Identification and Characterization of Leaf-Inhabiting Fungi from *Castanea* Plantations in China

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Abstract: Two *Castanea* plant species, *C. henryi* and *C. mollissima*, are cultivated in China to produce chestnut crops. Leaf spot diseases commonly occur in *Castanea* plantations, however, little is known about the fungal species associated with chestnut leaf spots. In this study, leaf samples of *C. henryi* and *C. mollissima* were collected from Beijing, Guizhou, Hunan, Sichuan and Yunnan Provinces, and leaf-inhabiting fungi were identified based on morphology and phylogeny. As a result, twenty-six fungal species were confirmed, including one new family, one new genus, and five new species. The new taxa are Pyrisporaceae fam. nov., *Pyrispora* gen. nov., *Aureobasidium castaneae* sp. nov., *Discosia castaneae* sp. nov., *Monochaetia castaneae* sp. nov., *Neopestalotiopsis sichuanensis* sp. nov. and *Pyrispora castaneae* sp. nov.

Keywords: *Castanea henryi*; *C. mollissima*; Diaporthales; phylogeny; Sporocadaceae; taxonomy

1. Introduction

*Castanea*, a plant genus well-known for edible chestnuts and hard timber, is distributed worldwide. The most four famous species are American chestnut (*C. dentata*), Chinese chestnut (*C. mollissima*), European chestnut (*C. sativa*) and Japanese chestnut (*C. crenata*). *C. mollissima* is widely cultivated as the crop in most provinces of China. In recent years, another *Castanea* species, *C. henryi*, was planted in Hunan Province of China to replace *C. mollissima* for its higher economic benefits.

In the long cultivation history of chestnut trees, fungal diseases have caused serious economic and ecological problems. Chestnut blight caused by *Cryptohonea parasitica* is the most notorious one, which devastated *Castanea dentata* forests in North America and weakened the other chestnut species in plantations [1–3]. Several emerging pathogens were recently reported in *Castanea sitiva* from Australia and Europe, viz. *Gnomoniopsis smithogilvyi* (syn. *G. castaneae*) [4,5], *Phytophthora cinnamomi* [6], *Sirococcus castanea* [7].

In China, previous studies have revealed a high fungal diversity associated with chestnut branch cankers. For example, three *Coryneum* species [8], two *Cryptohonea* species [2], six *Cytospora* species and seven *Dendrostroma* species were described from the cankered branches [9,10]. In addition, some cryptic species were discovered, such as *Aurantiosacculus castaneae* [2], *Endothia chinensis* [2], *Melanops castaneicola* and *Neopseudomelanconis castaneae* [11,12]. Due to the high fungal diversity on chestnut branches, we collected leaf samples from two chestnut species, *Castanea henryi* and *C. mollissima* in China. In the present study, fungal taxa associated with the symptomatic leaves were identified based on morphological and molecular approaches, which is a fundamental task for the subsequent research on chestnut leaf diseases.

2. Materials and Methods

2.1. Field Sampling and Isolation

From 2017 to 2020, we investigated *Castanea* plantations of *C. henryi* and *C. mollissima* in the Beijing, Guizhou, Hunan, Sichuan and Yunnan Provinces of China. The disease
symptoms were recorded (Figure 1), and 176 fresh leaf samples were collected and packed in sealed plastic bags. These leaf samples were transported to the laboratory for fungal isolation within ten days.

![Figure 1. Field Sampling. (A) A Castanea plantation; (B) Symptoms of twig canker; (C–E,G–I) Symptoms of leaf diseases; (F) Chestnuts.](image)

Fresh isolates were acquired by removing spore masses in fruiting bodies or surface-sterilized leaf tissues onto the surfaces of potato dextrose agar (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per L) using axenic syringe needles. Then plates were incubated in the dark at 25 °C until germination. Hyphal tips were cut and transported to new PDA plates, and incubated in the dark at 25 °C.

2.2. Morphological Identification and Characterization

Fungal species on Chinese chestnut leaves were initially observed based on ascomata and conidiomata formed on the leaf surface or PDA, under a dissecting stereomicroscope (AZ100, Nikon, Tokyo, Japan), then asci, ascospores, conidiogenous cells and conidia were photographed using a Leica compound microscope (DM 2500, Leica, Wetzlar, Germany). Cultural characteristics of isolates incubated on PDA in the dark at 25 °C were recorded.

2.3. DNA Extraction, Sequencing and Phylogenetic Analysis

Genomic DNA was extracted from mycelium grown on PDA using a CTAB (cetyltrimethylammonium bromide) method [13]. Then PCR (polymerase chain reaction) was conducted for each genus using selected genes and primers (Table 1). The PCR conditions were set 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 52 °C (ITS, LSU) or 54 °C (act, cal, chs-1, gapdh, his3, rpb2, tub2, tef1) and 1 min at 72 °C, and a final elongation step of 7 min at 72 °C. The PCR amplification products were sequenced using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

The sequences obtained in this study (Table 2) were supplemented with the additional sequences retrieved from GenBank. The sequences were aligned and checked manually using MEGA6. Ambiguous regions were excluded from the analyses and gaps were treated as missing data. Phylogenetic analyses were carried out with maximum likelihood analysis (ML), which was performed at the CIPRES web portal [14]. Bayesian inference analysis (BI) was performed in MrBayes v. 3.2.0 [15]. Phylogenetic trees were viewed in FigTree v1.4. The names of the isolates from present study are marked in blue in the trees. Maximum likelihood bootstrap support values ≥50% (BT) and Bayesian posterior probabilities ≥0.90 (PP) are given at the nodes respectively.
Table 1. Selected genes and primers for polymerase chain reaction of each genus.

| Genera                  | ITS [16] | LSU [16] | act [17] | cal [17] | chs-1 [17] | gapdh [18,19] | his3 [20,21] | rpb2 [22] | tef1 [17] | tub2 [20] |
|-------------------------|----------|----------|----------|----------|------------|---------------|--------------|-----------|----------|-----------|
| Aplosporella            | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Arthrinium              | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Aurobasidium            | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           |          |           |
| Bartalinia pini         | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           |          |           |
| Botryosphaeria          | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Colletotrichum          | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Coniella                | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Diaporthe               | ITS1/ITS4|          |          | CAL-228F | CAL-737R   |                |              |           |          |           |
| Didymella               | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           | RPB2-5F2  |           |
| Discosia                | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           |          |           |
| Gnomoniopsis            | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Monochaetia             | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           | RPB2-5F2  |           |
| Neopestalotiopsis       | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Pestalotiopsis          | ITS1/ITS4|          |          |          |            |                |              |           |          |           |
| Phyllosticta            | ITS1/ITS4|          |          |          |            | Gpd1-LM/Gpd2-LM |              |           |          |           |
| Pyrispora               | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           | RPB2-5F2  |           |
| Robillarda              | ITS1/ITS4| LR0R/LR5 |          |          |            |                |              |           | RPB2-5F2  |           |
| Tubakia                 | ITS1/ITS4|          |          |          |            |                |              |           | EFl-688F  |           |
Table 2. Isolates and GenBank accession numbers of sequences from this study.

| Species                  | Isolates       | GenBank Accession No. |
|--------------------------|----------------|-----------------------|
|                          |                | ITS | LSU | act | cal | cls-1 | gapdh | his3 | rpb2 | tef1 | tub2 |
| Aplosporella prunicola   | CFCC 54334 = SM18B | MW350059 | NA | NA | NA | NA | NA | NA | MW381858 | NA |
| Aplosporella prunicola   | SM18B-1         | MW350060 | NA | NA | NA | NA | NA | NA | MW381859 | NA |
| Arthrinium arundinis     | XT18-1          | MW364286 | NA | NA | NA | NA | NA | NA | NA | NA |
| Aureobasidium castaneae  | sp. nov.        | CFCC 54591 = JJ7-3 | MW364284 | MW364275 | NA | NA | NA | NA | NA | NA |
| Bartalinia pini          | CFCC 54574 = JJ4 | MW364285 | MW364276 | NA | NA | NA | NA | NA | NA | NA |
| Botryosphaeria dothidea  | JJ2B            | MW350061 | NA | NA | NA | NA | NA | NA | MW381860 | MW381864 |
| Botryosphaeria dothidea  | CFCC 54576 = JJ12 | MW350062 | NA | NA | NA | NA | NA | NA | MW381861 | MW381865 |
| Botryosphaeria dothidea  | JJ14            | MW350063 | NA | NA | NA | NA | NA | NA | MW381862 | MW381866 |
| Botryosphaeria dothidea  | JJ27-1          | MW350064 | NA | NA | NA | NA | NA | NA | MW381863 | MW381867 |
| Colletotrichum fructicola| SM6             | MW217249 | NA | MW227352 | NA | MW227370 | MW381824 | NA | NA | NA | MW227388 |
| Colletotrichum fructicola| SM4             | MW217250 | NA | MW227353 | NA | MW227371 | MW381825 | NA | NA | NA | MW227389 |
| Colletotrichum fructicola| CFCC 54363 = SM13 | MW217251 | NA | MW227354 | NA | MW227372 | MW381826 | NA | NA | NA | MW227390 |
| Colletotrichum fructicola| SM16            | MW217252 | NA | MW227355 | NA | MW227373 | MW381827 | NA | NA | NA | MW227391 |
| Colletotrichum fructicola| SM30            | MW217253 | NA | MW227356 | NA | MW227374 | MW381828 | NA | NA | NA | MW227392 |
| Colletotrichum fructicola| SM31            | MW217254 | NA | MW227357 | NA | MW227375 | MW381829 | NA | NA | NA | MW227393 |
| Colletotrichum henanense | CFCC 54364 = SM12 | MW217255 | NA | MW227358 | NA | MW227376 | MW381830 | NA | NA | NA | MW227394 |
| Colletotrichum henanense | SM22            | MW217256 | NA | MW227359 | NA | MW227377 | MW381831 | NA | NA | NA | MW227395 |
| Colletotrichum henanense | SM33            | MW217257 | NA | MW227360 | NA | MW227378 | MW381832 | NA | NA | NA | MW227396 |
| Colletotrichum henanense | ZX2-1           | MW217258 | NA | MW227361 | NA | MW227379 | MW381833 | NA | NA | NA | MW227397 |
| Colletotrichum jiangxiense | SM21         | MW217259 | NA | MW227362 | NA | MW227380 | MW381834 | NA | NA | NA | MW227398 |
| Colletotrichum jiangxiense| CFCC 54362 = ZX10-1 | MW217260 | NA | MW227363 | NA | MW227381 | MW381835 | NA | NA | NA | MW227399 |
| Colletotrichum jiangxiense| ZY12B          | MW217261 | NA | MW227364 | NA | MW227382 | MW381836 | NA | NA | NA | MW227400 |
| Colletotrichum jiangxiense| ZY12           | MW217262 | NA | MW227365 | NA | MW227383 | MW381837 | NA | NA | NA | MW227401 |
| Colletotrichum karsti   | CFCC 54365 = ZY3B | MW217263 | NA | MW227366 | NA | MW227384 | MW381838 | NA | NA | NA | NA |
| Colletotrichum karsti   | ZY3B-1          | MW217264 | NA | MW227367 | NA | MW227385 | MW381839 | NA | NA | NA | NA |
Table 2. Cont.

| Species                              | Isolates                  | GenBank Accession No. |
|--------------------------------------|---------------------------|-----------------------|
|                                      |                           | **ITS** | **LSU** | **act** | **cal** | **chl-1** | **gapdh** | **his3** | **rpb2** | **tef1** | **tub2** |
| Colletotrichum nymphaeae            | CFCC 54366 = SM26         | MW217265 | NA    | MW227368 | NA    | MW227386 | MW381840 | NA    | NA    | NA    | MW227402 |
| Colletotrichum nymphaeae            | SM26-1                    | MW217266 | NA    | MW227369 | NA    | MW227387 | MW381841 | NA    | NA    | NA    | MW227403 |
| Coniella castaneicola               | CFCC 54344 = ZY7-1       | MW208111 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227343 |
| Coniella castaneicola               | ZY7-2                     | MW208112 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227344 |
| Diaporthe lithocarpi                | CFCC 54573 = JJ3         | MW364281 | NA    | NA    | NA    | MW381842 | NA    | NA    | MW381845 | NA    | MW381851 |
| Diaporthe lithocarpi                | JJ3-2                     | MW364282 | NA    | NA    | NA    | MW381843 | NA    | NA    | MW381846 | NA    | MW381852 |
| Diaporthe lithocarpi                | JJ26B                     | MW364283 | NA    | NA    | NA    | MW381844 | NA    | NA    | MW381847 | NA    | MW381853 |
| Didymella coffeae-arabicae           | CFCC 54343 = SM24         | MW364357 | MW364277 | NA    | NA    | NA    | NA    | NA    | MW381854 | NA    | MW381856 |
| Didymella coffeae-arabicae           | SM24B                    | MW364358 | MW364278 | NA    | NA    | NA    | NA    | NA    | MW381855 | NA    | MW381857 |
| Discosia castaneae sp. nov.         | CFCC 54088 = CML1        | MN842798 | MN842796 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MN864778 |
| Discosia castaneae sp. nov.         | CML2                     | MN842799 | MN842797 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MN864779 |
| Gnomoniopsis daii                   | CFCC 54345 = ZY11        | MW208113 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227345 | MW218543 |
| Gnomoniopsis daii                   | ZY10-1                   | MW208114 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227346 | MW218544 |
| Gnomoniopsis daii                   | ZY10-3                   | MW208115 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227347 | MW218545 |
| Gnomoniopsis daii                   | ZY12A                    | MW208116 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227348 | MW218546 |
| Gnomoniopsis daii                   | ZX14-1                   | MW208117 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW227349 | MW218547 |
| Monochaetia castaneae sp. nov.      | CFCC 54354 = SM9-1       | MW166222 | MW166263 | NA    | NA    | NA    | NA    | NA    | MW199737 | MW199741 | MW218515 |
| Monochaetia castaneae sp. nov.      | SM9-2                    | MW166223 | MW166264 | NA    | NA    | NA    | NA    | NA    | MW199738 | MW199742 | MW218516 |
| Neopestalotiopsis asiatica          | CFCC 54339 = SM32        | MW166224 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199743 | MW218517 |
| Neopestalotiopsis asiatica          | SM7                      | MW166225 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199744 | MW218518 |
| Neopestalotiopsis asiatica          | SM8B                     | MW166226 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199745 | MW218519 |
| Neopestalotiopsis asiatica          | SM11                     | MW166227 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199746 | MW218520 |
| Neopestalotiopsis asiatica          | SM19B                    | MW166228 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199747 | MW218521 |
| Neopestalotiopsis brasiliensis      | CFCC 54341 = ZY4         | MW166229 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199748 | MW218522 |
| Neopestalotiopsis brasiliensis      | ZY4-2D                   | MW166230 | NA    | NA    | NA    | NA    | NA    | NA    | NA    | NA    | MW199749 | MW218523 |
| Species                        | Isolates             | GenBank Accession No. |
|-------------------------------|----------------------|----------------------|
| Neopestalotiopsis sichuansensis sp. nov. | CFCC 54338 = SM15-1  | MW166231 NA NA NA NA NA NA NA MW199750 MW218524 |
| Neopestalotiopsis sichuansensis sp. nov. | SM15-1C              | MW166232 NA NA NA NA NA NA NA MW199751 MW218525 |
| Neopestalotiopsis sp.1        | CFCC 54337 = ZX12A   | MW166233 NA NA NA NA NA NA NA MW199752 MW218526 |
| Neopestalotiopsis sp.1        | ZX12-1               | MW166234 NA NA NA NA NA NA NA MW199753 MW218527 |
| Neopestalotiopsis sp.2        | CFCC 54340 = SM14    | MW166235 NA NA NA NA NA NA NA MW199754 MW218528 |
| Neopestalotiopsis sp.2        | ZX22B                | MW166236 NA NA NA NA NA NA NA MW199755 MW218529 |
| Pestalotiopsis kenyana        | CFCC 54336 = ZX11    | MW166237 NA NA NA NA NA NA NA MW199756 MW218530 |
| Pestalotiopsis kenyana        | ZX3                  | MW166238 NA NA NA NA NA NA NA MW199757 MW218531 |
| Pestalotiopsis kenyana        | ZX7                  | MW166239 NA NA NA NA NA NA NA MW199758 MW218532 |
| Pestalotiopsis kenyana        | ZX9                  | MW166240 NA NA NA NA NA NA NA MW199759 MW218533 |
| Pestalotiopsis kenyana        | ZX18A                | MW166241 NA NA NA NA NA NA NA MW199760 MW218534 |
| Phyllosticta capitalensis     | CFCC 54577 = JJ16    | MW350068 NA MW381868 NA NA MW381879 NA NA NA NA |
| Phyllosticta capitalensis     | JJ20                 | MW350069 NA MW381869 NA NA MW381880 NA NA NA NA |
| Phyllosticta capitalensis     | SS07                 | MW350070 NA MW381870 NA NA MW381881 NA NA NA NA |
| Phyllosticta capitalensis     | SS10                 | MW350071 NA NA NA NA NA MW381882 NA NA NA NA |
| Phyllosticta capitalensis     | SS13                 | MW350072 NA MW381871 NA NA MW381883 NA NA NA NA |
| Phyllosticta capitalensis     | SS15                 | MW350073 NA MW381872 NA NA MW381884 NA NA NA NA |
| Phyllosticta capitalensis     | SS16-1               | MW350074 NA NA NA NA NA MW381885 NA NA NA NA |
| Phyllosticta capitalensis     | SS16-2               | MW350075 NA NA NA NA NA MW381886 NA NA NA NA |
| Phyllosticta capitalensis     | CFCC 54579 = XT10    | MW350076 NA MW381873 NA NA MW381887 NA NA NA NA |
| Phyllosticta capitalensis     | XT11                 | MW350077 NA MW381874 NA NA MW381888 NA NA NA NA |
| Phyllosticta capitalensis     | XT16-1               | MW350078 NA MW381875 NA NA MW381889 NA NA NA NA |
| Phyllosticta capitalensis     | XT16-2               | MW350079 NA MW381876 NA NA MW381890 NA NA NA NA |
| Phyllosticta capitalensis     | XT17                 | MW350080 NA MW381877 NA NA MW381891 NA NA NA NA |
| Phyllosticta capitalensis     | CFCC 54355 = ZX6     | MW350081 NA NA NA NA NA MW381892 NA NA NA NA |
| Phyllosticta capitalensis     | ZX11-1               | MW350082 NA NA NA NA NA MW381893 NA NA NA NA |
| Phyllosticta capitalensis     | CFCC 54356 = ZY6-1   | MW350083 NA MW381878 NA NA MW381894 NA NA NA NA |
| Species                    | Isolates | GenBank Accession No. |
|---------------------------|----------|-----------------------|
|                           | ITS      | LSU       | act | cal | clp-1 | gapdh | his3 | rpb2 | tef1 | tub2 |
| **Pyrispora castaneae**   | CFCC 54349 = SM17 | MW208108 | MW208105 | NA  | NA  | NA  | NA  | NA  | MW218535 | MW227340 | NA  |
| sp. nov.                  | CFCC 54350 = SM20 | MW208109 | MW208106 | NA  | NA  | NA  | NA  | NA  | MW218536 | MW227341 | NA  |
|                           | CFCC 54351 = SM29 | MW208110 | MW208107 | NA  | NA  | NA  | NA  | NA  | MW218537 | MW227342 | NA  |
| **Robillarda sessilis**   | CFCC 54353 = ZX5 | MW166242 | MW166265 | NA  | NA  | NA  | NA  | NA  | MW199739 | MW218550 | MW218553 |
|                            | ZX5-1   | MW166243 | MW166266 | NA  | NA  | NA  | NA  | NA  | MW199740 | MW218551 | MW218554 |
|                            | ZY5     | MW218478 | MW218479 | NA  | NA  | NA  | NA  | NA  | MW222613 | MW218552 | MW218555 |
| **Tubakia dryinoides**    | CFCC 54346 = SM10-1 | MW208118 | NA  | NA  | NA  | NA  | NA  | NA  | MW227350 | MW218548 |
|                           | SM10    | MW208119 | NA  | NA  | NA  | NA  | NA  | NA  | MW227351 | MW218549 |

NA: Not available.
3. Results

A total of 26 species were identified, which belonging to two classes, six orders, 13 families and 18 genera in Ascomycota (Table 3). The seven new taxa are Pyrisporaceae fam. nov., Pyrispora gen. nov., Aureobasidium castaneae sp. nov., Discosia castaneae sp. nov., Monochaetia castaneae sp. nov., Neopestalotiopsis sichuanensis sp. nov. and Pyrispora castaneae sp. nov.

3.1. Aplosporella Speg.

*Aplosporella prunicola* Damm & Crous, Fungal Divers. 27(1): 39 (2007)

Endophytic or pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Undetermined.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 5 days, flat with undulate edge, olivaceous to grey-olivaceous, aerial mycelium appressed, floccose, white to smoke-grey.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (living culture, CFCC 54334 = SM18B); *ibid*. (living culture, SM18B-1).

Notes—*Aplosporella prunicola* was initially recorded from *Prunus persica* var. *nucipersica* in South Africa [23]. Two new isolates from diseased chestnut leaves in present study are phylogenetically close to *Aplosporella prunicola* (Figure A1) and share similar culture characteristics on PDA. This is the first report of this fungus on the host *Castanea mollissima*, and in the Country China.

3.2. Botryosphaeria Ces. & De Not.

*Botryosphaeria dothidea* Ces. & De Not., Comm. Soc. crittog. Ital. 1(fasc. 4): 212 (1863) (Figure 2)

Pathogenic on *Castanea henryi* and *C. mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 100–250 µm diam., pycnidial, aggregated, globose, black. Conidiophores absent. Conidiogenous cells 3–11 × 1.5–3 µm, holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening. Conidia (18.3–)20–24.7(–26.4) × (6.3–)7.2–8.4(–8.7) µm (τ = 22.3 × 7.8 µm, n = 50), L/W = (2.3–)2.4–3.4(–3.9) (τ = 2.9, n = 50), hyaline, thin-walled, smooth with granular contents, unicellular, aseptate, ellipsoid to fusoid, base subtruncate to bluntly rounded.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 4 days, initially white with fluffy, aerial mycelium, becoming black on the surface after 5 days; reverse side of the colonies dark-brown.

Material examined—CHINA, Hunan Province, Changsha City, Changsha County, Jinjing Town, 28°58′52″ N, 113°34′38″ E, alt. 63 m, on leaf spots of *Castanea henryi*, 10 November 2020, C.M. Tian & N. Jiang (BJFC-S1823; living culture, JJ 2B); *ibid*. (living cultures, CFCC 54576 = JJ12, JJ14, JJ27-1); Hunan Province, Shaoshan City, Yintian Town, 27°52′04″ N, 112°35′03″ E, alt. 73 m, on leaf spots of *Castanea mollissima*, 9 November 2020, C.M. Tian & N. Jiang (living culture, CFCC 54585 = SS9-1); Beijing City, Haidian District, 40°00′30″ N, 116°20′26″ E, alt. 23 m, on leaf spots of *Castanea mollissima*, 28 August 2020, N. Jiang (BJFC-S1797).

Notes—The genus *Botryosphaeria* is presently not well separated phylogenetically [24–26]. In the phylogenetic inference of ITS, tef1 and tub2 sequence data (Figure A2), four new isolates from this study are close to *B. australasianum*, *B. dothidea*, *B. minutispermatia*, *B. qinlingensis*, *B. sinensis* and *B. wangensis*. Phylogenetic analyses based on more collections of this genus are necessary to clarify species concept in the future. In present study, we provide the morphology and phylogeny of samples from *Castanea* leaves, and identified these isolates as *Botryosphaeria dothidea s.l.*
Table 3. Fungal species from *Castanea* leaves.

| Phylum         | Class                | Order        | Family          | Species                        | Phgeny     | Morphology |
|----------------|----------------------|--------------|-----------------|--------------------------------|------------|------------|
| Ascomycota     | Dothideomycetes      | Botryosphaerales | Aplosporellaceae | *Aplosporella prunicola*        | Figure A1  | NA         |
|                |                      |              | Botryosphaerales | *Botryosphaeria dothidea*       | Figure A2  | Figure 2   |
|                |                      |              | Phyllostictaceae | *Phyllosticta capitulenta*      | Figure A3  | Figures 3 and 4 |
|                | Dothideales          | Saccotheciaceae | *Aureobasidium castaneae* |                  | Figure 5   | Figure 6   |
|                | Pleosporales         | Didymellaceae | *Didymella coffea-arabicae* |                  | Figure A4  | Figure 7   |
|                |                      |              | *Arthrinium arundinis* |                  | Figure A5  | NA         |
|                | Amphisphaerales      | Sporocadaceae | *Arthrinium arundinis* |                  | Figure A6  | Figure 8   |
|                |                      |              | *Discocasia castaneae* |                  | Figure 9   | Figure 10  |
|                |                      |              | *Monochaetia castaneae* |                  | Figure 11  | Figure 12  |
|                |                      |              | *Neopestalotiopsis asiatica* |                  | Figure 13  | Figure 14  |
|                |                      |              | *Neopestalotiopsis brasiliensis* |                  | Figure 13  | Figure 15  |
|                |                      |              | *Neopestalotiopsis sichuanensis* |                  | Figure 13  | Figure 16  |
|                |                      |              | *Neopestalotiopsis sp.1* |                  | Figure 13  | Figure 17  |
|                |                      |              | *Neopestalotiopsis sp.2* |                  | Figure 13  | Figure 18  |
|                | Sordariomycetes      |               | *Neopestalotiopsis kenjana* |                  | Figure A7  | Figure 19  |
|                |                      |              | *Robillarda sessilis* |                  | Figure A8  | Figure 20  |
|                | Diaporthales         | Diaporthaceae | *Diaporthe lithocarpi* |                  | Figure A9  | Figure 21  |
|                |                      | Gnemoniaceae | *Gnomoniopsis daei* |                  | Figure A10 | Figure 22  |
|                |                      | Pyrisporaceae | *Pyrispora castaneae* |                  | Figure 23  | Figures 24 and 25 |
|                |                      | Schizoparmaceae | *Coniella castaneicola* |                  | Figure A11 | Figure 26  |
|                | Glomerellaes         | Tubakiaceae  | *Tubakia dryinoides* |                  | Figure A12 | Figure 27  |
|                |                      |              | *Colletotrichum fructicola* |                  | Figure A13 | NA         |
|                |                      |              | *Colletotrichum henanense* |                  | Figure A13 | NA         |
|                |                      |              | *Colletotrichum jiangxiiense* |                  | Figure A13 | NA         |
|                |                      |              | *Colletotrichum karsti* |                  | Figure A14 | NA         |
|                |                      |              | *Colletotrichum nymphaeae* |                  | Figure A15 | NA         |
Figure 2. *Botryosphaeria dothidea* (BJFC-S1797). (A, B) Conidiomata on the diseased leaves of *Castanea mollissima*; (C) Section through the pycnidium; (D) Conidiogenous cells giving rise to conidia; (E, F) Conidia. Scale bars: (C) = 15 µm; (C–F) = 10 µm.

3.3. *Phyllosticta* Pers.

*Phyllostictacapitalensis* Henn., Hedwigia 48: 13 (1908) (Figures 3 and 4)

Pathogenic on *Castanea henryi* and *C. mollissima* leaves. Sexual morph: Ascomata 80–200 µm diam., globose to pyriform, black. Asci 62–85 × 8–12.5 µm, bitunicate, clavate to broadly fusoid-ellipsoid. Ascospores (13.2–)14.6–16.2(–17.4) × (3.7–)4.1–5.2(–6.8) µm (x = 15.1 × 4.5 µm, n = 30), L/W = (2.8–)2.9–4(–4.1) (x = 3.5, n = 30), bi-seriate, hyaline, smooth, 1-septate or aseptate, guttulate, straight and slightly curved, widest in the middle, limoniform with obtuse ends, with distinct hyaline gelatinous caps at both ends, 3–5 × 1.5–3 µm. Asexual morph: Conidiomata 100–250 µm diam., pycnidial, scattered or aggregated, globose to subglobose, black. Pycnidial wall of several layers, composed of cells of textura angularis, thick, inner wall of hyaline textura angularis cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 3–12 × 2.5–5.5 µm, terminal, subcylindrical to ampulliform to doliiform, hyaline, smooth, proliferating several times percurrently near apex. Conidia (9–)10–11.8(–12.4) × (6.8–)7.4–8.3(–8.6) µm (x = 10.9 × 7.8 µm, n = 50), L/W = (1.1–)1.3–1.5(–1.6) (x = 1.4, n = 50), solitary, hyaline, aseptate, thin and smooth walled, with a single large central guttulate, obovoid, tapering towards a narrow truncate base, enclosed in a persistent mucoid sheath, 2–7 µm thick.

Culture characteristics—Colonies on PDA reaching up to 40 mm in 10 days, flat, initially white-grey mycelium, gradually becoming greenish to dark brown, with white hyphae at the margin; reverse black.

Material examined—CHINA, Hunan Province, Changsha City, Changsha County, Jinjiejing Town, 28°58′52″ N, 113°34′38″ E, alt. 63 m, on leaf spots of *Castanea henryi*, 10 November 2020, C.M. Tian & N. Jiang (BJFC-S1821; living culture, JJ4); *ibid.* (BJFC-S1822; living culture, CFCC 54576 = JJ11); *ibid.* (living cultures, CFCC 54577 = JJ16, JJ20); Hunan Province, Shaoshan City, Yintian Town, 27°52′04″ N, 112°35′03″ E, alt. 73 m, on leaf spots of *Castanea mollissima*, 9 November 2020, C.M. Tian & N. Jiang (BJFC-S1820; living culture, SS5); *ibid.* (living cultures, SS10, SS13, SS15, SS16-1, SS16-2); Hunan Province, Xiangtan City, 27°48′51″ N, 112°71′42″ E, alt. 85 m, on leaf spots of *Castanea mollissima*, 9 November 2020, N. Jiang (BJFC-S1819; living culture, XT2); *ibid.* (living cultures, XT10, XT11, XT16-1, XT16-2, XT17); Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N,
105°10′35″ E, alt. 1280 m, on leaf spots of *Castanea mollissima*, 5 September 2020, N. Jiang (living cultures, ZX6, ZX11-1); Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of *Castanea mollissima*, 7 September 2020, N. Jiang (living culture, ZY6-1).

Notes—The genus *Phyllosticta* is a worldwide genus of pathogens, endophytes and saprobes, which was separated into six species complexes [27]. In the present study, we observed the sexual and asexual morphs on *Castanea* leaves, and identified them as *Phyllosticta capitalensis* s. s. based on the phylogenetic inference of ITS, LSU, *act*, *gapdh* and *tef1* sequence data (Figure A3).

**Figure 3.** *Phyllosticta capitalensis* (BJFC-S1821). (A,B) Ascostromata on the diseased leaves of *Castanea mollissima*; (C,D) Asci; (E,F) Ascospores. Scale bars: (C–F) = 10 μm.

**Figure 4.** *Phyllosticta capitalensis* (BJFC-S1820). (A,B) Conidiomata on the diseased leaves of *Castanea mollissima*; (C) Conidiogenous cells giving rise to conidia; (D–F) Conidia. Scale bars: (C–F) = 10 μm.

### 3.4. *Aureobasidium* Viala & G. Boyer

*Aureobasidium castaneae* C.M. Tian & N. Jiang, sp. nov. (Figure 6)
MycoBank: MB 838314

Etymology—named after the host genus, Castanea.

Holotype—BJFC-C007

Endophytic or pathogenic on Castanea henryi leaves. Sexual morph: Undetermined. Asexual morph was observed on PDA: Mycelium immersed, no aerial mycelium. Hyphae 3–7 µm wide, smooth, thin-walled, septe, hyaline or brown. Conidiogenous cells undifferentiated, hyaline or brown, intercalary or rarely terminal. Conidia (5.9–)7.1–10.6(–11.8) × (2.7–)2.7–4.7(–6.2) µm (\( \bar{x} = 8.9 \times 3.7 \mu m, n = 50 \)), L/W = (1.8–)2.1–2.8(–3.1) (\( \bar{x} = 2.4, n = 50 \)), hyaline or brown based on the color of conidiogenous cells, variable in size, ellipsoidal, straight, rarely slightly curved, with rounded to subtruncate base and a flat basal hilum, thin-walled, with two or more guttules.

Culture characteristics—Colonies on PDA reaching up to 40 mm in 7 days, spreading, smooth, flat, rapidly turning to olivaceous black, with dark green, irregular margins, covered with slimy masses of conidia.

Material examined—CHINA, Hunan Province, Changsha City, Changsha County, Jinjing Town, 28°58′52″ N, 113°34′38″ E, alt. 63 m, on leaf spots of Castanea henryi, 10 November 2020, C.M. Tian & N. Jiang (BJFC-C007, holotype; ex-type living culture, CFCC 54591 = JJ 7-3).

Notes—Aureobasidium is a well-known genus comprising saprophytes, endophytes and pathogens on various substrates [28–32]. A. castaneae from Castanea henryi in this study is closely related to A. thailandense from unknown plants based on phylogenetic inference of ITS and LSU sequence data (Figure 5). However, A. castaneae can be easily distinguished from A. thailandense by conidial size (7.1–10.6 × 2.7–4.7 µm in A. castaneae vs. 3–10 × 5–12 µm in A. thailandense) [31].

Figure 5. Phylogram generated from RAxML analysis based on combined ITS and LSU sequence data of Aureobasidium isolates. The tree was rooted to Sydowia polyspora (CBS 544.95). The scale bar indicates 0.03 nucleotide changes per site. Isolate from this study is marked in blue, and the identified species is marked in yellow.
3.5. Didymella Sacc.

*Didymella coffeae-arabicae* Qian Chen & L. Cai, Stud. Mycol. 82: 175 (2015) (Figure 7)

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 120–200 µm diam., 100–180 µm high, pycnidial, conspicuous, stromatic, scattered or aggregated, globose to subglobose, dark brown, with septe and dark hyphal outgrowths. Ostioles single, centric. Pycnidial wall pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, 8–20 µm thick. Conidiogenous cells phialidic, hyaline, simple, smooth, flask-shaped to globose. Conidia (3.1–)3.7–4.8(–4.9) × (1.8–)2.1–2.7(–2.8) µm (τ = 4.3 × 2.4 µm, n = 50), L/W = (1.3–)1.5–2.2(–2.6) (τ = 1.8, n = 50), ellipsoidal to ovoid, thin-walled, smooth, hyaline, always aseptate, eguttulate or with several minute apolar guttules.

Culture characteristics—Colonies on PDA reaching up to 70 mm in 7 days, margin regular, covered by felty aerial mycelium, olivaceous, mouse grey towards periphery, reverse dark mouse grey.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (BJFC-S1792; living culture, CFCC 54343 = SM24); *ibid.* (living culture, SM24B).

Notes—*Didymella* is a famous pathogenic genus mainly occurring on plant leaves [33–37]. Phoma-like species are difficult to identify to the genus and species level due to a lack of good characteristics, making molecular data are essential during species identification [34,35,37]. The new isolates (CFCC 54343 and SM24B) from *Castanea mollissima* grouped with *Didymella coffeae-arabicae* on Coffea arabica with high statistical support (Figure A4). Our collection also shared similar morphology in conidial size with *Didymella coffeae-arabicae* [33].
Figure 7. Didymella coffeae-arabicae (BJFC-S1792). (A, B) Conidiomata on the diseased leaves of Castanea mollissima; (C) Section through the pycnidium; (D) Conidiogenous cells giving rise to conidia (arrows); (E, F) Conidia. Scale bars: (C–F) = 10 µm.

3.6. Arthrinium Kunze

Arthrinium arundinis (Corda) Dyko & B. Sutton, Mycotaxon 8(1): 119 (1979)

Endophytic or pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph was observed on PDA: Mycelium consisting of smooth, hyaline, branched, septate, 1.5–3 µm diam hyphae. Conidiogenous cells 5.5–15 × 2.5–3.5 µm, aggregated in clusters on hyphae, pale brown, smooth, ampulliform. Conidia 3–4 µm diam. (X = 3.4 µm, n = 50), brown, smooth, globose, with pale equatorial slit.

Culture characteristics—Colonies on PDA reaching up to 70 mm in 7 days, flat, spreading, covered by iron-grey aerial mycelium, reverse grey.

Material examined—CHINA, Hunan Province, Xiangtan City, 27°48′51″ N, 112°71′42″ E, alt. 85 m, on leaf spots of Castanea mollissima, 9 November 2020, N. Jiang (living culture, XT18-1).

Notes—Arthrinium is a common genus on a wide range of substrates, including soil, plant debris, plants, lichens, marine algae, and human tissues [38–42]. The new isolate (XT18-1) from Castanea mollissima grouped with A. arundinis isolates with high statistical support (Figure A5). Therefore, we identify our new isolate as A. arundinis and Castanea mollissima as a new host record for the fungus.

3.7. Bartalinia Tassi

Bartalinia pini F. Liu, L. Cai & Crous, Stud. Mycol. 92: 309 (2019) (Figure 8)

Pathogenic on Castanea henryi leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 85–160 µm diam., 50–120 µm high, acervular, conspicuous, stromatic, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, septate, smooth, hyaline, invested in mucus. Conidiogenous cells 4.5–15 × 1.5–4 µm, annellidic, discrete, ampulliform. Conidia (18.2–)19–21.5(–22.1) × (3.4–)3.6–4.2(–4.5) µm (X = 20.1 × 3.8 µm, n = 50), L/W = (4.5–)4.7–5.9(–6.2) (X = 5.3, n = 50), cylindrical with acute or obtuse ends, straight or slightly curved, 4-septate, smooth; basal cell obconic with a truncate base, thin-walled, hyaline, 1.5–3.5 µm long; median cells 3, cylindrical, pale brown, each 3.5–7.5 µm long; apical cell conic with an acute apex, thin-walled, hyaline, 2–3.5 µm
long; apical appendage with three branches, tubular, filiform, flexuous, divergent, 5–25 µm long; basal appendage tubular, unbranched, excentric, 3–8 µm long.

Culture characteristics—Colonies on PDA reaching up to 70 mm in 10 days, flat with entire edge, glaucous grey to grey.

Material examined—CHINA, Hunan Province, Changsha City, Changsha County, Jinjing Town, 28°58'52" N, 113°34'38" E, alt. 63 m, on leaf spots of *Castanea henryi*, 10 November 2020, C.M. Tian & N. Jiang (BJFC-S1808; living culture, CFCC 54574 = JJ4).

Notes—*Bartalinia* is morphologically distinct from the other Sporocadaceous genera by its conidial characters [43]. The new isolate (CFCC 54574) from *Castanea henryi* grouped with *B. pini* isolates from *Pinus patula* (Pinaceae) needles and leaves of *Acacia koa* (Fabaceae) (Figure A6) and overlapped in morphology with *B. pini* [43]. Therefore, we identify our new isolate as *B. pini*, China as a new geographical record and *Castanea henryi* as a new host record for the fungus.

Figure 8. *Bartalinia pini* (BJFC-S1808). (A,B) Conidiomata on the diseased leaves of *Castanea henryi*; (C) Conidiogenous cells giving rise to conidia; (D–H) Conidia. Scale bars: (C–F) = 10 µm.

3.8. *Discosia* Lib. ex Durieu & Mont.

*Discosia castaneae* C.M. Tian & N. Jiang, sp. nov. (Figure 10)

Mycobank: MB837794

Etymology—named for the host genus, *Castanea*.

Holotype—BJFC-S1805

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 100–170 µm diam., 20–40 µm high, acervular, conspicuous, stromatic, scattered or aggregated, rounded, black, glabrous, epidermal. Conidiophores absent. Conidiogenous cells 3–7 × 1–3 µm, holoblastic to phialidic, ampulliform, integrated, hyaline, smooth-walled. Conidia (14.1–)15.4–17(–18.7) × (2.9–)3.1–3.8(–3.9) µm (x = 16.2 × 3.5 µm, n = 50), L/W = (3.9–)4.2–5.3(–5.5) (x = 4.8, n = 50), cylindrical to allantoid, initially hyaline, becoming pale brown at maturity, smooth-walled, guttulate, 3-euseptate, slightly constricted at septa, thin-walled; with basal cell obconic, slightly truncate at the base and appendaged; 2 median cells subcylindrical, with second cell from the base 5–8 µm long and third cell 3–6 µm long; apical cell subconical with a obtuse apex; apical and basal cells each with a single, simple, unbranched, filamentous appendage at the ends, apical appendage 5–12 µm and basal appendage 5–10 µm.

Culture characteristics—Colonies on PDA reaching up to 50 mm in 10 days, flat with entire edge, forming concentric circles, olivaceous black, sterile.
Material examined—CHINA, Beijing City, Miyun District, Xinchengzi Town, Potou Village, 40°60′28″ N, 117°36′02″ E, alt. 65 m, on leaf spots of *Castanea mollissima*, 29 October 2017, C.M. Tian & N. Jiang (BJFC-S1805, holotype; ex-type living cultures, CFCC 54088 = CML1, CML2). Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of *Castanea mollissima*, 5 September 2020, N. Jiang (living culture, CFCC 54352 = ZX22A). Hunan Province, Shaoshan City, Yintian Town, 27°52′04″ N, 112°35′03″ E, alt. 73 m, on leaf spots of *Castanea mollissima*, 9 November 2020, C.M. Tian & N. Jiang (BJFC-S1806, living culture, CFCC 54584 = SS3).

Notes—*Discosia* was recorded on leaf spots of Chinese chestnut leaves in the monograph of chestnut diseases [44]. However, the fungus was not identified to the species level. In present study, we collected *Discosia* samples from Beijing (North China), Hunan and Yunnan (South China), and found it different from any known species [43,45,46]. From the phylogram (Figure 9), *Discosia brasiliensis* was the closest species to *D. castaneae*, but they could be distinguished by their conidial width (3.5–4.5 µm in *D. castaneae* vs. 2–3 µm in *D. brasiliensis*) [46].

**Figure 9.** Phylogram generated from RAxML analysis based on combined ITS, LSU and *tub2* sequence data of *Discosia* isolates. The tree was rooted to *Immersidiscosia eucalypti* (CBS 544.95) and *Seimatosporium rosea* (CBS 139823). The scale bar indicates 0.03 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.
Figure 10. Discosia castaneae (BJFC-S1805). (A,B) Conidiomata on the diseased leaves of Castanea mollissima; (C) Section through the conidioma; (D,E) Conidiogenous cells giving rise to conidia; (F–I) Conidia. Scale bars: (C–I) = 10 μm.

3.9. Monochaetia (Sacc.) Allesch.

Monochaetia castaneae C.M. Tian & N. Jiang, sp. nov. (Figure 12)
MycoBank: MB837793
Etymology—named for the host genus, Castanea.
Holotype—BJFC-S1807
Pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 120–230 μm diam., 20–50 μm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores cylindrical, hyaline, smooth-walled. Conidiogenous cells 12–20 × 1.5–2.5 μm, phialidic, ampulliform, discrete, hyaline, smooth-walled. Conidia (18.8–)20–24(–27.3) × (4.7–)5.4–6.2(–6.6) μm (x = 22 × 5.8 μm, n = 50), L/W = (3.1–)3.2–4.5(–5.8) (x = 3.8, n = 50), fusiform, tapering at both ends, 4-septate; basal cell 2.5–4.0 μm long, conic, hyaline and smooth-walled; three median cells each 3.5–5.5 μm long, doliiform, light brown, rough-walled; apical cell 3.0–4.5 μm long, conic, hyaline and smooth-walled; apical appendage 17.5–35 μm long, single, tubular, filiform; basal appendage 10–20 μm long, single, central, tubular, filiform.

Culture characteristics—Colonies on PDA reaching up to 40 mm in 15 days, flat, spreading, with sparse aerial mycelium and smooth, lobate margin, cinnamon, reverse isabelline. Fruiting bodies were observed after 20 days.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″N, 102°21′27″E, alt. 978 m, on leaf spots of Castanea mollissima, 10 September 2020, N. Jiang (BJFC-S1807, holotype; ex-type living cultures, CFCC 54354 = SM9-1, SM9-2).

Notes—Monochaetia concentrica and M. kansensis were recorded to inhabit Castanea leaves, but Monochaetia castaneae from present study is narrower than them (4.7–6.6 μm in
M. castaneae vs. 6.5–8.5 µm in M. concentrica vs. 6.0–8.0 µm in M. kansensis) [47]. Monochaetia castaneae is phylogenetically close to M. junipericola from twigs of Juniperus communis (Figure 11), but they are distinguished by hosts and conidial sizes (18.8–27.3 × 4.7–6.6 µm in M. castaneae vs. 22–28 × 5.0–7.0 µm in M. junipericola) [48].

Figure 11. Phylogram generated from RAxML analysis based on combined ITS sequence data of Monochaeta isolates. The tree was rooted to Distononappendiculata banksia (CBS 131308). The scale bar indicates 0.02 nucleotide changes per site. Isolates from this study are highlighted. Isolates from this study are marked in blue, and the identified species is marked in yellow.
3.10. Neopestalotiopsis Maharachch., K.D. Hyde & Crous

*Neopestalotiopsis asiatica* Maharachch., K.D. Hyde & Crous, Stud. Mycol. 79: 136 (2014) (Figure 14)

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 80–200 µm diam., 20–150 µm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 4–10 × 1.5–4 µm, discrete, thin-walled, lageniform, subcylindrical or irregular. Conidia (15.4–)17.6–23.1(–25.5) × (4.6–)5.4–7.8(–8.4) µm (x = 19.4 × 6.2 µm, n = 50), L/W = (2.2–)2.7–3.5(–4) (x = 3.1, n = 50), basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 4–5.5 µm long; three median cells doliiform, versicolourous, second cell from base pale brown to olivaceous, 4.5–6 µm long; third cell honey brown, 5–6.5 µm long; fourth cell brown, 4.5–5.5 µm long; apical cell 3.5–5 µm long, hyaline, subcylindrical, rugose and thin-walled; with 3 (seldom 4) tubular apical appendages, arising from the apical crest, unbranched, filiform, 23–35 µm long; basal appendage present, 3.5–8.5 µm long.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 10 days, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 20 days.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (BJFC-S1789, living culture, CFCC 54339 = SM32); *ibid.* (living cultures, SM7, SM8B, SM11, SM19B).

Notes—*Neopestalotiopsis asiatica* was described based on the endophytic isolate from unknown trees in China under the name of *Pestalotiopsis asiatica* [49], but subsequently transferred to the genus *Neopestalotiopsis* [50]. In the present study, several isolates of *N. asiatica* were obtained from Chinese chestnut diseased leaves (Figure 13).
Figure 13. Phylogram generated from RAxML analysis based on combined ITS tef1 and tub2 sequence data of Neopestalotiopsis isolates. The tree was rooted to Pestalotiopsis trachicarpica (OP608) and Pseudopestalotiopsis cocos (CBS 272.39). The scale bar indicates 0.02 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species are marked in yellow.
3.10.1. *Neopestalotiopsis brasiliensis*

*Neopestalotiopsis brasiliensis* V.P. Abreu, A.W.C. Rosado & O.L. Pereira, Acta Bot. Brasilia 32(4): 661 (2018) (Figure 15)

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 50–150 µm diam., 30–90 µm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 6–15 × 1.5–3.5 µm, discrete, thin-walled, lageniform, subcylindrical or irregular. Conidia (18.5–)19.5–24.1(–26.1) × (5–)5.3–6.4(–6.8) µm (T = 21.8 × 5.9 µm, n = 50), L/W = (2.9–)3.2–4.3(–4.4) (T = 3.8, n = 50), fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 3–5.5 µm long; three median cells doliform, versicolourous, second cell from base pale brown to olivaceous, 4–6.5 µm long; third cell honey brown, 4.5–5.5 µm long; fourth cell brown, 4.5–6 µm long; apical cell 3.5–5.5 µm long, hyaline, subcylindrical, rugose and thin-walled; with 2 (seldom 3) tubular apical appendages, arising from the apical crest, unbranched, filiform, 8–20 µm long; basal appendage present, 2–5.5 µm long.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 15 days.

Material examined—CHINA, Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of *Castanea mollissima*, 7 September 2020, N. Jiang (BJFC-S1791, living culture, CFCC 54341 = ZY4); ibid. (living culture, ZY4-2D).

Notes—*Neopestalotiopsis brasiliensis* was described from rotted fruits of *Psidium guajava* in Brazil [51]. In present study, strains from diseased chestnut leaves formed a supported clade with the ex-type strain COAD 2166 (Figure 13), and shared similar morphology. Hence, we identified our strains as *N. brasiliensis*, which represented a new host and geographical record.
3.10.2. Neopestalotiopsis sichuanensis

C.M. Tian & N. Jiang, sp. nov. (Figure 16)

MycoBank: MB 837792

Etymology—named for the location of the type specimen.

Holotype—BJFC-S1788

Pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 100–250 µm diam., 50–150 µm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 7–20 × 2–6 µm, discrete, thin-walled, lageniform, subcylindrical or irregular. Conidia (23.2–)24.3–30.4(–32.8) × (5.7–)6.3–7.1(–7.5) µm (x = 27.3 × 6.7 µm, n = 50), L/W = (3.4–)3.5–4.6(–5.1) (x = 4.1, n = 50), fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 3.5–5 µm long; three median cells doliiform, versicolourous, second cell from base pale brown to olivaceous, 3.5–6 µm long; third cell honey brown, 4.5–6.5 µm long; fourth cell brown, 4.5–6 µm long; apical cell 3.5–6 µm long, hyaline, subcylindrical, rugose and thin-walled; with 2 or 3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 8–15 µm long; basal appendage present, 1.5–4 µm long.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 15 days.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of Castanea mollissima, 10 September 2020, N. Jiang (BJFC-S1788, holotype; ex-type living culture, CFCC 54338 = SM15-1); ibid. (living culture, SM15-1C).

Notes—Neopestalotiopsis sichuanensis was phylogenetically close to N. cubana (Figure 13) but differed in conidial width (5.7–7.5 µm in N. sichuanensis vs. 7.5–10 µm in N. cubana) [50].
3.10.3. *Neopestalotiopsis* sp.1

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 80–150 µm diam., 30–60 µm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 5.5–15.5 × 3–7 µm, discrete, thin-walled, lageniform, subcylindrical or irregular. Conidia (19.1–23.2(–24.7) × (5.4–)7.6(–8.6) µm (τ = 21.6 × 6.7 µm, n = 50), L/W = (2.9–)3.0–3.5(–3.7) (τ = 3.2, n = 50), fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 3–4.5 µm long; three median cells doliiform, versicolourous, second cell from base pale brown to olivaceous, 3–5 µm long; third cell honey brown, 4.5–6 µm long; fourth cell brown, 4–6 µm long; apical cell 3.5–5 µm long, hyaline, subcylindrical, rugose and thin-walled; with 2 or 3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 7.5–14 µm long; basal appendage present, 3–6.5 µm long (Figure 17).

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 15 days.

Material examined—CHINA, Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of *Castanea mollissima*, 5 September 2020, N. Jiang (BJFC-S1787, living culture, CFCC 54337 = ZX12A); *ibid.* (living culture, ZX12-1).

Notes—Although phylogenetically distinct, these two isolates were not proposed as a new species for lack of distinguished characters from close clades (Figure 13).
3.10.4. *Neopestalotiopsis* sp.2

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 75–175 μm diam., 30–75 μm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 3.5–8 × 2.5–5.5 μm, discrete, thin-walled, lageniform, subcylindrical or irregular. Conidia (21.4–)22–25.2(–26.2) × (5.1–)6.2–7.7(–8.7) μm (X = 23.6 × 7 μm, n = 50), L/W = (2.9–)3–3.9(–4.4) (X = 3.4, n = 50), fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 3–5 μm long; three median cells doliform, versicolourous, second cell from base pale brown to olivaceous, 4.5–6.5 μm long; third cell honey brown, 4.5–6 μm long; fourth cell brown, 4–6 μm long; apical cell 3.5–5 μm long, hyaline, subcylindrical, rugose and thin-walled; with 2 (seldom 3) tubular apical appendages, arising from the apical crest, unbranched, filiform, 10–25 μm long; basal appendage present, 1.5–5 μm long (Figure 18).

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 15 days.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (BJFC-S1790, living culture, CFCC 54340 = SM14); Yunnan Province, Zhaotong City, Zhenxiang County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of *Castanea mollissima*, 5 September 2020, N. Jiang (living culture, ZX22B).

Notes—Although phylogenetically distinct, these two isolates were not proposed as a new species for lack of distinguished characters from close clades (Figure 13).
3.11. Pestalotiopsis Steyaert

*Pestalotiopsis kenyana* Maharachch., K.D. Hyde & Crous, Stud. Mycol. 79: 166 (2014) (Figure 19)

Pathogenic on *Castanea henryi* and *C. mollissima* leaves. Sexual morph: Undetermined.
Asexual morph: Conidiomata 50–250 μm diam., 30–150 μm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 5–19 × 2.5–6.5 μm, discrete, thin-walled, lageniform, subcylindrical or irregular. Conidia (20.4–)21.8–26.2(–28) × (6.1–)6.3–7.4(–8) μm (T = 23.8 × 6.9 μm, n = 50), L/W = (3–)3.1–3.9(–4.2) (T = 3.5, n = 50), fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 3–5 μm long; three median cells doliform, concolourous, brown, second cell 3–5.5 μm long; third cell 4.5–6 μm long; fourth cell 4.5–6 μm long; apical cell 3.5–5.5 μm long, hyaline, subcylindrical, rugose and thin-walled; with 3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 3.5–15 μm long; basal appendage present, 1.5–3.5 μm long.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 15 days.

Material examined—CHINA, Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of *Castanea mollissima*, 5 September 2020, N. Jiang (BJFC-S1784, living culture, CFCC 54336 = ZX11); *ibid.* (living culture, ZX3, ZX7, ZX9, ZX18A); Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of *Castanea mollissima*, 7 September 2020, N. Jiang (BJFC-S1786, living culture, ZY6-2A); *ibid.* (living culture, ZY7); Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (BJFC-S1785, living culture, SM18); Hunan Province, Changsha City, Changsha County, Jinjing Town, 28°58′52″ N, 113°34′38″ E, alt. 63 m, on leaf spots of *Castanea henryi*, 10 November 2020, C.M. Tian & N. Jiang (BJFC-S1817; living culture, JJ 2A); *ibid.* (living cultures, JJ5, JJ10, JJ13, JJ15, JJ17, JJ18, JJ26).
Notes—Pestalotiopsis kenyana was proposed from Coffea sp. in Kenya [50]. Strains collected from Castanea mollissima in present study formed a supported clade with Pestalotiopsis kenyana (Figure A7), and shared similar morphology. Hence, we identified our strains as P. kenyana, which represented a new host record.

Figure 19. Pestalotiopsis kenyana (BJFC-S1784). (A,B) Conidiomata on the diseased leaves of Castanae mollissima; (C) Conidiogenous cells giving rise to conidia; (D–H) Conidia. Scale bars: (C–H) = 10 µm.

3.12. Robillarda Sacc.

Robillarda sessilis (Sacc.) Sacc., Michelia 2(no. 6): 8 (1880) (Figure 20)

Pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 100–200 µm diam., 20–60 µm high, acervular, conspicuous, scattered or aggregated, rounded, black. Conidiophores reduced to conidiogenous cells, smooth, hyaline. Conidiogenous cells 2.5–6 × 1.5–3 µm, discrete, thin-walled, guttulate or not, lageniform, ampulliform or irregular. Conidia composed of a 1-septate conidium body and a septate apical cell modified into a branched appendage. Conidium body (10.7–)11.2–13.1(=13.5) × (2.9–)3.1–3.8(=3.9) µm (x = 12.2 × 3.4 µm, n = 50), L/W = (2.9–)3.1–4.1(=4.5) (x = 3.6, n = 50), cylindrical, straight, 1-septate, smooth, hyaline to pale brown, guttulate, slightly constricted at the median septum; apical cell cylinindrical for 4.0–5.0 µm long, then dividing into 2–4 (mostly 3) divergent branches; apical appendages unbranched, attenuated, 15–28 µm long; basal appendages absent.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 15 days, flat with entire edge, white, aerial mycelia villiform. Fruiting bodies were observed after 20 days.

Material examined—CHINA, Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of Castanea mollissima, 5 September 2020, N. Jiang (BJFC-S1804, living culture, CFCC 54353 = ZX5); ibid. (living culture, ZX5-1); Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of Castanea mollissima, 7 September 2020, N. Jiang (living culture, ZY5-1).

Notes—Robillarda sessilis was documented from quite variable hosts, such as Bischofia, Cocos, Ficus, Fragaria, Fumana, Ludwigia, Magnolia, Paeonia, Quercus, Randia, Rosa, Rubus, and Vitis [52]. Strains from present study clustered with the ex-epitype strain (CBS 114312) of Robillarda sessilis (Figure A8). This is the first report of this fungus on the host Castanea mollissima, and in China as a country.
Figure 20. *Robillarda sessilis* (BJFC-S1804). (A, B) Conidiomata on the diseased leaves of *Castanea mollissima*; (C, D) Conidiogenous cells giving rise to conidia; (E–I) Conidia. Scale bars: (C–I) = 10 µm.

3.13. *Diaporthe* Nitschke

*Diaporthe lithocarpi* Y.H. Gao & L. Cai, Fungal Biology 119(5): 306 (2015) (Figure 21)

Pathogenic on *Castanea henryi* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 120–3000 µm diam., pycnidial, aggregated, globose to subglobose, black. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 2.5–12.5 × 1.5–3 µm, cylindrical, hyaline, phialidic, unbranched, straight. Conidia (7.6–)7.9–9.1(–9.8) × (2.5–)2.6–3(–3.1) µm (x = 8.5 × 2.8 µm, n = 50), L/W = (2.4–)2.7–3.4(–3.7) (x = 3.1, n = 50), aseptate, hyaline, ellipsoidal, biguttulate, mostly with one end obtuse and the other acute.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, flat, initially white mycelium, gradually becoming pale brownish, with cottony aerial mycelium and fringed margin; reverse pale yellowish.

Material examined—CHINA, Hunan Province, Changsha City, Changsha County, Jinjing Town, 28°58′52″ N, 113°34′38″ E, alt. 63 m, on leaf spots of *Castanea henryi*, 10 November 2020, C.M. Tian & N. Jiang (BJFC-S1809; living culture, CFCC 54573 = JJ3); ibid. (living cultures, JJ3-2, JJ26B).

Notes—*Diaporthe lithocarpi* was reported to cause leaf spots on *Lithocarpus glabra*, *Loropetalum chinensis*, *Smilax china*, *S. glabra* and *Ternstroemia gymnanthera* in Zhejiang Province of China [53]. The present study adds a new *Castanea henryi* host for the fungus based on the phylogenetic inference of ITS, *cal*, *his*, *tef1* and *tub2* sequence data (Figure A9), and exactly matched morphology.
Figure 21. *Diaporthe lithocarpi* (BJFC-S1809). (A, B) Conidiomata on the diseased leaves of *Castanea henryi*; (C) Conidiogenous cells giving rise to conidia; (D) Section through the pycnidium; (E, F) Conidia. Scale bars: (C–F) = 10 μm.

3.14. *Gnomoniopsis* Berl.

*Gnomoniopsis dainii* C.M. Tian & N. Jiang, Forests 10(11/1016): 6 (2019) (Figure 22)

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 30–100 μm diam., pycnidial, aggregated, globose to pulvinate, black, single ostiolate, forming long and yellow tendrils. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 2.5–18 × 1.5–3 μm, cylindrical, hyaline, phialidic, unbranched, straight. Conidia (4.9–)5.1–5.9(–6.2) × (2.4–)2.5–2.6(–2.7) μm (μ = 5.5 × 2.5 μm, n = 50), L/W = (1.9–)2–2.2(–2.5) (μ = 2.2, n = 50), aseptate, hyaline, ellipsoidal, guttulate.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, flat, velutinous to shortly woolly, dark brown in center, gradually lightening to pale grey at margin; margin diffuse; reverse dark brown to grey.

Material examined—CHINA, Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of *Castanea mollissima*, 5 September 2020, N. Jiang (BJFC-S1794, living culture, ZX14-1); Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of *Castanea mollissima*, 7 September 2020, N. Jiang (BJFC-S1795, living culture, CFCC 54345 = ZY11); ibid. (living cultures, ZY10-1, ZY10-3, ZY12A).

Notes—The fungus *Gnomoniopsis smithogilvyi* causes fruit rot and branch canker diseases on *Castanea sativa* in Australia and Europe [4,5]. Interestingly, similar symptoms on *Castanea mollissima* in China were caused by two different species. *Gnomoniopsis chinese* causes branch canker, and *G. dainii* causes fruit rot [54,55]. In this study, we confirmed the *Gnomoniopsis* pathogen on leaves in China as *G. dainii* based on the phylogenetic inference of ITS, *tef1* and *tub2* sequence data (Figure A10), and exactly matched morphology.
3.15. Pyrisporaceae C.M. Tian & N. Jiang

3.15.1. Pyrisporaceae C.M. Tian & N. Jiang, fam. nov.

MycoBank: MB 838315

Etymology—named from the type genus, *Pyrispora*.

Type genus—*Pyrispora* C.M. Tian & N. Jiang

Pathogenic or saprobic on *Castanea mollissima* leaves. Sexual morph: Ascomata semi-immersed, aggregated, globose to pulvinate, black, single ostiolate. Ostioles single, dark grey to black. Paraphyses deliquescent. Asci cylindrical to clavate, 8-spored, bi-seriate, with a distinct apical ring. Ascospores aseptate, hyaline, smooth, fusoid, multiguttulate, straight to slight curved. Asexual morph: Conidiomata pycnidial, aggregated, globose to subglobose, black, single ostiolate. Conidiophores reduced to conidiogenous cells. Conidiogenous cells pyriform base with long neck, hyaline, phialidic, unbranched, straight. Conidia aseptate, hyaline, smooth, ellipsoidal, multiguttulate.

Notes—The fungal order Diaporthales was well-classified based on both morphology and phylogeny in recent years (Figure 23) [56–59]. In this study, the sexual morph was observed on *Castanea* leaves, showing the typical characters of Diaporthales, the asci with distinct apical ring. Additionally, the asexual morph is distinctive based on the conidiogenous cells with pyriform base and long neck. Hence, we proposed a new family to accommodate this species.
Figure 23. Cont.
Figure 23. Phylogram generated from RAxML analysis based on combined ITS, LSU, tef1 and rpb2 sequence data of Diaporthales isolates. The tree was rooted to Pyricularia grisea (Ina 168) and Nakataea oryzae (CBS 243.76). The scale bar indicates 0.2 nucleotide changes per site.
3.15.2. *Pyrispora* C.M. Tian & N. Jiang, gen. nov.

*MycoBank:* MB 838316

**Etymology**—named for the pyriform base of the conidiogenous cells.

**Type species**—*Pyrispora castaneae* C.M. Tian & N. Jiang

Pathogenic or saprobic on *Castanea mollissima* leaves. Sexual morph: Ascomata semi-immersed, aggregated, globose to pulvinate, black, single ostiolate. Ostioles single, dark grey to black. Paraphyses deliquescent. Asci cylindrical to clavate, 8-spored, bi-seriate, with a distinct apical ring. Ascospores aseptate, hyaline, smooth, fusoid, multiguttulate, straight to slight curved. Asexual morph: Conidiomata pycnidial, aggregated, globose to subglobose, black, single ostiolate. Conidiophores reduced to conidiogenous cells. Conidiogenous cells pyriform base with long neck, hyaline, phialidic, unbranched, straight. Conidia aseptate, hyaline, smooth, ellipsoidal, multiguttulate.

3.15.3. *Pyrispora castaneae* C.M. Tian & N. Jiang, sp. nov.

*MycoBank:* MB 838317

**Etymology**—named for the host genus, *Castanea*.

**Holotype**—BJFC-S1798

Pathogenic or saprobic on *Castanea mollissima* leaves. Sexual morph: Ascomata 80–200 µm diam., semi-immersed, aggregated, globose to pulvinate, black, single ostiolate. Ostioles 30–75 µm, diam., single, dark grey to black. Paraphyses deliquescent. Asci (41–)44.5–52(–58) × (7–)8.5–10.5(–11) µm, cylindrical to clavate, 8-spored, bi-seriate, with a distinct apical ring. Ascospores (11.4–)12.2–14.5(–14.9) × (4.3–)4.4–4.9(–5.2) µm (\(\bar{x} = 13.3 \times 4.7 \mu m, n = 50\)), L/W = (2.2–)2.5–3(–3.2) (\(\bar{x} = 2.9, n = 50\)), aseptate, hyaline, smooth, fusoid, multiguttulate, straight to slight curved. Asexual morph: Conidiomata 60–250 µm diam., pycnidial, aggregated, globose to subglobose, black, single ostiolate. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 4–7.5 × 2–3.5 µm, pyriform base with long neck, necks up to 45 µm, hyaline, phialidic, unbranched, straight. Conidia (10.4–)11.7–13(–13.9) × (4.1–)4.4–4.9(–5.5) µm (\(\bar{x} = 12.3 \times 4.5 \mu m, n = 50\)), L/W = (2.2–)2.5–3.2(–3.4) (\(\bar{x} = 2.8, n = 50\)), aseptate, hyaline, smooth, ellipsoidal, multiguttulate (Figures 24 and 25).

*Figure 24.* *Pyrispora castaneae* (BJFC-S1800). (A,B) Ascomata on the diseased leaves of *Castanea mollissima*; (C–F) Asci and ascospores. Scale bars: (C–F) = 10 µm.
Figure 25. Pyrispora castaneae (BJFC-S1798). (A, B) Conidiomata on the diseased leaves of Castanea mollissima; (C–E) Conidiogenous cells giving rise to conidia; (F) Conidia. Scale bars: (C–F) = 10 μm.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, flat, white, with cottony aerial mycelium and fringed margin; reverse pale yellowish.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of Castanea mollissima, 10 September 2020, N. Jiang (BJFC-S1798, holotype; ex-type living culture, CFCC 54349 = SM17); ibid. (BJFC-S1799, living culture, CFCC 54350 = SM20); ibid. (BJFC-S1800, living culture, SM28); ibid. (BJFC-S1801, living culture, CFCC 54351 = SM29); ibid. (BJFC-S1802, living culture, SM30); ibid. (BJFC-S1803, living culture, SM31); Hunan Province, Xiangtan City, 27°48′51″ N, 112°71′42″ E, alt. 85 m, on leaf spots of Castanea mollissima, 9 November 2020, C.M. Tian & N. Jiang (living culture, CFCC 54578 = XT01).

3.16. Coniella Höhn.

Coniella castaneicola B. Sutton, The Coelomycetes (Kew): 420 (1980) (Figure 26)

Pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 100–150 μm diam., pycnidial, conspicuous, scattered, globose to subglobose, black, single ostiolate. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 4–13.5 × 1.5–3.5 μm, simple, hyaline, smooth, tapering. Conidia (16.7–)18.4–21.3–(22.3) × (2.7–)2.8–3.2/(−3.3) μm (x = 19.9 × 3 μm, n = 50), L/W = (5.1–)6–7.2/(−7.3) (x = 6.6, n = 50), aseptate, initially hyaline, becoming pale brown, smooth, cylindrical, linear, apex acute to nearly rounded, base truncate, smooth-walled, multiguttulate, enclosed in a persistent mucoid sheath.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 10 days, flat, white, aerial mycelium spreads in irregular concentric zones. Conidiomata were formed after 15 days.

Material examined—CHINA, Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of Castanea mollissima, 7 September 2020, N. Jiang (BJFC-S1793, living culture, CFCC 54344 = ZY7-1); ibid. (living culture, ZY7-2).

Notes—The genus Coniella was well classified recently [60]. However, Coniella castaneicola was not studied for lacking of fresh collections and DNA data. In present study, we obtained fresh Coniella isolates from Castanea mollissima, and found it distinct from others in the phylogram (Figure A11). However, specimen in present study shared similar conidial morphology with the original description of Coniella castaneicola (20 × 2–2.5 μm) [61], hence we temporarily assign it to C. castaneicola.
Figure 26. Coniella castaneicola (BJFC-S1793). (A–C) Conidiomata on the diseased leaves of Castanea mollissima; (D) Conidiogenous cells giving rise to conidia; (E,F) Conidia. Scale bars: (D–F) = 10 μm.

3.17. Tubakia B. Sutton

*Tubakia dryinoides* C. Nakash., Fungal Systematics and Evolution 1: 80 (2018) (Figure 27)

Pathogenic or saprobic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata 70–120 μm diam., pycnothyrial, conspicuous, aggregated, superficial, circular or subcircular, black. Conidiophores reduced to conidiogenous cells, arising from the underside of the scutella, around the columella, radiating. Conidiogenous cells 5–14 × 2.5–5 μm, cylindrical, conical, hyaline to brown, thin-walled, smooth, apex obtuse to truncate. Conidia (10.4–)11.5–15.6(–17.7) × (5.1–)5.3–6.1(–6.5) μm (ι = 13.6 × 5.7 μm, n = 50), L/W = (1.7–)1.9–2.9(–3.4) (ι = 2.4, n = 50), aseptate, hyaline to pale brown, smooth, cylindrical to obovoid.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 14 days, flat, creamy white, aerial mycelium forming concentric rings. Conidiomata were formed after 15 days.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31" N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (BJFC-S1796; living culture, CFCC 54346 = SM10-1); ibid. (living culture, = SM10).

Notes—*Tubakia dryinoides* was described from *Castanea crenata* and *Quercus phillyraeoides* in Japan [62]. In present study, strains from diseased chestnut leaves formed a supported clade with the ex-type strain NBRC 9267 (Figure A12), and shared similar morphology. Hence, we identified our strains as *T. dryinoides*, which represented a new host and geographical record.
3.18. Colletotrichum Corda

3.18.1. Colletotrichum fructicola

*Colletotrichum fructicola* Prihast., L. Cai & K.D. Hyde, Fungal Divers. 39: 96 (2009)

Pathogenic on *Castanea henryi* and *C. mollissima* leaves. Sexual morph: Undetermined. Asexual morph was observed on PDA: Conidiomata, acervular, aggregated, orange. Conidiophores hyaline, septate, branched. Conidiogenous cells 5–17.5 × 1.5–3 µm, hyaline, cylindrical to ampulliform. Conidia (10.8–)11.2–16.9(–17.9) × (3.1–)3.2–5.4(–5.9) µm (X = 14.7 × 4.3 µm, n = 50), L/W = (3.1–)3.2–3.5(–3.6) (X = 3.4, n = 50), aseptate, hyaline, smooth, cylindrical, both ends rounded.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 5 days, flat with entire edge, aerial mycelium dense, cottony, grey to dark grey in the centre, white at the margin; reverse greyish green.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13’31” N, 102°21’27” E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (living cultures, SM6, SM9, SM13, SM14, SM16, SM30, SM31); Hunan Province, Shaoshan City, Yintian Town, 27°52’04” N, 112°35’03” E, alt. 73 m, on leaf spots of *Castanea mollissima*, 9 November 2020, C.M. Tian & N. Jiang (living cultures, SS01, SS14); Hunan Province, Xiangtan City, 27°48’51” N, 112°71’42” E, alt. 85 m, on leaf spots of *Castanea mollissima*, 9 November 2020, N. Jiang (living cultures, XT08, XT12, XT14-2, XT15); Hunan Province, Changsha City, Changsha County, Jinjing Town, 28°58’52” N, 113°34’38” E, alt. 63 m, on leaf spots of *Castanea henryi*, 10 November 2020, C.M. Tian & N. Jiang (living culture, JJ21).

Notes—*Colletotrichum fructicola* was described from *Coffea arabica* in Thailand [63], and subsequently found to infect several economic plants in China, such as *Camellia sinensis*, *Citrus sinensis*, *Morus alba*, *Pyrus pyrifolia* and *Vitis vinifera* [64]. In present study, strains from diseased chestnut leaves formed a supported clade with *Colletotrichum fructicola* (Figure A13), and shared similar morphology. Hence, we identified our strains as *C. fructicola*, and *Castanea henryi* and *C. mollissima* represented two new host records.

3.18.2. Colletotrichum henanense

*Colletotrichum henanense* F. Liu & L. Cai, Persoonia 35: 80 (2015)

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph was observed on PDA: Conidiomata, acervular, aggregated, orange. Conidiophores
hyaline, septate, branched. Conidiogenous cells 4.5–15 × 1.5–2.5 μm, hyaline, cylindrical to ampulliform. Conidia (8.8–)11.5–13.2–(17.2) × (3–)3.4–5.1–(5.8) μm (π = 12.2 × 4.3 μm, n = 50), L/W = (2.5–)2.6–2.9–(3) (π = 2.7, n = 50), aseptate, hyaline, smooth, cylindrical, both ends rounded.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 5 days, flat with entire edge, aerial mycelium dense, cottony, grey to dark grey in the centre, white at the margin; reverse greyish green.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of Castanea mollissima, 10 September 2020, N. Jiang (living cultures, SM12, SM22, SM33); Hunan Province, Shaoshan City, Yintian Town, 27°52′04″ N, 112°35′03″ E, alt. 73 m, on leaf spots of Castanea mollissima, 9 November 2020, C.M. Tian & N. Jiang (living cultures, SS02, SS04); Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of Castanea mollissima, 5 September 2020, N. Jiang (living culture, ZX2-1); Hunan Province, Changsha City, Changsha County, Kaihui Town, 28°58′12″ N, 113°25′48″ E, alt. 65 m, on leaf spots of Castanea mollissima, 10 November 2020, C.M. Tian & N. Jiang (living culture, KH1).

Notes—Colletotrichum henanense was initially proposed as the leaf pathogen of Camellia sinensis and Cirsium japonicum in China [65]. Later, it was recorded to cause anthracnose of Camellia oleifera in China [66]. In the present study, strains from diseased chestnut leaves formed a supported clade with Colletotrichum henanense (Figure A13), and shared similar morphology. Hence, we identified our strains as C. henanense, and Castanea mollissima represented a new host record.

3.18.3. Colletotrichum jiangxiense

Colletotrichum jiangxiense F. Liu & L. Cai, Persoonia 35: 82 (2015)

Pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph: Undetermined.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 7 days, flat with entire edge, aerial mycelium dense, cottony, white to grey; reverse olivaceous.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of Castanea mollissima, 10 September 2020, N. Jiang (living culture, SM21); Yunnan Province, Zhaotong City, Zhenxiong County, 27°43′28″ N, 105°10′35″ E, alt. 1280 m, on leaf spots of Castanea mollissima, 5 September 2020, N. Jiang (living culture, ZX10-1); Hunan Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of Castanea mollissima, 7 September 2020, N. Jiang (living cultures, ZY12, ZY12B).

Notes—Colletotrichum jiangxiense was previously described from Camellia sinensis in China [65], and subsequently discovered from Citrus sinensis [67]. In present study, strains from diseased chestnut leaves formed a supported clade with Colletotrichum jiangxiense (Figure A13). Hence, we identified our strains as C. jiangxiense, and Castanea mollissima represented a new host record.

3.18.4. Colletotrichum karsti You L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, Cryptog. Mycol. 32(3): 241 (2011)

Pathogenic on Castanea mollissima leaves. Sexual morph: Undetermined. Asexual morph: Undetermined.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 6 days, flat with entire margin, aerial mycelium dense, cottony, initially white, becoming grey with age; reverse pale brown.

Material examined—CHINA, Guizhou Province, Zunyi City, Goujiang Town, 27°24′49″ N, 106°52′49″ E, alt. 1064 m, on leaf spots of Castanea mollissima, 7 September 2020, N. Jiang (living cultures, CFCC 54365 = ZY3B, ZY3B-1).

Notes—Colletotrichum karsti was described from Arundina graminifolia, Calanthe argenteostriata, Eria coronaria, Pleione bulbocodioides and Vanda sp. in China [68]. In the present study, strains from diseased chestnut leaves formed a supported clade with Colletotrichum
karsti (Figure A14). Hence, we identified our strains as *C. karsti*, and *Castanea mollissima* represented a new host record.

3.18.5. *Colletotrichum nymphaeae* (Pass.) Aa, Netherlands Journal of Plant Pathology, Supplement 1 84(3): 110 (1978)

Pathogenic on *Castanea mollissima* leaves. Sexual morph: Undetermined. Asexual morph: Undetermined.

Culture characteristics—Colonies on PDA reaching up to 60 mm in 8 days, flat with entire edge, aerial mycelium dense, cottony, white to grey; reverse olivaceous.

Material examined—CHINA, Sichuan Province, Yaan City, Shimian County, 29°13′31″ N, 102°21′27″ E, alt. 978 m, on leaf spots of *Castanea mollissima*, 10 September 2020, N. Jiang (living cultures, CFCC 54366 = SM26, SM26-1).

Notes—*Colletotrichum nymphaeae* was recorded to be associated with several hosts, including *Camellia oleifera*, *Citrus aurantifolia*, *Juglans regia*, *Malus domestica*, *Prunus salicina* and *Vitis vinifera* [69]. In the present study, strains from diseased chestnut leaves formed a supported clade with *Colletotrichum nymphaeae* (Figure A15). Hence, we identified our strains as *C. nymphaeae*, and *Castanea mollissima* represented a new host record.

4. Discussion

*Castanea henryi* and *C. mollissima* are two crops currently cultivated in plantations of China, and suffering from cankers, leaf spots and fruit rot diseases commonly. During our investigations in the past years, *Cryptonectria parasitica* and *Dendrostoma* spp. were commonly occurring in most plantations, causing mild to serious cankers depending on the management [2,10]. *Gnomoniopsis chinensis* caused fatal stem and branch canker disease in only Hebei Province [60]. Compared to the cankers, leaf spots are usually neglected. In present study, we focused on the leaf-inhabiting fungi, identified them to 26 fungal species using phenotypic characters and the multi-locus phylogeny.

From Table 3, most fungi (92.3%) belong to Sordariomycetes and the rest two species belong to Dothideomycetes. This result is nearly congruent as we expected, because Sordariomycetes is a species-rich class and contains many plant pathogens [69]. Within Sordariomycetes, Amphisphaeriales (45.8%), Botryosphaeriales (12.5%), Diaporthales (33.3%) and Glomerellales (20.8%) are identified. They contain famous plant pathogens such as pestalotoid taxa, Botryosphaeria-like taxa, diaporthalean fungi and *Colletotrichum* species. These fungi were documented in the monograph of chestnut disease by Xie in 1998 [44]. However, genus and species concepts have changed a lot in recent years. For example, the old name *Colletotrichum gloeosporioides* has been expanded to a group of species named *Colletotrichum gloeosporioides* species complex [70], hence the chestnut-inhabiting *Colletotrichum* needs to be re-identified to particular one or several species. The genus *Pestalotiopsis* s. l. was separated into three genera, namely *Pestalotiopsis* s. s., *Neopestalotiopsis* and *Pseudopestalotiopsis* based on phylogeny [50]. In addition, species with similar morphology from the same host, especially resulting into same symptoms, are not easy to be distinguished without molecular approach in previous studies. *Gnomoniopsis daii* from Chinese chestnut and *Gnomoniopsis smithogilvyi* (*G. castaneae*) from European chestnut were likely identified to *Phomopsis* (now *Diaporthe*) species for extremely similar morphology.

According to the filed investigation and sample observation, *Colletotrichum* spp., *Neopestalotiopsis* spp., *Pestalotiopsis keniana* and *Phylllosticta capitulensis* are now common pathogens in plantations of *Castanea henryi* and *C. mollissima*. Pathogenicity tests and disease control methods are required to be conducted in the future.

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Appendix A

Figure A1. Phylogram generated from RAxML analysis based on combined ITS and tef1 sequence data of Aplosporella isolates. The tree was rooted to Saccharata proteae (CBS 115206). The scale bar indicates 0.05 nucleotide changes per site. Isolates from this study are marked in blue, and the identified species is marked in yellow.
Figure A2. Phylogram generated from RAxML analysis based on combined ITS, tef1 and tub2 sequence data of Botryosphaeria isolates. The tree was rooted to Neofusicoccum luteum (CBS 110229). The scale bar indicates 0.02 nucleotide changes per site. Isolates from this study are marked in blue, and the identified species is marked in yellow.
Figure A3. Cont.
Figure A3. Phylogram generated from RAxML analysis based on combined ITS, LSU, act, gapdh and tef1 sequence data of *Phyllosticta* isolates. The tree was rooted to *Botryosphaeria obtusa* (CMW 8232) and *B. stevensii* (CBS 112553). The scale bar indicates 0.08 nucleotide changes per site. Isolates from this study are highlighted, and ex-type strains are marked with *. 
Figure A4. Phylogram generated from RAxML analysis based on combined ITS, LSU, rpb2 and tub2 sequence data of Didymella isolates. The tree was rooted to Epicoccum nigrum (CBS 125.82). The scale bar indicates 0.02 nucleotide changes per site. Isolates from this study are marked in blue, and the identified species is marked in yellow.
Figure A5. Phylogram generated from RAxML analysis based on combined ITS, tef1 and tub2 sequence data of *Arthrinium* isolates. The tree was rooted to *Nigrospora gorlenkoana* (CBS 480.73). The scale bar indicates 0.2 nucleotide changes per site. Isolate from this study is marked in blue, and the identified species is marked in yellow.
Figure A6. Phylogram generated from RAxML analysis based on combined ITS and LSU sequence data of Bartalinia isolates. Table 13794. The scale bar indicates 0.004 nucleotide changes per site. Isolate from this study is marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.
Figure A7. Phylogram generated from RAxML analysis based on combined ITS, tef1 and tub2 sequence data of Pestalotiopsis isolates. The tree was rooted to Neopestalotiopsis magna (MFLUCC 12-652). The scale bar indicates 0.04 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.
Figure A8. Phylogram generated from RAxML analysis based on combined ITS, LSU, rpb2, tef1, and tub2 sequence data of Robillarda isolates. The tree was rooted to Strickeria kochii (CBS 140411). The scale bar indicates 0.04 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.
Figure A9. Cont.
Figure A9. Cont.
Figure A9. Phylogram generated from RAxML analysis based on combined ITS, cal, his, tef1 and tub2 sequence data of Diaporthe isolates. The tree was rooted to Diaporthella corylina (CBS 121124). The scale bar indicates 0.1 nucleotide changes per site. Isolates from this study are marked in blue, and the identified species is marked in yellow.
Figure A10. Phylogram generated from RAxML analysis based on combined ITS, tef1 and tub2 sequence data of Gnomoniopsis isolates. The tree was rooted to Apiognomonia errabunda (CBS 342.86). The scale bar indicates 0.04 nucleotide changes per site. Isolates from this study are marked in blue, and the identified species is marked in yellow.
Figure A11. Phylogram generated from RAxML analysis based on combined ITS, tef1 and tub2 sequence data of Coniella isolates. The tree was rooted to Melanconiella sp. (CBS 110385). The scale bar indicates 0.05 nucleotide changes per site. Isolates from this study are marked in blue, and the identified species is marked in yellow.
Figure A12. Phylogram generated from RAxML analysis based on combined ITS, tef1 and tub2 sequence data of Tubakia isolates. The tree was rooted to *Sphaerosporithryium mexicanum* (CPC 33021). The scale bar indicates 0.03 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.
Figure A13. Cont.
Figure A13. Phylogram generated from RAxML analysis based on combined ITS, act, chs-1, gapdh and tub2 sequence data of Collectrichum gloeosporiodes species complex isolates. The tree was rooted to Collectrichum catinaense (CBS 142417) and C. boninense (CBS 123755). The scale bar indicates 0.03 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species are marked in yellow.
Figure A14. Phylogram generated from RAxML analysis based on combined ITS, act, chs-1, gapdh and tub2 sequence data of Collectrichum boninense species complex isolates. The tree was rooted to Collectrichum gloeosporioides (CBS 112999). The scale bar indicates 0.03 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.
Figure A15. Phylogram generated from RAxML analysis based on combined ITS, act, chs-1, gapdh and tub2 sequence data of Collectrichum acutatum species complex isolates. The tree was rooted to Collectrichum anthrisci (CBS 125334). The scale bar indicates 0.04 nucleotide changes per site. Isolates from this study are marked in blue, ex-type strains are marked with *, and the identified species is marked in yellow.

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