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Research Article

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 *Dactylonectria* sp., *D. hordeicola*, *Fusarium acuminatum*, *F. avenaceum*, *F. solani*, *F. 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*.

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

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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 [14,15]. 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. panacicola* and *C. obtusisporum* [9,16–18]. Furthermore, the taxon of *C. 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-1α* [30], and BT3 (CCCCTGATTCTACCCCGC) and BT4 (CTGACCGAAGACGAAGTTGTC) for partial *tub2* designed in this study. Sequences of polymerase chain reaction amplicons were assembled and edited with Chromas 1.5 (Technelysium 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-1α* 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

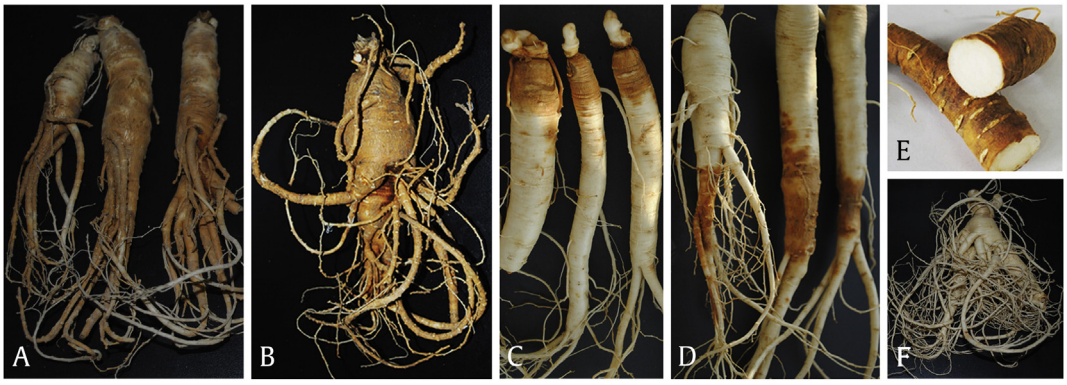

Fig. 1. Symptoms of red-skin disease of *Panax ginseng*. (A and B) On 6-year-old roots. (C and D) On 3-year-old roots. (E) Cross-section of a 3-year-old root (F) Comparison with a 6-year-old healthy ginseng root.

Table 1
Cylindrocarpon-like isolates used in the phylogenetic analyses.

Species	Isolate no.[1)]	Substrate	Locality	Collector	GenBank accession no.[2)]			
					ITS	*tub2*	*his3*	*tef1-α*
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
Cylindrodendrum album	**CBS 301.83**	*Fucus distichus*	Canada	R. C. Summerbell	KM231764	KM232021	KM231484	KM231889
C. album	CBS 110655	Soil	The Netherlands	F.X. Prenafeta-Boldú	KM231765	KM232022	KM231485	KM231890
C. alicantinum	**CBS 139518**	*Eriobotrya japonica*	Spain	J. Armengol	KP456014	KP400578	KP639555	KP452501
C. alicantinum	Cyl-8	*Eriobotrya japonica*	Spain	J. Armengol	KP456015	KP400579	KP639556	KP452502
C. hubeiensis	**CBS 124071**	*Rhododendron* sp.	China	W. P. Wu, W. Y. Zhuang, Y. Nong	FJ560439	FJ860056	KR909093	HM054090
C. hubeiensis	CBS 129.97	*Viscum album*	France	W. Gams	KM231766	KM232023	KM231486	KM231891
Dactylonectria alcacerensis	**CBS129087**	*Vitis vinifera*	Portugal	C. Rego, H. Oliveira	JF735333	AM419111	JF735630	JF735819
D. alcacerensis	Cy134	*Vitis vinifera*	Spain	J. Armengol	JF735332	AM419104	JF735629	JF735818
D. anthruriicola	**CBS 564.95**	*Anthurium* sp.	The Netherlands	R. Pieters	JF735302	JF735430	JF735579	JF735768
D. estremocensis	**CBS 129085**	*Vitis vinifera*	Portugal	C. Rego, T. Nascimento	JF735320	JF735448	JF735617	JF735806
D. estremocensis	CPC 13539	*Picea glauca*	Canada	R. C. Hamelin	JF735330	JF735458	JF735627	JF735816
D. hordeicola	**CBS 162.89**	*Hordeum vulgare*	The Netherlands	M. Barth	AM419060	AM419084	JF735610	JF735799
D. hordeicola	3S07	*Panax ginseng*	China	X. H. Lu	MF350482	MF350428	MF350455	MF350509
D. macrodidyma	**CBS 112615**	*Vitis vinifera*	South Africa	F. Halleen	AY677290	AY677233	JF735647	JF735836
D. macrodidyma	CBS 112601	*Vitis vinifera*	South Africa	F. Halleen	AY677284	AY677229	JF735644	JF735833
D. novozelandica	**CBS 113552**	*Vitis* sp.	New Zealand	R. Bonfiglioli	JF735334	AY677237	JF735633	JF735822
D. novozelandica	CBS 112608	*Vitis vinifera*	South Africa	F. Halleen	AY677288	AY677235	JF735632	JF735821
D. pauciseptata	**CBS 120171**	*Vitis* sp.	Slovenia	M. Žerjav	EF607089	EF607066	JF735587	JF735776
D. pauciseptata	CBS 100819	*Erica melanthera*	New Zealand	H. M. Dance	EF607090	EF607067	JF735582	JF735771
D. pinicola	**CBS 173.37**	*Pinus laricio*	Germany	H. W. Wollenweber	JF735319	JF735447	JF735614	JF735803
D. pinicola	CBS 159.34	*Pinus laricio*	UK: England	T. R. Peace	JF735318	JF735446	JF735613	JF735802
D. torresensis	**CBS 129086**	*Vitis vinifera*	Portugal	A. Cabral	JF735362	JF735492	JF735681	JF735870
D. torresensis	CBS 119.41	*Fragaria* sp.	The Netherlands	H. C. Koning	JF735349	JF735478	JF735657	JF735846
D. vitis	**CBS 129082**	*Vitis vinifera*	Portugal	C. Rego	JF735303	JF735431	JF735580	JF735769
Dactylonectria sp.	CGMCC 3.18786 = J711	*Panax ginseng*	China	X. H. Lu	MF350479	MF350425	MF350452	MF350506
Dactylonectria sp.	YJ212	*Panax quinquefolius*	China	X. H. Lu	MF350480	MF350426	MF350453	MF350507
Dactylonectria sp.	YJ515	*Panax quinquefolius*	China	X. H. Lu	MF350481	MF350427	MF350454	MF350508
Ilyonectria capensis	**CBS 132815**	*Protea* sp.	South Africa	C. M. Bezuidenhout	JX231151	JX231103	JX231135	JX231119
I. capensis	CBS 132816	*Protea* sp.	South Africa	C. M. Bezuidenhout	JX231160	JX231112	JX231144	JX231128
I. changbaiensis	**CGMCC 3.18789** = 4404	*Panax ginseng*	China	X. H. Lu	MF350464	MF350410	MF350437	MF350491
I. changbaiensis	72R2	*Panax ginseng*	China	X. H. Lu	MF350465	MF350411	MF350438	MF350492
I. changbaiensis	11R8	*Panax ginseng*	China	X. H. Lu	MF350466	MF350412	MF350439	MF350493
I. changbaiensis	1506	*Panax ginseng*	China	X. H. Lu	MF350467	MF350413	MF350440	MF350494
I. changbaiensis	1803	*Panax ginseng*	China	X. H. Lu	MF350468	MF350414	MF350441	MF350495
I. changbaiensis	306	*Panax ginseng*	China	X. H. Lu	MF350469	MF350415	MF350442	MF350496
I. changbaiensis	320	*Panax ginseng*	China	X. H. Lu	MF350470	MF350416	MF350443	MF350497
I. changbaiensis	3S10	*Panax ginseng*	China	X. H. Lu	MF350471	MF350417	MF350444	MF350498
I. communis	**CGMCC 3.18788** = 1512	*Panax ginseng*	China	X. H. Lu	MF350456	MF350402	MF350429	MF350483
I. communis	J410	*Panax ginseng*	China	X. H. Lu	MF350457	MF350403	MF350430	MF350484
I. communis	71R2	*Panax ginseng*	China	X. H. Lu	MF350458	MF350404	MF350431	MF350485
I. communis	J101	*Panax ginseng*	China	X. H. Lu	MF350459	MF350405	MF350432	MF350486
I. communis	301	*Panax ginseng*	China	X. H. Lu	MF350460	MF350406	MF350433	MF350487
I. communis	H207	*Panax ginseng*	China	X. H. Lu	MF350461	MF350407	MF350434	MF350488
I. communis	J710	*Panax ginseng*	China	X. H. Lu	MF350462	MF350408	MF350435	MF350489
I. communis	J305	*Panax ginseng*	China	X. H. Lu	MF350463	MF350409	MF350436	MF350490
I. coprosmae	**CBS 119606**	*Metrosideros* sp.	Canada	G. J. Samuels	JF735260	JF735373	JF735505	JF735694
I. crassa	**CBS 139.30**	*Lilium* sp.	The Netherlands	W. F. van Hell	JF735275	JF735393	JF735534	JF735723
I. crassa	CBS 158.31	*Narcissus* sp.	The Netherlands	W. F. van Hell	JF735276	JF735394	JF735535	JF735724
I. cyclaminicola	**CBS 302.93**	*Cyclamen* sp.	The Netherlands	M. Hooftman	JF735304	JF735432	JF735581	JF735770
I. destructans	**CBS 264.65**	*Cyclamen persicum*	Sweden	L. Nilsson	AY677273	AY677256	JF735506	JF735695
I. europaea	**CBS 129078**	*Vitis vinifera*	Portugal	C. Rego	JF735294	JF735421	JF735567	JF735756
I. europaea	CBS 537.92	*Aesculus hippocastanum*	Belgium	V. Demoulin	EF607079	EF607064	JF735568	JF735757
I. gamsii	**CBS 940.97**	Soil	The Netherlands	J. T. Poll	AM419065	AM419089	JF735577	JF735766

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. liliigena	**CBS 189.49**	*Lilium regale*	The Netherlands	M. A. A. Schippers	JF735297	JF735425	JF735573	JF735762
I. liliigena	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	DQ178164	DQ178171	JF735508	JF735697
I. lusitanica	**CBS 129080**	*Vitis vinifera*	Portugal	N. Cruz	JF735296	JF735423	JF735570	JF735759
I. mors-panacis	**CBS 306.35**	*Panax quinquefolium*	Canada	A. A. Hildebrand	JF735288	JF735414	JF735557	JF735746
I. mors-panacis	CBS 124662	*Panax ginseng*	Japan	Y. Myazawa	JF735290	JF735416	JF735559	JF735748
I. mors-panacis	11R9	*Panax ginseng*	China	X. H. Lu	MF350477	MF350423	MF350450	MF350504
I. palmarum	**CBS 135754**	*Howea forsteriana*	Italy	G. Polozzi	HF937431	HF922608	HF922620	HF922614
I. palmarum	CBS 135753	*Howea forsteriana*	Italy	G. Polozzi	HF937432	HF922609	HF922621	HF922615
I. panacis	**CBS 129079**	*Panax quinquefolium*	Canada	K. F. Chang	AY295316	JF735424	JF735572	JF735761
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	AJ875330	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. qitaiheensis	J919	*Panax ginseng*	China	X. H. Lu	MF350473	MF350419	MF350446	MF350500
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	KY304755	KY304621	KY304727
I. strelitziae	CBS 142254	*S. reginae*	Italy	D. Aiello	KY304651	KY304757	KY304623	KY304729
I. venezuelensis	**CBS 102032**	Bark	Venezuela	A. Y. Rossman	AM419059	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. *Campylocarpon fasciculare* and *C. pseudofascicul* 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 $20 \pm 1^{\circ}C$. 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 1×10^5 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 *Rhexocercosporidium 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*

Table 2
Fungal isolates recovered from *Panax ginseng* with red-skin disease symptoms in Northeastern China.

Location (county, city, province)	Number of isolation	*Dactylonectria* sp.	*D. hordeicola*	*Fusarium acuminatum*	*F. avenaceum*	*F. solani*	*F. torulosum*	*Ilyonectria changbaiensis*	*I. communis*	*I. mors-panacis*	*I. qitaiheensis*	*I. robusta*	*Rhexocercosporidium panacis*	Total
Tonghe, Harbin, Heilongjiang	9					7						2	2	11
Bei'an, Heihe, Heilongjiang	7			1		3						6		10
Qiezihe, Qitaihe, Heilongjiang	9					4					1	3		8
Fusong, Baishan, Jilin	20					12			8			3		23
Changbai, Baishan, Jilin	114		1	3	7	18	8	15	61	1		3		117
Jiaohe, Jilin, Jilin	5					2			3			2	2	9
Antu, Yanbian, Jilin	20	1				3			11			8		23
Hunchun, Yanbian, Jilin	6											6		6
Ji'an, Tonghua, Jilin	19					6					1	15	1	23
Total	209	1	1	4	7	55	8	15	83	1	2	48	5	230

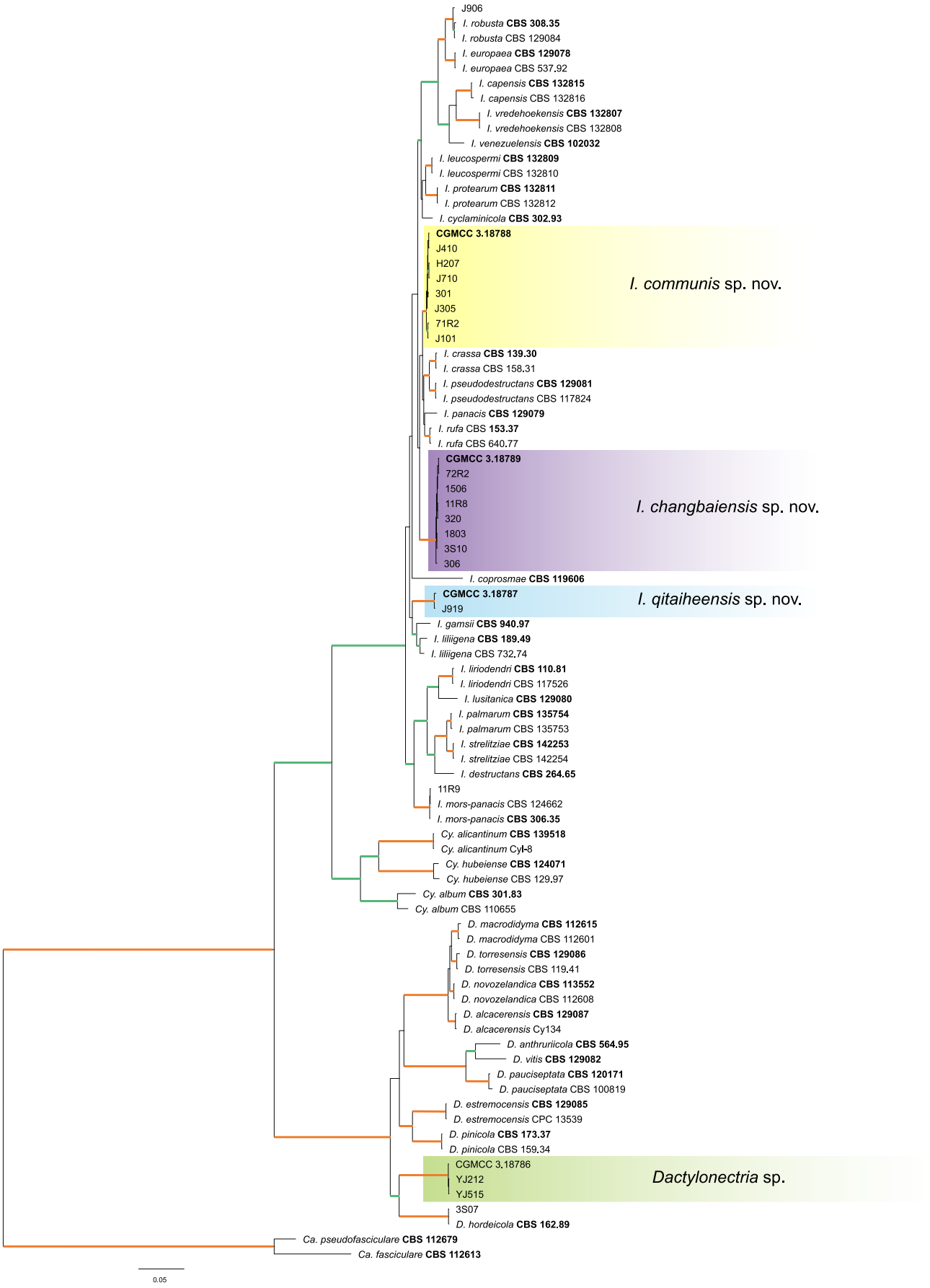

Fig. 2. Phylogenetic tree of *Cylindrocarpon*-like isolates based on the analysis of combined 4 genes. Branches with BS = 100% and PP = 1.00 are thickened and in red. Braches with BS ≥ 80% and PP ≥ 0.95 are thickened and in green. The phylogram is rooted with *Campylocarpon fasciculare* (CBS 112613) and *C. pseudofasciculare* (CBS 112679).

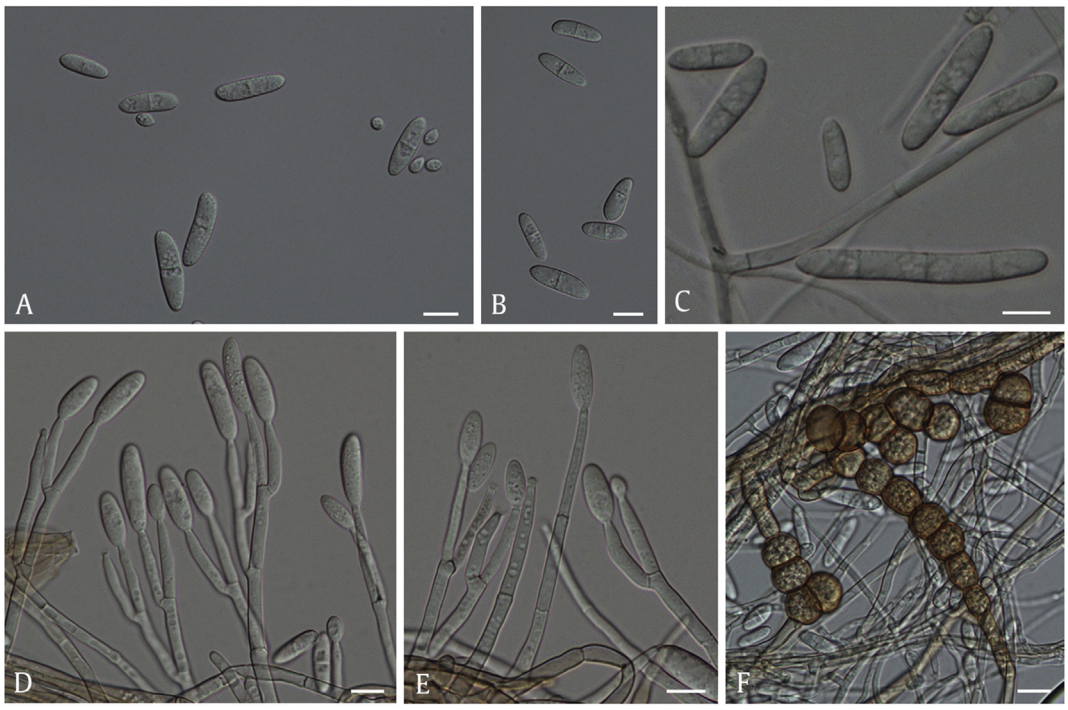

Fig. 3. Morphological characters of *Ilyonectria changbaiensis* (CGMCC 3.18789). (A–C) Macroconidia and microconidia. (D and E) Conidiophores. (F) Chlamydospores. Bar = 10 μm.

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).

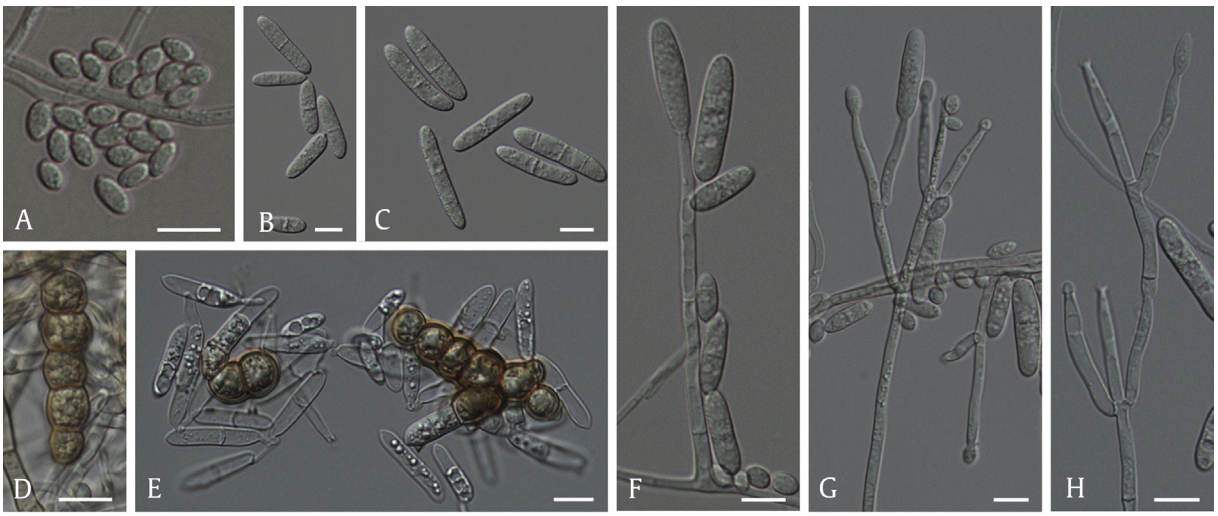

Fig. 4. Morphological characters of *Ilyonectria communis* (CGMCC 3.18788). (A–C) Microconidia and macroconidia. (D and E) Chlamydospores. (F and H) Conidiophores. Bar = 10 μm.

Fig. 5. Morphological characters of *Ilyonectria qitaiheensis* (CGMCC 3.18787). (A–C) Macroconidia and microconidia. (D and E) Conidiophores. (F and G) Chlamydospores. 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.
(Fig. 3)

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-)30.0-30.7-31.5(-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-skin 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.
Type: China: Jilin Province, Baishan, Changbai, on roots of *Panax ginseng*, Oct 2012, X. Lu (CGMCC 3.18788 = 1512 - holotype).
Description: Conidiophores simple or complex. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary, unbranched or frequently branched with up to four phialides, 0- to 3-septate, 58- to 94-μm long, phialides monophialidic, 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, 1.4–2.3 μm near the aperture. *Complex conidiophores* aggregated in small sporodochia, repeatedly and irregularly branched, phialides more or less cylindrical, tapering toward the apex, 16- to 33-μm long, 2–3 μm wide at the base, 1.5- to 2.5-μm wide at the apex. *Macroconidia* formed on both types of conidiophores, 1- to 3-septate, straight and frequently minutely curved, cylindrical or sometimes typically minutely widening toward the tip, mostly with a visible hilum; 1-septate, (13.0-)23.3-23.9-24.3(-34.0) × (4.0-)6.2-6.3-6.4(-9.0) μm, with a length:width ratio of 3.3–4.2; 2-septate, (20.0-)28.9-29.4-29.8(-38.0) × (5.0-)6.4-6.5-6.6(-9.0)μm, with a length:width ratio of 4.0–5.0; 3-septate, (23.0-)29.8-30.3-30.8(-42.0) × (5.0-)6.8-6.9-7.0(-9.0) 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; aseptate microconidia, (5.0-)8.7-8.9-9.1(-13.0) × (3.0-)4.0-4.1-4.2(-6.0) μm, with a length:width ratio of 1.7–2.5; one-septate microconidia, (6.0-)12.3-12.6-12.8(-18.0) × (3.0-)4.5-4.6-4.7(-7.0)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. Surface on PDA was gray yellow, and that on reverse was dark gray brown to light golden. Colony diameter was 46–56 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 (71R2, 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 (CGMCC 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, 1.2–2.2 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) × (4.0-)5.1-5.3-5.5(-7.0)μm, with a length:width ratio of 3.6–4.9; 2-septate, (21.0-)27.9-28.9-29.9(-37.0) × (4.0-)5.6-5.8-6.0(-8.0)μm, with a length:width ratio of 4.3–5.7; 3-septate, (22.0-)29.3-30.7-32.0(-44.0) × (5.0-)5.7-5.9-6.1(-8.0) 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-)7.9-8.4-8.8(-12.0) × (3.0-)3.4-3.6-3.8(-6.0)μm, with a length:width ratio of 1.0–3.7; one-septate microconidia, (9.0-)10.5-11.1-11.6(-14.0) × (3.0-)3.7-3.9-4.2(-6.0) μm, with a length:width ratio of 2.5–3.3. *Chlamydospores* globose to subglobose to ellipsoidal, sparely, 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 (J919).
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 rot 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

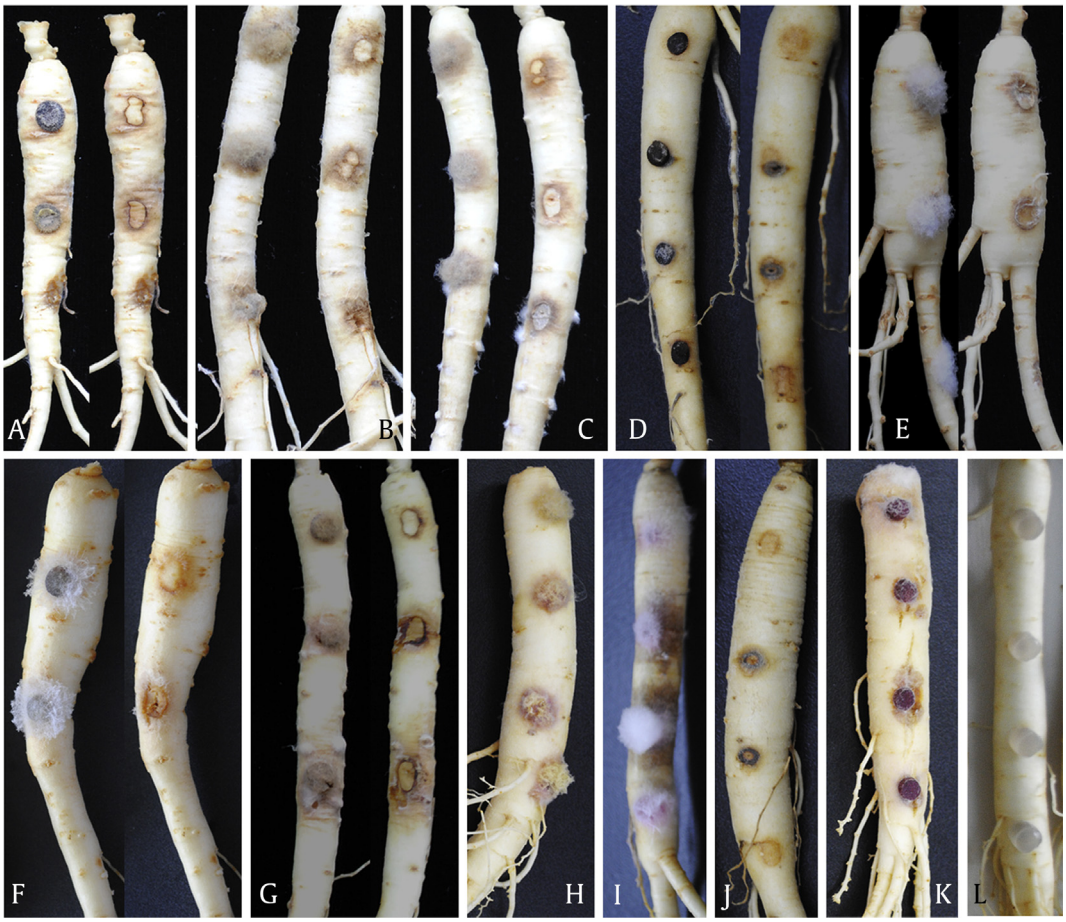

Fig. 6. Symptoms of red-skin root disease induced by *in vitro* inoculation on detached *Panax ginseng* roots with the fungi. (A) *Dactylonectria* sp. (B) *D. hordeicola*. (C) *Ilyonectria changbaiensis*. (D) *I. communis*. (E) *I. mors-panacis*. (F) *I. qitaiheensis*. (G) *I. robusta*. (H) *Fusarium acuminatum*. (I) *F. avenaceum*. (J) *F. solani*. (K) *F. torulosum*. (L) Mock-inoculated control. On each tap root, two to four inoculum plugs were placed in a line with same distance between each other. From the top and the tip of a root, the first and fourth plugs were directly placed on root surface and the second and the third plugs were placed on a punctured tissue, which was poked with an inoculation needle.

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 *Dactylonectria*, *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*. *Dactylonectria 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 *Lonicera 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.

Fig. 7. Symptoms of red-skin root disease of *Panax ginseng* roots inoculated with the fungi under greenhouse conditions. (A) *Dactylonectria* sp. (B) *D. hordeicola*. (C) *Ilyonectria changbaiensis*. (D) *I. communis*. (E) *I. mors-panacis*. (F) *I. qitaiheensis*. (G) *I. robusta*. (H) *Fusarium acuminatum*. (I) *F. avenaceum*. (J) *F. solani*. (K) *F. torulosum*. (L) sterilized water.

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 changbaiensis* and *I. qitaiheensis* were named by the only county where the isolates were collected from. *Ilyonectria changbaiensis* 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*

isolates were *F. acuminatum*, *F. avenaceum*, and *F. torulosum*. Contrastingly, *F. cerealis*, *F. redolens*, and *F. acuminatum* have been reported to cause Asian ginseng root rot [47–49]. In this study, *F. avenaceum* 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 condition. Probably, *F. acuminatum*, *F. avenaceum*, and *F. torulosum* could cause either red-skin disease or root rot depending on the environmental conditions. Similar results have been reported in *I. mors-panacis*, 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. avenaceum* is also a causal agent of rusty root in American ginseng, but *F. acuminatum F. solani* and *F. torulosum* did not cause disease on American ginseng [20,21]. Besides *F. avenaceum*, *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 *Rhexocercosporidium 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.

Acknowledgments

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Appendix A. Supplementary data

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

References

[1] Baranov A. Recent advances in our knowledge of the morphology, cultivation and uses of ginseng (*Panax ginseng* C.A. Meyer). Econ Bot 1966;20:403–6.
[2] Hu SY. A contribution to our knowledge of ginseng. Am J Chinese Med 1977;5:1–23.
[3] Zhou Y, Yang Z, Gao L, Liu W, Liu R, Zhao J, You J. Changes in element accumulation, phenolic metabolism, and antioxidative enzyme activities in the red-skin roots of *Panax ginseng*. J Ginseng Res 2017;41:307–15.
[4] Yu YH, Ohh SH. Research on ginseng diseases in Korea. Korean J Ginseng Sci 1993;19:61–8.
[5] Zhao Y, Li X, Hou Y. Biochemical changes in ginseng red coating root disease and comprehensive evaluation on the quality of disease roots. ACTA Phytopathol Sin 1997;27:175–9.
[6] Liu J, Zhao Y, Liu B. A study on the anatomical and morphological characteristics of the ginseng roots infected by the red root disease. ACTA Phytopathol Sin 1998;28:72.
[7] Shang Y, Chen Z, Si Y, Teng B. Research on the causes of red-skin disease of *Panax ginseng*. J Chinese Med Mater 1996;19:163–5.
[8] Zhao Y. Recent progress on diagnosis and integrated management of ginseng rusty root disease in Northeast China. Spec Wild Econ Anim Plant Res 1998:41–6.
[9] Wang Q, Jin R, Yun L, Wang J, Jiang X, Ma D, Bai Y. Occurrence investigation and pathogen study on ginseng rusty root rot. J Liaoning For Sci Technol 2014:15–7.
[10] Wang Y. A preliminary study on cause of ginseng red skin disease in the second ginseng farm of Jinyu county. Spec Wild Econ Anim Plant Res 1963;2:9–15.
[11] Li Z, Guo S, Tian S, Liu Z. Study on the causes for ginseng red skin sickness occurred in albic bed soil. ACTA Pedol Sin 1997;34:328–35.
[12] Sun S, Li Y, Lu J, Jiao B, Jiang X. Soil geochemical characterics with ginseng red skin sickness in Ji'an city, Jilin province. J Jilin U 2010;40:412–6.
[13] Wang Y, Li Z, Sun Y, Guo S, Tian S, Liu Z. Studies on the genesis of ginseng rust spots. Korean J Ginseng Sci 1997;21:69–77.
[14] Farh MEA, Kim YJ, Kim YJ, Yang DC. *Cylindrocarpon destructans/Ilyonectria radicicola*-species complex: causative agent of ginseng root-rot disease and rusty symptoms. J Ginseng Res 2018;42:9–15.
[15] Lee C, Kim KY, Lee JE, Kim S, Ryu D, Choi JE, An G. Enzymes hydrolyzing structural components and ferrous ion cause rusty-root symptom on ginseng (*Panax ginseng*). J Microbiol Biotechnol 2011;21:192–6.
[16] Yan X. The comparative biology studies of *Cylindrocarpon* on ginseng and American ginseng. In: Plant pathology. Shenyang, China: Shenyang Agricultural University; 2002.
[17] Sun J. The chemical induced resistant mechanism of Cylindrocarpon root rot of ginseng and molecular detection of *Cylindrocarpon destructans*. In: Pest and environment safety. China: Shenyang Agricultural University; 2013.
[18] Wang S, Zhang A, Chen X, Lei F, Xu Y, Zhang L. Pesticides screening against ginseng root rust rot. Agrochemicals 2011;50:449–51.
[19] Cabral A, Groenewald JZ, Rego C, Oliveira H, Crous PW. Cylindrocarpon root rot: multi-gene analysis reveals novel species within the *Ilyonectria radicicola* species complex. Mycol Prog 2012;11:655–88.
[20] Punja ZK, Wan A, Goswami RS, Verma N, Rahman M, Barasubiye T, Seifert KA, Lévesque CA. Diversity of *Fusarium* species associated with discolored ginseng roots in British Columbia. Can J Plant Pathol 2007;29:340–53.
[21] Punja ZK, Wan A, Rahman M. Role of *Fusarium* spp. in rusty-root development on ginseng in British Columbia. Can J Plant Pathol 2005;27:474.
[22] Reeleder RD. *Rhexocercosporidium panacis* sp. nov., a new anamorphic species causing rusted root of ginseng (*Panax quinquefolius*). Mycologia 2007;99:91–8.
[23] Reeleder RD, Brammall RA. Pathogenicity of *Pythium* species, *Cylindrocarpon destructans*, and *Rhizoctonia solani* to ginseng seedling in Onttario. Can J Plant Pathol 1994;16:311–6.
[24] Lu XH, Jiao XL, Hao JJ, Chen AJ, Gao WW. Characterization of resistance to multiple fungicides in *Botrytis cinerea* populations from Asian ginseng in northeastern China. Eur J Plant Pathol 2016;144:467–76.
[25] Crous PW, Verkleij GJM, Groenewald JZ, Samson RA. Fungal biodiversity. CBS Laboratory Manual Series 1. Utrecht: Centraalbureau voor Schimmelcultures; 2009.
[26] Rayner RW. A mycological colour chart. Great Britain: Commonwealth Mycological Institute; 1970.
[27] Nirenberg H. Untersuchungen uber die morphologische und biologische Differenzierung in der Fusarium-Sektion Liseola. Mitt Biol Bundesanst Land Forstwirtsch 1976;169:1–117.
[28] White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315-22. In: Innis MA, Gelfand D, Sninsky JJ, White TJ, editors. PCR Protocols: a guide to methods and applications. San Diego: Academic Press; 1990.
[29] Crous PW, Groenewald JZ, Risede JM, Simoneau P, Hywel-Jones NL. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Stud Mycol 2004:415–30.
[30] O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. P Natl Acad Sci USA 1998;95:2044–9.
[31] Santorum JM, Darriba D, Taboada GL, Posada D. jmodeltest.org: selection of nucleotide substitution models on the cloud. Bioinformatics 2014;30:1310–1.
[32] Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 2008;75:758–71.
[33] Ronquist F, Huelsenbeck JP. MrBayes version 3.0: Bayesian phylogenetic inference under mixed models. Bioinformatics 2013;19:1572–4.
[34] Lu XH, Chen AJ, Zhang XS, Jiao XL, Gao WW. First report of *Rhexocercosporidium panacis* causing rusty root of *Panax ginseng* in Northeastern China. Plant Dis 2014;98:1580.
[35] Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser D. Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 2000;31:21–32.
[36] Cabral A, Rego C, Nascimento T, Oliveira H, Groenewald JZ, Crous PW. Multigene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. Fungal Biol 2012;116:62–80.
[37] Song JY, Seo MW, Kim SI, Nam MH, Lim HS, Kim HG. Genetic diversity and pathogenicity of *Cylindrocarpon destructans* isolates obtained from Korean *Panax ginseng*. Mycobiology 2014;42:174–80.

[38] Chaverri P, Salgado C, Hirooka Y, Rossman AY, Samuels GJ. Delimitation of *Neonectria* and *Cylindrocarpon* (*Nectriaceae, Hypocreales, Ascomycota*) and related genera with *Cylindrocarpon*-like anamorphs. Studies in Mycology 2011;68:57–78.
[39] Aiello D, Guarnaccia V, Vitale A, Cirvilleri G, Granata G, Epifani F, Perrone G, Polizzi G, Groenewald JZ, Crous PW. *Ilyonectria palmarum* sp. nov. causing dry basal stem rot of Arecaceae. Eur J Plant Pathol 2014;138:347–59.
[40] Lombard L, Bezuidenhout CM, Crous PW. Ilyonectria black foot rot associated with *Proteaceae*. Australas Plant Path 2013;42:337–49.
[41] Aiello D, Polizzi G, Crous PW. *Pleiocarpon* gen. nov and a new species of *Ilyonectria* causing basal rot of *Strelitzia reginae* in Italy. IMA Fungus 2017;8:65–76.
[42] Lombard L, van Der Merwe NA, Groenewald JZ, Crous PW. Lineages in Nectriaceae: Re-evaluating the generic status of *Ilyonectria* and allied genera. Phytopathol Mediterranea 2014;53:515–32.
[43] Lu XH, Jiao XL, Chen AJ, Luo Y, Gao WW. First report of *Ilyonectria robusta* causing rusty root of Asian ginseng in China. Plant Dis 2015;99:156.
[44] Seifert KA, McMullen CR, Yee D, Reeleder RD, Dobinson KF. Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans*. Phytopathology 2003;93:1533–42.
[45] Farh MEA, Han JA, Kim YJ, Kim JC, Singh P, Yang DC. Discovery of a new primer set for detection and quantification of *Ilyonectria mors-panacis* in soils for ginseng cultivation. J Ginseng Res 2017.
[46] Farh MEA, Kim YJ, Singh P, Yang DC. Cross interaction between *Ilyonectriamors-panacis* isolates infectingkorean ginseng and ginseng saponins in correlation with their pathogenicity. Phytopathology 2017;107:561–9.
[47] Gao J, Wang Y, Guan YM, Chen CQ. *Fusarium cerealis*, a new pathogen causing ginseng (*Panax ginseng*) root rot in China. Plant Dis 2014;98:1433.
[48] Guan YM, Lu BH, Wang Y, Gao J, Wu LJ. First report of root rot caused by *Fusarium redolens* on ginseng (*Panax ginseng*) in Jilin province of China. Plant Dis 2013;98:844.
[49] Wang Y, Guan YM, Lu BH, Gao J. First report of ginseng (*Panax ginseng*) root rot caused by *Fusarium acuminatum* in China. Plant Dis 2015;100:525.