Venturia chinensis sp. nov., a new venturialean ascomycete from Khingan Mountains

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Abstract A new species of Venturia (V. chinensis) is described and illustrated from the leaves of Lonicera praeflorens collected from Lesser Khingan Mountains, the northeast China. It is characterized by habitat saprobic; ascomata small-sized, solitary or scattered, superficial, subglobose to citriform, wall black, papillate, ostiolate, covered with setae; peridium thin; hamathecium evanescent in mature ascomata; asc 8-spored, bitunicate, fissitunicate, oblong to oblack, with or without a short, knob-like pedicel; ascospores ellipsoidal, olivaceous pale brown, 1-septate, ascospore wall thin, smooth. Comparisons of V. chinensis with V. lonicerae (another species on Lonicera caerulea) and other species of Venturia lead to the conclusion that collected taxon is new. Its relationships with other species of Venturia are discussed based on morphology and 28S nrDNA and ITS nrDNA sequence comparisons.

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1. Introduction

Species of Venturia De Not. are widely distributed in north temperate area of the world, which are saprobic or parasitic on a large variety of plants. Some species of Venturia are notorious plant pathogens, such as the apple scab caused by V. inaequalis (Cooke) G. Winter and pear scab by V. pyrina Aderh. (Barr, 1968; Sivanesan, 1977). Venturia was first described by De Notaris (1844) to accommodate V. rosea De Not. and V. dianthi De Not. with no type designated. Subsequently, Cesati and De Notaris (1863) described another two species, i.e. V. dickiei (Berk. & Broome) Ces. & De Not. and V. erez (Berk. & Broome) Ces. & De Not. Saccardo (1882) emended the description of Venturia, excluded both V. rosea and V. dianthi, while accepted V. dickiei and V. erez. Venturia Sacc. was widely accepted, and was neotypified by V. inaequalis (Korf, 1956; Sivanesan, 1977). The diagnostic characteristics of Venturia Sacc. include habitat parasitic or saprobic on dicotyledonous leaves; ascomata small-sized, solitary, scattered, or gregarious, initially immersed, becoming

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erumpent, globose, subglobe, wall black, papillate, ostiolate; peridium thin, composed of a few layers of pigmented cells of textura angularis; hamathecium rare, evanescent in mature ascomata; asci 8-spored (rarely 4-spored), bitunicate, fissitunicate dehiscence unknown, oblong to obclavate, with a short, thick pedicel or pedicel lacking, with an inconspicuous ocular chamber; ascospores obliquely uniseriate and partially over-lapping to biserate, especially at the base, ellipsoidal, with broadly rounded ends, pale brown, 1-septate, slightly constricted at the septum, the upper cell shorter than the lower one, smooth-walled; having Fusicladium Bonord., Pollaccia E. Bald. & Cif. or Spilocaea Fr. anamorphs (Zhang et al., 2011).

One hundred and ninety-three species names are listed in the Index Fungorum, which were estimated to comprise 57 species (Kirk et al., 2008). Early studies of Venturia in China were conducted by Zhu (1927), and in the general summary of Chinese fungi by Tai (1941, 1979), five species were reported, i.e. V. geranii (Fr.) G. Winter, V. inaequalis, V. microsela Pat., V. pyrina and V. tremulae Aderh. Subsequently, Zhang (2003) revised the Fusicladium in China with 15 species of Fusicladium reported. In the summary of phytopathogens of woody plants in China conducted by Xu and He (2008), about 30 venturiaceous species were listed.

In the course of an ongoing survey of biodiversity of venturialean ascomycete in China initiated in 2014, a venturiaceous fungus was collected that appeared to fit Venturia s. s. well in morphological traits. This was supported by the comparisons of LSU and ITS nrDNA sequences of this new species with DNA sequences of other venturiaceous species deposited in GenBank. Based on the combination of subtle morphological and molecular differences a new taxon, Venturia chinensis is proposed.

2. Materials and methods

2.1. Morphological study

Leaf samples were collected from growing Lonicera praeflorens in August 2014, from Lesser Khingan Mountains in Heilongjiang province, Yichun, Wuyiling forestry station. Leaf samples were dried with absorbent paper in herbarium press, and studied directly under an Olympus SZ 61 dissecting microscope after preliminary incubation in a moist chamber. Microscopic observations of ascomatal contents were carried out from material mounted in water. Photomicrographs were taken on a Nikon Eclipse E600 Microscope fitted with a Nikon Digital Sight DS F11 digital camera and processed with NIS-Elements software. Measurements of asci, hamathecium, peridium thin, composed of a few layers of pigmented cells of textura angularis.

2.2. DNA extraction, PCR, sequencing

Sequencing of portions of rDNA was attempted from DNA extracted from the mycelium from the surface of MEA plates with CTAB plant genome DNA fast extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China). The LSU (large sub-unit, 28S) nrDNA region was amplified and sequenced with primers LROR and LR5. The ITS nrDNA amplifications and sequencing used primers ITS-1 and ITS-4. Comparisons to other nrDNA sequences were conducted with BLAST 2.2.24 queries (National Center for Biotechnology Information, National Institute of Health, Bethesda, Maryland). Representative sequences were deposited in GenBank.

2.3. Sequence alignment and phylogenetic analysis

Sequences generated were analysed with other sequences obtained from GenBank (Table 1). All of the other species included in the phylogeny were chosen based on the phylogeny of Zhang et al. (2011). A Multiple alignment was done in Mega 5 (Tamura et al., 2011) and analyses were performed in PAUP V. 4.0b10 (Swofford, 2002). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. ITS rDNA dataset was analysed in this study. Maximum likelihood (ML) and maximum parsimony (MP) were conducted using heuristic searches as implemented in PAUP, with the default options method. For the ML analysis, best-fit model of nucleotide evolution (GTR + I + G) was selected by Akaike information criterion (AIC) (Posada and Buckley, 2004) in MrModeltest 2.3. Bootstrap analysis with 1000 replicates was used to test the statistical support of the branches. With model parameters estimated from the data, a heuristic search with ten random taxon addition sequences and TBR branch swapping was performed. For MP analysis, clade stability was assessed in a bootstrap (BS) analysis with 1000 replicates, random sequence additions with maxtrees set to 5000 and other default parameters as implemented in PAUP. Trees were viewed in TREEVIEW. The nucleotide sequences reported in this paper were deposited in GenBank.

Table 1 Species and sequences database accession numbers used in this study (newly generated sequences are indicated in bold).

| Species          | Strain     | GenBank accession number |
|------------------|------------|--------------------------|
| V. chlorospora   | CBS 466.61 | EU035453 EU035453         |
| V. chinensis     | CGMCC 3.17685 | KP689595 KP689595         |
| V. helvetica     | CBS 474.61 | EU035458 EU035458         |
| V. loniceria     | CBS 445.54 | EU035461 EU035461         |
| V. minuta        | CBS 478.61 | EU035464 EU035464         |
| V. polygoni-vivipari | CBS 114207 | EU035466 EU035466         |
3. Results

**Taxonomy**

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**Mycobank:** MB 811424 (Fig. 1–3).

Etymology: The epithet “chinensis” refers to China, the country from which it is described.

Known distribution: China.

Asexual state: Unknown.

Ascomata 40–100 μm diam., solitary, scattered, initially immersed or slightly erumpent, becoming superficial, globose or citriform with a small papilla, ostiolate, wall black, upper one third of the ascomata covered with setae (Fig. 1–3). Setae dark brown, 0–1 septate, 23–61 × 5–7 μm, setae wall 1–1.5 μm (Fig. 1: 6, 11). *Peridium* 1-layered, composed of (1-) 2–3 layers of pigmented cells of textura angularis, cells 9–5 μm diam., cell wall 0.8–1 μm thick. *Hamathecium* rare, evanescent in mature ascomata (Fig. 1: 9–10). Asci 34–59 × 10–13 μm (\(\bar{x} = 48.3 \times 10.9 \) μm, \( n = 10 \)), 8-spored, bitunicate, fissitunicate, oblong to obclavate, with or without a short, knob-like pedicel or pedicel lacking, with an inconspicuous ocular chamber (Fig. 1: 5, 7). Ascospores 11–15(–20) × 4–5 μm (\( \bar{x} = 13.4 \times 4.5 \) μm, \( n = 20 \)), obliquely overlapping to biseriate at the base, ellipsoidal, with broadly rounded ends.

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**Fig. 1** *Venturia chinensis* (holotype HMAS246485). (1–3) Ascomata on the host surface. Note the long and black setae. (4) Section of an ascoma. (5,7) Obelavate asci. (6) Crushed ascoma showing the thick walled setae. (8) Ascus releasing ascospores. (9–10) Decomposing pseudoparaphyses. (11) Seta. Scale bars: (1) = 300 μm; (2–3) = 100 μm; (4–5) = 20 μm; (6–8) = 10 μm; (9–11) = 5 μm.
**Fig. 2** *Venturia chinensis* (extype CGMCC 3.17685). (1) Colony growing on MEA after two weeks. (2–9) Chlamydompores and hyphae. Scale bars: (1) = 1 mm; (2), (4), (6), (9) = 10 μm; (3), (5), (7–8) = 20 μm.

**Fig. 3** *Venturia chinensis* (extype CGMCC 3.17685). Upper (1) and reverse (2) view of colony on MEA 6 months after inoculation. bars: 1 mm.
olivaceous pale brown, 1-septate, slightly constricted at the septum, the upper cell shorter and wider than the lower one (length of upper/lower cell = (7:13–)5:7–1:1), ascospore wall thin, smooth (Fig. 1: 8).

Culture characteristics: Ascospore germinating on MEA (malt extract agar) after 2–3 d; colony growth is extremely slow, on MEA reaching up to 0.5 mm diameter in 14 days at 26–28°C in darkness, reaches 0.8–1.1 mm after 4 weeks and reaches 1.2–2.3 mm after 3 months. Colony first blackish, turning olivaceous black, reverse blackish; hyphae olivaceous brown, branched, 6–11 μm wide, constricted at septa, wall thickened, 1–1.5 μm, often giving rise to dark brown ellipsoidal chlamydospores, chlamydospores 11–18 × 7–14 μm, wall 1–2 μm; hyaline to pale brown thin-walled hyphae rarely produced, 4–7 μm wide, straight to coiled, and hyphae tips usually swollen or forming a pale brown to brown lemon-shaped cell, 19–30 × 10–18 μm; odour not detected. No conidiogenous structures were observed (see Figs. 2 and 3).

Type: CHINA. Heilongjiang province, Yichun, Wuyiling district, Wuyiling forestry station, 48°33′N, 129°30′E, ca. 330 m, on leaves of *L. praeflorens*, 26 August 2014 (HMAS246485, holotype).

Diagnosis: *V. chinensis* differs from other known species in the genus by the striking superficial ascomata covered with dark brown setae on the upper one-third part, and different ITS and LSU sequences.

Note: Ascomata scattered on decaying the leaf surface of *L. praeflorens* with no scab symptom observed. Thus *V. chinensis* should be a saprobic fungus.

4. Discussion

*Venturia chinensis* is characterized by saprobic habitat, small-sized, superficial ascomata, subglobose to citriform, with a striking papilla and covered with setae; evanescent hamathecium; oblong to obclavate asci with or without a short, knob-like pedicel; ellipsoidal, olivaceous pale brown, 1-septate and thin-walled ascospore. These morphological characteristics fit *Venturia* well, which are also strongly supported by DNA sequences data (Fig. 4).

Host specificity has been considered as one of the distinguishing characteristics of Venturieae, and one particular venturieae species usually restricted to one or a few closely related species of a host genus, or to members of closely related genera (Barr, 1989). Host species of one genus may bear more than one member of the *Venturia*, such as three species of *Venturia* has been reported in *Epilobium*, and 5 species of *Venturia in Salix* (Nüesch, 1960). *Venturia lonicerae* Sacc. has been reported from *Lonicera xylosteum* (Saccardo, 1882). CBS 445.54, an isolate of *V. lonicerae* from *Lonicera caerulea*, significantly differs from *V. chinensis* in ITS (identical rate: 98.6%) and LSU (identical rate: 99.8%) sequences (Table 2). In particular, the strikingly superficial and citriform ascomata of *V. chinensis* can be readily distinguished from *V. lonicerae* and other species of *Venturia*. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the ITS sequence are *V. chlorospora* (Ces.) P. Karst. (strain CBS 466.61, GenBank EU035453), *V. helvetica* Nüesch (strain CBS 474.61, GenBank...
Fusicladium or readily produced on the growing leaves of Salix in Switzerland by E. Müller. Both V. chlorospora and V. helvetica have been reported from Salix (Nüesch, 1960). The length/width index of V. chinensis is 2.97, which is much higher than that of V. chlorospora (2.1) and V. helvetica (2.4). In particular, the striking superficial ascomata of V. chinensis can be readily distinguished from the erumpent ascomata of V. chlorospora and V. helvetica. The identification status of these three isolates however, needs to be verified yet. In addition, the small-sized (40–60 μm diam.), immersed to erumpent ascomata of V. minuta can be readily distinguished from those of V. chinensis.

Venturia chinensis is saprobiuc and the ascomata of which is readily produced on the growing leaves of L. praeflorens in natural environment, and more ascomata could be obtained by inoculation in the lab. These characteristics differ from some parasitic species, the ascomata of which are usually produced during or after winter time (Nüesch, 1960). Comparable with other species of venturiaceous fungi, the colony of V. chinensis grows rather slowly in culture. The microstructures produced on the surface of media of V. chinensis derives from the normal asexual stage of Fusicladium or Spiloceua, Pollaccia in hyphae which are rather thick, heavily pigmented, producing chlamydospores and hyphal tips swollen. These characteristics, however, are unlike those encountered in saprobic fungi.

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