Morphological and phylogenetic analyses reveal two new species of Sporocadaceae from Hainan, China

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Abstract
Species of Sporocadaceae have often been reported as plant pathogens, endophytes or saprophytes and are commonly isolated from a wide range of plant hosts. The isolated fungi were studied through a complete examination, based on multilocus phylogenies from combined datasets of ITS/tub2/tef1, in conjunction with morphological characteristics. Nine strains were isolated from Ficus microcarpa, Ilex chinensis and Schima superba in China which represented four species, viz., Monochaetia schimae sp. nov., Neopestalotiopsis haikouensis sp. nov., Neopestalotiopsis piceana and Pestalotiopsis licualicola. Neopestalotiopsis piceana was a new country record for China and first host record from Ficus macrocarpa. Pestalotiopsis licualicola was first report from Ilex chinensis in China.

Keywords
Monochaetia, multigene phylogeny, Neopestalotiopsis, Pestalotiopsis

Introduction

The family Sporocadaceae was established by Corda in 1842 (type genus: Sporocadus). Species of Sporocadaceae are endophytic, plant pathogenic or saprobic, and associated with a wide range of host plants (Maharachch. et al. 2013; Jayawardena et al. 2015; Liu et al. 2019). Currently, the family comprises 35 genera including Monochaetia (Sacc.) Allesch., Neopestalotiopsis Maharachch. et al., Pestalotiopsis Steyaert, Pseudopestalotiopsis Maharachch.et al., and etc. Most genera have multi-septate and more or less fusiform
conidia with appendages at one or both ends, frequently with some melanised cells. Also known as pestalotioid fungi, resembling those taxa having affinities with *Pestalotia* (Liu et al. 2019).

Steyaert (1949) segregated two novel genera from *Pestalotia*, namely *Pestalotiopsis* (with 5-celled conidia) and *Truncatella* (with 4-celled conidia) based on the conidial forms. This resulted in apparent controversy from Guba (1956, 1961). He emphasised that there was no point in assembling species with similar numbers of conidial septa into distinct genera. Subsequently, Steyaert (1953, 1961, 1963) provided further evidence in support of splitting *Pestalotia*. Sutton (1980) accepted most of the genera discussed here (*Pestalotia*, *Pestalotiopsis*, *Truncatella*) which fitted into fairly well-defined groups and cited the electron microscope investigation of Griffiths and Swart (1974), which examined the conidial wall of *Pestalotia pezizoides* and two species of *Pestalotiopsis* (*P. funerea* and *P. triseta*) to support Steyaert’s division of *Pestalotiopsis*. Maharachch. et al. (2014) segregated two novel genera from *Pestalotiopsis*, namely *Neopestalotiopsis* and *Pseudopestalotiopsis*, based on conidia pigment colour, conidiophores and molecular phylogeny. *Neopestalotiopsis* can be easily distinguished from *Pseudopestalotiopsis* and *Pestalotiopsis* by its versicolourous median cells (Maharachch. et al. 2014). Saccardo (1884) introduced *Monochaetia* as a subgenus of *Pestalotia* (as *Pestolozzia*). The genus *Monochaetia* was introduced by Allescher (1902), which included 23 species. Allescher (1902) designated the type *Monochaetia monochaeta* which has a single apical appendage (Guba 1961; Maharachch. et al. 2014; Senanayake et al. 2015). Steyaert (1949) transferred numerous *Monochaetia* species to *Pestalotiopsis* or *Truncatella*. More than 40 species of *Monochaetia* were recognised by the monograph of Guba (1961). There are 127 *Monochaetia* epithets in the Index Fungorum (accession date: 31 March 2022) and most have been transferred to other genera such as *Sarcostroma*, *Seimatosporium* and *Seiridium* (Nag Raj 1993; Maharachch. et al. 2011, 2014, 2016). *Seridium* and *Monochaetia* have obvious morphological differences and show separate clades (de Silva et al. 2017).

To date, most phylogenetic studies addressing genera of Sporocadaceae have been based solely on ITS and LSU sequences (Barber et al. 2011; Tanaka et al. 2011; Jaklitsch et al. 2016), or on concatenated datasets of more genes but with incomplete datasets (Senanayake et al. 2015; Wijayawardene et al. 2016). In this study, we made a collection of the established genera *Monochaetia*, *Neopestalotiopsis* and *Pestalotiopsis* species from leaves of *Ficus microcarpa*, *Ilex chinensis* and *Schima superba* in Hainan Province, China. The inventories allowed establishing two new species that are described here.

### Materials and methods

#### Isolation and morphological studies

The samples were collected from Hainan Province, China. The strains were isolated from diseased leaves of *Ficus microcarpa*, *Ilex chinensis* and *Schima superba* using surface disinfected tissue fragments (0.5 × 0.5 cm) taken from the margin of leaf lesions
Surface disinfection consisted of steps including immersion in 75% ethanol for 30 s, 5% sodium hypochlorite (Aladdin, Shanghai, China) for 1 min, and sterile distilled water for 30 s. The pieces were dried with sterilized paper towels and placed on potato dextrose agar (PDA). All plates were incubated at 25 °C for 3–4 days. Then, hyphae were picked out of the periphery of the colonies and inoculated onto new PDA plates. Photographs of the colonies were taken at 7 and 15 days using a Powershot G7X mark II digital camera. Micromorphological characters were observed using an Olympus SZX10 stereomicroscope and Olympus BX53 microscope, all fitted with Olympus DP80 high definition colour digital cameras to photo-document fungal structures. The size of conidia was measured by software Digimizer (https://www.digimizer.com/), and thirty individual measurements were obtained for each character. All fungal strains were stored in 10% sterilised glycerin at 4 °C for further studies. The holotype specimens were deposited in the Herbarium of Plant Pathology, Shandong Agricultural University (HSAUP). Ex-type cultures were deposited in the Shandong Agricultural University Culture Collection (SAUCC). Taxonomic information on the new taxa was submitted to MycoBank (http://www.mycobank.org).

**DNA extraction and amplification**

Genomic DNA was extracted from fungal mycelium grown on PDA using cetyltrimethylammonium bromide (CTAB) protocol as described in Guo et al. (2000). The internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS) and partial beta-tubulin (tub2) and translation elongation factor 1-alpha (tef1) genes were amplified and sequenced by using primers pairs ITS5/ITS4 (White et al. 1990), T1/Bt2b (Glass and Donaldson 1995; O’Donnell and Cigelnik 1997), and EF1-728F/EF-2 (O’Donnell et al. 1998; Carbone and Kohn 1999).

PCR was performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification reactions were performed in a 50 μL reaction volume, which contained 25 μL Green Taq Mix (Vazyme, Nanjing, China), 2 μL of each forward and reverse primer (10 μM) (Tsingke, Beijing, China), and 2 μL template genomic DNA, to which distilled deionized water was added. PCR parameters were as follows: 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at a suitable temperature for 30 s, extension at 72 °C for 1 min and a final elongation step at 72 °C for 7 min. Annealing temperature was 55 °C for ITS, 54 °C for tub2, 52 °C for tef1. The PCR products were visualised on 1% agarose electrophoresis gel. Sequencing was done bi-directionally, conducted by the Tsingke Biotechnology Company Limited (Qingdao, China). Consensus sequences were obtained using MEGA 7.0 or MEGA-X (Kumar et al. 2016). All sequences generated in this study were deposited in GenBank (Table 1).

**Phylogeny**

Newly generated sequences in this study were aligned with additional related sequences downloaded from GenBank (Table 1) using MAFFT 7 online service with
the Auto strategy (Katoh et al. 2019, http://mafft.cbrc.jp/alignment/server/). To establish the identity of the isolates at the species level, phylogenetic analyses were conducted first individually for each locus and then as combined analyses of three loci (ITS, tub2 and tef1). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian inference (BI) for the multi-locus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. ML and BI were run on the CIPRES Science Gateway portal (https://www.phylo.org/) (Miller et al. 2012) using RaxML-HPC2 on XSEDE v. 8.2.12 (Stamatakis 2014) and MrBayes on XSEDE v. 3.2.7a (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012), respectively. Four Markov chains were run for two runs from random starting trees for 10,000,000 generations (ITS + tub2 + tef1) until the split deviation frequency value < 0.01, and trees were sampled every 1000 generation. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. The resulting trees were plotted using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CC 2019. New sequences generated in this study were deposited at GenBank (https://www.ncbi.nlm.nih.gov; Table 1). The final concatenated sequence alignments were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S29480).

Table 1. Species and GenBank accession numbers of DNA sequences used in this study. New sequences are in bold.

| Species               | Strain   | Host/substrate   | Country | GenBank accession number   | Reference          |
|-----------------------|----------|------------------|---------|---------------------------|--------------------|
| Bartalinia robillardoides | CBS 122705 T | Lepidopaus occidentalis | Italy   | LT853104 LT853202 LT853252 | Bonthond et al. 2018 |
| Ciliochorella phanericola | MFLUCC 14-0984 T | Phanera purpurea | Thailand | KX789680 – KX789682 | Jiang et al. 2021b |
| Monochaetia castaneae  | MFLUCC 12-0310 | Phanera purpurea | Thailand | KF827444 KF827477 KF827478 | Jiang et al. 2021b |
| M. dimorphospora      | MFLUCC 54354 – SM1-1 T | Castanea mollissima | China   | MW166222 MW199741 MW218515 | Jiang et al. 2021b |
| M. ilicis             | KUMCC 15-0520 T | Ilex sp.         | China   | KX984153 – –              | de Silva et al. 2017 |
| M. junipericola       | CBS 101009 | Air              | Japan   | MH553953 MH554371 MH554612 | Liu et al. 2019  |
| M. kansensis          | CBS 143391 T | Juniperus communis | Germany | MH107900 MH108021 MH108045 | Crous et al. 2018 |
| M. monochaeta         | CBS 546.80 Culture contaminant | Quercus pubescens | Netherlands | MH554056 MH554491 MH554732 | Liu et al. 2019 |
| M. quercus            | CBS 199.82 T | Quercus pubescens | Italy   | MH554018 – MH554694 | Liu et al. 2019  |
| M. schimae            | CBS 115004 | Quercus robur    | Netherland | IY853243 MH554398 MH554639 | Liu et al. 2019  |
| M. sinensis           | CBS 144045 T | Quercus edulis    | Mexico  | MH554171 MH554606 MH554844 | Liu et al. 2019  |
| Schima superba        | SAUCC212200 T | Schima superba | China   | MZ775565 OK104874 OK104867 | This study |
| M. sinensis           | HKAS 10065 T | Quercus sp.      | China   | MH115995 – MH115999 | de Silva et al. 2017 |
| Species | Strain | Host/substrate | Country | GenBank accession number | Reference |
|---------|--------|----------------|---------|--------------------------|-----------|
| Neopetalotiopsis acrostichi | MFLUCC 17-1754 T | Acrostichum aurum | Thailand | MK764272 MK764316 MK764338 | Norphanphou et al. 2019 |
| N. atapicalis | MFLUCC 17-2544 T | Rhizophora mucronata | Thailand | MK357772 MK463547 MK463545 | Kumar et al. 2019 |
| N. aotearoa | CBS 367.54 T | Canvas | New Zealand | KM199369 KM199526 KM199454 | Maharachch. et al. 2014 |
| N. asiatica | MFLUCC 12-0286 T | Unidentified tree | Mozambique | JX399097 JX399095 JX399014 | Maharachch. et al. 2012 |
| N. brachiata | MFLUCC 17-1555 T | Rhizophora apiculata | Thailand | MK764274 MK764318 MK764340 | Norphanphou et al. 2019 |
| N. braziliensis | COAD 2166 T | Psidium guajava | Brazil | MG686409 MG692402 MG692400 | Bezerra et al. 2018 |
| N. egyptiaca | CBS 367.54 T | Plant debris | France | KM199369 KM199526 KM199454 | Maharachch. et al. 2014 |
| N. ellipsospora | CBS 600.96 T | Leaf litter | Cuba | KM199369 KM199526 KM199454 | Maharachch. et al. 2014 |
| N. eucalypticola | CBS 114178 T | Leucospermum cuneiforme cv. "Sunbird" | Zimbabwe | JN712498 KM199542 KM199463 | Maharachch. et al. 2014 |
| Species         | Strain     | Host/substrate     | Country        | GenBank accession number | Reference             |
|-----------------|------------|--------------------|----------------|--------------------------|------------------------|
| *N. rhizophorae* | MFLUCC 17-1550 T | *Rhizophora mucronata* | Thailand       | MK764278 MK764322 MK764344 | Norphanphoun et al. 2019 |
| *N. mae*        | CBS 124745 | *Pemonia suffruticosa* | USA            | KM199360 KM199524 KM199430 | Maharachch. et al. 2014 |
|                 | CBS 101057 T | *Rosa sp.*         | New Zealand    | KM199359 KM199523 KM199429 | Maharachch. et al. 2014 |
| *N. micola*     | CFCC 51992 T | *Rosa chinensis*   | China          | KY885239 KY885243 KY885245 | Norphanphour et al. 2019 |
|                 | CFCC 51993 | *Rosa chinensis*   | China          | KY885240 KY885244 KY885246 | Norphanphour et al. 2019 |
| *N. sanarangensis* | MFLUCC 12-0233 T | *Syzygium samarangense* | Thailand       | JQ968609 JQ968611 JQ968610 | Maharachch. et al. 2012 |
| *N. rosae*      | CBS 124745 | *Paeonia suffruticosa* | USA            | KM199345 KM199538 KM199433 | Maharachch. et al. 2014 |
|                 | CBS 101057 T | *Rosa sp.*         | New Zealand    | MW166231 MW199750 MW218524 | Jiang et al. 2021b     |
| *N. rosicola*   | CFCC 51992 T | *Rosa chinensis*   | China          | KY885239 KY885243 KY885245 | Norphanphour et al. 2019 |
|                 | CFCC 51993 | *Rosa chinensis*   | China          | KY885240 KY885244 KY885246 | Norphanphour et al. 2019 |
| *N. samarangensis* | MFLUCC 12-0233 T | *Syzygium samarangense* | Thailand       | JQ968609 JQ968611 JQ968610 | Maharachch. et al. 2012 |
| *N. saprophytica* | MFLUCC 12-0282 T | *Magnolia sp.*     | China          | MK199345 KM199538 KM199433 | Maharachch. et al. 2014 |
| *N. sichuanensis* | CFCC 51993 | *Castanea mollissima* | China          | MW166231 MW199750 MW218524 | Jiang et al. 2021b     |
| *N. sonneratae* | MFLUCC 17-1745 T | *Sonneronata alba* | Thailand       | MK764280 MK764324 MK764346 | Norphanphour et al. 2019 |
| *N. steyaertii* | IMI 192475 | *Eucalytpus viminalis* | Australia      | KF582796 KF582792 KF582794 | Maharachch. et al. 2014 |
| *N. surinamensis* | CBS 450.74 T | *Soil under Elaeis guineensis* | Suriname       | KM199351 KM199518 KM199465 | Maharachch. et al. 2014 |
| *N. thailandica* | MFLUCC 17-1730 T | *Rhizophora mucronata* | Thailand       | MK764281 MK764325 MK764347 | Norphanphour et al. 2019 |
| *N. umbrinospora* | CBS 114193 T | *Eurafrax cinerea cv. “Summer black”* | Zimbabwe       | JX556231 KM199545 KM199456 | Maharachch. et al. 2014 |
| *N. vitis*      | MFLUCC 15-1265 T | *Vitis vinifera cv.* | China          | KU140694 KU140676 KU140685 | Jayawardena et al. 2016 |
| *N. zimbabwana* | CBS 111495 T | *Leucocarpus cuneiforme cv.* | Zimbabwe       | JX556231 KM199545 KM199456 | Maharachch. et al. 2014 |
| *Nonappendiculata quercina* | CBS 116061 T | *Quercus suber*     | Italy          | MH553982 MH554400 MH554641 | Liu et al. 2019         |
| *Pestalotiopsis australasiae* | CBS 114126 T | *Quercus pulcens*   | Italy          | MH554025 MH554459 MH554701 | Liu et al. 2019         |
| *P. australis*  | CBS 114193 T | *Grevillea sp.*     | Australia      | KM199332 KM199475 KM199383 | Maharachch. et al. 2014 |
| *P. grevilleae* | CBS 114127 T | *Grevillea sp.*     | Australia      | KM199300 KM199504 KM199407 | Maharachch. et al. 2014 |
| *P. hollandica* | CBS 265.33 T | *Sciadopitys verticillata* | The Netherlands | KM199328 KM199481 KM199388 | Maharachch. et al. 2014 |
| *P. kenyana*    | CBS 442.67 T | *Coffia sp.*        | Kenya          | KM199302 KM199502 KM199395 | Maharachch. et al. 2014 |
| *P. knightiae*  | CBS 114138 T | *Knightia sp.*      | New Zealand    | KM199310 KM199497 KM199408 | Maharachch. et al. 2014 |
| *P. licualicola* | HGUP4057 T | *Licuala grandis*   | China          | KC492509 KC481684 KC481683 | Geng et al. 2013        |
| *P. oryzae*     | CBS 279.82 | *Quercus suber*     | Italy          | MH554025 MH554459 MH554701 | Liu et al. 2019         |
| *P. parva*      | CBS 114126 T | *Knightia sp.*      | New Zealand    | KM199297 KM199499 KM199409 | Maharachch. et al. 2014 |
| *P. portugalica* | CBS 114193 T | *Grevillea sp.*     | Australia      | KM199332 KM199475 KM199383 | Maharachch. et al. 2014 |
| *P. spathuliappendiculata* | CBS 279.82 | *Quercus suber*     | Italy          | MH554025 MH554459 MH554701 | Liu et al. 2019         |
| *Pseudopestalotiopsis cocos* | CBS 278.35 | *Leucocarpus cuneiforme* | New Zealand    | KM199313 KM199509 KM199405 | Maharachch. et al. 2014 |
| *Seiridium papillatum* | CBS 393.48 T | *Eucalyptus delegatensis* | Indonesia      | KM199378 KM199553 KM199467 | Maharachch. et al. 2014 |
| *Seir. phylicae* | CBS 124745 | *Elaeis guineensis* | Nigeria        | MH554044 MH554479 MH554720 | Liu et al. 2019         |
|                 | CBS 459.78 T | *Rosa sinensis*     | India          | KM199381 KM199560 KM199470 | Maharachch. et al. 2014 |
| *Seir. phylicae* | CBS 340.97 T | *Eucalyptus delegatensis* | Australia      | LT853102 LT854468 LT853250 | Bonthond et al. 2018   |

Isolates marked with "T" are ex-type or ex-epitype strains.
Two new species of Sporocadaceae from Hainan, China

Figure 1. Phylogram of Sporocadaceae based on combined ITS, tub2 and tef1 sequences. The BI and ML bootstrap support values above 0.90 and 70% are shown at the first and second position, respectively. The tree is rooted to Bartalimia robillardoides (CBS 122705), ex-type or ex-epitype cultures are indicated in bold face. Strains from the current study are in red. Some branches were shortened according to the indicated multipliers.
Figure 1. Continued.
Result

Phylogenetic analyses

Nine strains of Sporocadaceae isolated from plant hosts from Hainan, China, were grown in culture and used for analyses of molecular sequence data. The combined dataset of ITS-tub2-tef1 has an aligned length of 2285 total characters (ITS: 1–638, tub2: 639–1558, tef1: 1559–2285) including gaps, of which 869 characters are constant, 292 variable and parsimony-uninformative, and 1124 parsimony-informative. For the BI and ML analyses, the substitution model GTR+G for ITS, HKY+I+G for tub2 and GTR+I+G for tef1 were selected and incorporated into the analyses. The MCMC analysis of the three concatenated genes run for 7,795,000 generations, resulting in 7796 trees. The ML tree topology confirmed the tree topologies obtained from the BI analyses, and therefore, only the ML tree is presented (Fig. 1).

Bayesian posterior probability (≥ 0.90) and ML bootstrap support values (≥ 70%) are shown as first and second position above nodes. The 96 strains were assigned to 75 species clades based on the three gene loci phylogeny (Fig. 1). Based on the multi-locus phylogeny and morphology, nine isolates were assigned to four species, including Monochaetia schimae sp. nov., Neopestalotiopsis haikouensis sp. nov., Neopestalotiopsis piceana and Pestalotiopsis licualicola.

Taxonomy

Monochaetia schimae Z. X. Zhang, J. W. Xia & X. G. Zhang, sp. nov.

MycoBank No: 841381

Fig. 2

Type. China, Hainan Province: East Harbour National Nature Reserve, on diseased leaves of Schima superba, 23 May 2021, Z.X. Zhang (holotype HSAUP212201; ex-type living culture SAUCC212201).

Etymology. Name refers to the genus of the host plant Schima superba.

Description. Leaf spots irregular, pale brown in centre, brown to tan at margin. Sexual morph not observed. Asexual morph on PDA: Conidiomata solitary, scattered, black, raising above surface of culture medium, subglobose, exuding black conidial droplets from central ostioles after 10 days in light at 25 °C. Conidiophores cylindrical, hyaline, smooth-walled. Conidiogenous cells 9.0–16.5 × 1.2–2.2 μm, phialidic, ampulliform, discrete, hyaline, smooth, thin-walled. Conidia 18–24 × 4.5–6.0 μm, mean ± SD = 20.5 ± 1.1 × 5.5 ± 0.4 μm, fusiform, tapering at both ends, 4-septate; apical cell 2.0–4.0 μm long, conical, hyaline and smooth-walled; three median cells doliiform, 12.5–15.5 μm long, mean ± SD = 14.2 ± 0.7 μm, olivaceous, rough-walled, upper second cell 3.8–5.3 μm long, upper third cell 3.4–5.0 μm
long, upper fourth cell 4.4–5.4 μm long; basal cell 2.2–4.5 μm long, conical, hyaline and smooth-walled; apical appendage 7.0–12.5 μm long (mean = 9.2 μm), single, unbranched, central, tubular, filiform; basal appendage 2.5–5.0 μm long, single, unbranched tubular, filiform.

Culture characteristics. Colonies on PDA 39.0–45.0 mm in diameter after 15 days at 25 °C in darkness, growth rate 2.5–3.0 mm/day, irregularly circular, raised, dense surface with lobate edge, zonate in different sectors, light brown at the margin, brown at the centre; reverse brown at the margin, dark brown at the centre.
**Additional specimen examined.** China, Hainan Province: East Harbour National Nature Reserve, 23 May 2021, Z.X. Zhang. On diseased leaves of *Schima superba*, paratype HSAUP212202, living culture SAUCC212202; on diseased leaves of *Schima superba*, paratype HSAUP212203, living culture SAUCC212203.

**Notes.** *Monochaetia schimae* is introduced based on the multi-locus phylogenetic analysis, with three isolates clustering separately in a well-supported clade (BI/ML = 0.99/96). *Monochaetia schimae* is phylogenetically close to *M. castaneae* from leaves of *Castanea mollissima*, *M. ilicis* from leaves of *Ilex* sp., and *M. junipericola* from twigs of *Juniperus communis*. However, *Monochaetia schimae* differs from *M. castaneae* by 148 nucleotides (11/463 in ITS, 89/743 in *tub2* and 48/403 in *tef1*), from *M. ilicis* by 94 nucleotides (18/526 in ITS, 32/698 in *tub2* and 44/456 in *tef1*), and from *M. junipericola* by 91 nucleotides (10/524 in ITS, 40/411 in *tub2* and 41/304 in *tef1*). Furthermore, they are distinguished by hosts and conidial sizes (18.0–24.0 × 4.5–6.0 μm in *M. schimae* vs. 18.8–27.3 × 4.7–6.6 μm in *M. castaneae* vs. 20.0–27.0 × 5.0–8.0 μm in *M. ilicis* vs. 22.0–28.0 × 5.0–7.0 μm in *M. junipericola*). In morphology, *Monochaetia castaneae* differs from *M. schimae* by the colour of colonies (cinnamon vs. brown), *Monochaetia ilicis* differs from *M. schimae* by the colour of median cells (brown vs. olivaceous), and *M. junipericola* differs from *M. schimae* by longer conidiogenous cells (10.0–30.0 μm vs. 9.0–16.5 μm) (de Silva et al. 2017; Crous et al. 2018; Jiang et al. 2021b).

**Neopestalotiopsis haikouensis** Z. X. Zhang, J. W. Xia & X. G. Zhang, sp. nov.

*Mycobank No: 841382*

**Fig. 3**

**Type.** China, Hainan Province, Haikou City: East Harbour National Nature Reserve, on diseased leaves of *Ilex chinensis*. 23 May 2021, Z.X. Zhang (holotype HSAUP212271; ex-type living culture SAUCC212271).

**Etymology.** Named after the host location, Haikou City.

**Description.** Leaf spots irregular, grey white in centre, brown to tan at margin. Sexual morph not observed. Asexual morph on PDA: Conidiomata globose to clavate, solitary or confluent, embedded or semi-immersed to erumpent, dark brown, exuding globose, dark brown to black conidial masses. Conidiophores indistinct, often reduced to conidiogenous cells. Conidiogenous cells discrete, subcylindrical to ampulliform, hyaline, 5.0–10.0 × 2.0–6.0 μm, apex 1.0–2.0 μm diam. Conidia fusoid, ellipsoid, straight to slightly curved, 4-septate, 16.0–22.0 × 4.5–7.0 μm, mean ± SD = 20.0 ± 1.8 × 5.5 ± 0.4 μm; basal cell conical with a truncate base, hyaline, rugose and thin-walled, 3.0–4.5 μm long; three median cells doliform, 11.5–15.0 μm long, mean ± SD = 13.2 ± 1.0 μm, wall rugose, septa darker than the rest of the cell, second cell from the base pale brown, 3.5–5.5 μm long; third cell honey-brown, 4.0–6.0 μm long; fourth cell brown, 3.8–5.7 μm long; apical cell 2.5–5.5 μm long, hyaline, cylindrical to subcylindrical, thin- and smooth-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, 13.5–24.0 μm long, mean ± SD = 19.1 ± 3.5 μm; basal appendage 2.0–7.0 μm long, single, tubular, unbranched, centric.
Culture characteristics. Colonies on PDA occupying an entire 90 mm petri dish in 7 days at 25 °C in darkness, growth rate of 7.0–14.0 mm/day, edge undulate, white to grey white, with moderate aerial mycelium on the surface, with black, gregarious conidiomata; reverse similar in colour.

Additional specimen examined. China, Hainan Province: East Harbour National Nature Reserve, 23 May 2021, Z.X. Zhang. On diseased leaves of *Ilex chinensis*, paratype HSAUP212272, living culture SAUCC212272.
Notes. Phylogenetic analysis of a combined three-gene ITS-tub2-tef1 showed that *Neopesataliotiopsis haikouensis* formed an independent clade with full-supported (BI/ML = 1/100, Fig. 1) and is phylogenetically distinct from *N. cocoensis* (MFLUCC 15-0152), *N. formicidarum* (CBS 362.72) and *N. sichuanensis* (CFCC 54338). *Neopesataliotiopsis haikouensis* can be distinguished from the phylogenetically most closely related species *N. cocoensis* by narrower conidia (4.5–7.0 vs. 7.5–9.5 μm), *N. formicidarum* by smaller conidia (16.0–22.0 × 4.5–7.0 vs. 20.0–29.0 × 7.5–9.5 μm), and *N. sichuanensis* by shorter conidia (16.0–22.0 vs. 23.2–32.8 μm). Furthermore, some species were reported from the same host genus *Ilex*, including *Pestalotia neglecta*, *Pestalotiopsis annulata*, *P. humicola* and *P. ilicis*. After comparison, *P. humicola* was closest to *N. haikouensis* in morphology, but with 78/588 differences in the ITS region (Maharachch. et al. 2014; Liu et al. 2019; Jiang et al. 2021b).

**Neopesataliotiopsis piceana** S.S.N. Maharachch., K.D. Hyde & P.W. Crous, Studies in Mycology 79:146. (2014)

Fig. 4

Description. Leaf spots irregular, pale brown in centre, brown to tan at margin. Asexual morph on PDA: Conidiomata solitary, globose to clavate, semi-immersed, brown to black; exuding globose, dark brown to black conidial masses. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, ampulliform to lageniform, hyaline, smooth and thin walled, simple, 4.0–12.0 × 2.0–10.0 μm, apex 2.0–5.0 μm diam. Conidia ellipsoid to clavate, straight to slightly curved, 4-septate, 19.5–26.5 × 5.5–7.0 μm, mean ± SD = 22.7 ± 0.8 × 6.1 ± 0.4 μm; somewhat constricted at septa; basal cell obconic with truncate base, rugose and thin-walled, 2.7–5.0 μm long; three median cells 12.0–16.0 μm long, mean ± SD = 14.7 ± 0.9 μm, doliiform, verruculose, versicoloured, septa darker than the rest of the cell, second cell from base pale brown, 4.0–5.7 μm long; third cell dark brown, 3.5–5.2 μm long; fourth cell brown, 3.8–5.8 μm long; apical cell obconic, hyaline, thin and smooth-walled, 2.5–5.2 μm long; with 1–3 tubular apical appendages, arising from the apical crest, flexuous, unbranched, 21.0–32.0 μm long, mean ± SD = 24.8 ± 3.5 μm; basal appendage single, tubular, unbranched, centric, 2.7–6.5 μm long.

Culture characteristics. Colonies on PDA incubated at 25 °C in the dark with an average radial growth rate of 9.0–14.0 mm/day and occupying an entire 90 mm petri dish in 7 d, with edge undulate, whitish, aerial mycelium on surface, fruiting bodies black, concentric; reverse of culture yellow to pale brown.

Specimen examined. China, Hainan Province: Five Fingers Group Scenic Area, 20 May 2021, Z.X. Zhang. On diseased leaves of *Ficus microcarpa*, HSAUP210112, living culture SAUCC210112; on diseased leaves of *Ficus microcarpa*, HSAUP210113, living culture SAUCC210113.

Notes. In the present study, two strains (SAUCC210112 and SAUCC210113) from symptomatic leaves of *Ficus microcarpa* were clustered with *Neopesataliotiopsis piceana*.
clade (Maharachch. et al. 2014) based on phylogeny (Fig. 1). Morphologically, our strains were the same as *N. piceana*, which was originally described with an asexual morph on wood of *Picea* sp., *Cocos nucifera* and fruit of *Mangifera indica*. The sexual morph of *N. piceana* was undetermined yet. *Neopestalotiopsis piceana* was a new record for China and first reported from *Ficus macrocarpa* (Moraceae).

**Figure 4.** *Neopestalotiopsis piceana* (SAUCC210112) a diseased leaf of *Ficus microcarpa* b surface of colony after 7 days on PDA c reverse of colony after 7 days on PDA d conidiomata e–g conidiogenous cells with conidia h–j conidia. Scale bars: 10 μm (e–j).

*Pestalotiopsis licualicola* K. Geng, Y. Song, K.D. Hyde & Yong Wang bis, *Phytotaxa* 88 (3):51. (2013)

Fig. 5

**Description.** Leaf spots irregular, pale brown in centre, brown to tan at margin. Asexual morph on PDA: Conidiomata solitary, scattered, black, raising above surface of culture
medium, subglobose. Conidiophores cylindrical, hyaline, smooth-walled. Conidiophores often indistinct. Conidiogenous cells discrete, hyaline, simple, filiform, 5.5–10.0 μm long. Conidia 18.0–24.5 × 4.0–5.5 μm, mean ± SD = 20.5 ± 1.9 × 5.3 ± 0.3 μm, fusiform, straight to slightly curved, 4-septate, smooth, greyish brown; basal cell conical, hyaline, thin-walled, 2.8–6.0 μm long; with three median cells, dark brown, concolorous, septa and periclinal walls darker than the rest of the cell, together 11.5–16.0 μm long, mean ± SD = 13.2 ± 1.2 μm; second cell from base 3.4–5.5 μm; third cell 3.3–4.7 μm; fourth cell 3.5–5.1 μm; apical cell hyaline, conic to subcylindrical, 3.1–5.3 μm; with 1–3

**Figure 5.** *Pestalotiopsis licualicola* (SAUCC210087) a diseased leaf of *Ilex chinensis* b surface of colony after 7 days on PDA c reverse of colony after 7 days on PDA d conidiomata f, g, j, k conidiogenous cells with conidia e, h, i, l, m conidia. Scale bars: 10 μm (e–m).
tubular apical appendages (mostly 1) without knobs, arising from the apex of the apical cell, 10.0–20.5 μm long, mean ± SD = 16.0 ± 4.0 μm; basal appendage filiform, short.

**Culture characteristics.** Colonies on PDA reaching 70.0–80.0 mm diam after 7 d at 25 °C, growth rate 9.0–12.0 mm/day, edge entire, whitish to pale honey coloured, with sparse aerial mycelium on the surface, with black, gregarious conidiomata; reverse similar in colour.

**Specimen examined.** China, Hainan Province: East Harbour National Nature Reserve, 23 May 2021, Z.X. Zhang. On diseased leaves of *Ilex chinensis*, HSAUP210087, living culture SAUCC210087; on diseased leaves of *Ilex chinensis*, HSAUP210088, living culture SAUCC210088.

**Notes.** In the present study, two strains (SAUCC210087 and SAUCC210088) from symptomatic leaves of *Ilex chinensis* were clustered to *Pestalotiopsis licualicola* clade (Geng et al. 2013) based on phylogeny (Fig. 1). Morphologically, our strains were the same as *P. licualicola*, which was originally described with an asexual morph on leaves of *Licuala grandis* in China. The sexual morph of *P. licualicola* was undetermined yet. This is the first time this species has been reported in *Ilex chinensis* (Aquifoliaceae) in China.

**Discussion**

Based on phylogeny and morphology, nine strains from three host species (*Ficus microcarpa*, *Ilex chinensis* and *Schima superba*) were described as well as two new species (*Monochaetia schima* sp. nov. and *Neopestalotiopsis baikouensis* sp. nov.) and two known species (*Neopestalotiopsis piceana* and *Pestalotiopsis licualicola*). In the genus *Monochaetia*, most species were found on Fagaceae hosts, including *Castanea pubinervis* (*Monochaetia dimorphospora*), *Castanea mollissima* (*Monochaetia castaneae*), *Quercus pubescens* (*Monochaetia monochaeta*) and etc. In our study, the species of *Monochaetia* (*M. schima*) was first reported from *Schima superba* (Theaceae). *Ilex* was widely grown as an evergreen tree all over the world and isolated many pathogens, endophytes or saprophytes (Alfieri et al. 1984; Maharachch. et al. 2014; de Silva et al. 2017; Solarte et al. 2018). More than 100 strains (Xylariales) have been isolated from the genus *Ilex*. Among these, there was 13 pestalotia-like fungi, and we compare morphology with my new collection. In morphology, the conidia size of *Pestalotiopsis humicola* is similar to *Neopestalotiopsis baikouensis*. Phylogenetic analyses of Maharachch. et al. (2014) and the current study show *Neopestalotiopsis* and *Pestalotiopsis* are different genus. The known species *Neopestalotiopsis piceana* was described from *Picea* sp. (Pinaceae) in United Kingdom (Maharachch. et al. 2014) and *Pestalotiopsis licualicola* was described from *Licuala grandis* (Palmaceae) in China (Geng et al. 2013). In this study, *Neopestalotiopsis piceana* was a new record for China and first reported from *Ficus macrocarpa* (Moraceae), *Pestalotiopsis licualicola* was first reported from *Ilex chinensis* (Aquifoliaceae) in China, so we described and illustrated *N. piceana* and *P. licualicola* again. Species in genera have multi-septate and more or less fusiform conidia with a single apical and basal appendage (*Monochaetia, Seiridium*); other genera do not form appendages (*Nonappendiculata*) or have 2–4 appendages (*Pestalotiopsis*, *Ciliochorella*, *Nonappendiculata*),
Neopestalotiopsis, Pseudopestalotiopsis) (Maharachch. et al. 2014; Bonthond et al. 2018; Liu et al. 2019). Our study supported this phenomenon.

As many pestalotioid species have overlapping morphological traits, sequence data is essential to resolve these three genera and introduce new species (Jeewon et al. 2002; de Silva et al. 2017; Norphanphoun et al. 2019). Combined gene sequences of ITS, tub2 and tef1 can provide a better resolution for Monochaetia. However, more genes are needed to provide better resolution and support in Neopestalotiopsis. In the previous studies, members of Sporocadaceae are of particular interest with regard to the production of secondary metabolites, e.g. Bartalinia, Morinia and Pestalotiopsis (Collado et al. 2006; Gangadevi and Muthumary 2008; Liu et al. 2009). Pestalotiopsis fici was shown to possess a very high number of gene clusters involved in bioactive compound synthesis (Wang et al. 2016). Owing to Pestalotiopsis and other genus in this family sharing the same evolutionary history, it is important to report novel species and screen for novel metabolites in future studies.

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Supplementary material 1

The combined ITS, tub2 and tef1 sequences

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Data type: phylogenetic

Explanation note: The combined ITS, tub2 and tef1 sequences.

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