Taxonomic Paper

Two new species of *Neopestalotiopsis* from southern China

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Abstract

Background

*Pestalotiopsis*-like fungi are widely distributed in many plants and include endophytes, pathogens and saprobes. Five strains of *Neopestalotiopsis* were isolated from diseased leaves of *Rhapis excelsa* (Principes, Palmae), *Rhododendron simsii* and *Rho. championiae* (Ericales, Ericaceae) and *Erythropalum scandens* (Santalales, Olacaceae) in southern China.

New information

Based on morphology and multi-gene (ITS, *tub2*, *tef1*) phylogeny, our five strains of *Neopestalotiopsis* represent two new species and one extant species. Descriptions, illustrations and notes are also provided for the new species.

Keywords

two new taxa, Sporocadaceae, taxonomy

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Introduction

Sporocadaceae was introduced by Corda (1842) and comprised abundant endophytic, plant pathogenic or saprobic taxa (Liu et al. 2019). A great part of Sporocadaceae species were reported as important plant pathogenic fungi that mainly harm various economic crops, such as tea, blueberry and elephant apple (Fernández et al. 2015, Banerjee et al. 2018, Tsai et al. 2020). Jaklitsch et al. (2016) synonymised Bartaliniaceae, Discosiaceae, Pestalotiopsidaceae and Robillardaceae under Sporocadaceae. Liu et al. (2019) studied the taxonomy of Sporocadaceae and accommodated 30 genera in it. Hyde et al. (2020) and Wijayawardene et al. (2020) placed Sporocadaceae in Amphisphaeriales and accepted 33 genera.

Neopestalotiopsis was introduced by Maharachchikumbura et al. (2014) to accommodate pestalotiopsis-like taxa that had versicolorous median cells and indistinct conidiophores. Until now, 49 taxa of Neopestalotiopsis are known (Mycobank 2021: https://www.mycobank.org/page/Home). This group commonly occurs on plants as endophytes, pathogens or saprobes (Jeewon et al. 2004, Liu et al. 2010, Hyde et al. 2016, Reddy et al. 2016, Shetty et al. 2016, Ran et al. 2017, Bezerra et al. 2018, Freitas et al. 2019). Recently, research showed them as plant pathogens causing stem blight, flower bight, twig dieback and fruit rot (Akinsanmi et al. 2016, Borrero et al. 2017, Mahapatra et al. 2018, Rodríguez-Gálvez et al. 2020). In the past few years, China and Thailand are places where most species of Neopestalotiopsis were found (Norphanphoun et al. 2019).

Amongst surveys of microfungi in southern China, we made five collections of Neopestalotiopsis from four host plants. Based on morphological descriptions and molecular analyses of three gene loci, our strains represent two new species and one known species.

Materials and methods

Sample collection and fungi isolation

Diseased leaf samples with fruiting bodies were collected from major botanical gardens in Yunnan, Guangxi and Guizhou Provinces in southern China. After surface disinfection of the diseased tissues (Zhang et al. 2020), the single-spore method was used for obtaining a pure culture (Senanayake et al. 2020). The isolates were transferred to new potato dextrose agar (PDA) plates to obtain a pure strain.

Morphology study

Cultures growing on potato dextrose agar (PDA) were incubated under moderate temperatures (28°C) for 2–4 weeks in 12 h daylight. The diameter of cultures was measured after 1 week and the colour was determined with the colour charts of Rayner (1970). The morphological features were noted and recorded following Hu et al. (2007). Microscopic preparations were prepared in lactophenol and over 30 measurements were
obtained per structure. Photographs were taken using a compound microscope (Olympus BX53, Japan). The holotype specimens were deposited in the Herbarium of Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). Ex-type cultures were deposited in the Culture Collection at the Department of Plant Pathology, Agriculture College, Guizhou University, China (GUCC).

DNA extraction and PCR amplification

DNA extraction and PCR amplification follow Dissanayake et al. (2020) with some minor changes. A Fungus Genomic DNA Extraction Kit (Biomiga#GD2416, San Diego, California, USA) was used to extract fungal genome DNA. DNA amplification was performed in a 25 µl reaction mixture which contains 2.5 µl 10 × PCR buffer, 1 µl of each primer (10 µM), 1 µl template DNA and 0.25 µl Taq DNA polymerase (Promega, Madison, WI, USA). The ITS rDNA region was amplified using primer pairs ITS4 and ITS5 (White et al. 1990). The partial tub2 gene region was amplified with primer pairs T1 and Bt2b (Glass and Donaldson 1995, O'Donnell and Cigelnik 1997). The tef1 gene fragment was amplified using the primer pairs EF1-728F and EF-2 (O'Donnell et al. 1998, Carbone and Kohn 1999). PCR amplification conditions were performed according to the methods described by Norphanphoun et al. (2019). The PCR products were sent to SinoGenoMax company (Beijing, China) which used the fluorescently-labelled Sanger method for sequencing. The resulting DNA sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and their accession numbers were provided in Table 1.

| Species name                     | Strain number | GenBank Accession numbers | Reference                          |
|----------------------------------|---------------|---------------------------|-----------------------------------|
| Neopestalotiopsis acrostichi     | MFLUCC 17-1754T | MK764272, MK764338, MK764316 | Norphanphoun et al. (2019)         |
|                                  | MFLUCC 17-1755 | MK764273, MK764339, MK764317 | Norphanphoun et al. (2019)         |
| N. alpapicalis                   | MFLUCC 17-2544 | MK357772, MK463545, MK463547 | Kumar et al. (2019)               |
|                                  | MFLUCC 17-2545 | MK357773, MK463546, MK463548 | Kumar et al. (2019)               |
| N. aotearoa                      | CBS 367.54T   | KM199369, KM199454, KM199526 | Maharachchikumbura et al. (2014)  |
|                                  | HNPeHNLD2001  | MT764947, MT796262, MT800516 | Direct submission                 |
| N. asiatica                     | MFLUCC 12-0286T | JX398983, JX399018, JX399049 | Maharachchikumbura et al. (2012)  |
| N. australis                     | CBS 114159T   | KM199348, KM199432, KM199537 | Maharachchikumbura et al. (2014)  |
| N. brachiata                     | MFLUCC 17-1555T | MK764274, MK764340, MK764318 | Norphanphoun et al. (2019)         |
| Species name           | Strain number | GenBank Accession numbers | Reference                  |
|-----------------------|---------------|---------------------------|----------------------------|
| **ITS**               |               |                           |                            |
| *N. brasiliensis*     | COAD 2166<sup>T</sup> | MG688469 MG692400 MG692402 | Bezerra et al. (2018)      |
| *N. chiangmaiensis*   | MFLUCC 18-0113<sup>T</sup> | - MH412725 MH388404 | Tibpromma et al. (2018)    |
| *N. chrysea*          | MFLUCC 12-0261<sup>T</sup> | JX398985 JX399020 JX399051 | Maharachchikumbura et al. (2012) |
| *N. clavispora*       | MFLUCC 12-0281<sup>T</sup> | JX398979 JX399014 JX399045 | Maharachchikumbura et al. (2012) |
| *N. egyptiaca*        | COAD 2167     | MG688470 MG692401 MG692403 | Silva et al. (2018)        |
| *N. ellipsospora*     | MFLUCC 12-0284 | JX398981 JX399015 JX399046 | Maharachchikumbura et al. (2012) |
| *N. eucalypticola*    | CBS 126.37<sup>T</sup> | KM199376 KM199431 KM199551 | Maharachchikumbura et al. (2014) |
| *N. foedans*          | CGMCC3.9178   | JX398989 JX399024 JX399055 | Maharachchikumbura et al. (2014) |
| *N. formicarum*       | CBS 362.72<sup>T</sup> | KM199358 KM199455 KM199517 | Maharachchikumbura et al. (2014) |
| *N. honoluluana*      | CBS 111535    | KM199363 KM199461 KM199546 | Maharachchikumbura et al. (2014) |
| *N. iranensis*        | CBS 137768<sup>T</sup> | KM074048 KM074057 KM074051 | Ayoubi and Soleimani (2016) |
| *N. javaensis*        | CBS 257.31<sup>T</sup> | KM199357 KM199437 KM199543 | Maharachchikumbura et al. (2014) |
| *N. ketleieeria*      | MFLUCC 12-0594 | KX816905 KX816933 KX816874 | Maharachchikumbura et al. (2014) |
| *N. macadamiae*       | BRIP 63737c<sup>T</sup> | KX186604 KX186654 KX186627 | Akinsanmi et al. (2017)     |
| Species name         | Strain number  | GenBank Accession numbers | Reference                        |
|----------------------|----------------|---------------------------|----------------------------------|
|                      |                | ITS | tub2  | tef1  |                                   |
| BRIP 63757a          |                | X186592 | X186674 | X186647 | Akinsanmi et al. (2017)          |
| *N. magna*           | MFLUCC 12-0652<sup>T</sup> | KF582795 | KF582793 | KF582791 | Maharachchikumbura et al. (2014) |
| *N. mesopotamica*    | CBS 336.88<sup>T</sup> | KM199362 | KM199441 | KM199555 | Maharachchikumbura et al. (2014) |
|                      | CBS 299.74     | KM199361 | KM199435 | KM199541 | Maharachchikumbura et al. (2014) |
| *N. musae*           | MFLUCC 15-0776<sup>T</sup> | KX789683 | KX789686 | KX789685 | Hyde et al. (2016)               |
| *N. natalensis*      | CBS 138.41<sup>T</sup> | KM199377 | KM199466 | KM199552 | Maharachchikumbura et al. (2014) |
| *N. nebuloides*      | BRIP 66617<sup>T</sup> | MK966338 | MK977632 | MK977633 | Crous et al. (2020)              |
| *N. pandanicola*     | KUMCC 17-0175<sup>T</sup> | - | MH412720 | MH388389 | Tibpromma et al. (2018)          |
| *N. pernambucana*    | URM7148        | - | - | KU306739 | Silvério et al. (2016)           |
| *N. petila*          | MFLUCC 17-1737<sup>T</sup> | MK764275 | MK764341 | MK764319 | Norphanphoun et al. (2019)       |
|                      | MFLUCC 17-1738 | MK764276 | MK764342 | MK764320 | Norphanphoun et al. (2019)       |
| *N. phangngaensis*   | MFLUCC 18-0119<sup>T</sup> | MH388354 | MH412721 | MH388390 | Tibpromma et al. (2018)          |
|                      | MFLUCC 19-2741 | - | - | MW148259 | MW192200 | Direct submission               |
| *N. piceana*         | CBS 394.48<sup>T</sup> | KM199368 | KM199453 | KM199527 | Maharachchikumbura et al. (2014) |
|                      | CBS 254.32     | KM199372 | KM199452 | KM199529 | Maharachchikumbura et al. (2014) |
| *N. protearum*       | CBS 114178<sup>T</sup> | JN712498 | KM199463 | KM199542 | Maharachchikumbura et al. (2014) |
|                      | CBS 111506     | MH553959 | MH554618 | MH554377 | Liu et al. (2019)                |
| *N. raphidis*        | GUCC 21501     | MW931620 | MW980441 | MW980442 | This study                       |
| *N. rhizophorae*     | MFLUCC 17-1551<sup>T</sup> | MK764277 | MK764343 | MK764321 | Norphanphoun et al. (2019)       |
|                      | MFLUCC 17-1550 | MK764278 | MK764344 | MK764322 | Norphanphoun et al. (2019)       |
| *N. rhododendri*     | GUCC 21504     | MW979577 | MW980443 | MW980444 | This study                       |
|                      | GUCC 21505     | MW979576 | MW980445 | MW980446 | This study                       |
| *N. rosae*           | CBS 101057<sup>T</sup> | KM199359 | KM199429 | KM199523 | Maharachchikumbura et al. (2014) |
|                      | CBS 124745     | KM199360 | KM199430 | KM199524 | Maharachchikumbura et al. (2014) |
| *N. rosicola*        | CFCC 51992     | KY885239 | KY885245 | KY885243 | Jiang et al. (2018)              |
| Species name      | Strain number | GenBank Accession numbers | Reference                          |
|-------------------|---------------|---------------------------|-----------------------------------|
| Neopestalotiopsis | CFCC 51993    | KY885240 KY885246 KY885244 | Jiang et al. (2018)               |
| N. samarangensis  | CBS 115451    | KM199365 KM199447 KM199556 | Maharachchikumbura et al. (2014) |
| N. saprophytica   | SS010         | JQ968609 JQ968610 JQ968611 | Direct Submission                 |
| N. sichuanensis   | SS010         | JQ968609 JQ968610 JQ968611 | Direct Submission                 |
| N. sonneratae     | MFLUCC 17-1744 | MK764279 MK764345 MK764323 | Norphanphoun et al. (2019)        |
| N. steyaertii     | IMI 192475T   | KF582796 KF582794 KF582792 | Jiang et al. (2021)               |
| N. surinamensis   | CBS 450.74T   | KM199351 KM199465 KM199518 | Maharachchikumbura et al. (2014) |
| N. thailandica    | MFLUCC 17-1730T | MK764281 MK764347 MK764325 | Norphanphoun et al. (2019)        |
| N. umbrinospora   | MFLUCC 12-0285T | JX399884 JX399019 JX399050 | Maharachchikumbura et al. (2014) |
| N. vitis          | MFLUCC 17-1108 | MG807045 MG859849 MG859769 | Jayawardena et al. (2016)         |
| N. zimbabwana     | CBS 111495T   | - KM199456 KM199545       | Maharachchikumbura et al. (2014)  |
| Pestalotiopsis    | MFLUCC 12-0287T | JX399009 JX399040 JX399073 | Maharachchikumbura et al. (2012)  |
| P. trachicarpica  | OP068 T       | JQ845947 JQ845945 JQ845946 | Zhang et al. (2012)               |
Sequence alignment and phylogenetic analyses

The reference sequences were downloaded from GenBank for phylogenetic analyses (Table 1). Multiple sequence alignments were generated with MAFFT v. 7.307 online version (Katoh and Standley 2016) and manually improved in MEGA v. 6.06, where necessary (Tamura et al. 2013). Concatenated multi-locus datasets for the three gene regions were aligned using Mesquite v. 2.75 (Maddison 2008). Manual improvement, when necessary, was done using AliView (Larsson 2014). Terminal ends and ambiguous regions of the alignment were deleted manually. Phylogenetic analyses were performed using concatenated sequences of the three loci (ITS, tub2 and tef1) with Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI).

Maximum Likelihood analysis was performed at the CIPRES Science Gateway web portal (Miller et al. 2010) using RAxML-HPC BlackBox v. 8.2.12 with the GTR+G+I model and 1,000 rapid bootstrap (BS) replicates (Stamatakis 2014).

Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001). Parameters of Bayesian analysis in MrBayes v. 3.2; Markov chains were run for 1000000 generations and trees were sampled every 100th generation (printfreq = 100) and 10000 trees were obtained. The last standard deviation of split frequencies was below 0.01. Initial trees were discarded (25% burn-in value) and the remaining trees were used to evaluate posterior probabilities (PP) in the majority rule consensus tree.

PAUP v. 4.0b10 (Swofford 2002) was used to perform Maximum Parsimony (MP) analyses. Trees were inferred by using the heuristic search option with 1,000 random sequence additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. The maxtrees were set as 5000. Descriptive tree statistics for parsimony (tree length (TL), consistency index (CI), retention index (RI), related consistency index (RC) and homoplasy index (HI)) were calculated.

Taxon treatments

*Neopestalotiopsis rhapidis* Qi Yang & Yong Wang bis, sp. nov.

- MycoBank 840065

**Material**

**Holotype:**

- scientificName: *Neopestalotiopsis rhapidis*; order: Amphisphaeriales; family: Sporocadaceae; genus: *Neopestalotiopsis*; country: China; stateProvince: Guangxi; locality: Nanning City, Guangxi Medicinal Botanical Garden; verbatimCoordinates: 108°19' E,22°51' N; recordedBy: Qi Yang; identifiedBy: Qi Yang; dateIdentified: 2021; collectionID: HGUP 332; occurrenceID: GUCC 21501
Description

**Disease symptom**: Pathogenic causing spots on leaves tip of *Rhapis excelsa*. Leaf spots shape irregular, brown, slightly sunken on leaves tip. Small brown spots appeared initially and then gradually enlarged, changing to dark brown spots with a yellow border and jagged edge.

*Colonies* on PDA reach 7.5–8 cm in diam. after 7 d at room temperature (28°C), under light 12 hr/dark. *Colonies* filamentous to circular, whitish, with clustered black fruiting bodies and filiform and fluffy margin, white from above and light yellow from the reverse. Sexual morph: undetermined. Asexual morph (Fig. 1): *Conidiomata* 560–1405 µm in diam., pycnidial, globose, solitary, black, semi-immersed on PDA, exuding brown to dark brown conidia. *Conidiophores* branched or unbranched, hyaline, thin-walled. *Conidiogenous cell* discrete to lageniform, obclavate, hyaline or rarely light brown, smooth-walled. *Conidia* (22–)25.5 × 4(–6) µm (x = 23 × 5.2 µm, n = 30), fusiform to clavate, straight to slightly curved, 4-septate; basal cell cylindrical to obconic, hyaline, thin-walled, smooth, 3–5 µm (x = 3.7 µm, n = 30); the three median cells 11.5–15 µm (x = 13.3 µm, n = 30), dark brown with septa darker than the rest of the cells, the second cell from base 3–5 µm (x = 4 µm, n = 30); the third cell 2.5–6 µm (x = 3.9 µm, n = 30); the fourth cell 3–4.5 µm (x = 3.8 µm, n = 30); apical cell 2–4.5 µm (x = 3.3 µm, n = 30), cylindrical, hyaline; 2–3 (mostly 3) tubular apical appendages, arising from the apex of the apical cell each at different points, flexuous, 11–16 µm (x = 13.6 µm, n = 30); basal appendage present, single, tubular, unbranched, 2–5.5 µm (x = 4 µm, n = 30).

Figure 1. *Neopestalotiopsis rhapidis* (GUCC 21501). a. Leaf spots of *Neopestalotiopsis rhapidis*; b, c. Culture on PDA (b-above, c-reverse); d. Colony sporulating on PDA; e–g. Conidiophores; h–k. Conidia. Scale bars: d = 1000 µm, e–k = 20 µm.
Etymology

Latin, raphidis, refers to the host plant (Rhapis excelsa) from which the fungus was isolated.

Notes

Neopestalotiopsis raphidis clustered with N. cocoes (MFLUCC 15-0152) with 85% ML support, although without enough MP and BI support. Within comparison of the three gene regions, there were only three character differences in the ITS region, but 27 in the tef1 region. Neopestalotiopsis raphidis has longer conidia and shorter apical appendages than those of N. cocoes (19–22.5 ×7.5–9.5 µm; 14.9–21 µm) (Hyde et al. 2016). Thus, Neopestalotiopsis raphidis (GUCC 21501) is introduced as a new species herein.

Neopestalotiopsis rhododendri Qi Yang & Yong Wang bis, sp. nov.

- MycoBank 840066

Materials

Holotype:

a. scientificName: Neopestalotiopsis rhododendri; order: Amphisphaeriales; family: Sporocadaceae; genus: Neopestalotiopsis; country: China; stateProvince: Yunnan; locality: Kunming; verbatimCoordinates: 102°72' E,25°05' N; recordedBy: Qi Yang; identifiedBy: Qi Yang; dateIdentified: 2021; collectionID: HGUP 134; occurrenceID: GUCC 21504

Other material:

a. scientificName: Neopestalotiopsis rhododendri; order: Amphisphaeriales; family: Sporocadaceae; genus: Neopestalotiopsis; country: China; stateProvince: Guizhou; locality: Kaili; verbatimCoordinates: 107°97' E,26°58' N; recordedBy: Qi Yang; identifiedBy: Qi Yang; dateIdentified: 2021; collectionID: HGUP 997; occurrenceID: GUCC 21505

Description

Disease symptom: Associated with leaf spots of Rhododendron simsii. The leaf spots are small irregular to subcircular shape, brown, slightly sunken spots appear on surface leaves of R. simsii, which scattered on the surface leaves tip and eventually develops into a large lesion. Small off-white spots appeared initially and then gradually enlarged, changing to light brown circular ring spots with a dark brown border.

Colonies on PDA reaching 6.5–7 cm in diam. after 7 d at room temperature (28°C), under light 12 hr/dark. Hyphae white, colonies filamentous to circular, slightly undulate at the edge, with black fruiting bodies clustered, has filiform and fluffy margin, white from above and light yellow from the reverse. Sexual morph: undetermined. Asexual morph ( Fig. 2 ) : Conidiomata 55–280 µm in diam., pycnidial, globose, solitary, black,
semi-immersed on PDA, exuding brown to dark brown mass of conidia. *Conidiophores* often reduced to conidiogenous cell, regularly septate and branched at the base. *Conidiogenous cells* mostly integrated, ampulliform, cylindrical, hyaline to light brown, smooth-walled. *Conidia* (25.5–)30 × 5(–6) µm (\( \bar{x} = 27.6 \times 5.5 \) µm, \( n = 30 \)), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic, hyaline, thin-walled, smooth, 3.5–6.5 µm (\( \bar{x} = 4.5 \) µm, \( n = 30 \)); the three median cells 13.5–19.5 µm (\( \bar{x} = 16.3 \) µm, \( n = 30 \)), light brown to dark brown with septa darker than the rest of the cells, the second cell from base 4–6 µm (\( \bar{x} = 5 \) µm, \( n = 30 \)); the third cell 3.5–5.5 µm (\( \bar{x} = 4.5 \) µm, \( n = 30 \)); the fourth cell 4–6.5 µm (\( \bar{x} = 4.8 \) µm, \( n = 30 \)); apical cell 3.5–6.3 µm (\( \bar{x} = 5 \) µm, \( n = 30 \)), cylindrical to sub-cylindrical, hyaline, 1–3 (mostly 2) tubular apical appendages, arising from the apex of the apical cell each at different points, 21–38.5 µm (\( \bar{x} = 29.2 \) µm, \( n = 30 \)); basal appendage present most of the time, single, tubular, unbranched, 6–11.5 µm (\( \bar{x} = 8.5 \) µm, \( n = 30 \)).

![Image of Neopestalotiopsis rhododendri](https://example.com/neopestalotiopsis-rhododendri)

**Etymology**

China, Yunnan Province, Kunming City, from leaves of *Rhododendron simsii*, 12 February 2018, Q. Zhang, HGUP 134, holotype, ex-type living culture GUCC 21504.
Notes

In the multi-gene analysis, strain GUCC 21504 formed a distinct clade with a sister strain GUCC 21505, but the node support values were 68/90/- (MP/ML/BI) and these two strains were close to *N. protearum* (CBS 114178). When comparing the polymorphic nucleotide differences of our two strains, there are 18 base pair differences, seven in ITS, two in *tub2* and nine in *tef1*, but without obvious distinction (higher than 98.5%). Compared with *N. protearum* and our ex-type strain (GUCC 21504), there were six character differences with *N. protearum* in the ITS region, three character differences with *N. protearum* in the *tub2* region, but 12 character differences from *N. protearum* in the *tef1* region; thus the DNA base pair differences were mainly in the *tef1* gene regions. The morphological differences between our strains and *N. protearum* were wider conidia (*N. protearum*: 24.8 ± 1.5 × 8.5 ± 0.6 µm), more apical appendages (*N. protearum*: 3–5) and shorter basal appendages (*N. protearum*: 5–8 µm) (Maharachchikumbura et al. 2014). Thus, *Neopestalotiopsis rhododendri* is introduced as a novel taxon, based on morphology and phylogeny.

**Neopestalotiopsis saprophytica** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, 2014

- MycoBank 809780

Materials

a. scientificName: *Neopestalotiopsis saprophytica*; order: **Amphisphaeriales**; family: Sporocadaceae; genus: *Neopestalotiopsis*; country: China; stateProvince: Guangxi; locality: Nanning City, Guangxi Medicinal Botanical Garden; verbatimCoordinates: 108°19' E,22°51' N; recordedBy: Qi Yang; identifiedBy: Qi Yang; dateIdentified: 2021; collectionID: HGUP 423; occurrenceID: GUCC 21506

b. scientificName: *Neopestalotiopsis saprophytica*; order: **Amphisphaeriales**; family: Sporocadaceae; genus: *Neopestalotiopsis*; country: China; stateProvince: Guangxi; locality: Nanning City, Guangxi Medicinal Botanical Garden; verbatimCoordinates: 108°19' E,22°51' N; recordedBy: Qi Yang; identifiedBy: Qi Yang; dateIdentified: 2021; collectionID: HGUP 133; occurrenceID: GUCC 21507

Description

**Disease symptom**: Pathogenic causing spots on leaves of *Erythropalum scandens*. Leaf spots shape irregular, brown to reddish-brown, slightly sunken spots appear on surface leaves of *E. scandens*, which scattered on the leaves tip. Small brown spots appeared initially and then gradually enlarged, changing to reddish-brown spots with a yellow border.

Colonies on PDA reaching 7.5–8 cm in diam. after 7 d at room temperature (28°C), under light 12 hr/dark. Hyphae change from light pink to off-white. Colonies filamentous to circular, slightly undulate at the edge, with black fruiting bodies clustered, filiform margin, light pink from above and light yellow from the reverse. Sexual morph: undetermined. Asexual morph (Fig. 3): Conidiomata up to 280 µm in diam., pycnidial,
globose, solitary, black, semi-immersed on PDA, exuding brown to dark brown mass of conidia. *Conidiophores* branched or unbranched, hyaline, thin-walled. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, thin-walled, smooth. *Conidia* (21.5–)26.5 × 4.5(–6.5) µm (x̄ = 23.2 × 5.2 µm, n = 30), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic, hyaline or sometimes pale brown, thin-walled, smooth, 3–5 µm (x̄ = 4 µm, n = 30); the three median cells 13–17 µm (x̄ = 14.9 µm, n = 30), pale brown to brown, dark brown with septa darker than the rest of the cells, the second cell from base 4–6.5 µm (x̄ = 4.9 µm, n = 30); the third cell 3–5 µm (x̄ = 4.1 µm, n = 30); the fourth cell 3.5–6 µm (x̄ = 4.8 µm, n = 30); apical cell 3–5 µm (x̄ = 3.9 µm, n = 30), cylindrical, hyaline; 1–4 (mostly 3) tubular apical appendages, arising from the apex of the apical cell each at different point, flexuous, 18–28.5 µm (x̄ = 22.4 µm, n = 30); basal appendage present most of the time, single, tubular, unbranched, 3.3–7 µm (x̄ = 4.3 µm, n = 30).

**Notes**

GUCC 21506 and GUCC 21507 with the same nucleotides sequences were related to *N. dendrobii* (MFLUCC 14-0106) and *N. saprophytica* (CBS 115452). There were ten character differences with *N. dendrobii* and 11 character differences with *N. saprophytica*, but the most differences (nine character differences) between our strains and *N. saprophytica* were only in the *tef1* region. Alternatively, collection differed to *N. dendrobii* in having more apical appendages (*N. dendrobii*: 2–3) and much longer apical appendages (*N. dendrobii*: 6 ± 0.9 µm) (Ma et al. 2019). Morphological
characters of our collections and \textit{N. saprophytica} overlapped (Maharachchikumbura et al. 2014). Thus, GUCC 21506 and GUCC 21507 are considered as \textit{N. saprophytica}.

Analysis

Phylogenetic analyses

The final dataset consists of 57 taxa, including \textit{Pestalotiopsis diversiseta} (MFLUCC 12-0287) and \textit{P. trachicarpicola} (OP068) as the outgroup taxa. It comprised 2052 characters including gaps (\textit{tef1}: 1–606, \textit{tub2}: 607–1443 and ITS: 1444–2052). There were 1426 constant, 284 parsimony uninformative and 342 parsimony informative characters (TL = 1225 steps, CI = 0.66, RI = 0.70, RC= 0.46 and HI= 0.34). The most parsimonious tree generated from combined ITS, \textit{tub2} and \textit{tef1} sequence data of species of \textit{Neopestalotiopsis} is shown in Fig. 4.

In the phylogenetic analyses, GUCC 21501 was sister to \textit{N. cocoes} (MFLUCC 15-0152\textsuperscript{T}), but only with a 85% ML bootstrap support. GUCC 21504 and GUCC 21505 formed an independent clade with MP and ML (68/90) supports and were close to \textit{N. protearum} (CBS 111506\textsuperscript{T}). GUCC 21506 and GUCC 21507 clustered with moderate and high supports (65/99/1: MP/ML/BI) and kept a very close relationship with \textit{N. saprophytica} (CBS 115452) by credible statistic support (100/67/1: MP/ML/BI). DNA sequence differences between our strains and related species are listed in Table 2.
Table 2.
DNA sequence differences of the three gene regions between our strains and related species.

| Species                   | Strain number | tef1 (characters: 1-606) | tub2 (characters: 607-1443) | ITS (characters: 1444-2052) |
|---------------------------|---------------|---------------------------|----------------------------|----------------------------|
| *N. rhapidis*             | GUCC 21501    | 0                         | 0                          | 0                          |
|                           | MFLUCC 15-0152T | 27 (gaps: 2)             | -                          | 3 (gaps: 3)                |
| *N. saprophytica*         | GUCC 21506    | 0                         | 0                          | 0                          |
|                           | GUCC 21507    | 0                         | 0                          | 0                          |
| *N. dendrobii*            | MFLUCC 14-0106T | 5 (gaps: 3)             | 4 (gap: 1)                 | 1 (gap: 0)                |
| *N. saprophytica*         | CBS 115452    | 9 (gaps: 3)               | 1 (gap: 0)                 | 1 (gap: 1)                |
| *N. rhododendri*          | GUCC 21504    | 0                         | 0                          | 0                          |
|                           | GUCC 21505    | 9 (gap: 0)                | 2 (gap: 0)                 | 7 (gap: 1)                |
| *N. protearum*            | CBS 114178T   | 12 (gaps: 6)              | 3 (gap: 0)                 | 9 (gaps: 2)                |

Discussion

Hu et al. (2007) believed that pestalotiopsis-like fungi had different phenotypes in conidial morphology. Maharachchikumbura et al. (2014) summarised some stable characteristics for determining pestaloids, such as the length and width of conidia, length of the apical appendages, presence or absence of knobbed apices and the position of the apical appendage attached to the conidial body. However, as these characteristics were often similar or overlapped, sequence data are crucial for the identification of pestalotioid, and as well as for the introduction of new species (Norphanphoun et al. 2019).

In this study, we describe two new species as *Neopestalotiopsis rhapidis* and *N. rhododendri*. The species were distinct from extant *Neopestalotiopsis* species, based on morphological and phylogenetic analyses. However, the statistical support of main nodes for the genus were very low (Fig. 4). The reason might be that the reference sequences we used were short, including the short *tef1* and *tub2* sequences (Ran et al. 2017). Longer sequences with more informative data are needed to solve this problem. Furthermore, our study also found that the evolutionary relationships amongst species of *Neopestalotiopsis are unstable* (Maharachchikumbura et al. 2014, Jiang et al. 2018, Kumar et al. 2019, Tsai et al. 2020). Therefore, other genes are needed to distinguish the inter-species
relationships in *Neopestalotiopsis* (Maharachchikumbura et al. 2014, Kumar et al. 2019, Norphanphoun et al. 2019).

Several indicators could be used in the classification of *Neopestalotiopsis* in this study, such as the size of conidia and the number and length of appendages (Maharachchikumbura et al. 2014, Freitas et al. 2019, Kumar et al. 2019). The differences in the colour of three median cells and the length of other cells, however, lacked significant variation to clearly distinguish the species of *Neopestalotiopsis*. Therefore, as the morphological identification alone cannot accurately identify the fungi of the genus *Neopestalotiopsis*, it must be combined with the phylogenetic tree (Liu et al. 2019, Norphanphoun et al. 2019, Tsai et al. 2020, Jiang et al. 2021).

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