



ORIGINAL ARTICLE

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



Jiaqi Zhang <sup>a</sup>, Zhipeng Dou <sup>b</sup>, Yupei Zhou <sup>a</sup>, Wei He <sup>b</sup>, Xiaodong Zhang <sup>c</sup>, Ying Zhang <sup>a,\*</sup>

<sup>a</sup> Institute of Microbiology, P.O. Box 61, Beijing Forestry University, Beijing 100083, PR China

<sup>b</sup> Beijing Key Laboratory for Forest Pest Control, Beijing Forestry University, Beijing 100083, PR China

<sup>c</sup> Plant Protection Unit, Juancheng County, Heze, Shandong 274600, PR China

Received 2 February 2015; revised 17 June 2015; accepted 21 June 2015

Available online 26 June 2015

## KEYWORDS

China;  
DNA sequences;  
Foliicolous fungi;  
Morphology;  
Taxonomy

**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; asci 8-spored, bitunicate, fissitunicate, oblong to obclavate, 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.

© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 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. eres* (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. eres*. *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

\* Corresponding author. Tel.: +86 10 62336309.

E-mail address: [yinghku@gmail.com](mailto:yinghku@gmail.com) (Y. Zhang).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

erumpent, globose, subglobose, 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 overlapping to biseriata, 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. microseta* 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 venturiacean 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 ascotal 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 F 3.2 software. Measurements of asci, hamathetical hyphae and ascospores were made from water mounts.

Isolations were made from single ascospores and grown on 2% water agar (WA) (Biolab, S.A.), and subsequently transferred to malt extract agar (MEA) with sterilized pine needles. Isolates were placed at ambient temperatures (about 26–28 °C) in the dark condition for establishing colony characteristics. Fungal isolates and herbarium specimens have been deposited at Beijing Forestry University (BJFU) with duplicates in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS).

### 2.2. DNA extraction, PCR, sequencing

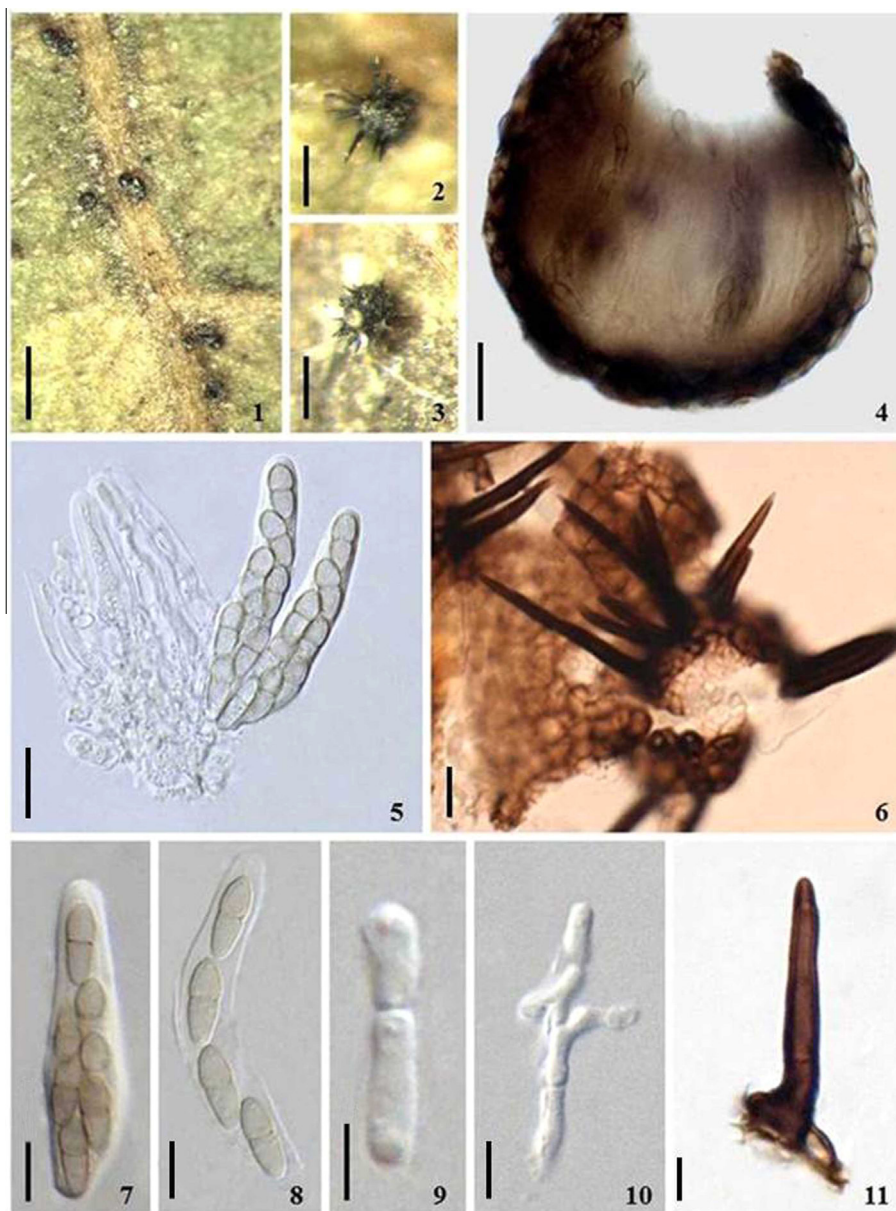
Sequencing of portions of rDNA was attempted from DNA extracted from the mycelium from the surface of MEA plates with CTAB plant genome DNA fast extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China). The LSU (large subunit, 28S) *nrDNA* region was amplified and sequenced with primers LROR 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	
		28S rDNA	ITS
<i>V. chlorospora</i>	CBS 466.61	EU035453	EU035453
<i>V. chinensis</i>	CGMCC 3.17685	<b>KP689595</b>	<b>KP689596</b>
<i>V. helvetica</i>	CBS 474.61	EU035458	EU035458
<i>V. lonicera</i>	CBS 445.54	EU035461	EU035461
<i>V. minuta</i>	CBS 478.61	EU035464	EU035464
<i>V. polygoni-vivipari</i>	CBS 114207	EU035466	EU035466



**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) Oblavate asci. (6) Crushed ascoma showing the thick walled setae. (8) Ascus releasing ascospores. (9–10) Decomposing pseudoparaphyses. (11) Seta. Scale bars: (1) = 300  $\mu\text{m}$ ; (2–3) = 100  $\mu\text{m}$ ; (4–5) = 20  $\mu\text{m}$ ; (6–8) = 10  $\mu\text{m}$ ; (9–11) = 5  $\mu\text{m}$ .

### 3. Results

#### Taxonomy

*Venturia chinensis* Y. Zhang ter & J.Q. Zhang, **sp. nov.**  
**Mycobank:** MB 811424 (Fig. 1–3).

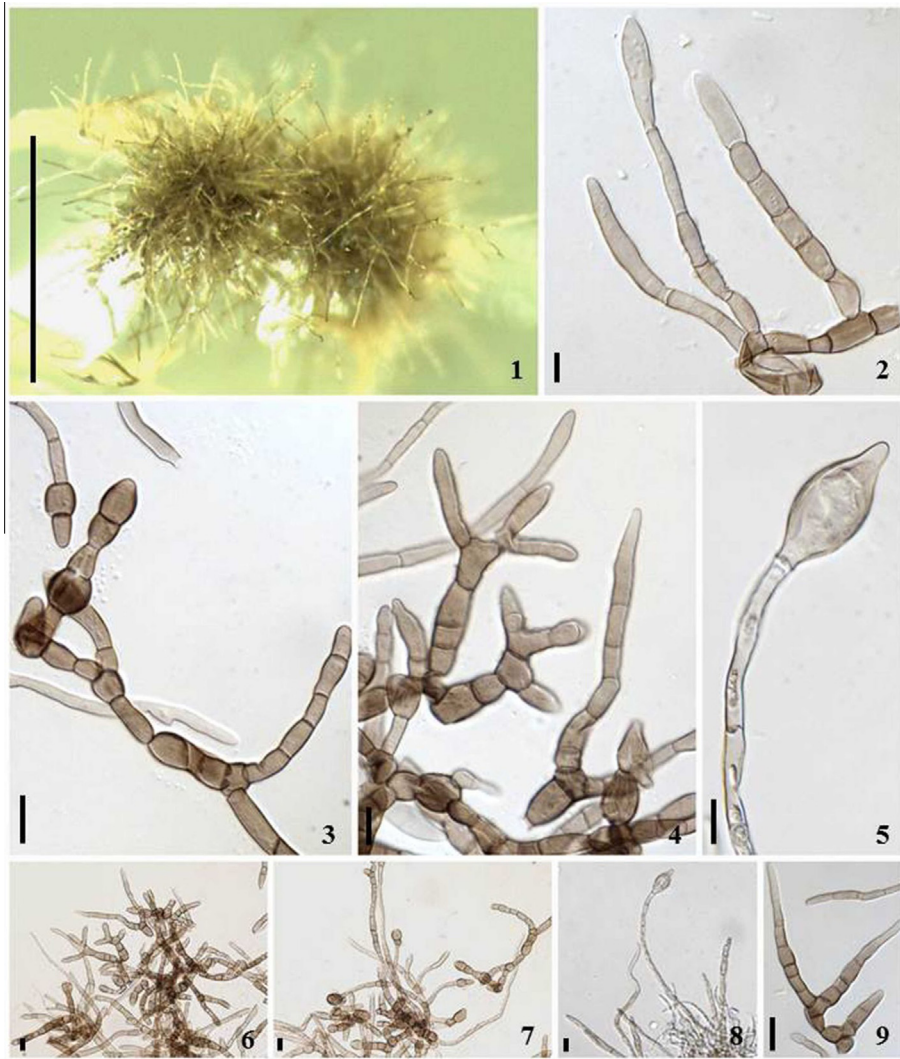
Etymology: — The epithet “*chinensis*” refers to China, the country from which it is described.

Known distribution: — China.

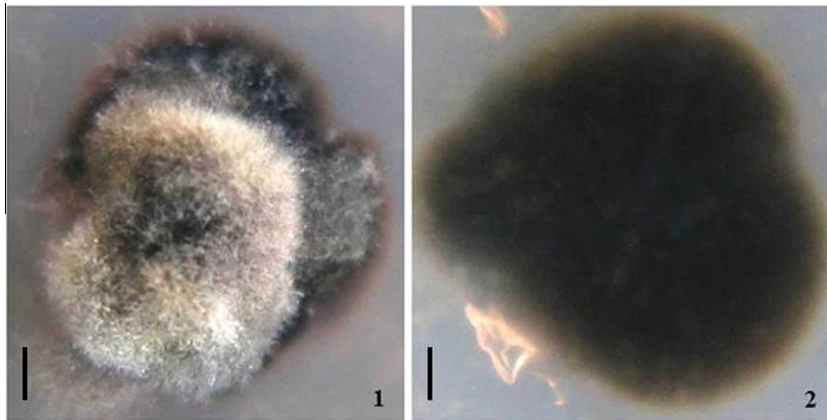
Asexual state: Unknown.

*Ascomata* 40–100  $\mu\text{m}$  diam., solitary, scattered, initially immersed or slightly erumpent, becoming superficial, globose

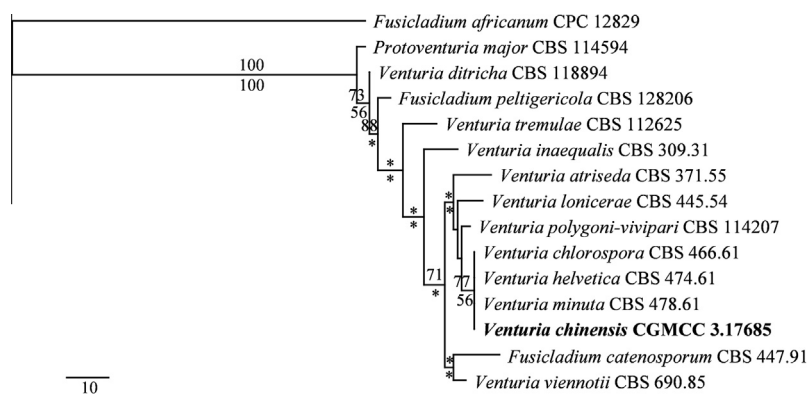
or citriform with a small papilla, ostiolate, wall black, upper one third of the ascomata covered with setae (Fig. 1–3). *Setae* dark brown, 0–1 septate, 23–61  $\times$  5–7  $\mu\text{m}$ , setae wall 1–1.5  $\mu\text{m}$  (Fig. 1: 6, 11). *Peridium* 1-layered, composed of (1–) 2–3 layers of pigmented cells of *textura angularis*, cells 9–5  $\mu\text{m}$  diam., cell wall 0.8–1  $\mu\text{m}$  thick. *Hamathecium* rare, evanescent in mature ascomata (Fig. 1: 9–10). *Asci* 34–59  $\times$  10–13  $\mu\text{m}$  ( $\bar{x}$  = 48.3  $\times$  10.9  $\mu\text{m}$ ,  $n$  = 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)  $\times$  4–5  $\mu\text{m}$  ( $\bar{x}$  = 13.4  $\times$  4.5  $\mu\text{m}$ ,  $n$  = 20), obliquely overlapping to biseriate at the base, ellipsoidal, with broadly rounded ends,



**Fig. 2** *Venturia chinensis* (extype CGMCC 3.17685). (1) Colony growing on MEA after two weeks. (2–9) Chlamydospores and hyphae. Scale bars: (1) = 1 mm; (2), (4), (6), (9) = 10  $\mu$ m; (3), (5), (7–8) = 20  $\mu$ m.



**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.



**Fig. 4** Maximum likelihood tree generated from sequence analysis of the ITS rDNA dataset. Designated outgroup taxon is *Fusicladium africanum*. Maximum likelihood bootstrap support values above 50% are shown under the branches and based on 1000 replicates. Maximum parsimony bootstrap support values above 70% are shown at the nodes.

**Table 2** Polymorphic nucleotides from sequence data of the ITS and 28S rDNA to show the relationship between *Venturia chinensis* and *V. lonicerae* and other related species.

Identity	Strain	ITS									28S rDNA	
		178	217	227	269	283	284	473	569	581	179	180
<i>V. lonicerae</i>	CBS 445.54	C	C	G	C	C	C	T	C	C	T	G
<i>V. chinensis</i>	CGMCC 3.17685	T	T	–	A	T	T	G	T	T	C	T
<i>V. minuta</i>	CBS 478.61	T	T	G	A	T	T	G	T	T	C	T
<i>V. helvetica</i>	CBS 474.61	T	T	G	A	T	T	G	T	T	C	T
<i>V. chlorospora</i>	CBS 466.61	T	T	G	A	T	T	G	T	T	C	T
<i>V. polygoni-vivipari</i>	CBS 114207	T	T	G	A	T	A	G	A	C	T	G

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 ellipsoid 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 Venturiaceae, and one particular venturiaceae 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 xylostium* (Saccardo, 1882). CBS 445.54, an isolate of *V. lonicera* 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 NCBI's 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

EU035458), and *V. minuta* M.E. Barr (strain CBS 478.61, GenBank EU035464) with identity rate as 655/656 (100%). What is amazing is that the ITS and LSU sequences of all these three strains (CBS 466.61, CBS 474.61, CBS 478.61) show no difference at all. All of these three strains are isolated from *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 saprobic, and the ascomata of which is readily produced on the growing leaves of *L. praeiflorens* 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 *Spilocaea*, *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.

#### Acknowledgements

This study is supported by the Fundamental Research Funds for the Central Universities (NO. YX2015-05), National Natural Science Foundation of China (General Program) (31370063), NSFC Projects of International Cooperation and Exchanges (31461143028) and National Science and Technology Foundation Project (2014FY210400). Prof. Dr. Pedro W. Crous (the Centraalbureau voor Schimmelcultures, the Netherlands (CBS)) is also acknowledged for his suggestions concerning the colony characteristics of *Venturia chinensis*.

#### References

- Barr, M.E., 1968. The Venturiaceae in North America. *Can. J. Bot.* 46, 799–864.
- Barr, M.E., 1989. The Venturiaceae in North America: Revisions and additions. *Sydowia* 41, 25–40.
- Cesati, V., De Notaris, G., 1863. Schema di classificazione degli sferiacei italici aschigeri. *Comm. Soc. Crittog. Ital.* 1, 177–240.
- De Notaris, G., 1844. Cenno sulla tribu de'pirenomiceti sferiacei e descrizione di alcuni nuovi generi. *Giorn. Bot. Ital.* 1, 322–335.
- Kirk, P.M., Cannon, P.F., Minter, D.W., Stappers, J.A., 2008. *Dictionary of the Fungi*, 10th ed. CABI Bioscience, UK.
- Korf, R.P., 1956. Nomenclatural Notes. I. Misuse of Neotypes for *Venturia* and *Phaeosphaerella*. *Mycologia* 48, 591–595.
- Nüesch, J., 1960. Beitrag zur Kenntnis der weidenbewohnenden Venturiaceae. *Phytopathol. Z.* 39, 329–360.
- Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systemat. Biol.* 53, 793–808.
- Saccardo, P.A., 1882. *Syll. Fung.* 1, 1–768.
- Sivanesan, A., 1977. *The taxonomy and pathology of Venturia species*. Lubrecht & Cramer Ltd, Vaduz.
- Swofford, D.L., 2002. PAUP\*. Phylogenetic Analysis Using Parsimony (\*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Tai, F.L., 1941. A preliminary survey of Plant pathogens of economical plants in Yunnan province. *Bull. Agric. Res. Inst. Nat. Tsing Hua Univ.* 6, 1–36 (in Chinese).
- Tai, F.L., 1979. *Sylloge Fungorum Sinicorum*. Science Press, Beijing, China, pp. 1–1527 (in Chinese).
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Xu, M.Q., He, P.X., 2008. *Sylloge of Phytopathogens on Woody Plants in China*. Press of Northeast Forestry University, Harbin, 1–1640 (in Chinese).
- Tai, F.L., 2003. *Flora fungorum sinicorum*. Vol. 14. *Cladosporium, Fusicladium, Pyricularia*. Science Press, Beijing, China, pp. 1–324 (in Chinese).
- Zhang, Y., Crous, P., Schoch, C., Bahkali, A., Guo, L., et al, 2011. A molecular, morphological and ecological re-appraisal of Venturiales – a new order of Dothideomycetes. *Fung. Div.* 51, 249–277.
- Zhu, F.M., 1927. Preliminary study phytopathogens in China. *Chin. Agric. Sci. Bull.* 54, 23–43 (in Chinese).