Article

Fungal Species from *Rhododendron* sp.: *Discosia rhododendricola* sp.nov, *Neopestalotiopsis rhododendrica* sp.nov and *Diaporthe nobilis* as a New Host Record.

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Abstract: In the present study, we report two new asexual fungal species (i.e., *Discosia rhododendricola, Neopestalotiopsis rhododendrica* (Sporocadaceae) and a new host for a previously described species (i.e., *Diaporthe nobilis; Diaporthaceae*). All species were isolated from *Rhododendron* spp. in Kunming, Yunnan Province, China. All taxa are described based on morphology, and phylogenetic relationships were inferred using a multigenic approach (LSU, ITS, RPB2, TEF1 and TUB2). The phylogenetic analyses indicated that *D. rhododendronicola* sp. nov. is phylogenetically related to *D. muscicola*, and *N. rhododendrica* sp. nov. is related to *N. sonnaratae*. *Diaporthe nobilis* is reported herein as a new host record from *Rhododendron* sp. for China, and its phylogeny is depicted based on ITS, TEF1 and TUB2 sequence data.

Keywords: leaf litter; multi-loci phylogenetic analyses; new taxa; saprobe; Sordariomycetes; taxonomy

1. Introduction

*Rhododendron*, a genus of shrub and small to large trees belonging to *Ericaceae*, is an indicator of health for forest areas [1], commonly found in low-quality acidic soil and sterile conditions. This plant is mainly distributed in India and southeastern Asia, extending from the northwest Himalayas (Arunachal Pradesh) to Bhutan, eastern Tibet, Nepal, north Myanmar, Sikkim, and west central China [2]. *Rhododendron* flowers are used as food, to produce fermented wine, and to make herbal tea due to their distinctive flavor and color [3,4]. Fungi colonizing *Rhododendron* include *Alternaria alternata, Aspergillus brasiliensis, Chrysosporium dietelii, C. succincta*, [5], *Diaporthe nobilis* [6], *Epicoccum nigrum, Mucor hiemalis, Pestalotiopsis sydowiana* and *Trichoderma koningii* [7]. However, given the economic importance of this plant, it is imperative to assess the fungal species associated with it.

*Discosia* was introduced in Discisiaceae by Maharachchikumbura et al. [8] to accommodate the type genus *Discosia* and the type species *D. artocreas*. Senanayake et al. [9,10] introduced *Adisciso, Discosia, Discostroma, Immersidiscosia, Sarcostroma* and *Seimatosporum* in the Discisiaceae family. Jaklitsch et al. [11] considered *Discisiaceae* a synonym of *Sporocadaceae* based on DNA sequence analyses with strong phylogenetic support.
Wijayawardene et al. [12] accepted *Discosia* species belonging to the family *Sporocadaceae*. Libert introduced *Discosia* in 1837, with *Discosia strobilina* being the lectotype [9,12]. Liu et al. [13] reviewed the generic description of *Discosia*, an updated morphology, and the phylogenetic relationships based on ITS sequence data [13]. There are 118 epithets of *Discosia* in Index Fungorum 2022 [14]. *Discosia* has been identified as an asexual fungus and is characterized by uni- to multilocular conidiomata with multi-layered walls. Conidiogenous cells are monoblastic and phialidic to annellidic. Conidial types are bipolar, polar and subpolar appendages, and usually hyaline to pale brown [9].

The genus *Neopestalotiopsis*, introduced by Maharachchikumbura et al., (2014) [15], belongs to the family *Sporocadaceae* (Amphisphaeriales, Sordariomycetes) [8,15,16], with *N. protearum* being the type species. *Neopestalotiopsis* species have been reported on saprobes, trees or plant pathogens causing postharvest diseases (fruit rots and leaf blights) [17,18]. The sexual morph of *Neopestalotiopsis* species remains unknown [15,18–20]. *Neopestalotiopsis* species have a worldwide distribution. This genus has also been reported in caves in China [15,17–22]. Studies related to the taxonomy of *Neopestalotiopsis* included DNA sequence analyses and phylogeny of the ITS, TEF1 and TUB2 [22].

The genus *Diaporthe* introduced by Nitschke [23] belongs to the families Diaporthaceae, Diaporthales, and Sordariomycetes [8,9]. *Diaporthe* species have a worldwide distribution [6,24–28]. This genus has been associated with several grapevine diseases in Europe [29] and was detected in Uruguayan deciduous fruit tree (Malus domestica ‘Gala’) wood disease [30]. Studies related to the taxonomy of *Diaporthe* included DNA sequence analyses and phylogeny of the ITS, TEF1, TUB2 and CAL loci [6,31]. Dissanayake et al. [32] provided phylogenetic relationships of 171 *Diaporthe* species currently known from culture or direct sequencing, and are linked to their holotype, epitype, isotype or neotype and that can now be recognized with DNA sequence data, essential to species identification [33].

In this study, we introduce the new species *D. rhododendricola*, *N. rhododendricola* and a new host record of *Diaporthe nobilis*, collected from dead leaves of *Rhododendron* species in China. We further provide descriptions, illustrations, and DNA sequence-based phylogeny to verify identification and placement.

2. Materials and Methods

2.1. Sample Collection, Morphological Observation, and Fungal Isolation

Isolation was performed as described by Senanayake et al. [34]. Dead leaves of *Rhododendron* spp. were collected from Kunming, Yunnan Province, China and brought to the laboratory in labelled paper envelopes. A light microscope (Nikon ECLIPSE 80i compound microscope, Melville, NY, USA) was used to observe the specimens. Spore mass fruiting bodies were isolated on potato dextrose agar (PDA) plates and incubated at 25 °C.

The isolates were transferred to new PDA plates, incubated at 25 °C, and photographed using a Canon EOS 600D digital camera fitted to the microscope. The Tarosoft (R) Image Frame Work program measured the morphological characteristics. The figures were processed using Adobe Photoshop CS6 Extended version 10.0 (Adobe Systems, San Jose, CA, USA).

The specimens were deposited at the Herbarium of Mae Fah Luang University (Herb. MFLU) and Herbarium of Kungming Institute of Botany (KUN), Chinese Academy of Science, Kunming, China. Living cultures were deposited at the Culture Collection of Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand and the Culture Collection of Kungming Institute of Botany (KUN), Chinese Academy of Science, Kunming, China. Faces of Fungi and Index Fungorum data are also provided [14,35]. New species were established based on guidelines provided by Jeewon and Hyde [36].

2.2. DNA Extraction, PCR Amplification, and Sequencing

Fungal cultures were grown on PDA at 25 °C for 2–4 weeks. The Biospin Fungus Genomic DNA Extraction Kit-BioFlux (BioFlux®, Hangzhou, China) was used to extract DNA from the mycelium. PCR amplification was performed using primer pairs, ITS4/ITS5 for
the internal transcribed spacer region of ribosomal DNA [37], LR0R/LR5 for large subunit nuclear ribosomal DNA [38], EF-728F/EF-986R for translation elongation factor 1-alpha gene [39], fRPB2-5f/fRPB2-7cR for the second largest subunit of RNA polymerase [40] and Bt2a/Bt2b for beta-tubulin [41]. The PCR conditions were based on the methodology as described by Chaiwan et al. [42].

2.3. Phylogenetic Analyses

The sequence alignment and phylogenetic analyses were performed as outlined by Dissanayake et al. [43] and Chaiwan et al. [42,44,45]. Phylogenetic analyses were performed using a combined Discosia dataset of ITS, LSU, RPB2, TEF1 and TUB2 sequence data and a combined Neopestalotiopsis and Diaporthe dataset of ITS, TEF1 and TUB2 sequence data. Taxa used in the analyses were obtained through recent publications [16,28,46]. The phylogenetic analyses were carried out using maximum parsimony (MP), maximum likelihood (ML) and Bayesian posterior probabilities (BYPP). PAUP v4.0b10 was used to conduct the parsimony analysis to obtain the phylogenetic trees [47]. Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were set to 1000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony—tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC) and homoplasy index (HI)—were calculated for trees generated following the Kishino-Hasegawa test (KHT) criteria [48], which was performed in order to determine whether trees were significantly different. Maximum-parsimony bootstrap values equal or greater than 60% are given as the second set of numbers above the nodes.

Maximum likelihood analysis was performed by using RAxML-HPC2, New Orleans, LA on XSEDE (8.2.8) [45,48–50]. The search strategy was set to rapid bootstrapping and the analysis was carried out using the GTRGAMMAI model of nucleotide substitution. Maximum likelihood bootstrap values equal to or greater than 60% are given as the first set of numbers above the nodes.

Bayesian inference (BI) analysis was conducted with MrBayes v. 3.1.2 to evaluate the posterior probabilities (BYPP) using Markov chain Monte Carlo sampling [51]. Two parallel runs were conducted using the default settings, but with the following adjustments: six simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 200 generations. The distribution of log-likelihood scores were examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.4 [52]. The first 10% of generated trees were discarded and the remaining 90% of trees were used to calculate posterior probabilities (PP) of the majority rule consensus tree. The phylogenetic trees were viewed in FigTree v. 1.4 [53] and edited using Microsoft Office Power Point 2007 and Adobe Photoshop CS6 Extended [42].

2.4. Genealogical Concordance Phylogenetic Species Recognition (GCPSR) Analysis

The related species were analyzed using the Genealogical Concordance Phylogenetic Species Recognition model. The pairwise homoplasy index (PHI) [54] is a model test based on the fact that multiple gene phylogenies will be concordant between species and discordant due to recombination and mutations within a species. The data were analyzed by the pairwise homoplasy index (PHI) test [54]. The test was performed in SplitsTree4 [55,56] as described by Quaedvlieg [57] to determine the recombination level within phylogenetically closely related species using a five-locus concatenated dataset to determine the recombination level within phylogenetically closely related species. If the PHI is below the 0.05 threshold ($\Phi_w < 0.05$), it indicates that there is significant recombination in the dataset. This means that related species in a group and recombination level are not different. If the PHI is above the 0.05 threshold ($\Phi_w > 0.05$), it indicates that it is not significant, which means the related species in a group level are different. The new species and its closely related species were analyzed using this model. The relationships between closely related species
were visualized by constructing a split graph, using both the LogDet transformation and splits decomposition options.

2.5. Discosia, Habitat and Known Distribution Checklist Associated with Rhododendron sp.

An updated checklist of Discosia based on the SMML database (https://nt.ars-grin.gov/fungaldatabases/) (accessed on 10 June 2022) is provided [58]. Those species for which molecular data are available are indicated. The distribution information regarding the type or original descriptions available and the locality from which Discosia have been recorded on Rhododendron spp. is provided, including all the specimens encountered during this study.

3. Results

3.1. Phylogenetic Analyses

The combined sequence alignments of Discosia comprised 54 taxa (Table 1), with Immersidiscosia eucalypti MFLU16-1372 and NBRC 104195 as the outgroup taxa. The dataset comprised 4364 characters including alignment gaps (LSU, ITS, RPB2, TEF1 and TUB2 sequence data). The MP analysis for the combined dataset had 430 parsimony-informative, 3522 constant, and 412 parsimony-uninformative characters, and yielded a single most parsimonious tree (TL = 1353, CI = 0.777, RI = 0.764, RC = 0.594; HI = 0.223). The RAxML analysis of the combined dataset yielded a best scoring tree with a final ML optimization likelihood value of −22,013.917605. The matrix had 840 distinct alignment patterns, with 66% undetermined characters or gaps. Bayesian posterior probabilities from Bayesian inference analysis were assessed with a final average standard deviation of split frequencies = 0.009983. The phylogenetic tree in this study showed that our strain (Discosia rhododendricola Kun-HKAS 123205 and MFLU20-0486) is related to D. muscicola with high support value in the phylogenetic tree (Figure 1). Sequence alignments are deposited in TreeBASE. The combined sequence alignments of Neopestalotiopsis comprised 89 taxa (Table 2), with Monochaetia monochaeta CBS115004 and M. ilex CBS101009 as the outgroup taxa. The dataset comprised 2634 characters including alignment gaps (ITS, TUB2 and TEF1 sequence data). The MP analysis for the combined dataset had 631 parsimony-informative, 1524 constant, and 479 parsimony-uninformative characters, and yielded a single most parsimonious tree (TL = 2304, CI = 0.679, RI = 0.813, RC = 0.552; HI = 0.321). The RAxML analysis of the combined dataset yielded a best scoring tree with a final ML optimization likelihood value of −24,500.881631. The matrix had 1268 distinct alignment patterns, with 35.77% undetermined characters or gaps. Bayesian posterior probabilities from Bayesian inference analysis were assessed with a standard deviation of split frequencies = 0.024223. The phylogenetic tree in this study showed that N. rhododendricola Kun-HKAS 123204 and MFLU20-0046 belonged to a separate clade, phylogenetically related to N. sonneratae, N. coffeae-arabicae and N. thailandica with 88% MP support (Figure 2). Sequence alignments are deposited in TreeBASE.
Figure 1. Phylogram generated from maximum parsimony analysis of LSU, ITS, RPB2, TEF1 and TUB2 gene regions. Bootstrap support values for MP and ML equal to or greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.90 are defined as MP/ML/PP above or below the nodes. Taxonomic novelty is indicated in red. The tree is rooted with *Immersidiscosia eucalypti* (MFLU 16-1372) and (NBRC 104195).
Figure 2. RAxML tree based on a combined dataset of ITS, TUB2 and TEF1 gene regions. Bootstrap support values for ML and MP equal to or greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.90 are defined as ML/MP/PP above or below the nodes. Our new taxon is indicated in red. The tree was rooted with Monochaeta monochaeta strains (CBS115004) and Monochaeta ilexae strains (CBS101009).
Table 1. Culture collection numbers and GenBank accession numbers for *Discosia* used in this study. The type species are indicated in bold. The newly generated sequences are indicated in red. Instances where the GenBank Accession No. did not show the molecular data are marked with a dash.

| Species Name | Culture Collection No. | Substrate/Host | Country | GenBank Accession No | References |
|--------------|------------------------|----------------|---------|----------------------|------------|
| *Discosia pleurochaeta* | KT 2179 | - | - | KT281912 KT284775 | [9] |
| *Discosia pleurochaeta* | KT 2188 | - | - | AB593713 AB594781 AB594179 | [9] |
| *Discosia pleurochaeta* | KT 2192 | - | - | AB593714 AB594782 AB594180 | [9] |
| *Discosia artocreas* | CBS 124848 | Fagus sylvatica | Germany | MH554213 MH553994 MH554662 MH554420 MH554903 | [13] |
| *Discosia brasiliensis* | MFLUCC 12-0429 | Dead leaf | Thailand | KF827436 KF827432 KF827469 KF827465 KF827473 | [59] |
| *Discosia brasiliensis* | MFLUCC 12-0431 | Dead leaf | Thailand | KF827437 KF827433 KF827470 KF827466 KF827474 | [59] |
| *Discosia brasiliensis* | MFLUCC 12-0435 | Dead leaf | Thailand | KF827438 KF827434 KF827471 KF827467 KF827475 | [59] |
| *Discosia fagi* | MFLU 14-0299A | Fagus sylvatica | Italy | KM678048 KM678040 | [60] |
| *Discosia fagi* | MFLU 14-0299B | Fagus sylvatica | Italy | KM678047 KM678039 | [60] |
| *Discosia fagi* | MFLU 14-0299C | Fagus sylvatica | Italy | KM678048 KM678040 | [60] |
| *Discosia italica* | MFLU 14-0298B | Fagus sylvatica | Italy | KM678045 KM678042 | [60] |
| *Discosia italica* | MFLU 14-0298C | Fagus sylvatica | Italy | KM678044 KM678041 | [60] |
| *Discosia macrozamiae* | CPC 32109 | - | - | MH327856 MH327820 MH327895 MH327884 | [61] |
| *Discosia muscicola* | CBS 109.48 | - | - | MH867828 | [62] |
| *Discosia neofraxinea* | NTIT 469 | Fagus sylvatica | Italy | KF827439 KF827435 KF827472 KF827468 KF827476 | [59] |
| *Discosia neofraxinea* | MFLUCC 13-0204 | Fagus sylvatica | Italy | KR072672 KR072673 | [10] |
| *Discosia pseudoartocreas* | CBS 136438 | Tilia sp. | Austria | KF777214 KF777161 MH554672 MH554430 MH554913 | [63] |
| *Discosia pini* | MAFF 410149 | Pinus densiflora | Japan | AB593708 AB594776 AB594174 | [9] |
| *Discosia querci* | MFLUCC 16-0642 | - | - | MG815830 MG815829 | [64] |
| *Discosia ravennica* | MFLU 18-0131 | Pyrus sp. | Italy | MT376617 MT376615 MT376394 | [46] |
| *Discosia rhododendricola* | KUN-HKAS 123205 | Rhododendrons. | China | MT741963 MT741959 | MW143037 This study |
| *Discosia rhododendricola* | MFLU20-0486 | Rhododendrons. | China | OP162409 OP162414 | OP169687 This study |
| Species Name | Culture Collection No. | Substrate/Host | Country | GenBank Accession No | References |
|--------------|------------------------|----------------|---------|---------------------|------------|
|              |                        |                | USA     | LSU                 |            |
| Discosia rubi| CBS 143893             | Rubus phoenicosius | USA     | MH554334           | [13]       |
| Discosia sp. | F 233                  | -              | -       | -                   | [13]       |
| Discosia sp. | 3T30CF                 | -              | -       | KU751876            | [13]       |
| Discosia sp. | 3T9A                   | -              | -       | FJ861385            | [65]       |
| Discosia sp. | 3T9C                   | -              | -       | FJ861386            | [65]       |
| Discosia sp. | FIHB 571               | -              | -       | DQ536523            | [66]       |
| Discosia sp. | HKUCC 6626             | -              | -       | AF382381            | [67]       |
| Discosia sp. | JSP0111c42             | -              | -       | KR093849            | [68]       |
| Discosia sp. | KT 2193                | -              | AB593706| AB594774            | [9]        |
| Discosia sp. | OT1 143c               | -              | -       | KT804147            | [13]       |
| Discosia sp. | OT2 143a               | -              | -       | KT804075            | [13]       |
| Discosia sp. | OT3 176b               | -              | -       | KT804146            | [13]       |
| Discosia sp. | P4 A7 53               | -              | -       | KU325138            | [13]       |
| Discosia sp. | P8 A7-852              | -              | -       | KU325418            | [13]       |
| Discosia sp. | R 158                  | -              | -       | JN689956            | [13]       |
| Discosia sp. | UNH ID260              | -              | -       | KX459431            | [13]       |
| Discosia sp. | UWR 012                | -              | -       | KX426948            | [13]       |
| Discosia sp. | UWR 040                | -              | -       | KX426977            | [13]       |
| Discosia sp. | KT 2109                | -              | -       | MT236494            | [69]       |
| Discosia sp. | SH 125                 | -              | -       | JF449727            | [13]       |
| Discosia sp. | SH 288                 | -              | -       | AB594783            | [9]        |
| Discosia sp. | MAFF 236709            | -              | -       | KU751876            | [13]       |
| Discosia sp. | CBS 241.66             | Acacia karroo | South Africa | MH554244 | [13] |

References: [13]
Table 1. Cont.

| Species Name                | Culture Collection No. | Substrate/Host              | Country           | GenBank Accession No | References |
|-----------------------------|------------------------|-----------------------------|-------------------|----------------------|------------|
| Discosia sp.                | CBS 684.70             | Aesculus hippocastanum      | Netherlands       | MH554277 MH554064 MH554740 - - | [13]       |
| Discosia tricellularis      | MAFF 237478            | -                           | -                 | AB593730 AB594798 AB594189 - - | [9]        |
| Discosia tricellularis      | NBRC 32705             | Rhododendron indicum        | Japan             | AB593728 AB594796 AB594188 - - | [9]        |
| Discosia yakushimensis      | MAFF 242774            | Symlocos prunifolia         | Japan             | AB593721 AB594789 AB594187 - - | [9]        |
| Sporocadus cornicola        | MFLUCC 14-0448         | Cornus sanguinea            | Italy             | - KU974967 - - - | [70]       |
| Sporocadus rosarum          | MFLUCC 14-0466         | Rosa canina                 | Italy             | KT281912 KT284775 - - | [70]       |

Table 2. Culture collection numbers and GenBank accession numbers for Neopestalotiopsis used in this study. The type species are indicated in bold. The newly generated sequences are indicated in red. Instances where the GenBank Accession No. did not show the molecular data are marked with the dash.

| Species Name             | Culture Collection No. | Substrate/Host              | Country              | GenBank Accession No | References |
|--------------------------|------------------------|-----------------------------|----------------------|----------------------|------------|
| Monochaetia ilexae       | CBS 101009             | Air                         | Japan                | MH55395 MH554612 MH554371 | [13]       |
| M. monochaeta            | CBS 115004             | Quercus robur               | Netherlands          | AY853243 MH554639 MH554398 | [13]       |
| Neopestalotiopsis acrostichi | MFLUCC 17-1754       | Acrostichum aureum          | Thailand             | MK764272 MK764338 MK764316 | [21]       |
| N. acrostichi            | MFLUCC 17-1755         | Acrostichum aureum          | Thailand             | MK764273 MK764339 MK764317 | [21]       |
| N. alpapicalis           | MFLUCC 17-2544         | Rhizophora mucronata        | Thailand             | MK357772 MK463545 MK463547 | [71]       |
| N. alpapicalis           | MFLUCC 17-2545         | Symptomatic Rhizophora apiculata leaves | Thailand | MK357773 MK463546 MK463548 | [71]       |
| N. aotearoa              | CBS 367.54             | Canvas                      | New Zealand          | KM199369 KM199454 KM199526 | [15]       |
| N. asiatica              | MFLUCC 12-0286         | Prunus dulcis               | China                | JX398983 JX399018 JX399049 | [15]       |
| Species Name            | Culture Collection No. | Substrate/Host          | Country     | GenBank Accession No | References |
|------------------------|------------------------|-------------------------|-------------|----------------------|------------|
|                       |                        |                         |             | ITS                  | TUB2       | TEF1       |            |
| *N. australis*         | CBS 114159             | Telopea sp.             | Australia   | KM199348             | KM199432   | KM199537   | [18]       |
| *N. brachiata*         | MFLUCC 17-555          | Rhizophora apiculata    | Thailand    | MK764274             | MK764340   | MK764318   | [21]       |
| *N. brasiliensis*      | COAD 2166              | Psidium guajava         | Brazil      | MG686469             | MG692400   | MG692402   | [72]       |
| *N. cavernicola*       | KUMCC 20-0269          | Cave                    | China       | MW545802             | MW557596   | MW550735   | [22]       |
| *N. chiangmaiensis*    | MFLUCC 18-0113         | Pandanus sp.            | Thailand    | -                    | MH412725   | MH388404   |            |
| *N. chrysea*           | MFLUCC 12-0261         | Dead leaves             | China       | JX398985             | JX399020   | JX399051   | [74]       |
| *N. chrysea*           | MFLUCC 12-0262         | Dead plant              | China       | JX398986             | JX399021   | JX399052   | [74]       |
| *N. clavispora*        | MFLUCC 12-0280         | Magnolia sp.            | China       | JX398978             | JX399013   | JX399044   | [74]       |
| *N. clavispora*        | MFLUCC 12-0281         | Magnolia sp.            | China       | JX398979             | JX399014   | JX399045   | [74]       |
| *N. cocoës*            | MFLUCC 15-0152         | Cocos nucifera          | Thailand    | KX789687             | -          | KX789689   | [75]       |
| *N. coffeae-arabicae*  | HGUP4015               | Coffea arabica          | China       | KF412647             | KF412641   | KF412644   | [75]       |
| *N. coffeae-arabicae*  | HGUP4019               | Coffea arabica          | China       | KF412649             | KF412643   | KF412646   | [75]       |
| *N. cubana*            | CBS 600.96             | Leaf Litter             | Cuba        | KM199347             | KM199438   | KM199521   | [15]       |
| *N. dendrobii*         | MFLUCC 14-0099         | Dendrobium cariniferum  | Thailand    | MK993570             | MK975834   | MK975828   | [76]       |
| *N. dendrobii*         | MFLUCC 14-0106         | Dendrobium cariniferum  | Thailand    | MK993571             | MK975835   | MK975829   | [76]       |
| *N. egypitaca*         | CBS H 22294            | Mangifera indica        | Egypt       | KP943747             | KP943746   | KP943748   | [77]       |
| *N. ellipsoidspora*    | MFLUCC 12-0283         | Dead plant materials    | China       | JX398980             | JX399016   | JX399047   | [74]       |
| *N. eucalypticola*     | CBS 264.37             | Eucalyptus globulus     | -           | KM199376             | KM199431   | KM199551   | [15]       |
| *N. foedans*           | CGMCC 3.9123           | Mangrove plant          | China       | JX398987             | JX399022   | JX399053   | [74]       |
| *N. foedans*           | CGMCC 3.9178           | Neodypsis decaryi       | China       | JX398989             | JX399024   | JX399055   | [74]       |
| *N. formicidarum*      | CBS 115.83             | Plant debris            | Cuba        | KM199344             | KM199444   | KM199519   | [78]       |
| *N. formicidarum*      | CBS 362.72             | Dead Formicidae (ant)   | Cuba        | KM199358             | KM199455   | KM199517   | [78]       |
| *N. honoluliana*       | CBS 111535             | Telopea sp.             | USA         | KM199363             | KM199461   | KM199546   | [15]       |
| *N. honoluliana*       | CBS 114495             | Telopea sp.             | USA         | KM199364             | KM199457   | KM199548   | [15]       |
Table 2. Cont.

| Species Name       | Culture Collection No. | Substrate/Host           | Country    | GenBank Accession No          | References |
|--------------------|------------------------|--------------------------|------------|------------------------------|------------|
|                    |                        |                          |            | ITS                          |            |
| N. hydeana         | MFLUCC 20-0132         | Artocarpus heterophyllus | Thailand   | MW266069                     | [79]       |
| N. iranensis       | CBS 137767             | Fragraia ananassa        | Iran       | KM074045                     | [80]       |
| N. iranensis       | CBS 137768             | Fragraia ananassa        | Iran       | KM074048                     | [81]       |
| N. javaensis       | CBS 257.31             | Cocos nucifera           | Java       | KM199357                     | [15]       |
| N. keteleeria      | MFLUCC 13-0915         | Keteleeria pubescens     | China      | KJ023087                     | [75]       |
| N. iranensis       | CBS 137768             | Fragraia ananassa        | Iran       | KM074048                     | [81]       |
|                    | CBS 257.31             | Cocos nucifera           | Java       | KM199357                     | [15]       |
| N. macadamiae      | BRIP 63737c            | Macadamia integrifolia   | Australia  | KX186604                     | [81]       |
| N. macadamiae      | BRIP 63742a            | Macadamia integrifolia   | Australia  | KX186599                     | [82]       |
| N. mesopotamica    | CBS 336.86             | Pinus brutia             | Iraq       | KM199362                     | [15]       |
| N. natalensis      | CBS 138.41             | Acacia mollissima        | South Africa | KM199377                    | [15]       |
| N. natalensis      | CBS 138.41             | Acacia mollissima        | South Africa | KM199466                    | [15]       |
| N. nebuloides      | BRIP 66617             | Sporobolus elongatus     | Australia  | MK966338                     | [82]       |
| N. pandanicola     | KUMCC 17-0175          | Pandanus sp.             | China      | -                            | [73]       |
| N. pernambucana    | URM7148                | Vismia guianensis        | Brazil     | KJ792466                     | [82]       |
| N. pernambucana    | RV02                   | Vismia guianensis        | Brazil     | KJ792467                     | [83]       |
| N. petilla         | MFLUCC 17-2373         | Rhizophora mucronata     | Thailand   | MK764275                     | [21]       |
| N. petilla         | MFLUCC 17-2378         | Rhizophora mucronata     | Thailand   | MK764276                     | [21]       |
| N. phangngaensis   | MFLUCC 18-0119         | Pandanus sp.             | Thailand   | MH388354                     | [73]       |
| N. piceana         | CBS 254.32             | Cocos nucifera           | Indonesia  | KM199372                     | [15]       |
| N. piceana         | CBS 394.48             | Picea sp.                | UK         | KM199368                     | [15]       |
| N. protearum       | CBS 111478             | Leucospernum cuneiforme cv. “Sunbird” | Zimbabwe | JN712498                     | [15]       |
| N. rhizophorae     | MFLUCC 17-1550         | Rhizophora mucronata     | Thailand   | MK764277                     | [21]       |
| N. rhizophorae     | MFLUCC 17-1551         | Rhizophora mucronata     | Thailand   | MK764278                     | [21]       |
| Species Name          | Culture Collection No. | Substrate/Host                  | Country       | GenBank Accession No | GenBank Accession No | GenBank Accession No | References |
|-----------------------|------------------------|---------------------------------|---------------|----------------------|----------------------|----------------------|------------|
| **Pestalotiopsis**    |                        |                                 |               |                      |                      |                      |            |
| adusta                | ICMP6088               | On refrigerator door PVC gasket  | Fiji          | JX399006             | JX399037             | JX399070             | [74]       |
| adusta                | MFLUCC10-0146          | Syzygium sp.                    | Thailand      | JX399007             | JX399038             | JX399071             | [74]       |
| anacardiacearum       | IFRDCC 2397            | Mangifera indica                | China         | KC247154             | KC247155             | KC247156             | [88]       |
| **N.**                |                        |                                 |               |                      |                      |                      |            |
| rhododendricola       | KUN-HKAS 123204        | Rhododendronsp.                 | China         | OK283069             | OK274147             | OK274148             | This study |
| N. rosae              | CBS 101057             | Rosa sp.                        | New Zealand   | KM199359             | KM199429             | KM199523             | [15]       |
| N. rosae              | CBS 124745             | Paeonia suffruticosa            | USA           | KM199360             | KM199430             | KM199524             | [15]       |
| N. rosicola           | CFCC 51992             | Rosa chinensis                  | China         | KY885239             | KY885245             | KY885244             | [84]       |
| N. rosicola           | CFCC 51993             | Rosa chinensis                  | China         | KY885240             | KY885246             | KY885244             | [84]       |
| N. samarangensis      | CBS 115451             | Unidentified tree               | China         | KM199365             | KM199447             | KM199556             | [85]       |
| N. saprophytica       | MFLUCC 12-0282         | Magnolia sp.                    | China         | JX398982             | JX399017             | JX399048             | [74]       |
| N. sichuanensis       | CFCC 54338             | Castanea mollissima             | China         | MW166231             | MW218524             | MW199750             | [86]       |
| N. sichuanensis       | SM15-1C                | Castanea mollissima             | China         | MW166232             | MW218525             | MW199751             | [86]       |
| N. sonneratae         | MFLUCC 17-1744         | Sonneronata alba                | Thailand      | MK764279             | MK764345             | MK764323             | [21]       |
| N. sonneratae         | MFLUCC 17-1745         | Sonneronata alba                | Thailand      | MK764280             | MK764346             | MK764324             | [21]       |
| N. steyaertii         | IMI 192475             | Eucalyptus viminalis            | Australia     | KF582796             | KF582794             | KF582792             | [15]       |
| N. surinamensis       | CBS 450.74             | Soil under Elaeis guineensis    | Suriname      | KM199351             | KM199465             | KM199518             | [15]       |
| N. thailandica        | MFLUCC 17-1730         | Rhizophora mucronata            | Thailand      | MK764281             | MK764347             | MK764325             | [21]       |
| N. thailandica        | MFLUCC 17-1731         | Rhizophora mucronata            | Thailand      | MK764282             | MK764348             | MK764326             | [21]       |
| N. umbrinospora       | MFLUCC 12-0285         | Unidentified plant              | China         | JX398984             | JX399019             | JX399050             | [74]       |
| N. vitis              | MFLUCC 15-1265         | Vitis vinifera cv. “Summer black” | China       | KU140694             | KU140685             | KU140676             | [87]       |
| N. vitis              | MFLUCC 15-1270         | Vitis vinifera cv. “Kyoho”      | China         | KU140699             | KU140690             | KU140681             | [87]       |
| N. zimbabwana         | CBS 111495             | Leucospermum cunciforme         | Zimbabwe      | JX556231             | KM199456             | KM199545             | [18]       |
Table 2. Cont.

| Species Name | Culture Collection No. | Substrate/Host | Country          | GenBank Accession No | References |
|--------------|------------------------|----------------|------------------|----------------------|------------|
| *P. humus*   | CBS 115450             | Ilex cinerea   | China            | KM199319 KM199418 KM199487 | [15]       |
| *P. humus*   | CBS 336.97             | Soil           | Papua New Guinea | KM199317 KM199420 KM199484 | [15]       |
| *P. hydei*   | MFLUCC 20135           | Litsea petiolata | Thailand        | MW266063 MW251112 MW251113 | [79]       |
| *N. thailandica* | MFLUCC 17-1730        | Rhizophora mucronata | Thailand      | MK764281 MK764347 MK764325 | [21]       |
| *N. thailandica* | MFLUCC 17-1731        | Rhizophora mucronata | Thailand      | MK764282 MK764348 MK764326 | [21]       |
| *N. umbrinospora* | MFLUCC 12-0285      | Unidentified plant | China          | JX398984 JX399019 JX399050 | [74]       |
| *P. hydei*   | E-72-02                | Eucalyptus grandis | Brazil         | KU926708 KU926716 KU926712 | [79]       |
| *P. inflexa* | MFLUCC12-0270          | Unidentified tree | China          | JX399008 JX399039 JX399072 | [74]       |
| *P. linearis* | MFLUCC12-0271          | Trachelospermum sp. | China          | JX398992 JX399027 JX399058 | [74]       |
| *Ps. cocos*  | CBS 272.29             | Cocos nucifera | Indonesia       | KM199378 KM199467 KM199553 | [15]       |
| *Ps. indica* | CBS 459.78             | Hibiscus rosa-sinensis | India        | KM199381 KM199470 KM199560 | [15]       |
| *Ps. theae*  | MFLUCC12-0055 T        | Camellia sinensis | Thailand      | JQ683727 JQ683711 JQ683743 | [15]       |
| *Ps. theae*  | SC011                  | Camellia sinensis | Thailand      | JQ683726 JQ683710 JQ683742 | [15]       |
The combined sequence alignments of Diaporthe comprised 56 taxa (Table 3), with Diaporthella corylina CBS 121124 used as the outgroup taxon. The dataset comprised 2350 characters, including alignment gaps (ITS, TEF1 and TUB2 sequence data). After alignment, 641 characters were derived from ITS, 916 from TEF1, and 793 from TUB2. The MP analysis for the combined dataset had 730 parsimony-informative, 1216 constant, and 404 parsimony-uninformative characters, and yielded a single most parsimonious tree (TL = 3968, CI = 0.480, RI = 0.622, RC = 0.298; HI = 0.520). The RAxML analysis of the combined dataset yielded a best scoring tree with a final ML optimization likelihood value of −21,299.667012. The matrix had 1319 distinct alignment patterns, with 37.51% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.226983, C = 0.316389, G = 0.231894, T = 0.224734; substitution rates AC = 1.153998, AG = 3.111864, AT = 1.039115, CG = 0.869376, CT = 4.271324, GT = 1.000000; gamma distribution shape parameter α = 0.376625. Bayesian posterior probabilities from Bayesian inference analysis were assessed with a standard deviation of split frequencies = 0.009867. The phylogenetic tree in this study showed that D. nobilis KUN-HKAS 123203 grouped with the ex-type strain of D. nobilis, and formed a supported clade with 0.99 PP (Figure 3). Sequence alignments are deposited in TreeBASE.

3.2. Taxonomy

3.2.1. Discosia rhododendricola Chaiwan & K.D. Hyde, sp. Nov. (Figure 4)

Mycobank number: 845145; Facesoffungi number: FoF 09452
Etymology: name reflects the host from which the fungus was isolated.

Holotype: KUN-HKAS 123205

Saprobic on dead leaves of Rhododendron sp. Sexual morph: Undetermined. Asexual morph: Conidiomata 200–250 × 30–75 µm, pycnidial, cervular, aplanelate to disc-like, partly immersed or superficial, black, rounded to irregular in outline, glabrous, unilocular or divided into several locules by tissue conspicuous at the surface. Conidiophores were observed arising from the base, hyaline, filiform to cylindrical, smooth, and reduced to conidiogenous cells. Conidiogenous cells appeared subcylindrical, flask-shaped, hyaline, smooth, phialidic, each producing a single unbranched conidium. Conidia 20–30 × 4–5 µm (X = 25 × 4.5 µm, n = 30), subcylindrical, slightly curved, 3-septate, with slight constrictions at the septa, brown, smooth-walled with unequal cells; bipolar appendages; with a long, tubular base, two median cells subcylindrical, second cell joined to the base, 10–15 µm (X = 12.5 µm) long, the third cell joined to the apex, 11–15 µm (X = 13 µm) long; apical cell subconical with a rounded apex; apical and basal cells each with a subapical, unbranched, filiform, straight appendage; apical appendage, 9–11 µm (X = 10 µm), basal appendage, 20–25 µm (X = 22.5 µm).

Culture characteristics: Colonies grown on PDA were filamentous, raised, filiform margin, reached 4–5 cm in 5 days at 25 °C, brown to black, mycelium superficial, branched, septate, white mycelium with aerial on the surface, and produced black spore mass.

Material examined: CHINA, Kunming Yunnan Province; on dead leaves of Rhododendron sp. (Ericaceae), 28 July 2018, Napalai Chaiwan, KIB009 (KUN-HKAS 123205, holotype; isolate MFLU20-0486; Ex-type living culture KUNCC22-10804, isolate MFLUCC21-0004.
Figure 3. RAxML tree based on a combined dataset of ITS, TEF1 and TUB2 gene regions. Bootstrap support values for ML and MP equal to or greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.90 are defined as ML/MP/PP above or below the nodes. Our new taxon is indicated in red. The tree was rooted with *Diaporthella corylina* (CBS 121124).
Figure 4. **Discosia rhododendricola** (KUN-HKAS 123205, holotype). (a–c) Appearance on host surface; (d) vertical section of conidioma; (e–i) conidiogenous cells and developing conidia; (j–o) conidia from holotype. Scale bars: (b) 500 μm; (c) 200 μm; (d) 100 μm; (g–i) 50 μm; (e,j–o) 20 μm; (f) 10 μm.

Notes: **Discosia rhododendricola** is similar to **D. macrozamiae** CPC 32109 [94] with regards to conidiomata size (**D. rhododendricola**: 200–250 μm diam., 30–75 μm high vs. **D. macrozamiae** CPC 32109: 250 μm diam, 50 μm height). **Discosia rhododendricola** and **D. artocreas** (type species) share similar conidiophores lining the inner cavity (0–2-septate, rarely branched at base). There are also similar in conidial characteristics (conidial dimensions between 30 and 32 μm; the second cell joining to the base was 10–15 μm in length (\(\bar{x} = 12.5 \mu m\)) in **D. rhododendricola**; 10–11 μm (\(\bar{x} = 10.5 \mu m\)) in **D. macrozamiae** CPC 32109; the third cell joining to the apex was 11–15 μm in length (\(\bar{x} = 13 \mu m\)) in **D. rhododendria** and 4–5 μm (\(\bar{x} = 4.5 \mu m\)) in **D. macrozamiae** CPC 32109. The apical appendage of **D. rhododendricola** was 9–11 μm in length (\(\bar{x} = 10 \mu m\)), while in **D. macrozamiae** (CPC 32109) it was 7–11 μm (\(\bar{x} = 9 \mu m\)). The basal appendage in **D. rhododendricola** was 20–25 μm (\(\bar{x} = 22.5 \mu m\)) in length, and in **D. macrozamiae** (CPC 32109) 10–16 μm (\(\bar{x} = 13 \mu m\)).

**Discosia rhododendricola** differs from the type species, **D. artocreas**, in ascomatal size (**D. rhododendricola**: 200–250 μm diam., 30–75 μm high; **D. artocreas**: 150–500 μm diam, 60 μm high). The two species share similar conidiophores and conidiogenous cells characteristics. However, **D. rhododendricola** has hyaline to pale brown conidiogenous cells and conidia, whereas **D. artocreas** has hyaline conidiogenous cells and conidia. The second cell joining to the base measured 10–15 μm in length (\(\bar{x} = 12.5 \mu m\)) in **D. rhododendricola** but 5–9 μm (\(\bar{x} = 7.5 \mu m\)) in **D. artocreas**. The third cell joining to the apex was 11–15 μm in length (\(\bar{x} = 13 \mu m\)) in **D. rhododendria** and 3–6 μm (\(\bar{x} = 4.5 \mu m\)) in **D. artocreas**. The apical appendage of **D. rhododendricola** was 9–11 μm in length (\(\bar{x} = 10 \mu m\)), while in **D. artocreas** it was 6–12 μm (\(\bar{x} = 10 \mu m\)). The basal appendage in **D. rhododendricola** was 20–25 μm in length (\(\bar{x} = 22.5 \mu m\)), while in **D. artocreas** it was 7–12 μm (\(\bar{x} = 10 \mu m\)).
Table 3. Culture collection numbers and GenBank accession numbers for Diaporthe used in this study. The type species are indicated in bold. The newly generated sequences are indicated in red. Instances where the GenBank Accession No. did not show the molecular data are marked with the dash.

| Species Name            | Culture Collection No. | Substrate/Host | Country     | GenBank Accession No. | ITS       | TUB2       | TEF1       | References |
|-------------------------|------------------------|----------------|-------------|-----------------------|-----------|------------|------------|------------|
| Diaporthe acaciigena    | CBS 129521             | *Acacia retinodes* | -           | KC343005              | KC343973  | KC343731   |            | [6]        |
| Diaporthe alleghaniensis| CBS 495.72             | *Betula alleghaniensis* | -           | KC343007              | KC343975  | KC343733   |            | [6]        |
| Diaporthe alnea         | CBS 146.46             | *Alnus sp.*      | -           | KC343008              | KC343976  | KC343734   |            | [6]        |
| Diaporthe ambigua       | CBS 187.87             | *Helianthus annuus* | Italy       | KC343015              | KC343983  | KC343741   |            | [6]        |
| Diaporthe ampelina      | CBS 111888             | *Vaccinium vinifera* | USA         | KC343016              | KC343984  | KC343742   |            | [6]        |
| Diaporthe amygdali      | CBS 126679             | *Prunus dulcis*  | -           | KC343022              | KC343990  | AY343748   |            | [6]        |
| Diaporthe anacardii     | CBS 720.97             | *Anacardium occidentale* | -           | KC343024              | KC343992  | KC343750   |            | [6]        |
| Diaporthe areca         | CBS 161.64             | *Areca catechu*  | -           | KC343032              | KC344000  | KC343758   |            | [6]        |
| Diaporthe arencae       | CBS 114979             | *Arenga engleri* | -           | KC343034              | KC344002  | KC343760   |            | [6]        |
| Diaporthe australafricana| CBS 111886             | *Vaccinium vinifera* | Australia  | KC343038              | KC344006  | KC343764   |            | [6]        |
| Diaporthe baccace       | CBS 136972             | *Vaccinium corymbosum* | -           | KJ160565              | -         | KJ160597   |            | [45]       |
| Diaporthe bicincta      | CBS 121004             | *Juglans sp.*    | -           | KC343134              | KC344102  | KC343860   |            | [6]        |
| Diaporthe bohemiensis   | CBS 143347             | *Vitis spp.*     | Czech Republic | MG281015          | MG281188  | MG281536   |            | [29]       |
| Diaporthe carpini       | CBS 114437             | *Carpinus betulus* | Sweden      | KC343044              | KC344012  | KC343770   |            | [6]        |
| Diaporthe celastrina    | CBS 139.27             | *Celastrus scandens* | -           | KC343047              | KC344015  | KC343773   |            | [6]        |
| Diaporthe celeris       | CBS 143349             | *Vaccinium vinifera* | UK         | KC343004              | KC343972  | KC343730   |            | [6]        |
| Diaporthe celoria       | CBS 121124             | *Corylus sp.*    | -           | KC843311              | KC843187  | KC843071   |            | [89]       |
| Diaporthe citri         | AR 3405                | -               | -           | -                     | -         | -          |            |            |
| Diaporthe cucurbitae    | DAO.M 42078            | *Cucumis sativus* | -           | KM453210              | KP118848  | KM453211   |            | [89]       |
| Diaporthe decedens      | CBS 109772             | *Corylus avellana* | Austria     | KC343059              | KC344027  | KC343785   |            | [6]        |
| Diaporthe detersa       | CBS 109770             | *Berberis vulgaris* | Austria     | KC343061              | KC344029  | KC343787   |            | [6]        |
| Diaporthe elaeagni      | CBS 504.72             | *Elaeagnus sp.*  | Netherlands | KC343064              | KC344032  | KC343790   |            | [6]        |
| Diaporthe nobilis       | KUN-HKAS 123203        | *Rhododendron sp.* | China       | MT741962              | MW150988  | MW248138   |            | This study |
| Diaporthe nobilis       | CBS 338.89             | *Hedera helix*   | -           | KC343152              | KC344120  | KC343878   |            | [6]        |
| Diaporthe nobilis       | CBS 200.39             | *Laurus nobilis* | Germany     | KC343151              | KC344119  | KC343877   |            | [6]        |
| Diaporthe nobilis       | CBS 113470             | -               | -           | KC343146              | -         | -          |            | [6]        |
| Diaporthe nobilis       | CBS 116953             | -               | -           | KC343147              | -         | -          |            | [6]        |
| Diaporthe nobilis       | CBS 124030             | -               | -           | KC343149              | -         | -          |            | [6]        |
| Diaporthe nobilis       | CBS 129167             | -               | -           | KC343150              | -         | -          |            | [6]        |
| Diaporthe nobilis       | CBS 587.79             | *Pinus pantepella* | -           | KC343153              | KC344121  | KC343879   |            | [6]        |
Table 3. Cont.

| Species Name          | Culture Collection No. | Substrate/Host                | Country | GenBank Accession No          | References |
|-----------------------|------------------------|-------------------------------|---------|-----------------------------|------------|
| Diaporthe fibrosa    | CBS 109751             | -                             | -       | KC343099, KC344067, KC343825 | [6]        |
| Diaporthe foeniculacea| CBS 187.27             | -                             | -       | KC343107, KC344075, KC343833 | [6]        |
| Diaporthe helianthi   | CBS 592.81             | *Helianthus annuus*           | -       | KC343115, KC344083, KC343841 | [6]        |
| Diaporthe nitschkei  | AR 5211                | *Hedera helix*                | -       | KJ210538, KJ420828, KJ210559 | [89]       |
| Diaporthe hispaniae   | CBS 143351             | -                             | -       | MG281124, MG281296, MG281645 | [29]       |
| Diaporthe hongkongensis| CBS 115448             | *Dichroa febrifuga*           | -       | KC343119, KC344087, KC343845 | [6]        |
| Diaporthe hungariae   | CPC 30129              | -                             | -       | KC343122, KC344089, KC343847 | [6]        |
| Diaporthe impulse     | CBS 114434             | -                             | -       | KC343122, KC344091, KC343849 | [6]        |
| Diaporthe inconspicua | CBS 133813             | *Maytenus ilicifolia*         | -       | KC343126, KC344094, KC343852 | [6]        |
| Diaporthe infecunda   | CBS 133812             | *Schinus terebinthifolius*    | -       | KC343126, KC344094, KC343852 | [6]        |
| Diaporthe neilliae    | CBS 144. 27            | *Spiraea sp.*                 | -       | KC343144, KC344112, KC343870 | [90]       |
| Diaporthe nothofagi   | BRIP 54801             | *Nothofagus cunninghamii*     | -       | JX862530, KF170922, JX862536 | [91]       |
| Diaporthe novem       | CBS 127271             | -                             | -       | HM347710, -                  | [6]        |
| Diaporthe oncostoma   | CBS 589.78             | -                             | -       | KC343162, KC344130, KC343888 | [6]        |
| Diaporthe perjuncta   | CBS 109745             | *Ulmus glabra*                | -       | KC343172, KC344140, KC343898 | [6]        |
| Diaporthe perseae     | CBS 151.73             | *Persea gratissima*           | -       | KC343173, KC344141, KC343899 | [6]        |
| Diaporthe pseudomangiferae | CBS 101339         | *Mangifera indica*            | -       | KC343181, KC344149, KC343907 | [6]        |
| Diaporthe pseudophoenicicola | CBS 462.69      | *Phoenix dactylifera*         | -       | KC343183, KC344151, KC343909 | [6]        |
| Diaporthe rudis       | CBS 2665               | -                             | -       | -                           | [6]        |
| Diaporthe saccarata   | CBS 116311             | *Protea repens*               | -       | KC343190, KC344158, KC343916 | [6]        |
| Diaporthe schini      | CBS 133181             | *Schinus terebinthifolius*    | -       | KC343191, KC344159, KC343917 | [6]        |
| Diaporthe sterilis    | CBS 136969             | *Vaccinium corymbosum*        | -       | KJ160579, KJ160528, KJ160611 | [92]       |
| Diaporthe subclavata  | ZJUD 95                | -                             | -       | KJ490630, KJ490541, KJ490509 | [93]       |
| Diaporthe toxica      | CBS 534.93             | *Lupinus angustifolius*       | -       | KC343220, KC344188, KC343946 | [6]        |
| Diaporthe vaccinii    | CBS 160.32             | *Vaccinium macrocarpon*       | -       | AF317578, JX270436, GQ250326 | [92]       |
| Phomopsis sp.         | FH 2012b              | -                             | -       | JQ954649, -                  | [93]       |
The NCBI BLAST search of ITS sequence *D. rhododendricola* presented 95.32% similarity with *Immersdiscosia eucalypti*. A comparison of the 542 ITS (+5.8S) nucleotides of *D. rhododendricola* sp. nov. and *I. eucalypti* reveals 21 (3.87%) nucleotides differences. We compared 876 LSU nucleotides of *D. rhododendricola* with *D. muscicola* CBS 109.48, and a 0.34% bp difference was observed (a difference of 3 bp in a total 879 bp) (Table 4).

When analyzing the sequences, *D. rhododendricola* sp. nov. (KUN-HKAS123205 and MFLU20-0486) were found to be phylogenetically related to *D. macrozamiae* CPC 32109, *D. muscicola* CBS 109.48, *D. pleurochaeta* KT2179, *D. pleurochae* KT 2188 and KT 2192, while *D. tricellularis* MAFF237478 and NCBR32705 and *D. yakushimensis* MAFF 242774 were found to be in a clade. The two isolates of the new taxon (KUN-HKAS123205 and MFLU20-0486) have a high support value in the phylogenetic tree in a distinct clade (Figure 1). The ITS and LSU base pair differences between *D. rhododendricola* and other related species are shown in Table 4.

Table 4. LSU and ITS nucleotides comparisons of Discosia species related to our new taxon.

| LSU                   | ITS                   |
|-----------------------|-----------------------|
| **Base Pair Positions** | **Base Pair Positions** |
| 70                    | G                     |
| 369                   | A                     |
| 379                   | T                     |
| 407                   | T                     |
| 502                   | G                     |
| 646                   | A                     |
| 872                   | C                     |
| 873                   | T                     |
| 939                   | A                     |
| 959                   | C                     |
| 1003                  | T                     |
| 1004                  | A                     |
| 1019                  | T                     |
| 1039                  | A                     |
| 1303                  | C                     |
| 1402                  | G                     |
| 1404                  | A                     |

Discosia rhododendricola KUN-HKAS 123205 is closely related to the clade consisting of *D. muscicola* CBS 109.48, *D. tricellularis* MAFF 237478, NBRC 32705, and *D. yakushimensis* MAFF 242774 (Figure 5). The results of molecular analyses based on the Genealogical Concordance Phylogenetic Species Recognition (GCP SR) also showed that *D. rhododendricola* KUN-HKAS 123205 can be distinguished as a separate species by genealogical concordance (PHI = 1.0).

3.2.2. Neopestalotiopsis Rhododendricola Chaiwan & K.D. Hyde, sp. Nov. (Figure 6)

*MycoBank number:* 845144; *Facesoffungi number:* FoF 10475

*Etymology:* Name reflects the host from which the fungus was isolated.

*Holotype:* KUN-HKAS 123204

*Saprobic on dead leaves of Rhododendron* sp. **Sexual morph:** Undetermined. **Asexual morph:** Conidiomata (on PDA) 60–80 × 50–75 µm, pycnidial, cervular, applanate to disc-like, partly immersed or superficial, globose to clavate, solitary or confluent, embedded or semi-immersed to erumpent, dark brown, exuding globose, dark brown to black conidial masses, rounded to irregular in outline, glabrous, and unilocular or divided into several locules by tissue cells. Conidiophores are indistinct, arising from the base, hyaline, filiform
to cylindrical, smooth, and are often reduced to conidiogenous cells. Conidiogenous cells appeared subcylindrical, flask-shaped, hyaline, smooth, and phialidic, with each producing a single conidium. Conidia 20–30 × 5–7 μm (x = 25 × 6 μm, n = 30), subcylindrical fusoid, ellipsoid, straight to slightly curved, 4-septate, (19–28) × 5–7 μm (x = 23.5 × 6 μm, n = 30), μm; basal cell conic with a truncate base, hyaline, rugose and thin-walled, with constrictions at the septa, hyaline, smooth-walled; with a long, tubular base, two median cells subcylindrical, second cell joined to the base, 10–15 μm (x = 12.5 μm) long, the third cell joined to the apex, 11–15 μm (x = 13 μm) long; apical cell subconical with a rounded apex; apical and basal cells each with a subapical, unbranched, filiform, straight appendage; apical appendage, 9–11 μm (x = 10 μm), basal appendage, 20–25 μm (x = 22.5 μm).

Figure 5. Results of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results (Φw) <0.05 indicate significant recombination within the dataset. The new taxon is in red bold type.

Culture characteristics: Colonies grown on PDA, with an undulating edge, reached 4–5 cm in 5 days at 25 °C, mycelium superficial, branched, septate, white mycelium with aerial on the surface, and produced black spore mass.

Material examined: CHINA, Kunming Yunnan Province; on dead leaves of Rhododendron sp. (Ericaceae), 28 July 2018, Napai Chaiwan, KIB008 (KUN-HKAS 123204, holotype; isolate MFLU20-0046; Ex-type living culture KUNCC22-10802; isolate MFLUCC22-0004).

Notes: Neopestalotiopsis rhododendrica (KUN-HKAS 123204 and MFLU20-0046) were isolated from a Rhododendron sp. in China. In the phylogenetic analyses, N. rhododendrica forms a distinct highly supported lineage sister to N. sonneratae (MFLUCC17-1745T, MFLUCC17-1744), N. coffeae-arabicae (HGUP4019T, HGUP4015), N. thailandica (MFLUCC17-1730T, MFLUCC17-1731) and N. macadamiae (Figure 2). Neopestalotiopsis sonneratae was reported on leaf spots from Sonneronata alba in Thailand [21], Neopestalotiopsis thailandica was reported from leaf spots on Rhizophora mucronata Lam. in Thailand [21], and N. coffeae-arabicae was found on leaves of Coffea arabica in China [75].

Neopestalotiopsis rhododendricola sp. nov. resembles N. thailandica in having similar conidial size [21], but the difference is that N. rhododendricola has two to three tubular appendages on the apical cell, while N. thailandica showed only one to two tubular appendages on the apical cell. Comparison of ITS sequence differences revealed 2 base pairs, comparison of TEF sequence differences revealed 15 base pairs, and comparison of TUB differences revealed 6 base pairs of N. rhododendricola and N. thailandica. Therefore, based on morphology and phylogeny, we justify the description of N. rhododendricola as a new species in the Neopestalotiopsis genus.
3.2.3. **Diaporthe nobilis** Tanaka & S. Endô, in Endô, J. Pl. Prot. Japan 13: (1927) (Figure 7)

Faces of Fungi number: FoF 02717

Saprobic on dead leaves of *Rhododendron* sp. **Sexual morph:** Undetermined. **Asexual morph:** Conidiomata pycnidial 50–100 × 25–75 μm. \( (\bar{x} = 75 \times 50 \, \mu\text{m}, \, n = 10) \), globose to stromatic, multilocular, dark brown to black, scattered. Conidiophores were observed arising from the base, hyaline, filiform to cylindrical, smooth, straight. Conidiogenous cells, 35–40 × 1–2 μm \( (\bar{x} = 37.5 \times 1.5 \, \mu\text{m}, \, n = 10) \), phialidic, cylindrical, terminal and lateral, slightly tapered towards the apex, with visible periclinal thickening, hyaline, and smooth-walled. Beta conidia 16–20 × 1–2 μm \( (\bar{x} = 18 \times 1.5 \, \mu\text{m}, \, n = 30) \), hyaline smooth, guttulate, fusoid to ellipsoid, straight, tapered towards both ends, apex sub obtuse, base sub truncate, and aseptate. Alpha conidia not found.

**Culture characteristics:** Colonies grew on PDA, filamentous, flattened, dense and felty, reaching 5–6 cm in 14 days at 25 °C, white to brown on the surface, mycelium superficial, branched, and septate.
Material examined: CHINA, Kunming Yunnan Province, on dead leaves of Rhododendron sp. (Ericaceae), 28 July 2018, Napalai Chaiwan, KIB003 (KUN-HKAS 123203, new host record; isolate MFLU20-0485; living culture KUNCC22-10803; isolate MFLUCC 18–1482. 

Notes: Diaporthe nobilis KUN-HKAS 123203 clustered with D. nobilis CBS 587.79 and CBS113470 with high 0.99 PP bootstrap support. Conidiomata from the MFLUCC 18–1482 strain was acervular, semi-immersed, globose to eustromatic, and multilocular, while D. nobilis CBS 587.79 has pycnidia subcuticular, scattered to confluent, and uniloculate. Our strain was observed to share similar morphological characteristics with other Diaporthe nobilis strains in having conidiogenous cells formed at the apex of the conidiophores, cylindric, straight or curved hyaline and smooth-walled. Comparison of ITS, TEF1 and TUB2 sequence data of isolate KUN-HKAS 123203 and D. nobilis CBS113470, revealed 9 bp (1.41%) in 637 ITS (+5.8S) nucleotides, 2 bp (0.40%) in 496 TEF1 nucleotides and 6 bp (0.71%) in 844 TUB2 nucleotides. Therefore, we consider our strain (KUN-HKAS 123203) as D. nobilis and as a new host record from Rhododendron sp. in China.

Figure 7. Diaporthe nobilis (KUN-HKAS 123203). (a) Habitat of host; (b,c) appearance of fungi on host surface; (d,e) culture characters on PDA; (g,h) conidiophore with attached conidium; (i–p) conidia. Scale bars: (b,c) 200 μm; (d–f,i–p) 10 μm.

4. Discussion

Discosia species are distributed on various vascular plants and a wide range of hosts, and occur primarily in their asexual state as endophytes, saprobes and pathogens [20,58]. Host-specificity of species in this genus has not yet been established. Discosia species can be found on Fagus sylvatica (Fagaceae), Gaultheria procumbens (Ericaceae), Platanus orientalis (Platanaceae), Quercus sp. (Fagaceae), Syzygium cumini (Myrtaceae), Smilax rotundifolia (Smilacaceae), and leaves of undetermined plants [60]. Discosia blumencronii Bubák was reported from Rhododendron ponicium [92], while other species can be found on leaves of Beilschmeidia tarairi (Lauraceae), Brachychiton populneus (Malvaceae), Ceanothus fiedleri


Neopestalotiopsis 123204, within the Discosia rhododendricola (Theaceae), Pyrus pyrifolia provided herein. A checklist for more collections of microfungi associated with Rhododendron, targeting a wide variety of geographical locations. A checklist for Discosia species associated with Rhododendron is also provided herein. 123204, within the Discosia rhododendricola (Theaceae), Pyrus pyrifolia provided herein. A checklist for more collections of microfungi associated with Rhododendron, targeting a wide variety of geographical locations. A checklist for Discosia species associated with Rhododendron is also provided herein.

The new taxon, D. rhododendricola, was phylogenetically related to D. muscicola, described by Nicot-Toulouse Morelet (1968), and isolated from Cephaloziaceae in France. However, no morphological data are available for comparison [94]. Discosia rhododendricola sp. nov. was isolated from Rhododendron sp. and its morphology was compared. The ascomata and conidia of D. rhododendricola were larger than those of D. artoceras, whereas the sizes of conidiophores, conidiogenous cells and apical appendage were similar.

Discosia rhododendricola is similar to D. macrozamiae (CPC 32109) [62], but the phylogenetic tree showed that our species was closely related to D. muscicola CBS 109.48. However, for D. muscicola CBS 109.48, only rDNA sequence data were available (Figure 1). It should be pointed out that when the ITS DNA sequences of Discosia muscicola were subjected to a blast search, the closest hits were Aspergillus species similar to A.avenaceus. Our novel species have DNA sequence data from three regions (LSU, ITS, and RPB2), but we can only compare the LSU region for D. muscicola CBS 109.48, as there are no sequence data of the protein coding gene available for comparison. Based on the previous study of Wijayarwede et al. [16], 34 genera are recognized in Sporocadaceae. In this study, we introduce Discosia rhododendricola as a new species based on phylogenetic analyses and the pairwise homoplasy index.

Discosia species share similar morphological characters, but most characters are not meaningful in species delineation. In this study, our new species constitutes a different branching pattern in our phylogeny and appears distinct from extant species. A relationship among species based on similar conidial characters does not necessarily correlate with our phylogenetic relationships, and this indicates that morphology has little significance for reliable species identification.

Herein, we introduce a new species, Neopestalotiopsis rhododendricola KUN-HKAS 123204, within the Neopestalotiopsis genus that was separated from the other Neopestalotiopsis clade based on morphological and molecular phylogenetic analyses (Figure 2). Neopestalotiopsis are characterized by their conidia with versicolor median cells, by indistinct conidiogenous cells [15] and the ITS, TUB2 and TEF1 sequences. The newly described species is phylogenetically related to the group of N. sonneratae, N. coffeae-arabicae and N. thailandica in the phylogenetic tree (Figure 2), and the relationship is not strongly supported. Our new species was found on a Rhododendron sp. plant host from China, while N. sonneratae was reported on leaf spots of Sonneronata alba L. [21] and Neopestalotiopsis thailandica was reported on leaf spots of Rhizophora macronut Lam. Both strains have been reported in Thailand [21], and N. coffeae-arabicae was found on leaves of Coffea arabica in China [75].

Diaporthe species have been reported as plant pathogens, saprobes and endophytes on many plant hosts [23,28,58]. Species of Diaporthe are not host-specific [6,28,40]. Substrates colonized by members of Diaporthe recorded to date are mainly dicotyledons of Ericaceae, Fagaceae, Pinaceae, Rhizophoraceae, Rosaceae and Theaceae. Some species of Diaporthe can be found on more than one host. For example, Diaporthe nobilis was reported on Camellia sinensis (Theaceae), Castanea sativa (Fagaceae), Malus pumila (Rosaceae), Pinus pantepella (Pinaceae), Pyrus pyrifolia (Rosaceae) and Rhododendron sp. (Ericaceae) [6,28,40,58]. Diaporthe is mostly presented in the asexual morph as coelomycetes [23]. Diaporthe nobilis complex [6] has alpha and beta conidia [28]. However, our strain was only found to have beta conidia.

Diaporthe have been reported on Rhododendron spp. from Europe (Latvia) [6]. The strain (KUN-HKAS 123203) was isolated from Asia (China), indicating that the species is distributed in different geographical locations on the host; however, there is a need for more collections of microfungi associated with Rhododendron, targeting a wide variety of geographical locations.

(Rhamnaceae), Eucalyptus sp. (Myrtaceae), Laurus nobilis (Lauraceae) and Phillyrea latifolia (Oleaceae) [9,60]. Discosia species is distributed in temperate regions, being previously reported in Algeria, Austria, Brazil, France, Germany, India, Italy, New Zealand, Portugal, the USA, Sweden, Tunisia and Turkey [9].
5. *Discosia* Species Associated with *Rhododendron* sp.: Habitat, Known Distribution and Checklist

The above information is based on the USDA Systematic Mycology and Microbiology Laboratory (SMML) database [58], relevant literature, data from this study while current names and fungal classifications used are according to Index Fungorum (2022) [14], and an outline of Ascomycota [16]. Species confirmed with DNA sequence data are marked with an asterisk.

1. *Discosia artocreas* (Tode) Fr., Summa veg. Scand., Sectio Post. (Stockholm): 423 (1849) = *Sphaeria artocreas* Tode, F. Meckl. 2: 77, 1791; Fries, Syst. Myc. 2: 523, 1823. Habitat: *Rhododendron arboretum*, *R. campylocarpum*, *R. nudiflorum* [95,96], *R. catawbiense*, *R. maximum* [97], *R. ponticum* [98,99] and *Rhododendron* sp. [95,96,100] Known distribution: Italy [98], Maryland [95–97], New York [97], United Kingdom [100], Turkey* [99], Washington [95,96].

2. *Discosia blumencronii* Bubá, in Handel-Mazzetti, Annln K. K. naturh. Hofmus. Wien 23: 106 (1910) Habitat: *Rhododendron ponticum* (on dead leaves) [101]

3. *Discosia himalayensis* Died., Annls mycol. 14(3/4): 218 (1916) = *Discosia strobilina* Lib. ex Sacc., Syll. Fung. (Abellini) 3: 656 (1884) Habitat: *Rhododendron arboretum*, *R. campanulatum* (on dead leaves) [101–103] Known distribution: India [101–103]

4. *Discosia rhododendri* (Spechnew, Monit. Jard. Bot. Tiflis 4: 10 (1906) Habitat: *Rhododendron albrechtii* (on dead leaves)* [104], *R. ponticum* [99], *Rhododendron* sp. (on leaves) [101] Known distribution: Japan* [104], Turkey* [99]

5. *Discosia rhododendricola* (This study *) Habitat: *Rhododendron* sp. (on dead leaves) (This study *) Known distribution: China (This study *)

6. *Discosia sp.* Habitat: *Rhododendron* sp.* [104] Known distribution: Japan* [104]

7. *Discosia tricellularis* (Okane, Nakagiri & Tad. Ito) F. Liu, L. Cai & Crous, in Liu, Bonthond, Groenewald, Cai & Crous, Stud. Mycol. 92: 322 (2018) (2019) Habitat: *Rhododendron indicum* [105] Known distribution: Japan [105]

8. *Discosia vagans* De Not., Atti Acad. Tor.: 354 (1849) Habitat: *Rhododendron arboretum*, *R. nilagiricum*, *R. veitchianum* [59,103], *R. ponticum* [61] Known distribution: India* [99,105], Scotland* [59]

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