Morphology, Phylogeny, and Pathogenicity of Pestalotioid Species on *Camellia oleifera* in China

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**Abstract:** Tea-oil tree (*Camellia oleifera*) is an important edible oil woody plant with a planting area of over 3,800,000 hectares in southern China. Pestalotioid fungi are associated with a wide variety of plants worldwide along with endophytes, pathogens, and saprobes. In this study, symptomatic leaves of *C. oleifera* were collected from Guangdong, Guangxi, Hainan, Hunan, and Jiangsu Provinces and pestalotioid fungi are characterized based on combined sequence data analyses of internal transcribed spacer (ITS), beta tubulin (*tub2*), and translation elongation factor 1-alpha (*tef-1α*) coupled with morphological characteristics. As a result, seven species were confirmed, of which five species are described as new viz. *N. camelliae-oleiferae*, *P. camelliae-oleiferae*, *P. hunanensis*, *P. nanjingensis*, *P. nanningensis*, while the other two are reported as known species, viz., *N. cubana* and *N. iberica*. Pathogenicity assays showed that all species except for *P. nanjingensis* developed brown lesions on healthy leaves and *P. camelliae-oleiferae* showed stronger virulence.

**Keywords:** five new taxa; Neopestalotiopsis; Pestalotiopsis; phylogeny; taxonomy

1. **Introduction**

Tea-oil tree (*Camellia oleifera* Abel.) is a unique woody edible oil species in China, mainly distributed in the Qinling-Huaihe River area. It has a long history of cultivation and utilization for more than 2300 years since ancient China [1]. Statistical data for 2014 indicated that these plantations comprise over 3,800,000 hectares and produce 518,000 tons of edible oil (State-owned Forest Farms and Nurseries Station, State Forestry Administration of China, 2016). Camellia oil, obtained from *C. oleifera* seeds, is rich in unsaturated fatty acids and unique flavors, and has become a rising high-quality edible vegetable oil in China [2]. Thus, the development of the *C. oleifera* industry is of great significance for the national economy and poverty alleviation of local farmers in China.

The expanding cultivation of *C. oleifera* over the last several decades has also attracted increasing attention from plant pathologists to infectious diseases on this crop. Anthracnose disease caused by *Colletotrichum* species is one of the foremost diseases in southern China, which can infect leaves and fruits of *C. oleifera*, causing up to a 40% fruit drop and up to 40% camellia seeds loss [3]. Several studies have focused on the diversity and the pathogenicity of fungi in this special habitat [3–5]. However, relatively little is known about the taxonomy, genetic diversity, and pathogenicity of pestalotioid species on *C. oleifera*.

Pestalotioid species represent a cosmopolitan group of fungi occupying diverse ecological behavior as plant pathogens, endophytes, or saprobes, and are widely distributed throughout tropical and temperate regions [6–8]. However, species identification in this
genus remains a major challenge because of overlapping conidial measurements [6,7,9,10]. Maharachchikumbura et al. [8] segregated Neopestalotiopsis and Pseudopestalotiopsis from Pestalotiopsis, based on conidial pigment color, conidiophores and multi-locus phylogenetic analyses. Neopestalotiopsis can be easily distinguished from Pseudopestalotiopsis and Pestalotiopsis by its versicolorous median cells [8]. Pseudopestalotiopsis differs from Pestalotiopsis by having three darker median cells and knobbed apical appendages [8]. Many novel species were introduced into this group during recent years through a polyphasic approaches together with morphology [11–21]. This study aimed to identify the pestalotioid fungi associated with Camellia oleifera in China based on both morphological characters and molecular phylogeny.

2. Materials and Methods

2.1. Sample Collection and Isolation

The isolates in this study were collected from Camellia oleifera with irregular, brownish-grey lesions on leaves, and accounted for 25% of the surveyed leaves. Samples were obtained from the main tea-oil camellia production fields in Guangdong, Guangxi, Hainan, Hunan, and Jiangsu Provinces in 2020. Small sections (3 × 3 mm) were cut from the margins of infected tissues, and surface-sterilized in 75% ethanol for 30 s, then sterilized in 5% (vol/vol) sodium hypochlorite for 1 min, followed by three rinses with sterilized water and finally dried on sterilized filter paper. The sections were then plated onto PDA plates and incubated at 25 °C. Fungal growth was examined daily for up to 7 d. Isolates were then transferred aseptically to fresh PDA and purified by single-spore culturing. All fungal isolates were placed on PDA slants and stored at 4 °C. Specimens and isolates of the new species have been deposited in the Central South University of Forestry and Technology Culture Collection (CSUFTCC).

2.2. Morphological and Cultural Characterization

Colony characteristics of cultures on potato dextrose agar (PDA) medium were recorded after 7 d incubation at 25 °C. Fungal morphology was recorded from colonies grown in the dark for 14 d at 25 °C on PDA. The morphological characteristics were examined by mounting fungal structures in clear lactic acid and 30 measurements at ×1000 magnification were determined for each isolate using a Leica compound microscope (DM 2500) with interference contrast (DIC) optics. Descriptions, nomenclature, and illustrations of taxonomic novelties are deposited in MycoBank [22].

2.3. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from colonies grown on cellophane-covered PDA using a CTAB [cetyltrimethylammonium bromide] method [23]. For PCR amplifications of phylogenetic markers, three different primer pairs were used [19]. The PCR conditions were: 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), 54 °C (tef-1α), or 55 °C (tub2), and 1 min at 72 °C, and a final elongation step of 7 min at 72 °C. PCR amplification products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with a BigDye Terminator Kit v.3.1 (Invitrogen, Waltham, MA, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

2.4. Phylogenetic Analyses

The quality of our amplified nucleotide sequences was checked and combined by SeqMan v.7.1.0 and reference sequences (Table 1) were retrieved from the National Center for Biotechnology Information (NCBI), according to recent publications of the genus [19–21]. Sequences were aligned using MAFFT v. 6 [24] and manually corrected using Bioedit 7.0.9.0 [25]. Phylogenetic analyses were carried out with maximum likelihood analysis (ML), which was performed at the CIPRES web portal [26], 1000 rapid bootstrap replicates were run with GTRGAMMA model of nucleotide evolution. Bayesian inference analysis
(BI) was performed in MrBayes v. 3.2.0 [27,28]. The best-fit nucleotide substitution models for each gene were selected using jModelTest v. 2.1.7 [29] under the Akaike Information Criterion. GTR + I model was selected a best-fit model for the ITS (Neopestalotiopsis), HKY + I + G was selected as the best-fit model for the ITS (Pestalotiopsis), GTR + I + G model was selected as the best-fit model for the \( \beta \)-tubulin, HKY + G was selected as the best-fit model for the \( tef-1\alpha \). Phylogenetic trees were viewed in FigTree v1.4. The names of the isolates from the present study are marked in blue in the trees. Maximum likelihood bootstrap support values \( \geq 50\% \) (BT) and Bayesian posterior probabilities \( \geq 0.90 \) (PP) are given at the nodes, respectively. Alignment and trees were deposited in TreeBASE (submission ID: S29114 and S29115).

2.5. Pathogenicity Testing

Young and healthy leaves of \textit{Camellia oleifera} were collected from trees growing in the greenhouse. The leaves were washed with tap water, then submerged in 70\% ethanol for 2 min, and finally rinsed in sterilized water twice. The petioles of leaves were wrapped with damp cotton wool and the leaves were placed into petri dishes, three leaves per dish. One piercing wounds of each leaf were made in the mid-region forming a tiny little dot using a sterilized needle. Three drops of 6 \( \mu \)L spore suspension (10\(^6\) conidia/mL) were individually placed directly onto the leaf upper surfaces. For the control group, 6 \( \mu \)L of sterilized water was used. Each set of three leaves per petri dish was incubated with a different isolate. The petri dishes were placed inside a plastic box and the leaves incubated at 25 \(^\circ\)C with humidity and 12/12 h fluorescent light/dark cycle. After 5 d, the leaves were examined for symptom development, and the diameter of diseased spot was measured.

| Species                     | Isolate | Host/Substrate          | Location       | GenBank Accessions Numbers | References |
|-----------------------------|---------|-------------------------|----------------|---------------------------|------------|
| Neopestalotiopsis acrostichi | MFLUCC 17-1754 * | Acrostichum aureum | Thailand       | MK764272 MK764338 MK764316 | [19]       |
|                             | MFLUCC 17-1755 | Acrostichum aureum | Thailand       | MK764273 MK764339 MK764317 | [19]       |
| Neophytopsila alpapalis      | MFLUCC 17-2544 * | Rhizophora mucronata | Thailand       | MK357772 MK463545 MK463547 | [30]       |
|                             | MFLUCC 17-2545 | Symbotic Rhizophora   | Thailand       | MK357773 MK463546 MK463548 | [30]       |
| N. aoteana                   | CBS 367.54 * | Canvas                 | New Zealand    | KM199369 KM199454 KM199526 | [6]        |
| N. asiatica                 | MFLUCC 12-0286 * | Prunus dulcis         | China          | JX398983 JX399018 JX399049 | [8]        |
| N. australis                 | CBS 114159 * | Telopea sp.            | Australia      | KM199348 KM199432 KM199537 | [8]        |
| N. brachiata                 | MFLUCC 17-1555 * | Rhizophora apiculata  | Thailand       | MK764274 MK764340 MK764318 | [19]       |
| N. brasiliensis              | COAD 2166 * | Psidium guajara       | Brazil         | MG686469 MG692400 MG692402 | [31]       |
| N. camelliae-oleifera        | CSUFTCC81 * | Camellia oleifera     | China          | OK493585 OK562360 OK507955 | This study |
|                             | CSUFTCC82 | Camellia oleifera     | China          | OK493586 OK562361 OK507956 | This study |
| N. cavernicola               | KUMCC 20-0269 * | Cave                  | China          | MW545802 MW557956 MW550735 | [32]       |
| N. chiangmaiensis            | MFLUCC 18-0113 * | Pandanus sp.          | Thailand       | NA MH412725 MH388404 | [18]       |
| Species          | Isolate     | Host/Substrate | Location  | GenBank Accessions Numbers | References |
|------------------|-------------|----------------|-----------|---------------------------|------------|
|                  |             |                |           | **ITS**                   |            |
|                  |             |                |           | **tub2**                  |            |
|                  |             |                |           | **tef-1a**                |            |
| *N. chrysea*     | MFLUCC 12-0261 * | Dead leaves | China     | JX398985 | JX399020 | JX399051 | [6] |
|                  | MFLUCC 12-0262 | Dead leaves | China     | JX398986 | JX399021 | JX399052 | [6] |
| *N. clavispora*  | MFLUCC 12-0281 * | Magnolia sp. | China     | JX398979 | JX399014 | JX399045 | [6] |
|                  | MFLUCC 12-0280 | Magnolia sp. | China     | JX398978 | JX399013 | JX399044 | [6] |
| *N. cocois*      | MFLUCC 15-0152 * | Cocos nucifera | Thailand | NR 156312 | NA | KX789689 | [19] |
| *N. coffeae- arabicae* | HGUP4015 | Coffea arabica | China | KF412647 | KF412641 | KF412644 | [33] |
|                  | HGUP4019 * | Coffea arabica | China | KF412649 | KF412643 | KF412646 | [33] |
| *N. cubana*      | CBS 600.96 * | Leaf litter | Cuba     | KM199347 | KM199438 | KM199521 | [8] |
| *N. cubana*      | CSUFTCC37 | Camellia oleifera | China | OK493583 | OK562358 | OK507953 | This study |
|                  | CSUFTCC42 | Camellia oleifera | China | OK493584 | OK562359 | OK507954 | This study |
| *N. dendrobii*   | MFLUCC 14-0106 * | Dendrobium cariniferum | Thailand | MK993571 | MK975835 | MK975829 | [34] |
|                  | MFLUCC 14-0099 | Dendrobium cariniferum | Thailand | MK993570 | MK975834 | MK975828 | [34] |
| *N. drenthii*    | BRIP 72263a | Macadamia integrifolia | Australia | MZ303786 | MZ312679 | MZ344171 | [21] |
|                  | BRIP 72264a * | Macadamia integrifolia | Australia | MZ303787 | MZ312680 | MZ344172 | [21] |
| *N. egyptiaca*   | CBS 1401628 | Mangifera indica | Egypt | KP943747 | KP943746 | KP943748 | [35] |
| *N. ellipsospora* | MFLUCC 12-02838 | Dead plant material | China | JX398980 | JX399016 | JX399047 | [6] |
| *N. eucalyptorum* | CBS 147684 * | Eucalyptus globulus | Portugal | MW794108 | MW802841 | MW805397 | [20] |
| *N. eucalyptica* | CBS 264.37 | Eucalyptus globulus | NA | KM199376 | KM199431 | KM199551 | [8] |
| *N. foedans*     | CGMCC 3.9123 * | Mangrove plant | China | JX398987 | JX399022 | JX399053 | [6] |
|                  | CGMCC 3.9178 | Neodypsis decaryi | China | JX398989 | JX399024 | JX399055 | [6] |
| *N. formicarum*  | CBS 362.72 * | Dead ant | Cuba | KM199358 | KM199455 | KM199517 | [8] |
|                  | CBS 115.83 | Plant debris | Cuba | KM199344 | KM199444 | KM199519 | [8] |
| *N. guajavae*    | FMBCC 11.1 * | Guava | Pakistan | MF783085 | MH460871 | MH460868 | [36] |
| *N. guajavicola* | FMBCC 11.4 * | Guava | Pakistan | MH209245 | MH460873 | MH460870 | [36] |
| *N. hadrolaeliae* | EHJ6a | Cattleya jongheana | Brazil | MK45709 | MK465120 | MK465122 | [37] |
| *N. hispanica*   | CBS 147686 * | Eucalyptus globulus | Portugal | MW794107 | MW802840 | MW805399 | [20] |
Table 1. Cont.

| Species       | Isolate         | Host/Substrate          | Location      | GenBank Accessions Numbers  | References |
|---------------|-----------------|-------------------------|---------------|-----------------------------|------------|
|               |                 |                         |               | **ITS** | **tub2** | **tef-1α** |               |
| N. honoluluana| CBS 114495 *    | Telopea sp.             | USA           | KM199364 | KM199457 | KM199548 | [8]          |
|               | CBS 111535      | Telopea sp.             | USA           | KM199363 | KM199461 | KM199546 | [8]          |
| N. hydeana    | MFLUCC 20-0132 *| Artocarpus heterophyllus| Thailand      | MW266069 | MW251119 | MW251129 | [38]         |
| N. iberica    | CSUFTCC91       | Camellia oleifera       | China         | OK493587 | OK562362 | OK507957 | This study   |
|               |                 |                         |               |         |         |         | [8]          |
|               | CSUFTCC92       | Camellia oleifera       | China         | OK493588 | OK562363 | OK507958 | This study   |
|               | CSUFTCC99       | Camellia oleifera       | China         | OK493589 | OK562364 | OK507959 | This study   |
| N. iraniensis | CBS 147688 *    | Eucalyptus globulus     | Portugal      | MW794111 | MW802844 | MW805402 | [20]         |
|               |                 |                         |               |         |         |         | [8]          |
| N. javaensis  | CBS 257.31 *    | Cocos nucifera          | Indonesia     | KM199357 | KM199457 | KM199548 | [8]          |
| N. keteleerii | MFLUCC 13-0915 *| Keteleeria pubescens    | China         | KJ503820 | KJ503821 | KJ503822 | [40]         |
| N. longiappendiculata | CBS 147690 * | Eucalyptus globulus | Portugal | MW794110 | MW802845 | MW805404 | [20]         |
| N. lusitanica | CBS 147692 *    | Eucalyptus globulus     | Portugal      | MW794112 | MW802843 | MW805406 | [20]         |
| N. macadamiae | BRIP 63737c *   | Macadamia integrifolia  | Australia     | KK186604 | KK186654 | KK186629 | [14]         |
|               | BRIP 63742a     | Macadamia integrifolia  | Australia     | KK186599 | KK186657 | KK186627 | [14]         |
| N. maddoxii   | BRI 72266a *    | Macadamia integrifolia  | Australia     | MZ303782 | MZ312675 | MZ344167 | [14]         |
| N. magna      | MFLUCC 12-0652 *| Pteridium sp.           | France        | KF582795 | KF582795 | KF582791 | [41]         |
| N. mesopotamica| CBS 336.86 *   | Pinus brutia            | Iraq          | KM199362 | KM199441 | KM199555 | [8]          |
|               | CBS 299.74      | Eucalyptus sp.          | Turkey        | KM199361 | KM199435 | KM199541 | [8]          |
| N. musae      | MFLUCC 15-0776 *| Musa sp.                | Thailand      | KX789683 | KX789686 | KX789685 | [19]         |
| N. natalensis | CBS 138.41 *    | Acacia mollissima       | South Africa  | KM199377 | KM199466 | KM199552 | [8]          |
| N. nebuloides | BRI 66617 *     | Sporobolus elongatus    | Australia     | MK966338 | MK977632 | MK977633 | [42]         |
| N. olumideae  | BRI 72273a *    | Macadamia integrifolia  | Australia     | MZ303790 | MZ312683 | MZ344175 | [21]         |
| N. pandanicola| KUMCC 17-0175 * | Pandanus sp.            | China         | NA       | MH412720 | MH388389 | [18]         |
| N. pernambucana| URM7148-01 *   | Vismia guianensis       | Brazil        | KJ792466 | NA       | KU306739 | [43]         |
|               | URM7148-02      | Vismia guianensis       | Brazil        | KJ792467 | NA       | KU306740 | [43]         |
| Species     | Isolate | Host/Substrate | Location       | GenBank Accessions Numbers | References |
|------------|---------|----------------|----------------|----------------------------|------------|
|            |         |                |                |                            |            |
| N. perukae | FMBCC 11.3 | Guava          | Pakistan       | MH209077 MH460876 MH523647 | [36]       |
| N. petila  | MFLUCC 17-1738 | Rhizophora mucronata | Thailand     | MK764275 MK764341 MK764319 | [19]       |
|            | MFLUCC 17-1737 | Rhizophora mucronata | Thailand     | MK764276 MK764342 MK764320 | [19]       |
| N. phangngaensis | MFLUCC 18-0119 | Pandanus sp. | Thailand       | MH388354 MH412721 MH388390 | [18]       |
| N. piceana | CBS 254.32 | Cocos nucifera | Indonesia     | KM199372 KM199452 KM199529 | [8]        |
|            | CBS 394.48 | Picea sp.      | UK             | KM199368 KM199453 KM199527 | [8]        |
| N. protearum | CBS 114178 | * Leucospermum cuneiforme cv. “Sunbird” | Zimbabwe | JN712498 KM199463 LT853201 | [44]       |
| N. psidii  | FMBCC 11.2 | Guava          | Pakistan       | MF783082 MH477870 MH460874 | [36]       |
| N. raphidis | GUCC 21501 | * Rhododendron simsii | China         | MW931620 MW980441 MW980442 | [45]       |
| N. rhizophorae | MFLUCC 17-1550 | Rhizophora mucronata | Thailand     | MK764277 MK764343 MK764321 | [19]       |
|            | MFLUCC 17-1551 | Rhizophora mucronata | Thailand     | MK764278 MK764344 MK764322 | [19]       |
| N. rhododendri | GUCC 21504 | * Rhododendron simsii | China         | MW979577 MW980443 MW980444 | [45]       |
|            | GUCC 21505 | Rhododendron simsii | China         | MW979576 MW980445 MW980446 | [45]       |
| N. rosae   | CBS 101057 | * Rosa sp.     | New Zealand    | KM199359 KM199429 KM199523 | [8]        |
|            | CBS 124745 | * Paeonia suffruticosa | USA           | KM199360 KM199430 KM199524 | [8]        |
| N. rosicola | CFCC 51992 | * Rosa chinensis | China         | KY885239 KY885245 KY885243 | [15]       |
|            | CFCC 51993 | Rosa chinensis | China         | KY885240 KY885246 KY885244 | [15]       |
| N. samaranensis | CBS 115451 | Unidentified tree | China         | KM199365 KM199447 KM199556 | [8]        |
| N. saprophytica | MFLUCC 12-0282 | Magnolia sp. | China         | JX399892 JX399017 JX399048 | [8]        |
| N. scalabiensis | MUM 21.34 | * Vaccinium corymbosum | Portugal      | MW969748 MW934611 MW959100 | [46]       |
| N. sichuanensis | CFCC 54338 | Castanea mollissima | China         | MW166231 MW218524 MW199750 | [16]       |
|            | SM15-1C  | Castanea mollissima | China         | MW166232 MW218525 MW199751 | [16]       |
| N. sonneratae | MFLUCC 17-1745 | Sonneronata alba | Thailand     | MK764279 MK764345 MK764323 | [19]       |
|            | MFLUCC 17-1744 | Sonneronata alba | Thailand     | MK764280 MK764346 MK764324 | [19]       |
Table 1. Cont.

| Species                  | Isolate       | Host/Substrate                  | Location | GenBank Accessions Numbers | References |
|--------------------------|---------------|---------------------------------|----------|---------------------------|------------|
| Neopestalotiopsis sp.1   | CSUFTCC61     | Camellia oleifera               | China    | OK493590 OK562365 OK507960 | This study |
|                          | CSUFTCC62     | Camellia oleifera               | China    | OK493591 OK562366 OK507961 | This study |
|                          | CSUFTCC63     | Camellia oleifera               | China    | OK493592 OK562367 OK507962 | This study |
| N. steaertii             | IMI 192475 *  | Eucalyptus viminalis            | Australia| KF582796 KF582794 KF582792 | [8]        |
| N. surinamensis          | CBS 450.74 *  | Soil under Elaeis guineensis    | Suriname | KM199351 KM199465 KM199518 | [8]        |
| N. thailandica           | MFLUCC 17-1730* | Rhizophora mucronata           | Thailand | MK764281 MK764347 MK764325 | [19]       |
|                          | MFLUCC 17-1731* | Rhizophora mucronata           | Thailand | MK764282 MK764348 MK764326 | [19]       |
| N. umbrinospora          | MFLUCC 12-0285* | Unidentified plant             | China    | JX398984 JX399019 JX399050 | [6]        |
| N. vaccinii              | MUM 21.36 *   | Vaccinium corymbosum           | Portugal | MW969747 MW934610 MW959099 | [46]       |
| N. vacciniicola          | MUM 21.35 *   | Vaccinium corymbosum           | Portugal | MW969751 MW934614 MW959103 | [46]       |
| N. wheenae               | BRIP 72293a * | Macadamia integrifolia         | Australia| MZ303792 MZ312685 MZ344177 | [21]       |
| N. vitis                 | MFLUCC 15-1265* | Vitis vinifera cv. “Summer black” | China    | KU140694 KU140685 KU140676 | [47]       |
|                          | MFLUCC 15-1270* | Vitis vinifera cv. “Kyoho”    | China    | KU140699 KU140690 KU140681 | [47]       |
| N. zakeelii              | BRIP 72282a * | Macadamia integrifolia         | Australia| MZ303789 MZ312682 MZ344174 | [21]       |
| N. zimbabwana            | CBS 111495 *  | Leucospermum cuneiforme        | Zimbabwe | JM56231 KM199456 KM199545 | [8]        |
| Pestalotiopsis abietis   | CFCC 53011 *  | Abies fargesii                  | China    | MK397013 MK622280 MK622277 | [48]       |
|                          | CFCC 53012 *  | Abies fargesii                  | China    | MK397014 MK622281 MK622278 | [48]       |
|                          | CFCC 53013 *  | Abies fargesii                  | China    | MK397015 MK622282 MK622279 | [48]       |
| P. adusta                | ICMP 6088 *   | Refrigerator door               | Fiji     | JX399006 JX399037 JX399070 | [6]        |
|                          | MFLUCC 10-146* | Syzygium sp.                   | Thailand | JX399007 JX399038 JX399071 | [6]        |
| P. aggestorum            | LC6301 *      | Camellia sinensis              | China    | KX895015 KX895348 KX895234 | [12]       |
|                          | LC8186        | Camellia sinensis              | China    | KY464140 KY464160 KY464150 | [12]       |
| P. anacardiacearum       | IFRDCC 2397 * | Mangifera indica               | China    | KC247154 KC247155 KC247156 | [8]        |
| P. arceuthobii           | CBS 434.65 *  | Arceuthobium campylosum        | USA      | KM199341 KM199427 KM199516 | [8]        |
| Species          | Isolate    | Host/Substrate                                      | Location          | GenBank Accessions Numbers | References |
|------------------|------------|-----------------------------------------------------|-------------------|---------------------------|------------|
| **P. arenga**    | CBS 331.92 * | *Arenga undulatifolia*                               | Singapore         | KM199340 KM199426 KM199515 | [8]        |
| **P. australis** | CBS 114126 * | *Knightia sp.*                                      | New Zealand       | KM199297 KM199409 KM199499 | [8]        |
|                  | CBS 114141 | *Protea sp.*                                        | New South Wales   | KM199298 KM199410 KM199501 | [8]        |
| **P. australis** | CBS 111503 | *Protea neriifolia × susannae cv. “Pink Ice”        | South Africa      | KM199331 KM199382 KM199557 | [8]        |
|                  | CBS 114193 *| *Grevillea sp.*                                      | New South Wales   | KM199332 KM199383 KM199475 | [8]        |
| **P. biciliata** | CBS 124463 *| *Platanus × hispanica*                              | Slovakia           | KM199308 KM199399 KM199505 | [8]        |
| **P. brachiata** | CBS 236.38 | *Paonia sp.*                                         | Italy             | KM199309 KM199401 KM199506 | [8]        |
| **P. brachiata** | LC2998 *   | *Camellia sp.*                                       | China             | KX894933 KX895265 KX895150 | [12]       |
|                  | LC8188     | *Camellia sp.*                                       | China             | KY464142 KY464162 KY464152 | [12]       |
|                  | LC8189     | *Camellia sp.*                                       | China             | KY464143 KY464163 KY464153 | [12]       |
| **P. brassicae** | CBS 170.26 *| *Brassica napus*                                     | New Zealand       | KM199379 NA KM199558     | [8]        |
| **P. camelliae** | MFLUCC 12-0277 * | *Camellia japonica*                           | China             | JX399010 JX399041 JX399074 | [6]        |
| **P. camelliae-**| CSUFTCC08 *| *Camellia oleifera*                                 | China             | OK493593 OK562368 OK507963 | In this study |
| oleifera**       | CSUFTCC09 *| *Camellia oleifera*                                 | China             | OK493594 OK562369 OK507964 | In this study |
|                 | CSUFTCC10 *| *Camellia oleifera*                                 | China             | OK493595 OK562370 OK507965 | In this study |
| **P. chamaeops** | CBS 186.71 *| *Chamaeops humilis*                                 | Italy             | KM199326 KM199391 KM199473 | [6]        |
|                 | LC3619     | *Camellia sp.*                                       | China             | KX894991 KX895322 KX895208 | [12]       |
| **P. clavata**   | MFLUCC 12-0268 * | *Buxus sp.*                                      | China             | JX398990 JX399025 JX399056 | [6]        |
| **P. colombiensis** | CBS 118553 * | *Eucalyptus europaoides*                           | Colombia          | KM199307 KM199421 KM199488 | [8]        |
| **P. digitalis** | MFLU 14-0208 * | *Digitalis purpurea*                                | New Zealand       | KP781879 KP781883 NA      | [49]       |
| **P. dilucida**  | LC3232 *   | *Camellia sinensis*                                 | China             | KX894961 KX895293 KX895178 | [12]       |
|                 | LC8184     | *Camellia sinensis*                                 | China             | KY464138 KY464158 KY464148 | [12]       |
| **P. diploclisiae** | CBS 115449 | *Psychotria tutcheri*                               | China             | KM199314 KM199416 KM199485 | [8]        |
|                 | CBS 115587 *| *Diploclisia glaucescens*                            | China             | KM199320 KM199419 KM199486 | [8]        |
| **P. disseminata** | CBS 118552 | *Eucalyptus botryoides*                             | New Zealand       | MH553986 MH554652 MH554410 | [12]       |
|                 | CBS 143904 | *Persea americana*                                  | New Zealand       | MH554152 MH554825 MH554587 | [12]       |
| Species          | Isolate       | Host/Substrate | Location       | GenBank Accessions Numbers | References |
|------------------|---------------|----------------|----------------|---------------------------|------------|
|                  |               |                |                | **ITS** | **tub2** | **tef-1a** |     |
| MEAN 1165        | Pinus pinea   | Portugal       |                | MT374687 | MT374712 | MT374699   | [50] |
| MEAN 1166        | Pinus pinea   | Portugal       |                | MT374688 | MT374713 | MT374700   | [50] |
| P. diversiseta   | MFLUCC 12-0287 * | Rhododendron sp. | China         | JX399009 | JX399040 | JX399073   | [6]  |
| P. doitungensis  | MFLUCC 14-0115 * | Dendrobium sp. | Thailand       | MK993574 | MK975837 | MK975832   | [34] |
| P. dracaenica    | MFLUCC 18-0913 * | Dracaena sp. | Thailand       | MN962731 | MN962733 | MN962732   | [51] |
| P. dracontomelonis | MFLU 14-0207 * | Dracontomelon dao | Thailand     | NA       | NA       | KP781880   | [49] |
| P. ericacearum   | IFRDCC 2439 * | Rhododendron delavayi | China         | KC537807 | KC537821 | KC537814   | [52] |
| P. etonensis     | BRIP 66615 * | Sporobolus jacquemontii | Australia     | MK966339 | MK977634 | MK977635   | [42] |
| P. formosana     | NTUCC 17-009 * | On dead grass | China          | MH809381 | MH809385 | MH809389   | [15] |
| P. farcata       | MFLUCC 12-0054 * | Camellia sinensis | Thailand     | JQ683724 | JQ683708 | JQ683740   | [53] |
|                  | LC6691        | Camellia sinensis | China         | KX895030 | KX895363 | KX895248   | [12] |
| P. gaultheria    | IFRD 411-014 * | Gaultheria forrestii | China         | KC537805 | KC537819 | KC537812   | [8]  |
| P. gibbosa       | NOF 3175 * | Gaultheria shallon | Canada          | LC311589 | LC311590 | LC311591   | [54] |
| P. grevilleae    | CBS 114127 * | Grevillea sp. | Australia       | KM199300 | KM199407 | KM199504   | [8]  |
| P. hawaiiensis   | CBS 114491 * | Leucospermum sp. | Hawaii         | KM199339 | KM199428 | KM199514   | [8]  |
| P. hollandica    | CBS 265.33 * | Sciadopitys verticillata | Netherlands  | KM199328 | KM199388 | KM199481   | [8]  |
| P. hispanica     | CBS 115391 * | Protea cv. 'Susara' | Spain         | MH553981 | MH554640 | MH554399   | [8]  |
| P. humus         | CBS 336.97 * | Soil            | Papua New Guinea | KM199317 | KM199420 | KM199484   | [8]  |
| P. hunanensis    | CSUFTCC15 * | Camellia oleifera | China         | OK493599 | OK562374 | OK507969   | In this study |
|                  | CSUFTCC18 | Camellia oleifera | China         | OK493600 | OK562375 | OK507970   | In this study |
|                  | CSUFTCC19 | Camellia oleifera | China         | OK493601 | OK562376 | OK507971   | In this study |
| P. inflexa       | MFLUCC 12-0270 * | Unidentified tree | China         | JX399008 | JX399039 | JX399072   | [6]  |
| P. intermedia    | MFLUCC 12-0259 * | Unidentified tree | China         | JX398993 | JX399028 | JX399059   | [6]  |
| P. italiana      | MFLU 14-0214 * | Cupressus glabra | Italy          | KP781878 | KP781882 | KP781881   | [49] |
| P. jesteri       | CBS 109350 * | Frangraea bodenii | Papua New Guinea | KM199380 | KM199468 | KM199554   | [8]  |
Table 1. Cont.

| Species       | Isolate     | Host/Substrate | Location       | GenBank Accessions Numbers | References |
|---------------|-------------|----------------|----------------|----------------------------|------------|
|               |             |                |                | **ITS** | **tub2** | **tef-1a** |                |            |
| *P. jiangxiensis* | LC4242      | Eurya sp.       | China          | KX895035 | KX895327 | KX895213 | [12]           |
|               | LC4399 *    | Camellia sp.    | China          | KX895009 | KX895341 | KX895227 | [12]           |
| *P. jinchangensis* | LC6636 *   | Camellia sinensis | China          | KX895028 | KX895361 | KX895247 | [12]           |
|               | LC8190      | Camellia sinensis | China          | KY464144 | KY464164 | KY464154 | [12]           |
| *P. kandelicola* | NCYU         | Kandelia candel | China          | MT560723 | MT563100 | MT563102 | [55]           |
| *P. kenyana*  | CBS 442.67 * | Coffea sp.      | Kenya          | KM199302 | KM199395 | KM199502 | [8]            |
|               | LC6633      | Camellia sinensis | China          | KX895027 | KX895360 | KX895246 | [8]            |
| *P. knightiae* | CBS 111963  | Knightia sp.    | New Zealand    | KM199311 | KM199406 | KM199495 | [8]            |
| CBS 114138 * | Knightia sp. | New Zealand    | KM199310 | KM199408 | KM199497 | [8] |
| *P. leucaedri* | CBS 121417 * | Leucadendron sp. | South Africa  | MH553987 | MH554654 | MH554412 | [56]          |
| *P. licualacola* | HGUP 4057 * | Licuala grandis | China          | KC492509 | KC481683 | KC481684 | [57]          |
| *P. linearis* | MFLUCC 12-0271 * | Trachelospermum sp. | China          | JX398992 | JX399027 | JX399058 | [6]           |
| *P. longiappendiculata* | LC3013 * | Camellia sinensis | China          | KX894939 | KX895271 | KX895156 | [12]          |
| *P. lushanensis* | LC4344 * | Camellia sp.    | China          | KX895005 | KX895337 | KX895223 | [12]           |
|               | LC8182      | Camellia sp.    | China          | KY464136 | KY464156 | KY464146 | [12]           |
|               | LC8183      | Camellia sp.    | China          | KY464137 | KY464157 | KY464147 | [12]           |
| *P. macadamiae* | BRIP 63738b * | Macadamia integrifolia | Australia    | KX186588 | KX186680 | KX186621 | [14]          |
| BRIP 63739a | Macadamia integrifolia | Australia | KX186589 | KX186681 | KX186622 | [14] |
| BRIP 63739b | Macadamia integrifolia | Australia | KX186587 | KX186679 | KX186620 | [14] |
| *P. malayana* | CBS 102220 * | Macaranga triloba | Malaysia       | KM199306 | KM199411 | KM199482 | [8]           |
| *P. monochaeta* | CBS 144.97 * | Quercus robur | Netherlands    | KM199327 | KM199386 | KM199479 | [8]           |
| CBS 440.83 | Taxis baccata | Netherlands     | KM199329 | KM199387 | KM199480 | [8] |
| *P. nanjingensis* | CSUFTCC16 * | Camellia oleifera | China          | OK493602 | OK562377 | OK507972 | This study |
| CSUFTCC20 | Camellia oleifera | China | OK493603 | OK562378 | OK507973 | This study |
| CSUFTCC04 | Camellia oleifera | China | OK493604 | OK562379 | OK507974 | This study |
| *P. nanningensis* | CSUFTCC10 * | Camellia oleifera | China          | OK493596 | OK562371 | OK507966 | This study |
| CSUFTCC11 | Camellia oleifera | China | OK493597 | OK562372 | OK507967 | This study |
| CSUFTCC12 | Camellia oleifera | China | OK493598 | OK562373 | OK507968 | This study |
| Species                  | Isolate         | Host/Substrate          | Location       | GenBank Accessions Numbers | References |
|-------------------------|-----------------|-------------------------|----------------|---------------------------|------------|
|                         |                 |                         |                | ITS | tub2 | tef-1s |                          |            |
| *P. neolitseae*          | NTUCC 17-011 *  | On leaf of *Neolitsea vilosa* | Taiwan         | MH809383 | MH809387 | MH809391 | [15]                      |            |
| *P. novachollandiae*     | CBS 130973 *    | *Banksia grandis*       | Australia      | KM199337 | KM199425 | KM199511 | [8]                       |            |
| *P. oryzae*              | CBS 111522      | *Telopea* sp.           | USA            | KM199294 | KM199394 | KM199493 | [8]                       |            |
|                         | CBS 171.26      | NA                      | Italy          | KM199304 | KM199397 | KM199494 | [8]                       |            |
|                         | CBS 353.69 *    | *Oryza sativa*          | Denmark        | KM199299 | KM199398 | KM199496 | [8]                       |            |
| *P. pandanicola*         | MFLUCC 16-0255 *| *Pandanus* sp.          | Thailand       | MH388361 | MH412723 | MH388396 | [18]                      |            |
| *P. papuana*             | CBS 331.96 *    | Coastal soil            | Papua New Guinea | KM199321 | KM199413 | KM199491 | [8]                       |            |
|                         | CBS 887.96      | *Cocos nucifera*        | Papua New Guinea | KM199318 | KM199415 | KM199492 | [8]                       |            |
| *P. pallidotoheae*       | MAFF 240993 *   | *Pieris japonica*       | Japan          | NR111022 | LC311584 | LC311585 | [58]                      |            |
| *P. parva*               | CBS 265.37 *    | *Delonix regia*         | NA             | KM199312 | KM199404 | KM199508 | [8]                       |            |
|                         | CBS 278.35      | *Leucothoe fontanesiana* | NA             | KM199313 | KM199405 | KM199509 | [8]                       |            |
| *P. photinicola*         | GZCC 16-0028 *  | *Photinia serrulata*    | China          | KY092404 | KY047663 | KY047662 | [59]                      |            |
| *P. portugalisca*        | CBS 393.48 *    | NA                      | Portugal       | KM199335 | KM199422 | KM199510 | [8]                       |            |
|                         | LC4324          | *Camellia chekiangoleosa* | China          | KX895001 | KX895333 | KX895219 | [12]                      |            |
| *P. pini*                | MEAN 1092 *     | *Pinus pinacea*         | Portugal       | MT374680 | MT374705 | MT374693 | [50]                      |            |
| *P. pinicola*            | KUMCC 19-0183 * | *Pinus armandii*        | China          | MN412636 | MN417507 | MN417509 | [60]                      |            |
| *P. rhododendri*         | IFRDCC 2399 *   | *Rhododendron sinogrande* | China          | KC537804 | KC537818 | KC537811 | [52]                      |            |
| *P. rhodomyrtus*         | HGUP4230 *      | *Rhodomyrtus tomentosa* | China          | KF412648 | KF412642 | KF412645 | [33]                      |            |
|                         | LC4458          | *Camellia sinensis*     | China          | KX895010 | KX895342 | KX895228 | [12]                      |            |
| *P. rhizophorae*         | MFLUCC 17-0416 *| *Rhizophora apiculata*  | Thailand       | MK764283 | MK764349 | MK764327 | [19]                      |            |
| *P. rosea*               | MFLUCC 12-0258 *| *Pinus sp.*             | China          | JX399005 | JX399036 | JX399069 | [6]                       |            |
| *P. scoparia*            | CBS 176.25 *    | *Chamaecyparis sp.*     | NA             | KM199330 | KM199393 | KM199478 | [8]                       |            |
| *P. sequoiae*            | MFLUCC 13-0399 *| *Sequoia sempervirens*  | Italy          | KX572339 | NA        | NA        | [61]                      |            |
| *P. spathulata*          | CBS 356.86 *    | *Gevuina avellana*      | Chile          | KM199338 | KM199423 | KM199513 | [8]                       |            |
| *P. spathulaependiculata*| CBS 144035 *    | *Phoenix canariensis*    | Australia      | MH554172 | MH554845 | MH554607 | [56]                      |            |
Table 1. Cont.

| Species      | Isolate         | Host/Substrate | Location     | GenBank Accessions Numbers | References |
|--------------|-----------------|----------------|--------------|----------------------------|------------|
|              |                 |                |              | ITS | tub2 | tef-1α | References |
| P. telopeae  | CBS 114137      | Protea sp.     | Australia    | KM199301 | KM199469 | KM199559     | [8]         |
|              | CBS 114161 *    | Telopea sp.    | Australia    | KM199296 | KM199403 | KM199500     | [8]         |
|              | CBS 113606      | Telopea sp.    | Australia    | KM199295 | KM199402 | KM199498     | [8]         |
| P. terricola | CBS 141.69 *    | Soil           | Pacific Islands | MH554004 | MH554680 | MH554438     | [56]        |
| P. thailandica | MFLUCC 17-1616 * | Rhizophora    | Thailand     | MK764285 | MK764351 | MK764329     | [19]        |
| P. trachicarpica | IFRDCC 2403     | Podocarpus     | China        | KC537809 | KC537823 | KC537816     | [52]        |
|              | LC4523          | Camellia sinensis | China       | KX895011 | KX895344 | KX895230     | [12]        |
|              | MFLUCC 12-0264  | Chrysophyllum sp. | China       | JX399004 | JX399035 | JX399068     | [6]         |
|              | OP068 *         | Trachycarpus fortunei | China       | JQ845947 | JQ845945 | JQ845946     | [62]        |
| P. unicolor  | MFLUCC 12-0276 * | Rhododendron sp. | China       | JX398999 | JX399030 | NA           | [6]         |
|              | MFLUCC 12-0275  | unidentified tree | China       | JX398998 | JX399029 | JX399063     | [6]         |
| P. verruculosa | MFLUCC 12-0274 * | Rhododendron sp. | China       | JX398996 | NA      | JX399061     | [6]         |
| P. yanglingensis | LC4553 *       | Camellia sinensis | China       | KX895012 | KX895345 | KX895231     | [12]        |
|              | LC3412          | Camellia sinensis | China       | KX894980 | KX895312 | KX895197     | [12]        |
| P. yunnanensis | HMAS 96359 *    | Podocarpus macrophyllus | China       | KA373375 | NA      | NA           | [63]        |

BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; COAD: Coleção Octávio Almeida Drummond, Universidade Federal de Viçosa, Brazil; CSUFTCC: Central South University of Forestry and Technology Culture Collection, Hunan, China; FMB: Fungal Molecular Biology Laboratory, Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan; GZCC: Guizhou Academy of Agricultural Sciences Culture Collection, Guizhou, China; HCAU: Plant Pathology Herbarium of Guizhou University; HMAS: Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IFRDCC: International Fungal Research and Development Culture Collection; IMI: Culture Collection of CABI Europe UK Centre, Egham, UK; KNU: Kyungpook National University, Daegu, Korea; KUMCC: Kunming Institute of Botany Culture Collection, Yunnan, China; LC: working collection of Lei Ca, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; MEAN: Instituto Nacional de Investigação Agrária e Veterinária I. P.; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MLM: Micoteca de Universidade do Minho, Portugal; NCYU: National Chiai University, Chiayi, Taiwan; NOF: The Fungus Culture Collection of the Northern Forestry Centre, Alberta, Canada; NTUCC: the Department of Plant Pathology and Microbiology, National Taiwan University Culture Collection; URM: Culture Collection of the Universidade Federal de Pernambuco, Brazil. Ex-type strains are labeled with *. NA: Not available.

3. Results

3.1. Phylogenetic Analyses

The first sequence datasets for the ITS, tef-1α and tub2, were analyzed in combination to infer the interspecific relationships within Neopestalotiopsis. The combined species phylogeny of the Neopestalotiopsis isolates consisted of 105 sequences, including the outgroup Pestalotiopsis trachicarpica (culture OP068). A total of 1389 characters including gaps (479 for ITS, 498 for tef-1α, and 412 for tub2) were included in the phylogenetic analysis.
Similar tree topologies were obtained by ML and BI methods, and the best scoring ML tree is shown in Figure 1. ML bootstrap values and BI posterior probabilities (MLBS/BIPP) are given at nodes of the phylogram (Figure 1). The phylogenetic tree inferred from the concatenated alignment resolved the ten Neopestalotiopsis isolates from symptomatic leaves of *Camellia oleifera* into four well-supported monophyletic clades that represent one novel species, one undetermined species and two known species of Neopestalotiopsis (Figure 1).

Figure 1. Cont.
Figure 1. Phylogram generated from RAxML analysis based on combined ITS, *tef-1* α and *tub2* sequence data of *Neopestalotiopsis* isolates. The tree was rooted to *Pestalotiopsis trachicarpica* (OP068). The scale bar indicates 0.04 nucleotide changes per site. Isolates from this study are marked in red and the identified species is marked in yellow. Ex-type strains are labeled with *.

The second sequence datasets for the ITS, *tef-1* α and *tub2* were analyzed in combination to infer the interspecific relationships within Pestalotiopsis. The combined species phylogeny of the Pestalotiopsis isolates consisted of 129 sequences, including the outgroup *Neopestalotiopsis magna* (culture MFLUCC 12-652). A total of 1557 characters including gaps (515 for ITS, 537 for *tef-1* α, and 505 for *tub2*) were included in the phylogenetic analysis. Similar tree topologies were obtained by ML and BI methods, and the best scoring ML tree is shown in Figure 2. ML bootstrap values and BI posterior probabilities (MLBS/BIPP) are given at nodes of the phylogram (Figure 2). The phylogenetic tree inferred from the concatenated alignment resolved the 12 Pestalotiopsis isolates from symptomatic leaves of *Camellia oleifera* into four well-supported monophyletic clades that represent four novel species of Pestalotiopsis (Figure 2).
Figure 2. Cont.
3.2. Taxonomy

**Neopestalotiopsis camelliae-oleiferae** Q. Yang & H. Li, sp. nov. (Figure 3).

**MycoBank**: MB841476.

**Etymology**: Named after the host species, *Camellia oleifera*.

**Holotype**: CSUFT081.

**Description**: Conidiomata acervular in culture on PDA, globose, 300–800 µm diam., solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells ampulliform, hyaline, smooth, annelidic. Conidia fusiform to clavate, straight or slightly curved, 22.5–24(−26.5) × (7–)8.5–10 μm, 4-septate; basal cell conical, 3.5–4.5 μm, hyaline or sometimes pale brown, smooth, thin-walled; with a single appendage filiform, unbranched, centric, (4.5–)6–8(−9) μm long; three median cells doliiform, 14–16(−18) μm long, smooth, versicoloured, septa darker than the rest of...
the cell (second cell from base pale brown, 4.5–5.5 μm long; third cell medium to dark brown, 5–5.5 (–6.5) μm long; fourth cell medium to dark brown, 4.5–6 μm long); apical cell conical, 2.5–4.5 μm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages unbranched, filiform, (13.5–)15.5–18.5 (–20.5) μm long. Sexual morph not observed.

**Culture characteristics:** Colonies on PDA reaching 55 mm diameter after seven days at 25 °C. Colonies filamentous to circular, with dense aerial mycelium on surface, fruiting bodies black.

**Material examined:** CHINA, Jiangsu Province, Nanjing City, from leaf spots of *Camellia oleifera*, 25 Oct. 2020, H. Li (CSUFT081, holotype); ex-type living culture CSUFTCC81, living culture CSUFTCC82.

**Notes:** *Neopestalotiopsis camelliae-oleiferae* was collected from symptomatic leaves of *C. oleifera* in Jiangsu Province, China. Two isolates (CSUFTCC81 and CSUFTCC82) representing *N. camelliae-oleiferae* clustered in a well-support clade (ML/BI = 100/1). *Neopestalotiopsis camelliae-oleiferae* was sister to a clade containing *N. longiappendiculata* and *N. vacciniicola*. *N. camelliae-oleiferae* can be distinguished from *N. longiappendiculata* based on ITS, *tef-1α* and *tub2* loci (3/449 in ITS, 3/450 in *tef-1α*, and 6/404 in *tub2*, no gaps). Morphologically, *N. camelliae-oleiferae* differs from *N. longiappendiculata* by wider conidia (8.5–10 vs. 7–7.8 μm); from *N. vacciniicola* by shorter apical tubular appendages (15.5–18.5 vs. 25.7–30.2 μm) [20]. Therefore, the collection in the present study is designated as a new species.

![Conidioma](image1.jpg)

**Figure 3.** *Neopestalotiopsis camelliae-oleiferae* (CSUFTCC81). (a) Conidioma formed on PDA, (b) conidiogenous cells, and (c–g) conidia. Scale bars: (a) = 1 mm, (b–g) = 10 μm.

*Neopestalotiopsis cubana* Maharachch, K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 138 (2014) (Figure 4).

**Description:** *Conidionema acervulare* in culture on PDA, globose, 800–1350 μm diam., solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. *Conidiogenous cells* ampulliform to cylindrical, hyaline, smooth, annelidic. *Conidia* fusoid to ellipsoidal, straight or slightly curved, (19.5–)21–25 (–26.5) × (5.5–)6.5–8 μm, 4-septate; basal cell conical, 3.5–4.5 μm, hyaline or sometimes pale brown, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 3.5–5.5 μm long; three median cells doliiform, 13.5–15 (–16) μm long, smooth, versicoloured, septa darker than the rest of the cell (second cell from base pale brown, 3.5–5.5 μm long; third cell medium to dark brown, 4–5 μm long; fourth cell medium to dark brown, 3.5–4.5 μm long);
apical cell conical, 3.5–4.5 μm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages, unbranched, filiform, (21–)24–29(−31) μm long. Sexual morph not observed.

**Culture characteristics:** Colonies on PDA reaching 70 mm diameter after seven days at 25 °C. Colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, pycnidia abundant, fruiting bodies black.

**Material examined:** CHINA, Hainan Province, Chengmai County, from leaf spots of *Camellia oleifera*, 9 Nov. 2020, H. Li (CSUFT042); living cultures CSUFTCC37 and CSUFTCC42.

**Notes:** *Neopestalotiopsis cubana* was originally described from leaf litter in Cuba [8]. In the present study, two isolates from leaves of symptomatic *C. oleifera* were congruent with *N. cubana* based on morphology and DNA sequences data (Figure 1). We therefore describe *N. cubana* as a known species for this clade.

![Figure 4. *Neopestalotiopsis cubana* (CSUFTCC37). (a) Conidiomata formed on PDA, (b) conidiogenous cells, and (c–f) conidia. Scale bars: (a) = 500 μm, (b–f) = 10 μm.](image)

*Neopestalotiopsis iberica* E. Diogo, M.H. Bragança & A.J.L. Phillips, in Diogo, Gonçalves, Silva, Valente, Bragança & Phillips, *Mycol. Progr.* 20(11): 1449 (2021) (Figure 5).

**Description:** Conidiomata acervular in culture on PDA, globose, 600–1500 μm diameter, solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells ampulliform, hyaline, smooth, annelidic. Conidia fusiform to ellipsoidal, straight or slightly curved, (21.5–)22.5–24(−26.5) × 7–9(−10.5) μm, 4-septate; basal cell conical, 3.5–4.5 μm, hyaline or sometimes pale brown, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 2.5–4 μm long; three median cells doliform, 12.5–14.5(−15.5) μm long, smooth, versicoloured, septa darker than the rest of the cell (second cell from base pale brown, 4.5–5 μm long; third cell medium to dark brown, 4.5–5.5(−6) μm long; fourth cell medium to dark brown, 4.5–5.5 μm long); apical cell conical, 2.5–4 μm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages, unbranched, filiform, 24–26(−29.5) μm long. Sexual morph not observed.

**Culture characteristics:** Colonies on PDA reaching 70 mm diameter after seven days at 25 °C. Colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin, fluffy, fruiting bodies black.

**Material examined:** CHINA, Jiangsu Province, Nanjing City, from leaf spots of *Camellia oleifera*, 25 Oct. 2020, H. Li (CSUFT091); living cultures LHNJ91, LHNJ92, and LHNJ93.

**Notes:** *Neopestalotiopsis iberica* was originally described from leaves and stems of *Eucalyptus globulus* in Portugal [30]. In the present study, three isolates from leaves of symptomatic *C. oleifera* were congruent with *N. iberica* based on morphology and DNA sequences data (Figure 1). We therefore describe *N. iberica* as a known species for this clade.
Notes: Neopestalotiopsis iberica was originally described from Portugal [30]. In the present study, three isolates from leaves of sympatric
Camellia oleifera were congruent with Neopestalotiopsis iberica based on morphology and DNA sequences data (Figure 1). We therefore describe
a new species.

Pestalotiopsis camelliae-oleiferae Q. Yang & H. Li, sp. nov. (Figure 6).
MycoBank: MB841478.
Etymology: Named after the host species, Camellia oleifera.
Holotype: CSUFT008.
Description: Conidiomata acervular in culture on PDA, globose, 1.0–2.6 mm diameter, solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete or integrated, cylindrical to subcylindrical, hyaline, smooth. Conidia fusoid, ellipsoid, straight or slightly curved, (19.5–)21.5–23(–25) μm, 4-septate; basal cell conic to obconic with a truncate base, 3.5–5.5 μm, hyaline, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 2.5–4.5 μm long; three median cells doliiform, 12.5–14 μm long, smooth, concolorous, brown, septa darker than the rest of the cell (second cell from base 4–4.5 μm long; third cell 4.5–5 μm long; fourth cell 3.5–4.5 μm long); apical cell conical, 2.5–4(–4.5) μm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages, unbranched, filiform, (11–)12.5–14.5(–16) μm long. Sexual morph not observed.
Culture characteristics: Colonies on PDA reaching 70 mm diameter after seven days at 25 °C. Colonies filamentous to circular, medium dense, with white sparse mycelium, fruiting bodies black.
Material examined: CHINA, Hunan Province, Changsha City, from leaf spots of Camellia oleifera, 30 Aug. 2020, H. Li (CSUFT008, holotype); ex-type living culture CSUFTCC08, living cultures CSUFTCC09 and CSUFTCC10.
Notes: Pestalotiopsis camelliae-oleiferae was sister to P. biciliata in a well-supported clade (ML/BI = 100/1) (Figure 2). Pestalotiopsis camelliae-oleiferae can be distinguished from P. biciliata based on ITS, tef-1α and tub2 loci (4/500 in ITS, 1/473 in tef-1α, and 6/443 in tub2, no gaps). Morphologically, P. camelliae-oleiferae differs from P. biciliata by shorter conidia (21.5–23 vs. 22–28 μm) [8]. Therefore, the collection in the present study is designated as a new species.
living cultures CSUFTCC18 and CSUFTCC19.

Colonies filamentous to circular, with sparse aerial mycelium, fruiting bodies black. Filamentous, (13.5–)15–22(33–)40–60 µm, smooth, concolorous, brown, septa darker than the rest of the cell (second cell from base 4–5 µm long; third cell 5–6.5 µm long; fourth cell 4.5–5.5 µm long); apical cell conical, 2.5–3 µm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages, unbranched, filiform, (13.5–)15–22(−26.5) µm long. Sexual morph not observed.

Colonies on PDA reaching 50 mm diameter after seven days at 25 °C. Colonies filamentous to circular, with sparse aerial mycelium, fruiting bodies black.

**Material examined:** CHINA, Hunan Province, Xiangtan City, from leaf spots of Camellia oleifera, 7 Nov. 2020, H. Li (CSUFT015, holotype); ex-type living culture CSUFTCC15, living cultures CSUFTCC18 and CSUFTCC19.

**Notes:** Pestalotiopsis hunanensis was sister to P. rosae in a well-supported clade (ML/BI = 100/1) (Figure 2). Pestalotiopsis hunanensis can be distinguished from P. rosae based on ITS, tef-1a and tub2 loci (6/501 in ITS, 13/475 in tef-1a, and 7/446 in tub2, 12 gaps). Morphologically, P. hunanensis differs from P. rosae by lager conidia (23–25 × 9–10.5 vs. 17.5–21.8 × 5.7–7 µm) [6]. Therefore, the collection in the present study is designated as a new species.

**Pestalotiopsis hunanensis**  Q. Yang & H. Li, sp. nov. (Figure 7).

MycoBank: MB841480.

**Etymology:** In reference to the Hunan Province, from where the fungus was first collected.

**Holotype:** CSUFT015.

**Description:** Conidomata acervular in culture on PDA, globose, 500–1000 µm diameter, solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete or integrated, cylindrical to subcylindrical, hyaline, smooth, annelidic. Conidia fusoid, ellipsoid, straight or slightly curved, (20.5–)23–25(−26.5) × (7–)9–10.5 µm, 4-septate; basal cell conic to obconic with a truncate base, 4–5.5 µm, hyaline, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 3–3.5 µm long; three median cells doliiform, (14–)15–18 µm long, smooth, concolorous, brown, septa darker than the rest of the cell (second cell from base 4–5 µm long; third cell 5–6.5 µm long; fourth cell 4.5–5.5 µm long); apical cell conical, 2.5–3 µm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages, unbranched, filiform, (13.5–)15–22(−26.5) µm long. Sexual morph not observed.

**Culture characteristics:** Colonies on PDA reaching 50 mm diameter after seven days at 25 °C. Colonies filamentous to circular, with sparse aerial mycelium, fruiting bodies black.

**Material examined:** CHINA, Hunan Province, Xiangtan City, from leaf spots of Camellia oleifera, 7 Nov. 2020, H. Li (CSUFT015, holotype); ex-type living culture CSUFTCC15, living cultures CSUFTCC18 and CSUFTCC19.

**Notes:** Pestalotiopsis hunanensis was sister to P. rosae in a well-supported clade (ML/BI = 100/1) (Figure 2). Pestalotiopsis hunanensis can be distinguished from P. rosae based on ITS, tef-1a and tub2 loci (6/501 in ITS, 13/475 in tef-1a, and 7/446 in tub2, 12 gaps). Morphologically, P. hunanensis differs from P. rosae by lager conidia (23–25 × 9–10.5 vs. 17.5–21.8 × 5.7–7 µm) [6]. Therefore, the collection in the present study is designated as a new species.

**Figure 6. Pestalotiopsis camelliae-oleiferae** (CSUFTCC08). (a) Conidioma formed on PDA, (b) conidiogenous cells, and (c–g) conidia. Scale bars: (a) = 1 mm, (b–g) = 10 µm.
Morphologically, the present study is designated as a new species. Living cultures CSUFTCC04 and CSUFTCC20.

Conidiomata acervular in culture on PDA, globose, 1000–1600 μm diameter, solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete or integrated, cylindrical to subcylindrical, hyaline, smooth, annelidic. Conidia fusoid, ellipsoid, straight or slightly curved, (19.5–)22–25 × (4.5–)5–6.5 μm, 4-septate; basal cell conic to obconic with a truncate base, 4.5–5 μm, hyaline, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 2.5–3.5 μm long; three median cells doliiform, 13–14.5(–16) μm long, smooth, concolorous, brown, septa darker than the rest of the cell (second cell from base 4.5–5.5 μm long; third cell 4.5–5.5 μm long; fourth cell 3.5–4.5 μm long); apical cell conical, 3.5–4 μm long, hyaline, smooth, thin-walled; with two apical tubular appendages, unbranched, filiform, (11–)13.5–18(–20) μm long. Sexual morph not observed.

Culture characteristics: Colonies on PDA reaching 60 mm diameter after seven days at 25 °C. Colonies filamentous to circular, medium dense, aerial mycelium on surface flat, fruiting bodies black.

Material examined: CHINA, Jiangsu Province, Nanjing city, from leaf spots of Camellia oleifera, 25 Oct. 2020, H. Li (CSUFT016, holotype); ex-type living culture CSUFTCC 16, living cultures CSUFTCC04 and CSUFTCC20.

Notes: Pestalotiopsis nanjingensis was sister to P. neolitseae in a well-supported clade (ML/BI = 100/1) (Figure 2). Pestalotiopsis nanjingensis can be distinguished from P. neolitseae based on ITS, tef-1α and tub2 loci (2/500 in ITS, 26/472 in tef-1α, and 2/442 in tub2, 5 gaps). Morphologically, P. nanjingensis differs from P. neolitseae by longer conidia (22–25 vs. 18–21 μm) and apical appendages (13.5–18 vs. 10–15 μm) [15]. Therefore, the collection in the present study is designated as a new species.

**Pestalotiopsis nanjingensis** Q. Yang & H. Li, sp. nov. (Figure 8).

MycoBank: MB841481.

Etymology: In reference to the Nanjing City, from where the fungus was first collected.

Holotype: CSUFT016.

Description: Conidiomata acervular in culture on PDA, globose, 1000–1600 μm diameter, solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete or integrated, cylindrical to subcylindrical, hyaline, smooth, annelidic. Conidia fusoid, ellipsoid, straight or slightly curved, (19.5–)22–25 × (4.5–)5–6.5 μm, 4-septate; basal cell conic to obconic with a truncate base, 4.5–5 μm, hyaline, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 2.5–3.5 μm long; three median cells doliiform, 13–14.5(–16) μm long, smooth, concolorous, brown, septa darker than the rest of the cell (second cell from base 4.5–5.5 μm long; third cell 4.5–5.5 μm long; fourth cell 3.5–4.5 μm long); apical cell conical, 3.5–4 μm long, hyaline, smooth, thin-walled; with two apical tubular appendages, unbranched, filiform, (11–)13.5–18(–20) μm long. Sexual morph not observed.

Culture characteristics: Colonies on PDA reaching 60 mm diameter after seven days at 25 °C. Colonies filamentous to circular, medium dense, aerial mycelium on surface flat, fruiting bodies black.

Material examined: CHINA, Jiangsu Province, Nanjing city, from leaf spots of Camellia oleifera, 25 Oct. 2020, H. Li (CSUFT016, holotype); ex-type living culture CSUFTCC 16, living cultures CSUFTCC04 and CSUFTCC20.

Notes: Pestalotiopsis nanjingensis was sister to P. neolitseae in a well-supported clade (ML/BI = 100/1) (Figure 2). Pestalotiopsis nanjingensis can be distinguished from P. neolitseae based on ITS, tef-1α and tub2 loci (2/500 in ITS, 26/472 in tef-1α, and 2/442 in tub2, 5 gaps). Morphologically, P. nanjingensis differs from P. neolitseae by longer conidia (22–25 vs. 18–21 μm) and apical appendages (13.5–18 vs. 10–15 μm) [15]. Therefore, the collection in the present study is designated as a new species.
Pestalotiopsis nanningensis Q. Yang & H. Li, sp. nov. (Figure 9).

MycoBank: MB841479.

Etymology: In reference to the Nanning City, from where the fungus was first collected.

Holotype: CSUFT011.

Description: Conidiomata acervular in culture on PDA, globose, 750–1200 μm diameter, solitary or aggregated in clusters, exuding black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete or integrated, cylindrical to subcylindrical, hyaline, smooth, annelidic. Conidia fusoid, ellipsoid, straight or slightly curved, (22–)24–26.5 × (6–)7–8(–9) μm, 4-septate; basal cell conical, 4.5–6 μm, hyaline, smooth, thin-walled; with a single appendage filiform, unbranched, centric, 4.5–6.5 μm long; three median cells doliiform, 13.5–15(–17) μm long, smooth, concolorous, brown, septa darker than the rest of the cell (second cell from base 4.5–5.5 μm long; third cell 5–6 μm long; fourth cell 4–5 μm long); apical cell conical, 3.5–4.5 μm long, hyaline, smooth, thin-walled; with 2–3 apical tubular appendages, unbranched, filiform, (13.5–)18–22.5(–26.5) μm long. Sexual morph not observed.

Culture characteristics: Colonies on PDA reaching 80 mm diameter after seven days at 25 °C. Colonies filamentous to circular, medium dense, white aerial mycelium on surface flat or raised.

Material examined: CHINA, Guangxi Province, Nanning City, from leaf spots of Camellia oleifera, 20 Oct. 2020, H. Li (CSUFT011, holotype); ex-type living culture CSUFTCC11, living cultures CSUFTCC12 and CSUFTCC13.

Notes: Pestalotiopsis nanningensis was sister to P. formosana in a well-supported clade (ML/BI = 100/1) (Figure 2). Pestalotiopsis nanningensis can be distinguished from P. formosana based on ITS and tef-1α loci (4/500 in ITS, 2/472 in tef-1α, and 1/442 in tub2, no gaps). Morphologically, P. nanningensis differs from P. formosana by larger conidia (24–26.5 × 7–8 μm) and longer apical appendages (18–22.5 vs. 11–16 μm) [15]. Therefore, the collection in the present study is designated as a new species.
3.3. Pathogenicity Assay

After five days, for the pathogenicity tests, *N. camelliae-oleiferae*, *N. cubana*, *N. iberica*, *Neopestalotiopsis* sp.1, *P. camelliae-oleiferae*, *P. hunanensis*, and *P. nanningensis* developed brown lesions on wounded leaves (right), whereas the controls showed no symptoms (left). *Neopestalotiopsis* sp.1 had the highest virulence, while *P. nanningensis* did not cause obvious symptoms (Figure 10). Koch’s postulates were fulfilled by reisolating the same fungi and verifying its colony and morphological characters.
Figure 10. Pathogenicity of eight pestalotioid species from tea-oil leaves. (a) Induced symptoms on tea-oil leaves after 5 days. (b). The virulence of the isolates was evaluated by measuring the diameters of the necrotic lesions on infected tea-oil leaves 5 days after wounding.

4. Discussion

In this study, an investigation of *C. oleifera* diseases in China was carried out and Camellia leaf disease caused by pestalotioid fungi was observed as a common disease. Identification of our collections was conducted, based on isolates from symptomatic leaves of *C. oleifera* using three combined loci (ITS, *tef-1α* and *tub2*), as well as morphological characteristics. It includes *N. cubana*, *N. iberica*, as well as five new species named *N. camelliae-oleiferae*, *P. camelliae-oleiferae*, *P. hunanensis*, *P. nanjingensis*, and *P. nanningensis*.

The expanding cultivation of *C. oleifera* over the last several decades has attracted increasing attention from plant pathologists to infectious diseases on this crop. Therein, pestalotioid species are more frequently regarded as endophytes or latent pathogens causing diseases only on specific situations [4,6,12,63,64]. Understanding the diversity of
pestalotioid species and the genetic variation within pathogen populations could help in developing sustainable disease management strategies.

Pestalotioid fungi (Pestalotiopsidaceae, Sordariomycetes) are species-rich asexual taxa, which are common pathogens that cause a variety of diseases, including leaf spots, shoot dieback, fruit rots and various post-harvest diseases [6,8,15,19,20,46,65]. As many pestalotioid species have overlapping morphological traits, sequence data is essential to resolve these three genera and introduce new species [8]. Combined gene sequence of ITS, tef-1α, and tub2 can provide a better resolution for Pestalotiopsis and Pseudopestalotiopsis. However, more genes are needed to provide better resolution and support in Neopestalotiopsis. Furthermore, this is the first systematic report of Neopestalotiopsis and Pestalotiopsis fungi associated with Camellia oleifera in China, which indicates that there may be a high undescribed diversity of fungi in this host.

Pathogenicity tests of eight pestalotioid species from Camellia oleifera showed that all species except for P. nanjingensis were capable of infecting wounded leaves. Neopestalotiopsis sp.1 and P. camelliae-oleiferae showed stronger virulence, with lesion diameters ranged from 14.7 to 17.8 mm on leaves of the Neopestalotiopsis sp.1 isolate (CSUFTCC61) and 13.5 to 15.5 mm on leaves of the P. camelliae-oleiferae isolate (CSUFTCC08). All pathogenicity tests were performed with a single C. camellia cultivar. Since different C. oleifera cultivars may have different resistance to pestalotioid species, more cultivars of C. oleifera should be studied for the variation of their resistance to pestalotioid pathogens. During the tests, the symptoms vary considerably with factors, such as relative humidity, temperature, and the inoculum concentration. In the future, field conditions with natural inoculum should be conducted rather than just in vitro artificial inoculation.

5. Conclusions

Seven pestalotioid species (two known species and five new species) were described and illustrated. This is the first systematic report of Neopestalotiopsis and Pestalotiopsis fungi associated with Camellia oleifera in China. The pathogenicity of these species on leaves were examined and showed that there were significant differences in the pathogenicity.

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