New geographical records of *Neopestalotiopsis* and *Pestalotiopsis* species in Guangdong Province, China

Senanayake IC¹-²,³, Lian TT¹, Mai XM¹, Jeewon R⁴, Maharachchikumbura SSN⁵, Hyde KD³, Zeng YJ², Tian SL¹, Xie N¹*

¹Guangdong Provincial Key Laboratory for Plant Epigenetics, College of Life Science and Oceanography, Shenzhen University, 3688, Nanhai Avenue, Nanshan, Shenzhen 518055, China
²Shenzhen Key Laboratory of Laser Engineering, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China
³Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
⁴Department of Health Sciences, Faculty of Science, University of Mauritius, Reduit, 80837, Mauritius
⁵School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 611731, China

Senanayake IC, Lian TT, Mai XM, Jeewon R, Maharachchikumbura SSN, Hyde KD, Zeng YJ, Tian SL, Xie N 2020 – New geographical records of *Neopestalotiopsis* and *Pestalotiopsis* species in Guangdong Province, China. Asian Journal of Mycology 3(1), 510–530, Doi 10.5943/ajom/3/1/19

Abstract

A study of monocotyledon inhabiting fungi in Guangdong Province, China resulted in the collection of several pestaloid taxa. Evidence from multi-locus phylogenies using ITS, BT and tef 1–α, together with morphology revealed *Neopestalotiopsis alpapicalis*, *Pestalotiopsis diplocisiae* and *P. parva* from living leaves of *Phoenix roebelenii*. *Pestalotiopsis parva* was also found on a dead petiole of *Phoenix* sp. and *P. diplocisiae* on dead leaves of *Butia* sp. *Pestalotiopsis foedans*, *P. lawsoniae*, *P. macadamia* and *P. virgatula* have been reported in Guangdong Province, and *Pestalotiopsis parva* and *P. diplocisiae* reported for the first time. This *Neopestalotiopsis alpapicalis* collection is the first species of the genus collected from this province. We provide descriptions and illustrations for these three isolates. Additionally, we provide a list of *Pestalotiopsis* and *Neopestalotiopsis* species recorded from China.

Key words – Appendage bearing conidia – Coelomycetes – Monocotyledons – Saprobes – Sporocadaceae

Introduction

*Pestalotiopsis* Steyaert was introduced to accommodate pestaloid species with 5-celled conidia (Steyaert 1949). Maharachchikumbura et al. (2014) re-examined *Pestalotiopsis* at the morphological and molecular levels and introduced two new genera, *Neopestalotiopsis* and *Pseudopestalotiopsis*. Currently, these three genera placed in Sporocadaceae (Amphisphaeriales) (Wijayawardene et al. 2018, Hyde et al. 2020). *Neopestalotiopsis* typified by *N. protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, is morphologically distinguished from other pestaloid genera by its variicolored median cells and indistinct conidiophores which are often reduced to conidiogenous cells. *Pestalotiopsis* typified by *P. guepinii* (Desm.) Steyaert and is easily
distinguished from other pestaloid genera as its conidia have concolourous median cells (Maharachchikumbura et al. 2014).

Species in both *Pestalotiopsis* and *Neopestalotiopsis* commonly occur as endophytes in leaves (Hu et al. 2007, Liu et al. 2010, Maharachchikumbura et al. 2012a, Debbab et al. 2013, Chen et al. 2018, Norphanphou et al. 2019), saprobes on dead leaves (Ariyawansa & Hyde 2018, Tsai et al. 2018), bark and twigs (Ellis & Ellis 1997) or human and animal pathogens (Monden et al. 2013). Some species found from soil, fabrics, wools and some are in the extreme environments (Guba 1961, Strobel et al. 1996, Tejesvi et al. 2007). Some *Pestalotiopsis* species can degrade plastics (Russell et al. 2011). Pestaloid endophytes produce chemical compounds, which use in therapeutic applications and agriculture (Aly et al. 2010, Xu et al. 2010, 2014). Therefore, investigation of novel pestaloid taxa and their chemical properties are of importance.

In this study, we collected three pestaloid taxa from Shenzhen, Guangdong Province, China, and their identifications, and phylogenetic relationships are investigated based on morphology and DNA sequence data of the internal transcribed spacer (ITS), β-tubulin (BT) and partial translation elongation factor 1-α gene (tef 1–α). Additionally, a list of *Pestalotiopsis* and *Neopestalotiopsis* species recorded from China is provided.

**Materials & Methods**

Sample collection and fungal isolation

Samples were collected in a survey of monocotyledon inhabiting fungi during 2018–2019 in Guangdong Province, China. The samples were brought to the laboratory in paper bags. They were examined and photographed using a Carl Zeiss Discovery V8 stereomicroscope fitted with Axiocam. The morphological characters were photographed using a Nikon Eclipse 80i compound microscope fitted with a Canon 450D digital camera. All microscopic measurements were made with Tarosoft image framework (v. 0.9.0.7). Colony characters were recorded from cultures grown on potato dextrose agar (PDA).

Single conidia isolation was carried out following the method described by Senanayake et al. (2018). Germinated conidia were aseptically transferred into fresh PDA plates, and incubated at 16°C to obtain pure cultures. Cultures were later transferred to PDA slants and stored at 4°C for further studies. All the voucher specimens are deposited in the fungaria of Mae Fah Luang University (MFLU), and living cultures are deposited at the Culture Collection of Kunming Institute of Botany (KUMCC).

DNA extraction, PCR amplification and DNA sequencing

Fungal mycelium grown on PDA for two weeks at 16°C in the dark and fruit bodies directly picked from the specimens were used for DNA extraction using M5 fungal Genomic DNA extraction kit. PCR reactions were carried out using ITS1/ITS4 for internal transcribed spacer nrDNA (ITS) (White et al. 1990), BT2a/BT2b for β-tubulin (BT) (Glass & Donaldson 1995), and EF1-728F/EF2 for translation elongation factor 1-α (tef 1–α) (Rehner 2001, Liu et al. 2017) genes according to the same protocol of Maharachchikumbura et al. (2014).

The amplification reactions were carried out with the following protocol: 25 μL reaction volume containing 1 μL of DNA template, 1 μL of each forward and reverse primers, 12.5 μL of 2×PCR Master Mix and 9.5 μL of double-distilled sterilized water (ddH2O). The PCR products were observed on 1% agarose electrophoresis gel stained with ethidium bromide. Purification and sequencing of PCR products were carried out at the Sunbiotech Company, Beijing, China. Sequence quality was checked and sequences were concatenated with DNASTAR Lasergene v.7.1. Sequences derived in this study were deposited in the GenBank, and accession numbers were obtained (Table 1).

Sequence alignment and phylogenetic analyses

BLASTn searches were made using the newly generated sequences to assist taxon sampling.
for phylogenetic analyses. All sequences obtained from GenBank and used by Maharachchikumbura et al. (2014, 2016), Liu et al. (2017), Nozawa et al. (2017), Ariyawansa & Hyde (2018), Chen et al. (2018), Tibpromma et al. (2018), Tsai et al. (2018), Watanabe et al. (2018), Norphanphoun et al. (2019), are listed in Table 1. DNA sequence data of the ITS, BT and tef 1–α sequence alignments were done using default settings of MAFFT v.7 (Katoh et al. 2017) and manually adjusted using BioEdit 7.1.3 (Hall 1999) to allow maximum alignment and minimum gaps. The evolutionary models for phylogenetic analyses were determined by MrModeltest v. 2.3 under the Akaike Information Criterion (AIC) was implemented in PAUP v. 4.0b10 (Nylander 2004).

Maximum likelihood analysis was performed by RAxML (Stamatakis & Alachiotis 2010) implemented in raxmlGUIv.1.3 (Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping, and the analysis carried out using the GTRGAMMAI model of nucleotide substitution with 1,000 replicates.

For the Bayesian inference (BI) analyses of the individual loci and concatenated ITS, BT and tef 1–α alignment, the above-mentioned model test was used to determine the best fitting nucleotide substitution model settings for MrBayes v. 3.0b4. Dirichlet base frequencies and the GTR+I+G model with inverse gamma-distributed rate were predicted by the MrModeltest analysis for all three data partitions and used in the Bayesian analysis.

The Markov Chain Monte Carlo sampling (MCMC) resulted in MrBayes v. 3.0b4 (Huelsenbeck et al. 2001) was used to calculate Posterior probability values (Zhaxybayeva & Gogarten 2002). Four simultaneous Markov chains were initially run for 10,000,000 generations, and every 500th generation was sampled. The distribution of log-likelihood scores was observed to check whether sampling is in stationary phase or not and Tracer v1.5 was used to check if further runs were required to reach convergence or not (Rambaut & Drummond 2007).

The Bayesian analysis lasted 10,000,000 generations (average standard deviation of split frequencies value = 0.0098), and the consensus tree and posterior probabilities were calculated after discarding the first 20% of sampled trees as burn-in. The remaining trees were used for calculating posterior probabilities in the majority rule consensus tree. The bootstrap values equal to or greater than 0.9 are given below or above each node (Figs 1, 2). The phylogram was visualized in FigTree v. 1.2.2 (Rambaut & Drummond 2008).

Table 1 Details of the isolates used in the phylogenetic tree. Newly generated sequences are bold.

| Taxon                                      | Culture accession number | Genbank number  |
|--------------------------------------------|--------------------------|-----------------|
| Neopestalotiopsis acrostichi               | MFLUCC 17-1754           |                 |
| Neopestalotiopsis alpapicalis              | MFLUCC 17-2544           |                 |
| Neopestalotiopsis alpapicalis              | KUMCC 20-0036            |                 |
| Neopestalotiopsis alpapicalis              | KUMCC 20-0037            |                 |
| Neopestalotiopsis aotearea                 | CBS 367.54               |                 |
| Neopestalotiopsis asiatica                | MFLUCC 12-0286           |                 |
| Neopestalotiopsis australis                | CBS 114159               |                 |
| Neopestalotiopsis brachiata               | MFLUCC 17-1555           |                 |
| Neopestalotiopsis brasiensis              | PA10                     |                 |
| Neopestalotiopsis chiangmaiensis           | MFLUCC 18-0113           |                 |
| Neopestalotiopsis chrysea                 | MFLUCC 12-0261           |                 |
| Neopestalotiopsis clavispora              | MFLUCC 12-0281           |                 |
| Neopestalotiopsis cocoes                   | MFLU 15-0220             |                 |
| Neopestalotiopsis coffea-arabicae          | HGUP 4015                |                 |
| Neopestalotiopsis cubana                   | CBS 600.96               |                 |
| Neopestalotiopsis egyptiaca               | PEST1                    |                 |
| Neopestalotiopsis ellipsospora             | MFLUCC 12-0283           |                 |
| Neopestalotiopsis eucalyptica             | CBS 264.37               |                 |
| Neopestalotiopsis foedans                  | CGMCC 3.9123             |                 |
| Neopestalotiopsis formicarum               | CBS 362.72               |                 |

    | ITS         | β-tubulin  | tef 1–α       |
|-------------|------------|--------------|
| MFLUCC 17-1754 | MK764272 | MK764338     | MK764316 |
| MFLUCC 17-2544 | MK357772 | MK463545     | MK463547 |
| KUMCC 20-0036 | MT222276 | MT135199     | MT175375 |
| KUMCC 20-0037 | MT222277 | MT135200     | MT175376 |
| CBS 367.54   | KM199369  | KM199454     | KM199526 |
| MFLUCC 12-0286 | JX399893 | JX399018     | JX399049 |
| CBS 114159   | KM199348  | KM199432     | KM199537 |
| MFLUCC 17-1555 | MK764274 | MK764340     | MK764340 |
| PA10         | N/A       | MK286948     | MK253112 |
| MFLUCC 18-0113 | N/A     | MH412725     | MH388404 |
| MFLUCC 12-0261 | JX399896 | JX399021     | JX399052 |
| MFLUCC 12-0281 | MN121843 | MN121844     | MN121845 |
| MFLU 15-0220  | NR-156312 | N/A          | N/A       |
| HGUP 4015    | KF412647  | KF412641     | KF412644 |
| CBS 600.96   | KM199347  | KM199438     | KM199521 |
| PEST1        | KP943747  | KP943746     | KP943748 |
| MFLUCC 12-0283 | JX399891 | JX399015     | JX399046 |
| CBS 264.37   | KM199376  | KM199431     | KM199551 |
| CGMCC 3.9123 | JX399876  | JX399022     | JX399053 |
| CBS 362.72   | KM199358  | KM199455     | KM199517 |
| Taxon                                           | Culture accession number | ITS          | β-tubulin       | tef 1-α     |
|------------------------------------------------|--------------------------|--------------|-----------------|-------------|
| Neopestalotiopsis honoluliana                   | CBS 111535               | N/A          | KM199461        | KM199546    |
| Neopestalotiopsis honoluliana                   | CBS 114495               | KM199364     | KM199457        | KM199548    |
| Neopestalotiopsis iraniensis                    | P815                     | N/A          | N/A             | N/A         |
| Neopestalotiopsis javaensis                     | CBS 257.31               | KM199357     | KM199457        | KM199548    |
| Neopestalotiopsis keteleeria                    | MFLUCC 13-0915           | KJ503820     | KJ503821        | KJ503822    |
| Neopestalotiopsis macademiae                    | BRIP 63737c              | NR-161002    | XK186654        | XK186627    |
| Neopestalotiopsis magna                         | MFLUCC 12-0055           | KF582795     | KF582793        | KF582791    |
| Neopestalotiopsis mesopotamica                  | CBS 464.69               | KM199353     | KM199436        | N/A         |
| Neopestalotiopsis musae                         | MFLUCC 15-0776           | NR-156311    | KX789686        | KX789685    |
| Neopestalotiopsis natalensis                    | CBS 138.41               | NR-15628     | KM199466        | KM199552    |
| Neopestalotiopsis pandanicola                   | KUMCC 17-0175            | N/A          | MH412720        | MH388389    |
| Neopestalotiopsis pernambucana                  | RV01                     | KJ792466     | N/A             | N/A         |
| Neopestalotiopsis petila                        | MFLUCC 17-1737           | MK764276     | MK764342        | MK764320    |
| Neopestalotiopsis phangngaensis                 | MFLUCC 18-0119           | MH388354     | MH412721        | MH388390    |
| Neopestalotiopsis piceana                       | CBS 225.30               | KM199371     | KM199451        | KM199535    |
| Neopestalotiopsis piceana                       | CBS 394.48               | KM199368     | KM199453        | KM199527    |
| Neopestalotiopsis protearum                     | CBS 114178               | JN712498     | KM199463        | KM199542    |
| Neopestalotiopsis rhizophorae                   | MFLUCC 17-1550           | MK764277     | MK764343        | MK764321    |
| Neopestalotiopsis rosae                        | CBS 124745               | KM199360     | KM199430        | KM199524    |
| Neopestalotiopsis rosicola                      | CFC 51992               | KY885239     | KY885245        | KY885243    |
| Neopestalotiopsis samarangensis                 | CBS 115451               | KM199365     | KM199447        | KM199556    |
| Neopestalotiopsis saprophytica                  | CBS 115452               | KM199345     | KM199433        | KM199538    |
| Neopestalotiopsis saprophytica                  | MFLUCC 12-0282           | JX398982     | JX399017        | JX399048    |
| Neopestalotiopsis sonneratae                    | MFLUCC 17-1744           | MK764280     | MK764346        | MK764324    |
| Neopestalotiopsis sp.                           | CBS 266.37               | KM199349     | KM199459        | KM199547    |
| Neopestalotiopsis sp.                           | CBS 323.76               | KM199350     | KM199458        | KM199550    |
| Neopestalotiopsis sp.                           | FMB 0127                 | N/A          | MH460876        | MH523647    |
| Neopestalotiopsis sp.                           | FMB 0128                 | N/A          | MH460875        | MH523646    |
| Neopestalotiopsis sp.                           | CBS 119.75               | KM199356     | KM199439        | KM199531    |
| Neopestalotiopsis sp.                           | LC3318                   | KX894964     | KX895296        | KX895181    |
| Neopestalotiopsis sp.                           | LC6285                   | KX895013     | KX895346        | KX895232    |
| Neopestalotiopsis sp.                           | LC6471                   | KX895019     | KX895352        | KX895238    |
| Neopestalotiopsis sp.                           | LPS61                    | MF379331     | N/A             | N/A         |
| Neopestalotiopsis sp.                           | SC2A3                    | KU252210     | KU252477        | KU252390    |
| Neopestalotiopsis sp.                           | SC2A4                    | KX146639     | KX146757        | KX146698    |
| Neopestalotiopsis sp.                           | SC3A3                    | KU252211     | KU252478        | KU252391    |
| Neopestalotiopsis sp.                           | SC5A9                    | KU252212     | KU252479        | KU252392    |
| Neopestalotiopsis sp.                           | YN1A5                    | KU252216     | KU252483        | KU252396    |
| Neopestalotiopsis sp.                           | ZJ1A2                    | KU252215     | KU252482        | KU252395    |
| Neopestalotiopsis sp.                           | CBS 274.29               | KM199375     | KM199448        | KM199534    |
| Neopestalotiopsis sp.                           | CBS 322.76               | KM199366     | KM199446        | KM199536    |
| Neopestalotiopsis sp.                           | CBS 360.61               | KM199346     | KM199440        | KM199522    |
| Neopestalotiopsis sp.                           | CBS 110.20               | KM199342     | KM199442        | KM199540    |
| Neopestalotiopsis sp.                           | CBS 164.42               | KM199367     | KM199434        | KM199520    |
| Neopestalotiopsis sp.                           | URM7148                  | N/A          | N/A             | KU306740    |
| Neopestalotiopsis steyaertii                    | IMI 192475               | KF582796     | KF582794        | KF582792    |
| Neopestalotiopsis surinamensis                  | CBS 111494               | KX894962     | KM199462        | KM199530    |
| Neopestalotiopsis surinamensis                  | CBS 450.74               | KM199351     | KM199465        | KM199518    |
| Neopestalotiopsis thailandica                   | MFLUCC 17-1730           | MK764281     | MK764347        | MK764325    |
| Neopestalotiopsis umbrinospora                   | MFLUCC 12-0285           | JX398984     | JX399019        | JX399050    |
| Neopestalotiopsis vitis                         | JZB340018                | KU140694     | KU140685        | KU140676    |
| Neopestalotiopsis zimbawana                     | CBS 111495               | JX556231     | KM199456        | KM199545    |
| Pestalotiopsis adusta                           | ICMP 6088                | JX399006     | JX399037        | JX399070    |
| Pestalotiopsis adusta                           | MFLUCC 10-0146           | JX399007     | JX399038        | JX399071    |
| Pestalotiopsis aggestorum                       | LC6301                   | KX895015     | KX895348        | KX895234    |
| Taxon                              | Culture accession number | Genbank number |
|-----------------------------------|--------------------------|----------------|
|                                   |                          | ITS           | β-tubulin   | tef 1–α      |
| Pestalotiopsis aggestorum         | LC8186                   | KY464140      | KY464160    | KY464150     |
| Pestalotiopsis anocardiaceanum    | IFRDCC 2397              | KC247154      | KC247155    | KC247156     |
| Pestalotiopsis arceuthobii        | CBS 434.65               | KM199341      | KM199427    | KM199516     |
| Pestalotiopsis arengae            | CBS 331.92               | KM199340      | KM199426    | KM199515     |
| Pestalotiopsis australasia        | CBS 114126               | KM199297      | KM199409    | KM199499     |
| Pestalotiopsis australasia        | CBS 114141               | KM199298      | KM199410    | KM199501     |
| Pestalotiopsis australis          | CBS 114193               | KM199334      | KM199385    | KM199477     |
| Pestalotiopsis australis          | CBS 119350               | KM199333      | KM199384    | KM199476     |
| Pestalotiopsis biciliata          | CBS 124463               | KM199308      | KM199399    | KM199505     |
| Pestalotiopsis biciliata          | CBS 790.68               | MH859228      | KM199400    | KM199507     |
| Pestalotiopsis brachiata          | LC2988                   | KY464142      | KY464162    | KY464152     |
| Pestalotiopsis brassicae          | CBS 170.26               | KM199379      | N/A         | KM199558     |
| Pestalotiopsis camelliae          | CBS 443.62               | KM199336      | KM199424    | KM199512     |
| Pestalotiopsis camelliae          | MFLUCC 12-0277           | KY319138      | KY363542    | KY432666     |
| Pestalotiopsis chamaeropsis       | CBS 113604               | KM199323      | KM199389    | KM199471     |
| Pestalotiopsis chamaeropsis       | CBS 186.71               | KM199325      | KM199390    | KM199472     |
| Pestalotiopsis chinensis          | LC3013                   | KX894939      | KX895271    | KX895156     |
| Pestalotiopsis clavata            | MFLUCC 12-0268           | JX398990      | JX399025    | JX399056     |
| Pestalotiopsis colombiensis       | CBS 118553               | KM199307      | KM199421    | KM199488     |
| Pestalotiopsis digitalis          | ICMP 5434                | KX895271      | KX895400    | KX895469     |
| Pestalotiopsis diplolociasiae     | CBS 115587               | KM199314      | KM199416    | KM199485     |
| *Pestalotiopsis* diploclisiae     | KUMCC 20–0035            | MT222272      | N/A         | MT175371     |
| Pestalotiopsis distincta          | LC3232                   | KX894961      | KX895293    | KX895178     |
| Pestalotiopsis diversiseta        | MFLUCC 12-0287           | NR_120187     | JX399040    | JX399073     |
| Pestalotiopsis dracontomelon      | MFLUCC 10-0149           | KP781877      | N/A         | KP781880     |
| Pestalotiopsis ericacearum        | OP023                    | KC537807      | KC537821    | KC537814     |
| Pestalotiopsis formosana          | NTUCC 17-0010            | MH809382      | MH809386    | MH809390     |
| Pestalotiopsis formosana          | NTUCC 17-0009            | MH809381      | MH809385    | MH809389     |
| Pestalotiopsis furarea            | ML4DY                    | EF055197      | EF055234    | N/A         |
| Pestalotiopsis furcata            | MFLUCC 12-0054           | JQ683724      | JQ683708    | JQ683740     |
| Pestalotiopsis gauthierina        | IFRD 411-014             | KC537805      | KC537819    | KC537812     |
| Pestalotiopsis gibbosa             | Pes6                     | LC311589      | LC311590    | LC311591     |
| Pestalotiopsis grevilleae         | CBS 114127               | KM199300      | KM199407    | KM199504     |
| Pestalotiopsis hawaiiensis        | CBS 114491               | KM199339      | KM199428    | KM199514     |
| Pestalotiopsis hollandica          | CBS 265.33               | KM199328      | KM199388    | KM199481     |
| Pestalotiopsis humus              | CBS 115450               | KM199319      | KM199418    | KM199487     |
| Pestalotiopsis humus              | CBS 336.97               | KM199317      | KM199420    | KM199484     |
| Pestalotiopsis inflexa            | MFLUCC 12-0270           | JX399008      | JX399039    | JX399072     |
| Pestalotiopsis intermedia         | MFLUCC 12-0259           | JX398993      | JX399028    | JX399059     |
| Pestalotiopsis italiana           | MFLUCC 12-0657           | KP781878      | KP781882    | KP781881     |
| Pestalotiopsis jesteri            | CBS 109350               | KM199380      | KM199468    | KM199554     |
| Pestalotiopsis jiangxiensis       | LC4399                   | KX895009      | KX895341    | KX895227     |
| Pestalotiopsis jinchanghensis     | LC6636                   | KX895028      | KX895361    | KX895247     |
| Pestalotiopsis jinchanghensis     | LC8190                   | KX895125      | KX895168    | KX895245     |
| Pestalotiopsis kenyana            | CBS 442.67               | KM199302      | KM199395    | KM199502     |
| Pestalotiopsis kenyana            | CBS 911.96               | KM199303      | KM199396    | KM199503     |
| Pestalotiopsis knightiae          | CBS 11963                | KM199311      | KM199406    | KM199495     |
| Pestalotiopsis knightiae          | CBS 114138               | KM199310      | KM199408    | KM199497     |
| Pestalotiopsis licuralcola        | HGUP 4057                | KC492509      | KC481683    | KC481684     |
| Pestalotiopsis linearis           | MFLUCC 12-0271           | JX398992      | JX399027    | JX399058     |
| Pestalotiopsis longiappendiculata | LC3013                   | KX894939      | KX895271    | KX895156     |
| Pestalotiopsis lusunensis         | LC4344                   | KX895005      | KX895337    | KX895223     |
| Pestalotiopsis lusunensis         | LC8182                   | KY461436      | KY461456    | KY461446     |
| Pestalotiopsis macadamiae         | BRIP 63738b              | KX186588      | KX186680    | KX186621     |
| Taxon                        | Culture accession number | Genbank number | ITS       | β-tubulin | tef 1–α |
|-----------------------------|--------------------------|----------------|-----------|-----------|---------|
| Pestalotiopsis malayana     | CBS 102220               |                | KM199306 | KM199411 | KM199482 |
| Pestalotiopsis microsora    | UMAS P15                 |                | KT337388 | N/A       | N/A     |
| Pestalotiopsis monochaeta   | CBS 144.97               |                | KM199327 | KM199386 | KM199479 |
| Pestalotiopsis monochaeta   | CBS 440.83               |                | KM199329 | KM199387 | KM199480 |
| Pestalotiopsis montellicia  | MFLUCC 12-0279           |                | JX399012 | JX399043 | JX399076 |
| Pestalotiopsis neglecta     | 1100                     |                | AB482220 | LC311599  | LC311600 |
| Pestalotiopsis neolitseae   | NTUCC 17-0111            |                | MH809383 | MH809387 | MH809391 |
| Pestalotiopsis novae-hollandiae | CBS 130973            |                | KM199337 | KM199425 | KM199511 |
| Pestalotiopsis oryzae       | CBS 111522               |                | KM199294 | KM199394 | KM199493 |
| Pestalotiopsis oryzae       | CBS 171.26               |                | MH854881 | KM199397 | KM199494 |
| Pestalotiopsis paenicola    | TR40                     |                | N/A       | KY930635 | N/A     |
| Pestalotiopsis papuana      | CBS 331.96               |                | KM199321 | KM199413 | KM199491 |
| Pestalotiopsis papuana      | CBS 887.96               |                | KM199318 | KM199415 | KM199492 |
| Pestalotiopsis parva        | KUMCC 20-0038            | MT122274       | MT135197 | MT175373  |
| Pestalotiopsis parva        | MFLU 20-0060             | MT122275       | MT135198 | MT175374  |
| Pestalotiopsis parva        | CBS 265.37               | KM199312       | KM199404 | KM199508  |
| Pestalotiopsis parva        | CBS 278.35               | KM199313       | KM199405 | KM199509  |
| Pestalotiopsis parva        | CBS 393.48               | KM199335       | KM199422 | KM199510  |
| Pestalotiopsis rhizophorae  | MFLUCC 17-0417           | MK76428     | MK764350 | MK764328  |
| Pestalotiopsis rhizosporae  | CBS 144424              | MH554109      | MH554782 | MH554543  |
| Pestalotiopsis rhododendri  | OP086                   | KC537804      | KC537818 | KC537811  |
| Pestalotiopsis rosea        | MFLUCC 12-0258           | JX399005      | JX399036 | JX399069  |
| Pestalotiopsis scoparia     | CBS 176.25              | KM199330       | KM199393 | KM199478  |
| Pestalotiopsis shorea       | MFLUCC 12-0314           | KI503811      | KI503814 | KI503817  |
| Pestalotiopsis sp.          | UMAS 1705               | KT337373      | N/A       | N/A       |
| Pestalotiopsis sp.          | CBS 263.33               | KM199316       | KM199414 | KM199489  |
| Pestalotiopsis sp.          | CBS 264.33               | KM199322       | KM199412 | KM199490  |
| Pestalotiopsis sp.          | HGUP 4057               | KC492509      | KC481683 | KC481684  |
| Pestalotiopsis spathulata   | CBS 356.86               | KM199338       | KM199423 | KM199513  |
| Pestalotiopsis telepaeae    | CBS 113606               | KM199295       | KM199402 | KM199498  |
| Pestalotiopsis telepaeae    | CBS 114161               | KM199301       | KM199469 | KM199559  |
| Pestalotiopsis thailandica  | MFLUCC 17-1616           | MK764285      | MK764351 | MK764329  |
| Pestalotiopsis theae        | CMU-ELA1                | JX205216       | N/A       | N/A       |
| Pestalotiopsis theae        | CPO Pe                  | JQ619652       | N/A       | N/A       |
| Pestalotiopsis trachicarpica| CBS 111507              | MH553960      | MH554619 | MH554378  |
| Pestalotiopsis trachicarpica| HGUP 56.2               | N/A           | MK360941 | MK512494  |
| Pestalotiopsis unicolor      | MFLUCC 12-0275           | JX398998       | JX399029 | MK512494  |
| Pestalotiopsis unicolor      | MFLUCC 12-0276           | JX398999       | JX399030 | N/A       |
| Pestalotiopsis verruculosa  | MFLUCC 12-0274           | JX398996       | N/A       | JX399061  |
| Pestalotiopsis yanglingensis| LC3067                  | KX894949       | KX895281 | KX895166  |
| Pestalotiopsis yanglingensis| LC4553                  | KX895012       | KX895345 | KX895231  |

Abbreviations: BRIP: The Plant Pathology Herbarium, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC: Chinese Forestry Culture Collection Center, Chinese Academy of Sciences, Beijing, China; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; HGUP: The Plant Pathology Herbarium of Guizhou University, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IFRDC: International Fungal Research & Development Centre Culture Collection, China; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; KUMCC: Culture Collection of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China; MFLU: Mae Fah Luang University Herbarium, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NTUCC: National Taiwan University culture collection, Taiwan; UMAS: Department of Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, University Malaysia Sarawak
Results

Phylogenetic inferences

The first combined BT, ITS and tef 1–α sequence dataset comprised 102 strains of Pestalotiopsis, and Neopestalotiopsis sp. (CBS 119.75) was the outgroup taxon. The second combined BT, ITS and tef 1–α sequence data set comprised 72 sequences of Neopestalotiopsis with Pestalotiopsis parva (CBS 265.37) as the outgroup taxon. Both concatenated data matrixes comprised 1527 characters (ITS: 566, BT: 469 and tef 1–α: 490). All individual trees generated under different criteria. Single-gene datasets were essentially similar in topology and not significantly different from the tree generated from the concatenated dataset (not discussed herein).

Maximum likelihood analysis for Pestalotiopsis with 1,000 bootstrap replicates yielded a tree with the likelihood value of ln: –13138.225580 and the following model parameters: alpha: 0.550988; Π(A): 0.239550, Π(C): 0.287070, Π(G): 0.215467 and Π(T): 0.257914. Maximum likelihood analysis for Neopestalotiopsis with 1,000 bootstrap replicates yielded a tree with the likelihood value of ln –6466.001103 and the following model parameters: alpha: 0.731256; Π(A): 0.234344, Π(C): 0.266539, Π(G): 0.216132 and Π(T): 0.282985. The ML analyses also resulted in similar tree topologies to those obtained in the Bayesian analyses. The best scoring RAxML trees derived from the analyses of the concatenated datasets for Pestalotiopsis (ingroup) and Neopestalotiopsis (ingroup) are shown in Figs 1, 2, respectively. Maximum likelihood bootstrap values ≥50% and Bayesian inference (BI) ≥0.9 are given at each node.

In our concatenated ML analyses, one Pestalotiopsis isolate (KUMCC 20–0035) form a distinct subclade with P. diplocilisiae (CBS 115449) with high statistical support (Fig. 1). In addition, the other two isolates of Pestalotiopsis (KUMCC 20–0038 and MFLU 20–0060) form a separate, high statistical supported lineage with Pestalotiopsis parva (CBS 278.35 and CBS 265.37). Hence, Pestalotiopsis strains of KUMCC 20–0038/MFLU 20–0060 is confirmed as Pestalotiopsis parva (Fig. 1). In the second dataset, two strains of Neopestalotiopsis form a distinct subclade with Neopestalotiopsis alpapicalis and N. rhizophorae with high statistical support. Hence, this collection is proposed here as Neopestalotiopsis alpapicalis (Fig. 2).

Taxonomy

Pestalotiopsis diplocilisiae Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 160 (2014)

Facesoffungi number: FoF 06982

Saprobic, associated with dead leaves of Butia sp. Sexual morph: Undetermined. Asexual morph: Conidiomata 500–900 μm diam., pycnidial, globose, blackish brown, immersed on substrate, semi-immersed in PDA, releasing conidia as a black, slimy, globose, glistening mass on culture media. Conidiophores indistinct or reduced to conidiogenous cells. Conidiogenous cells 5–20 × 2–3 μm (x̅ = 11 × 2.8 μm, n = 20), discrete, lageniform, hyaline, smooth-walled, anellidic, proliferating 2–3 times percurrently, collarette present, may not appears as flared. Conidia 18–25 × 5–7 μm (x̅ = 23 × 6 μm, n = 20), fusiform to clavate, straight to slightly curved, wall of one side curved than other side, 4-euseptate; basal cell obconic with a truncate base, hyaline or sometimes greenish brown, thick- and smooth-walled, 2–3.5 μm long (x̅ = 2.9 μm, n = 20); three median cells ± equal, each 4–5 μm long (x̅ = 4 μm), doliform or trapezoid, concolorous, pale brown, septa and periclinal walls darker than rest of the cell, wall smooth; apical cell 3–5 μm long (x̅ = 3.9 μm, n = 20), hyaline, conic to acute with truncate base; apical appendages 8–13 ×0.5–1 μm (x̅ = 11.5 × 0.5 μm, n = 40), 3–4 (mostly 3), tubular, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, rough; single basal appendage, tubular, unbranched, centric, 3–6 μm long (x̅ = 4.9 μm, n = 20).

Culture characteristics – Colonies on PDA reaching 2 cm diam., after 1 week at 18°C, under dark, colonies circular, medium dense, aerial mycelium on surface raised, white from above and reverse; fruiting bodies appears as black slimy bubbles.
Fig. 1 – Phylogram generated from maximum likelihood analysis based on combined ITS, BT and tef 1–α sequence data. Bootstrap support values for ML greater than 50% and Bayesian posterior
probabilities greater than 0.9 are given near nodes respectively. The tree is rooted with \textit{Neopestalotiopsis} sp (CBS 119.75). Ex-type strains are in black bold and the newly generated sequences are indicated in blue bold.

Fig. 1 – Continued.
Fig. 1 – Continued.
**Fig. 2** – Phylogram generated from maximum likelihood analysis based on combined ITS, BT and tef 1–α sequence data. Bootstrap support values for ML greater than 50% and Bayesian posterior probabilities greater than 0.9 are given near nodes respectively. The tree is rooted with *Pestalotiopsis parva* (CBS 265.37). Ex-type strains are in black bold and the newly generated sequences are indicated in blue bold.

**Fig. 3** – *Pestalotiopsis diploclisiae* (MFLU 20–0059). a Conidiomata on substrate. b-c Conidiogenous cells attached to conidia. d Upper surface of culture on PDA. e Lower surface of culture on PDA. f-j Conidia. Scale bars: b-c = 50 μm, f-j = 15 μm.
Material examined – CHINA, Guangdong Province, Shenzhen, Nanshan District, Mountain Yangtai Forest Park, 22°39′21.26″N 113°57′18.53″E, dead leaves of Butia sp. (Arecaceae), 5 September 2018, I. C. Senanayake, SI 66, (MFLU 20–0059; living culture KUMCC 20–0035).

Notes – Phylogenetically, our Pestalotiopsis diploclisiae collection (KUMCC 20–0035) is closely related to P. diploclisiae (CBS 115449) with high bootstrap support. Type strains of Pestalotiopsis diploclisiae were collected from fruits of Diplolisia glaucescens and Psychotria tutcheri in Hong Kong, while our P. diploclisiae strain (KUMCC 20–0035) collected from a dead leaf of Butia sp. in Shenzhen (China) closer to Hong Kong. The prologue provided by Maharachchikumbura et al. (2014) for Pestalotiopsis diploclisiae was based on the morphology derived from cultures. However, our description and illustration are based on the morphology derived from the specimen. Our strain produces smaller (5–11 × 2–3 μm), discrete, lageniform conidiogenous cells and smaller (17–24 × 6–7 μm), fusiform to clavate conidia with conic to acute apical cell and 3–4 apical appendages. Except for the size variation in conidia and conidiogenous cells, our collection of Pestalotiopsis diploclisiae (KUMCC 20–0035) is morphologically identical to its holotype. Comparison of the ITS regions DNA sequence of Pestalotiopsis diploclisiae (KUMCC 20–0035) with P. diploclisiae (CBS 115449) gives 0.94% base pair differences and therefore, our strain assigned as Pestalotiopsis diploclisiae.

Pestalotiopsis parva Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 175 (2014) Fig. 4
Facesoffungi number: FoF 07749

Saprobic, associated with dead petiole of Phoenix sp. Appears as black spots coming out from plant epidermis surface. Sexual morph: Undetermined. Asexual morph: Conidiomata pynidial, globose, immersed in substrate, semi-immersed on PDA, brown, releasing conidia in a black, slimy, globose mass. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 7–10 × 2–3 (x̅ = 8.9 × 2.4 μm, n = 20), discrete, subcylindrical to lageniform, hyaline, smooth, thin-walled, annellidic, proliferating once percurrently. Conidia 17–21 × 6–7 μm, (x̅ = 18.9 × 6.8 μm, n = 20), fusiform to mostly globose, straight, 4-septate; basal cell conic to acute with a truncate base, hyaline or sometimes pale brown, thin and smooth-walled, 2.5–4 μm long (x̅ = 3 μm, n = 20); three median cells ± equal, each 3–5 μm long (x̅ = 4 μm, n = 20), doliiform, pale brown, septa darker than rest of the cell, concolorous, wall rugose; apical cell 3–4.5 μm long (x̅ = 3.5 μm, n = 20), hyaline, subcylindrical to obconic, with 2–3 tubular appendages on apical cell, arising from the apical crest, unbranched, flexuous, 9–18 × 0.6–0.9 μm (x̅ = 13 × 1 μm, n = 20); basal appendage single, tubular, unbranched, centric, 3–4.5 μm long, (x̅ = 3.6 μm).

Culture characters – Colonies on PDA reaching 2.5 cm diam., within 1 week at 20°C, under dark, circular with several layers, medium dense, aerial mycelium clots concentrated along the colony margin, flat, filiform margin, white from above and reverse; fruiting bodies did not appear on cultures.

Material examined – CHINA, Guangdong Province, Shenzhen, Nanshan, Nanhai Avenue, Shenzhen University, dead petiole of Phoenix sp. (Arecaceae), 28 August 2018, I.C. Senanayake, SI 9, (MFLU 20–0060, living culture KUMCC 20–0038).

Notes – One of our Pestalotiopsis strain (KUMCC 20–0038) clusters with the type species of P. parva (CBS 265.37) with moderate bootstrap support in the phylogenetic analysis. Pestalotiopsis parva was introduced based on only two strains as CBS 265.37 and CBS 278.35 (Maharachchikumbura et al. 2014). However, the collected localities of those strains are unknown. Maharachchikumbura et al. (2014) described Pestalotiopsis parva based on the morphology derived from cultures. However, we obtained morphological characters of our Pestalotiopsis strain directly from the specimen, not from culture. Comparison of the ITS sequence of Pestalotiopsis parva KUMCC 20–0038 with P. parva CBS 265.37 and CBS 278.35 revealed that the base pair differences between them are less than 1% (0.88% and 0.88% respectively) which propose our strain as an existing species (Jeewon & Hyde 2016).
Fig. 4 – *Pestalotiopsis parva* (MFLU 20–0060). a Conidiomata on substrate. b Upper surface of culture on PDA. c Lower surface of culture on PDA. d-f Conidiogenous cells attached to conidia. g-j Conidia. Scale bars: d-j = 20 μm.

*Neopestalotiopsis alpapicalis* Vin. Kumar, Gentekaki & K.D. Hyde, in Kumar, Cheewangkoon, Gentekaki, Maharachchikumbura, Brahmange & Hyde, Phytotaxa 393(3): 253 (2019)  
Fig. 5  
Facesoffungi number: FoF 05753  
Saprobic or pathogenic, associated with living leaves of *Phoenix roebelenii*. Sexual morph: Undetermined. Asexual morph: Appears as swollen areas with split barks. *Conidiomata* 25–80 μm diam., pycnidial, globose, black, immersed in substrate, superficial in PDA, releasing conidia as a black, slimy, globose, mass on culture media. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4–6 × 3–4 μm (μ = 5 × 4 μm, n = 20), discrete, annellidic, globose to umbonate, short, hyaline, smooth-walled, simple, wide at the base. *Conidia* 24–28 × 9–11 μm (μ = 26 × 10 μm, n = 20), ellipsoid, straight to slightly curved, 4–(6)-septate; basal cell conic to obconic with a truncate base, hyaline, thin-and smooth-walled, 3–4 μm long (μ = 3.8 μm, n = 20); 3–(5) median cells, each 4.5–7 μm long (μ = 5.5 μm, n = 20), 4–7 μm long (μ = 5.6 μm, n = 20), 4–6 μm long (μ = 4.6 μm, n = 20), doliiform, concolorous, pale brown, septa and pericinial walls darker than rest of the cell, wall rugose; apical cell 3–6 μm long (μ = 4.6 μm, n = 20), long, hyaline, conic
to obtuse with truncate base; apical appendages 7–12 μm long ($\bar{x} = 10 \mu m$, $n = 20$), short, 1–4, more tubular, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous; single basal appendage, tubular, unbranched, rarely branched, centric, 4–6 μm long ($\bar{x} = 4.7 \mu m$, $n = 20$).

Culture characteristics – Colonies on PDA reaching 2 cm diam., within 10 days at 18°C, under dark, circular, medium dense, aerial mycelium clots scattered on PDA, flat, filiform margin, white from above and reverse; black, globose, sporulate on cultures after 4 weeks incubate at 20°C in dark.

Material examined – CHINA, Guangdong Province, Shenzhen, Luohu District, Fairy-lake botanical garden, 22°34′43″N 114°09′55.98″E, living leaves of *Phoenix roebelenii* O’Brien (Arecales), 26 July 2018, I.C. Senanayake, SI 100, (MFLU 20–0061, living culture KUMCC 20–0037); CHINA, Guangdong, Shenzhen, Luohu District, Fairy-lake botanical garden, 22°34′43.10″N 114°09′55.98″E, living leaves of *Musa* sp. (Musaceae), 26 July 2018, I.C. Senanayake, SI 103, (MFLU 20–0058, living culture KUMCC 20–0036).

**Fig. 5** – *Neopestalotiopsis alpapicalis* (MFLU 20–0058). a Conidiomata on substrate. b-d Conidiogenous cells attached to conidia. e-h Conidia from fruit bodies in substrate. i Upper surface of culture on PDA. j Lower surface of culture on PDA. k Conidioma on PDA. l-o Conidia derived from culture (l; unusual basal cell, m; conidia with six cells, o; wall ornamentations). Scale bars: b-h, l-o = 25 μm.

Notes – In the phylogenetic analysis (Fig. 2), our *Neopestalotiopsis* strain forms a distinct subclade basal to *N. alpapicalis* (MFLUCC 17–2544), and *N. rhizophorae* (MFLUCC 17–1550) with moderate bootstrap support. There are 1.27%, 1.07% and 1.02% base pair differences of the ITS (566bp), BT (469bp) and tef 1–α (490bp) sequences of our *Neopestalotiopsis* strains with *N. alpapicalis* (MFLUCC 17–2544), and these values are 1.59%, 0.85%, and 1.02% for *N. rhizophorae* (MFLUCC 17–1550). However, multi-locus gene regions use in this study may not enough to separate *Neopestalotiopsis* species well and there are no more gene regions available in GenBank. Therefore, determination of taxonomy of *Neopestalotiopsis* strains is challenging.

However, morphologically our *Neopestalotiopsis* collection is similar to *N. alpapicalis* more than *N. rhizophorae* in having highly pigmented, conidia with shorter, tubular, apical appendages
which are attached to the tip of apical cell. It is difficult to clarify and compare the morphological characters of fungi grown in different media and different growth conditions. Our Neopestalotiopsis strain is a saprobe collected from a terrestrial, monocotyledon plant in China, while Neopestalotiopsis alpapicalis collected with leaf spots of mangrove plants in Thailand. Therefore, based on available molecular data, morphology and ecological data we named this species as *N. alpapicalis*.

**Discussion**

*Pestalotiopsis parva* and *Neopestalotiopsis alpapicalis* collected from *Phoenix* are mostly dominant in northern and central Africa, Southeastern Europe, Southern Asia and east to Southern China (Chase et al. 2000). Fruits of some *Phoenix* species are edible and used as raw materials in the sugar industry. *Phoenix roebelenii* is widely grown for its ornamental value and its fruit used as food for livestock and poultry (Riffle & Craft 2003). *Phoenix* species have some resistant to pests and tolerance to soil variation and drought. Therefore, *Phoenix* species used for reforestation in swamps, deserts and mangrove coasts. *Pestalotiopsis diploclisiae* collected from dead leaves of *Butia*, which is an ornamental genus (Faria et al. 2011) and fruits of *Butia* species are used as foods, such as juices, liquor, marmalades and ice cream, while seeds are used to extract oil. *Musa* species are also important as a food source.

Even though fungal diversity associated with these plants have been studied, the micro-fungi inhabiting them are poorly known in Guangdong Province (Chobba et al. 2013, Shen et al. 2014, Wei et al. 2007, Zakaria & Aziz 2018). In this study, we collected several pestaloid taxa on *Butia*, *Phoenix* and *Musa* species and identified them through morpho-phylogenetic studies. Here, we provide taxonomic details for them.

Colonies of *Pestalotiopsis parva* grew faster on PDA than *Neopestalotiopsis alpapicalis* and *Pestalotiopsis diploclisiae* and did not sporulate in culture. *Pestalotiopsis diploclisiae* and *Neopestalotiopsis alpapicalis* sporulate in culture after four weeks of incubation at 20°C in the dark. Both *Pestalotiopsis diploclisiae* and *Neopestalotiopsis alpapicalis* initially formed copious aerial mycelia clots and those clots disappear with the formation of conidiomata on the PDA.

A checklist of *Neopestalotiopsis* and *Pestalotiopsis* fungi in China is given in Table 2. This includes seven species of *Neopestalotiopsis* and 69 species of *Pestalotiopsis*. Pestaloid fungi are common phytopathogens that cause a variety of diseases, including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scab, canker, severe chlorosis, fruit rots and various post-harvest diseases (Crous et al. 2011, Zhang et al. 2013, Maharachchikumbura et al. 2014). *Pestalotiopsis clavispora* and *P. anacardiacearum* have been reported to cause grey leaf spots and associated with the mango tip borer by *Penicillaria jocosatrix*, respectively in China. *Pestalotiopsis camelliae* was associated with grey leaf blight of *Camellia japonica* and *Pestalotiopsis ericacearum* with leaf spots of *Rhododendron delavayi*.

### Table 2 Checklist of *Neopestalotiopsis* and *Pestalotiopsis* fungi in China

| Taxon                  | Host/substrate                  | Province | References                                      |
|------------------------|---------------------------------|----------|-------------------------------------------------|
| *Neopestalotiopsis*    |                                 |          |                                                 |
| asiatica               | leaves of tree                  | Hunan    | Maharachchikumbura et al. (2014)                |
| *N. chrysea*           | dead leaves                     | Guangxi  | Maharachchikumbura et al. (2014)                |
| *N. clavispora*        | dead leaves of *Magnolia* sp.   | Guangxi  | Maharachchikumbura et al. (2014)                |
| *N. ellipsospora*      | dead plant material             | Yunnan   | Maharachchikumbura et al. (2014)                |
| *N. foedans*           | mangrove plant leaves           | Hainan   | Maharachchikumbura et al. (2014)                |
| *N. saprophytica*      | leaves of *Magnolia*            | Yunnan   | Maharachchikumbura et al. (2014)                |
| *N. umbrinospora*      | dead leaves                     | Guangxi  | Maharachchikumbura et al. (2014)                |
| *Pestalotiopsis adusta*| leaves of *Podocarpus macrophyllus* | Guangxi  | Wei et al. (2007)                              |
| *P. affinis*           | unknown                         | Yunnan   | Chen et al. (2002)                              |
| Taxon                        | Host/substrate                                      | Province | References                          |
|-----------------------------|-----------------------------------------------------|----------|-------------------------------------|
| *P. aggestorum*             | leaves of *Camellia sinensis*                       | Yunnan   | Liu et al. (2017)                   |
| *P. alpiniae*               | leaves of *Alpinia galanga*                         | Guangxi  | Chen et al. (2002)                  |
| *P. anacardiacearum*        | living leaf of *Mangifera indica*                   | Yunnan   | Maharachchikumbura et al. (2013)    |
| *P. antiaris*               | leaves of *Antiaris toxicaria*                      | Guangxi  | Chen et al. (2002)                  |
| *P. apiculata*              | trunk and leaves of *Cunninghamia lanceolata*       | Fujian   | Huang (1983)                        |
| *P. brideliae*              | living leaves of *Bridelia morcica*                 | China    | Chen & Wei (1997)                   |
| *P. briosiana*              | twigs of *Camellia sasanqua*                        | Yunnan   | Wei et al. (2007)                   |
| *P. camelliae*              | leaves of *Camellia japonica*                       | Yunnan   | Zhang et al. (2012a)                |
| *P. canarii*                | living leaves of *Canarium album*                   | Guangxi  | Chen et al. (2003)                  |
| *P. chinensis*              | leaves of *Taxus*                                  | Yunnan   | Maharachchikumbura et al. (2012b)   |
| *P. clavata*                | leaf of *Bixa*                                     | Yunnan   | Maharachchikumbura et al. (2014)    |
| *P. coffeae-arabicae*       | living leaves of *Coffee arabica*                   | Hainan   | Song et al. (2013)                  |
| *P. crassiuscula*           | leaves of *Podocarpus macrophyllus*                 | Zhejiang | Wei et al. (2007)                   |
| *P. dilleniæ*               | leaves of *Dillenia turbinata*                      | Guangxi  | Chen et al. (2002)                  |
| *P. dilucida*               | leaves of *Camellia sinensis*                       | Jiangxi  | Liu et al. (2017)                   |
| *P. diospyri*               | leaves of *Podocarpus macrophyllus*                 | Guizhou  | Wei et al. (2007)                   |
| *P. disseminata*            | leaves of *Podocarpus macrophyllus*                 | Guizhou  | Wei et al. (2007)                   |
| *P. diversiseta*            | leaves of *Rhododendron*                            | Yunnan   | Maharachchikumbura et al. (2014)    |
| *P. dracaenæ*               | leaves of *Dracaena fragrans*                       | Hainan   | Ariyawansa et al. (2015)            |
| *P. ericacearum*            | leaves of *Rhododendron delaveyi*                   | Zhejiang | Zhang et al. (2013)                 |
| *P. foedans*                | twigs of *Podocarpus massoniana*                    | Guangdong| Wei et al. (2007)                   |
| *P. gaultheriae*            | on *Gaultheria*                                     | Yunnan   | Maharachchikumbura et al. (2014)    |
| *P. hainanensis*            | stem of *Podocarpus macrophyllus*                   | Hainan   | Liu et al. (2007)                   |
| *P. heterocornis*           | fruit and bark of *Podocarpus macrophyllus*         | Zhejiang | Wei et al. (2007)                   |
| *P. inflexa*                | leaf of tree                                        | Hunan    | Maharachchikumbura et al. (2014)    |
| *P. intermedia*             | dead leaf of tree                                   | Hubei    | Maharachchikumbura et al. (2014)    |
| *P. jiangxiensis*           | on *Camellia*                                       | Jiangxi  | Liu et al. (2017)                   |
| *P. jinchanghensis*         | on leaves of *Camellia sinensis*                    | Yunnan   | Liu et al. (2017)                   |
| *P. keteleeriae*            | on leaves of *Keteleeria pubescens*                 | Guizhou  | Song et al. (2014)                  |
| *P. kunmingensis*           | leaves of *Podocarpus macrophyllus*                 | Yunnan   | Wei & Xu (2004)                     |
Table 2 Continued.

| Taxon               | Host/substrate                                                                 | Province | References                      |
|---------------------|-------------------------------------------------------------------------------|----------|---------------------------------|
| *P. kwangsiensis*   | leaves of *Sinopimelodendron kwangsiensis*                                   | Guangxi  | Chen et al. (2002)              |
| *P. lawsoniae*      | leaves of *Pinus massoniana*; twigs of *Podocarpus massoniana*                | Guangxi  | Wei et al. (2007)               |
| *P. licualicola*    | living leaves of *Licuala grandis*                                            | Hainan   | Geng et al. (2013)              |
| *P. lijiangensis*   | unknown                                                                       | Yunnan   | Zhou et al. (2008)              |
| *P. linearis*       | leaves of *Trachelospermum*                                                   | Yunnan   | Maharachchikumbura et al. (2014) |
| *P. longiappendiculata* | *Camellia sinensis*              | Fujian   | Liu et al. (2017)               |
| *P. lushanensis*    | *Camellia sp*                                                                 | Jiangxi  | Liu et al. (2017)               |
| *P. macadamii*      | living leaves of *Macadamia integrifolia*                                     | Guangdong| Akinsanmi et al. (2017)         |
| *P. menezesiana*    | leaves of *Podocarpus macrophyllus*                                          | Guangxi  | Wei et al. (2007)               |
| *P. microspora*     | twigs of *Podocarpus macrophyllus*                                           | Guangxi  | Wei et al. (2007)               |
| *P. nattrassioides* | unknown                                                                       | Yunnan   | Zhao & Zhao (2012)              |
| *P. neglecta*       | twigs of *Podocarpus nagi*                                                    | Guangxi  | Wei et al. (2007)               |
| *P. nelumbonis*     | leaves of *Nelumbo nucifera*                                                  | Guangxi  | Chen et al. (2002)              |
| *P. olivacea*       | leaves of *Podocarpus nagi*                                                   | Yunnan   | Wei et al. (2007)               |
| *P. oxyanthi*       | leaves of *Podocarpus macrophyllus*                                          | Zhejiang | Wei et al. (2007)               |
| *P. pachirae*       | living leaves of *Pachira macrocarpa*                                         | Yunnan   | Chen et al. (2003)              |
| *P. phaiii*         | living leaves of *Phaius tankervilleae*                                       | Yunnan   | Chen et al. (2003)              |
| *P. photinia*       | twigs of *Camellia japonica, Camellia sasanqua, Leaves of *Podocarpus massoniana* | Guangxi  | Wei et al. (2007)               |
| *P. photiniicola*   | leaves of *Photinia serrulata*                                                | Guizhou  | Chen et al. (2017)              |
| *P. pleurocrinita*  | unknown                                                                       | Yunnan   | Zhao & Zhao (2012)              |
| *P. rhododendri*    | dead parts of leaves of *Rhododendron sinogrande*                            | Yunnan   | Maharachchikumbura et al. (2014) |
| *P. rhodomyrtus*    | living leaves of *Rhodomyrtus tomentosa*                                     | Guangxi  | Zhang et al. (2013)             |
| *P. rosea*          | isolated from leaves of *Pinus*                                               | Yunnan   | Maharachchikumbura et al. (2014) |
| *P. schimae*        | leaves of *Schima superba*                                                    | Guangxi  | Chen et al. (2002)              |
| *P. subshorea*      | leaves of *Michelia hedyosperma*                                              | Guangxi  | Ariyawansa et al. (2015)        |
| *P. synsepali*      | leaves of *Synsepalum dulcificum*                                            | Hainan   | Chen et al. (2002)              |
| Taxon            | Host/substrate                                                                 | Province        | References               |
|------------------|--------------------------------------------------------------------------------|-----------------|--------------------------|
| *P. theae*       | leaves of *Camellia sinensis*, *Camellia reticulata* and *Camellia nitidissima*, twigs of *Podocarpus macrophyllus* | Zhejiang, Guangxi, Yunnan, Zhejiang | Wei et al. (2007) |
| *P. trachycarpicola* | leaves of *Trachycarpus fortune, Podocarpus macrophyllus*                       | Yunnan          | Zhang et al. (2012b)    |
| *P. unicolor*    | leaf of *Rhododendron*                                                          | Hunan           | Maharachchikumbura et al. (2012b) |
| *P. verruculosa* | leaf of *Rhododendron*                                                          | Yunnan           | Maharachchikumbura et al. (2012b) |
| *P. virgatula*   | leaves of *Podocarpus macrophyllus*, twigs of *Podocarpus massoniana*          | Zhejiang, Guangdong | Wei et al. (2007) |
| *P. vismiae*     | unknown                                                                         | Guangxi, Yunnan | Zhang et al. (2003)    |
| *P. yanglingensis* | on *Camellia sinensis*                                                         | Jiangxi         | Liu et al. (2017)       |
| *P. yunnanensis* | twigs of *Podocarpus macrophyllus* and grown on leaf segments of *Dianthus caryophyllus* | Yunnan           | Wei et al. (2013)       |
| *P. zonata*      | fruits of *Podocarpus macrophyllus*                                            | Zhejiang        | Wei et al. (2007)       |

**Acknowledgements**

Indunil C. Senanayake thanks to Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen, Guangdong, China for funding to molecular analysis. K.D. Hyde thanks the Thailand Research Funds for the grant “Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion grant number: RDG6130001”. Xie Ning thanks to technology Project of Shenzhen City, Shenzhen Bureau of Science, Technology and Information (JCYJ20180305123659726), and National Natural Science Foundation of China (No. 31601014).

**References**

Akinsanmi OA, Nisa S, Jeff-Ego OS, Shivas RG, Drench A. 2017 – Dry flower disease of Macadamia in Australia caused by *Neopestalotiopsis macadamiae* sp. nov. and *Pestalotiopsis macadamiae* sp. nov. Plant Disease 101(1), 45–53.

Aly AH, Debbab A, Kjer J, Proksch P. 2010 – Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Diversity 41, 1–16.

Ariyawansa HA, Hyde KD. 2018 – Additions to *Pestalotiopsis* in Taiwan. Mycosphere 9, 999–1013.

Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B et al. 2015 – Fungal diversity notes 111–252 – taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75, 27–274.

Chase MW, Soltis DE, Soltis PS, Rudall PJ et al. 2000 – Higher-level systematics of the monocotyledons: An assessment of current knowledge and a new classification. Monocots: Systematics and Evolution 3–16.

Chen YX, Wei G, Chen WP, Wang ZW, Lu ZH. 2003 – Three new species of *Pestalotiopsis* in China. Journal of Guangxi Agricultural and Biological Science 22, 1–4.

Chen YX, Wei G, Chen WP. 2002 – New species of *Pestalotiopsis*. Mycosystema. 21, 316–323.
Chen YX, Wei G. 1997 – Continuous notes on congeners of Pestalotiopsis in China. Journal of Guangxi Agricultural University 16: 1–9.
Chen YX, Zeng L, Shu N, Jiang M et al. 2018 – Pestalotiopsis like species causing gray blight disease on Camellia sinensis in China. Plant Disease 102, 98–106.
Chen YY, Maharachchikumbura SSN, Liu JK, Hyde KD et al. 2017 – Fungi from Asian Karst formations I. Pestalotiopsis photinica sp. nov., causing leaf spots of Photinia serrulata. Mycosphere 8(1), 103–110.
Chobba IB, Elleuch A, Ayadi I, Khannous L et al. 2013 – Fungal diversity in adult date palm (Phoenix dactylifera L.) revealed by culture-dependent and culture-independent approaches. Journal of Zhejiang University Science B 14, 1084–1099.
Crous PW, Summerell BA, Swart L. 2011 – Fungal pathogens of Proteaceae. Persoonia 27, 20–45.
Debbab A, Aly AH, Proksch P. 2013 – Mangrove derived fungal endophytes – a chemical and biological perception. Fungal Diversity 61, 1–27.
Ellis MB, Ellis JP. 1997 – Microfungi on land plants. an identification handbook. Richmond Publishing, England.
Faria JP, Siqueira E, Vieira R, Agostini-Costa TS. 2011 – Fruits of Butia capitata (Mart.) Becc as good sources of β-carotene and provitamina. Revista Brasileira de Fruticultura 33, 612–617.
Geng K, Zhang B, Song Y, Hyde KD et al. 2013 – A new species of Pestalotiopsis from leaf spots of Licuala grandis from Hainan, China. Phytotaxa 88(3): 49–54.
Glass NL, Donaldson GC. 1995 – Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61, 1323–1330.
Guba EF. 1961 – Monograph of Pestalotia and Monochaetia. Harvard University Press, Cambridge.
Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.
Hu HL, Jeewon R, Zhou DQ, Zhou TX, Hyde KD. 2007 – Phylogenetic diversity of endophytic Pestalotiopsis species in Pinus armandii and Ribes spp.: evidence from rDNA and β-tubulin gene phylogenies. Fungal Diversity 24, 1–22.
Huang TZ. 1983 – A preliminary report on dieback (shoot) disease in Chinese fir. Journal of North-Eastern Forestry Institute China 11(3), 45–50.
Huelsenbeck JP, Ronquist F, Hall B. 2003 – MrBayes: a program for the Bayesian inference of phylogeny. Version 3.0 b4.
Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ et al. 2020 – Refined Families of Sordariomycetes. Mycosphere 11(1): 305–1059.
Jeewon R, Hyde KD. 2016 – Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7, 1669–1677.
Katoh K, Rozewicki J, Yamada KD. 2017 – Mafft online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in bioinformatics. Doi 10.1093/bib/bbx108
Liu AR, Chen SC, Wu SY, Xu T et al. 2010 – Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in Pestalotiopsis taxonomy. Molecular Phylogenetics and Evolution 57, 528–535.
Liu AR, Xu T, Guo LD. 2007 – Molecular and morphological description of Pestalotiopsis hainanensis sp. nov., a new endophyte from a tropical region of China. Fungal Diversity 24, 23–36.
Liu F, Hou LW, Raza M, Cai L. 2017 – Pestalotiopsis and allied genera from Camellia, with description of 11 new species from China. Scientific Reports 7(866), 1–19.
Maharachchikumbura SSN, Guo LD, Cai L, Chukeatirote E et al. 2012a – A multi-locus backbone tree for Pestalotiopsis, with a polyphasic characterization of 14 new species. Fungal Diversity 56, 95–129.
Maharachchikumbura SSN, Guo LD, Lei C, Chukeatirote E et al. 2012b – A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Diversity 56(1), 95–129.

Maharachchikumbura SSN, Guo LD, Liu ZY, Hyde KD. 2016 – *Pseudopestalotiopsis ignota* and *Ps. camelliae* spp. nov. associated with grey blight disease of tea in China. Mycological Progress 15, 22.

Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. 2014 – *Pestalotiopsis* revisited. Studies Mycology 79, 121–186.

Maharachchikumbura SSN, Zhang YM, Hyde KD. 2013 – *Pestalotiopsis anacardiacearum*. sp. nov. has an intricate relationship with the mango tip borer. Phytotaxa 99, 49–57.

Monden Y, Yamamoto S, Yamakawa R. 2013 – First case of fungal keratitis caused by *Pestalotiopsis clavispora*. Clinical Ophthalmology 7, 2261–2264.

Norphanphoun C, Jayawardena RS, Chen Y, Wen TC. 2019 – Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand. Mycosphere 10, 531–578.

Nozawa S, Yamaguchi K, Van Hop D, Phay N et al. 2017 – Identification of two new species and asexual morph from the genus *Pseudopestalotiopsis*. Mycoscience 58, 328–337.

Nylander JAA. 2004 – MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Rambaut A, Drummond A. 2008 – FigTree: Tree figure drawing tool, version 1.2. 2. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut A, Drummond AJ. 2007 – Tracer v1, 4. Available from: http://beast.bio.ed.ac.uk/Tracer (Accessed on November 1, 2019).

Rehner S. 2001 – Primers for elongation factor 1-α (EF1-α). Available from: http://ocid.nacres.org/research/deephycphae/EF1primer. (Accessed on January 1, 2020).

Riffle RL, Wraith P. 2003 – An Encyclopedia of Cultivated Palms. Portland: Timber Press.

Russell JR, Huang J, Anand P, Kucera K et al. 2011 – Biodegradation of Polyester Polyurethane by Endophytic Fungi. Applied and Environmental Microbiology 77(17), 6076–6084.

Senanayake IC, Jeewon R, Chomnunti P, Wanasinhe DN et al. 2018 – Taxonomic circumscription of Diaporthales based on multigene phylogeny and morphology. Fungal Diversity 93, 241–443.

Shen HF, Zhang JX, Lin BR, Pu XM. 2014 – First Report of *Pestalotiopsis microspora* Causing Leaf Spot of Oil Palm (*Elaeis guineensis*) in China. Plant Disease 98, 1429.

Silvestro D, Michalak I. 2012 – raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12, 335–337.

Song Y, Geng K, Zhang B, Hyde KD et al. 2013 – Two new species of *Pestalotiopsis* from Southern China. Phytotaxa 126(1), 22–30.

Song Y, Maharachchikumbura SSN, Jiang YL, Hyde KD, Wang Y. 2014 – *Pestalotiopsis keteleeria* sp. nov., isolated from *Keteleeria pubescens* in China. Chiang Mai Journal of Science 41(4), 885–893.

Stamatakis A, Alachiotis N. 2010 – Time and memory efficient likelihood-based tree searches on phylogenomic alignments with missing data. Bioinformatics 26, 1132–1139.

Steyaert RL. 1949 – Contributions à l’étude monographique de *Pestalotia* de Not. et *Monochaetia* Sacc. (Truncatella* gen. nov. et Pestalotiopsis* gen. nov.). Bulletin Jardin Botanique Etat Bruxelles 19, 285–354.

Strobel G, Yang XS, Sears J, Kramer R et al. 1996 – Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*. Microbiology 142, 435–440.

Tejesvi MV, Nalini MS, Mahesh B, Prakash HS et al. 2007 – New hopes from endophytic fungal secondary metabolites. Boletín de la Sociedad Química de México 1, 19–26.

Tibpromma S, Hyde KD, McKenzie EHC, Bhat DJ et al. 2018 – Fungal diversity notes 840–928: micro-fungi associated with *Pandanaceae*. Fungal Diversity 92, 1–160.
Tsai I, Maharachchikumbura SSN, Hyde KD, Ariyawansa HA. 2018 – Molecular phylogeny, morphology and pathogenicity of *Pseudopestalotiopsis* species of *Ixora* in Taiwan. Mycological Progress 17, 941–952.

Watanabe K, Nozawa S, Hsiang T, Callan B. 2018 – The cup fungus *Pestalopecia brunneopruinosa* is *Pestalotiopsis gibbosa* and belongs to Sordariomycetes. PloS one 13, 6.

Wei JG, Phan CK, Wang L, Xu T et al. 2013 – *Pestalotiopsis yunnanensis* sp. nov., an endophyte from *Podocarpus macrophyllus* (Podocarpaceae) based on morphology and ITS sequence data. Mycological Progress 12, 563–568.

Wei JG, Xu T, Guo LD, Liu AR et al. 2007 – Endophytic *Pestalotiopsis* species associated with plants of Podocarpaceae, Theaceae and Taxaceae in southern China. Fungal Diversity 24, 55–74.

Wei JG, Xu T. 2004 – *Pestalotiopsis kunmingensis* sp. nov., an endophyte from *Podocarpus macrophyllus*. Fungal Diversity 15, 247–254.

White TJ, Bruns T, Lee SJWT, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–322.

Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK et al. 2018 – Outline of Ascomycota – 2017. Fungal Diversity 88, 167–263.

Xu J, Ebada SS, Proksch P 2010 – *Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites. Fungal Diversity 44, 15–31.

Xu J, Yang X, Lin Q. 2014 – Chemistry and biology of Pestalotiopsis-derived natural products. Fungal Diversity 66, 37–68.

Zakaria L, Aziz WNW. 2018 – Molecular Identification of Endophytic Fungi from Banana Leaves (*Musa* spp.). Trop Life Sci Res 29, 201–211.

Zhang J, Xu T, Ge Q. 2003 – Notes on *Pestalotiopsis* from southern China. Mycotaxon 85, 91–99.

Zhang YM, Maharachchikumbura SSN, McKenzie EHC, Hyde KD. 2012a – *Pestalotiopsis camelliae* sp. nov. associated with grey blight of *Camellia japonica* in China. Mycoscience 64, 335–344.

Zhang YM, Maharachchikumbura SSN, Tian Q, Hyde KD. 2012b – A novel species of *Pestalotiopsis* causing leaf spots of *Trachycarpus Fortunei*. Cryptogamie, Mycologie 33(3), 311–318.

Zhao GC, Zhao RL. 2012 – The higher microfungi from forests of Yunnan Province. book, 1–572.

Zhaxybayeva O, Gogarten JP. 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC genomics 3, 4.

Zhou YK, Li FP, Hou CL. 2018 – *Pestalotiopsis lijiangensis* sp. nov., a new endophytic fungus from Yunnan, China. Mycotaxon 133(3), 513–522.