

## Morphology and Molecular Characterization of a Fungus from the *Alternaria alternata* Species Complex Causing Black Spots on *Pyrus sinkiangensis* (Koerle pear)

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### ABSTRACT

A small-spored *Alternaria* was found from black spots of stored Koerle pear (*Pyrus sinkiangensis*), one of the economically important fruit in Xinjiang province, China. The morphology is similar to *A. limoniasperae* but obviously different in secondary conidiophores and conidial septa. A phylogenetic analysis using sequence datasets of ITS, GAPDH, TEF1, RPB2, Alt a1, OPA10–2, and EndoPG genes revealed that it belonged to the *Alternaria alternata* complex group. Pathogenicity tests illustrated that the fungus was the causal pathogen of black spot on Koerle pear fruit.

### ARTICLE HISTORY

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### KEYWORDS

*Alternaria*; black spot disease; phylogenetic analysis; pathogenicity

Koerle pear (*Pyrus sinkiangensis* synonymy *Pyrus* sp. nr. *communis*) is mainly distributed in north-western China, especially in Xinjiang Autonomous Region [1]. It is one of the important agricultural fruit and primarily exported to the international market because the fruit has a distinctive nice flavor and scent, thin skin, crisper and succulence, fewer dregs, and high volume sugary [2]. In 2011, the incidence of calyx-end black spot disease of Koerle pear reached from 14.7% to 34.8% with high yield loss in some orchards of Shayidong horticultural field, Bazhou, Xinjiang, China, of which casual pathogen is identified as *Alternaria alternata* based on morphology and sequence analyses of ITS, GAPDH, and TEF1 [3].

*Alternaria* is initially described by Nees (1816), which can be found as saprophytic, endophytic, and pathogenic species not only in agricultural products but also in soil and organic matter [4–6]. Two taxonomic sections of *Alternaria* including large-spored taxa and small-spored taxa are described by Simmons [7] based on conidial morphology and sporulation patterns. Most of small-spored *Alternaria* species are challenging because some morphological characters are difficult to clearly characterize [8]. Phylogenetically, a total of 27 sections are proposed by Lawrence et al. [9] after a review of biodiversity and taxonomy on *Alternaria*.

Among the phylogenetic sections, sect. *Alternaria* consists of 11 phylogenetic species and two species complexes, from which *A. alternata* species complex comprising 35 morphospecies [10]. Gannibal recommends that sec. *Alternaria* includes 59 species (1 type species, 21 phylogenetic species, and additional 37 morphospecies) [11].

Black spots of Koerle pear fruit were observed during storage in October 2017. An *Alternaria alternata*-like fungus was observed from the symptoms. The objectives of this study aim to test the pathogenicity of that fungus and clearly describe it based on morphology and sequence analyses of seven genes according to Simmons [7] and Woudenberg et al. [10], respectively. To get pure cultures, single spore was collected from infected fruit tissue segments and incubated on potato dextrose agar (PDA; Difco, Montreal, Canada) according to Luo et al. [12]. Five strains (YZU 171916, YZU 171918, YZU 171919, YZU 171920, and YZU 171921) were deposited in the Culture Collection at Yangtze University (YZU), Jingzhou, China (Table 1).

To determine the morphological characteristics, mycelia disks (6 mm diam.) were cut from 3-day-old colony and transferred on PDA kept at 25 °C for 7 days in darkness. The colony features were recorded and the colors were determined using the color chart of Rayner [13]. The fungus was

**Table 1.** Strains and their accession numbers used in the study.

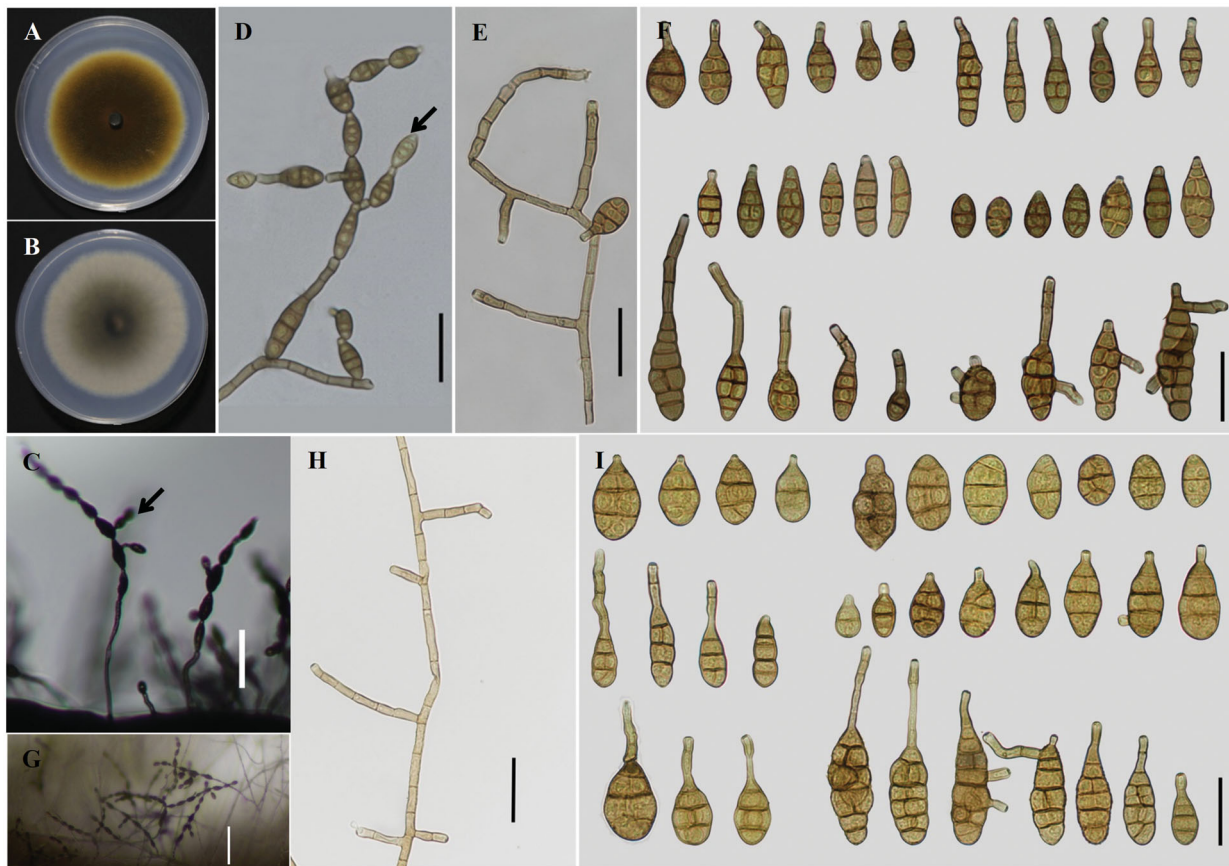
Morphospecies	Strain	Host	Location	GenBank accession No.						
				ITS	GAPDH	TEF1	RPB2	Alt a1	EndoPG	OPA10–2
<i>A. alternata</i>	CBS 916.96 T	<i>Arachis hypogaea</i>	India	AF347031	AY278808	KC584634	KC584375	AY563301	JQ811978	KP124632
<i>A. alternata</i>	CBS 110977 T	<i>Arachis hypogaea</i>	India	AF347031	AY278808	KC584634	KC584375	AY563301	JQ811978	KP124632
<i>A. astromeriae</i>	CBS 118808 R	<i>Astroemeria</i> sp.	United States	KP124296	KP124153	KP125071	KP124764	KP123845	KP123993	KP124601
<i>A. angustiovoidea</i>	CBS 195.86 T	<i>Euphorbia esula</i>	Canada	KP124317	KP124173	KP125093	KP124785	JQ646398	KP124017	KP124624
<i>A. arborescens</i>	CBS 115189	<i>Citrus clementina</i>	South Africa	KP124402	KP124254	KP125180	KP124872	KP123949	KP124106	KP124716
<i>A. arborescens</i>	CPC 25266	<i>Pyrus</i> sp.	Austria	KP124418	KP124269	KP125196	KP124887	KP123965	KP124122	KP124732
<i>A. arborescens</i>	CBS 124283	<i>Oryza</i> sp.	Russia	KP124416	KP124267	KP125194	KP124885	KP123963	KP124120	KP124730
<i>A. arborescens</i>	CBS 102605 T	<i>Solanum lycopersicum</i>	United States	AF347033	AY278810	KC584636	KC584377	AY563303	AY295028	KP124712
<i>A. astragali</i>	CBS 127672 T	<i>Astragalus bisulcatus</i>	United States	KP124382	KP124234	KP125160	KP124852	KP123930	KP124086	KP124695
<i>A. betae-kenyensis</i>	CBS 118810 T	<i>Beta vulgaris</i> var. <i>cicla</i>	Kenya	KP124419	KP124270	KP125197	KP124888	KP123966	KP124123	KP124733
<i>A. broussonetiae</i>	CBS 121455 T	<i>Broussonetia papyrifera</i>	China	KP124368	KP124220	KP125146	KP124838	KP123916	KP124072	KP124681
<i>A. brassicinae</i>	CBS 118811 T	<i>Brassica oleracea</i>	United States	KP124356	KP124210	KP125132	KP124824	KP123904	KP124057	KP124667
<i>A. burnsii</i>	CBS 107.38 T	<i>Cuminum cyminum</i>	India	KP124420	JQ646305	KP125198	KP124889	KP123967	KP124124	KP124734
<i>A. caudata</i>	CBS 121544 R	<i>Cucumis sativus</i>	United States	KP124371	KP124223	KP125149	KP124841	KP123919	KP124075	KP124684
<i>A. cerealis</i>	CBS 119544 T	<i>Avena sativa</i>	New Zealand	KP124408	JQ646321	KP125186	KP124878	KP123955	KP124112	KP124722
<i>A. cirtri</i>	CBS 102.47 R	<i>Citrus sinensis</i>	United States	KP124304	KP124161	KP125080	KP124773	KP123855	KP124002	KP124610
<i>A. citricancri</i>	CBS 119543 T	<i>Citrus paradisi</i>	United States	KP124363	KP124215	KP125139	KP124831	KP123911	KP124065	KP124674
<i>A. citriarbusti</i>	CBS 102598 T	<i>Minneola tangelo</i>	United States	KP124329	KP124184	KP125105	KP124797	KP123878	KP124031	KP124638
<i>A. citrimaculatis</i>	CBS 102596 T	<i>Citrus jambhiri</i>	United States	KP124328	KP124183	KP125104	KP124796	KP123877	KP124030	KP124637
<i>A. daucifolii</i>	CBS 118812 T	<i>Daucus carota</i>	United States	KC584193	KC584112	KC584652	KC584393	KP123905	KP124058	KP124668
<i>A. destruens</i>	CBS 121454 T	<i>Cuscuta gronovii</i>	United States	MH863109	AY278812	KP125145	KP124837	JQ646402	KP124071	KP124680
<i>A. dumosa</i>	CBS 102604 T	<i>Minneola tangelo</i>	Israel	KP124334	AY562410	KP125110	KP124802	AY563305	KP124035	KP124643
<i>A. eichhorniae</i>	CBS 489.92 T	<i>Eichhornia crassipes</i>	India	KC146356	KP124276	KP125204	KP124895	KP123973	KP124130	KP124740
<i>A. gaisen</i>	CBS 118488 R	<i>Pyrus pyrifolia</i>	Japan	KP124427	KP124278	KP125206	KP124897	KP123975	KP124132	KP124743
<i>A. gaisen</i>	CBS 118389 R	<i>Pyrus pyrifolia</i>	Japan	KP124407	KP124259	KP125185	KP124877	KP123954	KP124111	KP124721
<i>A. geophila</i>	CBS 101.13 T	Peat soil	Switzerland	KP124392	KP124244	KP125170	KP124862	KP123940	KP124096	KP124705
<i>A. gossypina</i>	CBS 104.32 T	<i>Gossypium</i> sp.	Zimbabwe	KP124430	JQ646312	KP125209	KP124900	JQ646395	KP124135	KP124746
<i>A. godetiae</i>	CBS 117.44 T	<i>Godetia</i> sp.	Denmark	KP124303	KP124160	KP125079	KP124772	KP123854	KP124001	KP124609
<i>A. herbiphorbicola</i>	CBS 119408 T	<i>Euphorbia esula</i>	United States	KP124362	JQ646326	KP125138	KP124830	JQ646410	KP124064	KP124673
<i>A. iridiauxtralis</i>	CBS 118404 R	<i>Iris</i> sp.	New Zealand	KP124434	KP124283	KP125213	KP124904	KP123980	KP124139	KP124750
<i>A. interrupta</i>	CBS 102603 T	<i>Minneola tangelo</i>	Israel	KP124333	KP124188	KP125109	KP124801	KP123882	KP124034	KP124642
<i>A. jacinthicola</i>	CBS 133751 T	<i>Eichhornia crassipes</i>	Mali	KP124438	KP124287	KP125217	KP124908	KP123984	KP124143	KP124754
<i>A. kikuchiana</i>	CBS 107.53	<i>Pyrus pyrifolia</i>	Japan	KP124305	KP124162	KP125081	KP124774	KP123858	KP124005	KP124613
<i>A. lini</i>	CBS 106.34 T	<i>Linum usitatissimum</i>	unknown	Y17071	JQ646308	KP125078	KP124771	KP123853	KP124000	KP124608
<i>A. limoniasperae</i>	CBS 102595 T	<i>Citrus jambhiri</i>	United States	FJ266476	AY562411	KC584666	KC584408	AY563306	KP124029	KP124636
<b>The present fungus</b>	<b>YZU 171916</b>	<b><i>Pyrus sinkiangensis</i></b>	<b>China</b>	<b>MK391581</b>	<b>MK391582</b>	<b>MK415954</b>	<b>MK391583</b>	<b>MK391585</b>	<b>MK415955</b>	<b>MK391584</b>
	<b>YZU 171918</b>	<b><i>Pyrus sinkiangensis</i></b>	<b>China</b>	<b>MK391594</b>	<b>MK415938</b>	<b>MK415946</b>	<b>MK391586</b>	<b>MK415950</b>	<b>MK415942</b>	<b>MK391590</b>
	<b>YZU 171919</b>	<b><i>Pyrus sinkiangensis</i></b>	<b>China</b>	<b>MK391596</b>	<b>MK415940</b>	<b>MK415948</b>	<b>MK391588</b>	<b>MK415952</b>	<b>MK415944</b>	<b>MK391592</b>
	<b>YZU 171920</b>	<b><i>Pyrus sinkiangensis</i></b>	<b>China</b>	<b>MK391595</b>	<b>MK415939</b>	<b>MK415947</b>	<b>MK391587</b>	<b>MK415951</b>	<b>MK415943</b>	<b>MK391591</b>
	<b>YZU 171921 T</b>	<b><i>Pyrus sinkiangensis</i></b>	<b>China</b>	<b>MK391597</b>	<b>MK415941</b>	<b>MK415949</b>	<b>MK391589</b>	<b>MK415953</b>	<b>MK415945</b>	<b>MK391593</b>
<i>A. longipes</i>	CBS 540.94 R	<i>Nicotiana tabacum</i>	United States	AY278835	AY278811	KC584667	KC584409	AY563304	KP124147	KP124758
<i>A. mali</i>	CBS 106.24 T	<i>Malus sylvestris</i>	United States	KP124298	KP124155	KP125073	KP124766	KP123847	AY295020	JQ800620
<i>A. palandui</i>	CBS 121336 T	<i>Allium</i> sp.	United States	KJ862254	KJ862255	KP125141	KP124833	KJ862259	KP124067	KP124676
<i>A. pellucida</i>	CBS 479.90 T	<i>Citrus unshiu</i>	Japan	KP124319	KP124174	KP125095	KP124787	KP123870	KP124019	KP124626
<i>A. perangusta</i>	CBS 102602 T	<i>Minneola tangelo</i>	Turkey	KP124332	KP124187	KP125108	KP124800	KP123881	AY295023	KP124641
<i>A. platycodonis</i>	CBS 121348 T	<i>Platycodon grandiflorus</i>	China	KP124367	KP124219	KP125144	KP124836	KP123915	KP124070	KP124679
<i>A. postmessia</i>	CBS 119399 T	<i>Minneola tangelo</i>	United States	KP124361	JQ646328	KP125137	KP124829	KP123910	KP124063	KP124672
<i>A. pulvinifungicola</i>	CBS 194.86 T	<i>Quercus</i> sp.	United States	KP124316	KP124172	KP125092	KP124784	KP123869	KP124016	KP124623
<i>A. rhadina</i>	CBS 595.93 T	<i>Pyrus pyrifolia</i>	Japan	KP124320	KP124175	KP125096	KP124788	JQ646399	KP124020	KP124627
<i>A. sanguisorbae</i>	CBS 121456 T	<i>Panglossia officinalis</i>	China	KP124369	KP124221	KP125147	KP124839	KP123917	KP124073	KP124682
<i>A. seleniiphila</i>	CBS 127671 T	<i>Stanleya pinnata</i>	United States	KP124381	KP124233	KP125159	KP124851	KP123929	KP124085	KP124694
<i>A. senecionicola</i>	CBS 119545 T	<i>Senecio skirrhodon</i>	New Zealand	KP124409	KP124260	KP125187	KP124879	KP123956	KP124113	KP124723
<i>A. soliaegyptiaca</i>	CBS 103.33 T	Soil	Egypt	KP124302	KP124159	KP125077	KP124770	KP123852	KP123999	KP124607
<i>Alternaria</i> sp.	CBS 632.93 R	<i>Pyrus pyrifolia</i>	Japan	KC584197	KC584116	KC584658	KC584399	KP123974	AY295033	KP124742
<i>A. tenuissima</i>	CBS 918.96 R	<i>Dianthus chinensis</i>	UK	AF347032	AY278809	KC584693	KC584435	AY563302	KP124026	KP124633
<i>A. tomato</i>	CBS 114.35	<i>Solanum lycopersium</i>	Unknown	KP124446	KP124295	KP125225	KP124916	KP123992	KP124152	KP124763
<i>A. tomaticola</i>	CBS 118815 R	<i>Solanum lycopersicum</i>	United States	KP124358	KP124212	KP125134	KP124826	KP123907	KP124060	KP124670
<i>A. tomaticola</i>	CBS 118814 T	<i>Solanum lycopersicum</i>	United States	KP124357	KP124211	KP125133	KP124825	KP123906	KP124059	KP124669
<i>A. turkisafria</i>	CBS 102599 T	<i>Minneola tangelo</i>	Turkey	KP124330	KP124185	KP125106	KP124798	KP123879	KP124032	KP124639
<i>A. turkisafria</i>	CBS 121344 R	<i>Minneola tangelo</i>	Israel	KP124365	KP124217	KP125142	KP124834	KP123913	KP124068	KP124677
<i>A. turkisafria</i>	CBS 121346 R	<i>Minneola tangelo</i>	South Africa	KP124366	KP124218	KP125143	KP124835	KP123914	KP124069	KP124678
<i>A. toxicogenica</i>	CBS 102600 T	<i>Citrus reticulata</i>	United States	KP124331	KP124186	KP125107	KP124799	KP123880	KP124033	KP124640
<i>A. vaccinii</i>	CBS 118818 T	<i>Vaccinium</i> sp.	United States	KP124359	KP124213	KP125135	KP124827	KP123908	KP124061	KP124671
<i>A. yali-inficiens</i>	CBS 121547 T	<i>Pyrus bretschneideri</i>	China	KP124372	KP124224	KP125150	KP124842	KP123920	KP124076	KP124685

T: ex-type strain; R: representative strain.

Bold contents are related to the present fungus generated in this study.

incubated on potato carrot agar (PCA) for 7 days at 22 °C under the daily fluorescent light/dark cycle of 8/16 h to describe the conidial morphology [7]. To describe the morphology from host, diseased tissue was incubated for 4 days under the same condition as conidial description on PCA. The

sporulation patterns and conidia were photographed using a compound light microscope (Nikon DS-Ri2, Tokyo, Japan). The conidia were mounted in lactophenol solution. Fifty conidia were investigated for the description of each of characteristics.



**Figure 1.** Morphological characteristics of *Alternaria* sp. YZU 171921 from *Pyrus sinkiangensis*. Colony on PDA (A) and (B); sporulation patterns, conidiophores and conidia on the PCA (C)–(F); sporulation patterns, conidiophores and conidia on the host plant (G)–(I). Bars: (D)–(F) = 25 μm, (H) and (I) = 25 μm, (C) = 100 μm, (G) = 100 μm.

After 7 days, the colonies reached to 65–66 mm in diam. on PDA with umber to olivaceous color surrounding with white margin (Figure 1(A,B)). On PCA (Figure 1(C–F)), primary conidiophores were 15–146 × 3–5 μm producing 4–10 units catenulate conidia and the secondary conidiophores to develop lateral intra-conidia were 3–20 × 3–4 μm forming branched chains of 1–4 units. Conidia comprising 1–7 transverse septa were narrow-ellipsoid (13–50 × 6–11 μm) or ovoid (6–23 × 4–13 μm) in the initial lower part of the chains, gradually becoming ovoid (7–22 × 5–9 μm) and considerably smaller in the distal part, with apical conidia (2–12 × 2–4 μm). On the host (Figure 1(G–I)), the primary conidiophores reached 3–107 × 2–4 μm producing catenulate conidia (3–10 units) and the secondary ones to produce lateral intra-conidia (catenulate with 1–4 units) were 3–23 × 2–4 μm in size. Normally, conidia were 13–44 × 2–28 μm, with 1–6 transverse septa and false beaks 2–32 × 2–7 μm in size. The present fungus was morphologically similar to the species *Alternaria limoniasperae*, *A. perangusta*, *A. interrupta*, and *A. turkisafria* (Table 2).

To test its pathogenicity, healthy Koerle pear fruits were obtained from the seller of Xinjiang market. Eighteen fruits were surface sterilized by

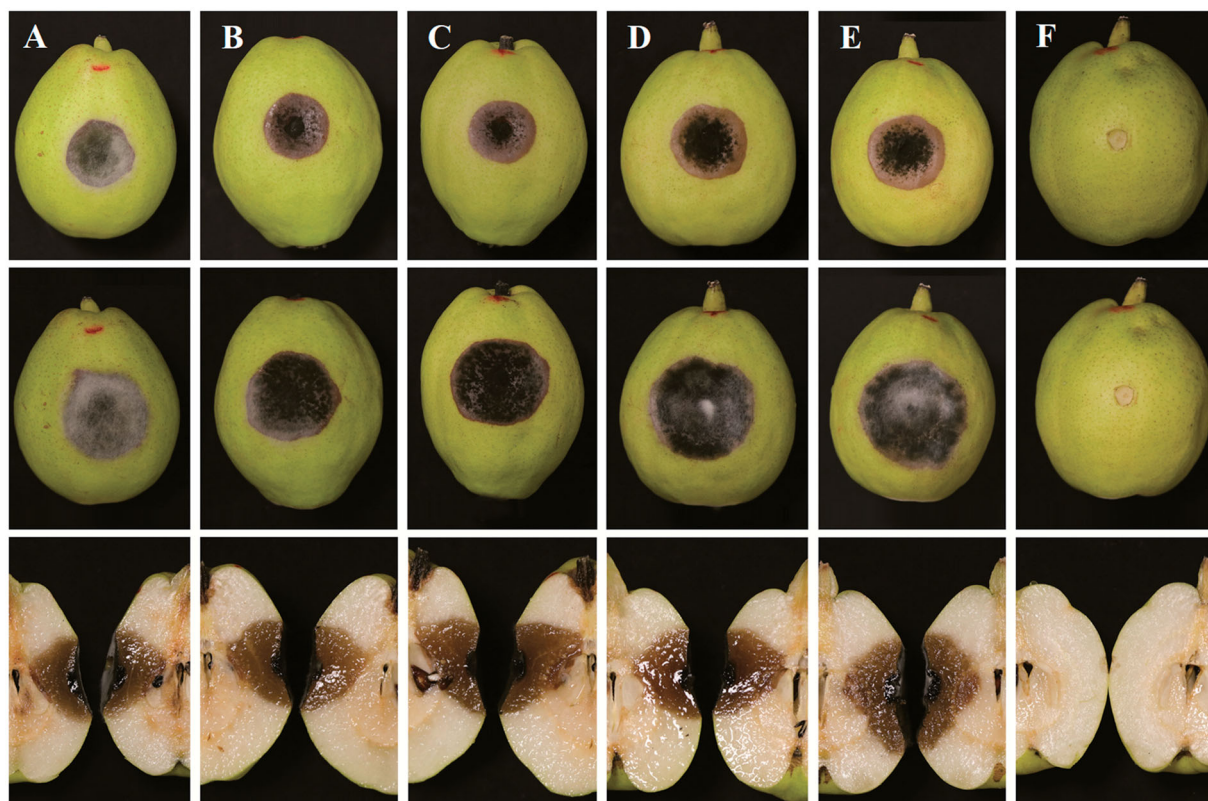
dipping in 1% sodium hypochlorite (NaOCl) for 2 min, and then, washed with sterilized distilled water for 3 times. Each fruit was wounded two sites (one for mycelia plug and another for spore suspension) by a puncher (4 mm diam.) and placed into moist containers maintained at 25 °C. Mycelia plugs (4 mm diam.) of each strain were cut from the edge of 3-day-old colonies and placed on wounded sites. Sterile PDA plugs were used as controls. Conidia were harvested from PCA to obtain the spore suspension (10<sup>6</sup> conidia/mL). A volume of 20 μL spore suspension was inoculated and distilled water was used as controls. Each strain was conducted with three replications and the experiment was repeated for three times. The disease development was checked daily.

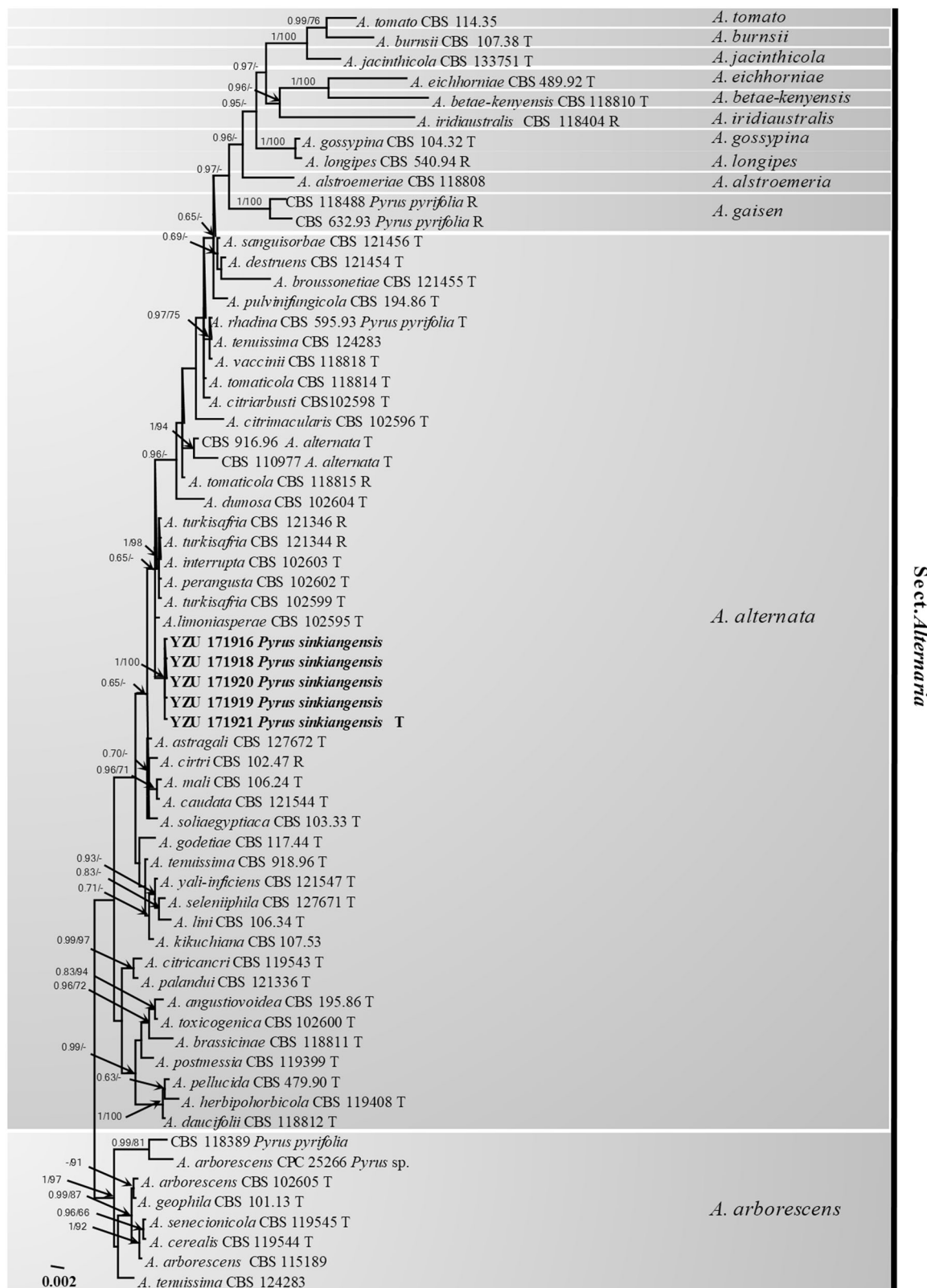
Necrotic symptoms were observed obviously at 3 days in both inoculations. After 7 days, the symptoms developed up to 23 mm (diam.) inoculated with mycelia plugs (Figure 2) and 14 mm with spore suspensions. After 14 days, the symptoms turned to be rotten reaching to 30 mm in mycelium block and 22 mm in spore suspension. Any control was symptomless during the experiment. By the way, unwounded fruits were symptomless either by mycelia plug or spore suspension (data not shown). The Koch's postulates were fulfilled by a re-isolation



**Table 2.** Morphological comparison of the present fungus and its closely related species described by Simmons [7].

Species	Conidia		Conidiophore ( $\mu\text{m}$ )	Secondary conidiophore ( $\mu\text{m}$ )	Conidia per primary chain (lateral branched chain)
	Shape and size ( $\mu\text{m}$ )	Septa			
<i>A. limoniasperae</i>	Narrow-ellipsoid 30–50 $\times$ 8–10 or ovoid 20–35 $\times$ 8–12 in the initial lower part of the chain, ovoid 8–12 $\times$ 4–8 in the distal part of the chain	1–4	100	2–4 $\times$ 2–3	up to 20 (4–10)
YZU 171921	Narrow-ellipsoid 13–50 $\times$ 6–11 or ovoid 6–23 $\times$ 4–13 in the initial lower part of the chain, ovoid 7–22 $\times$ 5–9 in the distal part of the chain	1–7	15–146 $\times$ 3–5	3–20 $\times$ 3–4	4–10 (1–4)
<i>A. turkisfria</i>	Narrow-ovoid to long-ovoid or long-ellipsoid 20–50 $\times$ 6–8, conidia have small 1-cell secondary conidiophores	3–8	30–60 $\times$ 4	3–5 $\times$ 2–3	8–20 (4–10)
<i>A. perangusta</i>	Long narrow-ellipsoid, rarely wide enough to be termed obclavate 15–40 $\times$ 3–7	3–7	100–200 $\times$ 3–4	3–5 $\times$ 2–3	10+ (unknown)
<i>A. interrupta</i>	Narrow-ellipsoid or narrow-obclavate 35–40 $\times$ 7–8	7–8	140 $\times$ 4	3–8 $\times$ 2–3	10–15 (unknown)

**Figure 2.** Pathogenicity tests on Koerle pear fruit (*Pyrus sinkiangensis*) inoculated with mycelia plugs of five strains (A–F) of the present fungus for 7 days (upper) and for 14 days (middle and down) at 25 °C. (A) YZU 171916; (B) YZU 171918; (C) YZU 171919; (D) YZU 171920; (E) YZU 171921; (F) Control.



**Figure 3.** The phylogenetic tree using 64 strains of sect. *Alternaria* based on ITS, GAPDH, TEF1, RPB2, Alt a1, OPA10-2, and EndoPG gene sequences. The Bayesian posterior probabilities (PP) > 0.65 and RAxML bootstrap support values (BP) > 65% are given at the nodes (PP/BP). The type strain or ex-type strain is indicated with T and representative strain is R.

from inoculated fruits. The results showed that the present fungus was the causal agent of black spot of Koerle pear fruit (Figure 2).

To confirm the phylogenetic position of the present fungus, the genomic DNA was extracted using

mycelia grown on PDA according to the method of Ceniz [14]. Seven genes including internal transcribed spacer rDNA regions (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), partial translation elongation factor 1 alpha (TEF1), RNA

polymerase second largest subunit (RPB2), *Alternaria* major allergen gene (Alt a1), an anonymous gene region (OPA10-2), and endopolygalacturonase gene (EndoPG) were amplified using the primer pairs ITS4/ITS5 [15], gpd1/gpd2 [16], EF1-728F/EF1-986R [17], RPB2-5F [18]/RPB2-7CR [19], Alt-for/Alt-rev [20], OPA 10-2L/OPA 10-2 R [8], and PG3/PG2b [8], respectively. PCR amplification was performed in a 25 µL reaction volume containing 8 µL ddH<sub>2</sub>O, 2 µL DNA solution, 1.25 µL each primer, and 12.5 µL 2 × Taq PCR StarMix (Genstar, Beijing, China). The PCR products were checked in 1% agarose gel, run in 0.5 × TBE buffer and visualized under UV illumination. Successfully amplified PCR products were sequenced by Beijing Genomics Institute (BGI, Beijing, China) using both primers. The resulting sequences were compared with those of morphospecies described by Simmons (2007) derived from Woudenberg et al. [10]. Each of seven gene sequences was aligned and combined using the MEGA v. 6.0.0 software [21]. The best-fit model GTRGAMMAI was selected by MrModeltest v. 2.3.6. [22]. Bayesian analyses were performed with MrBayes v. 3.1.2 [23]. The parameters including 2,000,000 Markov chain Monte Carlo (MCMC) generations and a sampling frequency of every 100 generations were set for the combined analysis of seven loci. And the run was stopped when the average standard deviation of split frequencies fell under 0.01. Burn-in was set to 25% after which the likelihood values were constant. A maximum-likelihood analysis was additionally run using RAxML v. 7.2.8 [24]. Bootstrap analysis was performed with 1000 replications for the combined analysis of seven loci. The *Alternaria arborescens* species complex (AASC) was used as root branch. The resulting tree was plotted and edited by FigTree v. 1.3.1 [25]. A total of 64 *Alternaria* isolates were included in the aligned sequence matrix. In the multigene phylogeny, 3445 characters were calculated including 502 of ITS, 446 of GAPDH, 240 of TEF1, 710 of RPB2, 472 of Alt a1, 633 of OPA10-2, and 442 of EndoPG. The Bayesian posterior probabilities (PP) >0.65 and RAxML bootstrap support values (BP) >65% were plotted in the phylogeny (Figure 3). Based on the seven genes, the five strains used in this study were identical to each other. The phylogenetic results showed that the present fungus was belonging to *Alternaria alternata* species complex (AALSC) group of *Alternaria* and fell into a monoclade highly supported by PP (1.00) and BP (100%) values. They were closely related to *Alternaria limoniasperae*, *A. perangusta*, *A. interrupta*, and *A. turkisafrica*. However, the present fungus was the closest to *A. limoniasperae* with seven nucleotide position differences: Alt a 1 position 350 (C), RPB2 position

546 (G), and OPA10-2 position 369 (T), 618 (C), 624 (G), 639 (C), 648 (G). Morphologically, the present fungus was obviously different from *A. limoniasperae* by producing more septa in conidia with shorter chains. All the previous results indicated that the species might be a new morphospecies in AALSC. All thirty-five morphospecies under one species *Alternaria alternata* [10] is not a great way for the taxonomy based on phylogeny. More works should be done to better understand the taxonomy of *Alternaria alternata* species complex based on morphology and molecular at the same times. In the present study, the fungus collected from black spot of Koerle pear fruit (*Pyrus sinkiangensis*) was found as a causal agent and illustrated clearly in morphology generated from authentic culture and host. Phylogenetically, it should be considered as a new member of AALSC.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

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