Description of the Sexual Morph of Seimatosporium vitis

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Description of the sexual morph of *Seimatosporium vitis*

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**Abstract** – *Seimatosporium vitis* was recently described based on the collection of its coelomycetous asexual morph on *Vitis vinifera* in Italy. In this study *Seimatosporium vitis* is introduced for the first time from grapevine in Iran. The sexual morph is illustrated and a full description is provided. The connection between two different morphs was proved in culture and based on ITS sequence data.

Iran / Pestalotioid fungi / Phylogeny / *Vitis* sp.

**INTRODUCTION**

*Seimatosporium* Corda is a member of family *Discosiaceae* in *Amphisphaeriales* (Senanayake et al., 2015) and typified by *S. rosae* (Corda, 1833). The genus is defined primarily based on conidial characteristics including size, septation, pigmentation, and presence or absence of appendages (Sutton, 1980; Nag Rag, 1993). Its members are considered so-called “pestalotioid fungi”, and their life styles range from pathogens to saprobes (Tanaka et al., 2011). This genus currently contains numerous species with more than 86 names recorded to date (Index Fungorum; http://www.indexfungorum.org/Names/Names.asp) and detailed descriptions of *Seimatosporium* spp. have been presented by several authors (Shoemaker, 1964; Pirozynski & Shoemaker, 1970; Sutton, 1980; Nag Raj, 1993; Hatakeyama & Harada, 2004; Tanaka et al., 2011; Barber et al., 2011; Norphanphoun et al., 2015; Senanayake et al., 2015; Goonasekara et al., 2016; Perera et al., 2016).

Tanaka et al. (2011) in a phylogenetic study based on LSU and ITS sequence data confirmed *Discostroma* Clem. (1909) as sexual morph of *Seimatosporium* species, while other related genera, including *Sporocadus*, *Sarcostroma*, *Diploceras* and *Vermisporium*, which lack sexual morphs, clustered in *Seimatosporium sensu stricto*, suggesting that they should be placed under the genus *Seimatosporium*. (Tanaka et al., 2011; Barber et al., 2011). Réblová et al., (2016) proposed to use *Seimatosporium* over *Discostroma*, because the former is the oldest name, has the greater number of species, and is more commonly used.

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There are very few publications on pestalotioid fungi from Iran. Arzanlou et al. (2012) have provided a check list for the known pestalotioid fungi from Iran, listing 3 Seimatosporium species, viz. S. fusisporum H.J. Swart & D.A. Griffiths (Aminaee & Ershad, 2008), S. lonicerae (Cooke) Shoemaker (Gräfenhan, 2006) and S. lichenicola (Corda) Shoemaker & Müll (Aghapour et al., 2010). More recently, Crous et al. (2014) described S. pistaciae from Iran. As mentioned by Norphanphoun et al. (2015) this species is similar to S. rosea based on phylogenetic analysis of LSU and ITS sequence data, but morphologically it appears to be distinct from S. rosea by having larger conidia, but sequence data from protein-coding genes may shed more light on the possible synonymy of these two species. More recently Ayoubi & Soleimani (2016) characterized a new species Neopestalotiopsis iranensis and N. mesopotamica on strawberry fruits from Kurdistan Province, Iran.

Through our work on the taxonomy of Diatrypaceae on trees native to the Arasbaran forest in Iran, Seimatosporium vitis was found as a new record for Iran. In this paper it is characterized morphologically and phylogenetically and a detailed description and illustration for both sexual and asexual morphs is provided.

MATERIALS AND METHODS

Morphological characterization. Samples were collected from Arasbaran forest in the East Azerbaijan on dead branch of Vitis sp. Isolation were made from single ascospores (IRAN2454C and IRAN2455C) and conidia (IRAN2427C). For microscopic examinations sections were cut freehand under an Olympus SZH stereo microscope. A Nikon Eclipse 80i light microscope with a Canon digital camera was used to capture micromorphological images. The measurements for each structure are in parentheses. Colony color were determined on potato dextrose agar (PDA) and malt extract agar (MEA) at 24°C using a color chart (Rayner, 1970). Dry specimens and fresh cultures were deposited in the herbarium and culture collection of the Iranian Research Institute of Plant Protection (IRAN, Tehran, Iran).

DNA extraction and sequencing. Isolates were grown in malt extract broth (1.5 % MEB) at room temperature for 14 d. Genomic DNA was extracted with an initial step of grinding the mycelia in liquid nitrogen as described by Liu et al. (2000). Polymerase chain reactions (PCR) were carried out using primer pairs of ITS1/ITS4 (White et al., 1990) to amplify the internal transcribed spacers (ITS) and LROR/LR7 (Rehner & Samuels, 1994; Vilgalys & Hester, 1990) to amplify part of large subunit rDNA (LSU). The PCR reaction mixtures 25 µL contained 2.5 µL 10x high yield PCR buffer (Jena Bioscience, Germany), 1 µL MgCl₂ (25 mM), 0.5 µL dNTPs (10 mM), 1 µL of each primer (10 pmol/µL, Takapouzist Inc.), 1.5 unit Taq polymerase (Jena Bioscience, Germany) and 1 µL genomic DNA (~30 ng/µL). PCR reactions were run on a PC-320 PCR System (ASTEC Co., Japan) machine with an initial denaturation step of 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 35 s at 58°C/57°C (ITS/LSU), 90 s at 72°C, and a final extension of 10 min at 72°C. PCR products were visualized in 1% agarose gel in 1xTBE buffer. Purification and sequencing of PCR products were performed by Macrogen (South Korea).

Phylogenetic analysis. The new sequences obtained in the present study were read and edited with FinchTV v. 1.4.0 (Geospiza Inc.). The sequences were compared with those in the GenBank database using the Megablast algorithm. Additional sequences were selected from Norphanphoun et al. (2015) and Senanayake
et al. (2015). *Pseudopestalotiopsis coccos* and *Pestalotiopsis hollandica* were included as outgroup taxa. The ITS and LSU sequence data (Table 1), were aligned with ClustalX with default settings (Thompson et al., 1997). Phylogenetic information contained in indels (insertions/deletions) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young & Healy, 2003). Phylogenetic analyses were performed with neighbor joining (NJ) and maximum parsimony (MP) as implemented in PAUP* v4.0b10 (Swofford, 2003). The neighbour-joining analysis was performed using Kimura-2-parameter nucleotide substitution model (Kimura, 1980). All characters were unordered and of equal weight. Bootstrap values were obtained from 1000 NJ bootstrap replicates. In MP analysis characters were equally weighted, and gaps were treated as missing data. Trees were inferred with the heuristic search option with TBR branch swapping and 1000 random sequence additions. Branches of zero length were collapsed and all parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis & Bull, 1993). Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI), Homoplasy index (HI). New sequences have been deposited in GenBank (Table 1).

**RESULTS**

**Phylogenetic analyses**

The LSU and ITS sequences for 30 isolates of 27 taxa were combined and aligned. Incomplete portions at the ends of the sequences were excluded from the analyses. The dataset consisted of 2120 characters after alignment, of which 1015 were excluded, 930 were constant, 58 were variable and parsimony uninformative and 117 were parsimony informative. A heuristic search of the 117 parsimony informative characters resulted in nine most parsimonious trees (TL = 290, CI = 0.69, RI = 0.77, HI = 0.31). NJ analysis produced a tree with the same topology as the MP trees. One of the MP trees with bootstrap support values is shown in Fig. 1. Isolates sequenced in this study were placed in a clade representative of *Seimatosporium vitis*.

**Taxonomy**

*Seimatosporium vitis* Y.P. Xiao, Camporesi & K.D. Hyde, *Fungal Diversity* 73: 103. 2015 (Fig. 2)

Lignicolous. **Sexual morph:** Ascomata perithecial, solitary, partly to completely immersed in the host tissue, globose to subglobose, blackish brown to black, 200-400 μm in diameter, 100-250 μm long. Paraphyses filiform, hyaline, unbranched, septate. Asci unitunicate, cylindrical, apex rounded, short stalked, 8-spored, 70-110 × 9-11.5 μm (x = 92.5 × 10.4 μm, n = 20). Ascospores uniseriate, elliptic to fusiform, hyaline, 2-celled, very rarely 3-4 celled, smooth, constricted at the septa when mature, (11.5-)14-20(-21) × 5-7(-7.5) μm (x = 17.3 × 6.5 μm, n = 20). **Asexual morph:** Conidiomata acervular, solitary or gregarious, immersed or erumpent, circular or irregular, dark brown to black, opening by a split in the host
Table 1. Isolates used for the phylogenetic analyses

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<th>Species</th>
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<th>Location</th>
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<td>KM199328</td>
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1 Sequences in bold face were generated during the present study.
2 n.s.: Not sequenced.
Fig. 1. One of the nine most parsimonious trees for *Seimatosporium* species based on a combined dataset of LSU and ITS sequence data. MP/NJ bootstrap support values from 1000 replicates higher than 50% are given at the nodes. Isolates from Iran are in boldface.
Fig. 2. *Seimatosporium vitis* from *Vitis* sp.: a. Habit of ascomata on bark; b. ASCOMA in longitudinal section; c. Habit of conidiomata on bark; d. CONIDIOMA in longitudinal section; e-f. Asci; g. Ascospore; h. Developing conidia attach to conidiogenous cells on natural substrate; i-k. Conidia on natural substrate; l-o. Conidia on MEA; p. Germinating spore on MEA; q. Colony morphology on MEA from above(left) and below(right); r. Colony morphology on PDA. Bars: a = 500 μm. b,d = 100 μm. c = 1 mm. e-l = 10 μm. m-o = 5 μm. p = 10 μm.
Sexual morph of *Seimatosporium vitis*

Septa, (11-)13-16(-17) × 5-6 (\( \bar{x} = 14.7 \times 5.6, n=30 \)) μm on natural substrate (on MEA; 15-25×4.5-5.5 μm, \( \bar{x} = 18.4 \times 4.8 \mu m, n=20 \)), basal cell obconic with a truncate base, with an appendage, hyaline to subhyaline, 2.5-3.7 (\( \bar{x} = 3.1 \)) μm long; 2 median cells subcylindrical to doliform, slightly thick-walled, smooth, brown to dark brown, with septa darker than the rest of the cell, together 7.3-9.8 (\( \bar{x} = 8.6 \)) μm long, (the second cell from the base 3.3-5(\( \bar{x} = 3.7 \)) μm long, the third cell 3.4-5.8 (\( \bar{x} = 4.2 \)) μm long); the apical cell conical with a rounded or acute apex, hyaline to subhyaline or concolorous with the central cells, 3-4 (\( \bar{x} = 3.6 \)) μm long; with or without 1 tubular apical filiform appendage (on MEA and PDA always present), sometimes branched, arising from the apex of the apical cell, 10-22 (\( \bar{x} = 16 \)) μm long, basal appendage present, filiform, tubular, single, sometime branched, eccentric, 7-30 (\( \bar{x} =19.7 \)) μm long.

**Cultural characteristics:** On MEA circular with regular margin, white to primrose (23”b”), cottony, reverse sienna (13i), reaching 20 cm in 7 days at 24°C. On PDA white to primrose (23”b”) from above, white to primrose (23”b”) from below, circular, cottony, with abundant black acervuli, reaching 10 cm in 7 days at 24°C.

**Specimen examined:** IRAN, East Azerbaijan Province, Arasbaran, on dead branches of *Vitis* sp., 11 July 2015, M. Mehrabi, IRAN 16717F, IRAN 2427C, IRAN 2454C, IRAN 2455C.

**DISCUSSION**

Based on phylogenetic analysis of LSU and ITS sequence data our isolates were placed in a strongly supported clade containing the type strain of *Seimatosporium vitis*. *Seimatosporium vitis* was recently described from *Vitis* plants in Italy based solely on the asexual morph, but although sequence data were produced, the species was not included in the phylogenetic analyses based on LSU and ITS sequence data (Senanayake *et al.* 2015). In terms of phylogeny we show here that it is completely distinct from all other *Seimatosporium* species (Fig. 1). *Seimatosporium vitis* resembles *D. ficicola* by having ellipsoid and hyaline ascospores of similar size and with one septum. *D. ficicola* was described based on sexual morph on leaves of *Ficus pleurocarpa* from Australia (Paulus *et al.*, 2006). Since there is no information on the asexual morph and no DNA sequence data for *D. ficicola*, it is not known whether they are conspecific. According to Paulus *et al.* (2006), *S. vitis* is also similar to *D. hyperboreum* in terms of ascospore size. However the latter has wider ascospores (14-17 × 7-8 μm vs. 14-20 × 5-7 μm).

In pestalotioid fungi conidial dimension, number of septa, shape and color and presence and morphology of the appendages are the most important characters to differentiate the isolates at the species level. In the absence of a standard procedure for morphological studies, it is important to realize that morphology is affected by the nature of substrates and environmental factors (Hatakeyama & Harada, 2004). Senanayake *et al.* (2015) have described *S. vitis* based on fungal structures on natural substrate. Dimensions of conidia on natural substrate in our isolates are distinctly smaller (13-16 × 5-6 μm) compared to the conidial dimensions (34-40 × 14-17 μm) measured by Senanayake *et al.* (2015). But, we strongly recommend checking the measures presented by Senanayake *et al.* (2015) because there is no compliance between scales and conidial size. Moreover they have mentioned that conidia bear a single appendage at the basal cell. In our isolates we observed both apical and basal appendages both on conidia from natural substrate and conidia on MEA and
PDA culture media, even though we observed rare conidia from natural substrate without any apical appendage. On MEA and PDA at 24°C, the fungus produced pale brown conidia with mostly 3 (rarely 4-6) septa, whereas on natural substrate conidia only had 3 septa. The conidia produced on artificial media were also longer than those on host tissue (15-25 × 4.5-5.5 µm vs. 13-16 × 5-6 µm). Our results suggest therefore that the number of septa and size of conidia are affected by the substrate (see Figs 2 i-o).

As mentioned by Senanayake et al. (2015) this species is a first *Sematosporium* member reported on *Vitis* and to the best of our knowledge this is the second report of this species on *Vitis* sp. and its first report from Iran. In this survey we have found the isolates as saprophyte on dead branches of *Vitis* sp., but it is important to examine possible pathogenicity of this species in future works.

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