Morphological and Phylogenetic Analyses Reveal Four New Species of Gnomoniopsis (Gnomoniaceae, Diaporthales) from China

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Abstract: The fungal genus Gnomoniopsis (Gnomoniaceae, Diaporthales) has been reported all around the world and isolated from multiple plant hosts. Based on multilocus phylogenies from a combined dataset of internal transcribed spacer (ITS) region, the ribosomal RNA gene cluster, and partial regions of translation elongation factor 1 alpha (tef1) and partial beta-tubulin (tub2), in conjunction with morphological characteristics, we describe and illustrate herein four new species, including Gnomoniopsis diaoluoshanensis sp. Nov., G. lithocarpi sp. Nov., G. mengyinensis sp. Nov. and G. yunnanensis sp. Nov. Alongside this, their similarity and dissimilarity to morphologically-allied and phylogenetically-related species are annotated and discussed. For facilitating future identification, we update the key to all species currently recognized in this genus.

Keywords: Sordariomycetes; taxonomy; multigene phylogeny; new taxon

1. Introduction

Diaporthales Nannf. is an important order in the perithecial ascomycetes Sordariomycetes Erikss. & Winka, accommodating not only saprophytes but also endophytes or phytopathogens on various hosts [1–5]. Gnomoniaceae Winter, which contains 60 genera and 919 species, the second largest family in this order, occurs on growing and overwintering leaves and twigs of hardwood trees, shrubs, and herbaceous plants [6,7]. This family was first established in 1886 [8] and conserved by Hawksworth and Eriksson in 1988 [9,10]. Gnomoniaceae was circumscribed by Sogonov et al. in 2008 [11], and since then, their concept has been followed by others. At the present time, besides morphology and molecular data, host specificity has become a key characteristic for species identification and a single species in the Gnomoniaceae is often associated with a single host genus or species [6,12–17].

Gnomoniopsis Berl. was initially described as a subgenus within Gnomonia Ces. & De Not. for species with multi-septate ascospores [11]. Subsequently, multiple septa were found not to be a stable characteristic; thus, the Gnomoniopsis was synonymized with Gnomonia [17]. Currently, Gnomoniopsis is accepted as a separate genus in the Gnomoniaceae and typified by Gnomoniopsis chamaemori (Fr.) Berl. This genus is characterized by having small, black perithecia immersed in the host tissue and one-septate, oval to fusiform ascospores [4]. Species in this genus are delimited by a combination of morphological and molecular data, and are known to inhabit three plant families only, viz. Fagaceae, Onagraceae and Rosaceae [4,5,11,15,18]. A total of 36 names are documented for Gnomoniopsis in the Index Fungorum (accessed on 20 June 2022) and 26 species possess sequence data.

Fungi associated with leaf spots were collected from Castanea mollissima Bl. (Fagaceae), Castanopsis chinensis Hance (Fagaceae), and Lithocarpus foehaiensis (Hu) A. Camus (Fagaceae). We obtained their respective morphological characteristics by separation and purification,
using sequences of three molecular markers, including the internal transcribed spacer of ribosomal RNA gene (ITS rDNA), the translation elongation factor 1 alpha gene (tef1), and the beta-tubulin gene (tub2); we identified these fungi as four species of the genus Gnomoniopsis, and proposed them herein.

2. Materials and Methods

2.1. Isolation and Morphology

Samples of Castanea mollissima, Castanopsis chinensis and Lithocarpus fohaiensis showing necrotic spots were collected from Hainan, Shandong and Yunnan Provinces in China during 2020 and 2021. We obtained a single strain using tissue isolation and single spore isolation. Fragments (5 × 5 mm) were taken from the edges of leaf lesions, surface-sterilized by immersing consecutively in 75% ethanol solution for 1 min and rinsed in sterile distilled water for 30 s, and in 5% sodium hypochlorite solution for 30 s, and then rinsed three times in sterile distilled water for 30 s. The sterilized pieces were placed on sterile filter paper to absorb moisture and then placed on the PDA (PDA: 200 g potato, 20 g dextrose, 20 g agar, 1000 mL distilled water, pH 7.0) and incubated at 25 °C for 2–4 days. Subsequently, portions of agar with fungal mycelia from the periphery of the colonies were transferred onto new PDA plates and photographed on the 7th and 15th days by a digital camera (Canon Powershot G7X).

Micromorphological characters from structures produced in culture were observed using an Olympus SZX10 stereomicroscope and Olympus BX53 microscope, all fitted with an Olympus DP80 high-definition color digital camera to photo-document fungal structures. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. Structural measurements were taken using the Digimizer software (https://www.digimizer.com/, accessed on 20 June 2022), with 30 measurements taken for each character [19]. Voucher specimens were deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). Ex-holotype living cultures were deposited in the Shandong Agricultural University Culture Collection (SAUCC). Taxonomic information of the new taxa was submitted to MycoBank (http://www.mycobank.org, accessed on 20 June 2022).

2.2. DNA Extraction and Amplification

Genomic DNA was extracted from mycelia grown on PDA using a CTAB (cetyltrimethylammonium bromide) method [20,21]. Three molecular markers, including an entire internal transcribed spacer region with intervening 5.8S rRNA gene (ITS), partial translation elongation factor 1-alpha gene (tef1) and partial beta-tubulin gene (tub2), were amplified with the primer pairs and polymerase chain reaction (PCR) programs listed in Table 1. PCR products were separated using the 1% agarose gel with GelRed and UV light was used to visualize the fragments [19]. Sequencing was carried out bidirectionally by the Biosune Company Limited (Shanghai, China). Consensus sequences were obtained using MEGA v. 7.0 [22]. All sequences generated in this study were deposited in GenBank under the accession numbers in Table 2.

Table 1. Molecular markers and their PCR primers and programs used in this study.

| Loci | PCR Primers   | Sequence (5′→3′)                                      | PCR Cycles                      | References |
|------|---------------|------------------------------------------------------|---------------------------------|------------|
| ITS  | ITS5          | GGA AGT AAA AGT CGT AAC AAG G                         | (95 °C: 30 s, 55 °C: 30 s) × 35 cycles | [23]       |
|      | ITS4          | TCC TCC GCT TAT TGA TAT GC                           | (95 °C: 1 min) × 35 cycles      |            |
|      | EF1-728F      | CAT CGA GAA GGT CGA GAA GG                           | (95 °C: 30 s, 48 °C: 30 s) × 35 cycles | [24,25]   |
|      | EF-2          | GGA RGT ACC AGT SAT CAT GGT                         | (95 °C: 1 min) × 35 cycles      |            |
| tub2 | Bt-2a         | GGT AAC CAA ATC GGT CCT GCT TTC                     | (95 °C: 30 s, 53 °C: 30 s) × 35 cycles | [26]       |
|      | Bt-2b         | ACC CTC AGT GTA GTG ACC CTG GGC                     | (95 °C: 1 min) × 35 cycles      |            |
Table 2. Information of specimens used in this study.

| Species                  | Voucher         | Host                        | Country | GenBank Accession Number |
|--------------------------|-----------------|-----------------------------|---------|--------------------------|
|                          |                 |                             |         | **ITS**                  |
|                          |                 |                             |         | **tef1**                  |
|                          |                 |                             |         | **tub2**                  |
| Gnomoniopsis alderdunensis | CBS 125680 *    | Rubus parviflorus (Rosaeace) | USA     | GU320825 GU320801 GU320787 |
|                          | CBS 125681      | Rubus parviflorus (Rosaeace) | USA     | GU320827 GU320802 GU320789 |
| G. castanopsidis         | CFCC 54437 *    | Castanopsis hystrix (Fagaceae) | China   | MZ902909 MZ936385 –      |
|                          | CFCC 54438      | Castanopsis hystrix (Fagaceae) | China   | MZ902910 MZ936386 –      |
| G. chamaemori            | CBS 804.79      | Rubus chamaemorus (Rosaeace) | Finland | GU320817 GU320809 GU320777 |
| G. chinensis             | CFCC 52286 *    | Castanopsis mollissima (Fagaceae) | China | MG866032 MH545370 MH545366 |
|                          | CFCC 52288      | Castanopsis mollissima (Fagaceae) | China | MG866034 MH545372 MH545368 |
|                          | CFCC 52287      | Castanopsis mollissima (Fagaceae) | China | MG866033 MH545371 MH545367 |
| G. clavulata             | CBS 121255      | Quercus falcata (Fagaceae)   | USA     | EU254818 EU221934 EU219211 |
| G. comari                | CBS 806.79      | Oryza sativa (Rosaeace)      | UK      | EU254821 GU320810 EU219156 |
| G. datii                 | CFCC 54043 *    | Castanopsis mollissima (Fagaceae) | China | MN598671 MN605517 MN605519 |
|                          | CFCC 55517      | Castanopsis mollissima (Fagaceae) | China | MN598671 MN605517 MN605519 |
| G. diaoluoshanensis      | SAUCC DL0963 *  | Castanopsis chinensis (Fagaceae) | China | ON753744 ON759769 ON759777 |
|                          | SAUCC DL0964    | Castanopsis chinensis (Fagaceae) | China | ON753743 ON759768 ON759776 |
|                          | SAUCC DL0961    | Castanopsis chinensis (Fagaceae) | China | ON753745 ON759770 ON759778 |
| G. fagacearum            | CFCC 54316 *    | Lithocarpus glaber (Fagaceae) | China   | MZ902916 MZ936392 MZ936408 |
|                          | CFCC 54288      | Castanopsis faberi (Fagaceae) | China   | MZ902913 MZ936389 MZ936405 |
| G. fragariae = G. fructicola | CBS 208.34   | Fragaria sp. (Rosaeace)    | USA     | EU254826 EU221968 EU219149 |
|                          | CBS 121226      | Fragaria vesca (Rosaeace)   | USA     | EU254824 EU221961 EU219144 |
| G. guangdongensis        | CFCC 54443 *    | Castanopsis fargesii (Fagaceae) | China | MZ902918 MZ936394 MZ936410 |
|                          | CFCC 54331      | Castanopsis fargesii (Fagaceae) | China | MZ902919 MZ936395 MZ936411 |
| G. guttulata             | MS 0312         | Agrimonia eupatoria (Rosaeace) | Bulgaria | EU254812 – – |
| G. hainanensis           | CFCC 54376 *    | Castanopsis hainanensis (Fagaceae) | China | MZ902921 MZ936397 MZ936413 |
|                          | CFCC 55877      | Castanopsis hainanensis (Fagaceae) | China | MZ902922 MZ936398 MZ936414 |
| G. idaeicola             | CBS 125672      | Rubus sp. (Rosaeace)        | USA     | GU320823 GU320797 GU320781 |
|                          | CBS 125673      | Rubus pedatus (Rosaeace)    | USA     | GU320824 GU320798 GU320782 |
|                          | CBS 125674      | Rubus sp. (Rosaeace)        | France  | GU320820 GU320796 GU320780 |
| G. lithocarpi            | SAUCC YN0743 *  | Lithocarpus fohaiensis (Fagaceae) | China | ON753749 ON759765 ON759783 |
|                          | SAUCC YN0742    | Lithocarpus fohaiensis (Fagaceae) | China | ON753750 ON759764 ON759782 |
| G. macounii              | CBS 121468      | Spiraea sp. (Rosaeace)      | USA     | EU254762 EU221979 EU219126 |
| G. mengyinensis          | SAUCC MY0293 *  | Castanopsis mollissima (Fagaceae) | China | ON753741 ON759766 ON759774 |
|                          | SAUCC MY0296    | Castanopsis mollissima (Fagaceae) | China | ON753742 ON759767 ON759775 |
| G. occulta               | CBS 125677      | Potentilla sp. (Rosaeace)   | USA     | GU320828 GU320812 GU320785 |
|                          | CBS 125678      | Potentilla sp. (Rosaeace)   | USA     | GU320829 GU320800 GU320786 |
Table 2. Cont.

| Species          | Voucher | Host                         | Country    | GenBank Accession Number |
|------------------|---------|------------------------------|------------|--------------------------|
|                  |         |                              |            | ITS | tef1 | tub2 |
| G. paraclavulata | CBS 123202 | Agrostis sp. (Fagaceae) | USA        | GU320830 | GU320815 | GU320775 |
| G. racemula      | CBS 121469 * | Triticum aestivum (Onagraceae) | USA | EU254841 | EU221889 | EU219125 |
| G. rossmaniae    | CFCC 54307 | Castanopsis hainanensis (Fagaceae) | China | MZ902923 | MZ936399 | MZ936415 |
| G. sanguisorbae  | CBS 858.79 | Sanguisorba minor (Rosaceae) | Switzerland | GU320818 | GU320805 | GU320790 |
| G. silvicola     | CFCC 54304 | Castanopsis hystrix (Fagaceae) | China | MZ902925 | MZ936401 | MZ936417 |
| G. smithogileyi | CFCC 54418 | Quercus serrata (Fagaceae) | China | MZ902926 | MZ936402 | MZ936418 |
|                  | CBS 130190 * | Castanea sp. (Fagaceae) | Australia | JQ910642 | JQ910645 | JQ910639 |
|                  | CBS 130189 | Castanea sp. (Fagaceae) | Australia | JQ910644 | JQ910647 | JQ910641 |
| G. tormentillae  | CBS 904.79 | Potentilla sp. (Rosaceae) | Switzerland | EU254856 | GU320795 | EU219165 |
| G. xunwuensis    | CFCC 53115 | Castanopsis fissa (Fagaceae) | China | MK432667 | MK578141 | MK578067 |
|                  | CFCC 53116 | Castanopsis fissa (Fagaceae) | China | MK432668 | MK578142 | MK578068 |
| G. yunnanensis   | SAUCC YN1659 * | Castanea mollissima (Fagaceae) | China | ON753746 | ON759771 | ON759779 |
|                  | SAUCC YN1657 | Castanea mollissima (Fagaceae) | China | ON753747 | ON759772 | ON759780 |
|                  | SAUCC YN1641 | Castanea mollissima (Fagaceae) | China | ON753748 | ON759773 | ON759781 |
| Melanconis stilbostoma | CBS 109778 | Betula pendula (Betulaceae) | Australia | DQ323524 | EU221886 | EU219104 |

Notes: New species established in this study are in bold. Ex-type or ex-epitype strains are marked with “*”.

2.3. Phylogenetic Analyses

The generated sequences for each gene were subjected to BLAST searches for identifying closely related sequences in the NCBI’s GenBank nucleotide database [27]. For the ITS- tef1- tub2 analysis, subsets of sequences from the alignments of Jiang et al. [4] were used as backbones. Newly generated sequences in this study were aligned with additional related sequences downloaded from GenBank (Table 1), using MAFFT 7 online service with the auto strategy (http://mafft.cbrc.jp/alignment/server/, accessed on 20 June 2022) [28]. To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each marker and then combinedly (ITS- tef1- tub2) (Supplementary File S1).

Phylogenetic analyses were conducted for the multi-marker data based on maximum likelihood (ML) and Bayesian inference (BI) algorithms. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 [29] and incorporated into the analyses. ML and BI run on the CIPRES Science Gateway portal (https://www.phylo.org/, accessed on 20 June 2022) [30]. ML was performed in RaxML-HPC2 on XSEDE (8.2.12) [31] and 1000 rapid bootstrap replicates were run with the GTR+ Gamma model of nucleotide evolution. BI was performed in MrBayes on XSEDE (3.2.7a) [32–34]. For ML analyses, the default parameters were used and BI was carried out using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included 4 parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency of 100 generations. The burn-in fraction was set to 0.25 and posterior probabilities (PP) were determined from the remaining trees. All resulted trees were plotted using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, accessed on 20 June 2022) and the layout of the trees was carried out in Adobe Illustrator CC 2019.
3. Results
3.1. Phylogenetic Analyses

The alignment contained 50 isolates representing *Gnomoniopsis* and allied taxa, and the strain CBS 109778 of *Melanconis stilbostoma* was used as the outgroup. A total of 1751 characters were used for phylogenetic analyses, viz. 1–550 (ITS), 551–1222 (tef1), 1223–1751 (tub2). Of these characters, 979 were constant, 69 were variable and parsimony-uninformative and 703 were parsimony-informative. MrModelTest recommended that the Bayesian inference should use the Dirichlet base frequencies and the GTR+I+G evolutionary mode for all the three partitions. The topology of the Bayesian tree was consistent with that of the ML tree, and therefore is shown as a representative for recapitulating evolutionary history within the genus *Gnomoniopsis* (Figure 1). The final ML optimization likelihood was -13036.518679. The 50 strains were assigned to 28 species clades on the phylogram (Figure 1).

Based on the phylogenetic resolution and morphological analyses, the present study reports four new species of the *Gnomoniopsis* species, viz. *Gnomoniopsis diaoluoshanensis* sp. nov., *G. lithocarpi* sp. nov., *G. mengyinensis* sp. nov. and *G. yunnanensis* sp. nov.

3.2. Taxonomy
3.2.1. *Gnomoniopsis diaoluoshanensis* S. Wang, Z.X. Zhang, X.Y. Liu and X.G. Zhang, sp. nov.

*MycoBank*—No: MB844512

*Etymology*—The epithet *diaoluoshanensis* pertains to the location of the holotype, Diaoluoshan National Silva Park.

*Type*—China, Hainan Province, Diaoluoshan National Silva Park (18°38′42″–18°50′22″ N, 109°41′38″–110°4′46″ E), on diseased leaves of *Castanopsis chinensis* (Fagaceae), 21 May 2021, Z.X. Zhang, holotype HMAS 352166, ex-holotype living culture SAUCC DL0963.

*Description*—Leaf is endogenic and associated with leaf spots. Conidiomata (pycnothyrria) are buried or attached to mycelia, aggregated or solitary, erumpent, exuding creamy yellow conidia after 7 days at 25 °C in dark. Conidiophores are indistinct, often reduced. Conidiogenous cells are hyaline, smooth, multi-guttulate, cylindrical to ampulliform, attenuate towards apex, phialidic, 8.0–12.0 × 1.0–2.0 μm. Conidia are hyaline, smooth, multi-guttulate, ellipsoid to broadly ellipsoid, base truncate, 3.8–7.0 × 1.2–2.0 μm, mean = (5.2 ± 0.7) × (1.6 ± 0.2) μm, see Figure 2. Sexual morph was not observed.

*Culture characteristics*—Colonies on PDA entirely occupy a 90 mm petri dish in 14 days at 25 °C in dark, with a growth rate of 6.0–6.5 mm/day, are grey-white to creamy white with an irregular margin, spreading out in circles in a similar way to petals and the reverse is similar in color.

*Additional specimen examined*—China, Hainan Province, Diaoluoshan National Silva Park, on diseased leaves of *Castanopsis chinensis* (Fagaceae), 21 May 2021, Z.X. Zhang, paratype HMAS 352168, ex-paratype living culture SAUCC DL0961; on diseased leaves of *Castanopsis chinensis* (Fagaceae), 21 May 2021, Z.X. Zhang, paratype HMAS 352167, ex-paratype living culture SAUCC DL0964.

*Notes*—Phylogenetic analyses of a combined three genes (ITS, tef1 and tub2) showed that *Gnomoniopsis diaoluoshanensis* sp. nov. formed an independent clade and is phylogenetically closely related to *G. daii*, *G. mengyinensis* sp. nov. and *G. yunnanensis* sp. nov. (Figure 1). In detail, *G. diaoluoshanensis* is distinguished from *G. daii* by 14/496, 25/314 and 32/445 characters in ITS, tef1 and tub2 sequences, respectively. It is distinguished from *G. mengyinensis* by 17/511, 46/638 and 27/467 characters, and from *G. yunnanensis* by 10/508, 28/638 and 6/466. Morphologically, *G. diaoluoshanensis* differs from *G. daii*, *G. mengyinensis* sp. nov. and *G. yunnanensis* sp. nov. mainly in conidia (3.8–7.0 × 1.2–2.0 μm vs. 5.5–7.0 × 2.1–2.5 μm vs. 4.5–6.5 × 1.8–2.8 μm vs. 4.1–5.5 × 1.3–2.0 μm) [4,35].
Figure 1. A Bayesian inference phylogram of *Gnomoniopsis* based on combined ITS, *tef1* and *tub2* gene sequences with CBS 109778 of *Melanconis stilbostoma* as the outgroup. At the nodes, the Bayesian inference posterior probability (left, BIPP ≥ 0.90) and the maximum likelihood bootstrap value (right, MLBV ≥ 50%) are separated by a slash. Strains marked with "*" are ex-types or ex-epitypes. Strains from the present study are in red. Some branches are shortened to fit to the page, which are indicated by double slashes and the number of fold times. The scale bar at the bottom middle indicates 0.03 substitutions per site.
Strains from the present study are in red. Some branches are shortened to fit to the page, which are indicated by double slashes and the number of fold times. The scale bar at the bottom middle indicates 0.03 substitutions per site.

Figure 2. *Gnomoniopsis diaoluoshanensis* (holotype HMAS 352166. (a) Leaves of host plant; (b,c) inverse and reverse sides of colony after 15 days on PDA; (d) colony overview; (e,f) conidiogenous cells and conidia; (g,h) conidia. Scale bars: (e–h) 10 μm.

3.2.2. *Gnomoniopsis lithocarpi* S. Wang, Z.X. Zhang, X.Y. Liu and X.G. Zhang, sp. nov.

Mycobank—No: MB844513

Etymology—The epithet *lithocarpi* pertains to the generic name of the host plant *Lithocarpus fohaiensis*.

Type—China, Yunnan Province, Xishuangbanna Tropical Botanical Garden (21°41′ N, 101°25′ E), Chinese Academy of Sciences, on diseased leaves of *Lithocarpus fohaiensis* (*Fagaceae*), 11 Sep 2020, Z. X. Zhang, holotype HMAS 352165, ex-holotype living culture SAUCC200743.
Description—Leaf is endogenic and associated with leaf spots. Conidiomata (pycnothyria) are buried or attached to mycelia, aggregated or solitary, erumpent, exuding pale yellow conidia after 14 days at 25 °C in dark. Conidiophores are indistinct, often reduced. Conidiogenous cells are hyaline, smooth, multi-guttulate, cylindrical to ampulliform, attenuate towards apex, phialidic, 6.0–13.0 × 1.5–2.5 μm. Conidia are hyaline, smooth, multi-guttulate, ellipsoid to ovoid, base circular, 4.0–5.8 × 1.7–2.4 μm, mean = (4.6 ± 0.5) × (2.1 ± 0.2) μm, see Figure 3. Sexual morph was not observed.

Figure 3. Gnomoniopsis lithocarpi (holotype HMAS 352165). (a) Leaves of host plant; (b,c) inverse and reverse sides of colony after 15 days on PDA; (d) colony overview; (e–g) conidiogenous cells and conidia; (h,i) conidia. Scale bars: (e–i) 10 μm.
Culture characteristics—Colonies on PDA at 25 °C for 14 days in dark reach 75–80 mm in diameter, are circular, with moderate aerial mycelia on the surface, light brown and sparse in the center, white and dense at the edge and the reverse is similar in color.

Additional specimen examined—China, Yunnan Province, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences on diseased leaves of *Lithocarpus fohaiensis* (*Fagaceae*), 11 Sep 2020, Z.X. Zhang, paratype HMAS 352164, ex-paratype living culture SAUCC YN0742.

Notes—Phylogenetic analyses of three combined genes (ITS, *tef1* and *tub2*) showed that *Gnomoniopsis lithocarpi* formed an independent clade closely related to *G. castanopsidis* and *G. silvicola*. The *G. lithocarpi* sp. nov. is distinguished from *G. castanopsidis* by 35/513, 41/325 and 48/478 characters in ITS, *tef1* and *tub2* sequences, respectively, and from *G. silvicola* by 38/517, 42/325 and 58/470 characters. Morphologically, *G. lithocarpi* differs from *G. castanopsidis* and *G. silvicola* in conidia (4.0–5.8 × 1.7–2.4 μm vs. 4.5–5.3 × 2.2–2.7 μm vs. 4.6–5.1 × 2.1–2.5 μm), and in colony texture (light brown to white on PDA and dense at the edge vs. dirty-white to fawn on PDA and undulate margin vs. dirty-white on PDA and undulate margin) [4,35].

3.2.3. *Gnomoniopsis mengyinensis* S. Wang, Z.X. Zhang, X.Y. Liu and X.G. Zhang, sp. nov.

**MycoBank No.:** MB844514

**Etymology**—The epithet *mengyinensis* pertains to the location where the holotype was collected, Mengyin County.

**Type**—China, Shandong Province, Mengyin County (35°71' N, 117°94' E), on diseased leaves of *Castanea mollissima* (*Fagaceae*), 25 July 2020, Z.X. Zhang, holotype HMAS 352160, ex-holotype living culture SAUCC MY0293.

**Description**—Leaf is endogenic and associated with leaf spots. Conidiomata (pycnothria) are aggregated or solitary, erumpent, globose to pulvinate, light brown, exuding creamy white or hyaline conidial after 10 days at 25 °C in dark. Conidiophores are indistinct, often reduced. Conidiogenous cells are hyaline, cylindrical, attenuate towards apex, phialidic, 8.0–11.5 × 1.3–2.2 μm. Conidia are hyaline, smooth, multi-guttulate, cylindrical, oval to fusoid, straight or slightly curved, truncate at the base, 4.5–6.5 × 1.8–2.8 μm, mean = (5.4 ± 0.4) × (2.2 ± 0.2) μm, see Figure 4. Sexual morph is unknown.

Culture characteristics—Cultures incubated on PDA at 25 °C in dark attain 82.0–86.0 mm in diameter after 14 days, with a growth rate of 5.8–6.2 mm diam/day and the colonies are flat, spreading with moderate aerial mycelia and lobate to undulate margins, grey-white to creamy, spreading out in a similar way to petals and the reverse is similar in color.

Additional specimen examined—China, Shandong Province, Mengyin County, on diseased leaves of *Castanea mollissima* (*Fagaceae*), 25 July 2020, Z.X. Zhang, paratype HMAS 352159, ex-paratype living culture SAUCC MY0296.

Notes—In the phylogenetic tree (Figure 1), *Gnomoniopsis mengyinensis* sp. nov. is closely related to *G. daii* (BIPP = 0.97, MLBS = 95%). This new species is distinguished from *G. daii* by a total of 65 characters in the concatenated sequence alignment (5/509 in the ITS, 29/313 in the *tef1* and 22/442 in the *tub2*). Morphologically, *Gnomoniopsis mengyinensis* differs from *G. daii* in conidia (4.5–6.5 × 1.8–2.8 μm vs. 5.1–6.3 × 2.8–3.2 μm), conidiogenous cells (4.5–6.5 × 1.8–2.8 μm vs. 5.6–6.1 × 2.8–3.2 μm), as well as conidiomatum color (light brown vs. dark brown) [4,35].
oval to fusoid, straight or slightly curved, truncate at the base, 4.5–6.5 × 1.8–2.8 μm, mean=(5.4 ± 0.4) × (2.2 ± 0.2) μm, see Figure 4. Sexual morph is unknown.

Figure 4. Gnomoniopsis mengyinensis (holotype HMAS 352160). (a) Leaves of host plant; (b,c) inverse and reverse sides of colony after 14 days on PDA; (d) colony overview; (e-h) conidiogenous cells and conidia; (i) conidia. Scale bars: (e–i) 10 μm.

3.2.4. Gnomoniopsis yunnanensis S. Wang, Z.X. Zhang, X.Y. Liu and X.G. Zhang, sp. nov.

Mycobank—No: MB844515

Etymology—The epithet yunnanensis pertains to the location where the holotype was collected, Yunnan Province.
Type—China, Yunnan Province, Xishuangbanna Tropical Botanical Garden (21°41’N, 101°25’E), Chinese Academy of Sciences, on diseased leaves of Castanea mollissima (Fagaceae), 11 Sep 2020, Z. X. Zhang, holotype HMAS 352161, ex-holotype living culture SAUCC YN1659.

Description—Leaf is endogenic and associated with leaf spots. Conidiomata (pycnothryia) are aggregated or solitary, erumpent, globose to pulvinate, light yellow, exuding creamy white or hyaline conidia after 14 days at 25 °C in dark. Conidiophores are indistinct, often reduced. Conidiogenous cells are hyaline, cylindrical, attenuate towards apex, phialidic, 9.0–18.0 × 0.5–1.57 μm. Conidia are hyaline, smooth, multi-guttulate, cylindrical, oblong to ellipsoid, straight or slightly curved, truncate at the base, 4.1–5.5 × 1.3–2.0 μm, mean = (4.9 ± 0.4) × (1.6 ± 0.2) μm, see Figure 5. Sexual morph is unknown.

Figure 5. Gnomoniopsis yunnanensis (holotype HMAS 352161). (a) Leaves of host plant; (b,c) inverse and reverse sides of colony after 15 days on PDA; (d) colony overview; (e–g) conidiogenous cells and conidia; (h,i) conidia. Scale bars: (e–i) 10 μm.
Culture characteristics—Cultures incubated on PDA at 25 °C for 14 days in dark attain 69.0–72.0 mm in diameter, with a growth rate of 4.9–5.2 mm diam/day, with moderate aerial mycelia and a lobate to undulate margin, grey-white to creamy, spreading out in a similar way to petals and the reverse is similar in color.

Additional specimen examined—China, Yunnan Province, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Castanea mollissima* (Fagaceae), 11 Sep 2020, Z.X. Zhang, paratype HMAS 352162, ex-paratype living culture SAUCC YN1657; on diseased leaves of *Castanea mollissima* (Fagaceae), 11 Sep 2020, Z.X. Zhang, paratype HMAS 352163, ex-paratype living culture SAUCC YN1641.

Notes—Strains SAUCC YN1659, SAUCC YN1657 and SAUCC YN1641 are identified to the same species *Gnomoniopsis yunnanensis* sp. nov. on the basis of similar morphology and molecular monophyly. For details, one can refer to the notes for *G. diaoluoshanensis*.

### 3.3. Key to the Species of *Gnomoniopsis*

Together with the 4 new species proposed in this study, we have currently accepted a worldwide total of 30 species in the genus *Gnomoniopsis*. In order to facilitate identification in the future, a key to the species of *Gnomoniopsis* is provided herein. Characteristics adopted in the key include perithecia, septa, asci, ascospores, conidiogenous cells, conidia, and chlamydoconidia.

1. Sexual morph known---------------------------------------------------------------2
2. Sexual morph unknown-------------------------------------------------------------16
3. Asci cylindrical---------------------------------------------------------------2
4. Asci fusiform---------------------------------------------------------------1
5. Ascosporangium size 10.0–12.0 × 2.0–3.0 µm----------------------------------*G. chamaemori*
6. Ascosporangium size 4.0–12.0 × 1.0–3.0 µm----------------------------------*G. smithogilvyi*
7. Perithecia without ascus-----------------------------------------------5
8. Perithecia with ascus-------------------------------------------------------------6
9. Perithecia immersed-------------------------------------------------------------7
10. Perithecia with stroma-----------------------------------------------------------8
11. Perithecia size 110.0–150.0 × 120.0–140.0 µm---------------------------------7
12. Perithecia groups---------------------------------------------------------------8
13. Ascospores size 4.0–12.0 × 1.0–3.0 µm-----------------------------------*G. racemula*
14. Ascospores size 10.0–12.0 × 1.5–3.0 µm-----------------------------------*G. tormentillae*
15. Ascospores size 7.0–8.0 × 1.8–2.2 µm----------------------------------*G. agrimoniae*
16. Perithecia solitary------------------------------------------------------------10
17. Perithecia immersed in leaves------------------------------------------1
18. Perithecia immersed in the stem------------------------------------------12
19. Perithecia size 280.0–375.0 × 327.0–490.0 µm-------------------------*G. alderdunensis*
20. Perithecia size 112–330.0 × 125–500.0 µm-----------------------------------*G. comari*
21. Perithecia immersed in leaves------------------------------------------13
22. Perithecia immersed in stem------------------------------------------15
23. Asci size 30.0–48.5 × 5.0–10.0---------------------------------*G. idaeicola*
24. Asci size 30.0–38.0 × 4.0–8.5---------------------------------*G. macounii*
25. Perithecia aggregated 2–4---------------------------------------------1
26. Perithecia solitary------------------------------------------------------------17
27. Conidiogenous cells guttulate---*G. lithocarpi* sp. nov.
28. Conidiogenous cells no guttulate-----------------------------------------24
29. Conidia base circular---------------------------------------------------------------20
30. Conidia base truncate----------------------------------------------------------18
31. Conidia ellipsoid or cylindrical-----------------------------------------19
32. Conidia oval or fusoid--------------------------------------------------------12
33. Conidiogenous cells size 8.0–12.0 × 1.0–2.0 µm-------------------------------*G. diaoluoshanensis* sp. nov.
was isolated from rotting chestnut kernels as an endophyte from asymptomatic flowers, Onagraceae (https://nt.ars-grin.gov/fungaldatabases/index.cfm, accessed on 20 June 2022). Among (Figures 2–5), and all these three hosts belong to the family Fagaceae. The phylogenetic data to gain insights into evolutionary relationships [37–39].

Jiang et al. [4] introduced six species in Gnomoniopsis, based on three gene loci encoding the internal transcribed spacer of ribosomal RNA (ITS), translation elongation factor 1 alpha (tef1), and beta-tubulin (tub2). They described and illustrated the Gnomoniopsis species from seven regions (Fujian, Guangdong, Hainan, Henan, Jiangxi and Shaanxi) in China. In sum, 13 species of Gnomoniopsis were recorded in more than 10 regions of China, and they are Gnomoniopsis castanopsidis, G. chinensis, G. daii, G. diaoluoshanensis, G. fagacearum, G. guangdongensis, G. hainanensis, G. lithocarpi, G. mengyinensis, G. rossmaniae, G. silvicola, G. xunwuensis and G. yunnanensis.

The Gnomoniopsis species were reported with 200 records in Fungal Databases (https://nt.ars-grin.gov/fungaldatabases/index.cfm, accessed on 20 June 2022). Among these, G. daii and G. chinensis were determined to be phytopathogenic, causing fruit rot and leaf spot diseases and branch canker of Chinese chestnut, respectively [40,41]. Gnomoniopsis smithogilvyi were illustrated and described in 12 countries (Australia, New Zealand, Chile, France, India, Ireland, Italy, Portugal, Spain, Switzerland, United Kingdom and USA) with 30 records in Fungal Databases, causing sweet chestnut branch canker and fruit rot in Australia, Europe and USA [42–44]. Apart from this, Linalcheddu et. al. revealed some fungi associated with branch diseases of hazelnut in Sardinia (Italy), including Dothiorella iberica, Do. omnivora, Do. symphoricarposiola and G. smithogilvyi. Gnomoniopsis smithogilvyi was isolated from rotting chestnut kernels as an endophyte from asymptomatic flowers, leaves and stems of the genus Chestnut [45]. The descriptions, pathogenicity testing and

4. Discussion

In the present study, four new species (Gnomoniopsis diaoluoshanensis, G. lithocarpi, G. mengyinensis, and G. yunnanensis) from three hosts (Castanea mollissima, Castanopsis chinensis, and Lithocarpus fohaiensis) in three provinces of China were described and illustrated (Figures 2–5), and all these three hosts belong to the family Fagaceae. Currently, Gnomoniopsis species were found from hosts that belong to three plant families (Fagaceae, Onagraceae and Rosaceae). Sixteen Gnomoniopsis species (including the four new species herein) were described from fagaceous hosts. Only one species (G. racemula) was described from the Onagraceae family [11,15,36]. The remaining 11 species were from the family Rosaceae. The Fagaceae, Onagraceae and Rosaceae plants are widely distributed in China, suggesting abundant potentially new Gnomoniopsis species.

Driven by recent developments in DNA sequence analyses, taxonomists have combined phylogenetic data to gain insights into evolutionary relationships [37–39]. Jiang et al. [4] introduced six species in Gnomoniopsis, based on three gene loci encoding the internal transcribed spacer of ribosomal RNA (ITS), translation elongation factor 1 alpha (tef1), and beta-tubulin (tub2). They described and illustrated the Gnomoniopsis species from seven regions (Fujian, Guangdong, Hainan, Henan, Jiangxi and Shaanxi) in China. In sum, 13 species of Gnomoniopsis were recorded in more than 10 regions of China, and they are Gnomoniopsis castanopsidis, G. chinensis, G. daii, G. diaoluoshanensis, G. fagacearum, G. guangdongensis, G. hainanensis, G. lithocarpi, G. mengyinensis, G. rossmaniae, G. silvicola, G. xunwuensis and G. yunnanensis.

The Gnomoniopsis species were reported with 200 records in Fungal Databases (https://nt.ars-grin.gov/fungaldatabases/index.cfm, accessed on 20 June 2022). Among these, G. daii and G. chinensis were determined to be phytopathogenic, causing fruit rot and leaf spot diseases and branch canker of Chinese chestnut, respectively [40,41]. Gnomoniopsis smithogilvyi were illustrated and described in 12 countries (Australia, New Zealand, Chile, France, India, Ireland, Italy, Portugal, Spain, Switzerland, United Kingdom and USA) with 30 records in Fungal Databases, causing sweet chestnut branch canker and fruit rot in Australia, Europe and USA [42–44]. Apart from this, Linalcheddu et. al. revealed some fungi associated with branch diseases of hazelnut in Sardinia (Italy), including Dothiorella iberica, Do. omnivora, Do. symphoricarposiola and G. smithogilvyi. Gnomoniopsis smithogilvyi was isolated from rotting chestnut kernels as an endophyte from asymptomatic flowers, leaves and stems of the genus Chestnut [45]. The descriptions, pathogenicity testing and
molecular data for species of Gnomoniopsis by taxonomists represent an important resource for plant pathologists and plant quarantine officials.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof8080770/s1, Supplementary File S1: The combined ITS, tef1 and tub2 sequences.

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

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