Endophytic Pestalotiopsis species associated with Rhododendron in Cangshan Mountain, Yunnan Province, China

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Rhododendron is an essential ornamental plant that is abundant in Yunnan province. In Cangshan Mountain, Yunnan, China, 61 species of Rhododendron have been reported. Endophytic fungi are internal plant tissue inhabitants that do not harm the host. It has emerged as an exciting research topic as they have the potential to provide numerous secondary metabolites. This study is focused on taxonomic novelties and new host records of endophytic fungi associated with Rhododendron plants collected from Cangshan Mountain in Yunnan Province, China. Pestalotiopsis fungi are associated with a vast array of plant species worldwide. In this study, fresh leaves of Rhododendron cyanocarpum, Rhododendron decorum, and Rhododendron delavayi were collected from Cangshan Mountain, Yunnan Province, China. Endophytic Pestalotiopsis fungi associated with Rhododendron were characterized based on phylogenetic analyses of combined ITS, TEF1-α, and TUB genes along with morphological characteristics. Six new species (Pestalotiopsis appendiculata, Pestalotiopsis cangshanensis, Pestalotiopsis daliensis, Pestalotiopsis fusioidea, Pestalotiopsis rosarioide, and Pestalotiopsis suae) and a new host record (Pestalotiopsis trachicarpicola) are described. Detailed descriptions and color photo plates of these species are provided. It is the first time that the endophytic fungi of Rhododendron plants in Cangshan Mountain have been studied.

KEYWORDS
six new species, endophytic fungi, Sordariomycetes, morphology, phylogeny
**Introduction**

*Rhododendron* is the largest genus of woody plants in the Northern Hemisphere and the largest genus in *Ericaceae* (Fang et al., 2005; Shrestha et al., 2018). *Rhododendron* is an important component of montane ecosystems, with more than 1,025 species and approximately 581 species in China (Cai et al., 2016; Ma et al., 2017; Cao et al., 2022). Moreover, Yunnan province in southwest China is a center of diversity for *Rhododendron* (Ma et al., 2021). *Rhododendron* is a popular landscape plant and a food source (Negi et al., 2013; Lin et al., 2016). Endophytic fungi benefit their host plants by increasing their drought resistance, disease resistance, and growth-promoting properties (Rodriguez et al., 2012). However, there is only one *Pestalotiopsis*-like taxon from fresh leaves of *Rhododendron*. Their taxonomic positions were established based on morphological descriptions and multi-locus phylogenetic analyses. The endophytic fungal strain resources were stored for future study on their secondary metabolites.

Endophytic fungi or endophytes exist widely inside the healthy tissues of living plants and are important components of plant micro-ecosystems (Jia et al., 2016). Endophytic fungi benefit their host plants by increasing their drought resistance, disease resistance, and growth-promoting properties (Rodriguez et al., 2009; De Silva et al., 2019; Rashmi et al., 2019). Endophytic fungi have the potential to produce metabolites with a wide range of biological activities, making them an appealing research topic (Huang et al., 2009; De Silva et al., 2019; Rashmi et al., 2019). More than 800 endophytic fungal genera have been reported worldwide, most speciose genera (>50 species) are *Penicillium* (103), *Colletotrichum* (78), *Alternaria* (61), *Fusarium* (59), *Pestalotiopsis* (53), and *Aspergillus* (52) (Rashmi et al., 2019). Amongst the different substrates, leaf endophytes have been studied and analyzed in more detail when compared to other parts (Rashmi et al., 2019).

Steyaert (1949) introduced *Pestalotiopsis* to accommodate species with fusiform conidia with three colored median cells and two colorless end cells, as well as two or more apical appendages. Traditionally, taxonomy and identification of *Pestalotiopsis* and allied genera were based mainly on conidial characters (Steyaert, 1949; Guba, 1961) and conidiogenesis (Sutton, 1980). Hu et al. (2007) reported that conidial characteristics, such as conidial length, median cell length, conidial width, and median cell color, were insignificantly different within *Pestalotiopsis*. Maharachchikumbura et al. (2014) selected internal transcribed spacer (ITS), partial β-tubulin (TUB), and partial translation elongation factor 1-alpha (TEF1-α), along with morphological characters to resolve the identification of *Pestalotiopsis*. Up to now, in a total of 92 *Pestalotiopsis* species have been introduced based on morphological and phylogenetic evidence (Maharachchikumbura et al., 2014; Liu et al., 2019; Rashmi et al., 2019; Shu et al., 2020; Monteiro et al., 2022). *Pestalotiopsis* is ordinarily isolable as endophytes in plants (Aly et al., 2010; Watanabe et al., 2010; Maharachchikumbura et al., 2012). However, there is only one *Pestalotiopsis* species viz., *Pestalotiopsis baurnensis* associated with *Rhododendron* (Rashmi et al., 2019).

As a part of the investigation on endophytic fungal diversity associated with *Rhododendron* plants in Cangshan Mountain, Yunnan province, China, we revealed seven *Pestalotiopsis*-like taxa from fresh leaves of *Rhododendron*. Their taxonomic positions were established based on morphological descriptions and multi-locus phylogenetic analyses. The endophytic fungal strain resources were stored for future study on their secondary metabolites.

**Materials and methods**

**Isolation and morphology**

Fresh *Rhododendron* (*Ericaceae*) leaves were obtained from Cangshan Mountain, Yunnan Province, China. The gathered leaves were placed in a sterile polyethylene bag and stored at 4°C. The symptomless leaves of each *Rhododendron* spp. were treated with gently running tap water to remove the surface debris. They were surface-sterilized by using 75% ethanol for 1 min, 0.1% *HgCl*₂ for 3 min, and washed five times using sterile distilled water, finally dried on sterile filter paper (Tao et al., 2013). The 5-mm diameter leaf discs treated as above were placed on potato dextrose agar (PDA) plates without antibiotics. The PDA plates were incubated in ambient light at 25°C. When colonies appeared, they were transferred onto new PDA plates and further incubated in ambient light at 25°C for morphological examination. Sporulation was induced on pine needle medium (‘pine needle’ and 1/10-strength PDA). Macromorphological characters of conidiomata on PDA were observed using an Optec SZ 760 compound stereomicroscope. Temporarily prepared microscope slides were placed under a Nikon ECLIPSE Ni-U compound stereomicroscope for observation and micro-morphological photography. Part of the pure culture that produced spores was removed and put into a water–agar medium (WA) with glycerol and air-dried at room temperature (De Silva et al., 2019). All endophytic isolates are stored at the Culture Collection of Kunming Institute of Botany, the Chinese Academy of Sciences (KUNCC), and the China General Microbiological Culture Collection Center (CGMCC). The Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (Herb. KUN-HKAS) housed the herbarium specimens. The MycoBank1 number was registered (Crous et al., 2004).

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1 https://www.mycobank.org
DNA extraction, PCR amplification, and sequencing

Genomic DNA extraction was carried out from fresh mycelium growing on PDA at 25°C using the Trelief™ Plant Genomic DNA Kit according to the manufacturer’s instructions. The primer pairs IT5S/IT5S4, EF1-728F/EF2, and Bt2a/Bt2b were used to amplify the ITS, TEF1-α, and TUB gene regions, respectively. The amplification was performed in a 25 μL reaction volume containing 12.5 μL of Master Mix (Tsingke Biotech, Yunnan, China), 1 μL of each primer (10 μM), 1 μL of template DNA, and 9.5 μL of deionized water. The PCR thermal cycles for three genes were performed under the following reaction conditions: an initial denaturing step for 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s for ITS and TEF1-α, and 56°C for 60 s for TUB, elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were verified on 1% agarose electrophoresis gels stained with ethidium bromide. Sequencing was carried out by Tsingke Biological Engineering Technology and Services Co., Ltd. (Yunnan, China).

Molecular phylogenetic analyses

Sequence alignment

Sequences with high similarity indices were assembled in BioEdit, and those with low similarity indices were identified through a BLAST search for the closest matches with Pestalotiopsis taxa and from recently published data (Li et al., 2021). All consensus and reference sequences were automatically aligned with MAFFT v.7 using the Auto strategy (Katoh and Standley, 2013). The aligned sequences from each gene region (ITS, TEF1-α, and TUB) were combined and manually improved using Sequence Matrix (Hall, 1999). Uncertain regions were omitted from the alignment, and gaps were treated as data that was missing. Maximum likelihood (ML) and Bayesian inference were used to conduct phylogenetic analyses.

Phylogenetic analyses

Maximum likelihood analysis was performed at the CIPRES Science Gateway v.3.3 (Miller et al., 2010) using RAxML v.8.2.8 as part of the “RAxML-HPC2 on XSEDE” tool (Stamatakis, 2006; Stamatakis et al., 2008). The optimal ML tree search was conducted with 1,000 separate runs using the default algorithm of the programme from a random starting tree for each run. The final tree was selected amongst suboptimal trees from each run by comparing the likelihood scores using the GTR+GAMMA substitution model. Maximum likelihood bootstrap values equal or greater than 60% were given as the first set of numbers above the nodes in the resulting ML tree.

Bayesian analysis was performed with MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) to evaluate posterior probabilities (Rannala and Yang, 1996) using Markov Chain Monte Carlo sampling (MCMC). The best-fit model of evolution was estimated using MrModeltest v.2.2 (Nylander, 2004). For Bayesian analysis, the best-fitting model of ITS, TEF1-α, and TUB was the GTR+I+G model. Posterior probabilities (PPs) (Rannala and Yang, 1996) were performed using Markov chain Monte Carlo sampling (BMCMC) in MrBayes v.3.1.2 (Liu et al., 2012). Six concurrent Markov chains were executed for 50 million generations, and samples of trees were taken every 5,000 generations (resulting in 10,000 trees). The initial 2,000 trees representing the burn-in phase of the analyses were discarded, while the remaining 8,000 trees were used to calculate PP in the majority rule consensus tree (Cai et al., 2006; Liu et al., 2012).

Phylogenetic trees were displayed in FigTree v. 1.4.4 (Rambaut, 2014) and edited in Adobe Illustrator CS5 (Adobe Systems, San Jose, CA, USA). Newly generated sequences were deposited in GenBank (Table 1).

Results

Phylogenetic analyses

The combined ITS, TEF1-α, and TUB sequence dataset included 154 ingroup taxa and two outgroup taxa (Neopestalotiopsis protearum and Pseudopestalotiopsis cocos) with 2,160 characters (ITS: 1–538 bp; TEF: 539–1,477 bp; TUB: 1,478–2,160 bp) overall post-alignment, including the gaps. The RAxML and Bayesian analyses of the combined dataset resulted in phylogenetic reconstructions with largely identical topologies and a ML analysis with a final likelihood value of −17482.622268, as shown in Figure 1. The matrix exhibited 993 distinct alignment patterns, with 24.14% undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.237874, C = 0.294954, G = 0.216783, T = 0.250389; substitution rates AC = 1.059763, AG = 3.258532, AT = 1.260093, CG = 0.980806, CT = 4.659318, GT = 1.000000; gamma distribution shape parameter α = 0.309168. The bootstrap support values for RAxML greater than 60% and the Bayesian posterior probabilities greater than 0.95 are given at each node (Figure 1).

In the phylogenetic analyses, all new strains were grouped with members of Pestalotiopsis, Pestalotiopsis rossiodoides, Pestalotiopsis intermedia, and Pestalotiopsis linearis were grouped together; however, P. rossiodoides has a separate branch with 84% ML and 0.96 BPP support. Pestalotiopsis appendiculata established a distinct lineage with 98% ML and 0.99 BPP bootstrap support. Pestalotiopsis suae was clustered as a sister taxon to Pestalotiopsis pinicola with a significant support (97% ML and 1 BPP). Pestalotiopsis daliensis was
TABLE 1 GenBank numbers and culture collection accession numbers of species included in the phylogenetic study.

| Taxa                          | Strain          | GenBank accession no.          | References                                      |
|-------------------------------|-----------------|--------------------------------|-------------------------------------------------|
|                               |                 | ITS   | TEF1-α  | TUB       |                                           |
| Pestalotiopsis adusta        | ICMP 6088       | AF409957 | JX399070 | JX399037  | Maharachchikumbura et al., 2012          |
| Pestalotiopsis adusta        | MFLUCC 10–0146  | JX399007 | JX399071 | JX399038  | Maharachchikumbura et al., 2012          |
| Pestalotiopsis aggestorum    | LC6301          | KX895015 | KX895234 | KX895348  | Liu et al., 2017                        |
| Pestalotiopsis aggestorum    | LC8186          | KY464140 | KY464150 | KY464160  | Liu et al., 2017                        |
| Pestalotiopsis anacardiacearum | IFRDCC 2397   | KC247154 | KC247156 | KC247155  | Maharachchikumbura et al., 2013          |
| Pestalotiopsis anacardiacearum | HN37–4        | -     | MK512485 | MK360932  | Shu et al., 2020                        |
| Pestalotiopsis anacardiacearum | YB41–2        | -     | MK512486 | MK360933  | Shu et al., 2020                        |
| Pestalotiopsis anacardiacearum | FY10–12       | -     | MK512484 | MK360931  | Shu et al., 2020                        |
| Pestalotiopsis appendiculata | CGMCC 3.23550   | OP082431 | OP185509 | OP185516  | This study                              |
| Pestalotiopsis areccuthobii  | CBS 434.65      | NR147561 | KM199516 | KM199427  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis arengae       | CBS 331.92      | NR147560 | KM199515 | KM199426  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis australasiae   | CBS 114142      | NR147546 | KM199499 | KM199409  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis australasiae   | CBS 114144      | KM199298 | KM199501 | KM199410  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis australis      | CBS 111503      | KM199331 | KM199557 | KM199382  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis australis      | CBS 114193      | KM199332 | KM199475 | KM199383  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis biciliata      | CBS 124463      | KM199308 | KM199505 | KM199399  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis biciliata      | CBS 236.38      | KM199309 | KM199506 | KM199401  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis biciliata      | CBS 790.68      | KM199305 | KM199507 | KM199400  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis brachiata      | LC2988          | KX894933 | KX895150 | KX895265  | Liu et al., 2017                        |
| Pestalotiopsis brachiata      | LC8188          | KY464142 | KY464152 | KY464162  | Liu et al., 2017                        |
| Pestalotiopsis brassicae      | CBS 170.26      | KM199379 | KM199558 | -         | Maharachchikumbura et al., 2014          |
| Pestalotiopsis camelliae      | CBS 443.62      | KM199336 | KM199512 | KM199424  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis camelliae-oleiferae | MFLUCC 12–0277 | NR120181 | JX399074 | JX399041  | Zhang et al., 2012a                     |
| Pestalotiopsis camelliae-oleiferae | LHLDK 08     | OK493593 | OK507963 | OK562368  | Li et al., 2021                         |
| Pestalotiopsis camelliae-oleiferae | LHLDK 09     | OK493594 | OK507964 | OK562369  | Li et al., 2021                         |
| Pestalotiopsis camelliae-oleiferae | LHLDK 10     | OK493595 | OK507965 | OK562370  | Li et al., 2021                         |
| Pestalotiopsis camelliae-oleiferae | CGMCC 3.23544 | OP082426 | OP185510 | OP185517  | This study                              |
| Pestalotiopsis chamaeaeopsis  | CBS 113607      | KM199325 | KM199472 | KM199390  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis chamaeaeopsis  | CBS 186.71      | KM199326 | KM199473 | KM199391  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis clavata        | MFLUCC 12–0268  | JX398990 | JX399056 | JX399025  | Maharachchikumbura et al., 2012          |
| Pestalotiopsis colombiensis   | CBS 118553      | NR147551 | KM199488 | KM199421  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis daleniensis    | CGMCC 3.23548   | OP082429 | OP185511 | OP185518  | This study                              |
| Pestalotiopsis digitalis      | ICMP 5344       | KITP81879 - | KITP81883 - | Maharachchikumbura et al., 2016          |
| Pestalotiopsis diploclisiae   | CBS 115585      | KM199315 | KM199483 | KM199417  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis diploclisiae   | CBS 115587      | KM199320 | KM199486 | KM199419  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis diploclisiae   | CBS 115449      | KM199314 | KM199485 | KM199416  | Maharachchikumbura et al., 2014          |
| Pestalotiopsis disseminata    | CBS 118552      | MH553986 | MH554410 | MH554652  | Liu et al., 2019                        |
| Pestalotiopsis disseminata    | CBS 143904      | MH554152 | MH554587 | MH554825  | Liu et al., 2019                        |
| Pestalotiopsis disseminata    | CPC 29351       | MH554166 | MH554601 | MH554839  | Liu et al., 2019                        |
| Pestalotiopsis distincta      | LC2323          | KX894961 | KX895178 | KX895293  | Liu et al., 2017                        |
| Pestalotiopsis distincta      | LC8184          | KY464138 | KY464148 | KY464158  | Liu et al., 2017                        |
| Pestalotiopsis diversiseta    | MFLUCC 12–0287  | JX399009 | JX399073 | JX399040  | Maharachchikumbura et al., 2012          |
| Pestalotiopsis doitungensis   | MFLUCC 14–0090  | MK953573 | MK975831 | MK975836  | Ma et al., 2019                         |
| Pestalotiopsis draconaeae     | HGUP4037        | MT596615 | MT598644 | MT598645  | Ariyawanana et al., 2015                |
| Pestalotiopsis draconaeola    | MFLUCC 18–0913  | MN962731 | -         | -         | Chaiwan et al., 2020                    |
| Pestalotiopsis draconaeola    | MFLUCC 18–0914  | MN962734 | -         | -         | Chaiwan et al., 2020                    |
| Pestalotiopsis dracocontomelum | MFLUCC 10–0149 | KITP81877 - | KITP81880 - | Maharachchikumbura et al., 2016          |
| Pestalotiopsis endophytic     | MFLUCC 18–0932  | NR 172439 | MW417119 | -         | De Silva et al., 2021                   |

(Continued)
| Taxa                     | Strain       | GenBank accession no. | References          |
|-------------------------|--------------|-----------------------|---------------------|
| Pestalotiopsis endophytic | MFLUCC 20–0142  | MW263948 - -        | De Silva et al., 2021 |
| Pestalotiopsis endophytic | MFLUCC 16–0946  | MW263947 MW29384 - - | De Silva et al., 2021 |
| Pestalotiopsis ericaeearum | IFRDC 2439  | KC337807 KC33784 KC57381 | Zhang et al., 2013 |
| Pestalotiopsis etonensis | BRIP 66615  | MK966339 MK97765 MK97764 | Crous et al., 2020 |
| Pestalotiopsis formosana | NTUCC 17–009  | MH809381 MH809389 MH809388 | Ariyawansa et al., 2015 |
| Pestalotiopsis formosana | NTUCC 17–010  | MH809382 MH809390 MH809386 | Ariyawansa et al., 2015 |
| Pestalotiopsis furcata | LC6303  | KX895016 KX895235 KX895349 | Liu et al., 2017 |
| Pestalotiopsis furcata | MFLUCC 12–0054  | IQ683724 IQ683740 IQ683708 | Maharachchikumbura et al., 2013 |
| **Pestalotiopsis fusioidea** | CGMCC 3.23545  | OP082427 OP155512 OP155519 | This study |
| Pestalotiopsis gaultheri | IFRD 411–014  | KC537805 KC537812 KC537819 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis gibbosa | NOF 3175  | LC311589 LC311591 LC311590 | Watanabe et al., 2018 |
| Pestalotiopsis hawaiiensis | CBS 114491  | NR147559 NR147560 NR147561 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis hawaiiensis | CBS 115391  | MH553981 MH554399 MH554640 | Liu et al., 2019 |
| Pestalotiopsis hunanensis | LHXT 15  | OK936000 OK936700 OK936270 | Li et al., 2021 |
| Pestalotiopsis hunanensis | LHXT 18  | OK936060 OK936700 OK936270 | Li et al., 2021 |
| Pestalotiopsis huangshanensis | CBS 336.97 | KM199317 KM199302 KM199395 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis hydei | MFLUCC 20–0135  | NR172003 MW251113 MW251112 | Huanalek et al., 2021 |
| Pestalotiopsis iberica | CBS 109350  | KM199380 KM199381 KM199382 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis iberica | CBS 111963  | KM199380 KM199381 KM199382 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis italiania | MFLUCC 12–0657  | KP78188 K78188 K781882 | Liu et al., 2015 |
| Pestalotiopsis jesteri | CBS 109350  | KM199380 KM199381 KM199382 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis jenkinsii | LC4399  | KX955090 KX955227 KX955341 | Liu et al., 2017 |
| Pestalotiopsis jenkinsii | LC6366  | KX955028 KX955247 KX955361 | Liu et al., 2017 |
| Pestalotiopsis kandelicola | LC8190  | KY641414 KY64154 KY64164 | Liu et al., 2017 |
| Pestalotiopsis kandelicola | NCYUCC 19–0355 | MT560722 MT560723 MT560724 | Hyde et al., 2020 |
| Pestalotiopsis kandelicola | NCYUCC 19–0355 | MT560722 MT560723 MT560724 | Hyde et al., 2020 |
| Pestalotiopsis kandelicola | KNU-PT-1804  | LC529593 LC525555 LC525594 | Das et al., 2020 |
| Pestalotiopsis kensuya | CBS 442.67  | KM199302 KM199303 KM199395 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis krahvienis | MFLUCC 16–0260  | MH388360 MH388395 MH388422 | Tiberamona et al., 2018 |
| Pestalotiopsis knightiae | CBS 114138  | KM199311 KM199349 KM199408 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis knightiae | CBS 111963  | KM199311 KM199349 KM199408 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis leucadendri | CBS 121417  | MH553987 MH554412 MH554645 | Liu et al., 2019 |
| Pestalotiopsis liculata | HGUP 4057  | KC492509 KC481684 KC481683 | Geng et al., 2013 |
| Pestalotiopsis linearis | MFLUCC 12–0271  | JX990094 JX990060 JX990027 | Maharachchikumbura et al., 2012 |
| Pestalotiopsis lushananensis | LC8182  | KY641416 KY641417 KY641416 | Liu et al., 2017 |
| Pestalotiopsis lushananensis | LC8182  | KY641416 KY641417 KY641416 | Liu et al., 2017 |
| Pestalotiopsis macadamiae | BRIP 63738B | KX186588 KX186620 KX186680 | Akinsanmi et al., 2017 |
| Pestalotiopsis malayantha | CBS 102220  | NR147550 KM199482 KM199441 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis monocella | CBS 144.97  | KM199327 KM199479 KM199386 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis monocella | CBS 144.97  | KM199327 KM199479 KM199386 | Maharachchikumbura et al., 2014 |
| Pestalotiopsis montellica | MFLUCC 12–0279  | JX990112 JX990076 JX990043 | Maharachchikumbura et al., 2012 |
| Pestalotiopsis nanjingensis | LHNJ 16  | OK936060 OK936700 OK936270 | Li et al., 2021 |
| Pestalotiopsis nanjingensis | LHNJ 20  | OK936060 OK936700 OK936270 | Li et al., 2021 |

(Continued)
| Taxa                        | Strain   | GenBank accession no. | References                      |
|-----------------------------|----------|-----------------------|---------------------------------|
| **Pestalotiopsis nanjingensis** | LHNJ 04  | OK493604 OK507974 OK562379 | Li et al., 2021                |
| **Pestalotiopsis nanjingensis** | LHGX 10  | OK493596 OK507966 OK562371 | Li et al., 2021                |
| **Pestalotiopsis nanjingensis** | LHGX 11  | OK493597 OK507967 OK562372 | Li et al., 2021                |
| **Pestalotiopsis neglecta**  | TAP1100  | AB482220 LC311600 LC311599 | Watanabe et al., 2018          |
| **Pestalotiopsis neolitseae** | NTUCC 17-011 | MH809383 MH809391 MH809387 | Aiyawansa and Hyde, 2018        |
| **Pestalotiopsis neolitseae** | NTUCC 17-012 | MH809384 MH809392 MH809388 | Aiyawansa and Hyde, 2018        |
| **Pestalotiopsis neolitseae** | KUMCC 19-0243 | MN625276 MN626741 MN626730 | Aiyawansa and Hyde, 2018        |
| **Pestalotiopsis novae-hollandiae** | CBS 130973 | NR147557 KM199511 KM199425 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis oryzae**     | CBS 111522 | KM199294 KM199493 KM199394 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis oryzae**     | CBS 353.69 | KM199299 KM199496 KM199398 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis pallidotheae** | MAFF 240993 | LC311585 LC311584 | Watanabe et al., 2010          |
| **Pestalotiopsis pandanicola** | MFLUCC 16-0255 | MH388361 MH388396 MH412723 | Töpbromma et al., 2018         |
| **Pestalotiopsis papuana**    | CBS 131.96 | KM199321 KM199491 KM199413 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis papuana**    | CBS 887.96 | KM199318 KM199492 KM199415 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis parva**      | CBS 265.37 | KM199312 KM199508 KM199404 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis parva**      | CBS 278.35 | MH855675 KM199509 KM199405 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis photinicola** | GZCC 16-0028 | KY092404 KY047662 KY047663 | Chen et al., 2017              |
| **Pestalotiopsis pini**       | CBS 146841 | MT374681 MT374694 MT374706 | Silva et al., 2020             |
| **Pestalotiopsis pini**       | CBS 146840 | MT374680 MT374693 MT374705 | Silva et al., 2020             |
| **Pestalotiopsis pini**       | CBS 146842 | MT374682 MT374695 MT374707 | Silva et al., 2020             |
| **Pestalotiopsis pini**       | MEAN 1167 | MT374689 MT374701 MT374714 | Silva et al., 2020             |
| **Pestalotiopsis pinicola**   | KUMCC 19-0203 | MN412637 MN417510 MN417508 | Töpbromma et al., 2019         |
| **Pestalotiopsis pinicola**   | KUMCC 19-0183 | MN412636 MN417509 MN417507 | Töpbromma et al., 2019         |
| **Pestalotiopsis portugalsica** | CBS 193.48 | KM199335 KM199510 KM199422 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis portugalsica** | LC2929  | KX894921 KX895138 KX895253 | Liu et al., 2017               |
| **Pestalotiopsis phyllophora** | MFLUCC 17-0416 | MK764283 MK764327 MK764349 | Norphanphoun et al., 2019      |
| **Pestalotiopsis phyllophora** | MFLUCC 17-0417 | MK764284 MK764328 MK764350 | Norphanphoun et al., 2019      |
| **Pestalotiopsis rhododendri** | OPI86   | KC537804 KC537811 KC537818 | Zhang et al., 2013             |
| **Pestalotiopsis rhodomyrtus** | LC4458  | KX894981 KX895198 KX895313 | Liu et al., 2017               |
| **Pestalotiopsis rhodomyrtus** | LC4458  | KX895010 KX895228 KX895342 | Liu et al., 2017               |
| **Pestalotiopsis rosea**      | MFLUCC 12-0258 | JX990005 JX990069 JX99036 | Maharachchikumbura et al., 2012 |
| **Pestalotiopsis rosarioides** | CGMCC 3.23549 | OP082430 OP185513 OP185520 | This study                     |
| **Pestalotiopsis scoparia**   | CBS 176.25 | KM199330 KM199478 KM199393 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis sequana**    | MFLUCC 13-0399 | KX572339 - - | Hyde et al., 2016              |
| **Pestalotiopsis shandongensis** | KUMCC 19-0241 | MN625275 MN626740 MN626729 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis shorea**     | MFLUCC 12-0314 | KJ503811 KJ503817 KJ503814 | Song et al., 2014              |
| **Pestalotiopsis spathulata**  | CBS 356.86 | NR147538 KM199513 KM199423 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis spathulataappendiculata** | CBS 144035 | MH554172 MH554607 MH554845 | Liu et al., 2019               |
| **Pestalotiopsis suae**       | CGMCC 3.23546 | OP082428 OP185514 OP185521 | This study                     |
| **Pestalotiopsis teleocep**   | CBS 113606 | KM199295 KM199498 KM199402 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis teleocep**   | CBS 114137 | KM199301 KM199559 KM199469 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis teleocep**   | CBS 114161 | KM199296 KM199500 KM199403 | Maharachchikumbura et al., 2014 |
| **Pestalotiopsis terricola**  | CBS 141.69 | MH554004 MH554438 MH554680 | Liu et al., 2019               |
| **Pestalotiopsis thailandica** | MFLUCC 17-1616 | MK764285 MK764329 MK764351 | Norphanphoun et al., 2019      |
| **Pestalotiopsis thailandica** | MFLUCC 17-1617 | MK764286 MK764330 MK764352 | Norphanphoun et al., 2019      |
| **Pestalotiopsis trachicarpicola** | OP068  | JQ845947 JQ845946 JQ845945 | Zhang et al., 2012b            |
clumped as a sister to Pestalotiopsis chamaeapertis with significant support (75% ML and 0.95 BPP). Pestalotiopsis fusoida, Pestalotiopsis cangshanensis, Pestalotiopsis pini, Pestalotiopsis lusahenensis, Pestalotiopsis rhodendri, and Pestalotiopsis clavata were grouped together in an independent clade within Pestalotiopsis, while P. fusoida and P. cangshanensis formed distinct branches. Pestalotiopsis trachicarpica was clustered with the ex-type of P. trachicarpica with strong support (98% ML and 0.99 BPP).

*Pestalotiopsis appendiculata* D.F. Bao, R. Gu and Z.L. Luo, sp. nov.

*MycoBank number:* 845187, *Figure 2.*

*Holotype—*KUN-HKAS 124571

*Etymology—“appendiculata,” denoting the fungus conidial appendages.

*Endophytic in fresh Rhododendron decorum leaves.* **Sexual morph:** *Conidiation pycnidial* in PDA culture, globose or clavate, aggregated or scattered, semi-immersed to erumpent, gray. There is no evidence of a conidiogenous cell. *Conidia* 19–24 × 5–6 μm (*n* = 30), fusoid, ellipsoid, straight to slightly curved, four-septate, slightly constricted at septa. Basal cell 2–4 μm long, conic to obconic with a truncate base, hyaline, verrucose, and thin-walled. Three-median cells doliiform, plicated, 13–15 μm (*n* = 14 μm, *n* = 30). Thin-walled, with a uniform light color on the third cell and the fourth cell relatively darker, the septa darker than the rest of the cells (second cell from the base, 4–6 μm long; third cell 5–6 μm long; fourth cell 4–6 μm long). Apical cell 2–4 μm long, hyaline, subcylindrical, or obconic with a truncate base, thin-walled, slightly rugose. With 2–3 tubular apical appendages arising from the apical crest, unbranched, filiform, 8–15 μm (*n* = 12 μm, *n* = 30). Basal appendage single, tubular, centric, or uncentred, 3–5 μm long.

*Material examined:* China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of *R. decorum* (2489 m, 24.3240°N, 101.0140°E), April 2021, Z.Q. Zhang, D-60 (KUN-HKAS 124571, *holotype*), ex-type culture, CGMCC 3.23550 = KUNCC 22-12405.

*Notes:* *P. appendiculata*, *P. intermedia*, *P. linearis*, and *P. rosarioides* were grouped in the same clade in the phylogenetic analysis. Comparisons of ITS, TEF-1α, and TUB gene regions of *P. appendiculata* with *P. linearis* (2/538 in ITS, 12/398 in TEF-1α, and 23/450 in TUB), *P. intermedia* (3/537 in ITS, 17/398 in TEF-1α, and 13/479 in TUB), and *P. rosarioides* (3/553 in ITS, 22/553 in TEF-1α, and 22/458 in TUB) indicated significant differences. However, *P. appendiculata* can be distinguished from *P. linearis* and *P. intermedia* with its smaller conidia (Table 2). Moreover, the three-median cells of conidia in *P. appendiculata* are light gray; in contrast, they are brown in *P. linearis*, *P. intermedia*, and *P. rosarioides*. Based on the combined ITS, TEF-1α, and TUB sequence data, our phylogenetic analysis revealed that they are clearly distinct in the phylogram. *P. appendiculata* formed a separate branch with strong support values (98 ML/0.99 PP, Figure 1). Therefore, based on phylogenetic analysis and its morphological characteristics, *P. appendiculata* is introduced as a new species.

*Pestalotiopsis cangshanensis* H.W. Shen, R. Gu and Z.L. Luo, sp. nov.

*MycoBank number:* 845188, *Figure 3.*

*Holotype—*KUN-HKAS 124573

*Etymology—“cangshanensis,” referring to the Cangshan Mountain, where the species was obtained.

*Endophytic in fresh Rhododendron delavayi leaves.* **Sexual morph:** Undetermined. **Asexual morph:** *Conidiation pycnidial* on PDA, dark brown to black conidial masses, globose, ink-shaped. *Conidiophores* indistinct and typically reduced to conidiogenous cells. Conidiogenous cells discrete, subcylindrical to ampulliform, hyaline, sometimes slightly wide at the base, truncated at the apex, 4–24 × 2–4 μm. *Conidia* 18–23 × 6–8 μm (*n* = 19 × 2 μm, *n* = 30), fusoid, straight to slightly curved, four-septate; three-median cells light or dark brown, 10–14 μm long (*n* = 12 μm, *n* = 30); second cell from the base pale-light brown 3–5 μm long; third cell 3–5 μm long.

**TABLE 1 (Continued)**

| Taxa                                | Strain     | GenBank accession no. | References                                      |
|-------------------------------------|------------|------------------------|-------------------------------------------------|
| *Pestalotiopsis trachicarpica*      | CGMCC 3.23547 | OP082432               | OP185515 | OP185522 | This study                                      |
| *Pestalotiopsis unicolor*            | MFLUCC 12–0275 | JX398998               | JX399063 | JX399029 | Maharachchikumbura et al., 2012                |
| *Pestalotiopsis unicolor*            | MFLUCC 12–0276 | JX398999               | JX399063 | JX399030 | Maharachchikumbura et al., 2012                |
| *Pestalotiopsis verruculosa*         | MFLUCC 12–0274 | JX398996               | JX399061 |          | Maharachchikumbura et al., 2012                |
| *Pestalotiopsis yunlingensis*        | LC3067     | KX895166               | KX895281 |          | Liu et al., 2017                              |
| *Pestalotiopsis yunlingensis*        | LC4553     | KX895012               | KX895231 | KX895345 | Liu et al., 2017                              |
| *Pseudopestalotiopsis cacao*         | CBS 272.29 | KM1999378              | KM1999467 |          | Maharachchikumbura et al., 2014                |
| *Neopestalotiopsis protearum*        | CBS 114178 | JN712498               | LT853201 | KM199463 | Maharachchikumbura et al., 2014                |

The newly generated sequences are in bold. “-” represent the sequences are unavailable.
long; fourth cell 3–5 µm long), wall verruculose, concolorous. Base cell inverted funnel-shaped with a truncated base, 3–4 µm long (x = 4 µm), hyaline, thin-walled. Apical cell 4–5 µm long (x = 4 µm), hyaline, cylindrical to subcylindrical, thin, and smooth-walled. With three tubular apical appendages 9–19 µm long (x = 15 µm, n = 30) arising from the apical crest, filiform, unbranched. Basal appendage single, tubular, unbranched, centric, 5–8 µm long (x = 7 µm, n = 30).

**Material examined:** China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of *R. delavayi* (2489 m, 25.4724°N, 99.5949°E), March 2021, Z.Q. Zhang, D-6 (KUN-HKAS 124573, holotype), ex-type culture, CGMCC 3.23544.

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**FIGURE 1**

(Continued)
FIGURE 1
Phylogenetic tree based on RAxML analyses of a combined internal transcribed spacer (ITS), translation elongation factor 1-alpha (TEF1-α), and β-tubulin (TUB) dataset. Bootstrap support values for maximum likelihood ≥60% and Bayesian posterior probabilities ≥0.95 are indicated above the nodes as Maximum likelihood/Posterior probability (ML/PP). The tree is rooted in Neopestalotiopsis protearum (CBS 114.178) and Pseudopestalotiopsis cocos (CBS 272.29). The new isolates are in blue.

Notes: P. cangshanensis, P. clavata, P. lushanensis, P. rhododendri, and P. pini were grouped together. Comparisons of ITS, TEF-1α, and TUB gene regions of P. cangshanensis with P. lushanensis (2/505 in ITS, 16/932 in TEF-1α, and 12/789 in TUB), P. pini (2/605 in ITS, 17/474 in TEF-1α, and 11/792 in TUB), P. rhododendri (2/538 in ITS, 17/941 in TEF-1α, and 11/458 in TUB), and P. clavata (1/539 in ITS, 10/947 in TEF-1α, and 19/457 in TUB) exhibited significant differences. Morphologically, P. cangshanensis has smaller conidia than P. pini, P. clavata, P. rhododendri, and P. lushanensis (Table 3). Moreover, P. cangshanensis has shorter apical appendages than those of P. rhododendri (21–28 vs. 9–19 μm) and P. lushanensis (17–26 vs. 9–19 μm). Based on combined ITS, TEF1-α, and TUB sequence data, P. cangshanensis is clearly separated in the phylogram as it forms an independent clade. It indicates that P. cangshanensis can be introduced as a new species.

Pestalotiopsis daliensis H.W. Shen, R. Gu and Z.L. Luo, sp. nov.
MycoBank number: 845189, Figure 4.
Holotype—KUN-HKAS 124576
Etymology—“daliensis,” referring to Dali City, where the species was obtained.
Endophytic in fresh R. decorum leaves. Sexual morph: Undetermined. Asexual morph: Conidiomata (on PDA)
FIGURE 2
Pestalotiopsis appendiculata (KUN–HKAS 124571, holotype). (A) Leaves of Rhododendron decorum. (B,C) Culture on potato dextrose agar (PDA) (upper and lower view). (D,E) Conidiomata on PDA. (F,G) Conidiophores, conidiogenous cells, and conidia. (H–O) Conidia. Scale bars: (F) 15 µm, (G) 10 µm, (H) 15 µm, (I–O) 10 µm.

TABLE 2 Compare the conidia size.

| Species             | Conidial size | References                      |
|---------------------|---------------|---------------------------------|
| Pestalotiopsis appendiculata | 19–24 × 5–6 µm | This study                     |
| P. linearis         | 24–33 × 5–6 µm | Maharachchikumbura et al., 2012|
| P. intermedia       | 24–28 × 6–7 µm | Maharachchikumbura et al., 2012|

Pycnidal, globose to clavate, solitary, exuding globose, dark-brown to black conidial masses. Conidiophores are usually indistinct and reduced to conidiogenous cells. Conidiogenous cells discrete, subcylindrical to ampulliform, hyaline, sometimes slightly wide at the base, truncated at the apex, 5–19 × 1–3 µm. Conidia 23–26 × 4–5 µm ($\bar{x} = 25 \times 5$ µm, $n = 30$), fusoid, ellipsoid, straight to slightly curved, four-septate; basal cell conic with a truncated base, hyaline or light-brown and thin-walled, 4–6 µm long ($\bar{x} = 5$ µm, $n = 30$). Three-median cells dark, 13–16 µm long ($\bar{x} = 15$ µm, $n = 30$), wall smooth, concolourous, septa darker than the rest of the cells (second cell from the base pale brown, 4–5 µm long; third cell, 4–5 µm long; fourth cell, 4–6 µm long). Apical cell 4–6 µm long ($\bar{x} = 5$ µm, $n = 30$), hyaline, subcylindrical, thin-walled, and smooth-walled. With 2–3 tubular apical appendages.
**FIGURE 3**
Pestalotiopsis cangshanensis (KUN-HKAS 124573, holotype). (A) Leaves of Rhododendron delavayi. (B,C) Culture on potato dextrose agar (PDA) (upper and lower view). (D,E) Conidiomata on PDA. (F) Conidiophores, conidiogenous cells, and conidia. (G–O) Conidia with appendages. Scale bars: (F–H) 10 µm, (I–M) 15 µm, (N,O) 10 µm.

### TABLE 3
Compare the conidia size.

| Species            | Conidial size | References                  |
|--------------------|---------------|-----------------------------|
| Pestalotiopsis cangshanensis | 18–23 × 6–8 µm   | This study                  |
| P. pini            | 23–25 × 7–8 µm   | Silva et al., 2020          |
| P. clavata         | 20–27 × 7–8 µm   | Maharachchikumbura et al., 2012 |
| P. rhododendri     | 24–26 × 6–7 µm   | Zhang et al., 2013          |
| P. lushanensis     | 20–27 × 8–10 µm  | Liu et al., 2017            |
| P. fusoidea        | 22–26 × 6–7 µm   | This study                  |

13–22 µm long (X = 18 µm, n = 30), arising from the apical crest, unbranched, filiform. Basal appendage 10–16 µm long (X = 13 µm, n = 30), single, tubular, unbranched, centric, straight, or slightly curved.

**Material examined:** China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of R. decorum (2470 m, 25.5044°N, 100.0542°E), March 2021, Z.Q. Zhang, D-33 (KUN-HKAS 124576, holotype), ex-type culture, CGMCC 3.23548 = KUNCC 22-12403.

**Notes:** In the phylogenetic analysis, P. chamaeropis and P. daliensis are closely associated. Comparisons of ITS, TEF-1α, and TUB gene regions of P. daliensis with P. chamaeropis (2/599 in ITS, 6/478 in TEF-1α, and 8/774 in TUB) exhibited significant differences. However, the conidia of P. daliensis are relatively narrower than P. chamaeropis (4–5 vs. 7–9 µm). Moreover, P. daliensis has a much longer conidial basal appendage (10–16 vs. 4–9 µm). Hence, *P. daliensis* is introduced as a new species.

**Pestalotiopsis fusoidea** D.F. Bao, R. Gu and Z.L. Luo, sp. nov.

**Mycobank number:** 845190, **Figure 5**.
FIGURE 4
Pestalotiopsis daliensis (KUN-HKAS 124576, holotype). (A) Leaves of Rhododendron decorum. (B,C) Culture on potato dextrose agar (PDA) (upper and lower view). (D,E) Conidiomata on PDA. (F) Conidiophores, conidiogenous cells, and conidia. (G–O) Conidia. Scale bars: (F) 25 µm, (G) 15 µm, and (H–O) 10 µm.

Holotype—KUN-HKAS 124579

Etymology—“fusoida,” referring to the fusoid conidia of this fungus.

Endophytic in fresh R. delavayi leaves. Sexual morph: Undetermined. Asexual morph: Colonies on PDA attaining 15–20 mm in diameter after 7 days at 25°C. Smooth edge, whitish, gregarious. Conidiomata formation black droplets, gregarious, reverse pale yellow. Conidia aggregate in culture to form black-droplet conidia masses. Conidiophores indistinct, typically reduced to conidiogenous cells. Conidiogenous cells discrete, subcylindrical to ampulliform, hyaline, and sometimes slightly wide at the base, 5–29 × 2–4 µm. Conidia 23–26 × 6–7 (X = 25 × 7 µm, n = 30), fusoid, four-septate, lightly, curved. Three-median cells 13–18 µm long (X = 16 µm, n = 30), brown or olive. Some of the third cells are the darkest, second cell 5–6 µm long (X = 5 µm, n = 30), third cell 4–6 µm long (X = 5 µm, n = 30), fourth cell 4–6 µm long (X = 5 µm, n = 30), apical cell 3–4 µm long (X = 4 µm, n = 30), hyaline, cylindrical to
FIGURE 5
Pestalotiopsis fusoidea (KUN-HKAS 124579, holotype). (A) Leaves of Rhododendron delavayi. (B,C) Culture on potato dextrose agar (PDA) (upper and lower view). (D,E) Conidiomata on PDA. (F–H) Conidiophores, conidiogenous cells, and conidia. (I–N) Conidia. Scale bars: (F–H) 20 µm, (J–N) 15 µm, and (I–N) 10 µm.

subcylindrical, with 2–4 (or mostly 3) tubular apical appendages 7–11 µm long (x̄ = 8 µm, n = 30) long arising from the apical crest, filiform. The base cell is an inverted triangle 4–6 µm long (x̄ = 4 µm, n = 30), with light brown or almost transparent hyaline. Basal appendage single, tubular, unbranched, centric, vertical, or curved, 4–6 µm long (x̄ = 6 µm, n = 30).

Material examined: China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of R. delavayi (2716 m, 25.5032°N, 100.4265°E), March 2021, Z.Q. Zhang. D-8 (KUN-HKAS 124579, holotype), ex-type culture CGMCC 3.23545 = KUNCC 22-12401.

Notes: Phylogenetically, P. fusoidea has a close with P. clavata, P. lushanensis, P. rhododendri, and P. pini. Comparisons of ITS, TEF-1α, and TUB gene regions of P. fusoidea with P. lushanensis (2/505 in ITS, 16/932 in TEF-1α, and 12/789 in TUB), P. rhododendri (2/538 in ITS, 13/941 in TEF-1α, and 11/458 in TUB), P. clavata (9/539 in ITS, 14/1031 in TEF-1α, and 12/947 in TUB).
14/947 in TEF-1α, and 11/457 in TUB), P. pini (2/571 in ITS, 17/512 in TEF-1α, and 11/514 in TUB) exhibited significant differences. However, P. fusoida has shorter apical appendages than P. pini (7–11 vs. 18–20 μm), P. clavata (7–11 vs. 20–25 μm), or P. rhododendri (7–11 vs. 21–29 μm). P. fusoida has smaller conidia than P. lusianensis (23–26 × 6–7 vs. 18–23 × 6–8 μm). Based on combined ITS, TEF1-α, and TUB sequence data, P. fusoida are apparently separated in the phylogram, forming a separate clade. It indicates that P. fusoida can be introduced as a new species.

**Pestalotiopsis rosarioides** H.W. Shen, R. Gu and Z.L. Luo, *sp. nov.*

*MycoBank number: 845191, Figure 6.*

Holotype—KUN-HKAS 124574

Etymology—“rosarioides,” referring to the rosary-like enlargement of the second and fourth cells of this fungus.

*Endophytic* in fresh *R. decorum* leaves. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* (on PDA) pycnidial, globose to clavate, solitary, exuding globose, dark-brown to black conidial masses. *Conidiogenous cells* are not obvious. *Conidia* 22–25 × 6–7 μm (x = 23 × 7 μm, n = 30), fusoid, ellipsoid, rosary, straight to slightly curved, four-septate. Basal cell conic with a truncated base, hyaline or light brown, and thin-walled, 4–5 μm long (x = 5 μm, n = 30). Three-median cells dark, 16–18 μm long (x = 17 μm, n = 30), smooth wall, concolourous, septa darker than the rest of the cells (second cell from the base pale brown and enlarged, 4–5 μm long; third cell 4–5 μm long; fourth cell expands to 3–6 μm long). Apical cell 4–7 (x = 5 μm, n = 30) long, hyaline, subcylindrical, smooth-walled. With 1–3 tubular apical appendages 5–9 μm long (x = 7 μm, n = 30) arising from the apical crest, unbranched, filiform. Basal appendage 4–5 μm long (x = 4 μm, n = 30), single, tubular, unbranched, centric. 

**Material examined:** China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of *R. decorum* (2784 m, 25°4.5'N, 99°9.15'E), July 2021, Z.Q. Zhang, D-46 (KUN-HKAS 124574, holotype), ex-type culture, CGMCC 3.23549 = KUNCC 22-12404.

Notes: From the phylogenetic analysis, *P. intermedia*, *P. linearis*, and *P. rosarioides* clustered within the same clade. Comparisons of ITS, TEF-1α, and TUB gene regions of *P. rosarioides* with *P. intermedia* (2/537 in ITS, 2/946 in TEF-1α, and 9/479 in TUB), and *P. linearis* (2/538 in ITS, 4/946 in TEF-1α, and 12/450 in TUB) exhibited significant differences. However, the second and fourth conidial cells of *P. rosarioides* are enlarged, which is distinct from other species in the genus. Moreover, *P. rosarioides* has much shorter apical appendages than *P. linearis* and *P. intermedia* (5–9 μm in *P. rosarioides* vs. 10–20 μm in *P. linearis* and 10–28 μm in *P. intermedia*). Furthermore, phylogenetic analysis indicates that *P. rosarioides* forms a distinct lineage within the clade (Figure 1), supporting it as a new species.

**Pestalotiopsis suae** H.W. Shen, R. Gu and Z.L. Luo, *sp. nov.*

MycoBank number: 845192, Figure 7.

Holotype—KUN-HKAS 124578

Etymology—“suae” in memory of the Chinese mycologist Prof. Hong-Yan Su, who kindly helped the authors in many ways and sadly passed away on 3 May 2022 during the preparation of the current article.

*Endophytic* in fresh *R. delavayi* leaves. **Sexual morph:** Undetermined. **Asexual morph:** Conidia aggregate in culture to form black-droplet conidia masses. *Conidiophores* indistinct and typically reduced to conidiogenous cells. *Conidiogenous cells* discrete, subcylindrical to ampulliform, hyaline, sometimes slightly wide at the base 5–19 × 1–3 μm. *Conidia* 17–24 × 4–8 μm (x = 23 × 7 μm, n = 30), fusoid, four-septate. A distinct dark separation exists between each cell, lightly curved, including three-median cells 7–16 μm long (x = 17 μm, n = 30), brown or olive, with the third cell darker. Apical cell 3–4 μm long (x = 4 μm, n = 30), hyaline, cylindrical to subcylindrical, with 2–3 tubular apical appendages (mostly 2), 7–11 μm long (x = 8 μm, n = 30), arising from the apical crest, filiform. Second cell 5–6 μm long (x = 5 μm, n = 30); third cell 4–6 μm long (x = 5 μm, n = 30); fourth cell 4–6 μm long (x = 5 μm, n = 30). Base cell is an inverted triangle, 4–6 μm long (x = 4 μm, n = 30), light brown or almost transparent hyaline. Basal appendage single, tubular, unbranched, centric, vertical, or curved, 4–6 μm long (x = 6 μm, n = 30).

**Material examined:** China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of *R. delavayi* (2489 m, 25°4.659°N, 99°58.29°E), March 2021, Z.Q. Zhang, D-14 (KUN-HKAS 124578, holotype), ex-type culture, CGMCC 3.23546 = KUNCC 22-12402.

Notes: Based on phylogenetic analysis, the newly discovered *P. suae* is closely related to *P. rosa* and *P. piniola*. Comparisons of ITS, TEF-1α, and TUB gene regions of *P. suae* with *P. rosa* (3/539 in ITS, 13/943 in TEF-1α, and 9/453 in TUB), and *P. piniola* (10/608 in ITS, 9/467 in TEF-1α, and 5/409 in TUB) exhibited significant differences. However, *P. suae* is different from *P. rosa* due to its brown conidia, while the conidia of *P. rosa* are slightly red. *P. suae* can be distinguished from *P. piniola* due to its size of apical and basal appendages; *P. suae* has shorter apical appendages (5–17 vs. 7–11 μm) and longer basal appendages (2–7 vs. 4–6 μm).

**Pestalotiopsis trachiparica** Y.M. Zhang and K.D. Hyde, Cryptog. Mycol. 33(3):311–318 (2012). **Figure 8.**

*Endophytic* in fresh *Rhododendron cyanocarpum* leaves. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* pycnidial in culture on PDA, globose or clavate, aggregated or scattered, semi-immersed to erumpent, dark-brown to black. *Conidiogenous cells* discrete, subcylindrical to ampulliform, hyaline, sometimes slightly wide at the base 5–19 × 1–3 μm. *Conidia* 17–24 × 4–8 μm (x = 23 × 7 μm, n = 30), fusoid, four-septate. A distinct dark separation exists between each cell, lightly curved, including three-median cells 7–16 μm long (x = 17 μm, n = 30), brown or olive, with the third cell darker. Apical cell 3–4 μm long (x = 4 μm, n = 30), hyaline, cylindrical to subcylindrical, with 2–3 tubular apical appendages (mostly 2), 7–11 μm long (x = 8 μm, n = 30), arising from the apical crest, filiform. Second cell 5–6 μm long (x = 5 μm, n = 30); third cell 4–6 μm long (x = 5 μm, n = 30); fourth cell 4–6 μm long (x = 5 μm, n = 30). Base cell is an inverted triangle, 4–6 μm long (x = 4 μm, n = 30), light brown or almost transparent hyaline. Basal appendage single, tubular, unbranched, centric, vertical, or curved, 4–6 μm long (x = 6 μm, n = 30).
Basal cell conic to obconic with a truncated base, hyaline, verruculose, and thin-walled, 2–4 μm long. Three median cells doliiform, 11–14 μm (X = 12 μm, n = 30). Wall thin, color uniform (light or dark brown), septa darker than the rest of the cells, and the conidium constructed at the septum (second cell from the base, 4–5 μm long; third cell, 5–6 μm long; fourth cell, 4–6 μm long). Apical cell 2–4 μm long, hyaline, subcylindrical, or obconic with a truncated base, thin-walled, slightly rugose. With 2–3 tubular apical appendages arising from the apical crest, unbranched, filiform, 13–23 μm (X = 18 μm, n = 30). Basal appendage single, tubular, centric, or uncentred, 4–8 μm long.

Material examined: China, Yunnan Province, Dali City, Cangshan Mountain, isolated from healthy leaves of R. cyanocarpum, March 2021, Z.Q. Zhang, D-20 (KUN-HKAS 124577), living culture, CGMCC 3.23547.

Notes: Based on the phylogenetic analysis, P. trachicarpicola can be grouped with P. trachicarpicola (OP068) with strong
Pestalotiopsis suae (KUN-HKAS 124578, holotype). (A) Leaves of *Rhododendron delavayi*. (B,C) Culture on potato dextrose agar (PDA) (upper and lower view). (D,E) Conidiomata on PDA. (F,G) Conidiophores, conidiogenous cells, and conidia. (H–N) Conidia with appendages. Scale bars: (F) 20 μm, (G–J) 15 μm, (K–N) 10 μm.

Support (98% ML and 0.99 BYPP). The morphologies of the two species are identical. For the first time, *P. trachicarpica* is isolated from *Rhododendron*.

**Discussion**

Many fungal groups, such as *Aspergillus*, *Ceratobasidium*, *Fusarium*, *Neocosmospora*, *Pestalotiopsis*, *Pyrenochaeta*, *Russula*, *Serendipita*, *Thanatephorus*, and *Trichoderma* have been reported as endophytic fungi (Fu et al., 2022). As an ornamental plant, *Rhododendron* has achieved worldwide recognition (Cox and Cox, 1997). Recent research has isolated fungi from the leaf spots, mycorrhizae, and rhizosphere of *Rhododendron* (Medeiros et al., 2022). However, few studies have been conducted on the endophytic fungi of *Rhododendron*. Yunnan Province is one of the world’s most significant distribution centers for *Rhododendron* (Tian et al., 2011). There are 61 species of *Rhododendron* in Cangshan Mountain, Yunnan Province, China (Zhang et al., 2021). Our investigation indicates high diversity of *Pestalotiopsis* species in *Rhododendron*. 
However, the current study collected the leaves of three *Rhododendron* species only. In future research, it is possible to expand the survey area and collect additional *Rhododendron* leaves to obtain more endophytic fungal resources.

Previous studies mentioned that the color intensities of the median conidial cell, differences in the size variation of conidia, and the presence or absence of basal appendages can be used as additional taxonomic characteristics for distinguishing *Pestalotiopsis* species (Jeewon et al., 2003; Liu et al., 2017). Apical appendage characteristics, such as branching pattern, number, and attachment position, are also useful at the species level, but not at the generic level of a generic character (Crous et al., 2012). Therefore, it was proposed to implement additional morphological characteristics for species identification. ITS, TUB, and TEF1-α gene sequences can be combined to provide greater resolution for *Pestalotiopsis* (Li et al., 2021). In our phylogenetic tree, branch lengths of *Pestalotiopsis cangshanensis*, *P. fusoidea*, and some other species in this genus were notably short and the support values were relatively low. Further studies...
of Pestalotiopsis are, therefore, required to reveal whether the less informative loci lead to the poorly resolved phylogram.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/ (OP082426, OP185510, OP185517, OP082429, OP185511, OP185518, OP082431, OP185509, OP185516, OP082427, OP185512, OP185519, OP082430, OP185513, OP185520, OP082428, OP185514, OP185521, OP082432, OP185515, and OP185522).

Author contributions

RG conducted the experiments, analyzed the data, and wrote the manuscript. D-FB, Z-LL, and H-WS revised the manuscript. H-WS planned the experiments and analyzed the data. Z-LL and X-JS planned and funded the experiments. X-JS and Y-XL helped the experiments. All authors revised the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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