Ascomycetes from the Qilian Mountains, China – Hypocreales

Zhao-Qing Zeng¹, Huan-Di Zheng¹, Xin-Cun Wang¹, Sheng-Long Wei², Wen-Ying Zhuang¹, ²

¹ State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
² Gansu Engineering Laboratory of Application Mycology, Hexi University, Zhangye 734000, China

Corresponding author: Wen-Ying Zhuang (zhuangwy@im.ac.cn)

Abstract
To investigate fungi from the Qilian Mountains in Gansu Province, ascomycetous specimens were collected and hypocrealean fungi were examined. Eighteen species belonging to six genera in the families Hypocreaceae and Nectriaceae were identified, including 11 species of Hypomyces and Trichoderma in Hypocreaceae and seven species of Nectria, Stylonectria, Thelonectria, and Thyronectria in Nectriaceae. Among them, Stylonectria qilianshanensis and Trichoderma gansuanaum are new to science. DNA sequence analyses of combined ACL1, ITS, RPB2, and TEF1 regions confirmed their taxonomic placements. Morphological distinctions between the new species and their close relatives are discussed. Hypomyces tremellicola is reported for the first time in China.

Keywords
Biodiversity, Hypocreaceae, Nectriaceae, sequence analysis, taxonomy

Introduction

The Qilian Mountains is located across the northeastern Qinghai and western Gansu provinces (35°50’–39°19’N, 94°10’–103°04’E) at elevations ranging from 4000 to 6000 m. The area has a temperate climate with mean annual precipitation around 400 mm. Forests are composed mainly of mixed broad-leaf and coniferous trees. The Qilian Mountain National Nature Reserve, where ascomycetes were surveyed, is ex-
tremely diverse in climate, vegetation, and geographic structure. Previous investigations of fungal resources have mainly focused on Basidiomycota (Gui et al. 2010; Xi et al. 2011). Our understanding of ascomycetes of the region needs to be broadened.

The order Hypocreales includes about 2700 species in 240 genera, which are divided into 12 families (Rossman et al. 1999; Lumbsch and Huhndorf 2007; Kirk et al. 2008; Lombard et al. 2015; Maharachchikumbura et al. 2016; Sun et al. 2017; Zhuang and Zeng 2017). Hypocrealean collections from the Qilian Mountains belong to the families Hypocreaceae and Nectriaceae. These families are ubiquitous in nature and exhibit very high species diversity in temperate and tropical regions (Rossman 1996; Rossman et al. 1999). They are economically important in fields of industry, environment protection, and agriculture. For instance, some species of *Trichoderma* Pers. play vital roles in production of industrial enzymes and antibiotics (Jangiret et al. 2017), while several species of *Hypomyces* (Fr.) Tul. & C. Tul. are pathogens of cultivated mushrooms (Tamm and Põldmaa 2013). Some members of *Nectria* (Fr.) Fr., *Thelonectria* P. Chaverri & C. Salgado, and *Thyronectria* Sacc. cause *Abies* and *Rubus* cankers (Hirooka et al. 2011, 2012; Salgado-Salazar et al. 2015). Therefore, discovery of fungi in Hypocreales is of theoretical and practical importance. Improvement and updating our knowledge of the group will provide useful information about sustainable utilization and conservation of natural resources.

Studies on fungi of this group in China dates back to 1895 when *Trichoderma cornu-damae* (Pat.) Z.X. Zhu & W.Y. Zhuang (as *Hypocrea cornu-damae* Pat.) was reported on rotten wood in Sichuan Province (Patouillard 1895). Early studies were initiated by Teng (1934, 1935, 1936, 1963), and recent studies are summarized by Liang (2007), Zhuang (2013), and Zhuang and Zeng (2017). A survey of ascomycetes in the Qilian Mountains was carried out in 2018. A total of 67 specimens were examined in this study. Eighteen taxa belonging to seven genera were identified, including 11 species of *Hypomyces* and *Trichoderma* in Hypocreaceae, and seven of *Nectria*, *Thelonectria*, *Thyronectria*, and *Stylonectria* Höhn. in Nectriaceae. *Stylonectria qilianshanensis* and *Trichoderma gansuanum* are described and illustrated as new species. *Hypomyces tremellicola* is reported for the first time from China.

**Materials and methods**

**Sampling and morphological studies**

Specimens were collected from the Qilian Mountains in Gansu Province, and they are deposited in the Herbarium Mycologicum Academia Sinica (HMAS). Cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences. The methods of Luo and Zhuang (2010) and Põldmaa et al. (2019) were followed for morphological observations. The ascomatal wall reactions to 3% potassium hydroxide (KOH) and 100% lactic acid (LA) were tested. To observe microscopic characteristics of perithecial walls, sections were made with a freezing microtome (YD-1508-III, Jinhua, China) at a thickness of 6–8 μm.
Cotton blue lactophenol solution and lactic acid solution were used as mounting media for examinations of anatomical structures and measurements of perithecia, asci, and ascospores. 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 AxioCamMRc 5 digital camera (Jena, Germany) attached to a Zeiss Axio Imager A2 microscope (Göttingen, Germany) for anatomy. Measurements of individual structures were based on $n = 30$, except as otherwise noted. The culture of *Hypomyces tremellicola* was isolated from conidia found on the surface of the host. To determine colony features and growth rates, strains were grown on malt extract agar [MEA, 2% (w/v) malt extract+ 2% (w/v) agar] and potato dextrose agar [PDA, 20% (w/v) potato + 2% (w/v) dextrose + 2% (w/v) agar] in 90 mm plastic Petri dishes at 25 °C for 7 d. For observation of conidiophores and conidia, cultures were grown on PDA at 25 °C with alternating periods of light and darkness (12 h/12 h).

**DNA extraction, PCR amplification, and sequencing**

Genomic DNA was extracted from dry specimens or fresh mycelia following the method of Wang and Zhuang (2004). Four primer pairs, acl1-230up/acl1-1220low (Nowrousian et al. 2000), ITS5/ITS4 (White et al. 1990), RPB2-5f/RPB2-7cR (Liu et al. 1999), EF1-728F/TEF1LLErev (Carbone and Kohn 1999; Jaklitsch et al. 2005) were used to amplify the ACL1, ITS, RPB2, and TEF1 gene regions, respectively. PCR reactions were performed using an ABI 2720 Thermal Cycler (Applied Biosciences, Foster City, USA) with a 25 μl reaction system consisting of 12.5 μl Taq MasterMix, 1 μl each primer (10 μM), 1 μl template DNA, and 9.5 μl ddH$_2$O. DNA sequencing was carried out in both directions on an ABI 3730XL DNA Sequencer (Applied Biosciences, Foster City, USA) based on the procedures detailed in Gräfenhan et al. (2011), Jaklitsch et al. (2005), and Chaverri et al. (2011).

**Sequence alignment and phylogenetic analyses**

Newly obtained sequences and those retrieved from GenBank are listed in Tables 1 and 2, respectively. The sequences were assembled, aligned and the primer sequences were trimmed using BioEdit 7.0.5 (Hall 1999), and converted to NEXUS files by ClustalX 1.83 (Thompson et al. 1997). The aligned sequences were combined in BioEdit and analyzed with Bayesian inference (BI) and maximum parsimony (MP) methods to determine the phylogenetic positions of the new species. The MP analysis was performed with PAUP 4.0b10 (Swofford 2002) using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR (tree bisection and reconnection) branch swapping. Topological confidence of the resulting trees was tested by maximum parsimony bootstrap proportion (BP) with 1000 replications, each with 10 replicates of random addition of taxa. The Bayesian inference (BI) analysis was conducted by
Table 1. List of *Stylonectria* species and the relatives, herbarium/strain numbers and GenBank accession numbers of materials used in this study.

| Species                                      | Herbarium/strain numbers | GenBank accession numbers |
|-----------------------------------------------|---------------------------|---------------------------|
| *Albonectria rigidiuscula* (Berk. & Broome) Rossman & Samuels | CBS 122570 | HQ897896  |
| *Clonostachys rosea* (Preuss) Mussat          | CML817/CBS 114056 | KX184866  |
| *Cyanopectria cyanostoma* (Sacc. & Flageolet) Samuels & P. Chaverri | CBS 101734 | HQ897895  |
| *Dialonectria episphaeria* (Tode) Cooke       | CBS 125494 | HQ897892  |
| *Fusarium sambucinum* Fuckel                  | CBS 14695  | KM231015  |
| *Fusicularia matuoi* (Hosoya & Tubaki) Gräfenhan & Seifert | CBS 58178 | HQ897754  |
| *Geefayessia cicatricum* (Berk.) Schroers     | CBS 125552 | HQ728171  |
| *Macroconia leptiophaeria* (Niesl) Gräfenhan & Schroers | CBS 100001 | HQ897891  |
| *Microcera coccophila* Desm.                  | CBS 31034  | HQ897843  |
| *Neocosmospora vainsfecta* E.F. Sm.           | CBS 32554  | KM231005  |
| *Stylonectria applanata* Höhn.                | CBS 125489 | HQ897875  |
| *Stylonectria carpini* Gräfenhan              | DAOM 235819 | HQ897909  |
| *Stylonectria norvegica* Lechat, J. Fourn. & Nordén | CBS 139239 | –          |
| *Stylonectria purtonii* (Grev.) Gräfenhan    | DAOM 235818 | HQ897919  |
| *Stylonectria qilianshanensis* Z.Q. Zeng & W.Y. Zhuang | HMAS 255803 | MT087289  |
| *Stylonectria wegeliniana* (Rehm) Gräfenhan, Voglmayr & Jaklitsch | CBS 125490 | HQ897890  |
| *Trichoderma parareesei* Atan., Jaklitsch, Komor-Zel., C.P. Kubicek & Druzhin. | CBS 125925 | KJ665112  |

Numbers in bold indicate the newly provided sequences.

MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a Markov chain Monte Carlo algorithm. MrModeltest v. 2.3 was used to determine the nucleotide substitution models (Nylander 2004). Four Markov chains were run simultaneously for 1000000 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’. Bayesian inference posterior probability (PP) was determined from the remaining trees. Branch support measures were calculated with 1000 bootstrap replicates. Trees were examined by TreeView 1.6.6 (Page 1996). The BIPP greater than 90% and MPBP greater than 70% were shown at the nodes.

Results

To determine taxonomic positions of the *Hypomyces* collections, sequences of ITS and 28S rDNA were searched against the NCBI GenBank database using BLASTN. Sequence comparisons showed that HMAS 247843 shares 99% sequence similarity with *H. tremellicola*, which confirmed its taxonomic position in the genus.

To place the *Stylonectria* specime n within a phylogenetic context, sequences of ACL1, ITS, and RPB2 regions from 15 species of the genus and relatives were analyzed
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using BI and MP methods. *Clonostachys rosea* (Preuss) Mussat and *Trichoderma parareesei* Atan., Jaklitsch, Komoń-Zel., C.P. Kubicek & Druzhin. were used as outgroup taxa. The partition homogeneity test (PHT) \( (P = 0.01) \) indicated that the individual partitions were not highly incongruent (Cunningham 1997), the three loci were thus combined for phylogenetic analyses. The combined datasets include 2197 characters, of which 964 were constant, 310 were variable and parsimony-uninformative and 923 were parsimony-informative. The MP analysis resulted in two most parsimonious trees (tree length = 4081, CI = 0.5285, HI = 0.4715, RI = 0.4459, RCI = 0.2357) with similar topology. The final matrix was deposited in TreeBASE with accession no. S25189. The BI tree is shown in Figure 1. The MP tree is similar to that of the BI tree in topology. HMAS 255803 was associated with other *Stylonectria* species forming a highly supported monophyletic group (BIPP/MPBP = 100%/100%), which confirmed its taxonomic position in the genus.

To place the *Trichoderma* collections with clavate fruit bodies within a phylogenetic context, the sequences of RPB2 and TEF1 from 15 species of the genus were analyzed using BI and MP. *Hypocrella discoidea* (Berk. & Broome) Sacc. and *Hypocrella nectrioides* Thaxt. were used as outgroup taxa. The PHT \( (P = 0.01) \) indicated that the individual parti-

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

| Species                            | Herbarium/strain numbers | GenBank accession numbers |
|------------------------------------|--------------------------|---------------------------|
| **Hypocrella discoidea** (Berk. & Broome) Sacc. | BCC 8237 | DQ452461 |
| **Hypocrella nectrioides** Thaxt. | GJS 8910 | DQ522448 |
| *Trichoderma alutacenum* Jaklitsch | CBS 120535 | FJ179600  | FJ179567 |
| *Trichoderma gansuanum* Z.Q. Zeng & W.Y. Zhuang | CBS 33269 | FJ179601  | FJ179568 |
| *Trichoderma gelatinosum* P. Chaverri & Samuels | CPK 1618 | FJ179604  | FJ179569 |
| *Trichoderma leucopas* Jaklitsch | CBS 122495 | FJ179606  | FJ179570 |
| *Trichoderma rosea* (Preuss) Mussat & Druzhin. | CBS 122499 | FJ179605  | FJ179571 |
| *Trichoderma alutacenum* Jaklitsch | CBS 121276 | FJ179610  | FJ179574 |
| *Trichoderma minutisporum* Bissett | CBS 122496 | FJ179612  | FJ179576 |
| *Trichoderma nitrosporum* Jaklitsch & Voglmayr | CBS 122500 | FJ179611  | FJ179575 |
| *Trichoderma parapiluliferum* (B.S. Lu, Druzhin. & Samuels) Jaklitsch & Voglmayr | CBS 20921 | FJ179614  | FJ179578 |
| *Trichoderma pezizoides* (Berk. & Broome) Samuels, Jaklitsch & Voglmayr | GJS 01257 | EU248608  | AY937438 |
| *Trichoderma piluliferum* J. Webster & Rifai | CBS 120927 | KJ842159  | FJ860674 |
| *Trichoderma placenta* Jaklitsch | CBS 120924 | FJ179616  | FJ179580 |
| *Trichoderma polysporum* (Link) Rifai | CPK 3131 | FJ860558  | FJ860661 |
| *Trichoderma poronioideum* (Möller) Samuels | GJS 01203 | –  | KP109823 |
| *Trichoderma seppoi* Jaklitsch | CBS 122497 | FJ179618  | FJ179582 |
| *Trichoderma strictipile* Bissett | CBS 122498 | FJ179617  | FJ179581 |
| *Trichoderma strictipile* Bissett | CPK 1601 | FJ860594  | FJ860704 |

*a* Numbers in bold indicate the newly provided sequences.
tions were not highly incongruent (Cunningham 1997), the two loci were thus combined for phylogenetic analyses. The combined datasets include 1436 characters, of which 688 were constant, 167 were variable and parsimony-uninformative and 581 were parsimony-informative. The MP analysis resulted in one most parsimonious tree (tree length = 1828, CI = 0.6510, HI = 0.3490, RI = 0.6706, RCI = 0.4366). The final matrix was deposited in TreeBASE with accession no. S25188. The BI tree is shown in Figure 2, which is similar

**Figure 1.** BI tree generated based on the combined datasets of ACL1, ITS and RPB2 sequences of *Stylopectria* and its relatives. Supporting values showing at branches: BIPP (left) and MPBP (right). BIPP greater than 90% and MPBP greater than 70% are shown at the nodes.
Figure 2. BI tree generated based on the combined datasets of RPB2 and TEF1 sequences of *Trichoderma* species. Supporting values showing at branches: BIPP (left) and MPBP (right). BIPP greater than 90% and MPBP greater than 70% are shown at the nodes.

To the MP tree in topology. Among the investigated species, HMAS 279687 was distinct from but associated with other *Trichoderma* species forming a highly supported monophyletic group (BIPP/MPBP = 100%/100%), which confirmed its taxonomic position.
Taxonomy

**Stylonectria qilianshanensis** Z.Q. Zeng & W.Y. Zhuang, sp. nov.

Fungal Names: FN 570729

Figure 3

**Holotype.** China. Gansu Province, Wuwei, Chashugou, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 26 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12155 (HMAS 255803).

**Paratypes.** China. Zhangye, Longchanghe, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 24 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12016, 12017 (HMAS 255804, 255805); Kangle, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 24 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12035, 12036, 12037, 12038 (HMAS 255806, 255807, 255808, 255809); Shandan, Yanzhishan, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 25 August 2018, H.D. Zheng, X.C. Wang & Z.Q. Zeng 12082 (HMAS 255810); Yanzhishan, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 25 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12086, 12087, 12088, 12089, 12090 (HMAS 255811, 255812, 255813, 255814, 255815); Wuwei, Chashugou, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 26 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12148, 12153, 12156, 12158 (HMAS 255816, 255817, 255818, 279708); Tianzhu, Kelacun, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 27 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12229 (HMAS 279709); Haxi, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 28 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12276, 12278 (HMAS 255819, 255820).

**Etymology.** The specific epithet refers to the type locality.

**Description.** Perithecia gregarious, up to 30 in a group, parasitic on decayed fruitbodies of an ascomycete, deep red to dark red, turning black red in 3% KOH and light yellow in 100% LA, subglobose to globose, not becoming cupulate upon drying, (216–)255–344 × (186–)206–304 μm (n = 12), apex broadly discoid, flattened, 50–70 μm high, 160–220 μm in diameter, slightly constricted below, with a tiny papilla. Perithecial wall of two layers, 25–38 μm thick, outer layer 20–31 μm, of textura angularis, cells 5–8 × 2–4 μm, walls 0.8–1.0 μm thick; inner layer 5–7 μm, of textura prismatica, cells 8–12.5 × 3–5 μm, walls 0.5–0.8 μm thick. Asci clavate, with an apical ring, 8-spored, 55–88 × 5–8(–10) μm. Ascospores ellipsoidal, ends rounded, 1-septate, light brown, smooth, uniseriate, 10–13 × 5–5.5 μm. Asexual state unknown.

**Distribution.** China.

**Notes.** *Stylonectria qilianshanensis* is morphologically similar to *S. wegeliniana* in having the perithecia with a broad, discoid apex, clavate asci with an apical ring, and ellipsoidal ascospores with rounded ends (Petch 1938). However, *S. qilianshanensis* differs in having smaller asci [55–88 × 5–8(–10) μm vs 90–100 × 9–10 μm] and ascospores (10–13 × 5–5.5 μm vs 10–18 × 6–9 μm) (Petch 1938). Sequence comparisons indicate that ITS of *S. qilianshanensis* differs from that of *S. wegeliniana* by 20 bp in a total length of 576 bp; ACL1 and RPB2 of the former differ from those of the
latter by 84 bp and 38 bp, respectively among 722 bp and of 869 bp in length. The asexual state of the fungus remains unknown until culture is available.

*Trichoderma gansuanum* Z.Q. Zeng & W.Y. Zhuang, sp. nov.
Fungal Names: FN 570730
Figure 4

**Holotype.** CHINA. Gansu Province, Shandan, Yanzhishan, on mossy humus, 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12100 (HMAS 279687).
Paratypes. China. Gansu Province, Shandan, Yanzhishan, on mossy humus, 25 August 2018, Z.Q. Zeng, H.D. Zheng 12043, 12044 (HMAS 279684, 279685), on mossy humus, 25 August 2018, X.Z. Liu & Z.Q. Zeng 12045 (HMAS 279686); Wuwei, Chashugou, on mossy humus, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12104, 12105 (HMAS 279688, 279689); Wuwei, Xiama, on mossy humus, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12162, 12163 (HMAS 279690, 279691).

Etymology. The specific epithet refers to the type locality.

Description. Stromata simple, rare dichotomously branched, clavate, 20–54 mm long. Fertile part clavate, 5–18 mm long, 1.2–4 mm wide at apex, only slightly broader than stipe, distinctly laterally compressed or longitudinally furrowed, gradually tapered downwards, reddish brown to brownish orange, KOH+; sterile part 15–36 mm long, 1–3 mm wide, beige to cream KOH+. Stromatal surface slight tuberculate from papil late perithecial elevations. Ostiolar openings visible, 30–58 μm high. In section, cortical tissue of textura angularis, 15–35 μm thick, cells hyaline to light yellow, 5–15 × 2–3 μm;
subcortical tissue of textura angularis, 8–28 μm thick, cell hyaline to light yellow, 8–15 × 3–5 μm; subperithecial tissue of textura epidermoidea, rare textura angularis, cells hyaline to light yellow. Perithecia globose to subglobose, 196–206 × 167–235 μm; peridium 8–18 μm thick at flanks, 9–20 μm thick at the base. Papilla prominent, blunt or truncate, brown, 15–35 μm high, 18–43 μm wide at the base. Asc subcylindrical, 50–80 × 3–4 μm. Part-ascospores green, spinulose, dimorphic, distal cells globose, rarely ellipsoidal, 2.5–4 × 2.5–4 μm; proximal cells ellipsoidal to oblong, 3–5.5 × 2.5–3 μm. Asexual state unknown.

**Distribution.** China.

**Notes.** Among the known stipitate species of *Trichoderma*, *T. gansuanum* resembles *T. nybergianum* in habitat and having simple, clavate, erect stromata, cylindrical asci and disarticulating ascospores (Chamberlain et al. 2004). *Trichoderma gansuanum* differs from the latter in shorter ascomatal stipe (20–54 mm vs 22–220 mm long), smaller perithecia (196–206 × 167–235 μm vs 180–450 × 65–315 μm), asci (50–80 × 3–4 μm vs 63–130 × 3.2–7.5 μm), and part-ascospores (distal: 2.5–4 × 2.5–4 μm vs 3–6 × 3–4.5 μm; proximal: 3–5.5 × 2.5–3 μm vs 3–6 × 2.5–4.5 μm) (Chamberlain et al. 2004). Sequence comparisons reveal that there are 25 bp and 16 bp divergences between the two species in the regions of RPB2 and TEF1. Both morphological and molecular data support distinguish them at the species level. The asexual state of *T. gansuanum* remains unknown until a culture is available.

**Hypomyces cervinigenus** Rogerson & Simms, *Mycologia* 63: 418, 1971.

**Specimens examined.** China, Gansu Province, Wuwei, Tianzhu, on *Helvella* sp., 26 August 2018, X.C. Wang, Z.Q. Zeng & H.D. Zheng 12103 (HMAS 279612); Wuwei, Tianzhu, on *Helvella* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12210, 12221, 12225 (HMAS 279667, 279668, 279669).

**Distribution.** Canada, China, United States.

**Hypomyces chrysospermus** Tul. & C. Tul., *Annls Sci. Nat.*, Bot., Sér. 4, 13: 16, 1860.

**Specimens examined.** China, Gansu Province, Tianzhu, Zhuchacun, on *Boletus* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12206 (HMAS 279670); Tianzhu, Kelacun, on *Boletus* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12220 (HMAS 279671).

**Distribution.** Australia, Belgium, Canada, China, French, Japan, New Zealand, United Kingdom, United States.

**Hypomyces lateritius** (Fr.) Tul. & C. Tul., *Annls Sci. Nat.*, Bot., Sér. 4, 13: 11, 1860.

**Specimens examined.** China, Gansu Province, Shandan, Yanzhishan, on *Lactarius* sp., 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12060, 12061 (HMAS 254608,
Hypomyces perniciosus Magnus, Bot. Ztg. 46: 394, 1888.

Specimen examined. China, Gansu Province, Tianzhu, Kelacun, on Lactarius sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12216, 12217, 12218, 12219 (HMAS 279672, 279673, 279674, 279675).

Sequences. ITS (MT676396) and LSU (MT669266).

Distribution. Austria, Belgium, Canada, China, Czech, Denmark, Finland, France, Germany, Italy, Mexico, New Zealand, Russia, Sweden, United Kingdom, United States.

Hypomyces rosellus (Alb. & Schwein.) Tul. & C. Tul., Annls Sci. Nat., Bot., Sér. 4 13: 12, 1860.

Specimen examined. China, Gansu Province, Shandan, Yanzhishan, on Lactarius sp., 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12056 (HMAS 279706).

Sequences. ITS (MT676395).

Distribution. China, Estonia, Poland, Ukraine, United States.

Hypomyces stephanomatis Rogerson & Samuels, Mycologia 77: 775, 1985.

Specimens examined. China, Gansu Province, Shandan, Yanzhishan, on Humaria sp., 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12063 (HMAS 279676); Tianzhu, Zhuchacun, on Humaria sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12205, 12207, 12208, 12209, 12211 (HMAS 279677, 279678, 279679, 279680, 279681); Tianzhu, Kelacun, on Humaria sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12223, 12226 (HMAS 279682, 279683).

Distribution. Canada, China, Germany, United States.

Hypomyces tremellicola (Ellis & Everh.) Rogerson, Mem. N. Y. Bot. Gdn. 26(3): 20, 1976.

Specimen examined. China, Gansu Province, Zhangye, Minyue, on (?) Agaricus sp., August 2018, C.H. Dong & S.J. Li 12287 (HMAS 247843).

Sequences. ITS (MT084414) and LSU (MT078664).

Description. On MEA, colony radius 33 mm after 7d at 25 °C, velvet, surface white, reverse light brown; aerial hyphae white. On PDA, colony radius 20 mm after 7d at 25 °C, floccose, surface grey white, reverse light sienna; aerial hyphae white. Simple branches of aerial hyphae terminating in 1–2-verticillate conidio-
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phores with terminal whorl of 2–4 phialides. Phialides subulate, tapering towards apex, smooth, 8–25 × 1.5–2 μm. Conidia ellipsoidal to rod-shaped, aseptate, hyaline, smooth, 2.5–8 × 1–3 μm. Chlamydospores globose, hyaline, smooth, 5–8 μm in diameter, rare ellipsoidal, 6–12 × 5–10 μm, formed singly or in chains in intercalary position.

**Distribution.** Canada, China, Germany, New Zealand, The Netherlands, United States, Venezuela.

**Notes.** *Hypomyces tremellicola* is a new record for China. This species was originally described as *Hypocrea tremellicola* Ellis & Everh. (Ellis and Everhart 1892), and later transferred to *Hypocreopsis* P. Karst. (Seaver 1910) and *Nectriopsis* Maire (Gams and Zaayen 1982). Samuels (1976) redescribed the species and assigned it to *Hypomyces*. It usually grows on *Crepidotus* spp., and less frequently on *Polyporus* spp. and *Pleurotus* spp. The shape and size of conidia and chlamydospores of the Chinese material match well with the description provided by Zare and Gams (2019). Sequence comparisons showed that 4 bp and 1 bp divergences existed in ITS and 28S rDNA between the Chinese material (HMAS 247843) and a collection from Germany (CBS 441.65). We treat them as infraspecific variations.

*Nectria asiatica* Hirooka, Rossman & P. Chaverri, Stud. Mycol. 68: 44, 2011.

**Specimen examined.** China, Gansu Province, Tianzhu, Zhuchacun, on rotten twigs, 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12214 (HMAS 254610).

**Distribution.** China, Japan.

*Nectria berberidicola* Hirooka, Lechat, Rossman & P. Chaverri, in Hirooka, Rossman, Samuels, Lechat & Chaverri, Stud. Mycol. 71: 48, 2012.

**Specimens examined.** China, Gansu Province, Shandan, Yanzhishan, on rotten twigs, 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12084 (HMAS 279707); Tianzhu, Zhuchacun, on rotten twigs of *Berberis* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12212 (HMAS 254611); Tianzhu, Haxia, on rotten twigs of *Berberis* sp., 28 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12279, 12280, 12281 (HMAS 254612, 254613, 255801).

**Sequences.** ITS from HMAS 254613 (MT676394).

**Distribution.** China, France.

*Nectria nigrescens* Cooke, Grevillea 7(no. 42): 50, 1878.

**Specimen examined.** China, Gansu Province, Shandan, Yanzhishan, on rotten twigs of broadleaf tree, 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12085 (HMAS 255802).
Sequences. ITS (MT676393) and LSU (MT669265).

Distribution. Canada, China, France, Germany, United Kingdom, United States.

*Thelonectria discophora* (Mont.) P. Chaverri & C. Salgado sensu lato, in Chaverri, Salgado, Hirooka, Rossman & Samuels, Stud. Mycol. 68: 76, 2011.

Specimen examined. China, Gansu Province, Wuwei, Chashugou, on rotten twigs, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12149 (HMAS 279710).

Distribution. Chile, China, United Kingdom.

*Thyronectria rosellinii* (Carestia) Jaklitsch & Voglmayr, Persoonia 33: 204, 2014.

Specimens examined. China, Gansu Province, Wuwei, Chashugou, on rotten twigs, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12150, 12151, 12154 (HMAS 279711, 255821, 255822).

Distribution. Canada, China, France, Germany, Italy, Japan, United States.

*Thyronectria zangii* (Z.Q. Zeng & W.Y. Zhuang) Voglmayr & Jaklitsch, in Voglmayr, Akulov & Jaklitsch, Mycol. Progr. 15: 934, 2016.

Specimens examined. China, Gansu Province, Wuwei, Chengshanqizu, on rotten twigs of *Populus* sp., 28 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12285, 12286 (HMAS 255823, 255824).

Sequences. ITS from HMAS 255824 (MT676392).

Distribution. China.

*Trichoderma alutaceum* Jaklitsch, Fungal Diversity 48: 69, 2011.

Specimen examined. China, Gansu Province, Zhangye, Dayekou, on mossy ground under *Picea asperata*, 3 September 1958, Q.M. Ma 890 (HMAS 23955).

Distribution. Austria, China, Denmark, Finland, Germany, Japan, Sweden, United Kingdom, United States.

*Trichoderma paraviridescens* Jaklitsch, Samuels & Voglmayr, Persoonia 31: 128, 2013.

Specimen examined. China, Gansu Province, Tianzhu, Haxi, on rotten twigs, 28 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12282 (HMAS 255825).
Sequences. ITS (MT676391) and TEF1 (MT674562).

Distribution. Austria, China, Colombia, France, Germany, Greece, Iran, Italy, Japan, Mexico, Spain, Switzerland, United States.

Trichoderma virens (J.H. Mill., Giddens & A.A. Foster) Arx, Beih. Nova Hedwigia 87: 288, 1987.

Specimen examined. China, Gansu Province, Yongchang, in soil, April 2016, K. Chen TC896.

Distribution. China, United States.

Discussion

The genus Stylonectria, typified by S. applanata, was established as a monotypic genus by Höhnel (1915). It was included in Nectria by Booth (1959) and then treated as a synonym of Cosmospora Rabenh. by Rossman et al. (1999). Gräfenhan et al. (2011) resurrected the generic name Stylonectria and accepted four species. Lechat et al. (2015) described S. norvegica Lechat, J. Fourn. & Nordén from Norway. The morphological features of S. qilianshanensis, such as red perithecia growing on other ascomycetes, with flattened discoid apices, and two-layered wall bearing thick-walled outer cells, fit well the generic concept defined by Gräfenhan et al. (2011), which was confirmed by sequence analyses of the ACL1, ITS, and RPB2 regions. Stylonectria qilianshanensis is associated with S. wegeliniana (BIPP/MPBP = 100%/76%). Stylonectria purtonii [as Cosmospora purtonii (Grev.) Rossman & Samuels] on carbonaceous pyrenomycetes was the only species of the genus reported from China (Zhang and Zhuang 2003).

For some nectriaceous fungi, substrate type was considered of taxonomic importance (Zeng and Zhuang 2016). Species of Stylonectria are fungicolous and sometimes host-specific. For example, S. applanata is known only from Melogramma bulliardii Tul. & C. Tul. on Corylus avellana, S. carpini is restricted to a pyrenomycete on Carpinus, and S. wegeliniana colonizes solely on Hapalocystis bicaudata Fuckel on Ulmus glabra (Gräfenhan et al. 2011; Lechat et al. 2015). Similarly, S. qilianshanensis occurs on decayed fruit bodies of an ascomycete on the bark of Picea asperata. However, S. norvegica and S. purtonii have slightly wider host ranges. The former occurs on pyrenomycetes on Betulaceae and Fagaceae, while the latter is found on pyrenomycetes on coniferous trees. Stylonectria is currently a poorly known genus. Further investigations may provide useful information about its selection of hosts or substrates.

Gross morphology like stroma, ascospore features and asexual states were stressed for generic delimitations of the hypocrealean fungi. Species of Hypocreaceae possessing clavate to cylindrical, fleshy, bright-colored stromata were previously accommodated in Podostroma P. Karst. (Karsten 1892; Rossman et al. 1999). The accumulated
molecular evidence argued that shape of fruit body is not phylogenetically distinctive within genus. Chamberlain et al. (2004) then synonymized *Podostroma* with *Hypocrea* Fr. (= *Trichoderma*). The diagnostic characteristics for *Trichoderma* include substrate, fruit body gross morphology, anatomy, extent of fertile region, surface pigmentation of stromata, and ascospore shape, size and ornamentation (Chamberlain et al. 2004). The genera *Aphysiostroma* Barrasa, A.T. Martinez & G. Moreno, *Pseudohypocrea* Yoshim. Doi, and *Sarawakus* Lloyd, which possess gliocladium-, trichoderma- and verticillium-like asexual states, were also synonymized with *Trichoderma* (Jaklitsch et al. 2014; Jaklitsch and Voglmayr 2015; Zeng and Zhuang 2017). The taxonomic position of *T. gansuanum* is confirmed by the combined sequence analyses of RPB2 and TEF1 regions and morphological characteristics, such as the stipitate, clavate, upright stromata, cylindrical asci, and disarticulating ascospores. A few stipitate species of *Trichoderma* have not been cultured or linked to asexual states (Chamberlain et al. 2004). Knowing the whole fungus is surely our future goal.

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