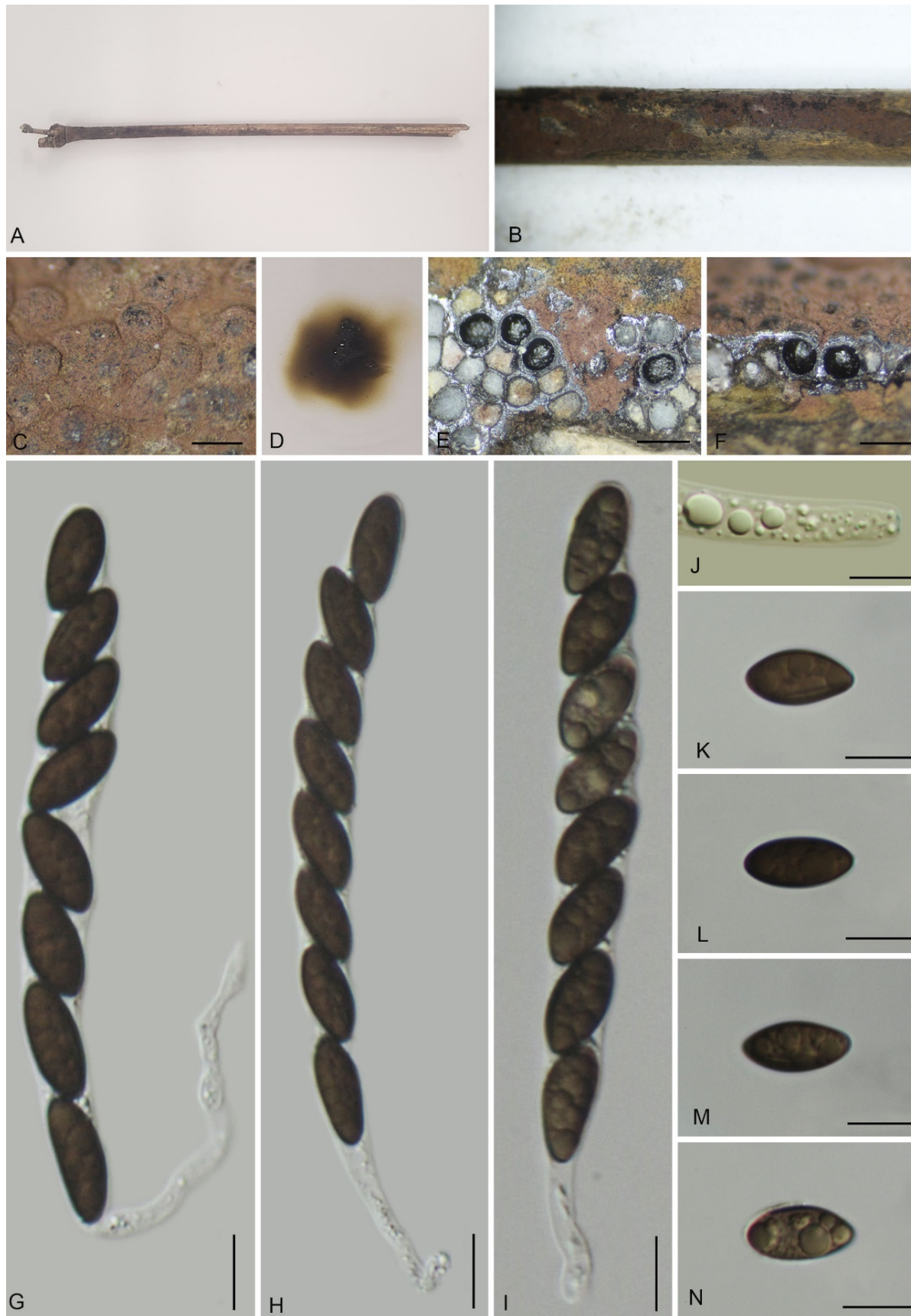


Fig. 9 *Hypoxylon guiense* (GMB4712, holotype). A Stromatal habit on bamboo culm. B Stromatal surface. C Ostioles. D Stromatal KOH-extractable pigment. E Cross section of stroma. F Longitudinal section of stroma. G–I Asci in distilled water. J Ascilar apical apparatus (stained in Melzer's reagent). K indehiscent perispore in 10% KOH. L–N Ascospores. Scale bars: E, F = 0.5 mm; G–N = 15 μ m.

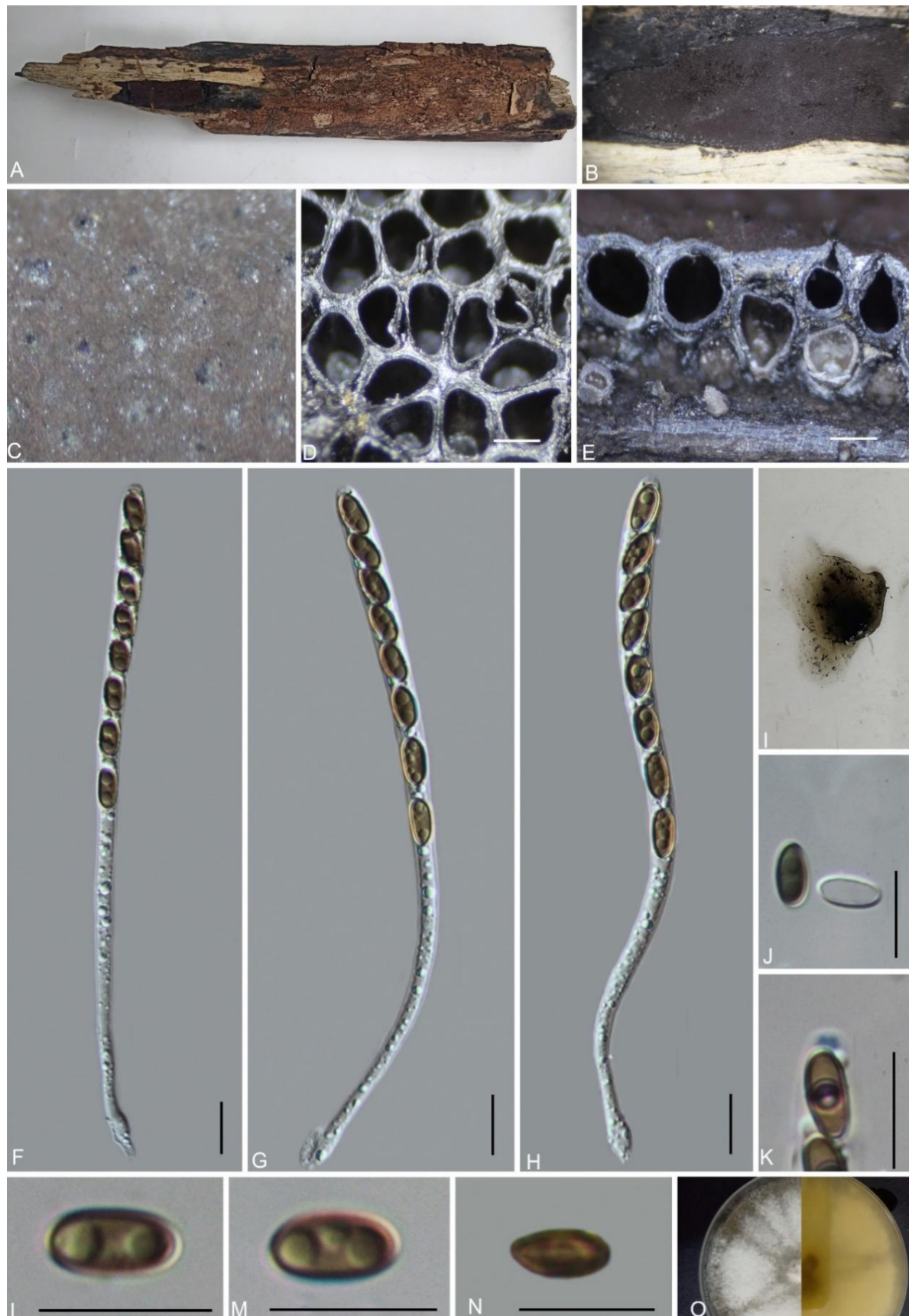


Fig. 10 *Hypoxylon huangjingense* (GMB4228, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles; D Cross section of stromata. E Longitudinal section of stroma. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascus apical apparatus (stained in Melzer's reagent). L–N Ascospores; O, Colonies on OA. Scale bars: D–E = 0.5 mm; F–H, J–N = 10 µm

Notes: Morphologically and phylogenetically (Fig. 1), *Hypoxylon huangjingense* is closely related to *H. investiens*, sharing similarly sized asci and ascospores with a germ slit slightly less than the spore length. However, *H. investiens* can be distinguished by its tubular to long-tubular ascomata (0.3–0.4 mm diam × 0.5–1 mm high vs. spherical 0.42–0.63 mm wide × 0.55–0.81 mm high), Greenish Yellow (16), Dull Green (70), or Dark Green (21) KOH-extractable pigments (vs. Grey Olivaceous [107] to Olivaceous Grey [121]), and its indehiscent perispore (vs. dehiscent) (Ju & Rogers 1996).

Morphologically, *H. huangjingense* also resembles *H. lenormandii* (= *H. riograndense*) in having the same surface coloration and spherical perithecia. However, *H. lenormandii* differs by its Hazel (88), Sienna (8), Cinnamon (62), Fulvous (43), Umber (9), or Ochreous (44) KOH-extractable pigments, and larger asci (123–170 µm total length vs. 120–140 µm total length) and ascospores (9.5–16 µm length vs. 6.5–9 µm length) (Ju & Rogers 1996).

Hypoxylon lancangense K. Habib, Y.H. Pi & Q.R. Li *sp. nov.*
Mycobank No.: MB859006; Fig. 11

Etymology: The epithet refers to the location of the type specimen, Lancang River.

Material examined: China, Yunnan Province, Baoshan City, Lancangjiang Nature Reserve (24°57'26.28"N, 99°44'23.08"E), altitude 1314 m, on dead branches of an unidentified plant, 2 Oct. 2019, Y. H. Pi, 2019LC110 (GMB0284 holotype; ex-type living culture GMB0284; KUN-HKAS 148619, isotype).

Teleomorph: Stromata on the surface of dead wood, 1–5 cm long × 2–4 cm wide × 0.4–1 mm thick (mean = 3.6 cm × 3.2 cm × 0.76 mm, n = 10), effused or effused-pulvinate, not carbonaceous, surface Brown Vinaceous (84), Umber (9), orange granules beneath the surface and between perithecia, with 10% KOH extractable pigments Ochreous (44) or Luteous (12) with Citrine (13) tone. Perithecia 0.24–0.58 mm wide × 0.32–0.61 mm high ($\bar{x}$ = 0.43 × 0.52 mm, n = 20), tubular. Ostioles umbilicate, lower than the stromatal surface, overlain with conspicuous white substance. Asci 95–130 × 8–9 µm ($\bar{x}$ = 114 × 8.5 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, spore-bearing part 65–90 µm ($\bar{x}$ = 76 µm, n = 30) long, stipes 30–75 µm (mean = 50 µm, n = 30) long, with apical apparatus bluing in Melzer's reagent, discoid, 2–2.5 µm broad × 0.7–1.4 µm high ($\bar{x}$ = 2.1 × 0.9 µm, n = 20). Ascospores 9–10.5 × 4–5 µm ($\bar{x}$ = 10 × 4.7 µm, n = 30), uniseriate, unicellular, inequilateral ellipsoidal with rounded ends, brown to black brown, with a faint straight germ slit nearly spore-length; perispore dehiscent in 10 % KOH, episore smooth.

Anamorph on OA: Conidiophores *Virgariella*-like, hyaline, smooth. Conidiogenous cells hyaline, smooth, 63–81 × 2.3–4.5 µm. Conidia hyaline, smooth to finely roughened, ovoid, 3.5–5.5 × 2.4–3.2 µm.

Culture characteristics: Colonies grown on OA at 25 °C for 3 weeks reached a diameter of 5 cm. Colonies surface white, azonate, velvety to felty, irregular entire margin; reverse pinkish white to hazel.

Additional materials examined: China, Guizhou Province, Guiyang City, Pingba (26°25'10.24"N, 106°24'25.21"E) altitude: 1052 m, on dead branches of an unidentified plant, 5 Sept. 2020, Y. H. Pi, 2020PB23 (GMB0286, culture GMB0286). China, Hunan Province, Yiyang City, Liubuoxi National Nature Reserve (28°23'53.73"N, 110°51'36.25"E),

altitude 836 m, on dead branches of an unidentified plant, 26 Aug. 2023, W. Y. Zeng, 2023LBX41 (GMB4249; culture GMB4249).

Notes: Morphologically, *Hypoxylon lancangense* is similar to *H. perforatum* in having the same surface coloration, ascomata overlain with a conspicuous white substance, and similarly sized asci. However, *H. perforatum* differs by its spherical ascomata (0.1–0.3 mm diam vs. tubular, 0.24–0.58 mm wide), smaller ascospores (8–13 × 4–6 µm vs. 9–10.5 × 4–5 µm), and larger conidia (3.5–4.8 × 2.8–3.5 µm vs. 3.5–5.5 × 2.4–3.2 µm) (Ju & Rogers 1996). Additionally, in the epitype description of *H. perforatum*, the ascospores are reported as larger (10–12.2 × 3.6–4.7 µm), while the asci are smaller (77.8–95.4 × 6.4–7.8 µm) compared to those of *H. lancangense*.

In surface coloration, ascospore size, and KOH-extractable pigments, *H. lancangense* is similar to *H. rubiginosum*. However, *H. rubiginosum* differs in having spherical to obovoid, larger ascomata (300–650 µm diam × 450–800 µm high vs. 0.24–0.58 mm wide × 0.32–0.61 mm high), larger asci (130–180 µm total length vs. 95–130 µm total length), and a *Nodulisporium*-like anamorph with larger conidia (5–6 × 3–4 µm vs. *Virgariella*-like anamorph conidia, 2.4–3.2 × 3.5–5.5 µm) (Ju & Rogers 1996).

In surface coloration, *Virgariella*-like anamorph, and ascospore size, *H. lancangense* is similar to *H. petriniae*. However, *H. petriniae* differs by its spherical to obovoid perithecia (vs. tubular), slightly longer asci (115–145 µm total length vs. 95–130 µm total length), and larger conidia (7–8 × 3–4 µm vs. 3.5–5.5 × 2.4–3.2 µm) (Stadler et al. 2004). In the phylogram, *H. lancangense* is also phylogenetically distant from these taxa.

Hypoxylon populi K. Habib, Y.H. Pi & Q.R. Li *sp. nov.*

Mycobank No.: MB859007; Fig. 12

Etymology: The epithet refers to the host plant genus "*Populus*".

Material examined: China, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Luodian County, Hongshui River (25°65'28.95"N, 107°30'21.74"E), altitude 480 m, on dead branches of *Populus* sp. 12 Sept. 2021, Y.H. Pi, 2021HSH7 (GMB4232, holotype; GMB4232, ex-type; KUN-HKAS 148620, isotype).

Teleomorph: Stromata on the surface of dead branches of *Populus* sp., 0.6–1 cm long × 0.2–1 cm wide × 0.7–0.9 mm thick ($\bar{x}$ = 0.8 cm × 0.4 cm × 0.78 mm, n = 10), glomerate, coalescent into larger pulvinate stromata, with conspicuous to inconspicuous perithecial mounds, surface dark vinaceous (82) to brown vinaceous (84); tissue between the perithecia black, tissue below the perithecial layer conspicuous, blackish brown, with 10% KOH extractable pigments Umber (9), Dark Purple (36). Perithecia 0.37–0.46 mm wide × 0.40–0.64 mm high ($\bar{x}$ = 0.41 × 0.51 mm, n = 20), spherical. Ostioles inconspicuous. Asci 104–125 × 8–9.5 µm ($\bar{x}$ = 113 × 9.2 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, spore-bearing part 60–90 µm ($\bar{x}$ = 81 µm, n = 30) long, with apical apparatus bluing in Melzer's reagent, discoid, 1.6–2.1 × 0.5–0.8 µm ($\bar{x}$ = 2 × 0.6 µm, n = 20). Ascospores 9.5–13 × 4.5–6.6 µm ($\bar{x}$ = 11.7 × 5.9 µm, n = 30), uniseriate, unicellular, dark brown, inequilateral, with rounded ends, with inconspicuous straight spore-length germ slit; perispore dehiscent in 10% KOH, with conspicuous coil-like ornamentation; episore smooth. Asexual morph: not formed.

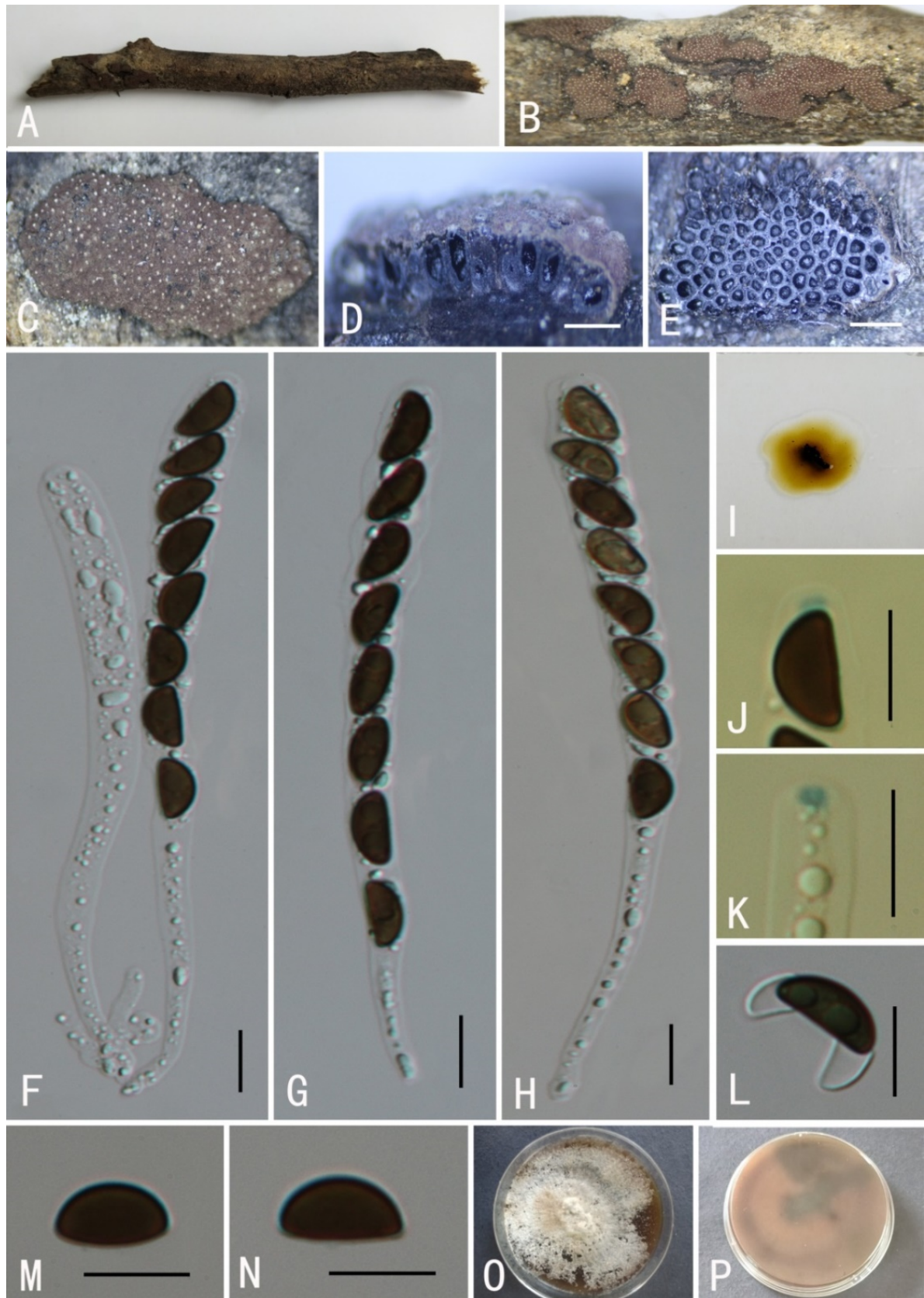


Fig. 11 *Hypoxylon lancangense* (GMB0284, holotype). A Stromatal habit on branch. B Stromatal surface. C Ostioles. D Longitudinal section of stroma. E Cross section of stromata. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J, K Ascilar apical apparatus (stained in Melzer's reagent). L Perispore dehiscent in 10% KOH. M, N Ascospores. O, P Colonies on OA (O-upper, P-lower). Scale bars: D, E = 0.5 mm; F–H, J–N = 10 µm.



Fig. 12 *Hypoxylon populi* (GMB4232, holotype). A Stromatal habit on branch. B Stromatal surface. C Ostioles. D Cross section of stromata. E Longitudinal section of stroma. F–H Asci in distilled water, I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascus apical apparatus (stained in Melzer's reagent). L–M Ascospores. N, O Colonies on OA (N-upper, O-lower). Scale bars: D, E = 0.5 mm; F–H, J–M = 10 µm.

Additional material examined: China, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Luodian County, Hongshui River (25°65'29.42"N, 107°30'23.38"E), altitude 514 m, on dead branches of *Populus* sp. L., 14 Sept. 2021, Y.H. Pi, 2021HSH300 (GMB0456).

Notes: Phylogenetically, *Hypoxylon populi* is closely related to *H. lienhwacheense*, which can be easily differentiated by Dark Violet (33), Livid Violet (79), or Dark Slate Blue (11) KOH-extractable pigments [vs. Umber (9) to Dark Purple (36)] and smaller ascospores (6–7.5 × 3–3.5 µm vs. 9.5–13 × 4.5–6.6 µm with smooth perispore (vs. with conspicuous coil-like ornamentation) (Ju & Rogers 1996).

In surface coloration and ascospore size, *H. populi* is similar to *H. lividicolor*. However, the latter differs in having taller perithecia (0.5–1.3 mm high vs. 0.40–0.64 mm high), larger asci (135–160 µm total length vs. 104–125 µm total length), and Dark Livid (80) KOH-extractable pigments [vs. Umber (9) to Dark Purple (36)] (Ju & Rogers 1996). Other closely related species, *H. lividipigmentum* and *H. hainanense*, differ in having effused–pulvinate stromata and Dark Livid (80), Pale Vinaceous (85) to Livid Vinaceous (83) and Vinaceous Purple (101) KOH-extractable pigments. Moreover, *H. hainanense* has light-brown to brown, smaller ascospore 6.1–9.6 × 3.2–5 µm and *H. lividipigmentum* has reddish brown stromatal granules (Ju & Rogers 1996; Fournier et al. 2014; Ma et al. 2022). Based on these differences and phylogeny, we introduce *Hypoxylon populi* as a new species.

Hypoxylon pseudorubiginosum K. Habib, Y.H. Pi & Q.R. Li sp. nov.

MycoBank No.: MB859010; Fig. 13

Etymology: The epithet refers to the morphological and phylogenetically closely related *Hypoxylon rubiginosum*.

Material examined: China, Guizhou Province, Rongjiang County, Leigongshan Nature Reserve (26°21'49.43"N, 108°16'8.05"E), altitude: 1165 m, on dead wood, 28 Aug. 2020, Y. H. Pi, 2020LGS190 (GMB0295, holotype; GMBC0295 ex-type culture; KUN-HKAS 148621, isotype)

Teleomorph: Stromata on surface of dead wood, 1.5–2.5 cm long × 0.6–0.8 cm wide × 0.8–0.9 mm thick effused, surface Brown Vinaceous (84), Sepia (63), or Dark Brick (60); widely effused margins black, weakly carbonaceous, with orange-yellow granules beneath surface and between perithecia, the tissue below the perithecial layer conspicuous, with 10% KOH extractable pigments Luteous (12) to Umber (9), Dark Brick (86). Perithecia 0.35–0.68 mm wide × 0.53–0.69 mm high ($\bar{x}$ = 0.53 × 0.60 mm, n = 20), spherical. Ostioles umbilicate, lower than the level of stromatal surface, surrounded by a ring of white substance. Asci 105–155 × 6.5–8.8 µm ($\bar{av}$. = 130 × 8 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 65–78 µm ($\bar{x}$ = 71 µm, n = 30) long, with apical apparatus bluing in Melzer's reagent, discoid, 2–2.8 µm broad × 0.8–1.4 µm high ($\bar{x}$ = 2.4 × 1.2 µm, n = 20). Ascospores 9.5–11.6 × 4.5–6 µm ($\bar{x}$ = 10.5 × 5 µm, n = 30), uniseriate, unicellular, ellipsoidal, inequilateral, with rounded ends, brown to blackish brown, with conspicuous straight germ slit covering the full spore length, perispore dehiscent in 10 % KOH, smooth, episporium smooth. Asexual morph: not formed.

Culture characteristics: Colonies on OA covering 4–5 cm diam. after 2 weeks at 25 °C. Colonies surface white, cottony, azonate with entire margins; after 4 weeks, covering a 9 cm

petri dish, become white grey, with yellow granules close to center; reverse black brown to hazel. No conidia were observed.

Additional material examined: China, Sichuan Province, Luzhou City, Huangjing Old Forest Nature Reserve, (28°6'6.2"N, 105°12'48.5"E), altitude 1000 m, on dead wood, 16 Aug. 2022, H.M. Hu, 2022HJLL25 (GMB4261).

Notes: *Hypoxylon pseudorubiginosum* is morphologically similar to *H. rubiginosum*, sharing comparable ascomatal and ascospore dimensions (Ju & Rogers 1996). Morphologically, *H. pseudorubiginosum* differs from *H. rubiginosum* (as described by Ju & Rogers 1996) in its KOH-extractable pigments Luteous (12) to Umber (9) or Dark Brick (86), contrasting with the orange (7) to rust (39) pigments of *H. rubiginosum*. Additionally, *H. pseudorubiginosum* possesses shorter asci (105–155 µm total length vs. 130–180 µm in *H. rubiginosum*). These distinctions are further corroborated by Pourmoghaddam et al. (2020), who reported *H. rubiginosum* yield Orange (7) to Scarlet (5) KOH pigments and asci stipes up to 180 µm long, further separating it from *H. pseudorubiginosum*. In the phylogram (Fig. 1), both species also showed deviating positions.

Hypoxylon pseudorubiginosum also shows superficial resemblance to *H. petriniae* due to shared vinaceous-brown stromata with black margins and ascospores of similar size. However, *H. petriniae* is distinguished by smaller ascomata (0.25–0.38 mm diam × 0.25–0.50 mm high vs. 0.35–0.68 mm diam × 0.53–0.69 mm high in *H. pseudorubiginosum*). Molecular data further confirm their distinction, with *H. pseudorubiginosum* exhibiting substantial genetic divergence from both *H. rubiginosum* and *H. petriniae*. Phylogenetically, it is distantly related, represent high molecular variation. Based on these differences and phylogenetic analysis, we introduce *H. pseudorubiginosum* as a new species.

Hypoxylon qiongense K. Habib, Y.H. Pi & Q.R. Li sp. nov.

MycoBank No.: MB859011; Fig. 14

Etymology: The specific epithet refers to Qiong, the traditional name for Hainan Province, the type locality.

Material examined: China, Hainan Province, Qiongzong County, Limushan Nature Reserve (19°12'28.95"N, 109°50'21.74"E), altitude 209 m, on dead wood, 12 Nov. 2020, Y.H. Pi, 2020QZ45 (GMB4229, holotype; GMBC0449, ex-type culture; KUN-HKAS 148622, isotype).

Teleomorph: Stromata on surface of dead wood, 0.8–6 cm long × 0.3–3 cm wide × 0.7–0.95 mm thick ($\bar{x}$ = 4 cm × 1.6 cm × 0.8 mm, n = 10), glomerate, coalescent into larger effused-pulvinate stromata, with conspicuous to inconspicuous perithecial mounds, noncarbonaceous, surface Blood Red (3) to Brown Vinaceous (84), with KOH-extractable pigments Fawn (87) to Sepia (63) after a while; tissue between the perithecia black, tissue below the perithecial layer conspicuous, blackish brown. Perithecia 0.46–0.64 mm wide × 0.61–0.94 mm high ($\bar{x}$ = 0.55 × 0.82 mm, n = 20), subglobose or tubular. Ostioles umbilicate, lower than the surface, inconspicuous. Asci 90–106 × 5–6.5 µm ($\bar{x}$ = 97 × 5.8 µm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 45–60 µm ($\bar{x}$ = 51 µm, n = 30) long, with apical apparatus bluing in Melzer's reagent, discoid, 1.2–1.6 × 0.6–0.9 µm ($\bar{x}$ = 1.5 × 0.8 µm, n = 20). Ascospores 6–8 × 3–3.5 µm ($\bar{x}$ = 7 × 3 µm, n = 30), uniseriate, unicellular, ellipsoid inequilateral to equilateral, with rounded ends, light brown to

brown, with an inconspicuous germ slit of spore length;
perispore indehiscent in 10% KOH; episporium smooth.

Asexual morph: not formed.

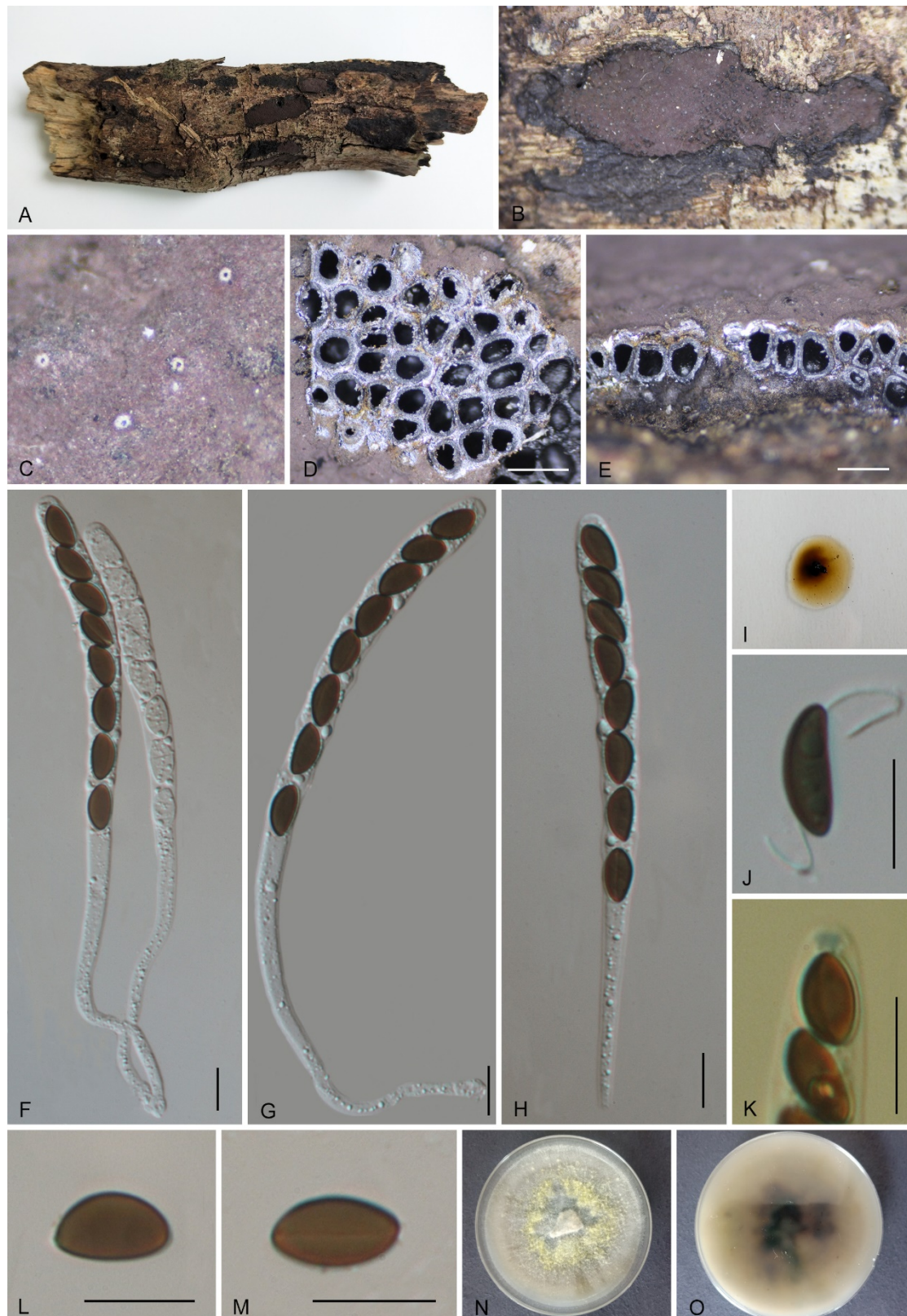


Fig. 13 *Hypoxylon pseudorubiginosum* (GMB0295). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross section of stromata. E Longitudinal section of stroma. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscence in 10% KOH. K Ascus apical apparatus (stained in Melzer's reagent). L, M Ascospores. N, O Culture on OA (N-above, O-below). Scale bars: D, E = 0.5 mm; F–H, J–M = 10 µm.

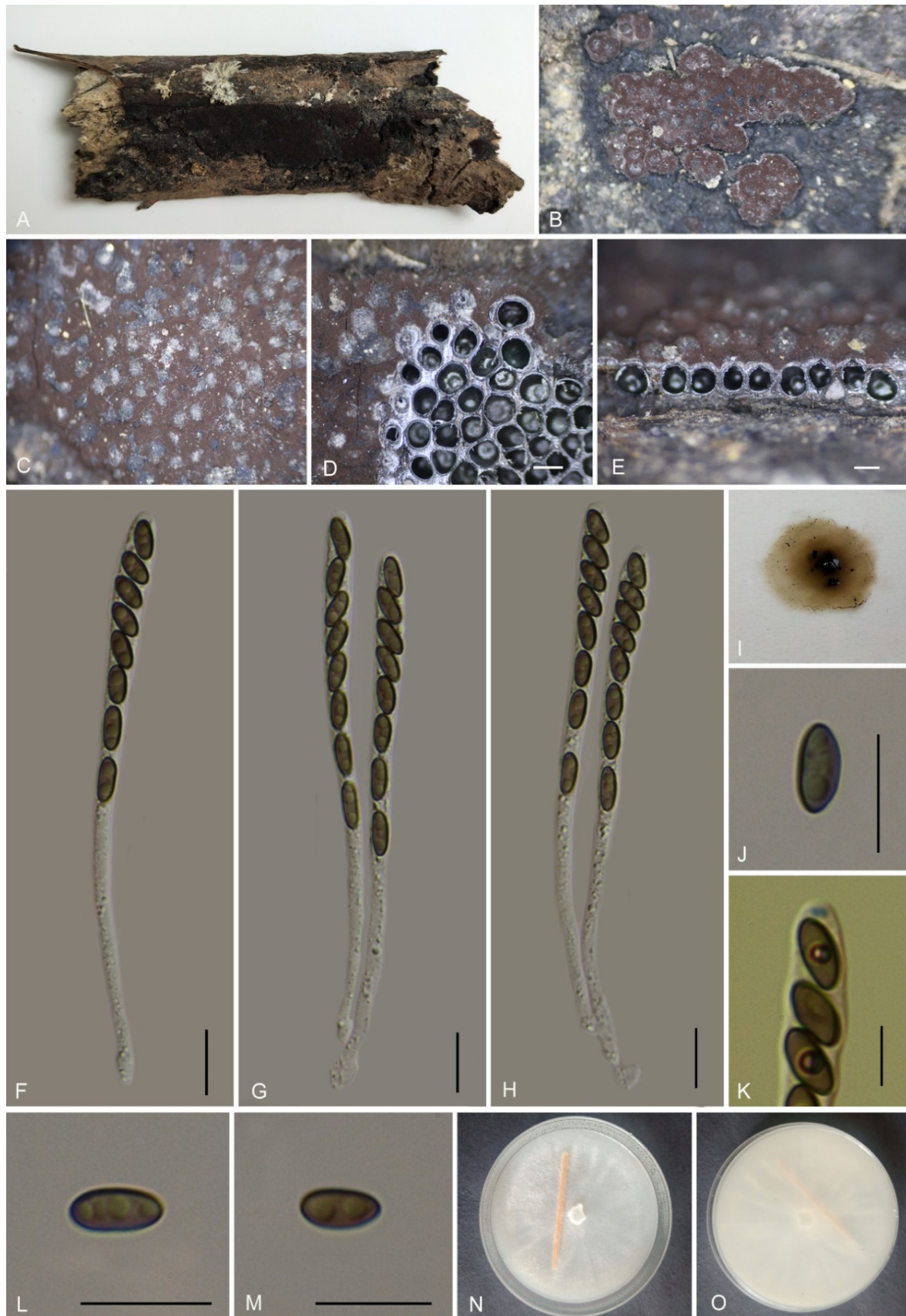


Fig. 14 *Hypoxylon qiongense* (GMB4229, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross section of stromata. E Longitudinal section of stromata. F–H Asci in distilled water. I KOH-extractable pigments. J Ascospores with indehiscent perispore in 10 % KOH. K Ascus apical apparatus (stained in Melzer's reagent). L, M Ascospores. N, O Colonies on OA (N-upper, O-lower). Scale bars: D, E = 0.5 mm; F–H, J–M = 10 μ m.

Culture characteristics: Colonies grown on OA at 25 °C for 3 weeks reached a diameter of 5 cm. Colonies surface white, felty, cottony, thin; reverse white. Not sporulating on OA nor on PDA.

Additional material examined: China, Hainan Province, Wuzhishan City, Atuoling Forest Park (18°48'26.79"N, 109°30'55.47"E), altitude 430 m, on dead wood, 15 Nov. 2020, Y.H. Pi and Q.R. Li, 2020ATL16-1 (GMB4265). China, Hainan Province, Wuzhishan City, Atuoling Forest Park (18°48'28.31"N, 109°30'58.63"E), altitude 571 m, on dead branches, 18 Nov. 2020, Y.H. Pi and Q.R. Li, 2020ATL306 (GMB0449).

Notes: Phylogenetically (Fig. 1) and morphologically, *Hypoxylon qiongense* is closely related to *H. pulicicidum*, sharing similar surface coloration, indehiscent perispore, and similarly sized asci. However, *H. pulicicidum* differs by its slightly larger ascospores (7.5–9.4 × 3.2–4.2 µm vs. 6–8 × 3–3.5 µm) and the production of Olivaceous Buff (89) pigments in 10% KOH [vs. Fawn (87) to Sepia (63)] (Bills et al. 2012). Sequence comparison also supports this differentiation, pairwise sequence identity between *H. qiongense* and *H. pulicicidum* (MUCL 49879) was 86.4% for ITS, 98.3% for LSU, and 95.5% for *tub2*.

Morphologically, *Hypoxylon qiongense* also resembles *H. investiens*, share effused-pulvinate stromata with a brown surface, an indehiscent perispore, and similarly sized asci. However, *H. investiens* differs in having tubular to long tubular perithecia, (vs. spherical in *H. qiongense*) and yield Greenish Yellow (16), Dull Green (70), or Dark Green (21) stromatal KOH-extractable pigments.

In perithecial, ascus, and ascospore size, *Hypoxylon qiongense* resembles *H. hinnuleum* and is also phylogenetically closely related. However, *H. hinnuleum* can be easily distinguished by its stromatal coloration, which ranges from Dark Vinaceous (82) to Sepia (63), becoming black at maturity, presence of orange-brown granules immediately beneath the surface and between perithecia (vs. black in *H. qiongense*) and yield Dark Brick (86) KOH-extractable pigments (Sir et al. 2019).

Based on these morphological differences and phylogeny, we introduce *Hypoxylon qiongense* as a new species.

Hypoxylon rongjiangense K. Habib, Y.H. Pi & Q.R. Li sp. nov.
Mycobank No.: MB859004; Fig. 15

Etymology: The epithet refers to Rongjiang County, where the holotype was collected.

Material examined: China, Guizhou Province, Rongjiang County, Leigongshan Nature Reserve (26°22'53.08"N, 108°21'49.71"E), altitude: 881 m, on dead wood, 29 Aug. 2020, Y.H. Pi, 2019LGS52 (GMB0303, holotype; GMBC0303, ex-type living culture; KUN-HKAS 148623, isotype).

Teleomorph: Stromata on the surface of dead wood, 2.5–6 cm long × 1–2 cm wide × 0.5–0.55 mm thick ($\bar{x}$ = 3 cm × 1.6 cm × 0.53 mm, n = 10), plane, effuse, surface Chestnut (40) or Dark Purple (36), orange granules beneath surface and between perithecia, tissue below the perithecial layer

brownish, with 10% KOH extractable pigments Orange (7) Sienna (8) or umber (9). *Perithecia* 0.27–0.52 mm wide × 0.45–0.61 mm high ($\bar{x}$ = 0.39 × 0.52 mm, n = 20), spherical. *Ostioles* lower than the level of stromatal surface, usually overlain with conspicuous white substance. *Asci* 80–110 × 6–9 µm ($\bar{x}$ = 93 × 7 µm, n = 30), 8-spored, unitunicate, cylindrical, short-stipitate, spore-bearing part 60–76 µm ($\bar{x}$ = 66 µm, n = 30) long, stipes 25–35 µm ($\bar{x}$ = 28 µm, n = 30) long, with apical apparatus bluing in Melzer's reagent, discoid 1.7–2 wide × 0.9–1.2 µm high ($\bar{x}$ = 1.8 × 1 µm, n = 20). *Ascospores* 8.5–9.7 × 4–5.5 µm ($\bar{x}$ = 9 × 5 µm, n = 30), uniseriate, unicellular, ellipsoidal, inequilateral, with broadly rounded ends, brown to dark brown, with conspicuous straight to slightly sigmoid germ slit covering the full spore length, perispore dehiscent in 10% KOH, smooth; episore smooth. Asexual morph: not formed.

Culture characteristics: Colonies on OA covering 4–5 cm diam. after 3 weeks at 25 °C. Colonies surface white, cottony, azonate with entire margins; after 6 weeks, covering a 9 cm Petri dish, become brownish, appressed with entire margins; reverse grey brown to hazel. No conidia were observed.

Additional material examined: China, Guizhou Province, Xishui County, Danxia Valley of Xishui National Nature Reserve (18°48'36.44"N, 109°34'12.26"E), altitude 1094 m, on dead wood, 18 Nov. 2022, H.M. Hu, 2022DXG13 (GMB4714). China, Guizhou Province, Rongjiang County, Leigongshan Nature Reserve (26°22'53.22"N, 108°21'49.31"E), altitude 865 m, on dead wood, 29 Aug. 2020, Y.H. Pi, 2019LGS69 (GMB0304).

Notes: Morphologically, *Hypoxylon rongjiangense* is similar to *H. zangii* in having the same surface coloration, ascomata overlain with a conspicuous white substance, and yielding similar KOH-extractable pigments. However, *H. zangii* differs in having smaller ascomata (0.20–0.40 mm wide × 0.30–0.50 mm high vs. 0.27–0.52 mm wide × 0.45–0.61 mm high), larger asci (85–145 µm total length vs. 80–110 µm), and larger ascospores (10.9–14.6 × 4.8–6.4 µm vs. 8.5–9.7 × 4–5.5 µm) (Song et al. 2022).

In surface coloration and ascospore size, *H. rongjiangense* also resembles *H. texense*. However, *H. texense* can be distinguished by its KOH-extractable pigments Rust (39) to Dark Brick (86), and its larger ascomata (0.3–0.6 mm high × 0.28–0.5 mm diam) (Sir et al. 2019).

Hypoxylon rongjiangense shares similar KOH-extractable pigmentation with *H. guilanense* but the latter differs by having hemispherical to pulvinate stromata with Sienna (8), UMBER (9) to Buff (45) surface colors, conspicuous perithecial mounds, and larger ascospores (12–15 × 5–6 µm) (Pourmoghaddam et al. 2020). There are sufficient nucleotide differences among the analyzed loci between *H. rongjiangense* and its closely related taxa, including *H. zangii*, *H. texense*, *H. guilanense*, *H. canariense*, and *H. laschii*. Both molecular and morphological evidence support the recognition of *H. rongjiangense* as a new species in the genus *Hypoxylon*.

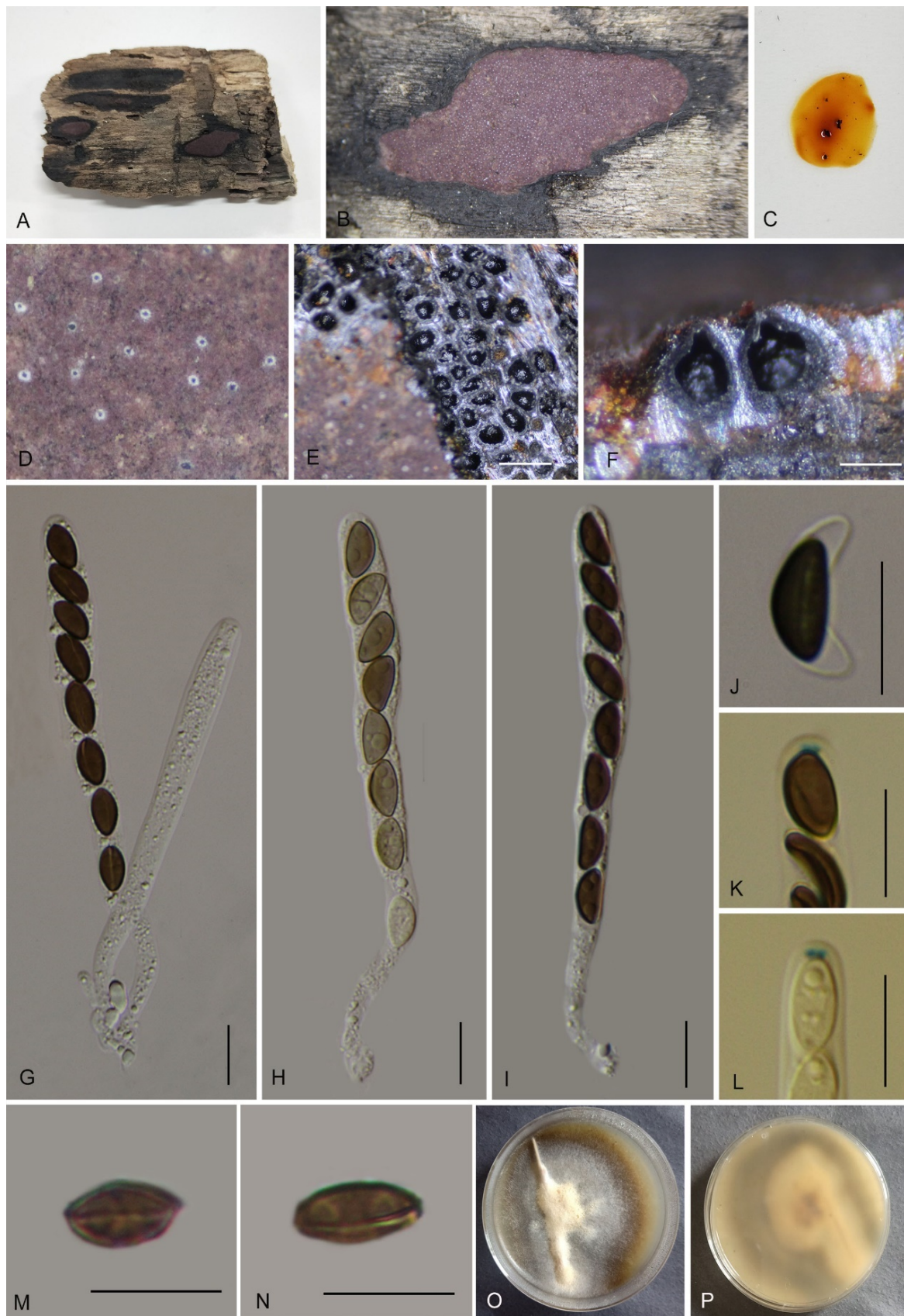


Fig. 15 *Hypoxylon rongliangense* (GMB0303, holotype). A Stromatal habit on wood. B Stromatal surface. C Stromatal KOH-extractable pigment. D Ostioles. E Cross section of stromata. F Longitudinal section of stroma. G–I Asci in distilled water. J Perispore dehiscent in 10% KOH. K, L Ascical apical apparatus (stained in Melzer's reagent). M, N Ascospores. O, P colonies on OA (O-upper, P-lower). Scale bars: E, F = 0.5 mm; G–N = 10 μ m.

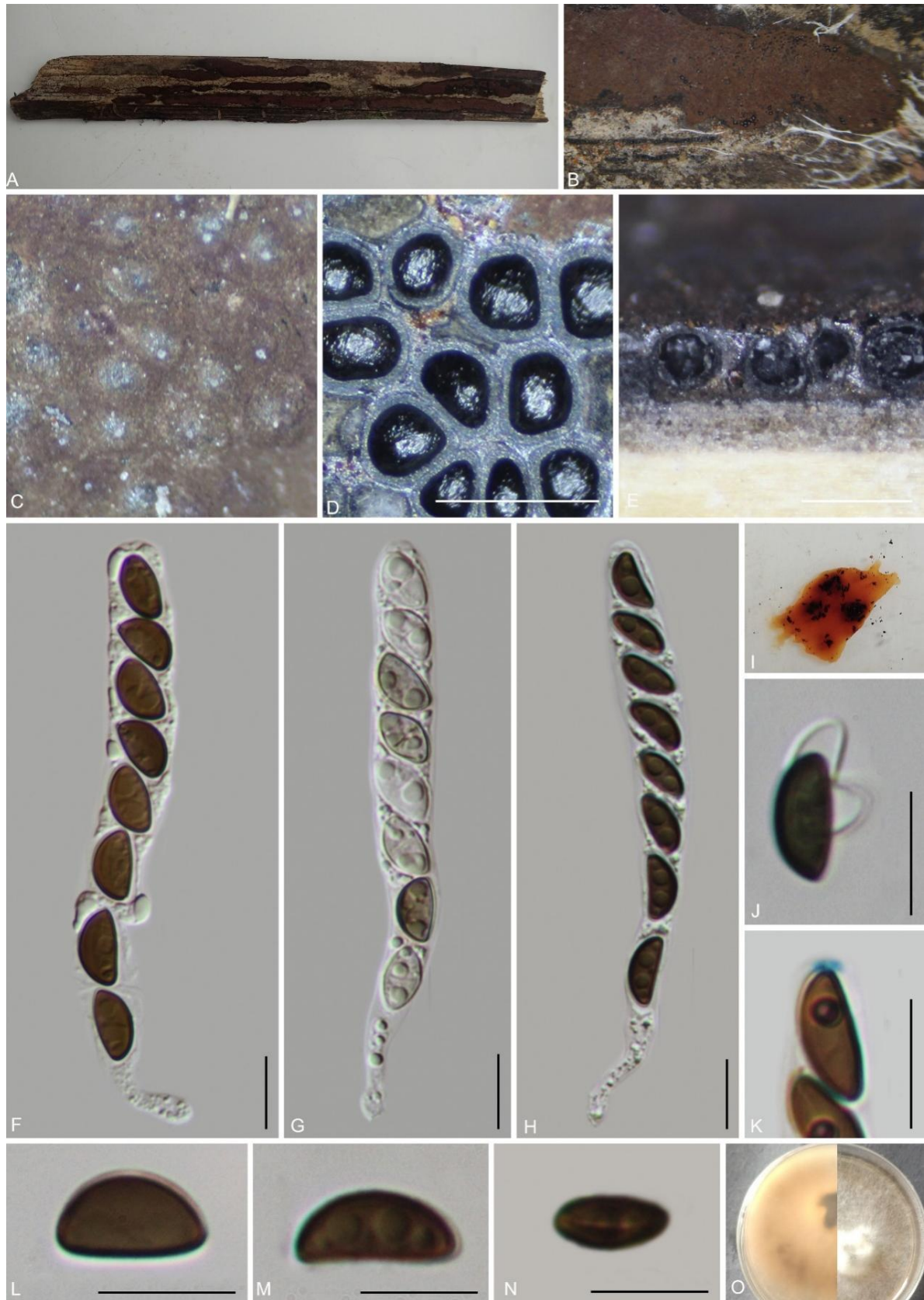


Fig. 16 *Hypoxylon sichuanense* (GMB4252, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross section of stroma. E Longitudinal section of stromata. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascus apical apparatus (bluing in Melzer's reagent). L–N Ascospores. O Culture on OA medium. Scale bars: D, E = 0.5 mm, F–H, J–N = 10 μ m.

Hypoxylon sichuanense H.M. Hu, K. Habib & Q.R. Li *sp. nov.*
Mycobank No.: MB859012; Fig. 16

Etymology: The specific epithet “*sichuanense*” refers to the type locality Sichuan Province, from where the specimen was collected.

Material examined: China, Sichuan Province, Huangjing Old Forest Nature Reserve, (28°6'54.9"N, 105°14'2.9"E), altitude 800 m, on dead bamboo culm, 16 Aug. 2022, H.M. Hu, 2022HJLL36 (GMB4252, holotype; GMBC4252 ex-type; KUN-HKAS 148624, isotype)

Teleomorph: *Stromata* on surface of dead bamboo culm, 1.2–6 cm long × 0.7–5 cm broad × 0.2–0.3 mm thick, effused or effused-pulvinate, confluent and forming larger elongate stromata, carbonaceous, surface Dark Vinaceous (82) or Sepia (63), yellowish-brown granules immediately beneath stromata, tissue black between perithecia, tissue below the perithecia inconspicuous, black, with 10% KOH extractable pigments Umber (9) to Rust (39). *Perithecia* 0.26–0.35 mm wide × 0.23–0.40 mm high ($\bar{x}$ = 0.32 × 0.34 mm, *n* = 20), globose to subglobose, black. *Ostioles* slightly lower than the level of stromatal surface, without a disc. *Asci* 86–110 × 9–11.5 μ m ($\bar{x}$ = 98 × 10.5 μ m, *n* = 30), 8-spored, unitunicate, cylindrical, spore-bearing part 68–77 μ m long ($\bar{x}$ = 71 μ m, *n* = 30), with apical apparatus bluing in Melzer's reagent, discoid, 2–3 × 1.5–2.2 μ m ($\bar{x}$ = 2.7 × 1.8 μ m, *n* = 20). *Ascospores* 10–11.5 × 5–6 μ m ($\bar{x}$ = 10.5 × 5.5 μ m, *n* = 30), uniseriate, unicellular, brown to dark brown, ellipsoid, inequilateral, with rounded ends, smooth, with an inconspicuous straight germ slit covering the full spore length; perispore dehiscent in 10% KOH, smooth; episore smooth. Asexual morph: Not observed.

Culture characteristics: Colonies on OA covering 4 cm diam. after 3 weeks at 25 °C, Colonies surface white, felty to cottony, azonate; after 6 weeks covering a 9 cm petri dish, becoming greyish white, dense; reverse brownish yellow. No conidia were observed.

Additional material examined: China, Sichuan Province, Luzhou City, Huangjing Old Forest Nature Reserve (28.115037°N, 105.234028°E), altitude: 583 m, on dead bamboo culm, 20 Aug. 2022, H.M. Hu, 2022HJLL301 (GMB4711).

Notes: Morphologically and phylogenetically (Fig. 1), *Hypoxylon sichuanense* is closely related to *H. pilgerianum*, which has also been reported on bamboo. However, *H. pilgerianum* differs in having dull orange-brown or dull reddish-brown granules immediately beneath the surface and between the perithecia (vs. black between the perithecia), smaller asci (68–97 μ m total length vs. 86–110 μ m total length), and slightly longer, narrower ascospores with narrowly rounded ends (8.5–13.5 × 4–5.5 μ m vs. 10–11.5 × 5–6 μ m with rounded ends) (Ju & Rogers 1996).

Hypoxylon pilgerianum was originally described from Brazil on *Chusquea* sp., with ascospores 10–12 × 4–5 μ m. Miller (1961), treated it as a synonym of *H. rubiginosum*. Later, Ju & Rogers (1996) reinstated the species based on collections from dead bamboo culms, reporting ascospores of 8.5–12(–13.5) × 4–5(–5.5) μ m. Fournier et al. (2015) documented two collections from Martinique, designating them as *H. cf. pilgerianum* sp. 1 and sp. 2, with ascospores

7.6–10 × 3.4–4.4 μ m and 10.3–12.8 × 4.9–6.7 μ m, respectively. In terms of chemical profiles, Ju & Rogers (1996) reported KOH-extractable pigments of Ochreous (44), Honey (64), or Amber (47), whereas Fournier et al. (2015) observed a pigment Sienna (8) turning Greyish Sepia (106). In contrast, *H. sichuanense* yields pigments Umber (9) to Rust (39).

Other species reported on bamboo, including *H. wuzhishanense*, *H. tibeticum*, and *H. pseudodefenderi*, share brownish stromata but all differ from *H. sichuanense* by the presence of yellowish-brown granules beneath the surface and between the perithecia (vs. black in *H. sichuanense*). Moreover, *H. wuzhishanense* has larger ascospores (9.5–14 × 5.4–6.7 μ m) and an indehiscent perispore (Ma et al. 2022). *Hypoxylon tibeticum* has larger ascospores (9.8–13 × 5.1–6.9 μ m). *Hypoxylon pseudodefenderi* has larger stromata (0.50–0.85 mm diam × 0.35–0.50 mm high), and shorter asci (70–90.5 μ m total length). There are no descriptions of its stromatal pigments in 10% KOH, and perispore detail, so these characteristics cannot be compared (Dai et al. 2017). Phylogenetic analyses also support the distinction of *H. sichuanense*. Hence, we introduce it as a new species.

Hypoxylon subjaklitschii H.M. Hu, K. Habib & Q.R. Li *sp. nov.*
Mycobank No.: MB859013; Fig. 17

Etymology: The epithet refers to the morphologically similar species, *H. jaklitschii*.

Material examined: China, Hainan Province, Anlong County (18°57'15.15"N, 109°03'45.21"E), altitude 1032 m, on dead wood, 22 July 2022, H.M. Hu, 2022BWL16 (GMB4241, holotype; GMBC4241, ex-type; KUN-HKAS 148625, isotype).

Teleomorph: *Stromata* on the surface of dead wood, 0.1–0.8 cm long × 0.1–0.4 cm wide × 0.5–1.0 mm thick ($\bar{x}$ = 0.38 cm × 0.22 cm × 0.89 mm, *n* = 10), pulvinate, glomerate, solitary to aggregated, spherical, united by thin stromatal tissue, non-carbonaceous, surface Sepia (63), Dark Brick (66) to black, black beneath surface and between perithecia, tissue below the perithecial layer inconspicuous, black, with KOH-extractable pigments Umber (9) or Brick (59). *Perithecia* 0.57–0.78 mm wide × 0.60–0.86 mm high ($\bar{x}$ = 0.67 × 0.71 mm, *n* = 20), spherical. *Ostioles* slightly higher than stromatal surface. *Asci* 130–170 × 8–10.5 μ m ($\bar{x}$ = 148 × 8.9 μ m, *n* = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 76–98 μ m ($\bar{x}$ = 88 μ m, *n* = 30) long, apical apparatus not bluing in Melzer's reagent. *Ascospores* 11–13.5 × 5.2–6.6 μ m ($\bar{x}$ = 12.2 × 6.2 μ m, *n* = 30), uniseriate, unicellular, ellipsoidal, inequilateral to nearly equilateral, with rounded ends, brown to dark brown, with a sigmoid spore-length germ slit, perispore dehiscent in 10% KOH with inconspicuous ornamentation; episore smooth. Asexual morph: not formed.

Culture characteristics: Colonies on OA covering 3 cm diam. after 2 weeks at 25 °C, Colonies surface white, felty, azonate, margin thin; after 6 weeks covering a 9 cm petri dish, becoming yellow-white, thick at center; reverse brown. No conidia were observed.

Additional material examined: China, Hainan Province, Anlong County (18°57'12.48"N, 109°03'42.59"E), altitude 985 m, on dead wood, 25 July 2022 H.M. Hu, 2022 BWL316 (GMB4722).



Fig. 17 *Hypoxylon subjaklitschii* (GMB4241, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostioles. D Cross section of stroma. E Longitudinal section of stromata. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascus apical apparatus (negative in Melzer's reagent). L–O Ascospores. Scale bars: D, E = 0.5 mm; F–H, J–O = 10 µm.



Fig. 18 *Jackrogersella maolanensis* (GMB0115, holotype). A Stromatal habit on wood. B Stromatal surface. C Ostiole. D Cross section of stromata. E Longitudinal section of stromata. F–H Asci in distilled water. I Stromatal KOH-extractable pigment. J Perispore dehiscent in 10% KOH. K Ascilar apical apparatus (stained in Melzer's reagent). L–O Ascospores. Scale bars: D, E = 0.5 mm, F–H, J–O = 10 µm.

Notes: Morphologically, *Hypoxylon subjaklitschii* resembles *H. jaklitschii* in having the same surface coloration and stromatal KOH-extractable pigments. Phylogenetically (Fig. 1), they also appear as sister species. However, *H. jaklitschii* differs by having orange-brown to dark brown granules immediately beneath the surface and between the perithecia (vs. black), amyloid ascal apical apparatus (vs. non-amyloid), smaller perithecia (0.3–0.4 mm wide vs. 0.57–0.78 mm wide), umbilicate ostioles lower than the stromatal surface, and smaller ascospores [$9.5\text{--}11.5(-12) \times 4\text{--}5.5 \mu\text{m}$ vs. $11\text{--}13.5 \times 5.2\text{--}6.6 \mu\text{m}$] (Kuhnert et al. 2015).

In having glomerate stromata and similar KOH-extractable pigments, *H. subjaklitschii* also resembles *H. lenormandii*. However, *H. lenormandii* is morphologically distinct with its Grayish Sepia (102), Fuscous (103), or Brown Vinaceous (84) surface coloration [vs. Sepia (63), Dark Brick (66) to black], smaller perithecia (0.3–0.5 mm diam.), and larger ascospores ($11\text{--}17 \times 4\text{--}6.5 \mu\text{m}$) (Kuhnert et al. 2015).

Phylogenetically, *H. subjaklitschii* is also closely related to *Hypoxylon mangrovei* and *H. teeravasati*. *Hypoxylon mangrovei* can be distinguished by its larger ascospores ($12\text{--}14.5 \times 5\text{--}6.5 \mu\text{m}$) and orange KOH-extractable pigments (Dayarathne et al. 2020). *Hypoxylon teeravasati* differs by having a burnt sienna stromatal surface, amyloid ascal apical apparatus and eye brown, KOH-extractable pigments (Phookamsak et al. 2019).

Jackrogersella maolanensis K. Habib, Y.H. Pi & Q.R. Li *sp. nov.*

Mycobank No.: MB859014; Fig. 18

Etymology: The epithet refers to Maolan National Nature Reserve, where the holotype was collected.

Material examined: China, Guizhou Province, Maolan National Nature Reserve, ($25^{\circ}10'54.6''\text{N}$, $108^{\circ}8'31.4''\text{E}$), altitude 586 m, on dead branch of an unidentified plant, 18 July 2022, H.M. Hu, 2022MLB32 (GMB0115, holotype; GMB0115, ex-type; KUN-HKAS 148626, isotype).

Teleomorph: Stromata on the surface of dead wood of unidentified plant, 0.1–2.7 cm length, 0.4–1.2 cm wide, and 0.2–0.8 mm high, pulvinate, solitary to confluent, spherical, with individual perithecial mounds prominent and occasionally approaching a rosellinoid appearance, united by thin stromatal tissue, surface blackish, carbonaceous, tissue beneath perithecial layer inconspicuous, black; with KOH extractable pigments Dark Brick (60), Vinaceous Grey (116) pigments in 10% KOH. Perithecia 0.54–0.71 mm wide $\times$ 0.62–0.87 mm high ($\bar{x} = 0.60 \times 0.71 \text{ mm}$, $n = 20$), spherical to obovoid. Ostioles papillate, black. Asci 100–135 $\times$ 3–4 μm ($\bar{x} = 122 \times 4 \mu\text{m}$, $n = 30$), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing part 40–52 μm ($\bar{x} = 48 \mu\text{m}$, $n = 30$) long, apical apparatus bluing in Melzer's reagent, discoid, 1.6–2.1 $\times$ 0.6–1 μm ($\bar{x} = 1.8 \times 0.8 \mu\text{m}$, $n = 20$). Ascospores 5.5–7.7 $\times$ 2.5–3.5 μm ($\bar{x} = 6.4 \times 3.1 \mu\text{m}$, $n = 30$), uniseriate, unicellular, ellipsoidal, inequilateral, with rounded ends, light brown, with an inconspicuous germ slit; perispore dehiscent in 10% KOH, smooth, with a thickening on the convex side, episporium smooth. Asexual morph: not formed.

Culture characteristics: Colonies on OA covering 2 cm diam. after 2 weeks at 25 °C, Colonies surface white, thin felty, fluffy azonate; after 6 weeks covering a 9 cm petri dish, becoming brownish white, dense; reverse greyish brown. No conidia were observed.

Additional material examined: China, Guizhou Province,

Kaili City, Leigongshan National Nature Reserve, ($26^{\circ}24'44.7''\text{N}$, $108^{\circ}12'4.5''\text{E}$), altitude 960 m, on dead branch of an unidentified plant, 16 Aug. 2022, H.M. Hu, 2022LGS5 (GMB0114).

Notes: Phylogenetically (Fig. 1), *Jackrogersella maolanensis* is closely related to *J. ilanensis*, with which it shares similar surface coloration, and ascospore size. However, *J. ilanensis* can be distinguished by its effused pulvinate stromata, ostiole encircled by a truncatum-type disc overlain with a white substance (vs. lack), and shorter asci (95–110 μm total length vs. 100–135 μm total length) (Ju & Rogers 1996).

Jackrogersella maolanensis stromata could be confused with *Jackrogersella minutella*. However, *J. minutella* differs in having inconspicuous perithecial mounds, smaller perithecia (0.40–0.50 mm wide vs. 0.54–0.71 mm wide), an inamyloid apical ring, and larger ascospores (6–9 $\times$ 3–4 μm vs. 5.5–7.7 $\times$ 2.5–3.5 μm) with a straight germ slit shorter than the spore length (as *H. cohaerens* var. *microsporum*; Ju & Rogers 1996; Wendt et al. 2018).

Species of the genus that lack molecular data, *J. gombakensis* and *J. nothofagi* can be easily distinguished by surface coloration and ascospore size. *Jackrogersella gombakensis* has a dark reddish-brown stromatal surface and larger ascospores (13–15 $\times$ 5–6 μm). *Jackrogersella nothofagi* has effused-pulvinate stromata with inconspicuous to planar perithecial mounds, a dark vinaceous to brown vinaceous surface, and larger ascospores 7–9.9 $\times$ 3.5–5 μm (Ju & Rogers 1996; Wendt et al. 2018).

Mitochondrial Genome

Characterization of the fifty Hypoxylaceae mitogenomes

The mitochondrial genomes of the 50 Hypoxylaceae strains represent four genera *Annulohypoxylon*, *Hypomontagnella*, *Hypoxylon*, and *Jackrogersella*. All mitogenomes are circular DNA molecules. Annotated mitochondrial diagrams of three representative species are shown in Figure 19. The remaining circular diagrams are in Supplementary Figure 7. A detailed overview of the mitochondrial genome composition across 50 strains within Hypoxylaceae is provided in Supplementary Tables 10 and 11.

The genomes are composed of circular DNA molecules with highly variable sizes across genera. In *Hypomontagnella*, genome sizes range from 27,637 bp (*H. vinacea* GMB0247) to 47,204 bp (*H. yinggelingsensis* GMB4715). *Hypoxylon* shows the widest variation, ranging from 26,809 bp (*H. lancangense* GMB4249) to 197,900 bp (*Hypoxylon* sp. GMB0308). *Annulohypoxylon* exhibits comparatively larger genomes, ranging from 130,895 bp (*A. baishaense* GMB4213) to 251,279 bp (*A. viridipigmentum* GMB0225). *Jackrogersella maolanensis* (GMB0115) has a genome size of 157,709 bp, which falls between the upper range of *Hypoxylon* and the lower range of *Annulohypoxylon* (Supplementary Fig. 7).

Protein coding genes and introns of the mitochondrial genomes

All 50 mitochondrial genomes of Hypoxylaceae contain 14 conserved core protein-coding genes (PCGs), including *atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6* (Fig. 19, Supplementary Table 11). Among these, *nad5* was the longest PCG, with an average length of 2,416.4 bp, while *atp8* was the shortest, averaging 153.6 bp.

The *atp8* gene was the shortest PCG across all 50 mitochondrial genomes. Its length was 153 bp in all strains except *Annulohypoxylon subyungense* GMB0226 and *A. pseudoalbidiscum* GMB0235, which showed 168 bp (Supplementary Table 11).

Gene length was most variable for *nad3*, ranging from 225 bp (*Hypoxylon* sp. GMB0308) to 2,631 bp (*Jackrogersella maolanensis* GMB0115). The gene *cox3* showed the second-

highest variation, spanning 483 bp to 1,872 bp across species. Of the 14 core PCGs, only *atp9* was invariant in length. The gene length of *nad1* in *Annulohypoxylon primevalense* GMB0240 (1,110 bp vs. 1,107 bp in others) and *nad4L* in *Hypoxylon* sp. GMB4719 (237 bp vs. 270 bp in others) deviated from those in the other 49 strains (Supplementary Table 11).



Fig. 19 Circular maps of the mitogenome from three species *Jackrogersella maolanensis* GMB0115; *Annulohypoxylon baishaense* GMB4213 and *Hypomontagnella vinacea* GMB0247.

Intron content varied significantly across the family, ranging from a complete absence in *Hypomontagnella vinacea* GMB0247 and *Hypoxylon lancangense* GMB0284 and GMB4249, to a maximum of 68 introns (with a cumulative intronic length of 64,743 bp) in *Annulohypoxylon linjiangense* GMB4216, which accounts for 45% of its total genome. Among the 14 core protein-coding mitochondrial genes (*cox1*, *cox2*, *cox3*, *cob*, *atp6*, *atp8*, *atp9*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*), introns were detected in all genes except *atp9*, *nad1*, *atp8* and *nad4L* (Supplementary Tables 10, 11). The *nad3* gene showed the highest intron diversity, with variable intron counts (225 bp in *Hypoxylon* sp. GMB0308 vs. 2631 bp in *Jackrogersella maolanensis* GMB0115), suggesting frequent intron gain or loss events.

Annulohypoxylon microdiscum GMB0234 has a short *cox1* (1,284 bp) and intron size of 81,032 bp, suggesting minimal intronic contribution to *cox1*. *Annulohypoxylon pseudoalbidiscum* GMB0235 has an exceptionally long *cox3* (1,872 bp) and intron size of 59,837 bp, implying a large intron within *cox3*.

Intron-rich species included *Annulohypoxylon linjiangense* GMB4216 (68 introns, 64,743 bp), *A. viridipigmentum* GMB0225 (50 introns, 120,275 bp), *A. yunnanense* GMB0462 (46 introns, 104,603 bp), *Hypoxylon duranii* GMB0265 (47 introns, 96,542 bp), and *Hypoxylon* sp. GMB0308 (43 introns, 110,871 bp).

Intergenic regions and rRNA and tRNA

The intergenic regions also showed high variation, ranging from 4,781 bp in *Hypomontagnella vinacea* GMB0247 (~17.3% of its 27,637 bp genome) to 62,450 bp in *Hypoxylon* sp. GMB0308 (~31.6% of its 197,900 bp genome). All strains encoded 25–35 tRNA genes (average ~26) and consistently 2 rRNA genes, with tRNA sizes ranging from 1,840 bp (*Annulohypoxylon subyungense* GMB0226) to 2,565 bp (*Hypoxylon* sp. GMB0297), and rRNA sizes ranging from 5,214 bp (*Hypoxylon qiongense* GMB4265) to 33,434 bp (*Annulohypoxylon areolatum* GMB4224) (Supplementary Table 10). This variability highlights the structural plasticity of Hypoxylaceae mitogenomes, indicating conserved rRNA gene numbers but significant divergence in intergenic regions and tRNA gene composition, likely reflecting evolutionary adaptations to diverse ecological niches, include expansions in non-coding regions for regulatory flexibility or tRNA/rRNA optimization to enhance metabolic efficiency.

Comparison of genes and similar species based on mitochondrial genomes

All 50 examined specimens possess the same set of 14 conserved mitochondrial protein-coding genes, indicating no variation in gene presence or absence across species. However, their mitochondrial genome sizes vary significantly, ranging from 26,809 bp (*Hypoxylon lancangense* GMB4249) to 251,279 bp (*Annulohypoxylon viridipigmentum* GMB0225). The lengths of the protein-coding genes show varying degrees of conservation and variability, which forms the basis of the correlation between genes and species.

Detailed analysis of gene lengths showed that some genes *nad1*, *atp8*, *atp9*, and *nad4L*, are highly conserved in length across species, suggesting strong functional constraints. For example, *nad1* gene of nearly all species has a length of 1,107 bp, with only *Annulohypoxylon*

primevalense GMB0240 at 1,110 bp (a difference of just 3 bp). Similarly, *atp8* is 153 bp in most species, with only two exceptions (*Annulohypoxylon pseudoalbidiscum* GMB0235 and *Annulohypoxylon subyungense* GMB0226) at 168 bp. The gene *atp9* is uniformly 225 bp across all species, showing no variation, representing the most conserved gene in the dataset.

In contrast, genes like *nad3* (225–2,631 bp) show extreme variability, driven largely by species-specific intron content. The gene length variation ranges overlap significantly across genera, and no consistent genus-specific pattern emerges, indicating that gene length variation is more species-specific than genus-specific.

The observed variability in gene lengths, particularly for genes like *cox1*, *cob*, and *nad3*, is likely influenced by the presence and size of introns within these genes. For example, *Annulohypoxylon microdiscum* GMB0234 has a short *cox1* (1284 bp) despite a large total intron content (81,032 bp), suggesting that *cox1* itself contains few or no introns and that most intronic sequence is located elsewhere in the mitogenome. *Hypomontagnella yinggelingsensis* GMB4715 has a longer *cox1* (1,833 bp) with a total intron size of 6,740 bp, possibly indicating the presence of introns within *cox1*. Similarly, *Annulohypoxylon pseudoalbidiscum* GMB0235 has an exceptionally long *cox3* (1872 bp) with a total intron size of 59,837 bp, implying the presence of a large intron within *cox3*.

Genes like *nad1*, *atp8*, *atp9*, and *nad4L* are highly conserved in length, showing minimal variation across species. Genes like *cox1*, *cob*, *nad4*, and especially *nad3* display greater length variability, likely due to species-specific differences in intronic content within these genes. This variability does not align with genus-level patterns but reflects individual species differences, possibly driven by evolutionary changes in intron presence or size. Significant mitochondrial genome size differences (26,809 to 251,279 bp), gene lengths remain relatively stable, indicating that genome size variation primarily attributed to non-coding regions (intergenic and intronic) rather than coding sequences.

The genus *Hypomontagnella* exemplifies mitochondrial diversity within a taxonomic group. *Hypomontagnella vinacea* (27,637 bp, no introns) and *H. yinggelingsensis* (47,204 bp, two introns) differ significantly in genome structure, supporting mitochondrial features as potential supplementary barcodes. *Hypoxylon lancangense* strains GMB0284 and GMB4249 display highly similar mitochondrial features. Their genome sizes (~26,813 bp and 26,809 bp), GC content (26.5%), intron absence, and conserved protein-coding gene content are nearly identical, indicating strong intraspecific consistency and genomic stability within this species.

Annulohypoxylon microdiscum strains GMB0233 and GMB0234 are morphologically indistinguishable. However, their mitochondrial genomes show much differences in size (171,360 bp vs. 162,152 bp), intron number (39 vs. 36), and intergenic region lengths (51,305 bp vs. 41,635 bp). These genomic differences suggest the possibility of cryptic speciation or divergence at the genomic level. Due to the absence of clear morphological distinctions, lack of a type strain, and a lack of detailed genomic analyses we tentatively identify both strains as *Annulohypoxylon microdiscum*. Refer Supplementary Tables 10 and 11 for a comparison of other species.

Mitochondrial genomes provide insights into evolutionary processes, however, their utility for species delimitation is constrained by overlapping length ranges across genera and insufficient functional gene analysis. Genome size variation (CV ~50%) far exceeds coding sequence variability (CV 0–15%), emphasizing that non-coding regions dominate mitochondrial evolution. A polyphasic approach combining mitogenomic data with nuclear markers (ITS, LSU, *rpb2*, *tub2*) and detailed morphological analyses will be essential to refine species boundaries and develop robust mitochondrial barcode markers. Pairwise comparisons of gene sequences between identical and closely related species are summarized in Supplementary Tables 1–7.

Evolutionary rates of mitochondrial genes

The largest K2P genetic distance was found in the *cob* gene among the 14 core PCGs and *rps3*, which indicate the *cob* gene varies greatly among the 50 Hypoxylaceae strains (Fig. 20). Less gene differentiation was also observed in the *cox1*, *nad1*, *nad2*, and *nad3* genes among the 50 Hypoxylaceae strains tested. The lowest mean K2P genetic distances were

observed in the *atp6* and *atp8* genes among the 14 core PCGs and *rps3*, indicating that these two genes are highly conserved among the 50 Hypoxylaceae species. The highest mean non-synonymous substitution rate (K_a) was observed in the *nad5* gene among the 14 core PCGs followed by the *cox1* and *rps3* genes (Figure 20), while the lowest mean K_a value was observed in the *nad4L* gene among the 14 core PCGs and *rps3* gene from the 50 Hypoxylaceae strains. The *nad3* genes in the 50 Hypoxylaceae strains exhibited the highest mean synonymous substitution rate (K_s), whereas the *atp8* gene exhibited the lowest mean K_s value among the the 14 core PCGs and *rps3* gene analyzed.

The K_a/K_s ratios for nine of the 14 core PCGs and *rps3* gene were less than 1, indicating that these nine genes were subjected to purifying selection during evolution. However, the K_a/K_s values of the *atp8*, *cox1*, *nad2*, *nad3*, and *nad5* genes were >1 between some Hypoxylaceae species, including members of the genera *Annulohypoxylon*, *Hypoxylon*, *Hypomontagnella* and *Jackrogersella* indicating that these genes may be under strong positive selection pressure in certain lineages within Hypoxylaceae.

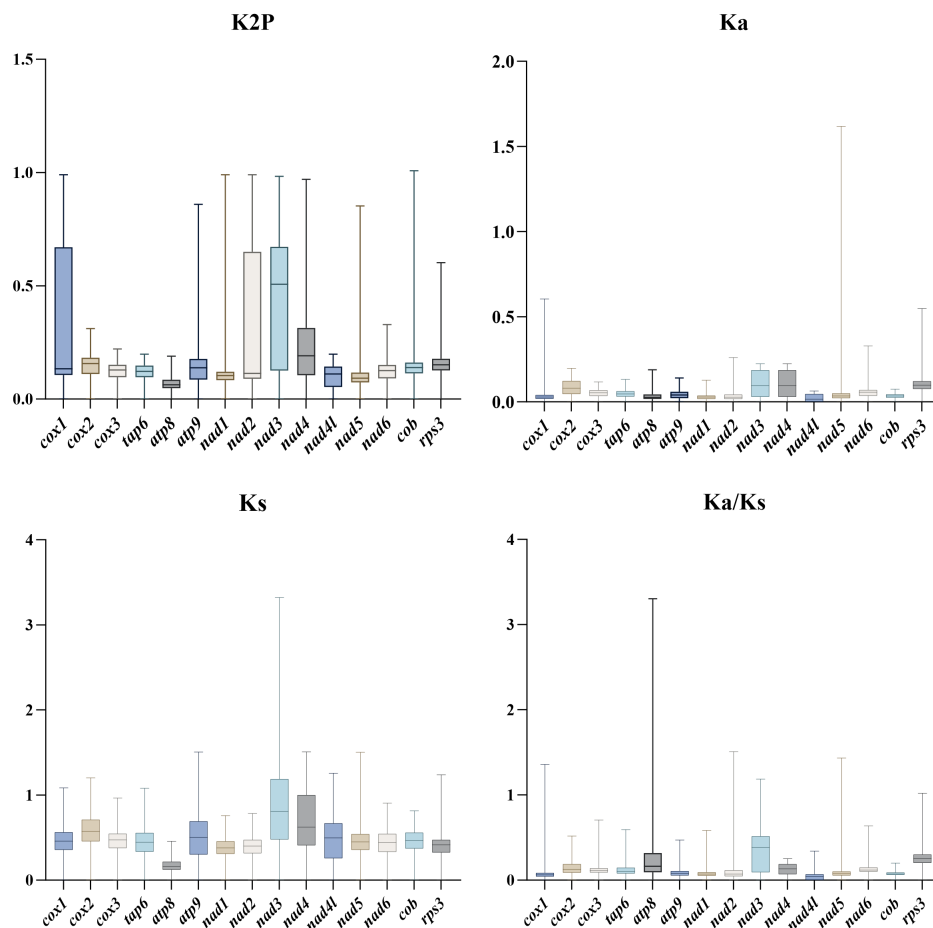


Fig. 20 Genetic analysis of 14 core protein coding genes and *rps3* conserved in the 50 Hypoxylaceae mitogenomes. K2P, pairwise genetic distances between each pair of the 14 core PCGs and *rps3* based on the Kimura-2-parameter model. K_a , non-synonymous substitution site; K_s , synonymous substitution site.

Analysis of Codon Preferences

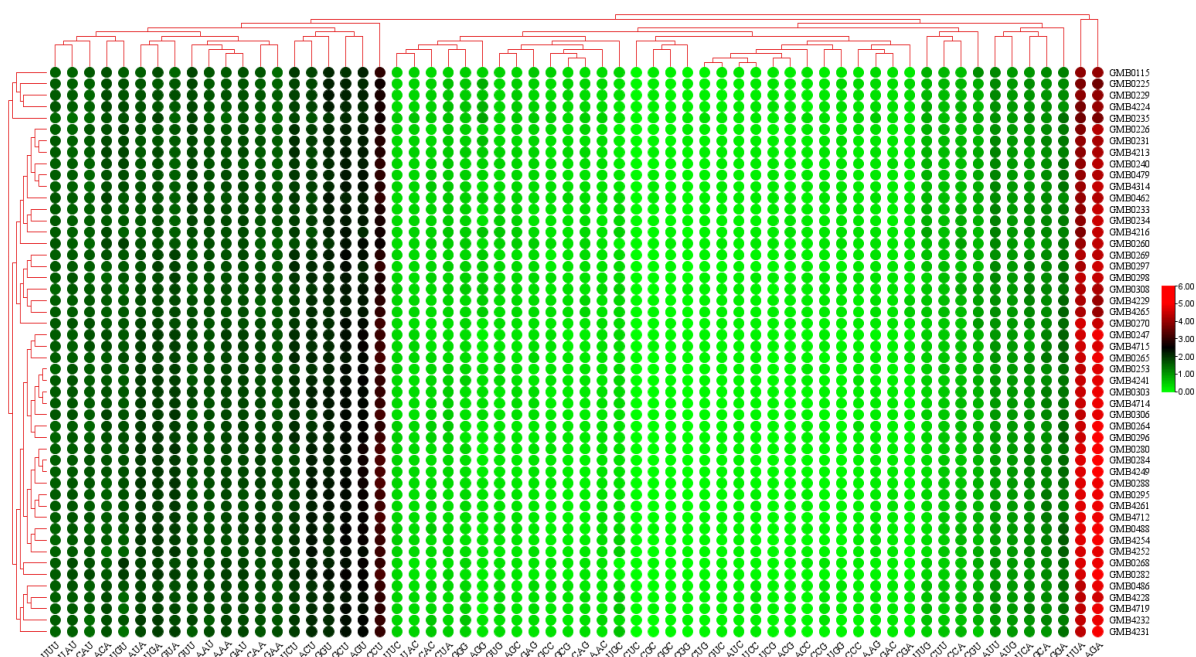
A comparative analysis of codon usage trends in 50 Hypoxylaceae mitogenomes were performed (Fig. 21). The

codons AGA (arginine) and UUA (leucine) were the most frequently utilized across the 50 Hypoxylaceae strains. In addition, the 50 Hypoxylaceae mitogenomes constantly

This consistent codon preference observed among Hypoxylaceae strains suggests a family-wide pattern. However, significant variations were noted in the start and stop codons across different strains (Supplementary Fig. 5). The pace of protein translation and impacts energy expenditure in most organelles is stimulated by codon usage. Codon usage analysis reveals substantial bias in synonymous codon selection within the mitochondrial coding sequences.

The average GC content of *atp9* (35.4%) and *cox1* (32.5%) exhibited consistently highest means, while *nad6* (21.5%) and *atp8* (20.8%) exhibited the lowest means of GC content, indicating relative conservation and structural constraints of these genes across the family. In a genus-level comparison, *Annulohypoxylon* strains exhibited slightly higher overall GC content (range 29.2–32.2%) than *Hypoxylon* and

The gene lengths of the 14 PCGs and *rps3* revealed a core pattern of high evolutionary conservation and genus- and species-specific dynamic variation. The gene lengths of *atp8* (153 bp), *atp6* (795 bp), *atp9* (225 bp), *nad4L* (270 bp), and *nad1* (1,107 bp) were highly stable and showed minimal to no variation (Fig. 23), indicating strong functional constraints. The genus *Hypoxylon* exhibited the highest gene lengths overall compared to other genera. In addition, the *nad3* gene exhibited massive size expansions in *Jackrogersella maolanensis* (GMB0115: 2,631 bp) and several *Hypoxylon* strains (GMB0298: 2,595 bp; GMB4252: 2,574 bp; GMB4265 and GMB4229: 2,502 bp; GMB0270: 2,487 bp) compared to its ordinary ~420 bp baseline. The GC skew for the 14 core PCGs was positive for all genes except *atp8*, which was consistently negative, revealing a unique evolutionary or mutational pressure on this gene (Fig. 24). The *atp9* and *rps3* exhibited the highest positive GC skews followed by *nad3*. *Annulohypoxylon* species exhibited higher and more homogenous GC skew values compared to other genera. In addition, higher variability or inverted GC skew was observed in *atp6* and *nad3* in *Hypoxylon* species. The AT skews across the 14 core PCGs were all negative across the 50 Hypoxylaceae mitogenomes. However, the *rps3* gene exhibited consistent positive AT skew across all genera, revealing a unique evolutionary trajectory (Fig. 25). Compared to other genera, *Annulohypoxylon* species exhibited moderate negative AT skews for core genes like *cox1* and *nad1*, indicating greater A/T compositional stability.



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rearrangements (Fig. 27). The size and location of homologous regions varied greatly among the 50 strains, even among species within the same genus. Variations in both the number and size of homologous regions across species and genera reflect the evolutionary and functional diversity within Hypoxylaceae. *Annulohypoxylon viridipigmentum* GMB0225 exhibited the highest number of homologous regions (39 regions) and the largest cumulative size, whereas *A. baishaense* GMB4213 exhibited a smaller size and the lowest number of homologous regions (35 regions) within the genus *Annulohypoxylon*. Similarly, *Hypoxylon* sp. GMB0308 exhibited the highest number of homologous regions (25) and the largest size, while *H.*

lancangense GMB0284 and *H. lancangense* GMB4249 exhibited the smallest sizes, each with only 8 homologous regions within the genus *Hypoxylon*. In the genus *Hypomontagnella*, *Hy. yinggelingsensis* GMB4715 exhibited larger genome size with 12 homologous regions compared to *Hy. vinacea* GMB0247 which exhibited the smallest size and 11 homologous regions. With only one sampled species in the genus *Jackrogersella*, *J. maolanensis* GMB0115 exhibited 34 homologous regions and a comparatively large size compared to several species in other genera among the 50 Hypoxylaceae. Variations in homologous regions across different species may be associated with differences in intron content.

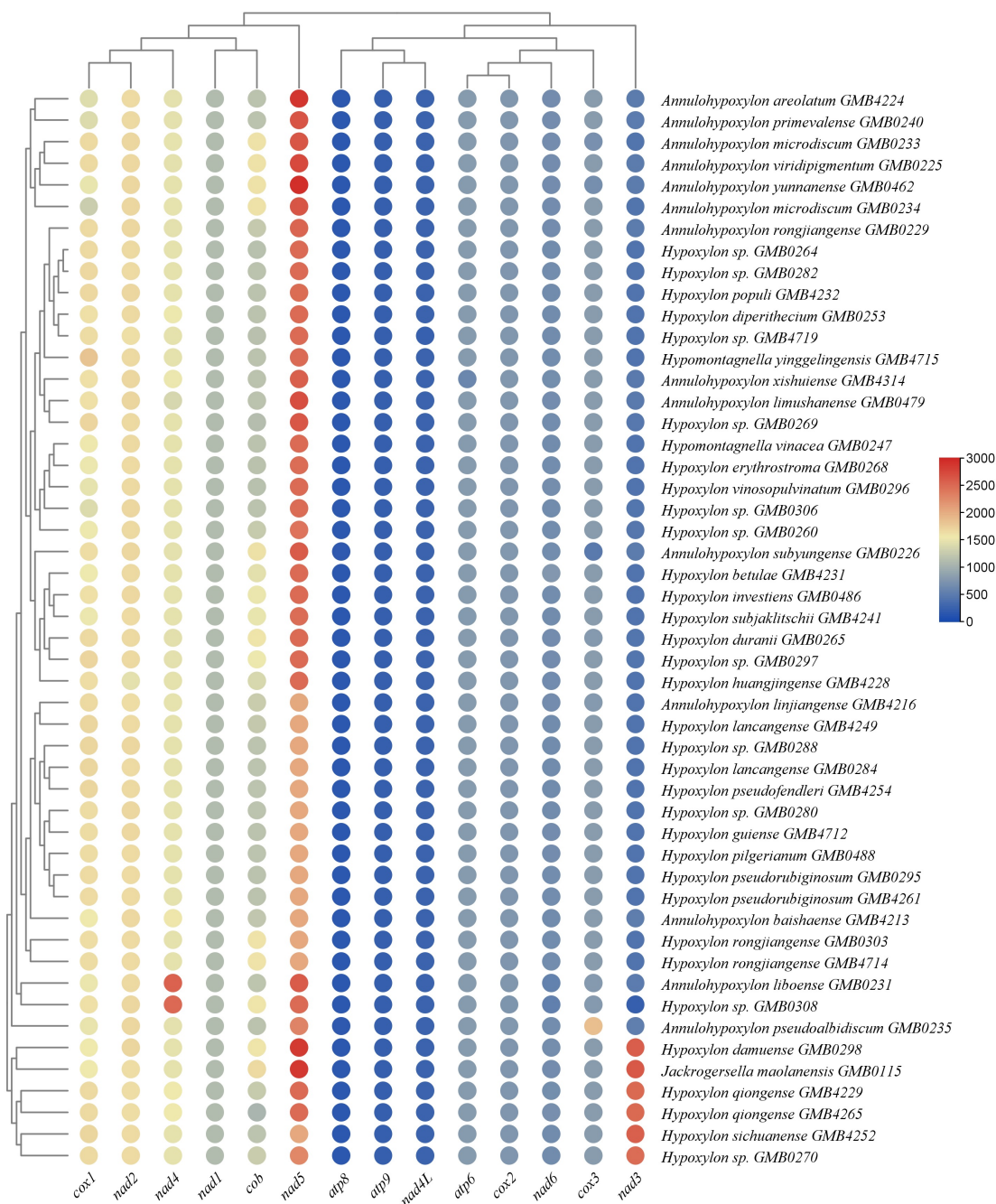


Fig 23 Heatmap showing the gene length across the mitogenomes of 50 Hypoxylaceae strains. The scale bar represents the percentage of the gene length (bp) where the blue (low), cream (mid) and red (high).

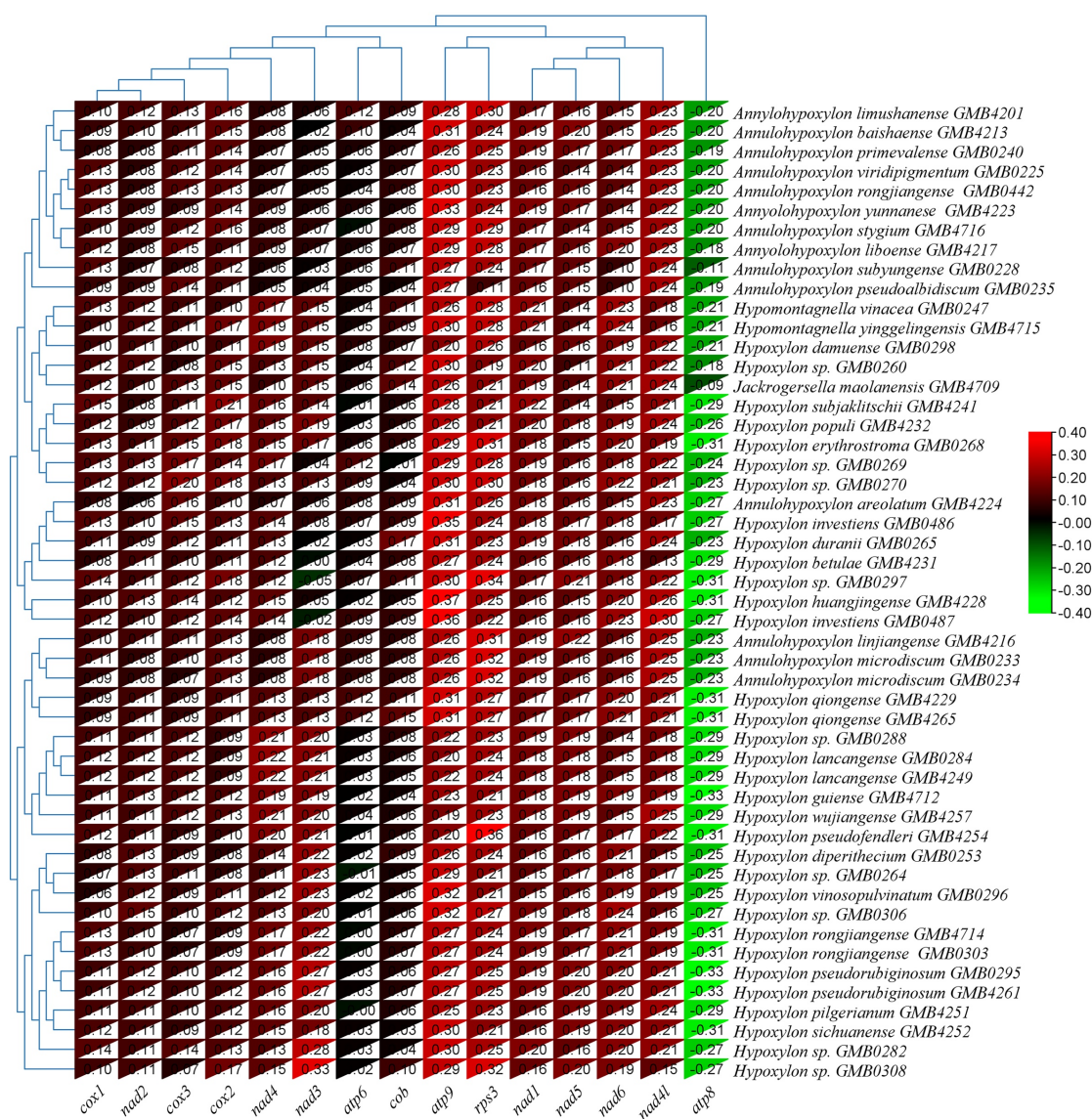


Fig. 24 Heatmap showing the GC skew across the mitogenomes of 50 Hypoxylaceae specimens. The scale bar represents the percentage of the skew values where the green (low), black (mid) and red (high).

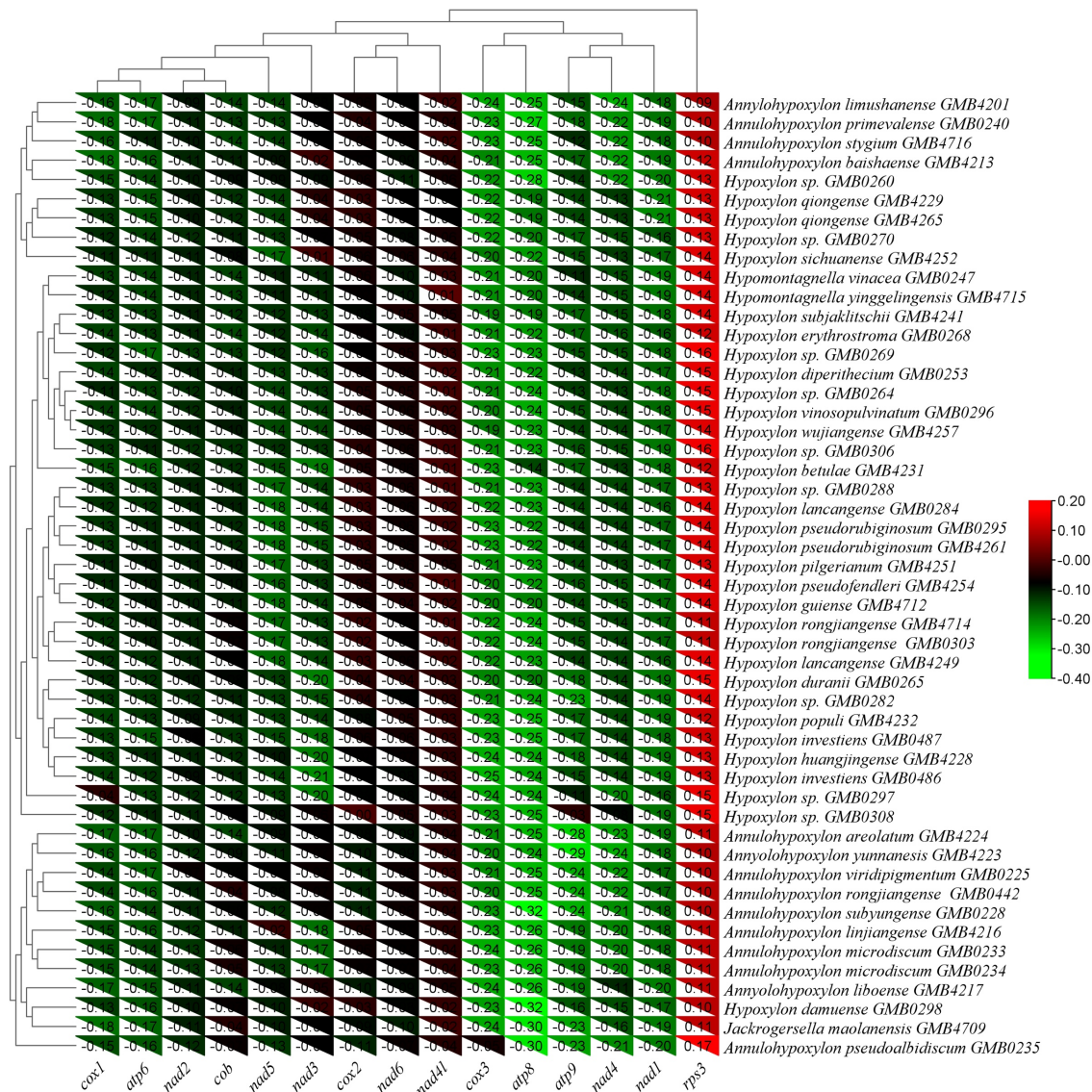


Fig. 25 Heatmap showing the AT skew across the mitogenomes of 50 Hypoxylaceae specimens. The scale bar represents the percentage of the skew values where the green (low), black (mid) and red (high).



Fig. 26 Mitochondrial gene arrangement analyses of the 50 Hypoxylaceae species. Genes with unchanged order, translocation and duplication are represented by blocks of different colors.

The structural patterns correlate with phylogenetic clades (Fig. 2). Clades M1, M2, and M3, which are predominantly composed of *Annulohypoxylon* species, contain the structurally complex mitogenomes. For example, *A. viridipigmentum* GMB0225 (Clade M1) has the highest number of homologous regions (39), suggesting an extensive history of break-and-rejoin events. In contrast, *A. baishaense* GMB4213 (Clade M2) shows reduced fragmentation (35 regions), indicating a possible shift toward structural stabilization in this lineage.

The genome size of *Jackrogersella maolanensis* GMB0115 (Fig. 2; Clade M4) is significantly larger than many species in *Hypoxylon* or *Hypomontagnella*. Structurally, *Jackrogersella* appears more similar to *Annulohypoxylon* than to its more immediate phylogenetic neighbors in the *Hypoxylon* complex. This high degree of fragmentation suggests that *Jackrogersella* represents a long-independent lineage where mitochondrial rearrangements have accumulated at a high rate, justifying its distinct classification at the genus level.

Species of *Hypomontagnella*, clustered in Clade M10 (Fig. 2), demonstrate a high degree of internal consistency. *Hypomontagnella yinggelingensis* GMB4715 (12 regions) and *H. vinacea* GMB0247 (11 regions) share nearly identical structural profiles. The slight variation in size and region count suggests a relatively recent divergence with minimal large-scale rearrangements. This stability in Clade M10 contrasts sharply with the volatility seen in the *Annulohypoxylon* clades (M1-M3), highlighting how different genera within the same family can follow distinct structural rules.

tRNA Genes in Hypoxylaceae Mitogenomes

The number of tRNA genes varied across the genera in the 50 Hypoxylaceae mitochondrial genomes. In the genus *Annulohypoxylon*, the number of tRNA genes ranged from 25 to 27. In the genus *Hypomontagnella*, 25 and 25 tRNA genes were detected in *Hy. vinacea* GMB0247 and *Hy. yinggelingensis* GMB4715 respectively. In the genus *Hypoxylon*, the number of tRNA genes ranged from 25 to 35. *Hypoxylon* sp. GMB0297 exhibited the highest number of tRNA genes (35), whereas *H. guinense* GMB4712, *Hypoxylon* sp. GMB0282, and *Hypoxylon* sp. GMB0306 each exhibited the lowest number, with 25 tRNA genes. In the genus *Jackrogersella*, *J. maolanensis* GMB0115 exhibited 25 tRNA genes. All tRNAs detected in the Hypoxylaceae mitogenomes were folded into classical cloverleaf structures (Supplementary Fig. 6). A representative example of the predicted tRNA secondary structure of *Annulohypoxylon areolatum* GMB4224 is shown in Fig. 28. The extra stem or loop regions were identified as the primary contributors to size variation among tRNA genes in Hypoxylaceae, with most variation occurring in the D-arm.

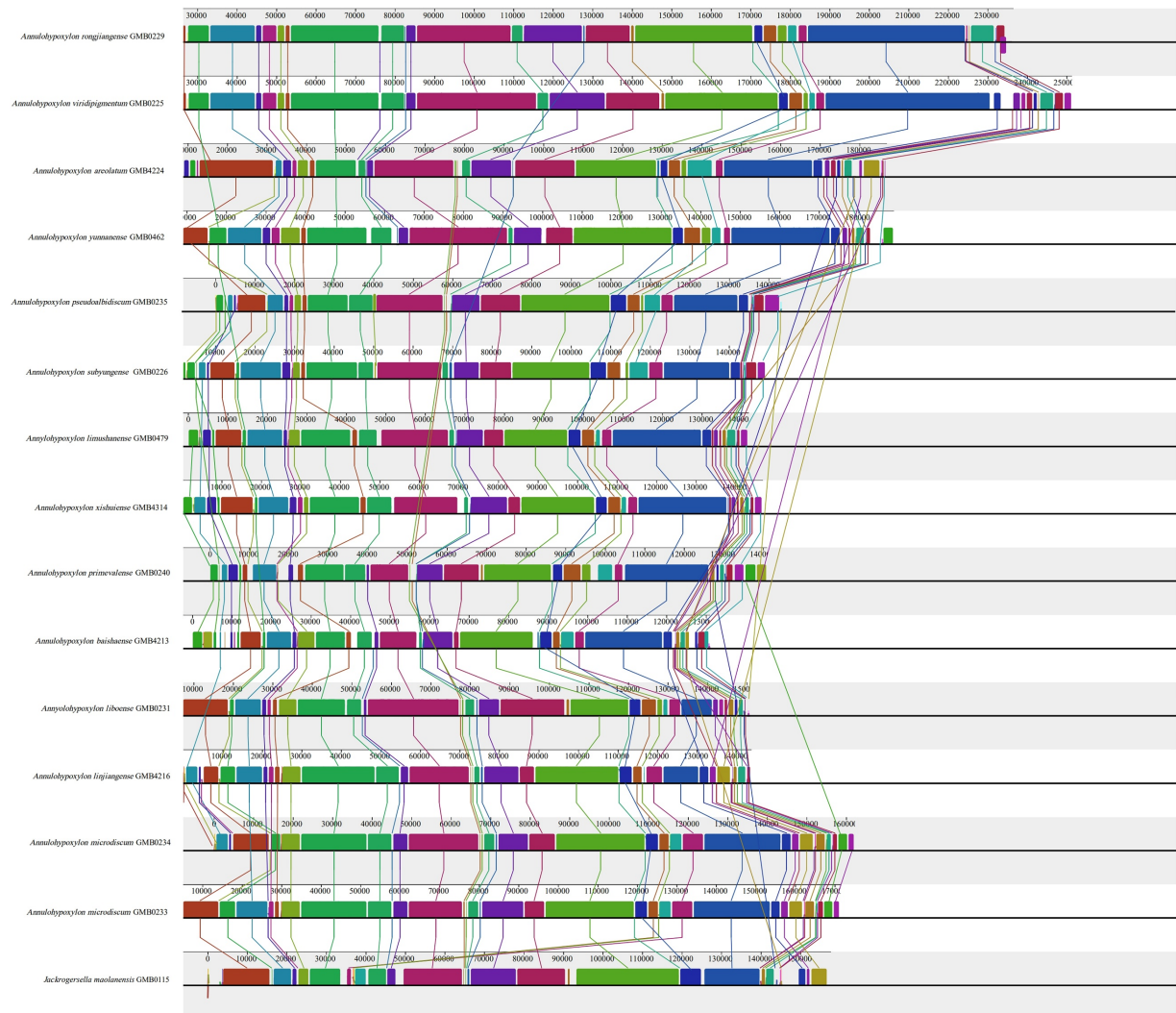


Fig. 27 Collinearity analysis map of the 50 Hypoxylaceae mitochondrial genomes. Homologous regions between different Hypoxylaceae species are represented by the same colour blocks and connected by the same colour lines.

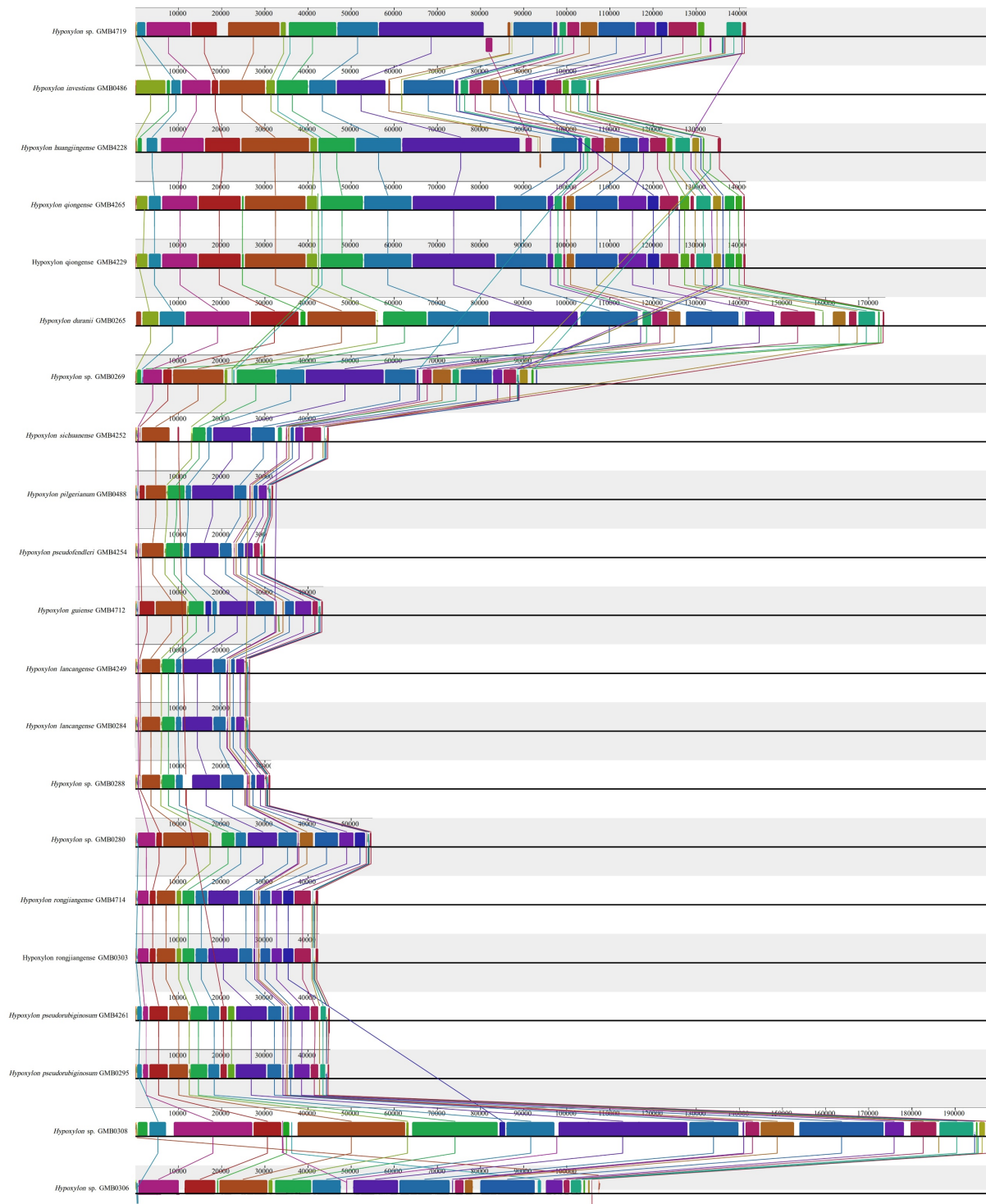


Fig. 27 (continued)

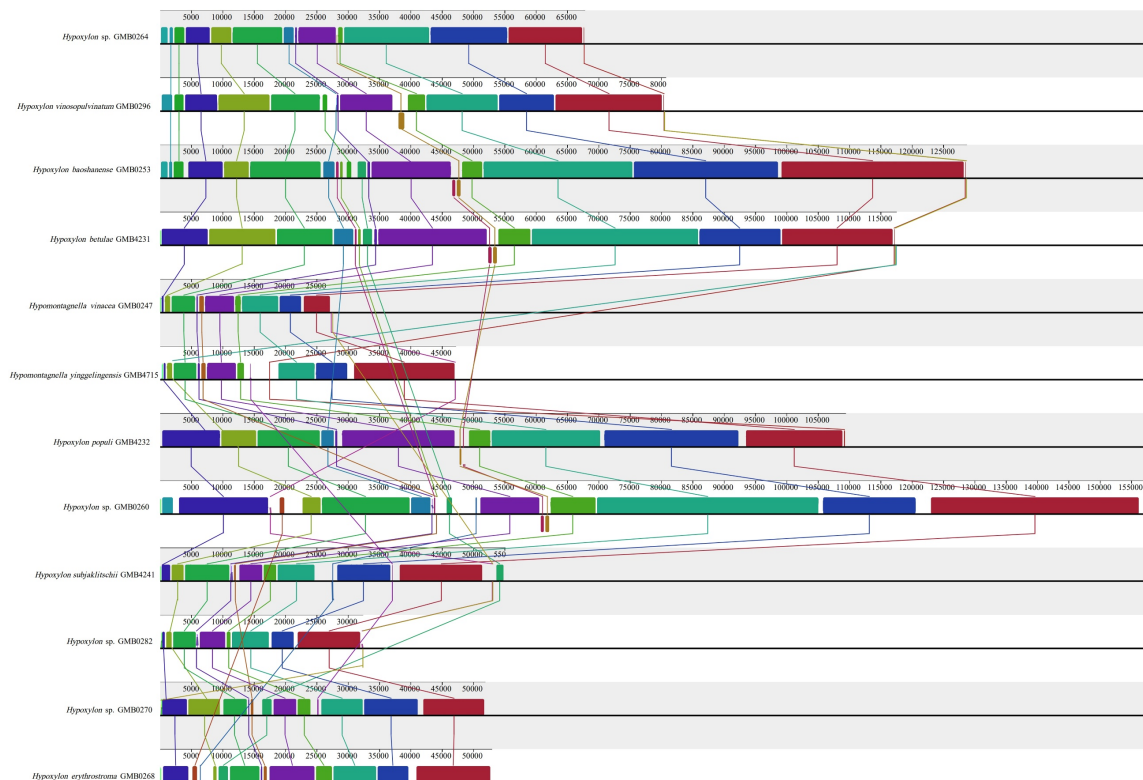


Fig. 27 (continued)

Discussion

The field of fungal taxonomy is dynamic and has been significantly impacted by technological and scientific advancements. In the seventeenth century, the invention of the microscope revolutionized the study of fungi by enabling researchers to observe previously inaccessible micromorphological features. By the twentieth century, physiological and biochemical assays such as color reactions and thin-layer chromatography (TLC) of secondary metabolites further refined species delimitations (Hsieh et al. 2005; Nilsson et al. 2008; Schoch et al. 2012; Houbraken et al. 2014).

The rise of molecular methods in the late twentieth century introduced DNA barcoding and phylogenetic reconstruction, which significantly improved taxonomic resolution. Early studies relied on single-locus datasets, typically ribosomal markers (18S rDNA, ITS1/2, 5.8S rDNA, 28S rDNA), but more recent multi-locus analyses including β -tubulin, translation elongation factor 1- α , and RNA polymerase II subunit *rpb2* have yielded more robust and stable tree topologies (Dornburg et al. 2017; Zhang et al. 2017; Hyde et al. 2017; Wendt et al. 2018; Li et al. 2023, 2024). Together, these combined morphological, biochemical, and molecular approaches continue to refine classification of Hypoxylaceae and reveal cryptic diversity within the family.

Despite these advancements, the classification of Hypoxylaceae remains with challenges. Morphologically cryptic species often show subtle or overlapping phenotypic characters, while significant molecular variations are observed in commonly used markers such as ITS, LSU, *rpb2*, and *tub2*. Stadler et al. (2020), Wibberg et al. (2021), and Cedeño-Sanchez et al. (2024a) argue that the universally

recognized ITS region, despite being the most used marker in fungal classification, has notable limitations in this group due to high intragenomic polymorphism and deep paralogy, which lead to ambiguous sequence alignments and conflicting phylogenetic interpretations. Consequently, Stadler and colleagues attempted to utilize the chemical composition of stroma as a basis for classification and published many articles (Lambert et al. 2019, Becker et al. 2020, Kuhnert et al. 2021, Cedeño-Sanchez et al. 2023). However, chemical classification has its own constraints, including high costs of chemical analysis equipment, more complex data analysis, the need for chemical experts' assistance in compound structure analysis, unavailability of standard compounds, and difficulties in international specimen transfer. These challenges make it difficult to adopt chemical profiling as a universal method for fungal classification.

This study proposes mitochondrial genome (mitogenome) data as a supplementary criterion for species delimitation in Hypoxylaceae, integrating it with traditional morphological and multi-locus molecular data. Mitogenomes offer distinct advantages, including high copy numbers, structural variability and conserved protein-coding regions that are less prone to homoplasy compared to nuclear markers. Our comprehensive analysis of 50 Hypoxylaceae mitogenomes revealed extraordinary variability in genome size (26,809–251,279 bp), intron content (0–68 introns), intergenic region length, gene arrangement, and tRNA composition, highlighting pronounced structural plasticity across the family. This structural diversity, including genus- and species-specific patterns in intron content, gene order, and tRNA composition, provides a rich source of comparative characters.

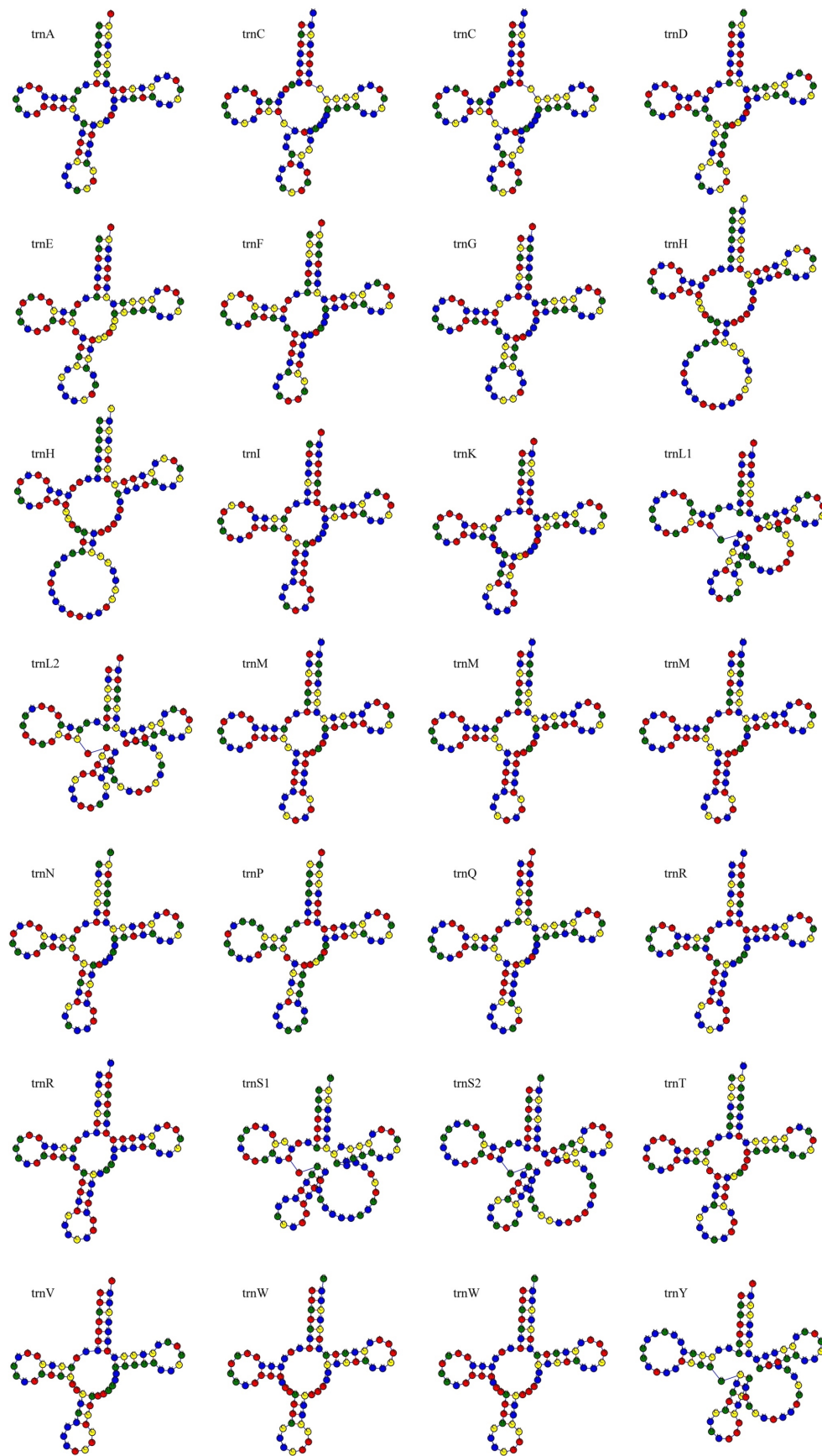


Fig. 28 An example of the secondary structures of tRNA genes of *Annulohypoxylon areolatum* GMB4224; Yellow C, Red A, Blue U, and Green G.

Several species of *Hypoxylon* have been provisionally identified using the term "cf." (confer), reflecting their ambiguous but close relationship to known species. For example, taxa closely resembling *H. ticinense* share nearly identical teleomorph, and many specimens previously assigned to *H. rubiginosum* (often reported as *H. cf. rubiginosum*) show clear genetic divergence yet lack consistent morphological or chemical differences (Pourmoghaddam et al. 2020). Our results demonstrate that mitogenomic features particularly intron profiles, genome size, and intergenic region expansion provide additional discriminatory power for such cryptic taxa.

To differentiate such cryptic or closely related taxa, mitochondrial genomes can serve as supplementary barcodes, providing additional resolution beyond nuclear markers and morphology. As shown in Supplementary Tables 10 and 11, mitogenomic data reveal significant variation in genome size, intron content, and intergenic region lengths. Genome size variation was driven primarily by non-coding regions rather than protein-coding genes, as coding sequence lengths remained relatively conserved (CV 0–15%) compared to total genome size (CV ~50%). This genomic divergence supports the utility of mitogenomes in resolving species boundaries within *Hypoxylon*. Specifically, the extreme variability in genes like *nad3* (225–2,631 bp) and the presence of unique, conserved gene rearrangements (e.g., the *cox2* translocation in *Jackrogersella maolanensis*) offer powerful synapomorphies for clade and species discrimination. To distinguish these cryptic or closely allied taxa, mitogenomic data provide a promising solution. As demonstrated in Supplementary Table 10 and 11 and Supplementary Tables 1–7, significant variation in genome size, intron content, and intergenic region length was observed across different species. These differences support the potential of mitogenomes as supplementary barcodes to enhance taxonomic resolution.

Phylogenetic trees constructed using both nuclear multi-gene datasets (ITS-LSU-*rpb2-tub2*) and mitogenomic data showed largely consistent topologies, except for the taxonomic placement of a few individual species. For example, *Hypomontagnella* forms a monophyletic clade in nuclear gene-based phylogenies (Fig. 1) but appears as a well-supported sister lineage to *Hypoxylon populi* in mitogenomic analyses (Fig. 2). This discrepancy may result from the limited availability of mitogenomic data for many taxa, underscoring the need for broader sampling to resolve conflicting evolutionary signals and refine taxonomic placements.

Comparative analysis of mitochondrial gene sequences revealed substantial intra- and inter-specific length polymorphisms, particularly in intron-rich regions, alongside conserved protein-coding domains. Genes such as *atp9*, *nad1*, *nad4L*, and *atp8* were highly conserved in length and showed low Ka/Ks ratios, indicating strong purifying selection, whereas genes *nad3*, *nad5*, *cox1*, and *cob* showed elevated variability and signatures of positive selection in certain lineages. These variations provide valuable information that can improve species delimitation, clarify evolutionary relationships, and enhance the resolution of taxonomic classifications within closely related or cryptic lineages. The case of the two *Annulohypoxylon microdiscum* strains (GMB0233 & GMB0234), which are morphologically identical yet show mitogenomic divergence, powerfully

illustrates the potential of mitogenomes to reveal hidden genetic divergence and flag potential cryptic species. Future studies should prioritize the generation of mitogenomes for underrepresented taxa, enabling the development of a unified taxonomic framework that synergizes genomic, morphological, and chemical data. Establishing a standardized pipeline for mitogenome annotation and comparative analysis will be crucial for its adoption as a routine tool in fungal taxonomy. This article presents preliminary mitogenomic data, and the in-depth comparative analysis will be published in subsequent articles.

Conclusion

This study advances the taxonomy of Hypoxylaceae by using mitochondrial genome data with traditional morphological and molecular approaches. Through comprehensive analyses of 50 specimens, we identified 16 novel species and generated the first mitogenomes for *Jackrogersella*, *Hypomontagnella*, and *Hypoxylon*. This represents the most extensive mitogenomic dataset for Hypoxylaceae to date. Our analyses revealed remarkable mitogenomic diversity in genome size, gene order, and intron content, and the 14-core protein-coding genes may serve as valuable supplementary barcodes. These mitogenomic variations, alongside collinearity and sequence divergence, provide robust support for distinguishing closely related taxa. Genes such as *nad3*, *nad5*, *cox1*, and *cob*, which show elevated variability and signatures of positive selection, emerge as promising supplementary barcode candidates. A more detailed analysis of these genes will be provided in a forthcoming publication. While nuclear multi-locus phylogenies and mitogenomic trees largely aligned, discrepancies in clade placements (e.g., *Hypomontagnella*) highlight the impact of limited mitogenome sampling and underscore the need for broader taxonomic representation across the family. The extensive structural diversity observed from compact, intron-less genomes in some *Hypoxylon* species to massive, intron-rich genomes in *Annulohypoxylon* demonstrates that mitogenome itself contains phylogenetically informative signals. To build a unified and stable classification for Hypoxylaceae, future work should prioritize expanding mitogenome sequencing alongside detailed morphological and chemical profiling across the family. Such an integrative framework will be essential for resolving cryptic species complexes, understanding mitochondrial evolution, and developing reliable mitochondrial barcode systems for fungal taxonomy.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (32170019 32460007, 31960005, and 32000009); the Funded Research Projects of the State Key Laboratory of Discovery and Utilization of Functional Components in Traditional Chinese Medicine 2025 (GMUSKL-202510); the Guizhou Medical University High-Level Talent Launch Fund Project (2023-058); the Guizhou Provincial Scientific and Technologic Innovation Base (No. [2023]003); the High-level Innovation Talents of Guizhou (No. GCC [2023]048); National Natural Science Foundation of China (12132006); the Guizhou Provincial Natural Science Foundation for High-Level Innovative Talents and Teams

(2016–5676, 2015–4021).

Author contributions

All authors contributed to the study conception and design. Conceptualization: J.-C. Kang, Q.-R. Li, Z.-B. Meng. Collection and morphological examinations: W.-Y. Zen, X. Zhou, Y.-L. Ren, Y.-H. Pi, H.-M. Hu, L.-L. Liu. Molecular sequencing, and phylogenetic analyses: X. Zhou, H.-M. Hu, K. Habib. Specimen identification: K. Habib, Q.-R. Li. Original draft preparation: K. Habib, Q.-R. Li. Review, supervision: Y.-Q. Kang, J.-C. Kang, X.-C. Shen. Editing: K. Habib, K.D. Hyde. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest statement

The author list includes members of the Editorial Board of Fungal Diversity. They were not involved in the journal's review of, or decisions related to, this manuscript. The authors declare no competing interests.

Data availability

The data sets generated during and/or analyzed during the current study are available in the MycoBank repository (included in the manuscript), and GenBank (included in Supplementary Table 9). And also, the data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplemental information

The online version contains supplemental information available at <https://doi.org/10.65390/fdiv.2026.136011>.

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