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New Species of Nectriaceae (Hypocreales) from China

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Abstract: Species of Nectriaceae commonly occur on living and decaying woody substrates, soil, fruitbodies of other fungi, and insects. Some of them are reported as endophytes, opportunistic pathogens of crops and humans, or producers of mycotoxins. To explore the species diversity of the family, specimens from different regions of China were collected and examined. Four novel taxa of Penicillifer, Pseudocosmospora, and Thelonectria were introduced on the basis of morphological characteristics and DNA sequence analyses of combined datasets of the act, ITS, LSU, rpb1, rpb2, tef1, and tub2 regions. Differences between the new species and their close relatives were compared and discussed.

Keywords: Ascomycota; morphology; multigene analyses; taxonomy

1. Introduction

The family Nectriaceae was established in 1865 to accommodate those fungi producing uniloculate perithecia that are yellow, orange-red to purple, or brown; often change color in 3% potassium hydroxide (KOH) and 100% lactic acid (LA); and have a tropical and subtropical distribution [1]. Approximately 69 genera are currently accepted [1–3], including genera such as Penicillifer Emden, Pseudocosmospora C.S. Herrera & P. Chaverri, and Thelonectria P. Chaverri & C. Salgado. The generic concepts and phylogenetic relationships of the family were comprehensively stated by Lombard et al. [2].

The genus Penicillifer, typified by Penicillifer pulcher Emden., was introduced by Emden [4]. It was treated as the asexual stage of Viridispora Samuels & Rossman [1] and recommended as the correct name for this group of fungi [2]. Seven species are currently known in the genus [1,2,5]. The genus Pseudocosmospora, typified by P. eutypellae C.S. Herrera & P. Chaverri, was established by Herrera et al. [6] to accommodate Cosmospora vilior (Starbäck) Rossman & Samuels and related species that are usually fungicolous. Sixteen species are recognized [6–9]. The genus Thelonectria, typified by T. discophora (Mont.) P. Chaverri & C. Salgado, was established by Chaverri et al. [10] to include the species formerly placed in the Nectria mammoida and N. veillotiana groups with a cosmopolitan distribution [11]. Forty-seven species are accepted in the genus [10–18].

In our study of the hypocrealean specimens from different regions of China, four unusual fungi were encountered. Judging by their perithecial gross morphology, anatomy, and culture characteristics, they represent four undescribed species of Penicillifer, Pseudocosmospora, and Thelonectria. Their taxonomic placements were further confirmed by multigene phylogenetic analyses of α-actin (act), nuclear ribosomal DNA ITS1-5.8S-ITS2 (ITS), large subunit of nuclear ribosomal DNA (LSU), the largest subunit of RNA polymerase II (rpb1), the second largest subunit of RNA polymerase II (rpb2), translation elongation factor 1-α (tef1), and β-tubulin (tub2). The differences between the novel taxa and their close relatives were compared.
2. Materials and Methods

2.1. Sampling and Morphological Studies

Specimens were collected from Beijing and the Guangxi Zhuang Autonomous Region, and they are preserved in Herbarium Mycologicum Academiae Sinicae (HMAS). Cultures were obtained by single ascospore isolation from the fresh perithecium and deposited in the China General Microbiological Culture Collection Center (CGMCC). The method of Lombard et al. [2] was followed for morphological observations. The ascomatal wall reactions to 3% KOH and 100% LA were tested. Sections were prepared with a freezing microtome (YD-1508-III, Jinhua, China) at a thickness of 6–8 µm for anatomic examination. Lactophenol cotton blue solution was used as a mounting medium for the measurements of the perithecia, asci, ascospores, conidiophores, and conidia. Photographs were taken with a Leica DFC450 digital camera (Wetzlar, Germany) attached to a Leica M125 stereomicroscope (Milton Keynes, UK) for gross morphology and a Zeiss AxioCam MRc 5 digital camera (Jena, Germany) attached to a Zeiss Axio Imager A2 microscope (Göttingen, Germany) for microscopic features. For colony morphology and growth rates, strains were grown on potato dextrose agar (PDA, 20% w/v potato + 2% w/v dextrose + 2% w/v agar) and synthetic nutrient-poor agar (SNA) [19] in 90 mm plastic Petri dishes at 25 °C for 14 d with alternating periods of light and darkness (12 h/12 h).

2.2. DNA Extraction, PCR Amplification, Sequencing, and Phylogenetic Analyses

Genomic DNA was extracted from fresh mycelium following the method of Lombard et al. [2]. Seven primer pairs, act1/act2 [20], ITS5/ITS4 [21], LR0R/LR5 [22,23], rpb1a/rpb1c [24], RPB2-5f/RPB2-7cR [25], 728F/EF2 [26,27], and T1/T22 [28], were used to amplify the sequences of the act, ITS, LSU, rpb1, rpb2, tef1, and tub2 regions, respectively. PCR reactions were performed using an ABI 2720 Thermal Cycler (Applied Biosciences, Foster City, CA, USA), and DNA sequencing was carried out in both directions on an ABI 3730XL DNA Sequencer (Applied Biosciences, Foster City, CA, USA).

Newly obtained sequences and those retrieved from GenBank are listed in Tables 1–3. The sequences were assembled and aligned, and the primer sequences were trimmed using BioEdit 7.0.5 [29] and converted to nexus files by ClustalX 1.83 [30]. A partition homogeneity test (PHT) was performed with 1000 replicates in PAUP*4.0b10 [31] to evaluate statistical congruence amongst these loci. The aligned sequences were combined in BioEdit and analyzed with Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) methods to determine the phylogenetic positions of the new species. The BI analysis was conducted by MrBayes 3.1.2 [32] using a Markov chain Monte Carlo algorithm. Nucleotide substitution models were determined by MrModeltest 2.3 [33]. Four Markov chains were run simultaneously for 1,000,000 generations, with the trees sampled every 100 generations. A 50% majority rule consensus tree was computed after excluding the first 2500 trees as ‘burn-in’. The Bayesian inference posterior probability (BIPP) was determined from the remaining trees. Branch support measures were calculated with 1000 bootstrap replicates. The ML analysis was performed via IQ-Tree 1.6.12 [34] using the best model for each locus chosen by ModelFinder [35]. The MP analysis was performed with PAUP 4.0b10 [31] using heuristic searches with 1000 replicates of random addition of sequences and subsequent TBR (tree bisection and reconnection) branch swapping. The topological confidence of the resulting trees and the statistical supports of the branches were tested by maximum parsimony bootstrap proportion (MPBP) with 1000 replications, each with 10 replicates of random addition of taxa. Trees were examined by TreeView 1.6.6 [36]. Maximum likelihood bootstrap proportion (MLBP) and MPBP greater than 70% and BIPP greater than 90% were shown at the nodes.
Table 1. List of *Penicillifer* species, herbarium/strain numbers, and GenBank accession numbers of materials used in this study.

| Species                  | Herbarium/Strain Numbers | GenBank Accession Numbers |
|--------------------------|--------------------------|---------------------------|
|                          |                          | ITS        | LSU     | rpb2    | tef1     |
| *Corallonectria jatrophae* | CBS 91396 *T*            | NR153873   | KM231611| KM232298| KM231863|
| *Dematiocladium celtidis* | CBS 115994 *T*           | AY793430   | AY793438|          | KM231864|
| *P. bipapillatus*         | CBS 42088 *T*            | KM231740   | KM231608| KM232295| KM231860|
| *P. dipartitisporus*      | CBS 37659 *T*            | NR154310   | MH869437| KM232296| KM231861|
| *P. macrosporus*          | CBS 42388 *T*            | MH862133   | KM231607| KM232294| KM231859|
| *P. martini*              | BRIP 59225 *T*           | NR168155   | NG068753|          | KJ696241|
| *P. pulcher*              | CBS 56067 *T*            | NR154311   | NG058093| KM232297| KM231862|
| *P. sinicus*              | CGMCC 3.24130 *T*        | OP223439 *a| OP223435| OP272863| OP272864|
| *Stachybotrys chartarum*  | CBS 12913                | MH854622   | MH866145| KM232434| KM231994|

*T* indicates the ex-type culture. *Numbers in bold indicate the newly provided sequences.

Table 2. List of *Pseudocosmospora* species, herbarium/strain numbers, and GenBank accession numbers of materials used in this study.

| Species                | Herbarium/Strain Number | GenBank Accession Numbers |
|------------------------|--------------------------|---------------------------|
|                        |                          | ITS        | LSU     | tub2     |
| *Corallonectella repens* | AR 4547                  | JF832594   | JF832679| JF832838|
| *Microcera larvarum*    | AR 4580                  | KC291751   | KC291759| KC291935|
| *P. beijingensis*       | CGMCC 3.24131 *T*        | OP223438 *a| OP223434| OP272862|
| *P. curvispora*         | CGMCC 3.20176 *T*        | MT592897   | MT592879| MT606156|
| *P. eutypae*            | CH 1101 *T*              | KC291735   | KC291766| KC291925|
|                        | IMI 73016                | KC291736   | KC291786| KC291909|
|                        | AR 4527                  | KC291720   | KC291756| KC291909|
| *P. eutypellae*         | AR 4562 *T*              | KC291721   | KC291757| KC291912|
|                        | GJS 10248                | KC291722   | KC291772| KC291911|
| *P. henanensis*         | HMAS 183528 *T*          | GU075856   | GU075863| HM054103|
| *P. hypoxylicola*       | cLL 19020 *T*            | MN886606   | MN886608| -        |
| *P. joca*               | AR 4779 *T*              | KC291746   | KC291762| KC291924|
| *P. metajoca*           | AR 4576 *T*              | KC291745   | KC291758| KC291923|
| *P. rogersonii*         | GJS 9056 *T*             | KC291729   | KC291780| KC291913|
|                        | GJS 10296                | KC291727   | KC291774| KC291917|
|                        | GJS 091384               | KC291726   | KC291770| KC291914|
| *P. shennongjiana*      | CGMCC 3.20177 *T*        | MT592898   | MT592880| MT606157|
| *P. vilior*             | AR 4810 *T*              | KC291737   | KC291763| KC291928|
|                        | AR 4771                  | KC291734   | KC291761| KC291926|
|                        | PC 1246                  | KC291738   | KC291791| KC291927|

*T* indicates the ex-type culture. *Numbers in bold indicate the newly provided sequences.

Table 3. List of *Thelonectria* species, herbarium/strain numbers, and GenBank accession numbers of materials used in this study.

| Species                  | Herbarium/Strain Numbers | GenBank Accession Numbers |
|--------------------------|--------------------------|---------------------------|
|                          |                          | act         | ITS      | LSU      | rpb1     | tub2     |
| *Cosmospora coccinea*    | CBS 114050               | GQ505967    | FJ474072 | GQ505990 | GQ506020 | DQ522501|
| *Nectria cinnabarina*    | AR 4477/AR 4302          | HM484627    | HM484548 | HM484562 | M484577  | HM484820|
| *T. acrotyla*            | GJS 90171                | JQ365047    | JQ403329 | JQ403368 | JQ403407 | JQ394720|
| *T. amamiensis*          | MAFF 239819              | JQ365054    | JQ403337 | JQ403375 | KJ02408   | JQ394727|
Table 3. Cont.

| Species          | Herbarium/Strain Numbers | GenBank Accession Numbers | act | ITS   | LSU   | rpb1 | tub2 |
|------------------|--------------------------|---------------------------|-----|-------|-------|------|------|
| T. chlamydospora | ST 121 ^T                | LC519560                  |     | LC509450 | LC509452 | -    | -    |
| T. globulosa     | CGMCC 3.24132 ^T         | OP272865 ^a               | OP223436 | OP223432 | OP227867 | OP586762 |
| T. gongylodes    | GJS 04171 ^T             | JQ365038                  |     | JQ403317 | JQ403357 | JQ403394 | JQ394710 |
| T. nodosa        | GJS 04155 ^T             | JQ365037                  |     | JQ403317 | JQ403357 | JQ403394 | -    |
| T. olida         | CBS 21567 ^T             | KJ021982                  | KJ022016 | KJ020258 | HM364334 | KM232024 |
| T. rubrococca    | IMI 324475 ^T            | KJ022275                  | KJ022008 | KJ020261 | KJ022439 | KJ022329 |
| T. spinulospora  | CGMCC 3.24133 ^T         | OP272866                  | OP223437 | OP223433 | OP227868 | OP586764 |
| T. torulosa      | AR 4768A                 | JQ365031                  |     | JQ403319 | JQ403359 | JQ403396 | JQ394702 |
| T. trachosa      | GJS 04357 ^T             | JQ365039                  |     | JQ403319 | JQ403359 | JQ403396 | JQ394702 |
| T. truncata      | GJS 04357 ^T             | JQ365039                  |     | JQ403319 | JQ403359 | JQ403396 | JQ394702 |
| T. veuillotiana  | AR 1751                  | JQ022273                  |      | JQ403345 | JQ403396 | JQ394698 |

^T indicates the ex-type culture. ^a Numbers in bold indicate the newly provided sequences.

3. Results

3.1. Phylogeny

The sequences of the ITS, LSU, rpb2, and tef1 regions from six *Penicillifer* species were analyzed. *Stachybotrys chartarum* (Ehrenb.) S. Hughes was used as the outgroup taxon. The partition homogeneity test (*p* = 0.01) indicated that the individual partitions were not highly incongruent [37]; thus, these four loci were combined for the phylogenetic analyses. The ML tree is shown in Figure 1. The topologies of the BI and MP trees were similar to that of the ML tree. The isolate CGMCC 3.24130 grouped with other members of *Penicillifer* and received high statistical support (MLBP/MLBP/BIPP = 96%/100%/100%).

![Figure 1](image-url)  

**Figure 1.** The maximum likelihood tree inferred from combined ITS, LSU, rpb2, and tef1 sequences of representative species of *Penicillifer*. MLBP (left) and MPBP (middle) values greater than 70% and BIPP (right) values greater than 90% are shown at the nodes.
The sequences of ITS, LSU, and tub2 regions from 11 *Pseudocosmospora* species were analyzed. *Corallomycetella repens* (Berk. & Broome) Rossman & Samuels and *Microcera larvarum* (Fuckel) Gräfenhan, Seifert & Schroers were used as outgroup taxa. The partition homogeneity test \( (p = 0.01) \) indicated that the individual partitions were not highly incongruent [37]; thus, these three loci were combined for the phylogenetic analyses. The ML tree is shown in Figure 2. The topologies of the BI and MP trees were similar to that of the ML tree. The isolate CGMCC 3.24131 grouped with other species of *Pseudocosmospora* and received high statistical support (MLBP/MLBP/BIPP = 92%/98%/100%).

Figure 1. The maximum likelihood tree inferred from combined ITS, LSU, rpb2, and tef1 sequences of representative species of *Penicillifer*. MLBP (left) and MPBP (middle) values greater than 70% and BIPP (right) values greater than 90% are shown at the nodes.

The sequences of the act, ITS, LSU, rpb1, and tub2 regions from 13 *Thelonectria* species were analyzed. *Cosmospora coccinea* Rabenh. and *Nectria cinnabarina* (Tode) Fr. were used as outgroup taxa. The partition homogeneity test \( (p = 0.01) \) indicated that the individual partitions were not highly incongruent [37]; thus, these five loci were combined for the phylogenetic analyses. The ML tree is shown in Figure 3. The topologies of the BI and MP trees were similar to that of ML tree. The isolates CGMCC 3.24132 and CGMCC 3.24133 were well-located among other *Thelonectria* species and received high supporting values (MLBP/MLBP/BIPP = 100%/100%/100%). The isolate CGMCC 3.24133 was related to *T. rubroscocca* (Brayford & Samuels) Salgado & P. Chaverri, receiving high statistic values (MLBP/MLBP/BIPP = 100%/100%/100%), and the strain CGMCC 3.24132 formed an independent lineage and was related to the *T. veuillotiana* complex (MLBP/MLBP/BIPP = 100%/72%/100%).

Figure 2. The maximum likelihood tree inferred from combined ITS, LSU, and tub2 sequences of representative species of *Pseudocosmospora*. MLBP (left) and MPBP (middle) values greater than 70% and BIPP (right) values greater than 90% were shown at the nodes.
3.2. Taxonomy

*Penicillifer sinicus* Z.Q. Zeng & W.Y. Zhuang, sp. nov. Figures 4 and 5.

**Fungal Names:** FN571297.

**Etymology:** The epithet refers to the country where the fungus was collected.

**Typification:** CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Mao’er Mountain, on rotten twigs, 7 December 2019, Z.Q. Zeng & H.D. Zheng 12496 (holotype HMAS 247865, ex-type strain CGMCC 3.24130).

**GenBank accession numbers:** ITS OP223439, LSU OP223435, rpb2 OP272863, tef1 OP272864, rpb1 OP586759, tub2 OP586763.

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**Figure 3.** The maximum likelihood tree inferred from combined act, ITS, LSU, rpb1, and tub2 sequences of representative species of Thelonectria. MLBP (left) and MPBP (middle) values greater than 70% and BIPP (right) values greater than 90% were shown at the nodes.

**Figure 4.** Macroscopic and microscopic morphology of *Penicillifer sinicus* (HMAS 247865). (a–c) Ascomata on natural substratum; (d) median section of perithecium in lactophenol cotton blue; (e–i) asci with ascospores in lactophenol cotton blue; (j–n) ascospore in lactophenol cotton blue. Bars: (a–c) = 1 mm; (d) = 50 μm; (e–n) = 10 μm.
Mycelium was not visible on the natural substratum. Perithecia were superficial, solitary, non-stromatic or with a basal stroma, and subglobose to globose with an acute to blunt papilla; the surface was warded; they did not collapse upon drying; they were yellowish brown to brown and did not change color in 3% KOH or 100% LA, and the size was 235–314 × 176–274 μm. The perithecial surface had warts 15–55 μm high. Perithecial walls were two-layered, 23–35 μm thick; the outer layer was of the textura angularis, 16–25 μm thick, with cell 5–9 × 4–8 μm, and cell walls 1–1.2 μm thick; the inner layer was of the textura prismaticia, 7–10 μm thick, with cell 5–13 × 2–3 μm, and cell walls 0.8–1 μm thick. Asci were unitunicate, cylindrical, and eight-spored with an apical ring, and 60–85 × 4.5–8 μm. Ascospores were ellipsoidal to fusiform, (0–)1-septate, constricted or not at septum, hyaline to light brown, smooth-walled, uniseriate, overlapping obliquely, and 10–15 × 4.5–5.3 μm.

**Colony characteristics:** On PDA, the colony was 20 mm in diam. after 1 week at 25 °C, the surface was cottony with dense, whitish aerial mycelium producing yellowish-brown pigments. On SNA, the colony was 26 mm in diam. after 1 week at 25 °C, the surface was velvet with sparse, whitish aerial mycelium. Conidiophores were verticillium-like, septate, and hyaline, with 1–2 whorls and a terminal whorl of 2–8 phialides, 30–120 μm long, and 2–3.5 μm wide at the base. Phialides were subulate, tapering toward the apex, 15–45 μm long, 1.5–2.5 μm wide at the base, and 0.2–0.3 μm wide at the apex. Macroconidia were ellipsoidal to fusiform or cylindrical, slightly curved, (0–)1(–3)-septate, smooth-walled, hyaline, and 10–28 × 3–5.5 μm.

**Notes:** Amongst the known species of the genus, *P. sinicus* is morphologically most similar to *P. macrosporus* Samuels in having superficial, solitary, non-stromatic, globose perithecia with warded surfaces; smooth-walled, 1-septate ascospores; and cylindrical, bicellular conidia [1]. However, *P. macrosporus* has a thicker perithecial wall (ca. 65 μm thick), clavate asci without apical rings, wider ascospores (5–7 μm wide), and longer conidia (33–47 μm long) [1]. In addition, there were 34 bp, 19 bp, 23 bp, 31 bp, and 23 bp divergences in the ITS, LSU, rpb1, rpb2, and tef1 regions between the ex-type cultures of
the two species (CGMCC 3.24130 and CBS 423.88). Thus, both the morphological and the molecular evidence support their separation at the species level.

*Pseudocosmospora beijingensis* Z.Q. Zeng & W.Y. Zhuang, sp. nov. Figures 6 and 7.

![Image of Pseudocosmospora beijingensis](image)

**Figure 6.** Macroscopic and microscopic morphology of *Pseudocosmospora beijingensis* (HMAS 290896). (a–d) Ascomata on natural substratum; (e,f) median section of perithecia in lactic acid; (g,h) ascus with ascospores in lactophenol cotton blue; (i–k) ascospore in lactophenol cotton blue. Bars: (a–d) = 1 mm; (e,f) = 50 μm; (g–k) = 10 μm.

![Image of Pseudocosmospora beijingensis](image)

**Figure 7.** Colonial and microscopic morphology of *Pseudocosmospora beijingensis* (CGMCC 3.24131). (a) Colony after 1 week at 25 °C on PDA; (b) colony after 1 week at 25 °C on SNA; (c–i) conidiophores, phialides, and microconidia in lactophenol cotton blue. Bars: (c–i) = 10 μm.

**Fungal Names:** FN571298.

**Etymology:** The epithet refers to the type locality of the fungus.

**Typification:** CHINA, Beijing, Beidagou forest, on rotten bark associated with other fungi, 10 August 2017, H.D. Zheng, X.C. Wang, Y.B. Zhang, C. Wang & P. Li 11339 (holotype HMAS 290896, ex-type strain CGMCC 3.24131).

**GenBank accession numbers:** ITS OP223438, LSU OP223434, tub2 OP272862.

Mycelium was not visible on the natural substratum. Perithecia were superficial and gregarious with a well-developed stroma, subglobose to globose, slightly roughened surface, laterally collapsed upon drying, orange-red to bright red, turning dark red in 3% KOH and light yellow in 100% LA, and 147–196 × 118–176 μm. Perithecial walls were two-layered, and 20–42 μm thick; the outer layer was of the textura globulosa to textura...
angularis, 15–25 μm thick, with cell 4–13 × 2.5–4.5 μm, and cell walls 1–1.2 μm thick; the inner layer was of the textura prismaticā, 5–8 μm thick, with cell 6–10 × 2.5–3.5 μm, and cell walls 0.8–1 μm thick. Ascii were unitunicate, cylindrical, with a simple apex, eight-spored, and 38–58 × 2.5–5 μm. Ascospores were ellipsoidal, 1-septate, not constricted at the septum, light yellow-brown, smooth-walled, uniseriate, and 8–10 × 2.5–4 μm.

**Colony characteristics:** On PDA, the colony was 25 mm in diam. after 1 week at 25 °C, surface crustose, producing yellowish-white pigments. On SNA, the colony was 15 mm in diam. after 1 week at 25 °C, surface velvet, with sparse, whitish aerial mycelium. Conidiophores were acremonium- to verticillium-like, septate, of indefinite length, and hyaline, with 1–2 whorls and a terminal whorl of 2–6 phialides. Phialides were subulate, tapering toward the apex, 10–55 μm wide, 0.9–1.2 μm wide at the base, and 0.2–0.3 μm wide at the tip. Conidia were allantoid, curved, unicellular, smooth-walled, hyaline, and 2.6–4.5 × 0.9–1.8 μm.

**Notes:** Among the known species of *Pseudocosmospora*, *P. beijingensis* most resembles *P. curvispora* Z.Q. Zeng & W.Y. Zhuang in having subglobose to globose perithecia that are laterally collapsed upon drying; having asci without an apical ring; having ellipsoidal, 1-septate, smooth-walled, and light yellow-brown ascospores; and producing acremonium- to verticillium-like conidiophores, and allantoid, unicellular, curved conidia [8]. However, *P. curvispora* differs in clavate and somewhat longer asci (53–68 μm long) and narrower conidia (0.8–1.2 μm wide) [8]. Sequence comparisons revealed that there were 30 bp, 22 bp, and 90 bp divergences detected for the ITS, LSU, and tub2 regions. Obviously, they are not conspecific.

**Thelonectria globulosa** Z.Q. Zeng & W.Y. Zhuang, sp. nov. Figures 8 and 9.

**Fungal Names:** FN571299.

**Etymology:** The epithet refers to the globose microconidia.

**Typification:** CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Mao’er Mountain, on rotten roots, 5 December 2019, Z.Q. Zeng & H.D. Zheng 12434 (holotype HMAS 255835, ex-type strain CGMCC 3.24132).

**GenBank accession numbers:** act OP272865, ITS OP223436, LSU OP223432, rpb1 OP272867, rpb2 OP586760, tub2 OP586762.

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Figure 8. Macroscopic and microscopic morphology of *Thelonectria globulosa* (HMAS 255835). (a–d) Ascomata on natural substratum; (e,f) median section of peritheium in lactophenol cotton blue; (g,h) ascus with ascospores in lactophenol cotton blue; (i–k) ascospore in lactophenol cotton blue. Bars: (a–d) = 1 mm; (e,f) = 50 μm; (g–k) = 10 μm.
Mycelium was not visible on the natural substratum. Perithecia were superficial, solitary to gregarious, with a basal or well-developed stroma, subglobose to globose, slightly roughened surface, with blunt papilla of 32–65 µm high and 52–75 µm wide at the base, did not collapse upon drying, orange-red to red, turning dark red in 3% KOH and light yellow in 100% LA, and 235–323 × 148–245 µm. Perithecial walls were two-layered, 20–40 µm thick; the outer layer was of the textura globulosa to textura angularis, 15–30 µm thick, with cell 5–15 × 4–12 µm, and cell walls 0.8–1 µm thick; the inner layer was of the textura prismatica, 5–10 µm thick, with cell 6–15 × 3–10 µm, and cell walls 1–1.2 µm thick. Ascii were unitunicate, cylindrical to clavate, eight-spored, with a simple apex, and 53–75 × 8–13 µm. Ascospores were ellipsoidal to fusiform, 1-septate, constricted at the septum, hyaline, smooth to spinulose, uniseriate or irregular biseriate in asci, and 13–20 × 5.5–8 µm.

**Colony characteristics:** The colony on PDA was 22 mm in diam. after 1 week at 25 °C and had a cottony surface with dense, whitish aerial mycelium producing yellowish brown pigments. The colony on SNA was 35 mm in diam. after 1 week at 25 °C and had a cottony surface with sparse, whitish aerial mycelium. Conidiophores were mostly unbranched; rarely had simple branches; and were septate, hyaline, 25–89 µm long, and 1.5–2.5 µm wide at the base. Macroconidia were cylindrical to rod-shaped, slightly curved, 1–3(–4)-septate, smooth-walled, hyaline, and 20–58 × 3.2–5.8 µm. Microconidia were globose, smooth-walled, hyaline, and 3–4.5 µm in diam. Chlamydospores were globose to subglobose, and 4–10 × 3–8 µm.

**Notes:** Among the known species of *Thelonectria*, *T. globulosa* is distinct because of its globose microconidia. Morphologically, *T. globulosa* resembles *T. nodosa* C.G. Salgado & P. Chaverri in having solitary to gregarious, globose perithecia that do not collapse upon drying; cylindrical to clavate asci; ellipsoidal to fusiform ascospores; and cylindrical macroconidia. However, *T. nodosa* differs because of its larger asci (68–115 × 10–17 µm) with an apical ring, macroconidia possessing more septa (up to six septa), and lack of microconidia formation [12]. Moreover, there are 38 bp, 96 bp, 37 bp, and 65 bp divergences.

**Figure 9.** Colonial and microscopic morphology of *Thelonectria globulosa* (CGMCC 3.24132). (a) Colony after 2 week at 25 °C on PDA; (b) colony after 2 week at 25 °C on SNA; (c) conidiophores in lactophenol cotton blue; (d–l) conidiophores and macroconidia in lactophenol cotton blue; (m) microconidia in lactophenol cotton blue; (m) chlamydospores in lactophenol cotton blue. Bars: (c–m) = 10 µm.
in the act, ITS, LSU, and rpb1 regions between the ex-type cultures of the two taxa (CGMCC 3.24132 and GJS 04155). Both the morphology and DNA sequence data distinguish them as different species.

*Thelonectria spinulosora* Z.Q. Zeng & W.Y. Zhuang, sp. nov. Figures 10 and 11.

**Figure 10.** Macroscopic and microscopic morphology of *Thelonectria spinulosora* (HMAS 290897). (a,b) Ascomata on natural substratum; (c) median section of perithecium in lactophenol cotton blue; (d–i) ascospore in lactophenol cotton blue. Bars: (a,b) = 1 mm; (c) = 50 μm; (d–i) = 10 μm.

**Figure 11.** Colonial and microscopic morphology of *Thelonectria spinulosora* (CGMCC 3.24133). (a) Colony after 2 week at 25 °C on PDA; (b) colony after 2 week at 25 °C on SNA; (c–e) conidiophores in lactophenol cotton blue; (f–j) conidiophores and macroconidia in lactophenol cotton blue; (k–o) macroconidia in lactophenol cotton blue; (p) chlamydospore in lactophenol cotton blue. Bars: (c–p) = 10 μm.
**Fungal Names:** FN571300.

**Etymology:** The specific epithet refers to the spinulose ascospores.

**Typification:** CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Mao’er Mountain, on rotten twigs, 7 December 2019, Z.Q. Zeng & H.D. Zheng 12499 (holotype HMAS 290897, ex-type strain CGMCC 3.24133).

**GenBank accession numbers:** act OP272866, ITS OP223437, LSU OP223433, rpb1 OP272868, rpb2 OP586761, tub2 OP586764.

Mycelium was not visible on the natural substratum. Perithecia were superficial, solitary, with a basal stroma, subglobose to broad-pyriform, surface slightly roughened, sometimes collapsed laterally upon drying, orange-red to red, turning dark red in 3% KOH and light yellow in 100% LA, and 123–195 × 143–212 µm. Perithecial walls were two-layered, 8–18 µm thick; the outer layer was of the textura globulosa to textura angularis, 5–13 µm thick, with cell 6–10 × 4–9 µm, and cell walls 1–1.2 µm thick; the inner layer was of the textura prismatica, 3–5 µm thick, with cell 2–8 × 3–10 µm, and cell walls 0.8–1 µm thick. Ascii were not observed. Ascospores were ellipsoidal, 1-septate, not constricted or slightly constricted at the septum, hyaline, spinulose, and 12–18 × 5.6–8 µm.

**Colony characteristics:** On PDA, the colony was 40 mm in diam. after 2 weeks at 25 ºC, the surface was cottony with dense, whitish aerial mycelium producing light yellow pigments. On SNA, the colony was 28 mm in diam. after 2 weeks at 25 ºC, surface velvet, with sparse, whitish aerial mycelium. Conidiophores were acremonium-like, rarely with simple branches, septate, hyaline, and 45–102 × 2.2–4 µm. Macroconidia were cylindrical, slightly curved, (1–2–)3-septate, smooth-walled, hyaline, and 28–62 × 2.8–4.5 µm. Chlamydospores were globose to subglobose, smooth-walled, hyaline, and 6–8 µm in diam.

**Notes:** The morphological features, such as the superficial, globose to subglobose, broad-pyriform perithecia that do not collapse when dry; ellipsoidal, two-celled, and hyaline ascospores; and curved macroconidia with rounded ends, indicate the placement of *T. spinulospora* in *Thelonectria*, which was confirmed by sequence analyses of the act, ITS, LSU, rpb1, and tub2 regions (Figure 3). Amongst the known species of the genus, the new species is morphologically similar and phylogenetically related to *T. rubrococca* (Brayford & Samuels) C.G. Salgado & P. Chaverri in having solitary to gregarious, globose perithecia that do not collapse upon drying, ellipsoidal ascospores, and cylindrical macroconidia. However, the latter differs in its larger perithecia (200–450 µm in diam.), smaller ascospores (8–14.5 × 3.6–6.6 µm), and macroconidia with more septa (up to five septa) [38]. Sequence comparisons between the ex-type cultures of the two species revealed that 24 bp, 8 bp, 0 bp, 22 bp, and 28 divergences were detected for the act, ITS, LSU, rpb1, and tub2 regions. Both the morphology and DNA sequence data support their distinction at the species level.

### 4. Discussion

The genus *Penicillifer* is proposed as the preferable name over *Viridispora* [2], following the International Code of Nomenclature for algae, fungi, and plants [39]. Our analyses, inferred from sequences of ITS, LSU, rpb2, and tef1 and including the new taxon, revealed a tree topology (Figure 1) similar to that given by Lombard et al. [2]. The phylogenetic tree shows that *Penicillifer* species forms a well-supported monophyletic clade (MLBS/MPBP/BIPP/ = 96%/100%/100%) (Figure 1). *Penicillifer sinicus* is closely related to *P. macrosorus* (MLBS/MPBP/BIPP/ = 100%/99%/100%). The sequence comparisons revealed that there were 34 bp, 19 bp, 23 bp, 31 bp, and 23 bp differences detected for the ITS, LSU, rpb1, rpb2, and tef1 regions. Therefore, both the molecular and the morphological evidence supports the separation of the two fungi at a specific level. Among the known species of *Penicillifer*, *P. martini* P. Wong, Y.P. Tan & R.G. Shivas is known solely by its sexual stage [5], and only asexual stages of *P. japonicus* Matsush. and *P. pulcher* have been discovered [4,40]; the rest species of the genus are holomorphic, including the newly added one.

Historically, nectriaceous species producing small, reddish, smooth, thin-walled perithecia were categorized as *Cosmospora* Rabenh. *sensu lato* [1]. The accumulated morpho-
logical and phylogenetic information indicated that the genus was not monophyletic [41,42]. Herrera et al. [6] established *Pseudocosmospora* to accommodate ten cosmospora-like fungi on *Eutypa* and *Eutypella* and with acremonium- to verticillium-like asexual stages. Since then, six additional taxa have joined the group [7–9]. The genus has become distributed worldwide and displays high species diversity in warm temperate and tropical regions [6]. Species of the genus have the following features in common: they are superficial perithecia, gregarious, KOH+, LA+, laterally collapsed upon drying, and usually less than 250 µm in height; and they have asci containing eight 1-septate ascospores, acremonium- to verticillium-like conidiophores, and non-septate conidia. *Pseudocosmospora beijingensis* fits well the generic concept. The multigene analyses indicated its distinctions from any other species of the genus (Figure 2).

Members of *Thelonectria* are often found on twigs and branches, trunks of recently killed or dying trees, and rotting roots; they occasionally cause small cankers and are mainly distributed in tropical, subtropical, and temperate regions [10,11,17]. Among the species of the genus, *T. coronata* (Penz. & Sacc.) P. Chaverri & C. Salgado, *T. discophora*, *T. lucida* (Höhn.) P. Chaverri & C. Salgado, and *T. veuillotiana* (Roum. & Sacc.) P. Chaverri & Salgado are cosmopolitan and are treated as species complexes [38,43,44]. Salgado-Salazar et al. [11–13] carried out a revisionary work on the above species complexes and described 30 cryptic species on the basis of genealogical concordance phylogenetic species recognition. Our phylogenetic results indicated that *T. globulosa* was associated with but clearly separated from members of the *T. veuillotiana* complex. *Thelonectria aurea*, known only by the asexual stage, can be easily distinguished in the absence of microconidia and chlamydospores in culture [17]. Moreover, there were 93 bp and 64 bp divergences in the ITS and tub2 regions between the ex-type culture of the two species.

There are 47 species currently known in this genus, of which 20 species have been reported in China [11,13,15]. Large-scale surveys of fungal resources in various regions with different climates, vegetation, geographic structures, and multiple niches will improve our understanding of the species diversity of nectriaceous fungi in the country.

5. Conclusions

The species diversity of the family Nectriaceae was investigated, and four novel taxa were discovered. With the joining of the new species, the phylogenetic relationships among species of these three genera were updated.

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