Taxonomy of fungal complex causing red-skin root of *Panax ginseng* in China

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**ABSTRACT**

Background: Red-skin root of Asian ginseng (*Panax ginseng*) significantly reduces the quality and limits the production of ginseng in China. The disease has long been thought to be a noninfectious physiological disease, except one report that proved it was an infectious disease. However, the causal agents have not been successfully determined. In the present study, we were to reveal the pathogens that cause red-skin disease.

Methods: Ginseng roots with red-skin root symptoms were collected from commercial fields in Northeast China. Fungi were isolated from the lesion and identified based on morphological characters along with multilocus sequence analyses on internal transcription spacer (*β*-tubulin (*tub2*), histone H3 (*his3*)), and translation elongation factor 1α (*tef-1α*). Pathogens were confirmed by inoculating the isolates in ginseng roots.

Results: A total of 230 isolates were obtained from 209 disease samples. These isolates were classified into 12 species, including *Hypomyces henrici*, *Dactylonectria* sp., *Fusarium* acuminatum, *Fusarium torulosum*, *Ilyonectria mors-panacis*, *I. robusta*, *Rhexocercosporidium panacis*, and three novel species *I. changbaiensis*, *I. communis*, and *I. qitaiheensis*. Among them, *I. communis*, *I. robusta*, and *F. solani* had the highest isolation frequencies, being 36.1%, 20.9%, and 23.9%, respectively. All these species isolated were pathogenic to ginseng roots and caused red-skin root disease under appropriate condition.

Conclusion: Fungal complex is the causal agent of red-skin root in *P. ginseng*. © 2019 The Korean Society of Ginseng. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**1. Introduction**

Asian ginseng (*Panax ginseng*) is a perennial herb, mainly cultivated for pharmaceutical purpose in China and Korea [1,2]. Dry roots of ginseng have been used for more than 4000 years to stimulate metabolism, hence maintaining and improving health of human beings [1,2]. The value of roots is determined by their size, shape, and overall appearance [3]. Ginseng cultivation requires multiple years, and generally four- to six-year-old roots are harvested for sale. In such a long time of cultivation in the field, roots are vulnerable to many soilborne diseases [4]. Red-skin root is the most common and serious problem in Northeast China, which is a major ginseng production area [5–7]. Red-skin root can occur in all ages of ginseng, but disease severity is more in later growing years, particularly after the fourth year [8]. Disease incidence can be up to 80% in heavily occurring fields. Red-skin root symptom greatly reduces root marketability by up to 40% [9].

Red-skin root is usually characterized by less fibrous roots and reddish-brown to orangish-brown discolored lesions with irregular shapes and margins at the crown of the tap root or areas forming lateral roots, sometimes even whole roots in the fields with heavy diseases. Typically, the superficial lesion can be easily scraped off, resulting in the exposure of inner white healthy tissue. Since red-skin root was first described in the 1960s in China [10], most researchers have treated it as a noninfectious physiological disease due to lack or excess of mineral nutrition and soil pH or moisture; they distinguished it from rusty root diseases [3,10–13]. However, Shang et al [7] reported that healthy ginseng roots can be infected...
by red-skin roots in the field. Meanwhile, the abiotic factors, including soil humidity and temperature, and fertilizers were not determinants but only accelerate the disease development [7]. Unfortunately, the causal agents have not been determined by the authors. The limited knowledge of red-skin root hinders the development of effective management strategies.

Rusty root of American ginseng has symptoms similar to red-skin root and has been well documented [14]. Rusty root is characterized by small or quite large reddish-brown areas at the crown of the tap root that can be easily scraped off that exposes the inner white healthy tissue [14]. Rusty root is caused by weak pathogens including Cylindrocarpon destructans/Ilyonectria radicicola species complex [14]. In China, pathogens of ginseng Cylindrocarpon root rot, rusty root rot, or rust rot diseases are divided into highly virulent species such as C. destructans and C. panacis and less virulent species such as C. panacici and C. obtusisporum [9,16–18]. Furthermore, the taxon of D. destructans species complex has been classified into 12 novel species by morphological and multigene analysis [19]. In addition to Cylindrocarpon species, Fusarium species and Rhexocercosporidium panacis have been reported to be the causal agents of American ginseng rusty root [20–23].

Our preliminary data led us to speculate that Asian ginseng red-skin root disease was an infectious disease caused by weak pathogens. To prove this hypothesis, we were to 1) isolate potential pathogenic microorganisms from ginseng grown in Northeast China, 2) identify the pathogen complex using multilocus analysis and morphological characteristics, and 3) confirm the pathogenesis of the isolates.

2. Materials and methods

2.1. Isolates

Two hundred and nine fresh ginseng roots with red-skin root symptoms (Fig. 1) were collected from 13 commercial fields in 9 counties of Northeast China between June 2012 and September 2013. Ginseng roots were washed in running tap water and blotted to dry. Small pieces of red-skin tissue were surface disinfested with 0.62% NaClO for 3 min, rinsed with sterile distilled water, air-dried, and cut into about 5-mm (in length) pieces. The tissue was placed on potato dextrose agar (PDA) amended with 100 μg/ml of chloramphenicol and 100 μg/ml of tetracycline [24]. Plates were incubated at 25°C for up to 2 weeks. Single spores or single hyphal tips were transferred to PDA plates for later use. All isolates were stored at ~80°C. Representative isolates were deposited in China General Microbiological Culture Collection Center (CGMCC, link: http://www.cgmcc.net/), Beijing, China.

2.2. Morphological observation

Fungal isolates were grown at 22°C on PDA and oatmeal agar in the dark for 2 weeks before observation. Culture characteristics, including texture, density, color, growth front, transparency, and zonation, were visually examined [25]. Colony colors observed from the surface and reverse, both top and back, were described using the color chart of Rayner [26].

Microscopic observation of morphology of fungal isolates was conducted using cultures grown on PDA and synthetic nutrient agar [27] under continuous n-UV light (400–315 nm). A Nikon Eclipse (D v4.50, Nikon, Tokyo) 80i light microscope equipped with a Digital Sight DS-L2 camera (Nikon, Tokyo) and NIS-Element software were used to capture digital images. For each isolate, at least 30 measurements were obtained for each structure. Measurements are given as minimum (lower limit of a 95% confidence interval), average, and maximum (upper limit of a 95% confidence interval). Based on morphology observation, Fusarium isolates were identified into genus level.

2.3. DNA extraction, polymerase chain reaction amplification, DNA sequencing, and multigene phylogenies

For each isolate, total genomic DNA was isolated from mycelium harvested from the 7-day-old colony grown on PDA at 25°C, using the FastDNA Plant Kit (Biomed Co. Ltd, Beijing, China) and the Precellys 24 Technology homogenizer (Bertin Technology, France) according to the manufacturer’s instructions.

Partial gene sequences were obtained by using the following protocols. Primers pair ITS1 and ITS4 were used for partial internal transcription spacer (ITS) [28], CYLH3F and CYLH3R for partial his3 [29], EF1 and EF2 for partial tef-1a [30], and BT3 (CCCCGATTCTACCCCGC) and BT4 (CTGACCGAAGACGATTGTGTC) for partial tub2 designed in this study. Sequences of polymerase chain reaction amplicons were assembled and edited with Chromas 1.5 (Technology Pty Ltd, Queensland, Australia) and DNAMAN 6.0 (Lynnon BioSoft, Quebec, Canada). Newly obtained sequences were deposited in GenBank (Table 1). Sequence alignments were generated using MAFFT, version 7 (Katoh & Standley 2013, Japan). For Fusarium isolates, only partial sequences of the tef-1a gene were amplified and blasted on the GenBank database for identification.

The most suitable substitution model was determined based on jModelTest [31]. Maximum likelihood (ML) analyses including 500 bootstrap replicates were run using RAxML BlackBox web server (Gamma model of rate heterogeneity) [32]. Bayesian analyses were performed using MrBayes, version 3.1.2 [33]. A Markov chain Monte Carlo algorithm of four chains was initiated in parallel from a
| Species | Isolate no. | Substrate | Locality | Collector | GenBank accession no. |
|---------|-------------|-----------|----------|-----------|----------------------|
| Campylocarpon fasciculare | CBS 112613 | Vitis vinifera | South Africa | F. Halleen | AY677301 AY677221 JF735502 JF735691 |
| C. pseudofasciculare | CBS 112679 | Vitis vinifera | South Africa | F. Halleen | AY677306 AY677214 JF735503 JF735692 |
| Cylindrocarpon -like isolates used in the phylogenetic analyses. | | | | | |
| C. album | CBS 110655 | Soil | The Netherlands | F. X. Prenafeta-Boldú | JF735695 JF735696 JF735697 JF735698 |
| C. alicantinum | CBS 139518 | Eriobotrya japonica | Spain | J. Armengol | KP456014 KP456015 KP456016 KP456017 |
| C. alicantinum | CY-8 | Eriobotrya japonica | Spain | J. Armengol | KP456015 KP456016 KP456017 KP456018 |
| C. huberensis | CBS 124071 | Vitis vinifera | Portugal | C. Rego | JF735303 JF735431 JF735579 JF735686 |
| C. huberensis | CBS 129.97 | Soil | The Netherlands | J. T. Poll | AY677273 AY677266 JF735577 JF735578 |
| Dactylonectria alicacerensis | CBS129087 | Vitis vinifera | Portugal | C. Rego, H. Oliveira | AM419110 AM419111 AM419112 AM419113 |
| D. alcacerensis | CBS 112615 | Vitis vinifera | South Africa | F. Halleen | JF735647 JF735648 JF735649 JF735650 |
| D. novozelandica | CBS 112608 | Vitis vinifera | South Africa | F. Halleen | JF735633 JF735634 JF735635 JF735636 |
| D. pinicola | CBS 129086 | Vitis vinifera | Portugal | C. Rego | JF735432 JF735433 JF735434 JF735435 |
| D. torresensis | CBS 124072 | Vitis vinifera | Portugal | C. Rego | JF735640 JF735641 JF735642 JF735643 |
| Dactylonectria sp. | CCMM 3.18786 – J711 | Panax ginseng | China | X. H. Lu | MF350479 MF350480 MF350481 MF350482 |
| Dactylonectria sp. | YJ12 | Panax ginseng | China | X. H. Lu | MF350480 MF350481 MF350482 MF350483 |
| I. communis | CBS 322816 | Protea sp. | South Africa | C. M. Bezuidenhout | JF735456 JF735457 JF735458 JF735459 |
| I. communis | CCMM 3.18789 – 4404 | Panax ginseng | China | X. H. Lu | MF350464 MF350465 MF350466 MF350467 |
| I. communis | 7282 | Panax ginseng | China | X. H. Lu | MF350465 MF350466 MF350467 MF350468 |
| I. communis | 1188 | Panax ginseng | China | X. H. Lu | MF350466 MF350467 MF350468 MF350469 |
| I. communis | 1506 | Panax ginseng | China | X. H. Lu | MF350467 MF350468 MF350469 MF350470 |
| I. communis | 1803 | Panax ginseng | China | X. H. Lu | MF350468 MF350469 MF350470 MF350471 |
| I. communis | 306 | Panax ginseng | China | X. H. Lu | MF350469 MF350470 MF350471 MF350472 |
| I. communis | 320 | Panax ginseng | China | X. H. Lu | MF350470 MF350471 MF350472 MF350473 |
| I. communis | 3510 | Panax ginseng | China | X. H. Lu | MF350471 MF350472 MF350473 MF350474 |
| I. communis | CCMM 3.18788 – 1512 | Panax ginseng | China | X. H. Lu | MF350472 MF350473 MF350474 MF350475 |
| I. communis | J410 | Panax ginseng | China | X. H. Lu | MF350473 MF350474 MF350475 MF350476 |
| I. communis | J710 | Panax ginseng | China | X. H. Lu | MF350474 MF350475 MF350476 MF350477 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350475 MF350476 MF350477 MF350478 |
| I. communis | H207 | Panax ginseng | China | X. H. Lu | MF350476 MF350477 MF350478 MF350479 |
| I. communis | J101 | Panax ginseng | China | X. H. Lu | MF350477 MF350478 MF350479 MF350480 |
| I. communis | J301 | Panax ginseng | China | X. H. Lu | MF350478 MF350479 MF350480 MF350481 |
| I. communis | J502 | Panax ginseng | China | X. H. Lu | MF350479 MF350480 MF350481 MF350482 |
| I. communis | J712 | Panax ginseng | China | X. H. Lu | MF350480 MF350481 MF350482 MF350483 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350481 MF350482 MF350483 MF350484 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350482 MF350483 MF350484 MF350485 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350483 MF350484 MF350485 MF350486 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350484 MF350485 MF350486 MF350487 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350485 MF350486 MF350487 MF350488 |
| I. communis | J305 | Panax ginseng | China | X. H. Lu | MF350486 MF350487 MF350488 MF350489 |
| Species       | CBS Number | Location/Origin                | Accession Numbers                  |
|--------------|------------|--------------------------------|-----------------------------------|
| *I. leucospermi* CBS 132809 | Leucospermum sp. South Africa | Zhuang, Y. Nong | JX231161, JX231113, JX231145, JX231129 |
| *I. leucospermi* CBS 132810 | Protea sp. South Africa | C. M. Bezuidenhout | JX231162, JX231114, JX231146, JX231130 |
| *I. liligena* CBS 189.49 | Lilium regale The Netherlands | M. A. A. Schippers | JF735297, JF735425, JF735573, JF735762 |
| *I. liligena* CBS 732.74 | Lilium sp. The Netherlands | G. J. Bollen | JF735298, JF735426, JF735574, JF735763 |
| *I. liriodendri* CBS 110.81 | Liriodendron tulipifera USA | J.D. MacDonald, E.E. Butler | DQ178163, DQ178170, JF735507, JF735696 |
| *I. liriodendri* CBS 117526 | Vitis vinifera Portugal | C. Rego | JF735296, JF735423, JF735570, JF735759 |
| *I. liliigena* CBS 306.35 | Panax quinquefolium Canada | A. A. Hildebrand | JF735288, JF735414, JF735557, JF735746 |
| *I. panacis* CBS 1189 | Panax ginseng China | Y. Myazawa | JF735290, JF735416, JF735559, JF735748 |
| *I. protearum* CBS 132811 | Protea sp. South Africa | C. M. Bezuidenhout | JX231157, JX231109, JX231141, JX231125 |
| *I. protearum* CBS 132812 | Protea sp. South Africa | C. M. Bezuidenhout | JX231165, JX231117, JX231149, JX231133 |
| *I. pseudodestructans* CBS 129081 | Vitis vinifera Portugal | C. Rego | AJ753330, AM419091, JF735563, JF735752 |
| *I. pseudodestructans* CBS 117824 | Quercus sp. Austria | E. Halmschlager | JF735292, JF735419, JF735562, JF735751 |
| *I. qitaiheensis* CGMCC 3.18787 – H309 | Panax ginseng China | X. H. Lu | MF350472, MF350418, MF350445, MF350499 |
| *I. robusta* CBS 308.35 | Panax quinquefolium Canada | A. A. Hildebrand | JF735264, JF735377, JF735518, JF735707 |
| *I. robusta* CBS 129084 | Vitis vinifera Portugal | N. Cruz | JF735273, JF735391, JF735532, JF735721 |
| *I. robusta* J906 | Panax ginseng China | X. H. Lu | KM015300, KM015297, KM015299, KM015298 |
| *I. rufa* CBS 153.37 | Sand dune France | F. Moreau | AY677271, AY677251, JF735540, JF735729 |
| *I. rufa* CBS 640.77 | Abies alba France | F. Gourbière | JF735277, JF735399, JF735542, JF735731 |
| *I. strelitziae* CBS 142253 | Strelitzia reginae Italy | D. Aiello | KY304649, KY304753, KY304621, KY304727 |
| *I. strelitziae* CBS 142254 | S. reginae Italy | D. Aiello | KY304651, KY304757, KY304623, KY304729 |
| *I. venezuelensis* CBS 102032 | Bark Venezuela | A. Y. Rossman | AM419095, AY677255, JF735571, JF735760 |
| *I. vredenhoekensis* CBS 132807 | Protea sp. South Africa | C. M. Bezuidenhout | JX231155, JX231107, JX231139, JX231123 |
| *I. vredenhoekensis* CBS 132808 | Protea sp. South Africa | C. M. Bezuidenhout | JX231159, JX231111, JX231143, JX231127 |

Epi-type and ex-type isolates indicated in **bold**. Sequences generated in this study indicated in *italics*

1) CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Beijing, China.

2) ITS: the internal transcribed spacer region and intervening 5.8S nrRNA; tub2: β-tubulin; his3: histone H3; tef1-α: translation elongation factor 1-alpha.
random tree topology with a heating parameter set at 0.2. The Markov chain Monte Carlo analyses lasted until the average standard deviation of split frequencies were below 0.01. The sample frequency was set to 100, and the first 25% of trees were removed as burn-in. *Campyllocarpon fasciculare* and *C. pseudofasciculare* were designated as the outgroup for all analyses. The resulting trees were obtained using FigTree, version 1.4.2, (Andrew Rambaut, UK) and annotated using Adobe Illustrator CS5.

2.4. Pathogenicity

Pathogenicity test was carried out on detached ginseng roots *in vitro* and also roots growing in potting soil inoculated with randomly selected isolates from each species. For test *in vitro*, fresh 3-year-old roots were dug from fields and gently washed with tap water, and roots with blemishes were discarded. Healthy roots were surface sterilized as described previously and placed on moist filter paper in an enamel tray. Mycelial plugs (5 mm in a diameter) cut from the margin of actively growing colonies were placed on ginseng roots with the mycelial side facing down to roots that had either a premade hole or not, about 2 to 4 plugs per root, and four replicated roots were inoculated for each isolate with noncolonized agar plugs as control. The tray was sealed with plastic film to prevent desiccation and incubated in the dark at 20 ± 1 °C. After 10 days of inoculation, pathogens were isolated from every root with symptomatic lesions and mock-inoculated control roots as described previously to confirm the inoculated isolates. For test in greenhouse, healthy, fresh, 2-year-old roots were obtained as described previously and planted in pots (2.5 L) with sterilized soil. Three ginseng plants were kept in each pot. Conidia suspensions were made by flooding actively sporulating cultures on PDA plates with sterile distilled water and filtering with sterilized lens-wiping paper to remove mycelia. Conidia concentrations were measured and adjusted to 1 × 10⁵ conidia/mL using a hemocytometer. Then, 10 μL of the suspension was drenched to one pot, and four pots were inoculated for each isolate. Sterile distilled water was used to drench control plants. The pots were maintained in greenhouse under 75% shade cloth. After 85 days, all roots were dug out and gently washed with tap water. Then, disease symptoms were observed, and pathogens were reisolated from roots with symptomatic lesions and also mock-inoculated control roots to confirm pathogen isolates.

3. Results

3.1. Isolation and identification

In total, 230 fungal isolates were obtained from ginseng roots with typical red-skin root symptoms (Fig. 1 and Table 2). In most cases, one species was isolated per lesion, but there were 21 isolations from which more than one species were obtained from a single lesion. Based on colony morphology and conidial characteristics, 74 isolates were preliminarily identified as *Fusarium* species, 151 isolates were *Cylindrocarpon*-like species (Figs. 2–5). The other 5 isolates had been described as *Rhoxecercospordium panacis* previously [34]. For *Fusarium* isolates, 4 were classified as *F. acuminatum*, 7 were *F. avenaceum*, 55 were *F. solani*, and 8 were *F. torulosum*, based on partial DNA sequences of tef1-α. For Cylindrocarpon-like isolates, 7 species were identified, including *Dactylonectria hordeicola*, *Dactylonectria sp.*, *I. mors-panacis*, *I. robusta*, *I. changbaiensis*, *I. communis* and *I. qitaiheensis*.

3.2. Phylogenetic analysis of Cylindrocarpon-like isolates

Polymerase chain reaction amplicons of approximately 450 bases for *tub2* and *his3*, 500 bases for ITS, and 800 bases for tef1-α were DNA-sequenced and analyzed for phylogenetic clustering. From the 7 species, 250 bases for *tub2*, 500 bases for *his3*, 800 bases for ITS, and 800 bases for tef1-α were sequenced. Cylindrocarpon-like isolates will be published in a separate paper.

### Table 2

| Location (county, city, province) | Number of *Dactylonectria* | Number of *Fusarium* | Number of *Cylindrocarpon*-like species | Total |
|---------------------------------|---------------------------|----------------------|----------------------------------------|-------|
| Tonghe, Harbin, Heilongjiang     | 1                         | 7                    | 24                                     | 32    |
| Benxi, Tiehe, Huludongjiang      | 2                         | 1                    | 5                                      | 8     |
| Qinghe, Dahei, Heilongjiang      | 1                         | 10                   | 10                                     | 21    |
| Qinghe, Dahei, Heilongjiang      | 3                         | 1                    | 11                                     | 15    |
| Jilin, Jilin, Jilin              | 1                         | 1                    | 3                                      | 5     |
| Antu, Yanbian, Jilin             | 1                         | 1                    | 2                                      | 4     |
| Hunchun, Yanbian, Jilin          | 2                         | 1                    | 2                                      | 5     |
| Yanbian, Jilin, Jilin            | 1                         | 1                    | 4                                      | 6     |
| Jiaohe, Jilin, Jilin             | 1                         | 1                    | 2                                      | 4     |
| Chongjin, Jilin, Jilin           | 1                         | 1                    | 5                                      | 7     |
| Total                           | 209                       | 1                   | 48                                     | 250   |

3.3. Number of fungal isolates recovered from Panax ginseng with red-skin disease symptoms in Northeastern China.
Fig. 2. Phylogenetic tree of Cylindrocarpon-like isolates based on the analysis of combined 4 genes. Branches with BS ≥ 100% and PP ≥ 1.00 are thickened and in red. Branches with BS ≥ 80% and PP ≥ 0.95 are thickened and in green. The phylogram is rooted with Campylocarpon fasciculare (CBS 112613) and C. pseudofasciculare (CBS 112679).
were obtained for 22 isolates sequenced. The combined alignment of the ITS, tub2, his3 and tef1-α had a total length of 1894 characters including alignment gaps (520 for ITS, 454 for tub2, 449 for his3, and 471 for tef1-α). An analysis by jModelTest proposed the best model TIM2+I+G. ML analysis resulted in a single best ML tree with likelihood \(=-13331.071129\) by using RAxML. Bayesian analysis lasted 330000 generations, and the consensus tree was calculated from 4689 trees left after 250 trees were discarded as burn-in.

The phylogenetic tree based on the combined analysis of four loci (Fig. 2) classified the 82 taxa into 39 species, fulfilling the requirements of genealogical concordance phylogenetic species recognition [35]. All the Cylindrocarpon-like isolates obtained from P. ginseng were grouped into seven highly supported clades (with maximum likelihood bootstrap (ML-BS) of 100% and bayesian inference posterior probabilities (BI-PP) 1.0). Three of the clades, I. robusta, I. mors-panacis, and D. hordeicola, have been described previously. The other four clades represent three novel Ilyonectria species, including I. communis, I. changbaiensis, and I. qitaiheensis, and one novel Dactylonectria species.

Phylogenetic analyses were also conducted on the individual locus and yielded trees with similar topology, but with rearrangement in the order of some clades. Of all loci used, ITS is the least informative region. The trees of both his3 and tub2 could separate all the species, but some clades had lower supporting values than those of the combined tree. Tree of tef1-α could resolve all species except I. communis and I. robusta, which were divided into two separate groups. The alignments and phylogenetic trees were deposited in TreeBASE (S23012).

Fig. 3. Morphological characters of Ilyonectria changbaiensis (CGMCC 3.18789). (A–C) Macroconidia and microconidia. (D and E) Conidiophores. (F) Chlamydospores. Bar = 10 μm.

Fig. 4. Morphological characters of Ilyonectria communis (CGMCC 3.18788). (A–C) Microconidia and macroconidia. (D and E) Chlamydospores. (F and H) Conidiophores. Bar = 10 μm.
3.3. Taxonomy

The morphological characteristics well supported by phylogenetic analyses revealed that isolates 3S07, 11R9, and J906 were *D. hordeicola*, *I. mors-panacis*, and *I. robusta*, respectively. Based on the phylogenetic and morphological data, three novel taxa in the genera *Ilyonectria* are named in this study, and one new species in *Dactylonectria* will be treated separately.

*Ilyonectria changbaiensis* X. Lu & W. Gao, sp. nov. MycoBank MB823893.

**Etymology:** Named after the county of Changbai, Jilin Province, China, where the isolates were collected.

**Diagnosis:** *Ilyonectria changbaiensis* can be distinguished from the phylogenetically closely related *I. communis*, *I. crassa*, *I. panacis*, *I. pseudodestructans*, and *I. rufa* in shorter and thicker 3-septate macroconidia.

**Type:** China: Jilin Province, Baishan, Changbai, on roots of *Panax ginseng*, Oct 2012, X. Lu (CGMCC 3.18789 = 4404 - holotype).

**Description:** Conidiophores simple or complex. Simple conidiophores arising laterally or terminally from aerial mycelium, solitary, dichotomously branched or unbranched or commonly branched with up to three phialides, 0- to 3-septate, 46- to 72-μm long, phialides monophialidic, cylindrical, tapering toward the apex, 16- to 62-μm long, 2.5- to 3.5-μm wide at base, 5 μm at the widest point, 1.5–2.5 μm near the aperture. Complex conidiophores aggregated in small sporodochia, repeatedly and irregularly branched, phialides more or less cylindrical, tapering toward the apex, 16- to 33-μm long, 2 to 3-μm wide at the base, 1.5–2.5 μm wide at the apex. *Macroconidia* formed on both types of conidiophores, 1- to 3-septate, straight, cylindrical with both ends more or less broadly rounded, mostly without a visible hilum; 1-septate, (16.0–)22.8–23.4–23.9(-33.0) × (4.0–)6.2–6.3–6.5(-8.0) μm, with a length:width ratio of 2.4–5.2; 2-septate, (22.0–)27.7–28.3–28.9(-36.0) × (5.0–)6.6–6.8–6.9(-8.0) μm, with a length:width ratio of 3.1–5.0; 3-septate, (25.0–)30.7–31.5(-38.0) × (6.0–)6.7–6.9–7.0(-8.0) μm, with a length:width ratio of 3.3–5.4. *Microconidia* 0- to 1-septate, more or less straight, with a laterally displaced hilum; aseptate microconidia globose to subglobose, (4.0–)7.4–7.7–8.1(-12.0) × (3.0–)3.8–3.9–4.0(-5.0) μm, with a length:width ratio of 1.3–3.3; one-septate microconidia ellipsoidal to ovoid, (9.0–)11.7–12.0–12.4(-16.0) × (3.0–)4.1–4.2–4.3(-5.0) μm, with a length:width ratio of 2.0–4.0. *Chlamydospores* globose to subglobose to ellipsoidal, 7–16 × 7–14 μm, smooth but often appearing rough due to deposits, thick-walled, terminal or intercalary, in chains or in clumps, hyaline, becoming medium brown, and formed abundantly in mature colonies. Sexual state not observed.

**Culture characteristics:** Mycelium felty with strong density. Surface on PDA was golden red, zonation was absent, and reverse was dark brown to yellow brown. Colony diameter was 51–61 mm at 22°C after 7 days. Hardly grew at 4°C and 30°C (no more than 3 mm colony diameter after 7 days).

**Additional culture examined:** China, Jilin Province, Baishan, Changbai, on roots of *Panax ginseng*, Oct 2012, X. Lu (320 &72R2).
**Ilyonectria communis** X. Lu & W. Gao, sp. nov.

MycoBank MB823894.

(Fig. 4)

Etymology: “communis” = Latin for “common”. The name is given because this is the commonest *Ilyonectria* species causing *Panax ginseng* red-skinned root disease in Northeast China.

Diagnosis: *Ilyonectria communis* can be distinguished from the phylogenetically closely related *I. crassa*, *I. pseudodestructans*, *I. rufa*, and *I. panacis*, with the former having more phialides of a simple conidiophore and thicker 3-septate macroconidia[19]. Two or three phialides of a simple conidiophore arising laterally or terminally from aerial mycelium, solitary, unbranched or frequently branched with up to four phialides, 0- to 3-septate, phialides more or less cylindrical, tapering toward the apex, 18- to 32-μm long, 2.1- to 3.3-μm wide at base, 5 μm at the widest point, 1.4- to 2.3 μm near the aperture. Complex conidiophores aggregated in small sporodochia, repeatedly and irregularly branched, phialides more or less cylindrical, tapering toward the apex, 15- to 40-μm long, 1.8- to 3.0-μm wide at base, 4.0 μm at the widest point, 1.2- to 2.2 μm near the aperture. Complex conidiophores aggregated in small sporodochia, repeatedly and irregularly branched, phialides more or less cylindrical, tapering toward the apex. *Macroconidia* formed on both types of conidiophores, 1- to 3-septate, straight or mostly minutely curved with the tip end, cylindrical or sometime typically minutely widening toward the tip, mostly with a visible hilum; 1-septate, (21.0-)21.8-23.9-35.2-34.0 μm, with a length:width ratio of 3.6-4.9; 2-septate, (210-279-289-29.9-370) μm, with a length:width ratio of 4.3-5.7; 3-septate, (220-)293.3-30.7-32.0(-44.0) μm, with a length:width ratio of 4.4-5.8. *Microconidia* 0- to 1-septate, globose to ellipsoidal to subcylindrical, more or less straight, mostly with a visible hilum; 1-septate, (130-)233-23.9-24.3-34.0 μm, with a length:width ratio of 3.3-4.2; 2-septate, (20.0-)28.9-29.4-29.8-38.0 μm, with a length:width ratio of 4.0-5.0; 3-septate, (230.0-298.3-30.3-30.8-42.0) μm, with a length:width ratio of 4.0-5.0. *Microconidia* 0- to 1-septate, ellipsoidal to ovoid to subcylindrical, more or less straight, without a visible hilum; 1-septate microconidia, (5.0-)8.7-8.9-9.1(-13.0) μm, with a length:width ratio of 1.7-2.5; one-septate microconidia, (6.0-)12.3-12.6-12.8(-18.0) μm, with a length:width ratio of 2.3-3.2. *Chlamydospores* globose to subglobose to ellipsoidal, 6-25×6-15 μm, smooth but often appearing rough due to deposits, thick-walled, terminal or intercalary, in chains or in clumps, and also in the cells of the macroconidia, becoming medium brown, and formed abundantly in mature colonies. Sexual state not observed.

Culture characteristics: Mycelium felty with average density and sparse mycelium. Surface on PDA was gray yellow, and that on reverse was gray brown to dark golden. Colony diameter was 52-60 mm at 22°C after 7 days. Hardly grew at 4°C and 30°C (no more than 2 mm colony diameter after 7 days).

Additional culture examined: China, Jilin Province, Baishan, Changbai, on roots of *Panax ginseng*, Oct 2012, X. Lu (J1R2, H207, J101, 314-2 & J710).

Notes: *Ilyonectria communis* differs from the phylogenetically closely related *I. crassa*, *I. pseudodestructans*, *I. rufa*, and *I. panacis* with respect to the number of phialides of a simple conidiophore and the diameter of 3-septate macroconidia[19]. Two or three phialides of a simple conidiophore are common for *I. communis*, but conidiophores are unbranched or sparsely branched, up to two phialides for *I. crassa*, *I. pseudodestructans*, *I. rufa*, and *I. panacis* [19]. The average thickness of the 3-septate macroconidia of *I. communis* (av. = 30.3 × 6.9 μm) was more than the average thickness of those of *I. crassa* (av. = 35.1 × 5.7 μm), *I. pseudodestructans* (av. = 35.2 × 6.0 μm), *I. rufa* (av. = 29.9 × 5.7 μm), and *I. panacis* (av. = 33.1 × 5.6 μm) [36].

**Ilyonectria qitaiheensis** X. Lu & W. Gao, sp. nov.

MycoBank MB823895

(Fig. 5)

Etymology: Named after the city of Qitaihe, Heilongjiang Province, China, where it was collected.

Diagnosis: *Ilyonectria qitaiheensis* can be distinguished from the phylogenetically closely related *I. liliigena* and *I. gamsii* in macroconidia mostly minutely curved with the tip end.

Type: China: Heilongjiang Province, Qitaihe, Qiezihe, on roots of *Panax ginseng*, Oct 2013, X. Lu (CGMC 3.18787 = H309 - holotype).

Description: Conidiophores simple or complex. Simple conidiophores arising laterally or terminally from aerial mycelium, solitary, unbranched or sparsely branched with up to two phialides, 0- to 3-septate, 46- to 132-μm long, phialides monophialidic, cylindrical, tapering toward the apex, 15- to 40-μm long, 1.8- to 3.0-μm wide at base, 4.0 μm at the widest point, 1.2- to 2.2 μm near the aperture. Complex conidiophores aggregated in small sporodochia, repeatedly and irregularly branched, phialides more or less cylindrical, tapering toward the apex. Macroconidia formed on both types of conidiophores, 1- to 3-septate, straight or mostly minutely curved with the tip end, cylindrical or sometime typically minutely widening toward the tip, mostly with a visible hilum; 1-septate, (15.0-)21.8-22.8-23.9(-34.0) μm, with a length:width ratio of 2.3-4.0; 2-septate, (210.0-279.9-289.2-29.9(-370) μm, with a length:width ratio of 3.6-4.9; 2-septate, (210.0-279.9-289.2-29.9(-370) μm, with a length:width ratio of 4.3-5.7; 3-septate, (220.0-293.3-30.7-32.0(-44.0) μm, with a length:width ratio of 4.4-5.8. *Microconidia* 0- to 1-septate, globose to ellipsoidal to subcylindrical, more or less straight, mostly with a visible hilum; aseptate microconidia, (3.0-)79.8-8.8(-12.0) μm, with a length:width ratio of 1.0-3.7; one-septate microconidia, (9.0-)10.5-11.1-11.6(-14.0) μm, with a length:width ratio of 2.5-3.3. *Chlamydospores* globose to subglobose to ellipsoidal, 8-14×7-20 μm, smooth but often appearing rough due to deposits, thick-walled, terminal or intercalary, in chains or in clumps, becoming medium brown, and formed abundantly in mature colonies. Sexual state not observed.

Culture characteristics: Mycelium felty with average density and sparse mycelium. Surface on PDA was gray yellow, and that on reverse was gray brown to dark golden. Colony diameter was 52-60 mm at 22°C after 7 days. Hardly grew at 4°C and 30°C (no more than 2 mm colony diameter after 7 days).

Additional culture examined: China, Jilin Province, Baishan, Changbai, on roots of *Panax ginseng*, Oct 2012, X. Lu (J19). Notes: *Ilyonectria qitaiheensis* differs from the phylogenetically closely related *I. liliigena* and *I. gamsii* with respect to macroconidia mostly minutely curved with the tip end [19].

3.4. Pathogenicity

For test in vitro, all the isolates tested in *Ilyonectria*, *Dactylonectria*, and *Fusarium* were pathogenic to ginseng roots (Fig. 6). For most isolates inoculated on punctured roots, rot lesions were restricted around the point of inoculation without expansion, and around root lesions, red-skin root symptoms showed. For most isolates on nonpunctured roots, only red-skin root symptoms were observed and the disease lesions were superficial and solid. For the isolates in *F. avenaceum* (Fig. 6I) and *F. torulosum* (Fig. 6K), soft rot symptoms expanded clearly and deep into the cortex. For test in whole plant, all the isolates tested were pathogenic to cause red-skin roots (Fig. 7). Roots infected by *I. mors-panacis* showed larger disease lesions and less lateral roots than roots infected by other pathogens (Fig. 7E). Besides red-skin root symptoms, root infected
by *F. acuminatum* showed dry rot lesion on taproots (Fig. 7H). All isolates were recovered from symptomatic roots and confirmed by analyzing DNA sequence of histone H3 gene separately. The mock-inoculated control roots remained symptomless, and no *Dactylo-nectria*, *Ilyonectria*, or *Fusarium* isolates were isolated. The inoculation experiments were repeated, and both trials showed the same results. Besides *Cylindrocarpon*-like species and *Fusarium* species, we have found that *R. panacis* is also a causal agent of red-skin root of ginseng in our previous report [34]. Among these species, *I. communis* (Fig. 6D), *I. robusta* (Fig. 6G), and *F. solani* (Fig. 6J) were the commonest species with isolation frequency of 36.1%, 20.9%, and 23.9%, respectively.

4. Discussion

By analyzing 230 fungal isolates, we have determined that Asian ginseng red-skin root disease was caused by a complex of fungi, which consisted of 12 species. These fungi are all weak pathogens, which only resulted in red-skin root symptoms under greenhouse condition. Even though ginseng roots were acupunctured before inoculation in vitro, the disease lesions were around the inoculated site without further expanding.

Root diseases of ginseng are mainly attributed to *Cylindrocarpon destructans* [14,37], the teleomorph of which is *Ilyonectria* spp. Most of them are soil inhabitants [19,36,38–41]. However, the limited number of *C. destructans* isolates from *Panax* spp. was deduced into *I. crassa*, *I. robusta*, *I. panacis*, and *I. mors-panacis* [19]. We have found that *Cylindrocarpon*-like isolates were the most frequent organisms causing root disease in ginseng, and they belonged to 7 species in 2 genera: *D. hordeicola, Dactylonectria* sp., *I. mors-panacis, I. robusta, I. changbaiensis, I. communis*, and *I. qitaiheensis*. *Dactyonectria hordeicola* was described as *Cylindrocarpon obtusisporum* previously [42], which caused rusty root rot disease of Asian ginseng in China and showed weak virulence [16]. As red-skin disease and rusty root rot disease of Asian ginseng in China had causal pathogens in common, we suggest treating red-skin disease as rusty root rot at early stage of Asian ginseng.

*Ilyonectria robusta* was isolated from *P. ginseng* for the first time recently in China but was widely distributed at a high frequency [43]. It has a broad host range, including herbaceous plants *Loroglossum hircinum* and *P. quinquefolium* and woody plants *Vitis vinifera*, *Prunus cerasus*, *Thymus* sp., *Quercus* spp., and *Tilia petiolaris* [19]. *Ramularia* *mors-panacis*, *Cylindrocarpon panacis*, and *Cylindrocarpon destructans* f. sp. *panacis* were the basionyms of *Ilyonectria mors-panacis* [19], and that was reported to be the strong pathogenic species causing root rot disease on *P. quinquefolium* and *P. ginseng* [44–46]. Similarly, the only one isolate of *I. mors-panacis* we obtained did show a higher virulence compared with other *Cylindrocarpon*-like species under greenhouse conditions.

*Ilyonectria crassa* and *I. panacis* have been isolated from American ginseng in Canada [19]. We did not find *I. crassa* and *I. panacis*, but their sister species *I. communis* was new and named.
Ilyonectria communis is characterized by branched conidiophores with up to four phialides, faster mycelial growth on PDA at 22°C in the dark and chlamydospores formed in the cells of microconidia, which can be clearly distinguished from the group I. pseudodestructans, I. crassa, I. rufa, and I. panacis. Ilyonectria changbaensis and I. qitaiheensis were named by the only county where the isolates were collected from. Ilyonectria changbaensis can be distinctly distinguished on frequently branched conidiophores with up to three phialides or wider 3-septate macroconidia, from the cluster I. qitaiheensis, I. gamsii, and I. liliigena. Ilyonectria qitaiheensis was characterized by faster mycelial growth on PDA at 22°C in the dark, longer 3-septate macroconidia and chlamydospores formed in the cells of microconidia. So far, the sister species I. gamsii and I. liliigena have not been isolated from Panax species [19]. Besides these Ilyonectria species, I. leucospermi was obtained from Korean ginseng roots recently [46], but we did not isolate I. leucospermi in this study.

Following Ilyonectria, Fusarium was the second most frequently isolated genus causing red-skin root disease on Asian ginseng. Among them, F. solani took 74.3% of the isolates. The rest of Fusarium...
isolate were F. acuminatum, F.avenaeum, and F. torulosum. Contrary to the fact, F. ceralis, F. redolens, and F. acuminatum have been reported to cause Asian ginseng root rot [47–49]. In this study, F.avenaeum and F. torulosum caused typical root rot symptoms on detached roots but caused red-skin symptoms after a growth season after inoculation under greenhouse condition. And, F. acuminatum caused both red-skin and root rot disease symptoms under greenhouse conditions. Probably, F. acuminatum, F.avenaeum, and F. torulosum could cause either red-skin disease or root rot depending on the environmental conditions. Similar results have been reported in L. mors-panaxis, which could cause root softening and also discoloration on Korean ginseng [46]. We suspect this may apply to other Cylindrocarpon-like species on Asian ginseng.

Among the Fusarium spp. causing red-skin root disease on Asian ginseng, F.avenaeum is also a causal agent of rooty rust in American ginseng, but F. acuminatum F. solani and F. torulosum did not cause disease on American ginseng [20,21]. Besides F.avenaeum, F. equiseti, F. sporotrichioides, and F. culmorum could infect American ginseng, and F. equiseti was a predominant pathogen causing discolored American ginseng roots [20,21]. These results suggested that the predominant Fusarium species causing root disease of Asian ginseng in China were distinctive from those on American ginseng in North America. Whether the cause of differences is attributed to host or geography remained to be confirmed in our ongoing work.

Besides Cylindrocarpon-like and Fusarium species, several other species were isolated from symptomatic ginseng roots, such as Plectosphaerella cucumerina, Phoma exigua, Mortierella sp., and Rhoxercosporidium panacis. However, only R. panacis caused red-skin root symptoms [34], and it is not clear whether these isolates were pathogens and how they contributed to the symptom development. The clarification that the red-skin root of Asian ginseng is an infectious disease caused by several weak pathogenic fungal species will help develop disease management strategies.

Conflicts of interest

The authors have no conflicts of interest to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jigr.2019.01.006.

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