Morphology and Phylogeny of Gnomoniopsis (Gnomoniaceae, Diaporthales) from Fagaceae Leaves in China

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Abstract: Gnomoniopsis (Gnomoniaceae, Diaporthales) is a well-classified genus inhabiting leaves, branches and fruits of the hosts in three plant families, namely Fagaceae, Onagraceae and Rosaceae. In the present study, eighteen Gnomoniopsis isolates were obtained from diseased leaves of Fagaceae hosts collected from Fujian, Guangdong, Hainan, Henan, Jiangxi and Shaanxi provinces in China. Morphology of the cultures and phylogeny based on the 5.8S nuclear ribosomal DNA gene with the two flanking internally transcribed spacer (ITS) regions, the translation elongation factor 1-alpha (tef1) and the beta-tubulin (tub2) genes were employed to identify these isolates. As a result, seven species were revealed, viz. Gnomoniopsis castanopsidis, G. fagacearum, G. guangdongensis, G. hainanensis, G. rossmaniae and G. silvicola spp. nov, as well as a known species G. daiti. In addition, G. daiti was firstly reported on the host Quercus alieta.

Keywords: Ascomycota; leaf disease; new species; oak; taxonomy

1. Introduction

Diaporthales is a species-rich fungal order usually associated with forest trees as endophytes, pathogens and saprotrophs [1–10]. Amongst the numerous tree pathogens, the most notorious one is Cryphonectria parasitica (Cryphonectriaceae) causing chestnut blight (Castanea spp.) blight worldwide [11–13]. An example for endophytic lifestyle is Diaporthe biconispora (Diaporthaceae) and an additional six Diaporthe species that are endophytic in healthy Citrus trees in China [14]. As an example of a saprophyte, Apiosporopsis carpinia (Apiosporopsidaceae) occurs on over-wintered leaves of Carpinus betulus [15].

Gnomoniaceae is a large family of the Diaporthales, with currently 38 accepted genera, including Gnomoniopsis [16–19]. Gnomoniopsis, based on the type species G. chamaemori, is a well-studied genus in regard to morphology, phylogeny and host associations. This genus is characterized by having small, black perithecia immersed in the host tissue and one-septate, oval to fusiform ascospores, and is well-distinguished by phylogenies based on the 5.8S nuclear ribosomal DNA gene with the two flanking internally transcribed spacer (ITS) regions, the translation elongation factor 1-alpha (tef1) and the beta-tubulin (tub2) genes [20,21]. Species of Gnomoniopsis are currently known to inhabit only members of three plant families as hosts, viz. Fagaceae, Onagraceae and Rosaceae [20–24].

Until now, thirty species epithets of Gnomoniopsis have been recorded in Index Fungorum, six of them were reported from fagaceous trees [22]. Two species, Gnomoniopsis clavulata and G. paraclavulata, were firstly discovered on overwintered leaves of Quercus trees in the USA [20,21]. Subsequently, Gnomoniopsis smithigolayi with its synonym G. castanea were proposed from rotten fruits of Castanea in Australia and Europe by two
independent studies [25,26]. However, these two names were proven to be a single species based on phylogeny and morphological characters [27]. Hence, G. castaneae becomes a synonym of G. smithogilvyi based on priority. In China, G. daii was described from rotten fruits and diseased leaves of Castanea mollissima [23,28]. Meanwhile, a different species named G. chinensis was reported to cause Chinese chestnut branch canker [29]. Later, Yang et al. described G. xunwuensis from leaf spots of Castanopsis fissa in China [24]. Since three Fagaceae-inhabiting species from China are now only known in the asexual morph, it is hard to separate them based on only morphological characters [23,24,29]. Hence, it is necessary to conduct phylogenetic analyses in order to recognize and identify the species [29].

Fagaceae is a common plant family widely distributed in the northern hemisphere, with seven genera namely Castanea, Castanopsis, Cyclobalanopsis, Fagus, Lithocarpus, Quercus and Trigonobalanus [30]. Previously, Gnomoniopsis has been reported from Castanea, Castanopsis and Quercus species [22]. The aims of present study are to investigate fagaceous hosts to collect Gnomoniopsis samples in China, and to identify them to species level based on combined morphology and phylogeny of ITS, tef1 and tub2 loci.

2. Materials and Methods
2.1. Field Sampling and Isolation

In the present study, we investigated leaf diseases of fagaceous trees in Fujian, Guangdong, Hainan, Henan, Jiangxi and Shaanxi provinces of China during 2018 and 2020. The diseased leaf samples were packed in paper bags and transferred to the laboratory for isolation. The infected leaves were firstly surface-sterilized for 1 min in 75% ethanol, 3 min in 1.25% sodium hypochlorite, and 1 min in 75% ethanol, then rinsed for 2 min in distilled water and blotted on dry sterile filter paper. Then samples were cut into 0.5 × 0.5 cm pieces using a double-edge blade, and transferred onto the surface of potato dextrose agar (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per L) and malt extract agar (MEA; 30 g malt extract, 5 g mycological peptone, 15 g agar per L), and incubated at 25°C to obtain the pure culture. The cultures were deposited in China Forestry Culture Collection Center (CFCC), and the specimens in the herbarium of the Chinese Academy of Forestry (CAF).

2.2. DNA Extraction, Sequencing and Phylogenetic Analyses

Genomic DNA was extracted from mycelia grown on cellophane-covered PDA using a cetyltrimethylammonium bromide (CTAB) method [31]. DNA was checked by electrophoresis in 1% agarose gel, and the quality and quantity were measured using a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). Three partial loci, ITS region, tef1 and tub2 genes were amplified by the following primer pairs: ITS1 and ITS4 for ITS [32], EF1-688F and EF2 for tef1 [33], and T1/Bt2a and Bt2b for tub2 [34,35]. The polymerase chain reaction (PCR) conditions were as follows: an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 50 s at 48°C (ITS) or 54°C (tub2) or 55°C (tef1), and 1 min at 72°C, and a final elongation step of 10 min at 72°C. PCR products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyser with a BigDye Terminator Kit v3.1 (Invitrogen, Waltham, MA, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

The sequences obtained in the present study were assembled using SeqMan v.7.1.0, and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI), based on recent publications on the genus Gnomoniopsis [20–24,29]. Sequences of an accession of Apiognomonia errabunda (AR 2813) were added to represent the outgroup. The sequences were aligned using MAFFT v.6 and corrected manually using MEGA 7.0.21 [36].

The phylogenetic analyses of the ITS region and of a combined matrix of the three loci (ITS-tef1-tub2) were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML was implemented on the CIPRES Science Gateway portal (https://www.phylo.org) using RAxML-HPC BlackBox 8.2.10 [37,38], employing a GTRGAMMA
substitution model with 1000 bootstrap replicates. Bayesian inference was performed using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.0 [39]. Two MCMC chains, starting from random trees for 1,000,000 generations and trees, were sampled every 100th generation, resulting in a total of 10,000 trees. The first 25% of trees were discarded as burn-in of each analysis. Branches with significant Bayesian Posterior Probabilities (BPP > 0.9) were estimated in the remaining 7500 trees. Phylogenetic trees were viewed with FigTree v.1.3.1 and processed by Adobe Illustrator CS5. The nucleotide sequence data of the new taxa were deposited in GenBank, and the GenBank accession numbers of all accessions included in the phylogenetic analyses are listed in Table 1.

Table 1. Strains and GenBank accession numbers used in this study.

| Species                  | Country    | Host               | Host Family | Strain | GenBank Accession Number |
|--------------------------|------------|--------------------|-------------|--------|--------------------------|
| Apiognomonia errabunda   | Switzerland| Fagus sylvatica    | Fagaceae    | AR 2813| DQ313525 DQ313565 DQ862014 |
| Gnomoniopsis alderdunensis | USA        | Rubus pedatus      | Rosaceae    | CBS 125679 GU320826 GU320813 GU320788 |
| Gnomoniopsis alderdunensis | USA        | Rubus parviflorus  | Rosaceae    | CBS 125680 GU320825 GU320801 GU320787 |
| Gnomoniopsis alderdunensis | USA        | Rubus parviflorus  | Rosaceae    | CBS 125681 GU320827 GU320802 GU320789 |
| Gnomoniopsis chamaeori   | Finland    | Rubus chamaemorus  | Rosaceae    | CBS 804.79 GU320817 GU320809 GU320777 |
| Gnomoniopsis chinensis   | China      | Castanea mollissima | Fagaceae  | CFCC 52286 * MG866032 MH545370 MH545366 |
| Gnomoniopsis chinensis   | China      | Castanea mollissima | Fagaceae  | CFCC 52287 MG866033 MH545371 MH545367 |
| Gnomoniopsis chinensis   | China      | Castanea mollissima | Fagaceae  | CFCC 52288 MG866034 MH545372 MH545368 |
| Gnomoniopsis chinensis   | China      | Castanea mollissima | Fagaceae  | CFCC 52289 MG866035 MH545373 MH545369 |
| Gnomoniopsis clavulata   | USA        | Quercus falcata    | Fagaceae    | CBS 121255 EU254818 GU320807 EU219211 |
| Gnomoniopsis castanopsidis | China     | Castanopsis hystrix | Fagaceae  | CFCC 54437 * MZ902909 MZ936385 NA |
| Gnomoniopsis castanopsidis | China     | Castanopsis hystrix | Fagaceae  | CFCC 55878 MZ902910 MZ936386 NA |
| Gnomoniopsis comari      | Finland    | Comarum palustre   | Rosaceae    | CBS 806.79 EU254821 GU320810 EU219156 |
| Gnomoniopsis comari      | Finland    | Comarum palustre   | Rosaceae    | CBS 807.79 EU254822 GU320814 GU320779 |
| Gnomoniopsis comari      | Switzerland| Comarum palustre   | Rosaceae    | CBS 809.79 EU254823 GU320794 GU320778 |
| Gnomoniopsis dati        | China      | Castanea mollissima | Fagaceae  | CFCC 54043 * MN598671 MN605517 MN605519 |
| Gnomoniopsis dati        | China      | Castanea mollissima | Fagaceae  | CMF002B MN598672 MN605518 MN605520 |
| Gnomoniopsis dati        | China      | Quercus aliena     | Fagaceae    | CFCC 55517 MZ902911 MZ936387 MZ936403 |
| Gnomoniopsis dati        | China      | Quercus aliena     | Fagaceae    | CFCC 55294B MZ902912 MZ936388 MZ936404 |
| Gnomoniopsis fagacearum  | China      | Castanopsis faberi | Fagaceae    | CFCC 54288 MZ902913 MZ936389 MZ936405 |
| Gnomoniopsis fagacearum  | China      | Quercus variabilis | Fagaceae    | CFCC 54439 MZ902914 MZ936390 MZ936406 |
| Gnomoniopsis fagacearum  | China      | Castanopsis eyrei  | Fagaceae    | CFCC 54414 MZ902915 MZ936391 MZ936407 |
| Gnomoniopsis fagacearum  | China      | Lithocarpus glaber | Fagaceae    | CFCC 54316 * MZ902916 MZ936392 MZ936408 |
| Gnomoniopsis fagacearum  | China      | Castanopsis chunti | Fagaceae    | CFCC 54412 MZ902917 MZ936393 MZ936409 |
| Species                  | Country | Host                  | Host Family | Strain       | GenBank Accession Number |
|--------------------------|---------|-----------------------|-------------|--------------|-------------------------|
| *Gnomoniopsis fragariae* = *G. fructicola* | USA     | *Fragaria vesca*     | Rosaceae    | CBS 121226   | EU254824 GU320792 EU219144 |
|                          | France  | *Fragaria sp.*       | Rosaceae    | CBS 208.34   | EU254826 GU320808 EU219149 |
|                          | USA     | *Fragaria sp.*       | Rosaceae    | CBS 125671   | GU320816 GU320793 GU320776 |
| *Gnomoniopsis guangdongensis* | China   | *Castanopsis fargesii* | Fagaceae    | CFCC 54443   | MZ902918 MZ936394 MZ936410 |
|                          | China   | *Castanopsis fargesii* | Fagaceae    | CFCC 54331   | MZ902919 MZ936395 MZ936411 |
| *Gnomoniopsis guangdongensis* | China   | *Castanopsis fargesii* | Agrimonia   | CFCC 54282   | MZ902920 MZ936396 MZ936412 |
| *Gnomoniopsis guttulata*   | Bulgaria| *Agrimonia eupatoria* |             | MS 0312      | EU254812 NA NA           |
| *Gnomoniopsis hainanensis* | China   | *Castanopsis hainanensis* | Fagaceae    | CFCC 54376   | MZ902921 MZ936397 MZ936413 |
| *Gnomoniopsis hainanensis* | China   | *Castanopsis hainanensis* | Fagaceae    | CFCC 55877   | MZ902922 MZ936398 MZ936414 |
| *Gnomoniopsis idacolica*   | USA     | *Rubus sp.*          | Rosaceae    | CBS 125672   | GU320823 GU320797 GU320781 |
| *Gnomoniopsis idacolica*   | USA     | *Rubus pedatus*      | Rosaceae    | CBS 125673   | GU320824 GU320798 GU320782 |
| *Gnomoniopsis idacolica*   | France  | *Rubus sp.*          | Rosaceae    | CBS 125674   | GU320820 GU320796 GU320780 |
| *Gnomoniopsis idacolica*   | USA     | *Rubus procerus*     | Rosaceae    | CBS 125675   | GU320822 GU320799 GU320783 |
| *Gnomoniopsis macounii*    | USA     | *Spiraea sp.*        | Rosaceae    | CBS 121468   | EU254762 GU320804 EU219126 |
| *Gnomoniopsis occulta*     | USA     | *Potentilla sp.*     | Rosaceae    | CBS 125677   | GU320828 GU320812 GU320785 |
| *Gnomoniopsis parachvulata*| USA     | *Potentilla sp.*     | Rosaceae    | CBS 125678   | GU320829 GU320800 GU320786 |
| *Gnomoniopsis racemula*    | USA     | *Quercus alba minor* | Fagaceae    | CBS 123202   | GU320830 GU320815 GU320775 |
| *Gnomoniopsis rossmaniae*  | China   | *Chamerion angustifolium* | Onagraceae | CBS 121469   | EU254841 GU320803 EU219125 |
| *Gnomoniopsis rossmaniae*  | China   | *Castanopsis hainanensis* | Fagaceae    | CFCC 54307   | MZ902923 MZ936399 MZ936415 |
| *Gnomoniopsis sanguisorbae*| Switzerland| *Sanguisorba serrata* | Rosaceae    | CBS 858.79   | GU320818 GU320805 GU320790 |
| *Gnomoniopsis silvicola*   | China   | *Castanopsis hystrix* | Fagaceae    | CFCC 54304   | MZ902925 MZ936401 MZ936417 |
| *Gnomoniopsis smithogilvyi*| China   | *Quercus serrata*    | Fagaceae    | CFCC 54418   | MZ902926 MZ936402 MZ936418 |
| *Gnomoniopsis smithogilvyi*| Australia| *Castanea sp.*       | Fagaceae    | CBS 130190   | JQ910642 KR072534 JQ910639 |
| *Gnomoniopsis smithogilvyi*| Australia| *Castanea sp.*       | Fagaceae    | CBS 130189   | JQ910644 KR072535 JQ910641 |
| *Gnomoniopsis smithogilvyi*| Australia| *Castanea sp.*       | Fagaceae    | CBS 130188   | JQ910643 KR072536 JQ910640 |
Table 1. Cont.

| Species               | Country  | Host          | Host Family | Strain       | GenBank Accession Number |
|-----------------------|----------|---------------|-------------|--------------|--------------------------|
|                       |          |               |             |              | ITS          | tef1        | tub2        |
| Gnomoniopsis smithogilvyi | Italy    | Castanea sativa | Fagaceae    | MUT 401      | HM142946     | KR072537    | KR072532    |
| Gnomoniopsis smithogilvyi | New Zealand | Castanea sativa | Fagaceae    | MUT 411      | HM142948     | KR072538    | KR072533    |
| Gnomoniopsis tormentillae | Switzerland | Potentilla sp.  | Rosaceae    | CBS 904.79   | EU254856     | GU320795    | EU219165    |
| Gnomoniopsis xunwuensis | China    | Castanopsis fissa | Fagaceae    | CFCC 53115 * | MK432667     | MK578141    | MK578067    |
| Gnomoniopsis xunwuensis | China    | Castanopsis fissa | Fagaceae    | CFCC 53116   | MK432668     | MK578142    | MK578068    |

Note: NA, not applicable. Ex-type strains are marked with *, and strains from present study are in black bold.

2.3. Morphological Identification and Characterization

The morphological data of the isolates collected in the present study were based on the cultures sporulating on PDA in the dark at 25 °C. The conidiomata were observed and photographed under a dissecting microscope (M205 C, Leica, Wetzlar, Germany). The conidiogenous cells and conidia were immersed in tap water, then the microscopic photographs were captured with an Axio Imager 2 microscope (Zeiss, Oberkochen, Germany) equipped with an Axiocam 506 color camera, using differential interference contrast (DIC) illumination. More than 50 conidia were randomly selected for measurement. Culture characteristics were recorded from PDA and MEA after 10 days incubation at 25 °C in the dark.

3. Results

3.1. Phylogeny

The sequence dataset of the ITS gene matrix was analysed to infer the interspecific relationships within *Gnomoniopsis*. The dataset consisted of 56 sequences including one outgroup taxon, *Apiognomonia errabunda* (CBS 342.86). A total of 538 characters including gaps were included in the phylogenetic analysis. The topologies resulting from ML and BI analyses of the concatenated dataset were congruent (Figure 1). Isolates from the present study formed seven individual clades representing seven species of *Gnomoniopsis*, including six new species and one known species.

The combined three-gene sequence dataset (ITS, *tef1* and *tub2*) was further analysed to compare with results of the phylogenetic analyses of the ITS gene. The dataset consisted of 56 sequences including one outgroup taxon, *Apiognomonia errabunda* (CBS 342.86). A total of 1426 characters including gaps (538 for ITS, 348 for *tef1* and 540 for *tub2*) were included in the phylogenetic analysis. The topologies resulting from ML and BI analyses of the concatenated combined dataset were congruent (Figure 2). Isolates from the present study formed seven individual clades which were congruent with those in Figure 1.
Figure 1. Phylogram of Gnomoniopsis resulting from a maximum likelihood analysis based on the ITS gene. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.9). The tree is rooted with Apiognomonia errabunda (CBS 342.86). Isolates from the present study are marked in blue, and taxa in bold face are studied in the present study.
Figure 2. Phylogram of *Gnomoniopsis* resulting from a maximum likelihood analysis based on a combined matrix of ITS, tef1 and tub2. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.9). The tree is rooted with *Apiognomonia errabunda* (CBS 342.86). Isolates from present study are marked in blue, and taxa in bold face are studied in the present study.

3.2. Taxonomy

*Gnomoniopsis castanopsidis* N. Jiang, sp. nov. Figure 3.
μm. Conidia aseptate, hyaline, smooth, multi-guttulate, oval to fusoid, straight or slightly curved, base truncate, (4.3–) 4.6–5.1 (–5.4) × (1.8–) 2.1–2.5 (–2.6) μm (n = 50), L/W = 1.8–2.6.

Culture characteristics—Colonies flat, spreading, with moderate aerial mycelium and undulate margin, fawn on MEA, dirty-white to fawn on PDA, forming abundant brown conidiomata with creamy conidial masses.

Material examined—CHINA, Hainan Province, Changjiang Li Autonomous County, on diseased leaves of Castanopsis hystrix, 16 November 2018, Yong Li (JNH0003 holotype; ex-type living culture, CFCC 54437); Ibid. (living culture CFCC 55878).

Notes—Two isolates from leaf spots of Castanopsis hystrix clustered into a well-supported clade named Gnomoniopsis castanopsidis, which is distinct from any known species phylogenetically (Figures 1 and 2). Morphologically, G. castanopsidis is similar to G. silvicola in conidial size and shape. However, G. castanopsidis is separated from G. silvicola in 36 bp differences in ITS.

Gnomoniopsis daii C.M. Tian & N. Jiang, Forests 10(11/1016): 6 (2019). Figure 4.
Culture characteristics—Colonies flat, spreading, with moderate aerial mycelium and undulate margin, dirty-white to sienna on MEA, dirty-white to fawn on PDA, forming abundant brown conidiomata with creamy conidial masses.

Material examined—CHINA, Henan Province, Xinyang City, Shihe District, on diseased leaves of Quercus aliena, 7 August 2019, Yong Li (JNH0004; living culture, CFCC 55517); Ibid. (living culture CFCC 55294B).

Notes—Gnomoniopsis daii was initially described as the pathogen of Chinese chestnut (Castanea mollissima) fruit rot [23], and subsequently discovered to be the leaf spot pathogen of Chinese chestnut [28]. In the present study, two isolates from diseased leaves of Quercus aliena formed a well-supported clade with the ex-type strain of G. daii (Figures 1 and 2). Hence, Gnomoniopsis daii is for the first time reported on the host genus Quercus.

Gnomoniopsis fagacearum N. Jiang, sp. nov. Figure 5.

Description—Conidiomata pycnidial, aggregated or solitary, erumpent, globose to pulvinate, brown, 200–600 μm diam., exuding a creamy conidial mass. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, multi-guttulate, cylindrical, attenuate towards apex, phialidic, 7.5–19.5 × 2–3.5 μm. Conidia aseptate, hyaline, smooth, multi-guttulate, oval to fusoid, straight or slightly curved, base truncate, (5.1–) 5.6–6.1 (–6.3) × (2.3–) 2.8–3.2 (–3.6) μm (n = 50), L/W = 1.4–2.5.

Culture characteristics—Colonies flat, spreading, with moderate aerial mycelium and undulate margin, dirty-white to sienna on MEA, dirty-white to fawn on PDA, forming abundant brown conidiomata with creamy conidial masses.

Material examined—CHINA, Henan Province, Xinyang City, Shihe District, on diseased leaves of Quercus aliena, 7 August 2019, Yong Li (JNH0004; living culture, CFCC 55517); Ibid. (living culture CFCC 55294B).

Notes—Gnomoniopsis daii was initially described as the pathogen of Chinese chestnut (Castanea mollissima) fruit rot [23], and subsequently discovered to be the leaf spot pathogen of Chinese chestnut [28]. In the present study, two isolates from diseased leaves of Quercus aliena formed a well-supported clade with the ex-type strain of G. daii (Figures 1 and 2). Hence, Gnomoniopsis daii is for the first time reported on the host genus Quercus.

Gnomoniopsis fagacearum N. Jiang, sp. nov. Figure 5.
Morphology of *Gnomoniopsis fagacearum* (CFCC 54316). (A) Conidioma formed on PDA; (B) Conidiogenous cells giving rise to conidia; (C–F) Conidia. Scale bars: A = 300 µm; (B–F) = 10 µm.

**Mycobank No.:** 840970.

**Etymology**—Named after the host family, *Fagaceae*.

**Description**—Conidiomata acervular, solitary, erumpent, pulvinate, red-brown, 250–450 µm diam. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells red-brown, smooth, multi-guttulate, cylindrical, slightly curved, attenuate towards apex, phialidic, 16–33.5 × 2–5 µm. Conidia aseptate, hyaline or seldom red-brown, smooth, multi-guttulate, fusoid, straight or curved, base truncate, (9–) 9.6–11.4 (–12.6) × (2.8–) 3.1–4 (–4.5) µm (n = 50), L/W = 2.1–4.2.

**Culture characteristics**—Colonies flat, spreading, with moderate aerial mycelium, folded surface and lobate margin, sienna to red-brown on MEA, dirty-white to slightly red-brown on PDA, occasionally forming red-brown conidiomata.

**Material examined**—CHINA, Guangdong Province, Qingyuan City, Yangshan County, on diseased leaves of *Lithocarpus glaber*, 26 November 2019, Dan-Ran Bian (JNH0005 holotype; ex-type living culture, CFCC 54316); Jiangxi Province, Xinyu City, Fenyi County, on diseased leaves of *Castanopsis faberi*, 20 October 2019, Yong Li (living culture, CFCC 54288); Shaanxi Province, Hanzhong City, Foping County, on diseased leaves of *Quercus variabilis*, 13 August 2019, Yong Li (living culture, CFCC 54439); Fujian Province, Nanping City, Yanping County, on diseased leaves of *Castanopsis eyrei*, 13 July 2019, Dan-Ran Bian (living culture, CFCC 54414); Guangdong Province, Qingyuan City, Yangshan County, on diseased leaves of *Castanopsis chunii*, 26 November 2019, Dan-Ran Bian (living culture, CFCC 54412).

**Notes**—Five isolates from leaf spots of *Castanopsis chunii*, *C. eyrei*, *C. faberi*, *Lithocarpus glaber* and *Quercus variabilis* clustered into a well-supported clade here newly described as *Gnomoniopsis fagacearum*, which is distinct from any known species phylogenetically.
(Figures 1 and 2). Morphologically, *G. guangdongensis* can be distinguished from the other *Gnomoniopsis* species by red-brown conidiogenous cells.

*Gnomoniopsis guangdongensis* N. Jiang, sp. nov. Figure 6.

**Mycobank No.:** 840971.

**Etymology**—Named after the collection site, Guangdong Province.

**Description**—Conidiomata pycnidial, aggregated or solitary, erumpent, globose to pulvinate, dark brown, 150–600 μm diam., exuding a creamy conidial mass. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, multi-guttulate, cylindrical to ampulliform, attenuate towards apex, phialidic, 12.5–24 × 1.5–3 μm. *Conidia* aseptate, hyaline, smooth, multi-guttulate, cylindrical, constricted at the middle, straight or slightly curved, base truncate, (4.3–) 4.6–5 (–5.2) × (1.4–) 1.6–1.8 (–2) μm (n = 50), L/W = 2.4–3.3.

**Culture characteristics**—Colonies flat, spreading, with sparse to moderate aerial mycelium and diffuse margin, buff to fawn on MEA, dirty-white on PDA, with age forming narrow concentric zones, forming abundant dark brown conidiomata with creamy conidial masses.

**Material examined**—CHINA, Guangdong Province, Qingyuan City, Yangshan County, on diseased leaves of *Castanopsis fargesii*, 26 November 2019, Dan-Ran Bian (JNH0006 holotype; ex-type living culture, CFCC 54443); Ibid. (living cultures CFCC 54331 and CFCC 54282).

**Notes**—Three isolates from leaf spots of *Castanopsis fargesii* clustered into a well-supported clade named *Gnomoniopsis guangdongensis*, which is distinct from any known species phylogenetically (Figures 1 and 2). Morphologically, *G. guangdongensis* can be distinguished from the other *Gnomoniopsis* species by its conidia constricted at the middle.
Gnomoniopsis hainanensis N. Jiang, sp. nov. Figure 7.

Figure 7. Morphology of Gnomoniopsis hainanensis (CFCC 54376). (A) Conidioma formed on PDA; (B) Conidiogenous cells giving rise to conidia; (C–F) Conidia. Scale bars: A = 300 μm; (B–F) = 10 μm.

Mycobank No.: 840972.
Etymology—Named after the collection site, Hainan Province.
Description—Conidiomata pycnidial, solitary, erumpent, globose to pulvinate, light brown, 100–300 μm diam., exuding a creamy conidial mass. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, multi-guttulate, cylindrical, attenuate towards apex, phialidic, 16.5–26 × 2.5–4.5 μm. Conidia aseptate, hyaline, smooth, multi-guttulate, fusoid, straight, base truncate, (7.3–) 8–10 (–12.2) × (3.3–) 3.4–3.9 (–4.2) μm (n = 50), L/W = 1.9–3.3.

Culture characteristics—Colonies flat, spreading, with sparse aerial mycelium and lobate to undulate margin, sienna to luteous on MEA, luteous on PDA, with age forming narrow concentric zones, forming abundant light brown conidiomata with creamy conidial masses.

Material examined—CHINA, Hainan Province, Changjiang Li Autonomous County, on diseased leaves of Castanopsis hainanensis, 16 November 2018, Yong Li (JNH0007 holotype; ex-type living culture, CFCC 54376); Ibid. (living culture CFCC 55877).

Notes—Two isolates from leaf spots of Castanopsis hainanensis clustered into a well-supported clade here newly described as Gnomoniopsis hainanensis, which is distinct from any known species phylogenetically (Figures 1 and 2). G. guangdongensis is different from the phylogenetically close species G. fagacearum by its conidial size and length-width ratio (7.3–12.2 × 3.3–4.2 μm, L/W = 1.9–3.3 in G. guangdongensis vs. 9–12.6 × 2.8–4.5 μm, L/W = 2.1–4.2 in G. fagacearum).

Gnomoniopsis rossmaniae N. Jiang, sp. nov. Figure 8.
Culture characteristics—Colonies flat, spreading, with sparse aerial mycelium and lobate to undulate margin, hazel on MEA, dirty-white on PDA, seldom forming dark brown conidiomata with brown conidial masses.

Material examined—CHINA, Hainan Province, Changjiang Li Autonomous County, on diseased leaves of *Castanopsis hainanensis*, 16 November 2018, Yong Li (JNH0008 holotype; ex-type living culture, CFCC 54307); Ibid. (living culture CFCC 55876).

Notes—Two isolates from leaf spots of *Castanopsis hainanensis* clustered into a well-supported clade here newly described as *Gnomoniopsis rossmaniae*, which is distinct from any known species phylogenetically (Figures 1 and 2). Morphologically, *G. rossmaniae* can be distinguished from the other *Gnomoniopsis* species by its aseptate to 1-septate, elongate-fusoid conidia.

*Gnomoniopsis silvicola* N. Jiang, sp. nov. Figure 9.

Mycobank No.: 840973.

Etymology—In honor of Amy Y. Rossman for her contributions to the study of the fungal order *Diaporthales*.

Description—Conidiomata pycnidial, solitary, erumpent, pulvinate, dark brown, 250–650 µm diam., exuding a brown conidial mass. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, multi-guttulate, cylindrical to ampulliform, attenuate towards apex, phialidic, 9–19 × 2–3 µm. Conidia aseptate to 1-septate, slightly constricted at septum, hyaline, smooth, multi-guttulate, elongate-fusoid, straight, base truncate, (10–) 11.6–14.6 (–16.1) × (3.1–) 3.3–3.9 (–4.1) µm (n = 50), L/W = 2.8–4.5.

Culture characteristics—Colonies flat, spreading, with sparse aerial mycelium and lobate to undulate margin, hazel on MEA, dirty-white on PDA, seldom forming dark brown conidiomata with brown conidial masses.

Material examined—CHINA, Hainan Province, Changjiang Li Autonomous County, on diseased leaves of *Castanopsis hainanensis*, 16 November 2018, Yong Li (JNH0008 holotype; ex-type living culture, CFCC 54307); Ibid. (living culture CFCC 55876).

Notes—Two isolates from leaf spots of *Castanopsis hainanensis* clustered into a well-supported clade here newly described as *Gnomoniopsis rossmaniae*, which is distinct from any known species phylogenetically (Figures 1 and 2). Morphologically, *G. rossmaniae* can be distinguished from the other *Gnomoniopsis* species by its aseptate to 1-septate, elongate-fusoid conidia.

*Gnomoniopsis silvicola* N. Jiang, sp. nov. Figure 9.

Figure 8. Morphology of *Gnomoniopsis rossmaniae* (CFCC 54307). (A) Conidioma formed on PDA; (B) Conidiogenous cells giving rise to conidia; (C–F) Conidia. Scale bars: A = 300 µm; (B–F) = 10 µm.
Morphology of Gnomoniopsis silvicola (CFCC 54418). (A) Conidiomata formed on PDA; (B) Conidiogenous cells giving rise to conidia; (C–F) Conidia. Scale bars: A = 500 μm; (B–F) = 5 μm.

Mycobank No.: 840974.

Etymology—Name from “silva” = forest and “-cola” = inhabiting; with reference to its woody host.

Description—Conidiomata pycnidial, aggregated or solitary, erumpent, globose to pulvinate, brown, 250–650 μm diam., exuding a creamy conidial mass. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, multi-guttulate, cylindrical to ampulliform, attenuate towards apex, phialidic, 7–15 × 1.5–2.5 μm. Conidia aseptate, hyaline, smooth, multi-guttulate, oval to fusoid, straight or slightly curved, base truncate, (4.3–) 4.5–5.3 (–5.9) × (1.9–) 2.2–2.6 (–2.7) μm (n = 50), L/W = 1.7–2.5.

Culture characteristics—Colonies flat, spreading, with moderate aerial mycelium and undulate margin, luteous to brown on MEA, dirty-white on PDA, forming abundant brown conidiomata with creamy conidial masses.

Material examined—CHINA, Shaanxi Province, Hanzhong City, Foping County, on diseased leaves of Quercus serrata, 13 August 2019, Yong Li (JNH0009 holotype; ex-type living culture, CFCC 54418); Guangdong Province, Shaoguan City, Lechang County, on diseased leaves of Castanopsis hystrix, 4 December 2019, Dan-Ran Bian (living culture, CFCC 54304).

Notes—Two isolates from leaf spots of Castanopsis hystrix and Quercus serrata clustered into a well-supported clade here described as the new species Gnomoniopsis silovicola, which is distinct from any known species phylogenetically (Figures 1 and 2). Morphologically, G. silvicola has a bit smaller conidia than its phylogenetically close species G. daii (4.3–5.9 × 1.9–2.7 μm in G. silvicola vs. 5.1–6.3 × 2.3–3.6 μm in G. daii). In addition, G. silvicola is separated from G. daiii in 34 bp differences in ITS.
4. Discussion

In the present study, six new *Gnomoniopsis* species (viz. *G. castanopsidis*, *G. fagacearum*, *G. guangdongensis*, *G. hainanensis*, *G. rossmaniae* and *G. silvicola*) are described and illustrated (Figures 3–10), and a new host, *Quercus aliena*, is reported for the known species *G. daii*. As noted in previous studies, the fungal genus *Gnomoniopsis* is so far only known from hosts of three plant families, *Fagaceae*, *Onagraceae* and *Rosaceae* [20,21,40], of which only one species, *G. racemula* was described from the family *Onagraceae* [20]. Hence, *Fagaceae* and *Rosaceae* are the main hosts for *Gnomoniopsis* species. Although several new species and host records are reported from *Fagaceae* in China herein, numerous additional hidden species might remain to be revealed from the widely spread fagaceous species in China.

Figure 10. *Gnomoniopsis* cultures at 10 days. (A) *G. silvicola* (CFCC 54304) on MEA; (B) *G. silvicola* (CFCC 54304) on PDA; (C) *G. rossmaniae* (CFCC 54307) on MEA; (D) *G. rossmaniae* (CFCC 54307) on PDA; (E) *G. guangdongensis* (CFCC 54443) on MEA; (F) *G. guangdongensis* (CFCC 54443) on PDA; (G) *G. hainanensis* (CFCC 54376) on MEA; (H) *G. hainanensis* (CFCC 54376) on PDA; (I) *G. fagacearum* (CFCC 54316) on MEA; (J) *G. fagacearum* (CFCC 54316) on PDA; (K) *G. silvicola* (CFCC 54418) on MEA; (L) *G. silvicola* (CFCC 54418) on PDA; (M) *G. castanopsidis* (CFCC 54437) on MEA; (N) *G. castanopsidis* (CFCC 54437) on PDA; (O) *G. daii* (CFCC 55517) on MEA; (P) *G. daii* (CFCC 55517) on PDA.
So far, eleven *Gnomoniopsis* species were reported from fagaceous hosts, of which *G. clavulata* and *G. paraclavulata* were described from *Quercus* in the USA [20]. *Gnomoniopsis smithogilvyi* was reported as causal agent of sweet chestnut fruit rot in Australia, Europe and North America [25,26,41–45]. The remaining eight species are only known from China. They were well distinguished in phylogenetic analyses of the ITS gene and of combined matrices of ITS, *tef1* and *tub2* genes (Figures 1 and 2). The conidial characters as well as the hosts and distribution provide useful information for species delimitation (Table 2).

### Table 2. Comparison of *Gnomoniopsis* species on hosts belonging to Fagaceae.

| Species          | Host                                      | Conidial Length (µm) | Conidial Width (µm) | L/W Ratio | Reference |
|------------------|-------------------------------------------|----------------------|---------------------|-----------|-----------|
| *G. castanopsis*| *Castanopsis hystrix*                     | (4.3–) 4.6–5.1 (–5.4) | (1.8–) 2.1–2.5 (–2.6) | 1.8–2.6   | This study |
| *G. chinensis*   | *Castanea mollissima*                     | (6.0–) 6.5–8.5 (–9.0) | (2.2–) 2.7–3 (–3.5)  | NA        | [29]      |
| *G. clavulata*   | *Quercus falcata*                         | (5–) 6–6.5 (–8)      | (2–) 2.5–3 (–4)     | 1.4–3.7   | [20]      |
| *G. daii*        | *Castanea mollissima*                     | (5.0–) 5.5–7.0 (–8.0) | 2.0–3.5            | NA        | [23,28]   |
| *G. fagacearum*  | *Castanea mollissima*; *C. eyrei, C. faberi,* | (5.1–) 5.6–6.1 (–6.3) | (2.3–) 2.8–3.2 (–3.6) | 1.4–2.5   | This study |
| *G. guangdongensis* | *Lithocarpus glaber* and *Quercus variabilis* | (9–) 9.6–11.4 (–12.6) | (2.8–) 3.1–4 (–4.5) | 2.1–4.2   | This study |
| *G. hainanensis* | *Castanopsis hystrix*                     | (4.3–) 4.6–5 (–5.2)  | (1.4–) 1.6–1.8 (–2) | 2.4–3.3   | This study |
| *G. paraclavulata* | *Quercus alba*                            | (7.3–) 8–10 (–12.2) | (3.3–) 3.4–3.9 (–4.2) | 1.9–3.3   | This study |
| *G. rossmaniae*  | *Castanopsis hainanensis*                 | (6–) 7.5–8 (–9.5)    | (2–) 3–3 (–3.5)     | 1.6–4.2   | [20]      |
| *G. silvicola*   | *Castanopsis hystrix* and *Quercus serrata* | (10–) 11.6–14.6 (–16.1) | (3.1–) 3.3–3.9 (–4.1) | 2.8–4.5   | This study |
| *G. smithogilvyi* | *Castanea sativa*                         | (4.3–) 4.5–5.3 (–5.9) | (1.9–) 2.2–2.6 (–2.7) | 1.7–2.5   | This study |

Several *Gnomoniopsis* species are pathogens of leaves, branches or fruits [29,46]. For example, *G. smithogilvyi* causes sweet chestnut branch canker and fruit rot in Australia, Europe and the USA [26,42,45], whereas in China *G. daii* is one of the main pathogens of Chinese chestnut causing fruit rot and leaf spot diseases [23,28]. In addition, *G. chinensis* causes branch canker of Chinese chestnut in China [29]. The newly described species of the present study were isolated from diseased leaves; however, additional studies are required to confirm their pathogenicity.

### 5. Conclusions

Eight *Gnomoniopsis* species are known from fagaceous hosts in China based on morphology and phylogeny, viz. *G. chinensis on Castanea mollissima, G. castanopsis on Castanopsis hystrix, G. daii on Castanea mollissima and Quercus aliena, G. fagacearum on Castanopsis chunii, Castanopsis eyrei, Castanopsis faberi, Lithocarpus glaber and Quercus variabilis, G. guangdongensis on Castanopsis fargesii, G. hainanensis on Castanopsis hainanensis, G. rossmaniae on Castanopsis hainanensis and G. silvicola on Castanopsis hystrix and Quercus serrata*. They can be well distinguished by the combined approaches of morphology and phylogeny based on ITS, *tef1* and *tub2* genes.

### Author Contributions

Conceptualization, Y.L. and N.J.; methodology, N.J. and D.-R.B.; software, N.J.; validation, N.J., S.-K.W., Y.L. and C.-G.P.; formal analysis, N.J.; investigation, Y.L.; resources, Y.L.; data curation, N.J.; writing—original draft preparation, N.J.; writing—review and editing, H.V.; visualization, N.J.; supervision, C.-G.P.; project administration, C.-G.P.; funding acquisition, C.-G.P. All authors have read and agreed to the published version of the manuscript.

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