



Pestalotioid Species Associated with Medicinal Plants in Southwest China and Thailand

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ABSTRACT In this paper, a total of 26 pestalotioid isolates associated with different medicinal plants from southwest China and Thailand were studied. Based on morphological examinations and multigene analyses of three gene loci (*ITS*, *tef1- α* , and *tub2*), these 26 isolates represent 17 species distributed in three genera, including seven new species and eight new records. The concatenated three loci tree was used to infer the occurrence of sexual recombination within each pestalotioid genus through the pairwise homoplasmy index (PHI) test implemented in SplitsTree. Further, simplifying the description of pestalotioid species is discussed, and a checklist for pestalotioid species associated with medicinal plants worldwide is provided.

IMPORTANCE Pestalotioid species are an important fungal group, occurring commonly as plant pathogens, endophytes, and saprophytes. The study of pestalotioid species associated with medicinal plants is significant for agriculture, industry, and pharmaceutical industry but remains poorly studied. In this study, we report 17 pestalotioid species related to medicinal plants based on morphology and molecular analyses. Our study significantly enriches the species richness of pestalotioids and provides a basis for follow-up studies.

KEYWORDS 7 new species, 8 new records, diversity, endophytes, plant pathogens, phylogeny, saprophytes, taxonomy

Medicinal plants play a crucial role in the development of human cultures and are a rich source of natural products with both biological and chemical properties. Medicinal plants are used in health care, serve as treatments for various diseases, and have been used since prehistoric times across the world (1, 2). It is estimated that more than 70% of the world's population relies on medicinal plants (3). Microfungi can affect the growth and quality of medicinal plants. Some endophytes isolated from medicinal plants have broad developmental prospects (4, 5). Microfungi associated with medicinal plants are research hot spots (6–9).

Pestalotioid species are a very common group of fungi that form important associations with different plants as pathogens, endophytes, or saprophytes and are widely distributed in tropical and temperate regions (10–17). Traditional taxonomy of pestalotioid species mainly depend on their hosts and color intensities of the median conidial cell (10, 18–21). With the development of DNA-based phylogenetic analysis, the traditional classification system based on hosts and conidial colors is controversial. The use of molecular data in resolving pestalotioid species was revisited by Maharachchikumbura et al. (13), who separated this group into three genera, viz. *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis*. *Neopestalotiopsis* differs from *Pseudopestalotiopsis* and *Pestalotiopsis* by its versicolorous (two upper median cells darker than the lowest median cell) median cells and indistinct conidiophores, while *Pseudopestalotiopsis* can be easily distinguished

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from *Pestalotiopsis* by darker-colored concolorous (for those possessing equally pigmented median cells) median cells (13).

As important plant pathogens, pestalotioid species are almost ubiquitous in agricultural and noncultivated ecosystems, causing multiple diseases and huge economic losses (22–30). For example, gray blight disease of tea plants is caused by *Pseudopestalotiopsis* spp. and *Pestalotiopsis* spp. and accounts for at least 17% production damage in southern India (31) and 10 to 20% yield loss in Japan (32). *Neopestalotiopsis clavispora* caused the leaf blight of *Elettaria cardamomum* in India (33) and leaf spot of *Taxus chinensis* in China (34). Diogo et al. (22) reported that pestalotioid fungi caused stem girdling and dieback in young eucalyptus plants in Portugal. Li et al. (35) identified five new pestalotioid species associated with symptomatic leaves of *Camellia oleifera* in China. Thus, it is necessary to study the pathogenic pestalotioid species related to medicinal plants, which could provide the research foundation for the prevention and treatment of diseases and reduce economic losses.

The study of endophytic fungi in medicinal plants is of great significance for elucidating their distribution, growth and developmental characteristics, and resource regeneration (6, 7, 9, 36, 37). Many pestalotioid fungi have been found as endophytes from different medicinal plants with rich secondary metabolites (5, 36, 38–41). For example, the endophytic fungus *Pestalotiopsis versicolor* was isolated from the healthy leaves of *Taxus cuspidata*, and it is an excellent candidate for an alternate source of Paclitaxel supply (42). Therefore, the study of endophytic pestalotioid species related to medicinal plants could be of great importance to pharmaceuticals and therapeutic medicine.

This study aims to identify the pestalotioid fungi associated with medicinal plants in southwest China and Thailand based on morphology and molecular analyses. This paper describes, illustrates, and compares seven new species and eight new records with allied species. In addition, we provide a checklist for pestalotioid species associated with medicinal plants worldwide.

RESULTS

***Neopestalotiopsis* Maharachch., K.D. Hyde, and Crous, Stud. Mycol. 79:135 (2014).**

(i) Phylogenetic analyses. The combined data sets consist of 100 *Neopestalotiopsis* strains along with the outgroup *Pestalotiopsis diversiseta* (MFLUCC 12–0287) and *P. spathulata* (CBS 356.86), which were analyzed to infer the interspecific relationships within *Neopestalotiopsis*. The aligned sequence matrix comprised internal transcribed spacers (*ITS*; 1 to 485), translation elongation factor 1 (*tef1- α* ; 486 to 982), and partial β -tubulin region (*tub2*; 983 to 1,423), sequence data for a total of 1,423 sites, including coded alignment gaps. Similar tree topologies were obtained by maximum likelihood (ML) and Bayesian posterior probability (BYPP) methods, and the most likely tree ($-\ln = 7671.251111$) is presented in Fig. 1. The phylogenetic tree, which analyzed the 15 *Neopestalotiopsis* isolates from medicinal plants, indicated four novel species and three new records.

(ii) Genealogical concordance phylogenetic species recognition analysis. The pairwise homoplasy index (PHI) test revealed that there is no significant recombination ($\Phi_w = 0.06$), between *N. amomi* and its closely related taxa *N. eucalypticola* (CBS 264.37), *N. magna* (MFLUCC 12-0652), and *N. zingiberis* (GUCC 21001) (Fig. 2a). Additionally, based on the PHI test results, there is no significant recombination ($\Phi_w = 0.11$) between *N. hyperici* and its closely related taxa *N. acrostichi* (MFLUCC 17–1754), *N. lusitanica* (MEAN 1320), *N. protearum* (CBS 114178), *N. rhododendricola* (KUN-HKAS 123204), and *N. rhododendri* (GUCC 21504) (Fig. 2b). Similar result also occurred in *N. photiniae* ($\Phi_w = 1.0$) (Fig. 2c) and *N. suphanburiensis* ($\Phi_w = 1.0$) (Fig. 2d), indicating there is no significant recombination between them and their closely related taxa.

(iii) Taxonomy. (a) *Neopestalotiopsis amomi* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571230; Facesoffungi number: FoF 12912 (Fig. 3).

Etymology: refers to the name of the host plant from which the fungus was isolated.

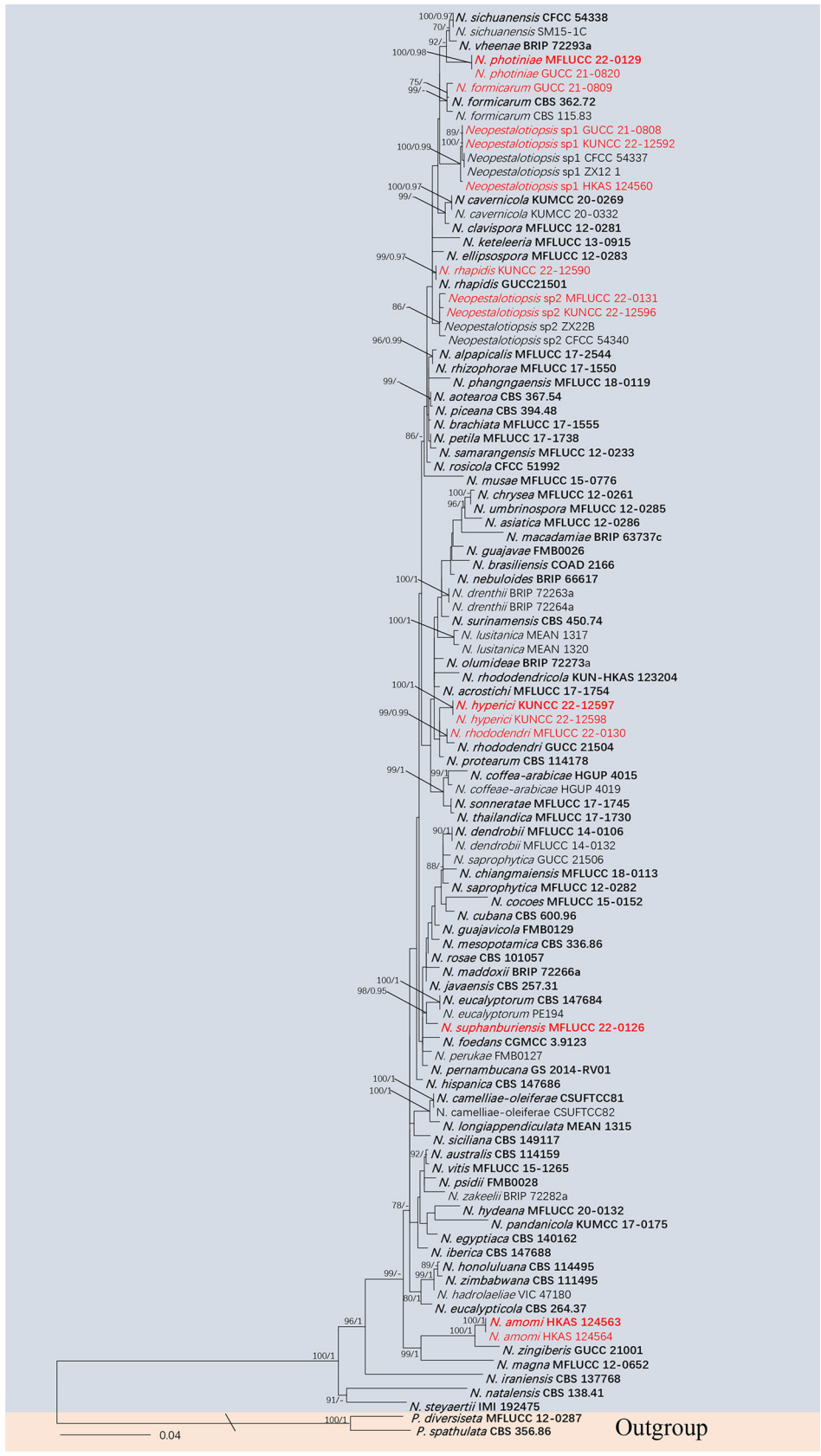


FIG 1 Maximum likelihood (RAxML) tree for *Neopestalotiopsis* based on the analysis of a combined data set of *ITS*, *tef1-α*, and *tub2* sequence data. The tree is rooted with *Pestalotiopsis diversiseta* (MFLUCC 12-0287) and *P. spathulata* (CBS 356.86). Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. The new isolates are in red, and ex-type strains are in bold.



FIG 2 (a, b, c and d) Split graphs showing the results of a PHI test of new *Neopestalotiopsis* species with their most closely related species using Log-Det transformation and splits decomposition options. The new taxon in each graph is shown in red font.

Holotype: HKAS 124563.

Associated with leaf blight of *Amomum villosum*. Symptoms: irregular shape, pale to brown, slightly sunken spots appear on the leaves of *Amomum villosum*, which later expand outward. Sexual morph: not observed. Asexual morph: conidiomata solitary, subglobose to globose, unilocular, brown, semi-immersed on leaves. Conidiophores 3 to 5 μm long, often reduced to conidiogenous cells. Conidiogenous cells 1 to 2 μm wide, subcylindrical, ampulliform, hyaline. Conidia 18 to 30 \times 4 to 7 μm (\bar{x} = 25 \times 6 μm , n = 40), length/width (L/W) ratio of 4.2, fusiform, straight to slightly curved, 4 septate; basal cell obconic with a truncate base, hyaline, smooth walled, 3 to 7 μm long; three median cells 12 to 19 μm long (\bar{x} = 16 μm , n = 40), pale brown to brown, concolorous, wall rugose, septa darker than the rest of the cell; second cell from base pale brown to brown, 3 to 8 μm long; third cell pale brown to brown, 3 to 7 μm long; fourth cell pale brown to brown, 3 to 7 μm long; apical cell 2 to 5 μm long, hyaline, conic to acute; with 2 to 3 tubular appendages on the apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 7 to 17 μm long; single basal appendage, unbranched, tubular, centric, 2 to 5 μm long.

Culture characteristics: conidia germinated on potato dextrose agar (PDA) within 12 h from single-spore isolation. Colony diameter reached 8 cm after 2 weeks at 25°C on PDA medium and appeared circular, with a flat, rough surface and was white from above and below.

Material examined: China, Guizhou Province, Qiannan Bouyei and Miao Autonomous Prefecture, Luodian District, leaf blight of *Amomum villosum* (Zingiberaceae), 3 September 2021, Y.R. Sun, L8 (HKAS 124563, holotype); *ibid.*, on leaf blight of *Amomum villosum*, 3 September 2021, Y.R. Sun, L8-1 (HKAS 124564).

Notes: *Neopestalotiopsis amomi* was isolated from the diseased leaves of *Amomum villosum* in China. Two collections HKAS 124563 and HKAS 124564 clustered together

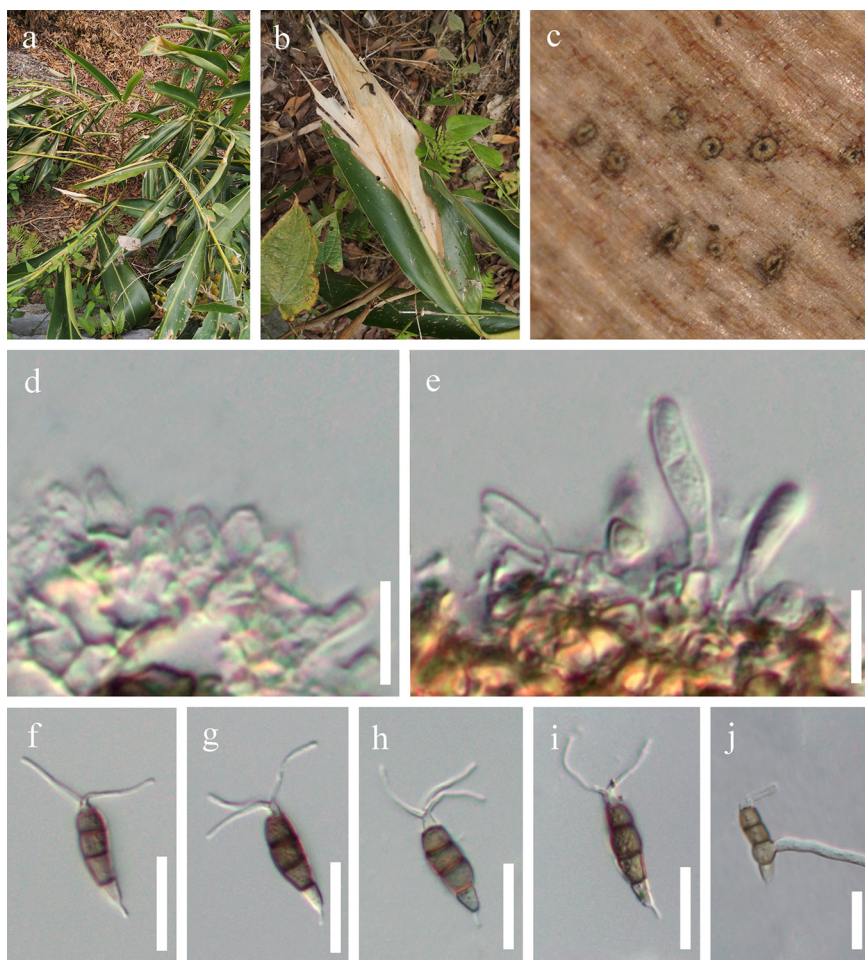


FIG 3 *Neopestalotiopsis amomi* (HKAS 124563, holotype). (a) Host. (b) Leaf blight on *Acrostichum aureum*. (c) Close-up view of conidiomata. (d) Conidiogenous cells. (e) Immature conidia attached to conidiogenous cells. (f to i) Conidia. (j) Germinated conidium; scale bars: 10 μm (d and e) and 20 μm (f to j).

with good support (ML-BS = 100%, BYPP = 1) and formed a sister clade to *N. zingiberis* (GUCC 21001), which was also isolated from a *Zingiberaceae* plant (43). The former differs in producing thinner conidia (4 to 7 μm in *N. amomi* versus 6 to 9.5 μm in *N. zingiberis*) and shorter conidiophores (3 to 5 μm in *N. amomi* versus 12 to 25 μm in *N. zingiberis*). In addition, there are 4 bp different between HKAS 124563 and GUCC 21001 in the *ITS* gene and 10 bp different in the *tef1- α* gene. *Neopestalotiopsis amomi* also differs by smaller conidia (18 to 30 \times 4 to 7 μm versus 42 to 46 \times 9.5 to 12 μm) from *N. magna*. The PHI test on *N. amomi* indicated that there is no significant recombination ($\Phi_w = 0.06$) between *N. amomi* and its closely related taxa. Thus, we introduce *N. amomi* as a new species.

(b) *Neopestalotiopsis hyperici* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571228; Facesoffungi number: FoF 12913 (Fig. 4).

Etymology: the specific epithet is referring to *Hypericum*, the host plant that the fungus was isolated from.

Holotype: HKAS 124561.

Associated with leaf spots of *Hypericum monogynum*. Symptoms: irregular shape, pale to brown, slightly sunken spots appear on the leaves of *Hypericum* sp., which later expand outward. Sexual morph: not observed. Asexual morph: conidiomata solitary, unilocular, dark. Conidiophores often reduced to conidiogenous cells. Conidiogenous cells indistinct. Conidia 17 to 22 (to 24) \times 5 to 8 μm ($\bar{x} = 19 \times 7 \mu\text{m}$, $n = 30$), L/W ratio of 2.8, fusoid, subcylindrical, straight to slightly curved, 4 septate; basal cell conic to obconic with a truncate

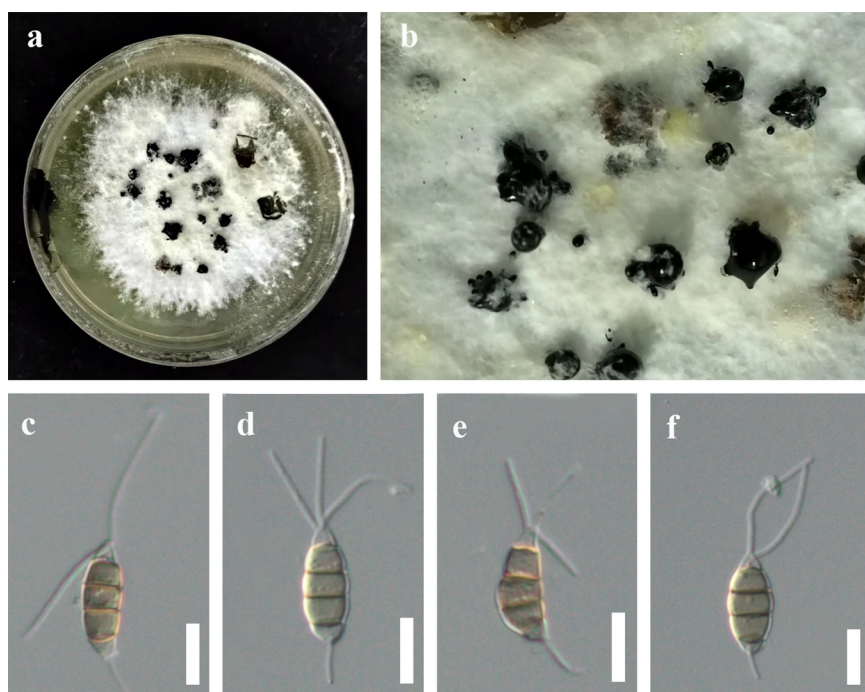


FIG 4 *Neopestalotiopsis hyperici* (HKAS 124561, holotype). (a) Culture. (b) Close-up view of conidiomata. (c to f) Conidia; scale bars: 10 μm (c to f).

base, hyaline to subhyaline, 2 to 4 μm long; three median cells 10 to 14 (to 17) μm long (\bar{x} = 12 μm , n = 30), wall rugose, concolorous; second cell from base pale brown to brown, 3 to 5 μm long; third cell pale brown to brown, 3 to 6 μm long; fourth cell pale brown to brown, 2 to 6 μm long; apical cell 1 to 4 μm long, hyaline, rugose and thin walled; with 2 to 3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 11 to 23 μm long; single basal appendage 4 to 7 μm long, unbranched, tubular, centric.

Culture characteristics: colonies on PDA reached up to 10 cm after 2 weeks, dense mycelium was on the surface, and colonies appeared white from above and below. Fruiting bodies were observed after 14 days.

Material examined: China, Guizhou Province, Guiyang City, Baiyun District, Changpoling National Forest Park, leaf spot of *Hypericum monogynum* (*Clusiaceae*), 20 August 2021, Y.R. Sun, CL5-1 (HKAS 124561, holotype); ex-type culture, KUNCC 22-12597 = GUCC 21-0812; *ibid.*, on leaf spots of *Hypericum monogynum*, 20 August 2021, Y.R. Sun, CL5-1-1, living culture KUNCC 22-12598 = GUCC 21-0811.

Notes: *Neopestalotiopsis hyperici* is related to *N. rhododendri* and *N. protearum* in the phylogenetic analysis (Fig. 1), but they can be distinct from concolorous conidia and the size of their median cells (10 to 14 μm in *N. hyperici* versus 14 to 20 μm in *N. rhododendri* versus 16 to 17 μm in *N. protearum*) (13, 44). In addition, there are 13 bp different between *N. hyperici* and *N. rhododendri* and 10 bp different between *N. hyperici* and *N. protearum* in the *tef1- α* region. Moreover, the PHI test on *N. hyperici* indicated that there is no significant recombination (Φ_w = 0.11) between *N. hyperici* and its closely related taxa (Fig. 2b). Thus, we introduce *N. hyperici* as a new species.

(c) *Neopestalotiopsis photiniae* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571231; Facesoffungi number: FoF 12914 (Fig. 5).

Etymology: referring to the host plant from which the fungus was isolated.

Holotype: HKAS 125895.

Associated with leaf spots of *Photinia serratifolia*. Symptoms: irregular shape, pale to brown, slightly sunken spots appear on the leaves of *Photinia serratifolia*, which later expand outward. Small spots gradually enlarged, changing to brown circular ring spots

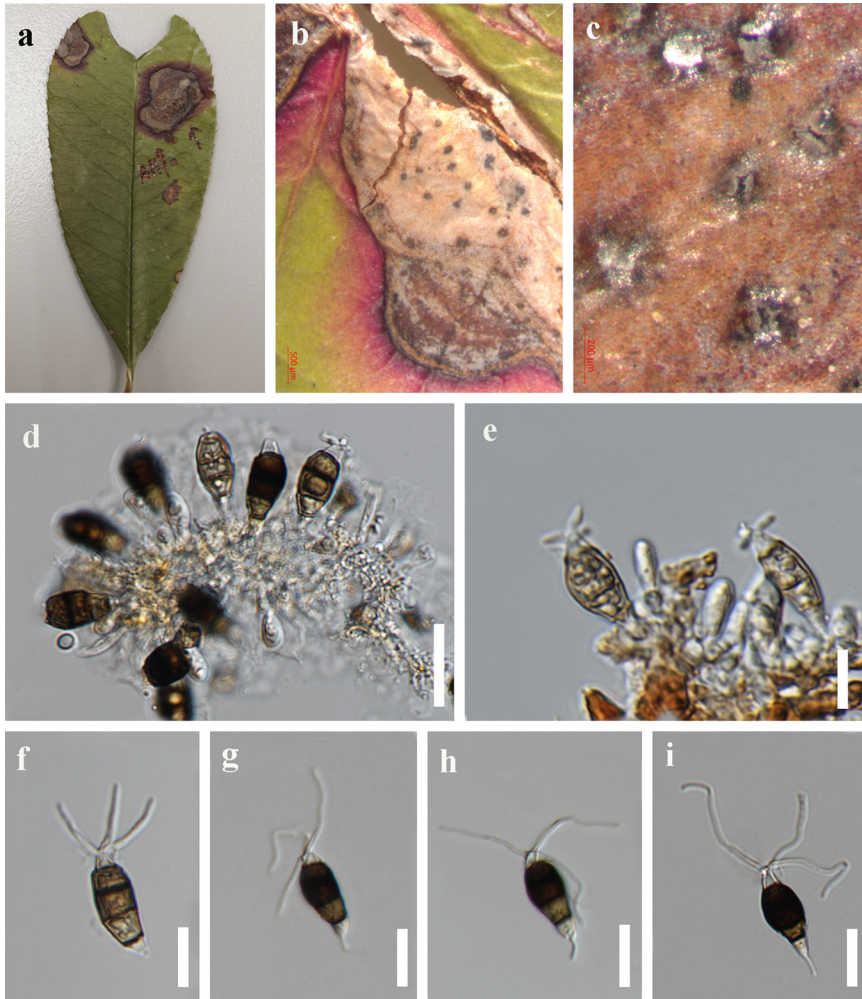


FIG 5 *Neopestalotiopsis photiniae* (HKAS 125895, holotype). (a) Host. (b) Leaf spot on *Photinia serratifolia*. (c) Close-up view of conidiomata. (d and e) Conidia attached to conidiogenous cells. (f to i) Conidia; scale bars, 200 μm (c), 20 μm (d), and 10 μm (f to i).

with a dark brown border. Sexual morph: not observed. Asexual morph: conidiomata solitary, subglobose to globose, unilocular, dark brown, semi-immersed on leaves. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells 1 to 3 \times 2 to 4 μm , subcylindrical, ampulliform, hyaline. Conidia 20 to 29 \times 5 to 12 μm (\bar{x} = 23 \times 9 μm , n = 40), L/W ratio of 2.6, broadly fusiform, straight to slightly curved, 4 septate; basal cell obconic with a truncate base, hyaline to pale brown, 1 to 5 μm long; three median cells 13 to 19 μm long (\bar{x} = 16 μm , n = 40), brown to dark, wall rugose, versicolorous; second cell from base pale brown to brown, 4 to 6 μm long; the third and fourth cells, dark brown to black, are not easily distinguished, septate indistinct, 10 to 13 μm long; apical cell 2 to 4 μm long, hyaline, conic to acute; with 2 to 3 tubular appendages on the apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 17 to 33 μm long; single basal appendage, unbranched, tubular, centric, 1 to 6 μm long.

Culture characteristics: conidia germinated on PDA within 12 h at 25°C from single-spore isolation. Apical cells produced germ tubes. Colony diameter reached 80 mm after 3 weeks at 25°C on PDA medium, were circular with a rough, flat surface, and appeared white from above and below.

Material examined: China, Guizhou Province, Guiyang City, Nanming District, Xiaochehe Road, Guiyang Ahahu National Wetland Park, on leaf spots of *Photinia serratifolia* (*Rosaceae*), 21 September 2019, Y.R. Sun, AH9 (HKAS 125895, holotype); ex-type

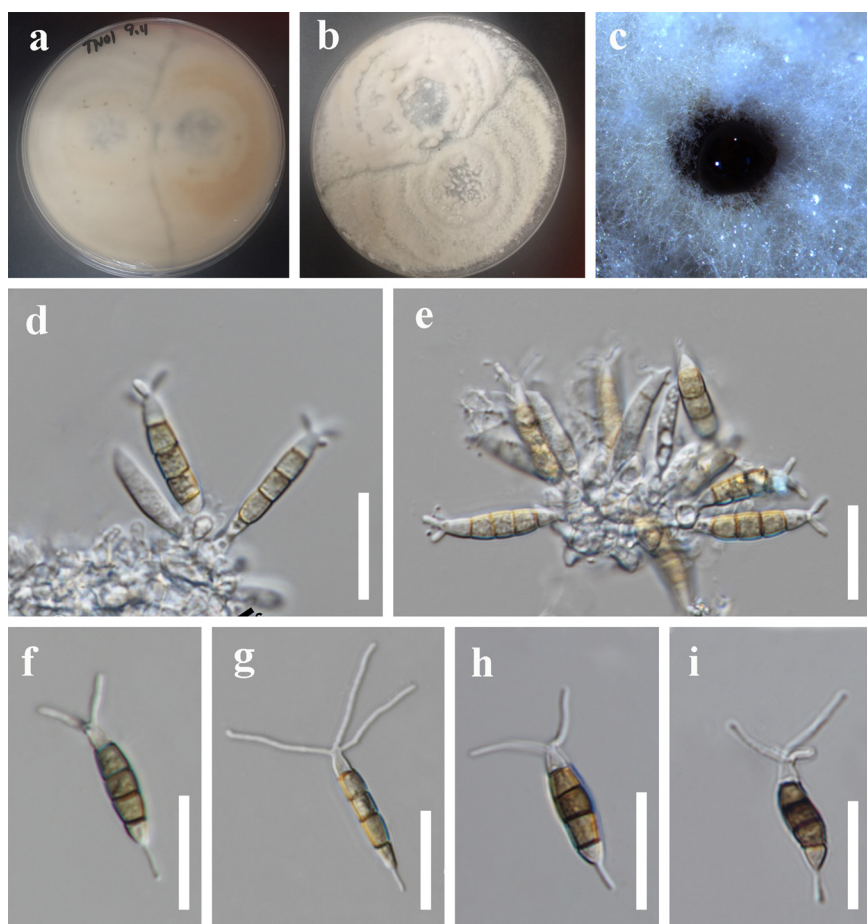


FIG 6 *Neopestalotiopsis suphanburiensis* (MFLU 22-0168, holotype). (a and b) Cultures. (c) Colony in culture. (d and e) Conidia attached to conidiogenous cells. (f to i) Conidia; scale bars, 10 μm (d to i).

culture, MFLUCC 22-0129; *ibid.*, on leaf spots of *Photinia* sp. (*Rosaceae*), 21 September 2019, Y.R. Sun, AH9-1, living culture, GUCC 21-0820.

Notes: *Neopestalotiopsis photiniae* is phylogenetically sister to *N. sichuanensis* and *N. vheena* (Fig. 1). *Neopestalotiopsis photiniae* differs by its thinner conidia (L/W ratio = 2.6 versus L/W ratio = 4.1) from *N. sichuanensis* (45). *Neopestalotiopsis photiniae* is morphologically distinguishable from *N. vheena* in shorter conidiogenous cells (1 to 3 \times 2 to 4 μm versus 5 to 10 \times 3 to 5 μm) (23). The result of the PHI test showed that there is no obvious recombination ($\Phi_w = 1.0$) between *N. photiniae* and its closely related taxa (Fig. 2c). Therefore, *N. photiniae* is introduced as a new species.

(d) *Neopestalotiopsis suphanburiensis* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571232; Facesoffungi number: FoF 12916 (Fig. 6).

Etymology: refers to the province where the fungus was collected, Suphan Buri Province.

Holotype: Mae Fah Luang University (MFLU) 22-0168.

Saprobic on stems of an unidentified plant. Sexual morph: not observed. Asexual morph: conidiomata solitary, subglobose to globose, unilocular, brown to dark, immersed on stems. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells subcylindrical, ampulliform, hyaline. Conidia 19 to 29 \times 4 to 7 μm ($\bar{x} = 25 \times 5 \mu\text{m}$, $n = 40$), L/W ratio = 4.9, fusiform, straight to slightly curved, 4 septate; basal cell obconic with a truncate base, hyaline, smooth walled, 3 to 7 μm long; three median cells 12 to 19 μm long ($\bar{x} = 16 \mu\text{m}$, $n = 40$), pale brown to brown, wall rugose, concolor, septa darker than the rest of the cell, versiculous; second cell from base pale brown to brown, 3 to 7 μm long; third cell pale brown to brown, 3 to 6 μm long;

fourth cell pale brown to brown, 4 to 6 μm long; apical cell 3 to 6 μm long, hyaline, conic to acute; with 2 to 3 tubular appendages on the apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 9 to 21 μm long; single basal appendage, unbranched, tubular, centric, 2 to 11 μm long.

Culture characteristics: colony diameter reached 8 cm after 2 weeks at 25°C on PDA medium. Colonies were circular with a rough, flat surface and appeared white from above and white to pale gray from below.

Material examined: Thailand, Suphan Buri Province, dead stem of an unidentified plant, 5 September 2020, S Wang, TN01 (MFLU 22-0168, holotype); ex-type culture, MFLUCC 22-0126.

Notes: *Neopestalotiopsis suphanburiensis* is phylogenetically sister to *N. eucalyptorum*, which was isolated from leaves and stems of *Eucalyptus globulus* (Fig. 1). In morphology, *N. suphanburiensis* differs from *N. eucalyptorum* in having thinner conidia (4 to 7 μm versus 7.6 to 8.1 μm). In addition, there are 10 bp different (without gap, 445 bp) in the *tef1- α* region. The PHI test on *N. suphanburiensis* also indicated that there is no significant recombination ($\Phi_w = 1.0$) between *N. suphanburiensis* and its closely related taxa (Fig. 2d). We thus introduce *N. suphanburiensis* as a new species.

(e) *Neopestalotiopsis* sp. 1. Associated with leaf spot of *Cyrtomium fortunei*, *Lithocarpus* sp., and *Smilax scobinicaulis* (Fig. 7). Symptoms: irregular shape, pale brown, small spots gradually enlarged, changing to brown circular ring spots with a dark brown border. Sexual morph: not observed. Asexual morph: conidiomata solitary, subglobose to globose, unilocular, dark brown, semi-immersed on leaves. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells subcylindrical or ampulliform, hyaline. Conidia 21 to 31 \times 4 to 7 μm ($\bar{x} = 26 \times 6 \mu\text{m}$, $n = 30$), L/W ratio of 4.4, fusiform, straight to slightly curved, 4 septate; basal cell obconic with a truncate base, hyaline, 3 to 7 μm long; three median cells doliiform to cylindrical, 11 to 18 μm long ($\bar{x} = 15 \mu\text{m}$, $n = 30$), yellow to brown, concolorous, septa darker than the rest of the cell; second cell from base yellow to brown, 3 to 6 μm long; third cell yellow to brown, 3 to 7 μm long; fourth cell yellow to brown, 4 to 7 μm long; apical cell 2 to 6 μm long, hyaline, conic to acute; with 1 to 4 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 13 to 26 μm long; single basal appendage, unbranched, tubular, centric, 2 to 7 μm long.

Culture characteristics: conidia germinated on PDA within 12 h at 25°C from single-spore isolation. Apical cells produced germ tubes. Colony diameter reached 80 mm after 2 weeks at 25°C on PDA medium. Colonies were circular with a rough, flat surface and appeared white from above and yellow from below.

Material examined: China, Guizhou Province, Tongren City, Jiangkou District, Yamugou Parkland, leaf spot of *Lithocarpus* sp. (*Fagaceae*), 20 May 2022, Y.R. Sun, JK15-2 (HKAS 124565); living culture, KUNCC 22-12592 = GUCC 21-0808; China, Guizhou Province, Qiannan Bouyei and Miao Autonomous Prefecture, Libo District, leaf spot of *Smilax china* (*Liliaceae*), 12 March 2022, Y.R. Sun, bb1 (HKAS 124560); China, Guizhou Province, Guiyang City, Baiyun District, Changpoling National Forest Park, leaf spot of *Dryopteris crassirhizoma* (*Dryopteridaceae*), 20 August 2021, Y.R. Sun, CL1-2, living culture, GUCC 21-0813.

Notes: three strains (KUNCC 22-12592, HKAS 124560, and GUCC 21-0813) have identical *ITS*, *tef1- α* , and *tub2* sequences as isolates CFCC-54337 and ZX12-1, which were previously provided by Jiang et al. (45). However, they did not introduce it as a new species due to lack of distinguished characters from close clades. In this study, these five strains clustered together and formed a distinct clade in the tree. We keep these five strains as *Neopestalotiopsis* sp.1 for the same reasons as before. We speculate that *Neopestalotiopsis* sp.1 could be a common phytopathogen, as it has been found in leaf spots on different plants.

(f) *Neopestalotiopsis* sp. 2. Saprobic on *Ceiba pentandra* leaves and endophytic from *Pinellia ternata* (Fig. 8). Sexual morph: not observed. Asexual morph: conidiomata solitary, unilocular, dark, immersed on stems. Conidiophores indistinct, often reduced to

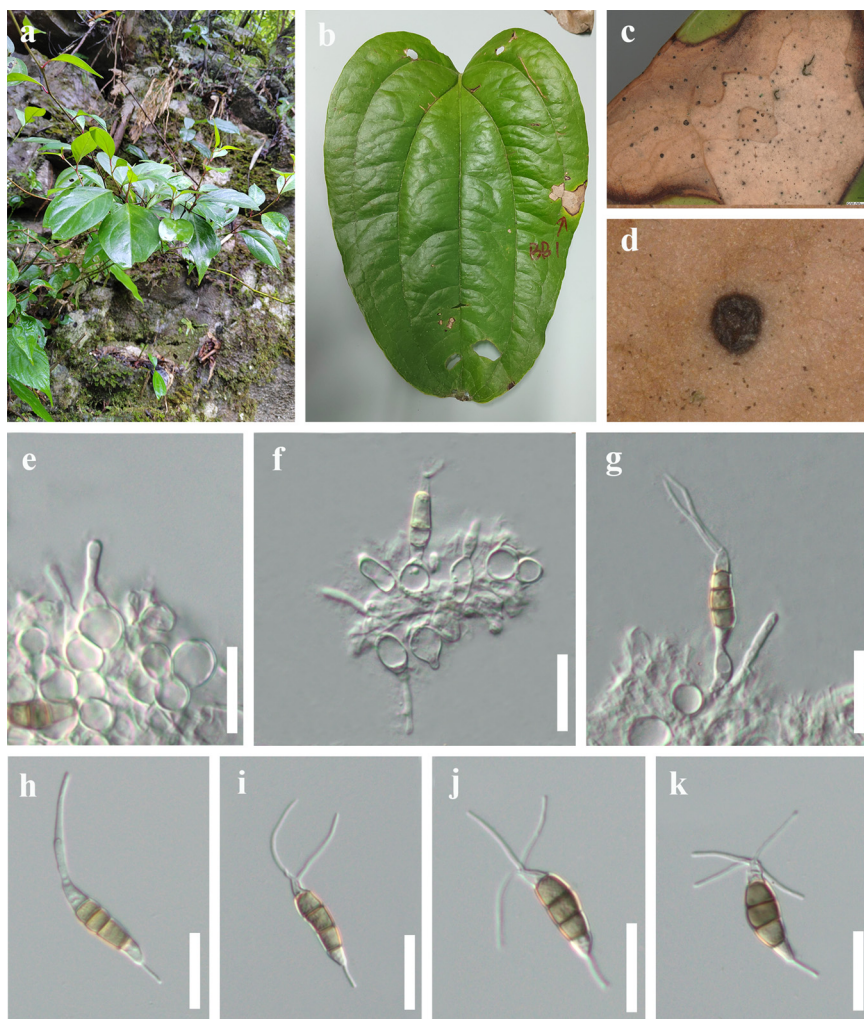


FIG 7 *Neopestalotiopsis* sp.1 (HKAS 124560). (a) Host. (b) Leaf spot on *Smilax scobinicaulis*. (c and d) Close-up view of conidiomata. (e to g) Conidia attached to conidiogenous cells. (h to k) Conidia; scale bars, 20 μm (e to k).

conidiogenous cells. Conidiogenous cells indistinct. Conidia 19 to 25 \times 6 to 8 μm (\bar{x} = 22 \times 7 μm , n = 30), L/W ratio of 3.1, fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4 septate; basal cell conic to obconic with a truncate base, hyaline to subhyaline, 3 to 6 μm long; three median cells 13 to 15 μm long (\bar{x} = 14 μm , n = 30), wall rugose, versicolorous, septa darker than the rest of the cell; second cell from base pale brown to brown, 3 to 5 μm long; third cell brown, 3 to 6 μm long; fourth cell brown, 3 to 6 μm long; apical cell 2 to 4 μm long, hyaline, rugose and thin walled; with 2 (seldom 3) tubular apical appendages, arising from the apical crest, unbranched, filiform, 11 and 20 μm long; single basal appendage 2 to 5 μm long, unbranched, tubular, centric.

Culture characteristics: colonies on PDA reached up to 8 cm in 2 weeks, with dense aerial mycelium on the surface with undulate edge; white. Fruiting bodies were observed after 14 days.

Material examined: Thailand, Chiang Rai Province, dead leaves of *Ceiba pentandra* (*Bombacaceae*), 16 Jan 2021, Y.R. Sun, CR20 (MFLU 22-0170); living culture, MFLUCC 22-0131; China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy leaves of *Pinellia ternata* (*Araceae*), 1 May 2022, Y.R. Sun, E2, living culture KUNCC 22-12596 = GUCC 21-0805.

Notes: our isolates KUNCC 22-12596 and MFLUCC 22-0170 clustered together with

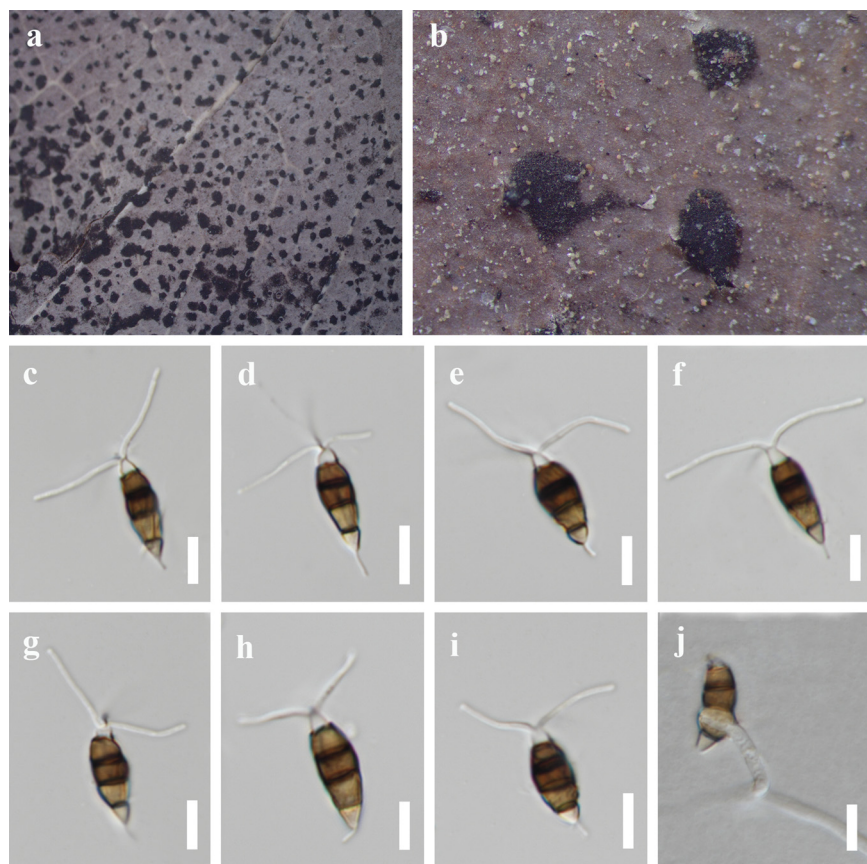


FIG 8 *Neopestalotiopsis* sp. 2 (MFLU 22-0170). (a and b) Conidiomata on the host. (c to i) Conidia. (j) Germinated conidium; scale bars, 10 μ m (c to j).

Neopestalotiopsis sp. 2 (CFCC 54340 and ZX22B), and these four isolates formed a distinct clade in the phylogenetic tree (Fig. 1). Four isolates have similar characteristics. We keep these four strains as *Neopestalotiopsis* sp. 2 as explained above. Interestingly, KUNCC 22-12596, MFLUCC 22-0170, ZX22B, and CFCC 54340 have different habitats. KUNCC 22-12596 was endophytic in healthy leaves of *Pinellia ternata*, MFLUCC 22-0170 was saprobic on decaying leaves of *Ceiba pentandra*, and CFCC 54340 and ZX22B were isolated from leaf spots of *Castanea mollissima*.

(g) *Neopestalotiopsis formicarum* Maharachch., K.D. Hyde, and Crous. Material examined: China, Guizhou Province, Guiyang City, Nanming District, Xiaochehe Road, Guiyang Ahahu National Wetland Park, on leaf spots of *Photinia serrulate* (*Rosaceae*), 21 September 2019, Y.R. Sun, AH11, living culture, GUCC 21-0809.

Notes: *Neopestalotiopsis formicarum* was introduced by Maharachchikumbura et al. (13) as a saprobic species isolated from dead ants in Ghana and plant debris from Cuba. Later, many studies have proven that *N. formicarum* is a serious phytopathogen, which can cause leaf fall disease in rubber trees in Thailand (46), leaf spot pathogens of the guarana plant in Brazil (47), and leaf brown blight of jabuticaba in Taiwan province, China (48). In this study, a new *N. formicarum* taxon was isolated from leaf spots of *Photinia serrulate* in China.

(h) *Neopestalotiopsis rhapsidis* Qi Yang and Yong Wang bis. Material examined: China, Guizhou Province, Qiannan Bouyei and Miao Autonomous Prefecture, Libo District, leaf spots of *Podocarpus macrophyllus* (*Podocarpaceae*), 12 March 2022, Y.R. Sun, ML3 (HKAS 124559); living culture, KUNCC 22-12590 = GUCC 21-0806.

Notes: *Neopestalotiopsis rhapsidis* was introduced by Yang et al. (44) from leaf spot of *Rhapis excelsa* (*Arecaceae*) in China. Our isolate KUNCC 22-12590 clustered together with *N. rhapsidis* (GUCC 21501) in the phylogenetic tree. These two species have

overlapping conidial measurements (17 to 25×5 to $8 \mu\text{m}$ for KUNCC 22 to 12590 versus (22 to) 25.5×4 (to $6 \mu\text{m}$ for GUCC 21501) (44). Both isolates were associated with leaf spots in China. Therefore, we identify KUNCC 22-12590 and GUCC 21501 to be conspecific species, and KUNCC 22-12590 represents a new host record.

(i) *Neopestalotiopsis rhododendri* Qi Yang and Yong Wang bis. Material examined: Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf spots of *Dracaena fragrans* (*Liliaceae*), 15 September 2020, S Wang, LD1, living culture, MFLUCC 22-0130.

Notes: *Neopestalotiopsis rhododendri* was introduced by Yang et al. (44) from the diseased leaf of *Rhododendron simsii* (*Ericaceae*) in China. Based on our phylogenetic analysis of combined *ITS*, *tef1- α* , and *tub2* sequence data, our isolate MFLUCC 22-0130 clustered with the type species *N. rhododendri* (GUCC 21504) with good support (ML-BS = 99% and BYPP = 0.99). Our collection also shares similar morphological features with the holotype of *N. rhododendri* (GUCC 21504). Both isolates were associated with leaf spots. Therefore, we identify our collection as *N. rhododendri*, which represents a new host and geographical record.

***Pestalotiopsis Steyaert*, Bull. Jard. bot. État Brux. 19:300 (1949). (i) Phylogenetic analyses.** The phylogenetic tree (*Pestalotiopsis*) comprised 120 ingroups and two outgroups, *Neopestalotiopsis protearum* (CBS 114178), and *N. cubana* (CBS 600.96). A total of 1,496 characters including gaps (543 for *ITS*, 516 for *tef1- α* , and 437 for *tub2*) were included in the phylogenetic analysis. Similar tree topologies were obtained by ML and BYPP methods, and the most likely tree ($-\ln = 12,403.616855$) is presented (Fig. 9). The phylogenetic tree analyzed 10 *Pestalotiopsis* taxa isolated from medicinal plants and revealed three novel species and three new records of *Pestalotiopsis*.

(ii) **Genealogical concordance phylogenetic species recognition analysis.** The PHI test revealed that there is no significant recombination ($\Phi_w = 0.26$) between *P. chiangmaiensis* and its closely related taxa *P. smilacicola* (MFLUCC 22-0125), *P. dracontomelon* (MFLUCC 10-0149), and *P. rhizophorae* (MFLUCC 17-0416) (Fig. 10a). The *P. loeiana* (MFLUCC 22-0123)-based PHI test confirmed that there is no significant recombination ($\Phi_w = 0.13$) between *P. loeiana* and its closely related taxa *P. chiangmaiensis* (MFLUCC 22-0127), *P. nanningensis* (CSUFTCC10), *P. rhizophorae* (MFLUCC 17-0416), and *P. thailandica* (MFLUCC 17-1616) (Fig. 10b).

(iii) **Taxonomy.** (a) *Pestalotiopsis chiangmaiensis* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571225; Facesoffungi number: FoF 04525 (Fig. 11).

Etymology: refers to the location where the fungus was encountered.

Holotype: MFLU 22-0164.

Associated with leaf strips of *Phyllostachys edulis*. Sexual morph: not observed. Asexual morph: conidiomata on PDA pycnidial, subglobose to globose, solitary or aggregated, dark, semi-immersed or partly erumpent; exuding black conidial masses. Conidiophores hyaline, smooth, simple, reduced to conidiogenous cells. Conidiogenous cells 5 to 11×1 to $3 \mu\text{m}$, cylindrical to subcylindrical or ampulliform to lageniform, hyaline, smooth. Conidia pale brown, fusiform, straight to slightly curved, (3 to) 4 septate, 16 to 26×4 to $7 \mu\text{m}$ ($\bar{x} = 21 \times 5 \mu\text{m}$, $n = 40$), L/W ratio of 4.2; basal cell obconic with a truncate base, hyaline or sometimes pale brown, smooth walled, 2 to $6 \mu\text{m}$ long; three median cells 10 to $16 \mu\text{m}$ long ($\bar{x} = 14 \mu\text{m}$, $n = 40$), pale brown, concolorous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base pale brown, 3 to $6 \mu\text{m}$ long; third cell brown, 3 to $6 \mu\text{m}$ long; fourth cell brown, 3 to $6 \mu\text{m}$ long; apical cell 2 to $5 \mu\text{m}$ long, hyaline, conic to acute; with 2 (to 3) tubular appendages on the apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 8 to $13 \mu\text{m}$ long; single basal appendage 2 to $7 \mu\text{m}$, unbranched, tubular, centric.

Culture characteristics: colonies on PDA reached 5 to 6 cm in diameter after 7 days at 25°C , colonies were filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform (curled) margin, fluffy, white from above and below; fruiting bodies black.

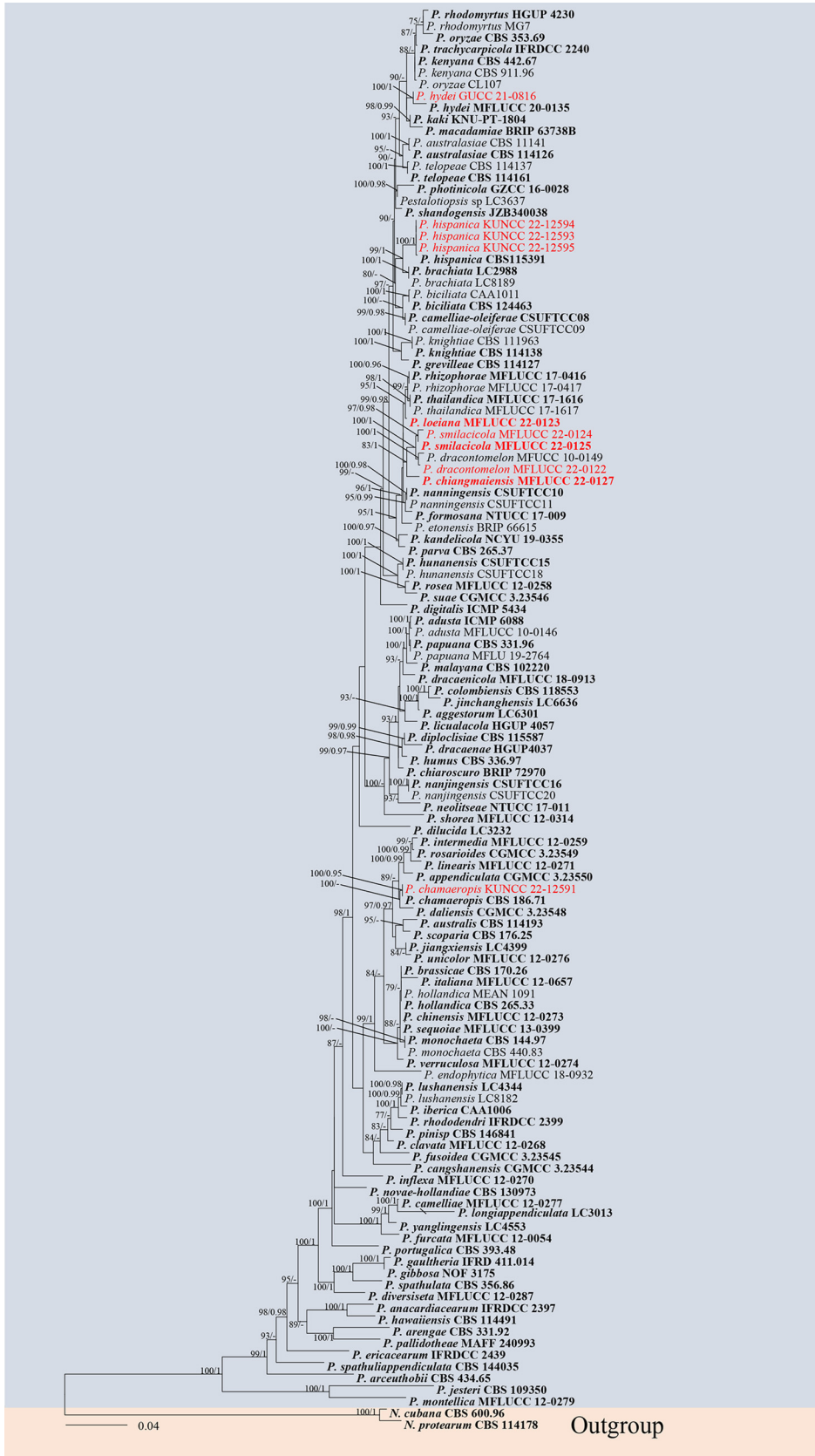


FIG 9 Maximum likelihood (RAxML) tree for *Pestalotiopsis* based on analysis of a combined data set of *ITS*, *tef1-α*, and *tub2* sequence data. The tree is rooted with *Neopestalotiopsis protearum* (CBS 114178) and *N. cubana* (CBS 600.96). Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. The new isolates are in red, and the ex-type strains are in bold.

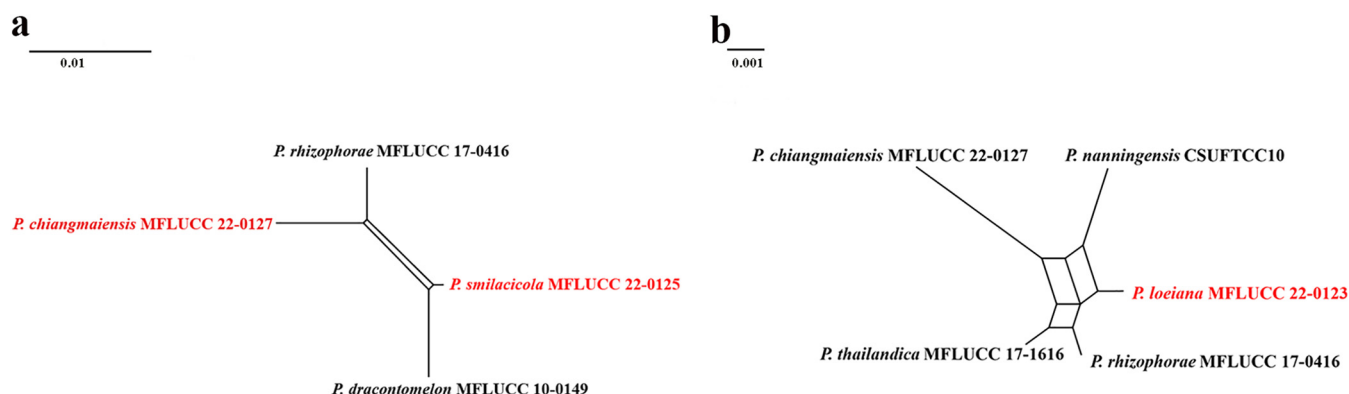


FIG 10 (a and b) Split graphs showing the results of the PHI test of new *Pestalotiopsis* species with their most closely related species using Log-Det transformation and split decomposition options. The new taxon in each graph is shown in red font.

Material examined: Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf strip of *Phyllostachys edulis* (*Poaceae*), 15 July 2020, Y.R. Sun, M18 (MFLU 22-0164, holotype); ex-type culture, MFLUCC 22-0127.

Notes: *Pestalotiopsis chiangmaiensis* formed a distinct lineage and was sister to *P. smilacicola* and *P. dracontomelon* in the phylogenetic tree (Fig. 11). It differs by longer conidiogenous cells (5 to 11 μm versus 1 to 4 μm) than *P. smilacicola* and shorter apical appendages (8 to 13 μm versus 10 to 22 μm) than *P. dracontomelon*. In addition, there are 14 bp different (without gap, 474 bp) in the *tef1- α* region between *P. chiangmaiensis* (MFLUCC 22-0127) and *P. smilacicola* (MFLUCC 22-0125) and 15 bp different (without gap, 464 bp) between *P. chiangmaiensis* (MFLUCC 22-0127) and *P. dracontomelon* (MFLUCC 10-0149). The PHI test on *P. chiangmaiensis* also showed that there is no significant recombination ($\Phi_w = 0.26$) between *P. chiangmaiensis* and its closely related taxa (Fig. 10a). Therefore, we introduce *P. chiangmaiensis* as a new species.

(b) *Pestalotiopsis loeiana* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571226; Facesoffungi number: FoF 12919 (Fig. 12).

Etymology: refers to the collected site, Loei Province.

Holotype: MFLU 22-0167.

Saprobic on dead leaves. Sexual morph: not observed. Asexual morph: conidiomata solitary, black, semi-immersed on leaves. Conidiophores indistinct and conidiogenous cells indistinct. Conidia 17 and 22 \times 4 and 6 μm ($\bar{x} = 19 \times 5 \mu\text{m}$, $n = 40$), L/W ratio of 3.7, fusiform, straight to slightly curved, 4 septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, rugose walled, 3 to 6 μm long, with 1 to 3 basal appendages, unbranched, tubular, centric, 3 to 13 μm long ($\bar{x} = 9 \mu\text{m}$); three median cells 10 to 14 μm ($\bar{x} = 12$, $n = 40$), doliiform to cylindrical, brown, concolorous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base brown, 3 to 6 μm long; third cell brown, 3 to 5 μm long; fourth cell brown, 2 to 5 μm long; apical cell 3 to 5 μm long, hyaline, conic to acute; with 1 to 3 tubular appendages on the apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 13 to 24 μm long.

Culture characteristics: colonies on PDA reached 8 cm in diameter after 2 weeks at 25°C, colonies filamentous to circular, medium dense, mycelium on surface flat or raised, with filiform margin, fluffy, yellow circle in the middle surrounded by white mycelium from above, light yellow to pale brown from the reverse.

Material examined: Thailand, Loei Province, dead leaves of an identified plant, 27 February 2020, J.Y. Zhang, JY1 (MFLU 22-0167, holotype); ex-type culture, MFLUCC 22-0123.

Notes: *Pestalotiopsis loeiana* (MFLUCC 22-0123) is phylogenetically sister to *P. rhizophorae* and *P. thailandica*, which were isolated from leaf spots of mangroves (Fig. 11). Morphologically, *P. loeiana* is distinguishable by its more than one basal appendage

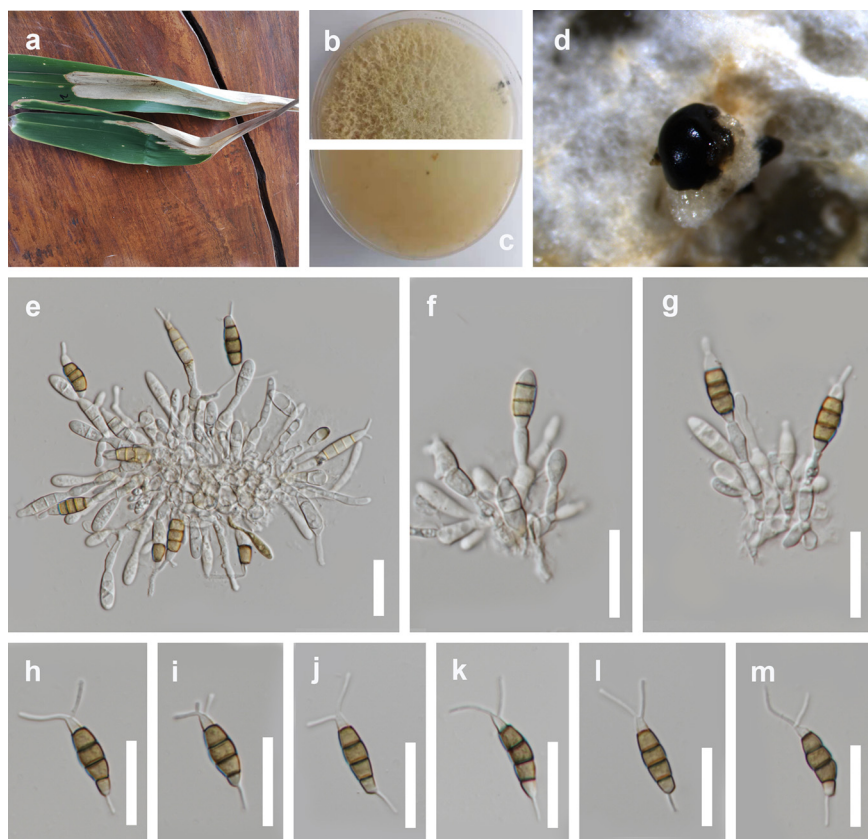


FIG 11 *Pestalotiopsis chiangmaiensis* (MFLU 22-0164, holotype). (a) Host. (b and c) Cultures. (d) Colonies on PDA. (e to g) Conidiogenous cells and developing conidia. (h to m) Conidia; scale bars, 20 μm (e to m).

and smaller conidia (17 to 22 \times 4 to 6 μm in *P. loeiana* versus 27 \times 6.5 to 7.5 μm in *P. rhizophorae* versus 21 to 25 \times 6 to 7 in *P. thailandica*). The result of the PHI test ($\Phi_w = 0.13$) also showed that there is no significant recombination between *P. loeiana* and its closely related taxa (Fig. 10b). Therefore, we introduce *P. loeiana* as a new species.

(c) *Pestalotiopsis smilacicola* Y.R. Sun and Yong Wang bis, sp. nov. Fungal names number: FN 571227; Facesoffungi number: FoF 12921 (Fig. 13).

Etymology: refers to the host plant from which the fungus was isolated.

Holotype: MFLU 22-0165.

Associated with leaf spots of *Smilax* sp. Symptoms subcircular to irregular shape, brown, slightly sunken spots appear on the leaves of *Smilax china*, which later expand outward. Small auburn spots appeared initially and then gradually enlarged. Sexual morph: not observed. Asexual morph: conidiomata solitary, subglobose, unilocular, black, semi-immersed on leaves. Conidiomatal wall 7 to 10 μm wide, thin walled, pale brown. Conidiophores indistinct. Conidiogenous cells 1 to 4 \times 1 to 3 μm , subcylindrical to ampulliform, hyaline, smooth. Conidia 18 to 22 \times 4 to 7 μm ($\bar{x} = 20 \times 5 \mu\text{m}$, $n = 40$), L/W ratio of 3.8, fusiform, straight to slightly curved, 4 septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, smooth walled, 3 to 5 μm long; three median cells 9 to 15 μm long ($\bar{x} = 12 \mu\text{m}$, $n = 40$), pale brown to brown, concolorous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base pale brown to brown, 3 to 5 μm long; third cell brown, 2 to 5 μm long; fourth cell brown, 3 to 5 μm long; apical cell 2 to 5 μm long, hyaline, conic to acute; with 2 to 3 tubular appendages on the apical cell, inserted at different

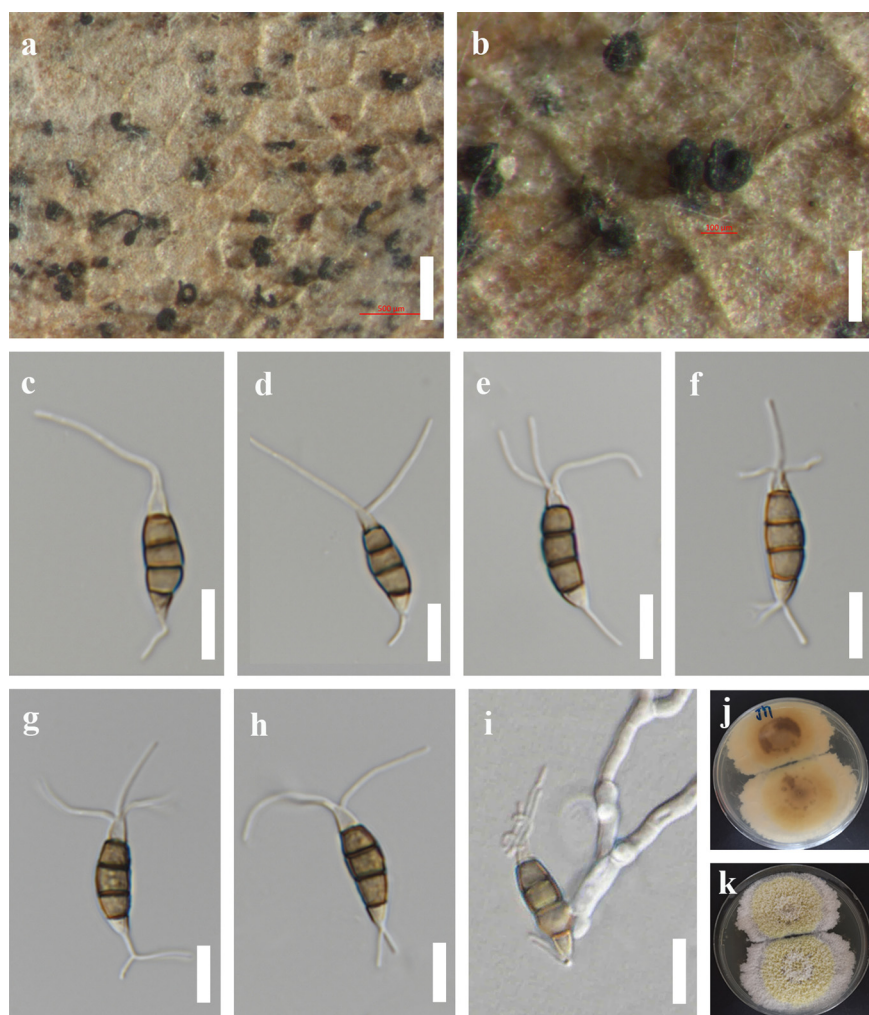


FIG 12 *Pestalotiopsis loeiana* (MFLU 22-0167, holotype). (a and b) Conidiomata on the host. (c to h) Conidia. (i) Germinated conidium. (j and k) Colonies on PDA; scale bars, 500 μm (a), 200 μm (b), and 10 μm (c to i).

loci in a crest at the apex of the apical cell, unbranched, 6 to 14 μm long; single basal appendage, unbranched, tubular, centric, 2 to 6 μm long.

Culture characteristics: colonies on PDA reached 10 cm in diameter after 2 weeks at 25°C, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin, fluffy, white from above and reverse.

Material examined: Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf spots of *Smilax* sp. (*Liliaceae*), 16 July 2020, Y.R. Sun, M26 (MFLU 22-0165, holotype), ex-type culture, MFLUCC 22-0125. *ibid.*, leaf spots of *Smilax china* (*Liliaceae*), 15 July 2020, Y.R. Sun, M13 living culture, MFLUCC 22-0124.

Notes: two isolates MFLUCC 22-0125 and MFLUCC 22-0124 share similar morphology. These two isolates clustered together and formed a sister clade to *P. dracontomeilon* (MFLUCC 10-0149) in the phylogenetic tree. There is only 1 bp different in *tef1- α* and *tub2* genes and 3 bp different in the *ITS* gene between these two isolates. For the differences between *P. smilacicola* and its related species see the notes of *P. chiang-maiensis* (this study). Therefore, these two isolates are identified as conspecific, representing a new species.

(d) *Pestalotiopsis chamaeropsis* Maharachch., K.D. Hyde, and Crous. Material examined: China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy leaves of *Peristrophe japonica* (*Acanthaceae*), 1 May 2022, Y.R. Sun, E33, living culture KUNCC 22-12591 = GUCC 21-0800.

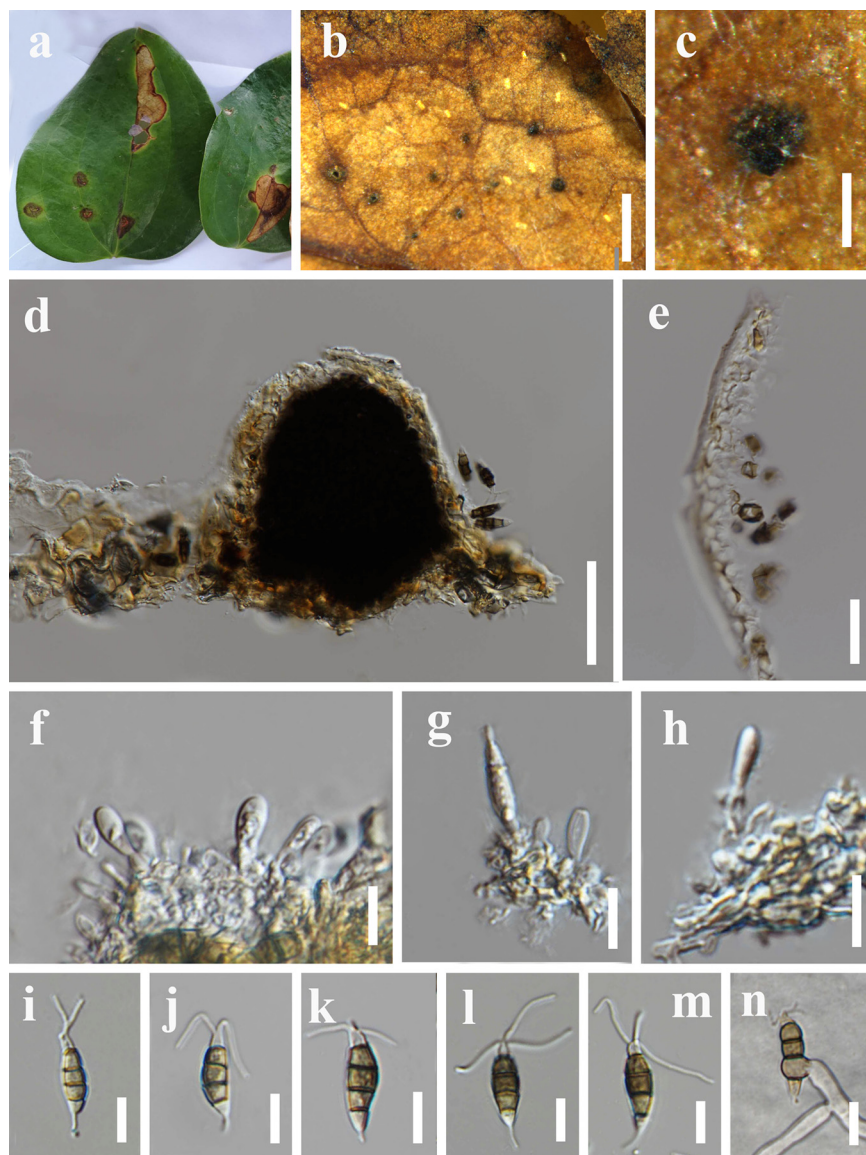


FIG 13 *Pestalotiopsis smilacicola* (MFLU 22-0165, holotype). (a) Host. (b and c) Close-up view of conidiomata. (d) Section through conidiomata. (e) Section through pycnidial wall. (f to h) Immature conidia attached to conidiogenous cells. (i to m) Conidia. (n) Germinated conidium; scale bars, 1,000 μm (b), 200 μm (c), 50 μm (d), 20 μm (e), 10 μm (f to n).

Notes: *Pestalotiopsis chamaeropsis* was originally reported on leaves of *Chamaerops humilis* in Italy by Maharachchikumbura et al. (13). Subsequently, many studies have proven that *P. chamaeropsis* is a serious phytopathogen, which can cause diseases of *Camellia sinensis* and *Camellia oleifera* and *Eurya nitida* (in China), *Erica arborea* (in Tunisia), Japanese andromeda (in Japan), and *Prostanthera rotundifolia* (in Australia) (34, 49–56). Park et al. (57) reported *P. chamaeropsis* as an endophyte from the leaves of woody plants in Korea. In this study, our strain KUNCC 22-12591 is phylogenetically clustered with *P. chamaeropsis* CBS 186.71 with good support (ML-BS = 100% and BYPP = 0.95), and it has overlapping characteristics with *P. chamaeropsis* (CBS 186.71). Thus, we identify KUNCC 22-12591 as *P. chamaeropsis*, representing a new host record.

(e) *Pestalotiopsis dracontomelon* Maharachch and K.D. Hyde. Material examined: Thailand, Chiang Rai Province, Mae Fah Luang University, leaf spots of *Podocarpus* sp. (*Podocarpaceae*), 15 January 2019, Y.R. Sun, S18 (MFLU 22-0166); living culture, MFLUCC 22-0122.

Notes: *Pestalotiopsis dracontomelon* was isolated from diseased leaves of *Dracontomelon mangifera* (Anacardiaceae) in Thailand (58). Our isolate MFLUCC 22-0122 was grouped with *P. dracontomelon* (MFLUCC 10-0149) in the phylogenetic tree. Morphologically, they have overlapping conidial measurements (19 to 26×5.5 to $8 \mu\text{m}$ for MFLUCC 22-0122 versus 18 to 23×5.5 to $7.5 \mu\text{m}$ for MFLUCC 10-0149). Therefore, we identify MFLUCC 22-0122 as the new host record of *P. dracontomelon*.

(f) *Pestalotiopsis hispanica* F. Liu, L. Cai, and Crous. Material examined: China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy leaves of *Peristrophe japonica* (Acanthaceae), 1 May 2022, Y.R. Sun, E53, living culture KUNCC 22-12595 = GUCC 21-0803; *ibid.*, on healthy leaves of *Peristrophe japonica*, 1 May 2022, Y.R. Sun, E55, living culture KUNCC 22-12593 = GUCC 21-0802; *ibid.*, on healthy leaves of *Peristrophe japonica*, 1 May 2022, Y.R. Sun, E52, living culture KUNCC 22-12594 = GUCC 21-0804.

Notes: *Pestalotiopsis hispanica* was originally reported on *Proteaceae* plants in Spain by Liu et al. (59). In this study, three strains were obtained from healthy leaves of *Peristrophe japonica* in China. These three strains grouped with *P. hispanica* (CBS 115391) with maximum support (ML-BS = 100% and BYPP = 1), and it has overlapping characteristics with *P. hispanica* (CBS 115391). Thus, we identify KUNCC 22-12594, KUNCC 22-12595, and KUNCC 22-12593 as *P. hispanica*, representing a new record.

(g) *Pestalotiopsis hydei* Huanraluek and Jayaward. Material examined: China, Guizhou Province, Qiannan Bouyei and Miao Autonomous Prefecture, Libo District, on dead twigs, 12 March 2022, J.E. Sun, L19-1, living culture, GUCC 21-0819.

Notes: *Pestalotiopsis hydei* was isolated from the leaf spots of *Litsea petiolate* in Thailand (60). Our isolate GUCC 21-0819 clustered together with *P. hydei* (MFLUCC 20-0135) in the phylogenetic tree. GUCC 21-0819 also has a similar conidial measurement to MFLUCC 20-0135 (19 to 26×4 to $6 \mu\text{m}$ in GUCC 21-0819, 18 to 35×3 to $6 \mu\text{m}$ in MFLUCC 20-0135). Additionally, there are only 3 bp different in the *ITS* and *tef1- α* genes. Therefore, we identify GUCC 21-0819 as a new geographical record of *P. hydei*.

***Pseudopestalotiopsis* Maharachch., K.D. Hyde, and Crous, Stud. Mycol. 79: 180 (2014).** (i) **Phylogenetic analyses.** The sequence data sets for *ITS*, *tef1- α* , and *tub2* were analyzed in combination to infer the interspecific relationships within *Pseudopestalotiopsis*. The aligned sequence matrix consisted of 27 sequences, including two outgroups *Pestalotiopsis trachycarpicola* (IFRDCC 2240) and *P. linearis* (MFLUCC 12-0271). Similar tree topologies were obtained by ML and BYPP methods, and the most likely tree ($-\ln = 66,531.894$) is presented (Fig. 14). Our collection is clustered with the type species of *Pseudopestalotiopsis*, *P. theae*, in the phylogenetic tree (Fig. 14).

(ii) **Taxonomy.** (a) *Pseudopestalotiopsis theae* (Sawada) Maharachch., K.D. Hyde, and Crous. Material examined: Thailand, Suphan Buri Province, dead leaf of *Ceriops tagal* (Rhizophoraceae), 5 September 2020, S Wang, TN07 (MFLU 22-0169); living culture, MFLUCC 22-0128.

Notes: our isolate MFLUCC 22-0128 is phylogenetically grouped with the type species of *Pseudopestalotiopsis*, *Ps. theae*. Morphologically, our new collection MFLU 22-0169 resembles *Ps. theae* (MFLUCC 12-0055) in color and size of the conidiogenous cells, conidia, and appendages. Therefore, we report this isolate as a new host record of *Ps. theae* from *Ceriops tagal*.

DISCUSSION

During research of microfungi on medicinal plants in southwest China and Thailand, 26 pestalotioid strains representing 17 species were isolated from 16 medicinal plants. Four new *Neopestalotiopsis* species, namely, *N. amomi*, *N. photinae*, *N. suphanburiensis*, and *N. hyperici*, three new *Pestalotiopsis* species, namely, *P. chiangmaiensis*, *P. loeiana*, and *P. smilacicola*, and eight new records are introduced. Among them, 10 species are related to leaf diseases of medicinal plants, 3 species are saprobes, 2 species are endophytes, and 1 species has various lifestyles. A worldwide checklist of pestalotioid species

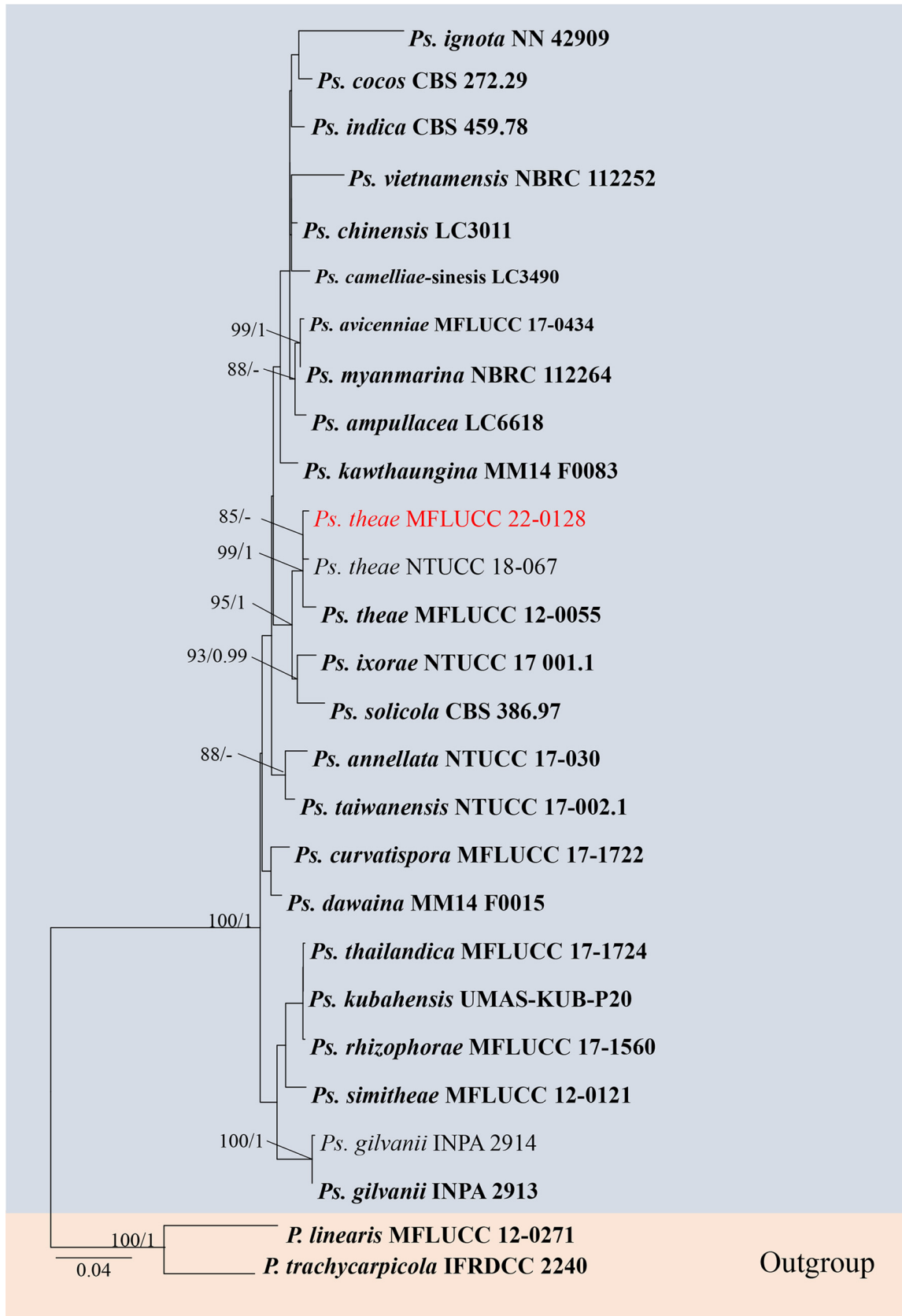


FIG 14 Maximum likelihood (RAxML) tree for *Pseudopestalotiopsis* based on analysis of a combined data set of *ITS*, *tef1- α* , and *tub2* and sequence data. The tree is rooted with *P. trachycarpicola* (IFRDCC 2240) and *P. linearis* (MFLUCC 12-0271). Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. The new isolates are in red, and the ex-type strains are in bold.

associated with medicinal plants is provided (Table 1). Among them, most are related to diseases of medicinal plants, and 47 (a total of 79) were found in China and Thailand. The analysis showed that the majority of pestalotioid species are associated with dicotyledonous medicinal plants (Fig. 15).

In this study, *Neopestalotiopsis* sp. 1 was isolated from healthy leaves of *Pinellia ternata* and dead leaves of *Ceiba pentandra*, and it was previously isolated from the leaf spots of *Castanea mollissima* (45). *Pestalotiopsis chamaeropsis* was found as an endophyte from an important medicinal plant here. However, it was reported as a serious phytopathogen in different countries (34, 49–51, 53–56). *Pestalotiopsis photiniae* was reported as an endophytic fungus on the branches of *Roystonea regia*, and it also has been isolated from the diseased leaves of blueberries (61, 62). These seem to indicate that one fungus can be endophytic, pathogenic, or saprophytic in different (or the same) plants or organs. Understanding the external factors that influence the fungal lifestyle could have major implications for agriculture, industrial development, and human health.

It is worth noting that two *Neopestalotiopsis* species, *N. amomi* and *N. hyperici* introduced in this paper, do not match the characteristics of versicolorous median cells described in *Neopestalotiopsis* (13). This phenomenon was also mentioned by Liu et al. (27). The reason for this phenomenon probably could be the type of medium or the time of cultivation. However, there are not enough data to explain this phenomenon.

Neopestalotiopsis and *Pseudopestalotiopsis* were separated from *Pestalotiopsis* by Maharachchikumbura et al. (13) based on phylogenetic analyses and conidial color. After that, the number of species in *Neopestalotiopsis* increased from 24 to 73, and the number of species in *Pseudopestalotiopsis* increased from 3 to 23 (<http://www.indexfungorum.org/>, 10 Nov 2022). In recent years, there have been more reports on diseases caused by *Neopestalotiopsis*; for example, *N. vitis* caused grapevine leaf spots in China, *N. rosicola* caused stem canker of *Rosa chinensis* in China, *N. clavispora* caused leaf spots and fruit rot of strawberry in India, *N. maddoxii* caused flower diseases of *Macadamia integrifolia* in Australia, *N. eucalyptorum* was associated with disease of *Eucalyptus* plants in Portugal, and *N. siciliana* caused stem lesion and dieback on avocado plants in Italy (22, 23, 26, 29, 30, 63). However, only six *Pseudopestalotiopsis* species were reported as plant pathogens (47, 64–67). In this study, there are nine *Neopestalotiopsis* species, and only one *Pseudopestalotiopsis* species was encountered. This seems to indicate that *Neopestalotiopsis* has richer species diversity, and the *Neopestalotiopsis* species are more likely to infect the plant and cause disease than *Pseudopestalotiopsis*. Comparing the differences between the two genera through whole-genome sequence analysis and finding related disease-causing genes would probably explain this phenomenon.

The interspecific morphological differences of pestalotioid species have been unclear. In their attempt to find a reliable criterion for interspecific differences, taxonomists have complicated the description of pestalotioid species (11, 13, 60, 68). In the past, the conidia were divided into apical cells, basal cells, and the three median cells when describing them. The three median cells were divided into the second cells from the base, third cells, and fourth cells, and every cell is measured. Obviously, the length of the three median cells is the sum length of the second, third, and fourth cells. Therefore, the descriptions are repeated. In addition, the characteristics of each cell were not treated as criteria for interspecies differences (11, 26, 27, 69). Therefore, we suggest removing the measurement for each cell to simplify the descriptions.

MATERIALS AND METHODS

Collection, examination, and isolation. Fresh healthy leaves, diseased leaves, and twigs of different medicinal plants were collected from terrestrial habitats in southwest China and Thailand from 2019 to 2022. Samples were brought to the laboratory in Ziploc plastic bags or paper envelopes for examination. The fruiting bodies on natural substrates were observed and photographed using a stereomicroscope

TABLE 1 Checklist of pestalotioid species associated with medicinal plants

Species ^a	Life mode ^b	Disease (if any)	Host ^c	Location	Reference
<i>Neopestalotiopsis acrostichi</i>	P	Leaf spot	<i>Acrostichum aureum</i> (F)	Thailand	67
<i>Neopestalotiopsis alpapicalis</i>	E		<i>Rhizophora mucronate</i> (D)	Thailand	96
<i>Neopestalotiopsis amomi</i>	P	Leaf spot	<i>Amomum villosum</i> (M)	China	This study
<i>Neopestalotiopsis brachiata</i>	P	Leaf spot	<i>Rhizophora apiculata</i> (D)	Thailand	67
<i>Neopestalotiopsis clavispora</i>	P	Leaf spot, branch blight	<i>Dendrobium officinale</i> , <i>Taxus × media</i> (M/G)	China	97, 98
<i>Neopestalotiopsis cubana</i>	P	Leaf blight	<i>Ixora chinensis</i> (D)	Malaysia	99
<i>Neopestalotiopsis dendrobii</i>	E		<i>Dendrobium cariniferum</i> (M)	Thailand	41
<i>Neopestalotiopsis ellipospor</i>	Unknown		<i>Ardisia crenata</i> (D)	Hong Kong, China	13
<i>Neopestalotiopsis eucalypticola</i>	Unknown		<i>Eucalyptus globulus</i> (D)	Unknown	13
<i>Neopestalotiopsis eucalyptorum</i>	P	Leaf necrosis, stem basal cankers	<i>Eucalyptus globulus</i> (D)	Fundão/Guarda/ Portugal	22
<i>Neopestalotiopsis formicarum</i>	P	Leaf spot	<i>Photinia serratifolia</i>	China	This study
<i>Neopestalotiopsis haikouensis</i>	P	Leaf spot	<i>Ilex chinensis</i> (D)	China	17
<i>Neopestalotiopsis hispanica</i>	P	Leaves and stem necrosis	<i>Eucalyptus globulus</i> (D)	Fundão/Guarda/Spain	22
<i>Neopestalotiopsis hydeana</i>	P	Leaf spot	<i>Alpinia malaccensis</i> (M)	Thailand	60
<i>Neopestalotiopsis hyperici</i>	P	Leaf spot	<i>Hypericum monogynum</i> (D)	China	This study
<i>Neopestalotiopsis iberica</i>	P	Leaves and stem necrosis	<i>Eucalyptus globulus</i> (D)	Pegões/Portugal/Spain	22
<i>Neopestalotiopsis longiappendiculata</i>	P	Leaves and stem necrosis	<i>Eucalyptus globulus</i> (D)	Pegões/Portugal	22
<i>Neopestalotiopsis lusitanica</i>	Unknown		<i>Vismia guianensis</i> (D)	Brazil	100
<i>Neopestalotiopsis pernambucana</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	67
<i>Neopestalotiopsis photiniae</i>	P	Leaf spot	<i>Photinia serrulate</i> (D)	China	This study
<i>Neopestalotiopsis rhapsidis</i>	P	Leaf spot	<i>Podocarpus macrophyllus</i> (G)	China	This study
<i>Neopestalotiopsis rhizophorae</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	67
<i>Neopestalotiopsis rhododendri</i>	P	Leaf spot	<i>Dracaena fragrans</i> (M)	Thailand	This study
<i>Neopestalotiopsis rosae</i>	Unknown		<i>Paeonia suffruticosa</i> (D)	United States	13
<i>Neopestalotiopsis rosicola</i>	P*	Stem canker	<i>Rosa chinensis</i> (D)	China	63
<i>Neopestalotiopsis saprophytica</i>	S		<i>Litsea rotundifolia</i> (D)	Hong Kong, China	13
<i>Neopestalotiopsis surinamensis</i>	E		<i>Scurrula atropurpurea</i> (D)	Indonesia	101, 102
<i>Neopestalotiopsis thailandica</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	67
<i>Pestalotiopsis adusta</i>	E, P*	Leaf spot	<i>Clerodendrum canescens</i> / <i>Sinopodophyllum hexandrum</i> / <i>Rubus idaeus</i> (D)	China	103–105
<i>Pestalotiopsis affinis</i>	P	Leaf spot	<i>Taxus chinensis</i> (G)	China	106
<i>Pestalotiopsis alpiniae</i>	P	Leaf spot	<i>Alpinia galanga</i> (M)	China	106
<i>Pestalotiopsis antiaris</i>	P	Leaf spot	<i>Antiaris toxicaria</i> (M)	China	106
<i>Pestalotiopsis bicilia</i>	S		<i>Viburnum opulus</i> (D)	Canada	13
<i>Pestalotiopsis biciliata</i>	P*	Stem canker	<i>Pistacia lentiscus</i> (D)	Tunisia	107
<i>Pestalotiopsis bicolor</i>	S		<i>Smilax sp.</i> (M)	United States	108
<i>Pestalotiopsis bruguierae</i>	Unknown		<i>Bruguiera gymnorhiza</i> (D)	India	109
<i>Pestalotiopsis bulbophylli</i>	S		<i>Bulbophyllum thouars</i> (M)	China	110
<i>Pestalotiopsis chamaeropsis</i>	E, P*	Leaf spot	<i>Eurya nitida</i> , <i>Peristrophe japonica</i> (D)	China	55, this study
<i>Pestalotiopsis chiangmaiensis</i>	P	Leaf strip	<i>Bamboo</i> (M)	Thailand	This study
<i>Pestalotiopsis cruenta</i>	Unknown		<i>Polygonum lasianthum</i> (D)	Japan	Index Fungorum (2022)
<i>Pestalotiopsis digitalis</i>	P	Leaf spot	<i>Digitalis purpurea</i> (D)	New Zealand	58
<i>Pestalotiopsis dilleniae</i>	P	Leaf spot	<i>Dillenia turbinata</i> (M)	China	106
<i>Pestalotiopsis diploclisiae</i>	Unknown		<i>Diploclisia glaucescens</i> (D)	Hong Kong, China	13
<i>Pestalotiopsis dracaenae</i>	S		<i>Dracaena fragrans</i> (M)	China	111
<i>Pestalotiopsis ellipospora</i>	P*	Stem canker	<i>Acanthopanax divaricatus</i> (D)	Korea	112
<i>Pestalotiopsis gibbosa</i>	S		<i>Gaultheria shallon</i> (D)	United States	113
<i>Pestalotiopsis heucherae</i>	Unknown		<i>Heuchera parviflora</i>	United States	114
<i>Pestalotiopsis hispanica</i>	E		<i>Peristrophe japonica</i>	China	This study
<i>Pestalotiopsis hughesii</i>	Unknown		<i>Cyperus articulatus</i> (M)	Ghana	115
<i>Pestalotiopsis japonica</i>	Unknown		<i>Cedrela sinensis</i> (D)	Japan	19
<i>Pestalotiopsis jinchanghensis</i>	E		<i>Vaccinium dunalianum</i> (D)	China	116

(Continued on next page)

TABLE 1 (Continued)

Species ^a	Life mode ^b	Disease (if any)	Host ^c	Location	Reference
<i>Pestalotiopsis kenyana</i>	P*	Leaf spot	<i>Zanthoxylum schinifolium</i> (D)	China	117
<i>Pestalotiopsis kunmingensis</i>	E		<i>Podocarpus macrophyllus</i> (G)	China	68
<i>Pestalotiopsis kwangsiensis</i>	P	Leaf spot	<i>Sinopimelodendron kuwangsensis</i> (D)	China	106
<i>Pestalotiopsis lespedezae</i>	Unknown		<i>Lepedeza bicolor</i> (D)	Japan	118
<i>Pestalotiopsis linearis</i>	E		<i>Trachelospermum</i> sp. (D)	China	24
<i>Pestalotiopsis lushanensis</i>	P*	Brown leaf spot, leaf blight	<i>Sarcandra glabra</i> , <i>Podocarpus macrophyllus</i> (G)	China	119
<i>Pestalotiopsis microspora</i>	S		<i>Hedera helix</i> (D)	Argentina	120
<i>Pestalotiopsis moluccensis</i>	Unknown		<i>Xylocarpus moluccensis</i> (D)	India	109
<i>Pestalotiopsis neolitsea</i>	P*	Leaf spot	<i>Neolitsea villosa</i> (D)	Taiwan, China	51
<i>Pestalotiopsis oenotherae</i>	Unknown		<i>Oenothera laciniata</i> (D)	United States	121
<i>Pestalotiopsis pandani</i>	Unknown		<i>Pandanus odoratissimus</i> (M)	Taiwan, China	108
<i>Pestalotiopsis paraguariensis</i>	Unknown		<i>Ilex paraguariensis</i> (D)	Brazil	122
<i>Pestalotiopsis pestalozzioides</i>	Unknown		<i>Clematis ligusticifolia</i> (D)	New Mexico	11
<i>Pestalotiopsis pipericola</i>	Unknown		<i>Piper nigrum</i> (D)	India	123
<i>Pestalotiopsis quadriciliata</i>	Unknown		<i>Vitis vulpine</i> (D)	Canada	124
<i>Pestalotiopsis rhodomyrtus</i>	Unknown		<i>Rhodomyrtus tomentosa</i> (D)	China	125
<i>Pestalotiopsis smilacicola</i>	P	Leaf spot	<i>Smilax china</i> , <i>Dioscorea</i> sp. (M)	Thailand	This study
<i>Pestalotiopsis sinensis</i>	Unknown		<i>Ginkgo biloba</i> (G)	China	126
<i>Pestalotiopsis tecomicola</i>	Unknown		<i>Tecoma radicans</i> (D)	United States	11
<i>Pestalotiopsis thailandica</i>	P	Leaf spot	<i>Rhizophora apiculata</i> (D)	Thailand	67
<i>Pseudopestalotiopsis ampullace</i>	E		<i>Magnolia candolli</i> (D)	China	76
<i>Pseudopestalotiopsis curvatipora</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	67
<i>Pseudopestalotiopsis gilvanii</i>	P*	Leaf spot	<i>Paullinia cupana</i> (D)	Brazil	47
<i>Pseudopestalotiopsis indica</i>	Unknown		<i>Hibiscus rosa-sinensis</i> (D)	India	13
<i>Pseudopestalotiopsis simitheae</i>	S, E		<i>Pandanus odoratissimus</i> / <i>Magnolia candolli</i> (M/D)	Thailand/China	76, 127
<i>Pseudopestalotiopsis thailandica</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	67
<i>Pseudoestalotiopsis theae</i>	S		<i>Ceriops tagal</i> (D)	Thailand	This study

^aThe checklist includes species names, life modes, disease names (if any), hosts, locations, and references. The current name is used according to Index Fungorum (2022).

^bThe mode of life is given as endophyte (E), pathogen (P), and saprobe (S). For the species, those with confirmed pathogenicity data are marked with an asterisk (*).

^cThe taxonomic status of the host is given as dicotyledons (D), ferns (F), gymnosperms (G), and monocotyledons (M).

(SteREO Discovery, V12, Carl Zeiss Microscopy GmbH, Germany; VHX-7000, Keyence, Japan). Morphological characteristics were observed using a Nikon Eclipse Ni compound microscope (Nikon, Japan) and photographed with a Nikon DS-Ri2 digital camera (Nikon, Japan) or using a Carl Zeiss compound microscope (Carl Zeiss AG, Germany) and an Axiocam 208 color digital camera (Carl Zeiss AG, Germany). The photo plates were made with Adobe Photoshop CS6 Extended v. 13.0 software. Measurements were obtained with the Tarosoft (R) Image Frame Work software.

For endophytes, materials were washed under running tap water and immersed in 70% ethanol for 1 min, followed by soaking in 4% NaOCl for 1 min, rinsing three times in sterile distilled water, and drying on sterile filter paper. For the control, the final sterile water rinse was plated and observed during the postincubation period. The absence of any fungal (microbial) growth indicated that the leaf surface was sterile (70). The sterilized materials were cut into 2- to 5-mm² segments and placed on PDA containing 50 μg/mL penicillin and 50 μg/mL streptomycin (71). The plates were observed daily, and the mycelial growth on the edge of the fungal colonies was transferred to fresh PDA dishes to obtain pure cultures. For other samples, single-spore isolations were used to obtain pure cultures following the methods described by Senanayake et al. (72). Germinated conidia were transferred to fresh PDA plates and incubated at 25°C for 4 weeks. The pure cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand, the Culture Collection of Kunming Institute of Botany, the Chinese Academy of Sciences (KUNCC), Kunming, China, and the Culture Collection of the Department of Plant Pathology, Agriculture College, Guizhou University (GUCC), Guiyang, China. Specimens were deposited in the herbarium of Mae Fah Luang University (MFLU) Chiang Rai, Thailand, and the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS), Kunming, China. Facesoffungi (FoF) numbers were acquired as described by Jayasiri et al. (73). Taxonomic descriptions and nomenclature were deposited at Fungal Names (<https://nmdc.cn/fungalnames/registre>) following the description in reference 74.

DNA extraction, PCR amplification, and sequencing. A Biomiga fungus genomic DNA extraction kit (Biomiga, USA) was used to extract DNA from fresh fungal mycelia, which were grown on PDA medium for 4 weeks at 25°C. PrepMan ultra sample preparation reagent (Thermo Fisher Scientific, Japan) was used to extract DNA directly from fruiting bodies. Three genes were selected in this study: the

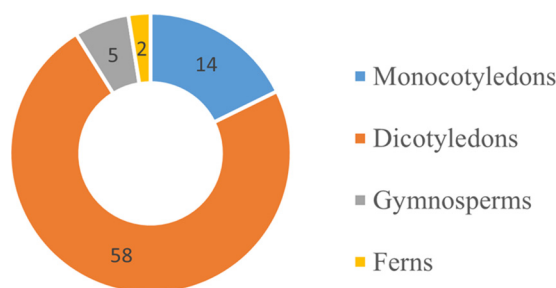


FIG 15 Distribution of pestalotioid species in different medicinal plants.

internal transcribed spacers (*ITS*), the translation elongation factor 1 (*tef1- α*), and the partial β -tubulin region (*tub2*). PCR was carried out in a 20- μ L reaction volume, which contained 10 μ L of 2 \times PCR master mix, 7 μ L of double-distilled water (ddH₂O), 1 μ L of each primer, and 1 μ L of template DNA. The PCR thermal cycle program and primers are given in Table 2. Purification and sequencing of PCR products were carried out at SinoGenoMax (Beijing) Co., China.

Phylogenetic analyses. Phylogenetic analyses and the preparatory work were carried out according to the method described in Dissanayake et al. (75). The sequences were compared against the NCBI GenBank nucleotide database using BLASTn to evaluate the closest phylogenetic matches. All sequences used in this study were downloaded from GenBank referring to previous studies (16, 23, 27, 30, 44, 65, 67, 76–79) (Table 3). The single-gene sequences were checked with BioEdit v. 7.0.9.0 (80). Alignments for each locus were generated using MAFFT v.7 (<https://mafft.cbrc.jp/alignment/server/>) and were manually improved using AliView (81). The final single-gene alignments were combined by SequenceMatrix 1.7.8 (82). For the final alignment, see Data S1 in the supplemental material.

The maximum likelihood (ML) analyses were carried out using IQ-TREE (83, 84) on the IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at>, 1 Nov 2022) under partitioned models. The best-fit substitution models (Table 4) were determined by WIQ-TREE (85). Ultrafast bootstrap (BS) analyses were implemented with 1,000 replicates (86, 87).

The Bayesian posterior probability (BYPP) analyses were performed in CIPRES (88) with MrBayes on XSEDE 3.2.7a (89). MrModeltest 2.2 (90) was used to evaluate the best nucleotide substitution models (Table 4) for the data. The Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP) (91). Six simultaneous Markov chains were run for 10 million generations, and trees were sampled every 1,000th generation. The first 20% of trees, representing the burn-in phase of the analyses, were discarded, and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree.

The final phylogenetic trees were viewed with FigTree v1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) and were modified in Microsoft Office PowerPoint 2010.

Genealogical concordance phylogenetic species recognition analysis. Genealogical concordance phylogenetic species recognition was used to analyze the new species, their species boundaries, and their most closely related taxa, as described by Quaedvlieg et al. (92). The pairwise homoplasy index (PHI) test (93) was performed in SplitsTree4 (94, 95). If the PHI is above 0.05 threshold ($\Phi_w > 0.05$), it indicates that there is no significant recombination present in the data set. The relationships between closely related species were visualized in splits graphs with both the Log-Det transformation and splits decomposition options.

Data availability. The sequences generated in this study can be found in GenBank. The accession numbers of the sequences deposited in GenBank are listed in Table 3.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

TABLE 2 Primers and PCR procedures used in this study

Locus	Primers		PCR procedures	Reference
	Name	Sequence (5' to 3')		
<i>ITS</i>	ITS5	GGAAGTAAAAGTCGTAACAAGG	94°C 3 min; 94°C 30 s; 52°C 30 s; 72°C 1 min; repeat 2 to 4 for 35 cycles; 72°C 8 min; 4°C on hold	128
	ITS4	TCCTCCGCTTATTGATATGC		
<i>tef1-α</i>	EF1-728F	CAT CGA GAA GTT CGA GAA GG	94°C 5 min; 94°C 30 s; 52°C 30 s; 72°C 1 min; repeat 2 to 4 for 40 cycles; 72°C 8 min; 4°C on hold	129, 130
	EF2	GGA RGT ACC AGT SAT CAT GTT		
<i>tub2</i>	T1	AACATGCGTGAGATTGTAAGT	95°C 3 min; 94°C 30 s; 55°C 50 s; 72°C 1 min; repeat 2 to 4 for 40 cycles; 72°C 8 min; 4°C on hold	131, 132
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		

TABLE 3 Taxa of the three genera *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis* used in the phylogenetic analysis with the corresponding GenBank accession numbers

Taxa ^a	Strain/voucher no.	<i>tef1</i> - α^b	<i>tub2</i> ^b	<i>ITS</i> ^b
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	MK764316	MK764338	MK764272
<i>N. alpapicalis</i>	MFLUCC 17-2544	MK463547	MK463545	MK357772
<i>N. amomi</i>	HKAS 124563	OP653489	OP752133	OP498012
<i>N. amomi</i>	HKAS 124564	OP753382	OP765913	OP498013
<i>N. aotearoa</i>	CBS 367.54	KM199526	KM199454	KM199369
<i>N. asiatica</i>	MFLUCC 12-0286	JX399049	JX399018	JX398983
<i>N. australis</i>	CBS 114159	KM199537	KM199432	KM199348
<i>N. brachiata</i>	MFLUCC 17-1555	MK764318	MK764340	MK764274
<i>N. brasiliensis</i>	COAD 2166	MG692402	MG692400	MG686469
<i>N. camelliae-oleiferae</i>	CSUFTCC81	OK507955	OK562360	OK493585
<i>N. camelliae-oleiferae</i>	CSUFTCC82	OK507956	OK562361	OK493586
<i>N. cavernicola</i>	KUMCC 20-0269	MW550735	MW557596	MW545802
<i>N. cavernicola</i>	KUMCC 20-0332	MW590327	MW590328	MW581238
<i>N. Chiangmaiensis</i>	MFLUCC 18-0113	MH388404	MH412725	NA
<i>N. chrysea</i>	MFLUCC 12-0261	JX399051	JX399020	JX398985
<i>N. clavispora</i>	MFLUCC 12-0281	JX399045	JX399014	JX398979
<i>N. cocoes</i>	MFLUCC 15-0152	KX789689	NA	NR_156312
<i>N. coffea-arabicae</i>	HGUP4015	KF412644	KF412641	KF412647
<i>N. coffea-arabicae</i>	HGUP4109	KF412646	KF412643	KF412649
<i>N. cubana</i>	CBS 600.96	KM199521	KM199438	KM199347
<i>N. dendrobii</i>	MFLUCC 14-0106	MK975829	MK975835	MK993571
<i>N. dendrobii</i>	MFLUCC 14-0132	MK975830	NA	MK993572
<i>N. drenthii</i>	BRIP 72264a	MZ344172	MZ312680	MZ303787
<i>N. drenthii</i>	BRIP 72263a	MZ344171	MZ312679	MZ303786
<i>N. ellipsospora</i>	MFLUCC 12-0283	JX399047	JX399016	JX398980
<i>N. egyptiaca</i>	CBS 140162	KP943748	KP943746	KP943747
<i>N. eucalypticola</i>	CBS 264.37	KM199551	KM199431	KM199376
<i>N. eucalyptorum</i>	PE194	MW805398	MW802831	MW794098
<i>N. eucalyptorum</i>	CBS 147684	MW805397	MW802841	MW794108
<i>N. foedans</i>	CGMCC 3.9123	JX399053	JX399022	JX398987
<i>N. formicarum</i>	CBS 362.72	KM199517	KM199455	KM199358
<i>N. formicarum</i>	CBS 115.83	KM199519	KM199444	KM199344
<i>N. formicarum</i>	GUCC 21-0809	OP753367	OP752132	OP498007
<i>N. guajavae</i>	FMB0026	MH460868	MH460871	MF783085
<i>N. guajavicola</i>	FMB0129	MH460870	MH460873	MH209245
<i>N. haikouensis</i>	SAUCC212271	OK104877	OK104870	OK087294
<i>N. haikouensis</i>	SAUCC212272	OK104878	OK104871	OK087295
<i>N. hadrolaeliae</i>	VIC 47180	MK465122	MK465120	MK454709
<i>N. hispanica</i>	CBS 147686	MW805399	MW802840	MW794107
<i>N. honoluluana</i>	CBS 114495	KM199548	KM199457	NR_145245
<i>N. hydeana</i>	MFLUCC 20-0132	MW251129	MW251119	MW266069
<i>N. hypericin</i>	KUNCC 22-12597	OP713768	OP765908	OP498010
<i>N. hypericin</i>	KUNCC 22-12598	OP737880	OP737883	OP498009
<i>N. iberica</i>	CBS 147688	MW805402	MW802844	MW794111
<i>N. iraniensis</i>	CBS 137768	KM074051	KM074057	KM074048
<i>N. javaensis</i>	CBS 257 31	KM199543	KM199437	NR_145241
<i>N. keteleeria</i>	MFLUCC 13-0915	KJ503822	KJ503821	KJ503820
<i>N. longiappendiculata</i>	MEAN 1315	MW805404	MW802845	MW794112
<i>N. lusitanica</i>	MEAN 1317	MW805406	MW802843	MW794110
<i>N. lusitanica</i>	MEAN 1320	MW805409	MW802830	MW794097
<i>N. macadamiae</i>	BRIP 63737c	KX186627	KX186654	NR_161002
<i>N. maddoxii</i>	BRIP 72266a	MZ344167	MZ312675	MZ303782
<i>N. magna</i>	MFLUCC 12-0652	KF582791	KF582793	KF582795
<i>N. mesopotamica</i>	CBS 336.86	KM199555	KM199441	KM199362
<i>N. musae</i>	MFLUCC 15-0776	KX789685	KX789686	NR_156311
<i>N. natalensis</i>	CBS 138.41	KM199552	KM199466	NR_156288
<i>N. nebuloides</i>	BRIP 66617	MK977633	MK977632	MK966338
<i>N. olumideae</i>	BRIP 72273a	MZ344175	MZ312683	MZ303790
<i>N. pandanicola</i>	KUMCC 17-0175	MH388389	MH412720	NA
<i>N. pernambucana</i>	GS 2014-RV01	KU306739	NA	KJ792466
<i>N. petila</i>	MFLUCC 17-1738	MK764319	MK764341	MK764275

(Continued on next page)

TABLE 3 (Continued)

Taxa ^a	Strain/voucher no.	<i>tef1-α^b</i>	<i>tub2^b</i>	<i>ITS^b</i>
<i>N. phangngaensis</i>	MFLUCC 18-0119	MH388390	MH412721	MH388354
<i>N. photiniae</i>	MFLUCC 22-0129	OP753368	OP752131	OP498008
<i>N. photiniae</i>	GUCC 21-0820	OP828691	OP896200	OP806524
<i>N. perukae</i>	FMB0127	MH523647	MH460876	MH209077
<i>N. piceana</i>	CBS 394.48	KM199527	KM199453	KM199368
<i>N. protearum</i>	CBS 114178	KM199542	KM199463	JN712498
<i>N. psidii</i>	FMB0028	MH460874	MH477870	MF783082
<i>N. rhapsidis</i>	GUCC21501	MW980442	MW980441	MW931620
<i>N. rhapsidis</i>	KUNCC 22-12590	OP753369	OP752134	OP498004
<i>N. rhizophorae</i>	MFLUCC 17-1550	MK764321	MK764343	MK764277
<i>N. rhododendri</i>	GUCC 21504	MW980444	MW980443	MW979577
<i>N. rhododendri</i>	MFLUCC 22-0130	OP753370	OP762671	OP497995
<i>N. rhododendricola</i>	KUN-HKAS-123204	OK274148	OK274147	OK283069
<i>N. rosae</i>	CBS 101057	KM199523	KM199429	KM199359
<i>N. rosicola</i>	CFCC 51992	KY885243	KY885245	KY885239
<i>N. samarangensis</i>	MFLUCC 12-0233	JQ968611	JQ968610	JQ968609
<i>N. saprophytica</i>	MFLUCC 12-0282	KM199538	KM199433	KM199345
<i>N. saprophytica</i>	GUCC 21506	MW980449	MW980447	MW979578
<i>N. sichuanensis</i>	CFCC 54338	MW199750	MW218524	MW166231
<i>N. sichuanensis</i>	SM15-1C	MW199751	MW218525	MW166232
<i>N. siciliana</i>	CBS 149117	ON107273	ON209162	ON117813
<i>N. sonneratae</i>	MFLUCC 17-1745	MK764323	MK764345	MK764279
<i>N. steyaertii</i>	IMI 192475	KF582792	KF582794	KF582796
<i>N. suphanburiensis</i>	MFLUCC 22-0126-	OP753372	OP752135	OP497994
<i>N. surinamensis</i>	CBS 450.74	KM199518	KM199465	KM199351
<i>N. thailandica</i>	MFLUCC 17-1730	MK764325	MK764347	MK764281
<i>N. umbrinospora</i>	MFLUCC 12-0285	JX399050	JX399019	JX398984
<i>N. vheenae</i>	BRIP 72293a	MZ344177	MZ312685	MZ303792
<i>N. vitis</i>	MFLUCC 15-1265	KU140676	KU140685	KU140694
<i>N. zakeelii</i>	BRIP 72282a	MZ344174	MZ312682	MZ303789
<i>N. zimbabwana</i>	CBS 111495	KM199545	KM199456	MH554855
<i>Neopestalotiopsis</i> sp. 1	CFCC 54337	MW199752	MW218526	MW166233
<i>Neopestalotiopsis</i> sp. 1	ZX12-1	MW199753	NA	MW166234
<i>Neopestalotiopsis</i> sp. 1	HKAS 124560	OP753364	OP752138	OP498005
<i>Neopestalotiopsis</i> sp. 1	KUNCC 22-12592	OP753365	OP752140	OP498006
<i>Neopestalotiopsis</i> sp. 1	GUCC 21-0808	OP753366	OP752139	OP498011
<i>Neopestalotiopsis</i> sp. 2	CFCC 54340	MW199754	MW218528	MW166235
<i>Neopestalotiopsis</i> sp. 2	ZX22B	MW199755	MW218529	MW166236
<i>Neopestalotiopsis</i> sp. 2	MFLUCC 22-0131	OP753371	OP752141	OP497996
<i>Neopestalotiopsis</i> sp. 2	KUNCC 22-12596	OP797834	OP752142	OP498003
<i>Pestalotiopsis adusta</i>	ICMP 6088	JX399070	JX399037	JX399006
<i>P. adusta</i>	MFLUCC 10-0146	JX399071	JX399038	JX399007
<i>P. aggestorum</i>	LC6301	KX895234	KX895348	KX895015
<i>P. anacardiacearum</i>	IFRDCC 2397	KC247156	KC247155	KC247154
<i>P. arceuthobii</i>	CBS 434.65	KM199516	KM199427	KM199341
<i>P. appendiculata</i>	CGMCC 3.23550	OP185509	OP185516	OP082431
<i>P. arengae</i>	CBS 331.92	KM199515	KM199426	KM199340
<i>P. australasiae</i>	CBS 114126	KM199499	KM199409	KM199297
<i>P. australasiae</i>	CBS 11141	KM199501	KM199410	KM199298
<i>P. australis</i>	CBS 114193	KM199475	KM199383	KM199332
<i>P. biciliata</i>	CBS 124463	KM199505	KM199399	KM199308
<i>P. biciliata</i>	CAA1011	MW959090	MW934601	MW969738
<i>P. brachiata</i>	LC2988	KX895150	KX895265	KX894933
<i>P. brachiata</i>	LC8189	KY464153	KY464163	KY464143
<i>P. brassicae</i>	CBS 170.26	KM199558	NA	KM199379
<i>P. camelliae</i>	MFLUCC 12-0277	JX399074	JX399041	JX399010
<i>P. camelliae-oleiferae</i>	CSUFTCC08	OK507963	OK562368	OK493593
<i>P. camelliae-oleiferae</i>	CSUFTCC09	OK507964	OK562369	OK493594
<i>P. cangshanensis</i>	CGMCC 3.23544	OP185510	OP185517	OP082426
<i>P. chamaeropsis</i>	CBS 186.71	KM199473	KM199391	KM199326
<i>P. chamaeropsis</i>	KUNCC 22-12591	OP753373	OP752130	OP497998
<i>P. chiangmaiensis</i>	MFLUCC 22-0127	OP753374	OP752137	OP497990
<i>P. chioscuro</i>	BRIP 72970	OK423753	OK423752	OK422510

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TABLE 3 (Continued)

Taxa ^a	Strain/voucher no.	<i>tef1-α</i> ^b	<i>tub2</i> ^b	ITS ^b
<i>P. chinensis</i>	MFLUCC 12-0273	NA	NA	NR_111786
<i>P. clavata</i>	MFLUCC 12-0268	JX399056	JX399025	JX398990
<i>P. colombiensis</i>	CBS 118553	KM199488	KM199421	KM199307
<i>P. daliensis</i>	CGMCC 3.23548	OP185511	OP185518	OP082429
<i>P. digitalis</i>	ICMP 5434	NA	KP781883	KP781879
<i>P. diploclisiae</i>	CBS 115587	KM199486	KM199419	KM199320
<i>P. dilucida</i>	LC3232	KX895178	KX895293	KX894961
<i>P. diversiseta</i>	MFLUCC 12-0287	JX399073	JX399040	NR_120187
<i>P. dracaenae</i>	HGUP4037	MT598644	MT598645	NA
<i>P. dracaenicola</i>	MFLUCC 18-0913	MN962732	MN962733	MN962731
<i>P. dracontomelon</i>	MFUCC 10-0149	KP781880	NA	KP781877
<i>P. dracontomelon</i>	MFLUCC 22-0122	OP753375	OP762672	NA
<i>P. endophytica</i>	MFLUCC 18-0932	MW417119	NA	NR_172439
<i>P. ericacearum</i>	IFRDCC 2439	KC537814	KC537821	KC537807
<i>P. etonensis</i>	BRIP 66615	MK977635	MK977634	MK966339
<i>P. formosana</i>	NTUCC 17-009	MH809389	MH809385	MH809381
<i>P. furcata</i>	MFLUCC 12-0054	JQ683740	JQ683708	JQ683724
<i>P. fusoides</i>	CGMCC 3.23545	OP185512	OP185519	OP082427
<i>P. gaultheria</i>	IFRD 411.014	KC537812	KC537819	KC537805
<i>P. gibbosa</i>	NOF 3175	LC311591	LC311590	LC311589
<i>P. grevilleae</i>	CBS 114127	KM199504	KM199407	KM199300
<i>P. hawaiiensis</i>	CBS 114491	KM199514	KM199428	KM199339
<i>P. hispanica</i>	CBS 115391	MH554399	MH554640	MH553981
<i>P. peristrophes</i>	KUNCC 22-12595	OP753381	OP765910	OP498001
<i>P. peristrophes</i>	KUNCC 22-12593	OP753378	OP737882	OP498000
<i>P. peristrophes</i>	KUNCC 22-12594	OP753380	OP765912	OP498002
<i>P. hydei</i>	MFLUCC 20-0135	MW251113	MW251112	NR_172003
<i>P. hydei</i>	GUCC 21-0816	OP753383	OP765909	OP753660
<i>P. hollandica</i>	CBS 265.33	KM199481	KM199388	KM199328
<i>P. hollandica</i>	MEAN 1091	MT374691	MT374703	MT374678
<i>P. humus</i>	CBS 336.97	KM199484	KM199420	KM199317
<i>P. hunanensis</i>	CSUFTCC15	OK507969	OK562374	OK493599
<i>P. hunanensis</i>	CSUFTCC18	OK507970	OK562375	OK493600
<i>P. iberica</i>	CAA1006	MW759039	MW759036	MW732249
<i>P. inflexa</i>	MFLUCC 12-0270	JX399072	JX399039	JX399008
<i>P. intermedia</i>	MFLUCC 12-0259	JX399059	JX399028	JX398993
<i>P. italiana</i>	MFLUCC 12-0657	KP781881	KP781882	KP781878
<i>P. jesteri</i>	CBS 109350	KM199554	KM199468	KM199380
<i>P. jiangxiensis</i>	LC4399	KX895227	KX895341	KX895009
<i>P. jinchanghensis</i>	LC6636	KX895247	KX895361	KX895028
<i>P. kandelicola</i>	NCYU 19-0355	MT563101	MT563099	MT560722
<i>P. kaki</i>	KNU-PT-1804	LC553555	LC552954	LC552953
<i>P. kenyana</i>	CBS 442.67	KM199502	KM199395	KM199302
<i>P. kenyana</i>	CBS 911.96	KM199503	KM199396	KM199303
<i>P. knightiae</i>	CBS 114138	KM199497	KM199408	KM199310
<i>P. knightiae</i>	CBS 111963	KM199495	KM199406	KM199311
<i>P. licualacola</i>	HGUP4057	KC481684	KC481683	KC492509
<i>P. linearis</i>	MFLUCC 12-0271	JX399058	JX399027	JX398992
<i>P. loeiana</i>	MFLUCC 22-0123	OP737881	OP713769	OP497988
<i>P. longiappendiculata</i>	LC3013	KX895156	KX895271	KX894939
<i>P. lushanensis</i>	LC4344	KX895223	KX895337	KX895005
<i>P. lushanensis</i>	LC8182	KY464146	KY464156	KY464136
<i>P. macadamiae</i>	BRIP 63738B	KX186621	KX186680	KX186588
<i>P. malayana</i>	CBS 102220	KM199482	KM199411	KM199306
<i>P. monochaeta</i>	CBS 144.97	KM199479	KM199386	KM199327
<i>P. monochaeta</i>	CBS 440.83	KM199480	KM199387	KM199329
<i>P. montellica</i>	MFLUCC 12-0279	JX399076	JX399043	JX399012
<i>P. nanjingensis</i>	CSUFTCC16	OK507972	OK562377	OK493602
<i>P. nanjingensis</i>	CSUFTCC20	OK507973	OK562378	OK493603
<i>P. nanningensis</i>	CSUFTCC10	OK507966	OK562371	OK493596
<i>P. nanningensis</i>	CSUFTCC11	OK507967	OK562372	OK493597
<i>P. neolitseae</i>	NTUCC 17-011	MH809391	MH809387	MH809383
<i>P. novae-hollandiae</i>	CBS 130973	KM199511	KM199425	KM199337

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TABLE 3 (Continued)

Taxa ^a	Strain/voucher no.	<i>tef1-α</i> ^b	<i>tub2</i> ^b	<i>ITS</i> ^b
<i>P. oryzae</i>	CBS 353.69	KM199496	KM199398	KM199299
<i>P. oryzae</i>	CL107	MN022941	MN015425	MK156295
<i>P. papuana</i>	CBS 331.96	KM199491	KM199413	KM199321
<i>P. papuana</i>	MFLU 19-2764	MW192204	MW296942	MW114337
<i>P. parva</i>	CBS 265.37	KM199508	KM199404	KM199312
<i>P. pallidotheae</i>	MAFF 240993	LC311585	LC311584	NR_111022
<i>P. photinicola</i>	GZCC 16-0028	KY047662	KY047663	KY092404
<i>P. pinisp</i>	CBS 146841	MT374694	MT374706	MT374681
<i>P. portugalia</i>	CBS 393.48	KM199510	KM199422	KM199335
<i>P. rhizophorae</i>	MFLUCC 17-0416	MK764327	MK764349	MK764283
<i>P. rhizophorae</i>	MFLUCC 17-0417	MK764328	MK764350	MK764284
<i>P. rhododendri</i>	IFRDCC 2399	KC537811	KC537818	NR_120265
<i>P. rhodomyrtus</i>	HGUP4230	KF412645	KF412642	KF412648
<i>P. rhodomyrtus</i>	MG7	MZ126725	MZ126718	MZ089458
<i>P. rosarioides</i>	CGMCC 3.23549	OP185513	OP185520	OP082430
<i>P. rosea</i>	MFLUCC 12-0258	JX399069	JX399036	JX399005
<i>P. scoparia</i>	CBS 176.25	KM199478	KM199393	KM199330
<i>P. sequoiae</i>	MFLUCC 13-0399	NA	NA	NR_153271
<i>P. shandogensis</i>	JZB340038	MN626740	MN626729	MN625275
<i>P. shorea</i>	MFLUCC 12-0314	KJ503817	KJ503814	KJ503811
<i>P. smilacicola</i>	<u>MFLUCC 22-0124</u>	<u>OP737879</u>	<u>OP762674</u>	<u>OP497989</u>
<i>P. smilacicola</i>	<u>MFLUCC 22-0125</u>	<u>OP753376</u>	<u>OP762673</u>	<u>OP497991</u>
<i>P. spathulata</i>	CBS 356.86	KM199513	KM199423	KM199338
<i>P. spathuliappendiculata</i>	CBS 144035	MH554607	MH554845	MH554172
<i>P. suae</i>	CGMCC 3.23546	OP185514	OP185521	OP082428
<i>P. telopeae</i>	CBS 114161	KM199500	KM199403	KM199296
<i>P. telopeae</i>	CBS 114137	KM199559	KM199469	KM199301
<i>P. thailandica</i>	MFLUCC 17-1616	MK764329	MK764351	MK764285
<i>P. thailandica</i>	MFLUCC 17-1617	MK764329	MK764351	MK764285
<i>P. trachycarpicola</i>	IFRDCC 2240	JQ845946	JQ845945	NR_120109
<i>P. unicolor</i>	MFLUCC 12-0276	NA	JX399030	JX398999
<i>P. verruculosa</i>	MFLUCC 12-0274	JX399061	NA	JX398996
<i>P. yanglingensis</i>	LC4553	KX895231	KX895345	KX895012
<i>Pestalotiopsis</i> sp.	LC3637	KX895210	KX895324	KX894993
<i>Pseudopestalotiopsis ampullacea</i>	LC6618	KX895244	KX895358	KX895025
<i>Ps. annellata</i>	NTUCC 17-030	MT321988	MT321889	MT322087
<i>Ps. avicenniae</i>	MFLUCC 17-0434	MK764331	MK764353	MK764287
<i>Ps. camelliae-sinesis</i>	LC3490	KX895202	KX895316	KX894985
<i>Ps. chinensis</i>	LC3011	KX895154	KX895269	KX894937
<i>Ps. curvatispora</i>	MFLUCC 17-1722	MK764332	MK764354	MK764288
<i>Ps. cocos</i>	CBS 272.29	KM199553	KM199467	KM199378
<i>Ps. dawaina</i>	MM14 F0015	LC324752	LC324751	LC324750
<i>Ps. gilvanii</i>	INPA 2913	MN385957	MN385954	MN385951
<i>Ps. gilvanii</i>	INPA 2914	MN385958	MN385955	MN385952
<i>Ps. ignota</i>	NN 42909	KU500016	NA	KU500020
<i>Ps. indica</i>	CBS 459.78	KM199560	KM199470	KM199381
<i>Ps. ixorae</i>	NTUCC 17-001.1	MG816336	MG816326	MG816316
<i>Ps. kawthaungina</i>	MM14 F0083	LC324755	LC324754	LC324753
<i>Ps. kubahensis</i>	UMAS-KUB-P20	NA	NA	KT006749
<i>Ps. myanmarina</i>	NBRC 112264	LC114065	LC114045	LC114025
<i>Ps. rhizophorae</i>	MFLUCC 17-1560	MK764335	MK764357	MK764291
<i>Ps. simitheae</i>	MFLUCC 12-0121	KJ503818	KJ503815	KJ503812
<i>Ps. solicola</i>	CBS 386.97	MH554474	MH554715	NR_161086
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	MG816339	MG816329	MG816319
<i>Ps. thailandica</i>	MFLUCC 17-1724	MK764336	MK764358	MK764292
<i>Ps. theae</i>	MFLUCC 12-0055	JQ683743	JQ683711	JQ683727
<i>Ps. theae</i>	NTUCC 18-067	MT321987	MT321888	MT322086
<i>Ps. theae</i>	<u>MFLUCC 22-0128</u>	<u>OP753377</u>	<u>OP752136</u>	<u>OP497993</u>
<i>Ps. vietnamensis</i>	NBRC 112252	LC114074	LC114054	LC114034

^aEx-type strains are in bold, and the newly generated strains are indicated with underlining.^bNA, not available.

TABLE 4 The best-fit evolutionary models used in our phylogenetic analyses

Data set	Method	Model		
		<i>tef1-α</i>	<i>tub2</i>	<i>ITS</i>
<i>Neopestalotiopsis</i>	ML	HKY+F+G4	TNe+I+G4	TIM2+F+I+G4
	BYPP	GTR+I+G	GTR+I+G	GTR+I+G
<i>Pestalotiopsis</i>	ML	TN+F+I+G4	K2P+I+G4	TPM3u+F+I+G4
	BYPP	GTR+I+G	GTR+I+G	GTR+I+G
<i>Pseudopestalotiopsis</i>	ML	TIM+F+I+G4	GTR+F+I+G4	TIM2+I+G4
	BYPP	GTR+I+G	GTR+I+G	GTR+I+G

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We declare no conflicts of interest.

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