Multi-Locus Phylogeny and Taxonomy of the Fungal Complex Associated With Rusty Root Rot of Panax ginseng in China

Yi Ming Guan1,2, Ying Ying Ma2, Qiao Jin2, Qiu Xia Wang2, Ning Liu2, Yong Ping Fu1, Ya Yu Zhang2 and Yu Li1*

1 Engineering Research Center, Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, China, 2 Institute of Special Wild Economic Animal and Plant Science, Chinese Academy of Agricultural Sciences, Changchun, China

Panax ginseng rusty root rot caused by the Ilyonectria species complex is a devastating disease, and it is one of the main factors contributing to the difficulty in continual cropping. Rusty root rot occurs in all ginseng fields, but little is known about the taxonomy of the fungal pathogen complex, especially Ilyonectria and Ilyonectria-like species. Rusty root rot samples were collected from commercial ginseng cultivation areas of China, and the pathogens were isolated and purified as single spores. Based on the combination analysis of multiple loci (rDNA-ITS, TUB, HIS3, TEF, ACT, LSU, RPB1, RPB2, and SSU) and morphological characteristics, the pathogens causing ginseng rusty root rot were determined. Fungal isolates were obtained from infected roots in 56 locations within main cultivation areas in China. A total of 766 strains were identified as Ilyonectria, Ilyonectria-like species, including I. robusta (55.0%), I. communis (21.7%), I. mors-panacis (10.9%), I. pseudodestructans (2.0%), I. changbaiensis (1.3%), I. qitaiheensis (1.3%), Neonectria obtusispora (2.0%), Dactylonectria torresensis (0.5%), D. sp. (0.5%), and R. panacis (1.5%), and four novel species, Thelonectria ginsengicola (1.0%), T. jixiensis (1.0%), T. mulanensis (0.8%) and T. fusongensis (0.3%), with a total of 14 species. As the pathogen present in the highest proportion, I. robusta was the most prevalent and damaging species, unlike the pathogens reported previously. All of the examined strains were proven to cause ginseng rusty root rot. Our results indicate that the taxonomy of the fungal complex associated with ginseng rusty root rot includes Ilyonectria, Ilyonectria-like genera (Dactylonectria, Neonectria, and Thelonectria) and Rheocercosporidium.

Keywords: Dactylonectria, Ilyonectria, Ilyonectria-like, Neonectria, Panax ginseng, Rheocercosporidium, rusty root rot, Thelonectria

INTRODUCTION

Panax ginseng is one of the most cultivated medicinal plants in China, and the quality of ginseng greatly influences the quality of cosmetics, health care products and medicines that use ginseng as a raw material (Baranov, 1966; Hu, 1977). Ginseng rusty root rot is caused by Ilyonectria/Cylindrocarpon or Ilyonectria/Cylindrocarpon-like species, and it is the most
devastating chronic disease and the greatest threat to ginseng (Panax ginseng) cultivation (Guan et al., 2020). Rusty root rot may cause 30% loss of ginseng, and it can be found in all ginseng planting areas. Rusty root rot has always been one of the important factors that interferes with the maintenance of continuous ginseng cropping. The incidence of rusty root rot in 2-year-old ginseng continuous cropping is 95.8% (Cho et al., 1995).

All parts of the ginseng root may be infected, and the infected root exhibits reddish brown dry rot with a gully appearing on most of the root. The infection stops with increased temperature, and some parts of the ginseng root may undergo self-healing, but the gully or dry rot scars remain. The appearance of ginseng root rot and the degree of damage greatly influence the value of ginseng (Zhou et al., 2017). Ilyonectria and Ilyonectria-like species are common soil fungi, opportunistic plant root pathogens, or asymptomatic root endophytes (Seifert et al., 2003). Ginseng seeds also carry Ilyonectria pathogens that may pose a threat to ginseng cultivation (Guan et al., 2019). Ilyonectria fungi play an important role in black foot rot in grapevines (Halleen et al., 2004; Halleen et al., 2006a,b), apple replant disease (Tewoldemedhin et al., 2011), and beech cankers (Castlebury et al., 2006); the affected plants are representative hosts of economic importance, especially the lignified roots of perennials. Thus, Ilyonectria and Ilyonectria-like species are commonly associated with rot and decay of woody and herbaceous plants (Domsch et al., 2007).

The pathogen associated with root rot disease of Panax quinguefalcum was first extracted by Zinnsmeister (1918), and it was designated Ramularia destructans. Similar diseases were found by Chou and Chung on ginseng in China and Korea (Chung, 1979; Wang, 2001). Scholten established the new species C. destructans, and R. destructans was treated as a synonym (Scholten, 1964).

Neonectria/Cylindrocarpon is a paraphyletic based on phylogenetic analysis (Mantiri et al., 2001; Halleen et al., 2004; Hirooka et al., 2005; Castlebury et al., 2006). The Neonectria complex was divided into four genera (Ilyonectria, Neonectria/Cylindrocarpon, Rugenectria and Thelonectria) based on a combination of morphological characteristics. Ilyonectria replaced the N. radicicola group based on the description by Booth (1966); Seifert et al. (2003), and the corresponding Cylindrocarpon also became Ilyonectria (Mantiri et al., 2001).

I. destructans was linked to the teleomorph I. radicicola (Booth, 1966; Samuels and Brayford, 1990; Chaverri et al., 2011). Based on a phylogenetic analysis of nuclear ribosomal internal transcribed spacer (rDNA-ITS) gene sequences, Schroers et al. concluded that the I. radicicola complex included C. destructans, C. destructans var. crassum, I. coprosmae, I. lirioidendri, N. austrotredicola and N. macroconidialis (Schroers et al., 2007). The rDNA-ITS, β-tubulin (TUB), histone H3 (HIS3) and translation elongation factor 1-α (TEF) genes were used to support the I. radicicola species complex, and HIS3 was the most contributing gene (Cabral et al., 2012b). Sixty-eight strains of pathogenic fungi from ginseng and other hosts were once considered C. destructans within four groups: I. mors-panacis, I. robusta, I. panacis, and I. crassa (Cabral et al., 2012a). Lu et al. (2020) obtained 230 isolates and believed that ginseng red-sin root was caused by 12 species, including Fusarium, Dactylonectria, Ilyonectria and others.

Thelonectria was first classified in Cylindrocarpon and first described in 2011 to accommodate grouping in the Cylindrocarpon classification (Chaverri et al., 2011). However, the forms isolated from soil were the asexual states of Thelonectria. Thelonectria and related species with Cylindrocarpon-like morphology were redefined using molecular phylogenetic techniques (Chaverri et al., 2011; Salgado-Salazar et al., 2012, 2013, 2015). The observation that R. panacis causes ginseng rusted root rot was reported for the first time in 2006, after more than 70 years of the disease (Reelered et al., 2006; Reelered, 2007).

In this work, we report the population structure of pathogens associated with ginseng rusty root rot. In the commercial ginseng cultivation areas in China, 90% of rusty root rot is thought to be caused by I. destructans (Wang, 2001), but the experimental data support different conclusions. The present study determined that the pathogens that cause rusty root rot disease of ginseng are consistently associated with a fungal complex species, and management strategies must be developed.

MATERIALS AND METHODS

Sample Collection, Fungal Isolation, and Morphological Observation

Fresh ginseng roots with rusty root rot symptoms (Figure 1) were collected between 2017 and 2019 from 56 sampling locations in northeast China that corresponded to the commercial ginseng-producing areas of China (Figure 2). The junction of the healthy and diseased parts was cut into 25-mm³ pieces, and the surfaces of the pieces were disinfected by immersion in 1% NaOCl for 2 min and washing three times with sterile water. The pieces were evenly placed on water agar (WA) medium cultured in the dark at 22°C. Colonies were collected for 15-21 days, purified and cultured from single spores (Punja, 1997). The cultures were transferred to potato dextrose agar (PDA) medium and synthetic nutrient-poor agar (SNA) and cultured in the dark for 20-60 days, and the characteristics of the resulting cultures were recorded. The isolates were cultivated on PDA supplemented with ginseng roots under continuous near-ultraviolet light (n-UV, 315-400 nm), and the characteristics of conidiophore cells and the shape and size of chlamydospores and conidia were observed. Images and measurements were acquired using a KEYENCE VXH-5000 digital microscope system (Osaka, Japan).

DNA Extraction and PCR Amplification

Colonies grown for 15 days were collected for DNA extraction. DNA was obtained by following the procedures described in the Qiagen DNeasy Plant Mini Kit (Qiagen 69104, Qiagen, Hilden, Germany). Each 50-µl PCR aliquot contained 25 ml
of premix (Takara R045, Kusatsu, Japan; the error rate of the DNA polymerase is 0.02%), 2 μl of each primer (10 μM), 1 μl of DNA (500 ng/μl), and 20 μl of double-distilled H₂O. The following PCR program was used to obtain the sequences of the actin gene (ACT), rDNA-ITS, the 28S ribosomal RNA gene (LSU), the gene encoding RNA polymerase II largest subunit (RPB1), the gene encoding RNA polymerase II second-largest subunit (RPB2), 18S ribosomal RNA (SSU), TEF, TUB and HIS3: 95°C for 3 min; 30 cycles of 95°C for 15 s, annealing temperature for 15 s, 72°C for 60 s, and 72°C for 10 min; the sample was then held at 4°C. The amplicons were analyzed using 0.8% agarose gel electrophoresis in 0.5 × TAE buffer.
TABLE 1 | Details regarding the PCR primers used in species identification and gene sequencing.

| Gene region                               | Primer  | Direction | Annealing temperature (°C) | References                                      |
|-------------------------------------------|---------|-----------|-----------------------------|-------------------------------------------------|
| Nuclear ribosomal internal transcribed spacer | V9G     | sense     | 51                          | de Hoog and van den Ende, 1998                  |
|                                           | ITS4    | antisense |                             |                                                  |
| Actin                                     | ACT512F | sense     | 51                          | Carbone and Kohn, 1999; Groenewald et al., 2013 |
|                                           | ACT1RD  | antisense |                             |                                                  |
| 28S ribosomal RNA gene                    | LROR    | sense     | 48                          | Vilgalys and Hester, 1990; Moncalvo et al., 2000 |
| RNA polymerase II largest subunit         | LR5     | antisense |                             |                                                  |
|                                           | RPB1a   | sense     | 55                          | Castlebury et al., 2004                         |
| Histone H3 gene                           | RPB1c   | antisense |                             |                                                  |
| RNA polymerase II second-largest subunit  | CYLH3F  | sense     | 50                          | Crous et al., 2004                              |
|                                           | CYLH3R  | antisense |                             |                                                  |
|                                           | RPB2-7cf| sense     | 54                          | Liu et al., 1999                               |
| 18S ribosomal RNA gene                    | NS1     | sense     | 55                          | Gargas and Taylor, 1992                         |
| β-tubulin                                 | BT3     | sense     | 52                          | Lu et al., 2020; O’Donnell and Cigelnik, 1997   |
|                                           | BT4     | antisense |                             |                                                  |
|                                           | T1      | sense     |                             |                                                  |
|                                           | T2      | antisense |                             |                                                  |
| Translation elongation factor 1-alpha     | CYLEF-1 | sense     | 55                          | Carbone and Kohn, 1999; Crous et al., 2004      |
|                                           | CYLEF-R2| antisense |                             |                                                  |
|                                           | EF728   | sense     |                             |                                                  |
|                                           | EF1567  | antisense |                             |                                                  |

Details regarding the PCR primers and annealing temperatures are listed in Table 1.

DNA Sequencing and Phylogenetic Analysis

The purified amplicons were sequenced in both directions by Sangon Biotech (Shanghai, China), and the sequences were assembled using Seqman (v8.1). Phylogenetic trees were constructed using the combined rDNA-ITS, TUB, HIS3 and TEF genes for analyses of Dactylonectria, Ilyonectria and Neonectria, and the analysis of Thelonectria was based on a combination of the ACT, rDNA-ITS, LSU, RPB1, RPB2, SSU, TEF and TUB genes. All sequences were homologously aligned by mega 7.06. A maximum parsimony (MP) method was used to construct a phylogenetic tree in the PAUP 4.0b program. The MP analyses were performed with the heuristic search option, and 100 random sequence additions were used to find the global optimum tree. The gaps were treated as missing data, and the strength of the internal branches of the resulting trees was tested with bootstrap analysis using 1000 replications. The consistency index (CI), retention index (RI), and rescaled consistency index (RC) of the tree were also calculated. Detailed information on the sequences of the genes whose accession numbers were applied for in the NCBI database, including all strains used for phylogeny, is shown in Tables 2, 3.

Pathogenicity Tests

The 26 newly sequenced strains were also subjected to pathogenicity testing (Table 3). The pathogenicity of the isolates was evaluated on 3-year-old detached ginseng roots (cultivar: Damaya) using an improved published method in vitro (Lu et al., 2020). The fresh ginseng roots were washed, wiped with 75% alcohol, and rinsed with sterile water. Healthy ginseng roots were inoculated by placing 50 µl of 1 × 10⁵/ml spore suspension taken from the edges of actively growing colonies on PDA plates into premade holes 4 mm in diameter and 1 mm deep. Two to three holes per root and four replicated roots were inoculated for each isolate, and sterile water was used as the control. The ginseng roots were cultured in a fresh-keeping box at 25°C in the dark and evaluated after 7 days. The same strain and disinfection method were used to inoculate whole plants in the greenhouse (25°C, 78% humidity). After disinfection, 3-year-old ginseng seedlings were dipped into a 1 × 10⁵/ml spore suspension for 20 min and then transferred to pots containing sterile sand, with 6 plants per pot. Each
**TABLE 2** | Strains analyzed in this study.

| Species                  | Strain a | Origin       | GenBank accession number b |
|--------------------------|----------|--------------|----------------------------|
|                          |          |              | rDNA-ITS | TUB | HIS3 | TEF1-α | ACT | LSU | RPB1 | RPB2 | SSU |
| D. alcacerensis          | IAFM Cy20-1 | Spain       | JF735332 | AM419104 | JF735629 | JF735818 |
| D. alcacerensis          | 129087   | Portugal     | JF735333 | AM419111 | JF735630 | JF735819 |
| D. anthruriicola         | CBS 564.95 | Netherlands  | JF735302 | JF735430 | JF735579 | JF735768 |
| D. estremencensis        | Cy135    | Portugal     | AM419069 | AM419105 | JF735615 | JF735804 |
| D. estremencensis        | CBS 129085 | Portugal     | JF735320 | JF735448 | JF735617 | JF735806 |
| D. hordeicola           | CBS 162.89 | Portugal     | AM419060 | AM419084 | JF735610 | JF735799 |
| D. hordeicola           | 3S07     | China        | MF350482 | MF350428 | MF350455 | MF350509 |
| D. macrodidyma           | CBS 12615 | South Africa | AY677290 | AY677233 | JF735647 | JF735836 |
| D. macrodidyma           | CBS 112601 | South Africa | AY677284 | AY677229 | JF735644 | JF735833 |
| D. novozelandica         | CBS 112608 | South Africa | AY677288 | AY677235 | JF735632 | JF735821 |
| D. novozelandica         | CBS 113552 | New Zealand  | JF735334 | JF735633 | JF735822 | JF735822 |
| D. pinicola             | CBS 159.34 | UK, England  | JF735318 | JF735446 | JF735613 | JF735802 |
| D. pinicola             | CBS 173.37 | Germany      | JF735319 | JF735447 | JF735614 | JF735803 |
| D. sp.                  | Q18-22   | China        | MT678572* | MT810745* | MT800966* | MT800973* |
| D. sp.                  | Q18-23   | China        | MT678573* | MT810748* | MT800967* | MT800974* |
| D. torresensis          | CBS 129086 | Portugal     | JF735362 | JF735492 | JF735681 | JF735870 |
| D. torresensis          | CBS 113555 | New Zealand  | JF735350 | AY677234 | JF735661 | JF735850 |
| D. torresensis          | DT2      | China        | MT678571* | MT810744* | MT800965* | MT800972* |
| D. vitis                | CBS 129082 | Portugal     | JF735303 | JF735431 | JF735580 | JF735769 |
| I. changbaiensis         | CGMCC 3.18789 | China     | MF350464 | MF350410 | MF350437 | MF350491 |
| I. changbaiensis         | 72R2     | China        | MF350465 | MF350411 | MF350438 | MF350492 |
| I. changbaiensis         | CB4-7    | China        | MT678567* | MT810740* | MT800951* | MT800968* |
| I. changbaiensis         | Q24-5    | China        | MT678568* | MT810741* | MT800952* | MT800969* |
| I. communis             | CGMCC 3.18788 | China     | MF350402 | MF350402 | MF350429 | MF350483 |
| I. communis             | J410     | China        | MF350457 | MF350403 | MF350430 | MF350464 |
| I. communis             | CB4-2    | China        | MT678565* | MT810738* | MT800949* | MT800966* |
| I. communis             | H1-9     | China        | MT678566* | MT810739* | MT800960* | MT800967* |
| I. coprosmae            | CBS 119606 | Canada       | JF735360 | JF735373 | JF735505 | JF735694 |
| I. crassa               | CBS 129083 | Canada       | AY295311 | JF735395 | JF735536 | JF735725 |
| I. crassa               | CBS 158.31 | Canada       | JF735694 | JF735376 | JF735535 | JF735724 |
| I. cyclaminicola        | CBS 302.93 | Netherlands  | JF735304 | JF735432 | JF735581 | JF735770 |
| I. cyclaminicola        | EFA-444  | Spain        | MF440369 | MF797792 | MF471472 | MH070096 |
| I. destructans          | CBS 264.65 | Sweden       | AY677273 | AY677256 | JF735506 | JF735695 |
| I. europaea             | CBS 102892 | Germany      | JF735295 | JF735422 | JF735669 | JF735758 |
| I. europaea             | CBS 129078 | Portugal     | JF735294 | JF735421 | JF735657 | JF735756 |
| I. gamsii               | CBS 940.97 | Netherlands  | AM419065 | AM419089 | JF735577 | JF735766 |
| I. leucospermi          | CBS 13289 | South Africa | JX231161 | JX231113 | JX231145 | JX231129 |

(Continued)
| Species          | Strain\(^a\) | Origin         | GenBank accession number\(^b\) |
|------------------|--------------|----------------|--------------------------------|
|                   |              |                | rDNA-ITS | TUB  | HIS3 | TEF1-\(\alpha\) | ACT | LSU | RPB1 | RPB2 | SSU |
| I. leucospermi   | CBS 132810   | South Africa   | JX231162 | JX231114 | JX231146 | JX231130 |
| I. liliiigena    | CBS 732.74   | Netherlands    | JF735298 | JF735426 | JF735574 | JF735763 |
| I. liliiigena    | CBS 189.49   | Netherlands    | JF735297 | JF735425 | JF735573 | JF735762 |
| I. liriodendri   | CBS 117526   | Portugal       | DQ178164 | DQ178171 | JF735506 | JF735697 |
| I. liriodendri   | CBS 110.81   | United States  | DQ178163 | DQ178170 | JF735507 | JF735696 |
| I. lusitanica    | CBS 129080   | Portugal       | JF735296 | JF735423 | JF735570 | JF735759 |
| I. mors-panasis  | CBS 306.35   | Canada         | JF735288 | JF735414 | JF735557 | JF735746 |
| I. mors-panasis  | H-1          | China          | MT678563* | MT810736* | MT800947* | MT800964* |
| I. mors-panasis  | XFC1         | China          | MT678564* | MT810737* | MT800948* | MT800965* |
| I. panasis       | CBS 129079   | Canada         | AJ295318  | JF735424 | JF735572 | JF735761 |
| I. palmarum      | CBS 135753   | Italy          | HF937432  | HF922609 | HF922621 | HF922615 |
| I. palmarum      | CBS 135754   | Italy          | HF937431  | HF922608 | HF922620 | HF922614 |
| I. proteanum     | CBS 132812   | South Africa   | JX231165  | JX231117 | JX231149 | JX231133 |
| I. proteanum     | CBS 132811   | South Africa   | JX231157  | JX231109 | JX231141 | JX231125 |
| I. pseudodestructans | CBS 129081 | Portugal       | AJ875330  | AM419091 | JF735563 | JF735752 |
| I. pseudodestructans | CBS 117824 | Austria        | JF735292  | JF735419 | JF735562 | JF735751 |
| I. pseudodestructans | ZP2     | China          | MT678561* | MT810734* | MT800945* | MT800962* |
| I. pseudodestructans | PR20-11  | China          | MT678562* | MT810735* | MT800946* | MT800963* |
| I. qitaheensis   | CGMCC 3.18787 | China         | MF504072  | MF350418 | MF350445 | MF350499 |
| I. qitaheensis   | J919         | China          | MF504073  | MF350419 | MF350446 | MF350500 |
| I. qitaheensis   | R3-2         | China          | MT678569* | MT810742* | MT800953* | MT800970* |
| I. robusta       | CBS 117818   | Austria        | JF735267  | JF735382 | JF735523 | JF735712 |
| I. robusta       | CBS 129084   | Portugal       | JF735273  | JF735391 | JF735532 | JF735721 |
| I. robusta       | H-5          | China          | MT678559* | MT810732* | MT800943* | MT800960* |
| I. robusta       | CB13-12      | China          | MT678560* | MT810733* | MT800944* | MT800961* |
| I. rufa          | CBS 153.37   | France         | AY677271  | AY677251 | JF735450 | JF735729 |
| I. rufa          | CBS 640.77   | France         | JF735277  | JF735399 | JF735542 | JF735731 |
| I. g             | CBS 142253   | Italy          | KY304649  | KY304755 | KY304621 | KY304727 |
| I. streitziiae   | CBS 142254   | Italy          | KY304651  | KY304757 | KY304623 | KY304729 |
| I. vredehoekensis| CBS 132807   | South Africa   | JX231155  | JX231107 | JX231139 | JX231123 |
| I. vredehoekensis| CBS 132808   | South Africa   | JX231159  | JX231111 | JX231143 | JX231127 |
| N. coccinea      | CBS 119158   | Germany        | JF288759  | KC660273 | JF288734 |
| N. faginata      | CBS 217.67   | Canada         | HQ840385  | JF268730 | JF268746 |
| N. faginata      | CBS 119160   | United States  | HQ840384  | DQ789883 | DQ789740 |
| N. lugdunensis   | CBS 1254585  | China          | KM231762  | KM232019 | KM231482 | KM231187 |
| N. obtusispora   | CBS 183.36   | Germany        | AM419061  | AM419085 | JF735607 | JF735796 |
| Species                  | Strain\(^a\) | Origin      | GenBank accession number\(^b\) |
|-------------------------|--------------|-------------|--------------------------------|
|                         |              |             | rDNA-ITS | TUB     | HIS3 | TEFl-\(\alpha\) | ACT | LSU | RPB1 | RPB2 | SSU |
| *N. obtusispora*        | CPC 13544    | Canada      | AY295306 | JF735443 | JF735608 | JF735797 |
| *N. obtusispora*        | H7-6         | China       | MT678754V | MT810747* | MT800958* | MT800975* |
| *N. obtusispora*        | Q12-4        | China       | MT678575* | MT810748* | MT800959V | MT800976* |
| *N. punicea*            | CBS 242.29   | Germany     | KC660522 | DQ789673 |          |          |
| *N. punicea*            | 119724       | Austria     | KC660469 | DQ789824 |          |          |
| *N. ramulariae*         | CBS 151.29   | England     | AY677291 | JF735438 | JF735602 | JF735791 |
| *N. ramulariae*         | 182.36       | Unknown     | HMO54157 | JF735439 | JF735603 | JF735792 |
| *N. shennongjiana*      | CBS 127475   | China       | MH864598 | KJ022346 |          |          |
| *N. shennongjiana*      | HMAS 183185  | China       | FJ860440 | FJ860057 |          |          |
| *Rhexocercosporidium*   | RP17         | China       | MT814852* | MT822282* | MT822281* |          |
| *T. acrotyla*           | IMI 345086   | Venezuela   | KJ021971 | KJ022293 | KJ022347 | KJ022238 |
| *T. acrotyla*           | CBS 123766   | Venezuela   | JQ403329 | JQ394720 | JQ394751 | JQ365047 |
| *T. amamiensis*         | MAFF 239820  | Japan       | JQ403338 | JQ394728 | KJ022349 | JQ365055 |
| *T. amamiensis*         | MAFF 239819  | Japan       | JQ403337 | JQ394727 | KJ022348 | JQ365054 |
| *T. blackeriella*       | CBS 142200   | Italy       | KX778111 | KX778702 | KX77887 | KX778690 |
| *T. ciliaria*           | CBS 132324   | Costa Rica  | KJ021972 | JQ394715 | KJ022351 | JQ365043 |
| *T. ciliaria*           | IMI 325844   | Jamaica     | KJ021973 | JQ394707 | KJ022439 | JQ365037 |
| *T. coronalis*          | CBS 132337   | China       | JQ403343 | JQ394732 | KJ022348 | JQ365054 |
| *T. coronalis*          | CBS 132338   | China       | JQ403344 | JQ394733 | KJ022352 | JQ365044 |
| *T. coronata*           | IMI 325241   | Indonesia   | JQ403326 | JQ394717 | KJ024736 | JQ365040 |
| *T. coronata*           | CBS 132322   | Costa Rica  | JQ403230 | JQ394711 | KJ024736 | JQ365040 |
| *T. diademata*          | CBS 132331   | Argentina   | JQ403306 | JQ394700 | KJ022328 | JQ365029 |
| *T. diademata*          | CBS 132332   | Argentina   | JQ403351 | JQ394736 | KJ022383 | JQ365032 |
| *T. gongylodes*         | CBS 124611   | United States | JQ403318 | JQ394710 | JQ403401 | JQ403395 |
| *T. gongylodes*         | IMI 343571   | United States | JQ403331 | JQ394721 | JQ403401 | JQ403395 |
| *T. nodosa*             | CBS 124742   | United States | JQ403306 | JQ394699 |          |          |
| *T. nodosa*             | CBS 123272   | United States | JQ403317 | JQ394709 |          |          |
| *T. olida*              | CBS 215. 67  | Germany     | KJ021982 | KM232024 |            |          |
| *T. stemmata*           | CBS 112468   | Jamaica     | JQ403312 | JQ394704 | JQ403739 | JQ403352 |
| *T. stemmata*           | CBS 123236   | Jamaica     | JQ403313 | JQ394705 | KJ022384 | JQ403353 |
| *T. torulosa*           | CBS 136782   | Cameroon    | KJ02007  |            | KJ023900 | JQ365008 |
| *T. torulosa*           | CBS 132339   | Argentina   | JQ403309 | JQ394701 | KJ022389 | JQ365030 |
| *T. truncata*           | MAFF 241521  | Japan       | JQ403339 |            | KJ022325 | JQ365066 |
| *T. veillotiana*        | CBS 132341   | Azores Island | JQ403305 | JQ394698 | KJ022373 | JQ365067 |
| *T. veillotiana*        | CBS 124114   | France      | JQ403335 | JQ394726 | KJ022737 | JQ365064 |
| *T. westlandica*        | ICMP 10387   | New Zealand | KF569844 | KJ569871 | KF569861 | KF569834 |

*Continued*
Continued

| Species          | Strain | GenBank accession number | GenBank accession number | GenBank accession number | GenBank accession number | GenBank accession number | GenBank accession number | GenBank accession number | GenBank accession number |
|------------------|--------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| T. ginsengicola  | IMI 255610 | New Zealand | KF569843  | KF569870  | KF569862  | KF569833  | KF569853  | KF569881  | KJ022581  |
| T. mulanensis    | CGMCC 3.20154; R9 China | MT742968* | MT742970* | MT792252* | MT792254* | MT792256* | MT792260* | MT792262* | MT792264* |
| T. mulanensis    | CGMCC 3.20155; Q20-8 China | MT742970* | MT742972* | MT792254* | MT792256* | MT792258* | MT792260* | MT792262* | MT792264* |
| T. fusongensis   | CGMCC 3.20153; R1-8 China | MT742972* | MT742974* | MT792256* | MT792258* | MT792260* | MT792262* | MT792264* | MT792266* |
| T. jixiensis     | P4 China | MT742973* | MT742975* | MT792257* | MT792259* | MT792260* | MT792262* | MT792264* | MT792266* |
| Cinnamomeonectria| CBS 133756 French Guiana | KJ021979  | KJ022341  | KJ022393  | KJ0221286 | KJ022072  | KJ022451  | KJ022584  | KJ022206  |

*Sequences newly generated in this study.

rDNA-ITS, Nuclear ribosomal internal transcribed spacer; HIS3, histone H3 gene; TEF-1α, translation elongation factor 1-alpha gene; TUB, β-tubulin gene; ACT, actin gene; LSU, 28S ribosomal RNA gene; RPB1, RNA polymerase II largest subunit gene; RPB2, RNA polymerase II second-largest subunit gene; SSU, 18S ribosomal RNA gene.

RESULTS

Population Structure of Fungal Complex Associated With Ginseng Rusty Root Rot

A total of 766 isolates were obtained from ginseng roots that showed typical signs of rusty root rot disease. All of these strains were determined by sequencing and morphological characteristics, and 26 strains from each genus were randomly selected for phylogenetic analysis. A total of 14 species (Figures 3, 4) were identified as Ilyonectria, Ilyonectria-like and Rhexocercosporidium species.

The Ilyonectria species complex included I. robusta, I. communis, I. mors-panacis, I. pseudodestructans, I. changhaiensis and I. qitaheensis, and the isolate proportions were 55.0%, 21.7%, 10.9%, 2.0%, 1.3% and 1.3%, respectively. Ilyonectria-like contained three genera that were also members of the Nectriaceae family: Dactylonectria, Neonectria and Thelonectria. N. obtusispora, D. torresensis and D. sp. accounted for 2.0%, 0.5% and 0.5% of the population, respectively. Four novel species are named in this article: T. ginsengicola (1.0%), the type strain is R9; T. jixiensis (1.0%), the type strain is Q21-5; T. mulanensis (0.8%), the type strain is Q20-8 and T. fusongensis (0.5%), the type strain is R1-8. R. panacis was the only pathogen except Ilyonectria-like that was present at an abundance of 1.5%. I. communis, I. pseudodestructans, I. changhaiensis, I. qitaheensis, N. obtusispora, D. torresensis, T. jixiensis, T. mulanensis and T. fusongensis were first reported to cause ginseng rusty root rot. Thelonectria is also the first Ilyonectria-like genus reported to cause ginseng rusty root rot. I. robusta was the dominant pathogen and appeared in the largest proportion; this is the greatest difference between our results and previous conclusions. The number of fungal isolates recovered from each sampling location in China is shown in Table 4.

Molecular Phylogenetic Analysis

The sequences of rDNA-ITS, TEF, TUB and HIS3 were obtained using polymerase chain reaction amplicons and were analyzed in Dactylonectria, Ilyonectria, and Neonectria. Low-quality sequences were removed after base alignment to obtain the sequences of rDNA-ITS (491 bp), HIS3 (472 bp), TEF (507 bp), and TUB (467 bp). The sequences were combined, and a sequence with a total length of 2231 bp, including alignment gaps, was used to build a phylogenetic tree (Figure 3). Similarly, ACT (575 bp), rDNA-ITS (533 bp), LSU (700 bp), RPB1 (630 bp), RPB2 (856 bp), SSU (521 bp), TEF (858 bp), and TUB (479 bp) were combined, resulting in a total length of 4422 bp including alignment gaps, and analyzed for Thelonectria (Figure 4). All of the sequences were topologically congruent, and the results indicated that Dactylonectria, Ilyonectria, Neonectria and Thelonectria formed a single clade. The isolated strains were divided into 14 highly supported clades; the group was grown in triplicate. The inoculum was treated with sterile water as a control. After 2 months, plant infection was evaluated.
FIGURE 3 | Phylogenetic tree based on the combined rDNA-ITS, TUB, HIS3 and TEF gene sequences constructed using the maximum parsimony method of the PAUP 4.0b program. *Rhizocerocordylophora panacias* R59 was used as an outgroup. Bold font indicates the strains isolated in this study.
FIGURE 4 | Phylogenetic tree based on the combined ACT, rDNA-ITS, LSU, RPB1, RPB2, SSU, TEF and TUB gene sequences constructed using the maximum parsimony method of the PAUP 4.0b program. Cinnmomeonectria cinammomea CBS 133756 was used as an outgroup. Bold font indicates the strains isolated in this study.
Brackets contain I. rubosta, I. mors-panacis and other known species were highly consistent with species type or verified species.

Four novel species were divided into independent clades, and they were supported with 90-100% bootstrap support for T. mulanensis, T. jixiensis, T. fusongensis and T. ginsengcola (Figure 4). For D. sp., we observed only a few macroconidia, and the specific sporulation structure and other morphological characteristics were not clear. Therefore, the species is not named here. Similarly, a separate gene phylogenetic tree was constructed for Thelonectria, with the least information obtained from the SSU and TUB genes, and the lowest contribution. ACT and RP2 were the most informative and were key to distinguishing the phylogenetic tree of all of the known candidate strains and four novel species. The combined use of rDNA-ITS, TUB, TEF, and LSU genes will aid in the classification and analysis of all candidate species. The four novel species of Thelonectria belong to the T. coronata complex classification reported in 2016 (Salgado-Salazar et al., 2016). The three closely related species T. mulanensis, T. jixiensis, and T. fusongensis have nucleotide sequence differences in their ACT, rDNA-ITS, LSU, RP2, RP2, SSU, TEF, and TUB genes (Table 5). There is no nucleotide difference in the SSU gene, 1 bp difference in the rDNA-ITS and LSU genes, and 2 bp differences in the RP2 gene, thus, these three genes are ineffective for distinguishing the three species. These three species have 5 bp differences in ACT, 14 bp differences in RP2, 19 bp differences in TEF and 4 bp differences in TUB. T. mulanensis and T. fusongensis have 3 bp differences in RP2, 5 bp differences in TEF and 4 bp differences in TUB. Phylogenetic analysis combining the RP2, TEF and TUB genes is most effective at distinguishing T. mulanensis and T. fusongensis. T. mulanensis and T. fusongensis were clustered on a branch with a close genetic relationship and may form a complex group. However, due to their distinct morphological characteristics of conidia size and number of septa, they were considered two novel species.

### Brief Introduction to the Morphological Characteristics of Ilyonectria Species

Morphological characteristics also support the classification of phylogenetic trees. The morphological characteristics of I. robusta, I. communis, I. mors-panacis, I. pseudodestructans, I. changbaiensis, I. qitaiheensis, N. obtusispora, D. torresensis and R. panacis were consistent with previously reported strains. Four novel species were confirmed by phylogeny and are described as follows.

*Thelonectria ginsengcola* Y. M. Guan & Y. Li, sp. nov. MycoBank MB836525 (Figure 5).

**Etymology:** Named after the host Panax ginseng. “Ginsengcola” means “born on ginseng.”

### TABLE 3 | Detailed information on the collection of isolates used in sequencing and pathogenicity testing.

| Species         | Strain | Origin                                      | Collection date | Host      | Years |
|-----------------|--------|---------------------------------------------|-----------------|-----------|-------|
| D. sp.          | Q18-22 | Yongqiang Town, Antu City, Jilin Province, China | 08, 2019        | P. ginseng| 3     |
| D. sp.          | Q18-23 | Xinhe Town, Antu City, Jilin Province, China | 09, 2018        | P. ginseng| 4     |
| D. torresensis  | DT2    | Duling Town, Tonghua City, Jilin Province, China | 06, 2017        | P. ginseng| 4     |
| I. changbaiensis| CB4-7  | Beigang Town, Baishan City, Jilin Province, China | 07, 2019        | P. ginseng| 4     |
| I. changbaiensis| Q24-5  | Datougu Town, Wangqiang City, Jilin Province, China | 08, 2018        | P. ginseng| 3     |
| I. communis     | CB4-2  | Dongbeicha Town, Baishan City, Jilin Province, China | 08, 2018        | P. ginseng| 3     |
| I. communis     | H1-9   | Hulin Town, Jixi City, Heilongjiang Province, China | 09, 2017        | P. ginseng| 4     |
| I. mors-panasis | H6-1   | Hallun Town, Hallun City, Heilongjiang Province, China | 09, 2019        | P. ginseng| 5     |
| I. mors-panasis | XFC1   | Xigang Town, Baishan City, Jilin Province, China | 06, 2018        | P. ginseng| 3     |
| I. pseudodestructans | ZP2 | Zuoja Town, Jil City, Jilin Province, China | 06, 2018        | P. ginseng| 4     |
| I. pseudodestructans | PR20-11 | Datougu Town, Wangqiang City, Jilin Province, China | 06, 2018        | P. ginseng| 5     |
| I. qitaheensis  | R3-2   | Longquan Town, Baishan City, Jilin Province, China | 06, 2018        | P. ginseng| 3     |
| I. qitaheensis  | PR14 25 | Fusong County, Baishan City, Jilin Province, China | 06, 2018        | P. ginseng| 3     |
| I. robusta      | H8-5   | Hallin Town, Mudanjiang City, Heilongjiang Province, China | 08, 2019        | P. ginseng| 4     |
| I. robusta      | CB13-12 | Wanliang Town, Fusong County, Baishan City, Jilin Province, China | 08, 2019        | P. ginseng| 2     |
| N. obtusispora  | H7-6   | Zhanhe Town, Heihe City, Heilongjiang | 08, 2019        | P. ginseng| 3     |
| N. obtusispora  | Q12-4  | Longquan Town, Baishan City, Jilin Province, China | 08, 2019        | P. ginseng| 4     |
| Rhexocercosporidium panacis | RP17 | Yulin Town, Jia City, Jilin Province, China | 08, 2019        | P. ginseng| 3     |
| T. ginsengcola  | CGMCC 3.20154; R9 | Dongbeicha Town, Baishan City, Jilin Province, China | 07, 2018        | P. ginseng| 4     |
| T. ginsengcola  | R4     | Quanyang Town, Baishan City, Jilin Province, China | 07, 2018        | P. ginseng| 3     |
| T. mulanensis   | CGMCC 3.20155; Q20-8 | Bayan Town, Mulan County, Heilongjiang, China | 06, 2017        | P. ginseng| 4     |
| T. mulanensis   | Q20-5  | Mulan Town, Mulan County, Heilongjiang, China | 06, 2017        | P. ginseng| 4     |
| T. fusongensis  | CGMCC 3.20153; R1-8 | Fusong Town, Fusong County, Baishan City, Jilin Province, China | 06, 2017        | P. ginseng| 3     |
| T. fusongensis  | P4     | Wanliang Town, Fusong County, Baishan City, Jilin Province, China | 06, 2017        | P. ginseng| 3     |
| T. jixiens      | CGMCC 3.20156; Q21-5 | Hulin Town, Jixi City, Heilongjiang Province, China | 07, 2018        | P. ginseng| 4     |
| T. jixiens      | Q21-1  | Hulin Town, Jixi City, Heilongjiang Province, China | 07, 2018        | P. ginseng| 3     |
### TABLE 4 | Fungal isolates recovered from *Panax ginseng* with rusty root rot symptoms in China.

| Location                                                                 | A | B | C | D | E | F | G | H | I | J | K | L | M | N |
|-------------------------------------------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jindou Town, Tonghua City, Jilin Province, China                      | 12| 2 |   |   |   |   |   |   |   |   |   |   |   |   |
| Chaoyang Forestry Centre, Tonghua City, Jilin Province, China        | 7 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |
| Sanyuanpu Town, Jian City, Jilin Province, China                    | 9 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |
| Duling Town, Tonghua City, Jilin Province, China                    | 5 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |
| Fujian Town, Tonghua City, Jilin Province, China                    | 4 | 4 |   |   |   |   |   |   |   |   |   |   |   |   |
| Jianglianzhu Town, Tonghua City, Jilin Province, China               | 6 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |
| Qinghe Town, Jian City, Jilin Province, China                       | 10|   |   |   |   |   |   |   |   |   |   |   |   |   |
| Yulin Town, Jian City, Jilin Province, China                        | 7 | 3 |   |   |   |   |   |   |   |   |   |   |   |   |
| Shuangchua Town, Jian City, Jilin Province, China                  | 8 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Maxian Town, Jian City, Jilin Province, China                       | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Linghou Town, Jian City, Jilin Province, China                     | 7 | 5 |   |   |   |   |   |   |   |   |   |   |   |   |
| Taishang Town, Jian City, Jilin Province, China                    | 11|   |   |   |   |   |   |   |   |   |   |   |   |   |
| Sankeyushu Town, Jian City, Jilin Province, China                  | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Zuojia Town, Jili City, Jilin Province, China                       | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Longfeng Forestry Centre, Jiaohe City, Jilin Province, China       | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Wulin Town, Jiaohe City, Jilin Province, China                     | 4 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Qianjin Town, Jiaohe City, Jilin Province, China                  | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Dongbeichua Town, Baishan City, Jilin Province, China              | 17| 12| 3 |   |   |   |   |   |   |   |   |   |   |   |
| Longquan Town, Baishan City, Jilin Province, China                 | 8 | 11| 4 | 2 | 2 | 4 |   |   |   |   |   |   |   |   |
| Xingshen Town, Baishan City, Jilin Province, China                 | 19| 3 | 1 |   |   |   |   |   |   |   |   |   |   |   |
| Beigang Town, Baishan City, Jilin Province, China                  | 11| 9 | 4 | 1 | 1 | 1 |   |   |   |   |   |   |   |   |
| Quanyang Town, Baishan City, Jilin Province, China                 | 10| 6 | 5 | 1 |   |   |   |   |   |   |   |   |   |   |
| Erdaogang Town, Baishan City, Jilin Province, China                | 17| 8 | 3 | 2 |   |   |   |   |   |   |   |   |   |   |
| Donggang Town, Baishan City, Jilin Province, China                 | 13| 5 | 5 | 2 |   |   |   |   |   |   |   |   |   |   |
| Xigang Town, Baishan City, Jilin Province, China                   | 7 | 5 | 6 | 2 |   |   |   |   |   |   |   |   |   |   |
| Fusong County, Baishan City, Jilin Province, China                 | 6 | 12| 2 | 3 | 2 |   |   |   |   |   |   |   |   |   |
| Wanliang Town, Fusong County, Baishan City, Jilin Province, China  | 2 | 6 | 4 |   |   |   |   |   |   |   |   |   |   |   |
| Touduo Town, Helong City, Jilin Province, China                    | 3 | 5 |   |   |   |   |   |   |   |   |   |   |   |   |
| Datougu Town, Wanggeng City, Jilin Province, China                  | 7 | 2 | 2 | 1 |   |   |   |   |   |   |   |   |   |   |
| Fuxing Town, Wanggeng City, Jilin Province, China                   | 8 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Luezigou Town, Wanggeng City, Jilin Province, China                | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Manzu Town, Hunchun City, Jilin Province, China                    | 5 | 3 | 5 | 2 |   |   |   |   |   |   |   |   |   |   |
| Yangpao Town, Hunchun City, Jilin Province, China                   | 4 | 3 |   |   |   |   |   |   |   |   |   |   |   |   |
| Yingtian Town, Dunhau City, Jilin Province, China                  | 8 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |
| Quilgou Town, Dunhau City, Jilin Province, China                   | 4 | 5 | 3 |   |   |   |   |   |   |   |   |   |   |   |
| Dashitou Town, Dunhau City, Jilin Province, China                  | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Yongqing Town, Antu City, Jilin Province, China                    | 11|   | 2 |   |   |   |   |   |   |   |   |   |   |   |
| Yongqing Town, Antu City, Jilin Province, China                    | 2 | 2 | 2 |   |   |   |   |   |   |   |   |   |   |   |
| Xinhe Town, Antu City, Jilin Province, China                      | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Fuxing Town, Fuxin City, Liaoning Province, China                  | 10|   |   |   |   |   |   |   |   |   |   |   |   |   |
| Huanren Town, Huanren City, Liaoning Province, China               | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Xinbin Town, Xinbin City, Liaoning Province, China                | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Hulun Town, Jixi City, Heilongjiang Province, China               | 5 | 5 | 3 | 1 | 3 |   |   |   |   |   |   |   |   |   |
| Hulun Town, Jixi City, Heilongjiang Province, China               | 5 | 2 | 6 | 2 |   |   |   |   |   |   |   |   |   |   |
| Halin Town, Mudanjia City, Heilongjiang Province, China           | 8 | 4 | 2 |   |   |   |   |   |   |   |   |   |   |   |
| Ningan Town, Mudanjia City, Heilongjiang Province, China          | 6 | 2 | 1 |   |   |   |   |   |   |   |   |   |   |   |
| Dongjing Town, Mudanjia City, Heilongjiang Province, China       | 3 | 1 | 1 | 3 |   |   |   |   |   |   |   |   |   |   |
| Yongshun Town, Qitaite City, Heilongjiang Province, China        | 6 | 5 | 3 |   |   |   |   |   |   |   |   |   |   |   |
| Hailun Town, Hailun City, Heilongjiang Province, China           | 11| 4 | 6 |   |   |   |   |   |   |   |   |   |   |   |
| Binzhou Town, Haerbin City, Heilongjiang Province, China         | 2 | 2 | 2 | 1 |   |   |   |   |   |   |   |   |   |   |
| Bayan Town, Muhan County, Heilongjiang, China                    | 9 | 3 | 3 | 1 | 3 |   |   |   |   |   |   |   |   |   |
| Mulan Town, Mulan County, Heilongjiang, China                    | 9 | 5 | 4 |   |   |   |   |   |   |   |   |   |   |   |
(Continued)
TABLE 4 | Continued

| Location | A | B | C | D | E | F | G | H | I | J | K | L | M | N |
|----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shangzhi Town, Shangzhi City, Heilongjiang, China | 8 | 6 | 2 | | | | | | | | | | | |
| Zhanhe Town, Heihe City, Heilongjiang, China | 11 | 8 | 1 | 2 | | | | | | | | | | |
| Qingan Town, Suihua City, Heilongjiang, China | 12 | | | | | | | | | | | | | |
| Tieli Town, Yichun City, Heilongjiang, China | 11 | 5 | 1 | | | | | | | | | | | |
| Total | 421 | 166 | 84 | 15 | 10 | 10 | 15 | 4 | 4 | 11 | 8 | 8 | 6 | 4 |

A, I. robusta; B, I. communis; C, I. mors-panacis; D, I. pseudodestructans; E, I. changbaiensis; F, I. qitaiheensis; G, N. obtusispora; H, D. torresensis; I, D. sp.; J, R. panacis; K, T. ginsengicola; L, T. jixiensis; M, T. mulanensis; N, T. fusongensis.

TABLE 5 | Nucleotide differences in the partial gene sequences of rDNA-ITS, ACT, LSU, RPB1, RPB2, TEF, and TUB for T. mulanensis, T. fusongensis and T. jixiensis.

| Species | Position (bp) |
|---------|--------------|
| rDNA-ITS | ACT | LSU | RPB1 | RPB2 |
| 141 | 1 | 223 | 226 | 334 | 343 | 36 | 370 | 400 | 9 | 12 | 13 | 20 | 21 | 26 | 27 | 28 | 29 | 30 | 31 | 57 | 687 | 699 |
| T. mulanensis | C | T | T | C | C | – | G | G | A | C | A | G | C | C | – | C | A | T | C | G | T | G |
| T. fusongensis | C | T | T | C | T | – | G | G | G | C | A | G | C | C | – | C | A | T | A | G | C | G |
| T. jixiensis | T | C | C | T | C | A | A | C | A | C | A | T | C | C | A | G | A | T | C | C | A | T | C | T |

| Species | Position (bp) |
|---------|--------------|
| TEF | TUB |
| 23 | 52 | 72 | 134 | 164 | 167 | 175 | 195 | 372 | 403 | 411 | 416 | 419 | 423 | 424 | 448 | 558 | 788 | 833 | 59 | 60 | 300 | 318 |
| T. mulanensis | C | A | C | – | G | – | G | – | C | A | C | T | A | C | A | T | C | T | C | G | T | G |
| T. fusongensis | T | A | G | A | G | – | G | – | C | A | T | C | A | T | C | C | C | A | C | C | C | A | C | C |
| T. jixiensis | T | T | C | – | A | A | A | T | T | G | T | C | C | – | – | C | T | T | T | G | T | G |

Position refers to the nucleotide position in each sequence of rDNA-ITS, ACT, LSU, RPB1, RPB2, TEF and TUB of type isolates.

Type: China, Jilin Province, Changbai City, county of Fusong, on roots of Panax ginseng, Oct 2018, Y. Guan. (CGMCC3.20154 = R9 - holotype).

Description: Sexual morph: Undetermined. Asexual morph: Conidiophores were simple or complex with 2-3 branches, monophaillides. Mycelial cells were thick-walled and septate. Hyphae hyaline to yellow. Chlamydospores were borne apically or intermittently in hyphae and were more commonly single or in pairs. Macroconidia were the majority, with 1-5 septa; most had 3-4 septa. The shape was a sickle-shaped curve; the base foot was blunt and became gradually thicker toward the top, and the top was also blunt. The size was (38.4) 44.3 (57.7) µm × (4.8) 5.7 (6.9) µm, and the length/width ratio was (5.9) 8.1 (10.3). Microconidia were elliptical, stick or sickle-shaped curves with an irregular shape and 0-1 septa. The size of microconidia was (12.5) 9.69 (12.5) µm × (4.8) 4.1 (6.9) µm, and the length/width ratio was (1.4) 2.7 (3.7). The size of one septate microconidium was (19.5) 24.3 (29.3) µm × (4.1) 4.8 (6.5) µm, and its length/width ratio was (3.9) 5.5 (6.4).

Culture characteristics: On PDA, the color was initially white, later becoming dark yellow with a wavy margin; the reverse side was reddish brown. The colony grows outward in a wave shape. The mycelium grew vigorously, and it was difficult to produce conidia on PDA and SNA. After the addition of ginseng roots and continuous n-UV irradiation, pionnotes were produced on the mycelium or on the vertical wall of the petri dish. The growth of the colony formed concentric rings, and the growth was very slow. The size of the colony reached 22 mm in 10 days. On SNA, the colonies were sparse and white to transparent with regular margins and chlamydosporae; macroconidia and microconidia were not observed.

Note: T. ginsengicola and T. truncata were grouped together on a branch. The greatest difference was the presence of microconidia and chlamydospores. Macroconidia were smaller than those of T. truncate (Salgado-Salazar et al., 2012).

Thelonectria jixiensis Y. M. Guan & Y. Li, sp. nov.

MycoBank MB 836520 (Figure 6).

Etymology: Named after the city of Jixi, Heilongjiang Province, China, where the strain type was collected.

Type: China, Heilongjiang Province, Jixi City, on roots of Panax ginseng, Oct 2018, Y. Guan. (CGMCC3.20156 = Q21-5 - holotype).

Description: Sexual morph: Undetermined. Asexual morph: Conidiophores simple or complex, spiral branches, monophaillides and straight macroconidia or slightly curved head. The body is widest at one-quarter of the head; the basal cells with tips are thinner and narrower, with 0-4 septa. Most had 2-3 septa, and very few had 0 or 4 septa; sizes were in the range of (27.3) 35.8 (41.7) µm × (5.5) 6.9 (8.9) µm, with a length/width ratio of (4.3) 5.1 (7.9). Microconidia were not
observed. Chlamydospores were mostly borne apically on the mycelium, and solitary was more common.

**Culture characteristics:** On PDA, the colonies produced were white at the beginning with a sparsely floccose to fluffy aerial mycelium and vigorous growth. The hyphae at the margins of the colony were thin, and the center hyphae were denser. Light yellow color appeared occasionally on the reverse side over time. The colony grew slowly to a size of 38 mm in 10 days. On SNA, the colonies were sparse, with regular margins, and the mycelium obviously degenerated and gradually disappeared as the number of breeding generations increased. Chlamydospores and macroconidia were not observed on SNA.

**Note:** The phylogenetic tree shows that *T. jixiensis* is closely related to *T. blackeriella*. *T. jixiensis* produced simple or complex conidiophores with spiral branches, and its macroconidia were slightly larger. The simple conidiophores of *T. blackeriella* and the macroconidia were relatively small. The shapes of the macroconidia of the two are very easily distinguished, and neither species produces microconidia (Carlucci et al., 2017).

**Thelonectria mulanensis** Y. M. Guan & Y. Li, sp. nov.

*MycoBank MB 836521 (Figure 7).*

**Etymology:** Named after the County of Mulan, Heilongjiang Province, China, where the strain type was collected.

**Type:** China, Heilongjiang Province, Mulan County, on roots of *Panax ginseng*, Oct 2018, Y. Guan. (CGMCC3.20155 = Q20-8 - holotype).

**Description:**

- **Sexual morph:** Undetermined.
- **Asexual morph:** Conidiophores simple or complex, with 2-3 branches, monophialides. The mycelial cells were thick-walled, transparent and septate. The chlamydospores were produced from the mycelium, and spores were intercalary or terminal and single, in pairs or multiple bunches. Macroconidia were the majority, with 3-7 septa, but most were 5 septa, and lengths ranged from (39.1) 76.5 (96.6) µm × (2.7) 8.3 (5.6) µm. Fungi were a sickle-shaped curve. The base foot was blunt and became gradually thicker toward the top, and the top was also blunt. The microconidia were elliptical, stick or sickle-shaped curved of an irregular shape with 0-1 septum, and lengths ranged from (4.6) 9.5 (18.3) µm × (2.7) 3.9 (5.6) µm. Microsclerotia were produced after more than 8 weeks.

**Culture characteristics:** On PDA, the colonies produced a white, cottony dense floccose to fluffy aerial mycelium. The margins of the colony were irregular, and the hyphae were sparse and radial. The reverse color was white to yellow. In PDA culture, colonies grew slowly and reached 25 mm in 10 days. On SNA, the colonies were sparse, white to transparent, with regular margins and chlamydospores; macroconidia and microconidia were not observed.

**Note:** This species is closely related to *T. jixiensis*, but it produces macroconidia and microconidia, and *T. jixiensis* has no microconidia. The macroconidia of *T. mulanensis* were twice the size of those of *T. jixiensis*, had more septa and were thinner. The conidiophores and chlamydospores of the two species are similar.

**Thelonectria fusongensis** Y. M. Guan & Y. Li, sp. nov.

*MycoBank MB 836524 (Figure 8).*

**Etymology:** Named after the county of Fusong, Changbai City, Jilin Province, China, where the strain type was collected.
**Type:** China, Jilin Province, Changbai City, county of Fusong, on roots of *Panax ginseng*, Oct 2018, Y. Guan. (CGMCC3.20153 = R1-8 - holotype).

**Description:** Sexual morph: Undetermined. Asexual morph: Conidiophores were simple or complex, with 2-3 branches, monophialides. The mycelial cells were thick-walled, transparent, and septate. Chlamydospores were borne apically or intercalary in hyphae and were solitary or in pairs. Macroconidia were in the majority, with 1-5 septa, but most had 2-3 septa. The body was a sickle-shaped curve, and the base was blunt and thickened gradually toward the top. The top was also blunt, not sharp, with sizes in the range of (40.2) 52.3 (62.2) µm × (4.2) 5.9 (7.9) µm. Microconidia were in the minority and exhibited irregular elliptical, stick or curved sickle shapes and 0-1 septa, with sizes in the range of (7.4) 13.5 (23.3) µm × (2.9) 4.1 (6.1) µm.

**Culture characteristics:** On PDA, the colonies grew in a concentric wavy pattern and presented a velvety surface; they were initially white, then brownish yellow, and brownish red on the reverse side. The mycelium became increasingly sparse from the center to the edge. It was difficult to produce conidia on PDA and SNA. The addition of ginseng roots and continuous n-UV light cultivation caused the surface of the mycelium to produce yellow pionnotes at more than 8 weeks. The colonies reached 30 mm in diameter in 10 days. On SNA, the colonies were sparse, white to transparent, with regular margins and chlamydospores; macroconidia and microconidia were not observed.

**Note:** Phylogenetic inference revealed that *T. fusongensis* is closely related to *T. mulanensis*, but the microconidia of the former were larger than those of the latter, there were fewer septa, the microconidia were straighter, and the bending arc was smaller.

**Pathogenicity Tests**

For *in vitro* inoculation using spore suspension, evaluation after 7 days showed that all of the strains infected ginseng roots. The mycelium grew on the ginseng root, and the longitudinal portion of the ginseng root also showed infection. Reddish-brown areas formed on the shallow ginseng roots near the inoculation point. The control showed no symptoms (Figure 9).
For whole plant inoculation in the greenhouse, all of the strains used in the test caused rusty root rot. 2 months after inoculation with the spore suspension, the inoculated 3-year-old ginseng roots showed symptoms similar to rusty root rot and to natural disease in the field. Some ginseng roots were rust-colored, but no gully like symptoms formed (Figure 10). Ginseng roots inoculated with sterile water were asymptomatic. Recovery of the diseased samples and sequencing of all of the genes described above yielded results that were the same as those obtained for the isolates used in the inoculations, satisfying Koch’s postulates, and the identity of the species was determined. Ginseng roots inoculated with *R. panacis* showed a darker color with grayish-black symptoms.

**DISCUSSION**

Ginseng rusty root rot is the root disease with the highest incidence in China’s main ginseng cultivation areas. The identities of the varieties of diseased ginseng samples collected in the experiments were not clear. Most of the currently grown Chinese cultivars are domesticated varieties, and Damaya ginseng is commercially cultivated. Therefore, the correlation between the presence of fungi on ginseng roots and ginseng variety was not analyzed. The identification and pathogenicity analysis of 766 isolates revealed that ginseng rusty root rot was caused by a complex of *Ilyonectria*, *Ilyonectria*-like, and *Rhexocercosporidium* fungi. The *Ilyonectria*-like fungi included three genera of *Ilyonectria*, *Dactylonectria* and *Thelonectria*, all of which belonged to Nectriaceae, except *Rhexocercosporidium*. *I. robusta* and *I. communis* were the dominant pathogens with the highest proportion of ginseng rusty root rot pathogens that were recovered from ginseng rusty rot roots.

Ginseng rusty root rot has been attributed to the *Ilyonectria* fungus in China and South Korea (Wang, 2001; Farh et al., 2018). The 766 isolates we isolated were divided into 14 species, of which *R. panacis* was the only pathogen other than *Ilyonectria* and *Ilyonectria*-like species previously reported to cause rusty root rot.
rot symptoms. *I. robusta* was the most widely isolated dominant pathogen; this is inconsistent with a previous report that 90% of Chinese ginseng rusty root rot is caused by *I. radicicola/C. destructans* (Wang, 2001). *I. radicicola/C. destructans* may be a huge complex group in terms of morphological similarity (Cabral et al., 2012a). The species found later in various plants were not closely related to the original strain type CBS 264.5 (*I. destructans*), and *I/C. destructans* has generally not been found to be as common as when it was first proposed and discovered.

Most of the *Ilyonectria* strains infecting ginseng were obtained from *P. quinquefolius*, and *P. ginseng* and *P. quinquefolius* differ significantly with respect to the proportions of pathogens present. *I. crassa* and *I. panacis* were isolated from *P. quinquefolius* in Canada (Cabral et al., 2012a). The sampling collections of the present study covered the main cultivated areas of Chinese ginseng, and *I. panacis*, *I. rufa* and *I. crassa* strains were not isolated. In contrast, the dominant pathogen *I. robusta* was once considered to exhibit low pathogenicity. The pathogenicity was not directly proportional to the separation ratio. *I. robusta* isolated from *P. quinquefolium* was first described by Hildebrand as *Ramularia robusta* (Hildebrand, 1935). *I. robusta* was first reported in 2014 to cause ginseng root disease in China (Lu et al., 2014). Sixty-eight strains were derived from many hosts. Twenty-one of the isolates obtained from ginseng plants were segregated phylogenetically into four different groups, each of which was considered a different species of *Ilyonectria*: *I. mors-panacis*, *I. robusta*, *I. panacis*, and *I. crassa* (Seifert et al., 2003; Cabral et al., 2012a). These strains were grouped according to the strength of their pathogenicity; the weakly aggressive strains also originated from other hosts, but the highly aggressive strains were derived from a specific host ginseng (Cabral et al., 2012a). *I. mors-panacis* is not limited to infecting ginseng (Mi et al., 2017). *I. robusta* is considered a weakly aggressive strain based on the phylogenetic analysis and on *I. robusta*'s and *I. crassa*'s host and other factors. It is speculated that American and Korean ginseng are not susceptible to *I. robusta* or *I. crassa* (Cabral et al., 2012a), and the present article confirmed that *I. robusta* was the dominant pathogen with the highest isolation ratio. The prevention and control of rusty root rot in cultivation should be dominated by attention to *I. rubosta*.

Ginseng red-skin root is a non-infectious disease that differs from ginseng rusty root rot in China. Lu et al. reported that ginseng red-skin root is caused by a complex group of *Fusarium*, *Ilyonectria* and *Dactylonectria* and that *Ilyonectria* fungi were the dominant pathogens. *I. communis*, *I. changbaiensis* and *I. qitaiheensis* were newly reported species, but the same species were obtained in the present study. The types of pathogenic populations obtained in the present research are similar to the previously identified pathogens. Red-skin root and rusty root rot may not be separated. These conditions are primarily caused by *Ilyonectria* and *Ilyonectria*-like fungi, and they may represent different infection stages or may be caused by different species of *Ilyonectria* and *Ilyonectria*-like fungi (Lu et al., 2020). However, this hypothesis must be clarified. The uniform name “rusty root rot” is more acceptable because most of the infected ginseng roots have dry rot that is similar to the symptoms of decayed wood, not merely red skin; it is possible that the pathogens that cause rusty root rot also cause red-skin root and that red-skin roots are
only in the early stages of the disease. *I. communis* and *I. robusta* were also the two pathogens present in the highest proportions in red-skin root isolates. Differences in pathogenicity may be the most widely accepted cause of the different symptoms, but if the disease is inferred based only on the apparent form of the agents infecting the ginseng root, the diagnosis will not be acceptable. The lifestyle of each microorganism is different, and different effects may not result from differences in pathogenicity. *Fusarium* was not reported as a pathogen that causes dry rot symptoms alone. *Fusarium* may infect the roots of ginseng via multi-infection in tissues infected by primary intruders that are not the direct cause of rusty root rot or red-skin root (Guan et al., 2014; Gao et al., 2014; Wang et al., 2016).

*I. mors-panacis* was discovered on *P. quinquefolium* by Hildebrand (1935), who described it as “*R. mors-panacis*” (Hypocreales, 1935). This species was also found in Japan on *P. ginseng*, and it was collected as “*C. destructans*” f. sp. *Panacis* (ex-type CBS 124662) is treated as a synonym. This species is considered a strong, aggressive species in *Ilyonectria*, but the isolation ratio was only 10.9%. Sufficient time to complete the infection is necessary for the chronic pathogenic *Ilyonectria* population to cause damage to the roots of perennials (Guan et al., 2020). Of course, the relationship between the degree of epidemic disease and pathogenicity was not studied in depth, and this relationship is determined by many factors. Although *I. communis* is a new species that was only recently reported (Lu et al., 2020), it is one of the dominant pathogens that cause ginseng rusty root rot. *I. robusta* and *I. communis* should be the main targets for controlling ginseng rusty root rot in cultivation.

The four novel species of *Thelonecrtia* were identified based on the conclusion that closely related species were being compared. Multi-locus studies are feasible and effective for the identification of novel species. None of the individual genes studied completely matched the same gene in another species, and the combined phylogenetic analysis of multiple gene sequences may result in a suitable classification. Among the studied genes, SSU contributed very little to the strains listed in the present article, but its role cannot be ignored in *Thelonecrtia* (Salgado-Salazar et al., 2016). *ACT* and *RPB2* provided the most information, and analysis
of these genes could fully resolve all of the strains described in this article. Comparison of the RPB2, TEF and TUB genes is the most effective way to distinguish *T. mulanensis* from *T. fusongensis*. The use of all of the gene sequences studied in this work makes the phylogenetic tree more credible. For *Ilyonectria*, rDNA-ITS was not a good marker for distinguishing strains such as *I. rufa* and *I. robusta*, and the HIS3 gene may be used alone to initially distinguish species of *Ilyonectria* (Cabral et al., 2012b).

Many novel *Ilyonectria* and *Ilyonectria*-like species have been discovered recently (Carlucci et al., 2017; Lawrence et al., 2019). Due to the slow growth of some fungi, it is not easy to obtain species using separation techniques. Novel species of *Ilyonectria* and *Ilyonectria*-like fungi that infect the roots of ginseng will continue to be discovered in soil. *Thelonectria* fungi have saprophytic properties or weak pathogenicity, and their host specificity has not been determined. Evaluation after inoculation also showed that *Thelonectria* fungi had weak pathogenicity, and these fungi may also be opportunistically pathogenic, similar to *Ilyonectria*-like fungi.

The present research revealed pathogens that cause ginseng rusty root rot but have remained unknown for many years. These results, which were obtained using a multi-locus combined approach, update previous conclusions and identify the species that should be targeted for the prevention and control of rusty root rot in ginseng cultivation. However, due to the continuous cropping properties of ginseng and continuous land use changes, *Ilyonectria* and *Ilyonectria*-like pathogens are soil-borne diseases, and the population structure may also change.

### DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: [https://www.ncbi.nlm.nih.gov/genbank/](https://www.ncbi.nlm.nih.gov/genbank/), MT678559 etc.

### AUTHOR CONTRIBUTIONS

YG conceived and designed the study and wrote the manuscript. YG, YM, and QJ performed the experiments. QW, NL, and YF analyzed the sequences. YZ and YL reviewed and edited the manuscript. All authors read and approved the manuscript.

### FUNDING

This work was supported by the Key project at the central government level: The Establishment of Sustainable Use for Valuable Chinese Medicine Resources (2060302), the Agricultural Science and Technology Innovation Program (CAAS-XTCX20190025-6), the China Agriculture Research System (CARS-21), Central the Public-interest Scientific Institution Basal Research Fund (Nos. 1610342016010 and 1610342020004), the Science and Technology Development Plan Project of Jilin Province (20191001021XH), and the National Natural Science Foundation of China (81903755 and 31501828).
REFERENCES

Baranov, A. (1966). Recent advances in our knowledge of the morphology, cultivation and uses of ginseng (Panax ginseng C. A. Meyer). Econ. Bot. 20, 407–417. doi: 10.1007/BF02904062

Booth, C. (1966). The genus Cylindrocarpon. Mycol. Papers 104, 1–56. doi: 10.1007/978-3-319-23534-9_1

Cabral, A., Groenewald, J. Z., Rego, C., Oliveira, H., and Crous, P. W. (2018). Multigene phylogenetic analyses of the fungus Taxonomy of fungal complex causing red-skin root of Panax ginseng in China. Planta 2014;104, 330–339. doi: 10.1007/PHYSIO-96-1243

Guan, Y. M., Lu, B. H., Wang, Y., Gao, J., and Wu, L. J. (2014). First report of root rot caused by Fusarium redolens on ginseng (Panax ginseng) in Jilin province of China. Plant Dis. 98:844. doi: 10.1094/PDIS-09-13-0922-PDN

Halleen, F., Fournier, P. H., and Crous, P. W. (2006a). A review of black-foot disease of grapevines. Phytopathol. Mediterr. 45, 555–567. doi: 10.1520/STP37480S

Halleen, F., Schroers, H. J., Groenewald, J. Z., Rego, C., Oliveira, H., and Crous, P. W. (2006b). Neoectria liorindieri sp. nov., the main causal agent of black foot disease of grapevines. Stud. Mycol. 55, 227–234. doi: 10.3114/smy.55.1.227

Halleen, F., Schroers, H. J., Groenewald, J. Z., and Crous, P. W. (2004). Novel species of Cylindrocarpon (Neoclysmata) and Campylocarpogen. gen. nov. associated with black foot disease. Stud. Mycol. 50, 431–445. doi: 10.1023/B:MYCO.0000012225.7966.29

Hildebrand, A. (1935). Root rot of ginseng in Ontario caused by members of the genus Ramularia. Can. J. Res. 12, 82–114. doi: 10.1139/cj3-350-007

Hirooka, Y., Kobayashi, T., and Natsukari, K. T. (2005). Neoectria castaneicola and Neo. rugulosa in Japan. Mycologia 97, 1058–1066. doi: 10.2307/115557-011-0777-7

Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H., and Lee, H. S. (1995). Root-rot disease and rusty symptoms. J. Ginseng Res. 19, 175–180.

Chung, H. S. (1979). Ginseng Disease. Research Reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection.

Crous, P. W., Groenewald, J. Z., Risse, J., and Hyweljones, N. (2004). Caloectoria species and their Cylindrocladium anamorph: species with sphearopeculdate vesicles. Stud. Mycol. 50, 415–429. doi: 10.1016/S0378-8797(03)80017-1

Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H., and Lee, H. S. (1995). Root-rot development of 2-year old ginseng (Panax ginseng CA Meyer) caused by Cylindrocarpon destructans (Zimm.) Schollen in the continuous cultivation field. J. Ginseng Res. 19, 175–180.

Chung, H. S. (1979). Ginseng Disease. Research Reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection.

Crous, P. W., Groenewald, J. Z., Risse, J., and Hyweljones, N. (2004). Caloectoria species and their Cylindrocladium anamorph: species with sphearopeculdate vesicles. Stud. Mycol. 50, 415–429. doi: 10.1016/S0378-8797(03)80017-1

Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H., and Lee, H. S. (1995). Root-rot development of 2-year old ginseng (Panax ginseng CA Meyer) caused by Cylindrocarpon destructans (Zimm.) Schollen in the continuous cultivation field. J. Ginseng Res. 19, 175–180.

Chung, H. S. (1979). Ginseng Disease. Research Reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection.

Crous, P. W., Groenewald, J. Z., Risse, J., and Hyweljones, N. (2004). Caloectoria species and their Cylindrocladium anamorph: species with sphearopeculdate vesicles. Stud. Mycol. 50, 415–429. doi: 10.1016/S0378-8797(03)80017-1

Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H., and Lee, H. S. (1995). Root-rot development of 2-year old ginseng (Panax ginseng CA Meyer) caused by Cylindrocarpon destructans (Zimm.) Schollen in the continuous cultivation field. J. Ginseng Res. 19, 175–180.

Chung, H. S. (1979). Ginseng Disease. Research Reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection.

Crous, P. W., Groenewald, J. Z., Risse, J., and Hyweljones, N. (2004). Caloectoria species and their Cylindrocladium anamorph: species with sphearopeculdate vesicles. Stud. Mycol. 50, 415–429. doi: 10.1016/S0378-8797(03)80017-1

Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H., and Lee, H. S. (1995). Root-rot development of 2-year old ginseng (Panax ginseng CA Meyer) caused by Cylindrocarpon destructans (Zimm.) Schollen in the continuous cultivation field. J. Ginseng Res. 19, 175–180.

Chung, H. S. (1979). Ginseng Disease. Research Reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection.

Crous, P. W., Groenewald, J. Z., Risse, J., and Hyweljones, N. (2004). Caloectoria species and their Cylindrocladium anamorph: species with sphearopeculdate vesicles. Stud. Mycol. 50, 415–429. doi: 10.1016/S0378-8797(03)80017-1

Cho, D. H., Park, K. J., Yu, Y. H., Ohh, S. H., and Lee, H. S. (1995). Root-rot development of 2-year old ginseng (Panax ginseng CA Meyer) caused by Cylindrocarpon destructans (Zimm.) Schollen in the continuous cultivation field. J. Ginseng Res. 19, 175–180.

Chung, H. S. (1979). Ginseng Disease. Research Reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection.
Guan et al. Taxonomy of Rusty Root Rot Pathogens

Salgado-Salazar, C., Rossman, A. Y., Samuels, G. J., Hirooka, Y., Sanchez, R. M., and Chaverri, P. (2015). Phylogeny and taxonomic revision of *Thelonectria discophora* (Ascomycota, Hypocreales, Nectriaceae) species complex. *Fungal Divers.* 70, 1–29. doi: 10.1007/s13225-014-0280-y

Samuels, G. J., and Brayford, D. (1990). Variation in *Nectria radicicola* and its anamorph, *Cylindrocarpon destructans*. *Mycol. Res.* 94, 433–442. doi: 10.1016/S0953-7562(10)80001-2

Scholten, G. (1964). *Nectria radicicola* en *Thielaviopsis basicola* als parasieten van *Cyclamen persicum*. *Neth. J. Plant Pathol.* 70, 1–68. doi: 10.1007/BF02001771

Schroers, H. J., Žerjav, M., Munda, A., Halleen, F., and Crous, P. W. (2007). *Cylindrocarpon pauciseptatum* sp. nov. with notes on *Cylindrocarpon* species with wide, predominantly 3-septate macroconidia. *Mycol. Res.* 112, 82–92. doi: 10.1016/j.mycres.2007.10.004

Seifert, K. A., McMullen, C. R., Yee, D., Reeleder, R. D., and Dobinson, K. F. (2003). Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans*. *Phytopathology* 93, 1533–1542. doi: 10.1094/PHYTO.2003.93.12.1533

Tewoldemedhin, Y. T., Mazzola, M., Mostert, L., and McLeod, A. (2011). *Cylindrocarpon* species associated with apple tree roots in South Africa and their quantification using real-time PCR. *Eur. J. Plant Pathol.* 129, 637–651. doi: 10.1007/s10658-010-9728-4

Vilgalys, R., and Hester, M. (1990). Rapid genetic identification, and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 172, 4238–4246. doi: 10.1128/JB.172.8.4238-4246

Wang, T. S. (2001). *China Ginseng*. Liaoning: Science and Technology Press.

Wang, Y., Guan, Y. M., Lu, B. H., and Gao, J. (2016). First report of ginseng (*Panax ginseng*) root rot caused by *Fusarium acuminatum* in China. *Plant Dis.* 100:525. doi: 10.1094/PDIS-03-15-0273-PDN

Zhou, Y., Yang, Z., Gao, L., Liu, W., Liu, R., Zhao, J., et al. (2017). Changes in element accumulation, phenolic metabolism, and antioxidative enzyme activities in the red-skin roots of *Panax ginseng*. *J. Ginseng Res.* 41, 307–315. doi: 10.1016/j.jgr.2016.06.001

Zinnsmeister, C. L. (1918). Ramularia root-rots of ginseng. *Phytopathology* 8, 557–571.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Guan, Ma, Jin, Wang, Liu, Fu, Zhang and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.