

Contribution to the knowledge of pestalotioid fungi of Iran

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Pestalotioid fungi, generally comprising *Bartalinia*, *Monochaetia*, *Pestalotia*, *Pestalotiopsis*, *Sarcostroma*, *Seimatosporium*, *Truncatella*, are coelomycetous genera with saprobic, endophytic or plant pathogenic life styles residing in the Amphisphaeriaceae (Xylariales). Little is known about the biodiversity of pestalotioid fungi in Iran. We provide a literature-based checklist for the pestalotioid fungi known to occur on different plant species in Iran. Two species, *Bartalinia pondoensis* and *Pestalotiopsis neglecta* are characterised based on morphological and molecular data from bamboo and rock samples, respectively. This is the first record of the genus *Bartalinia* from Iran and first report on the occurrence of *B. pondoensis* on bamboo and first report of *P. neglecta* on rock sample worldwide.

Key words – appendage – coelomycetes – *Pestalotiopsis* – *Seimatosporium*

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Introduction

Pestalotioid fungi are anamorphic forms in the family Amphisphaeriaceae (Xylariales), which are characterized by appendage-bearing conidia (coelomycetes) (Barr 1975, 1990, Kang et al. 1998, 1999) and encompass several genera *viz.* *Bartalinia* Tassi, *Ciliochorella* Sydow, *Discosia* Libert, *Monochaetia* (Saccardo) Allescher, *Monochaetiopsis* Jeewon & K.D. Hyde, *Pestalotia* De Notaris, *Pestalotiopsis* Steyaert, *Sarcostroma* Cooke, *Seimatosporium* Corda, *Seiridium* and *Truncatella* Steyaert (Tanaka et al. 2011). Members of these genera possess diverse life styles ranging from pathogens on plant species

(Espinoza et al. 2008, Tanaka et al. 2011, Arzanlou et al. 2012), endophytes or with both endophytic and pathogenic stages in their life cycle (Wei et al. 2005, 2007, Liu et al. 2006, Tejesvi et al. 2009, Watanabe et al. 2010), saprobes (Liu et al. 2008), or rarely causing diseases in humans (Sutton 1999, De Hoog et al. 2000).

For most pestalotioid genera the teleomorph remains unknown and taxonomy mainly relies on morphological criteria of conidia. The most important features for generic delineation include conidium septation (number of septa), lack or presence / shape and



Figs 1–6 – *Bartalinia pondoensis*. **1** Colony morphology on PDA. **2** Conidia and conidiogenous cells. **3–6** Conidia. – Scale bars (3, 6 = 20 μ m, 4–5 = 10 μ m).

branching pattern of appendages, pigmentation of median cell (Jeewon et al. 2002, 2003, 2004, Kang et al. 1998, 1999, Barber et al. 2011). However, the morphological criteria used for the delineation of pestalotioid fungi are insufficient and overlap among different genera (Lee et al. 2006, Barber et al. 2011). With the aid of DNA sequence data, taxonomy of pestalotioid fungi has undergone drastic revision (Jeewon et al. 2002, 2003, 2004, Kang et al. 1998, 1999, Lee et al. 2006) and now the boundaries of the genera are more clear (Lee et al. 2006, Tanaka et al. 2011).

Little is known on the biodiversity of pestalotioid fungi of Iran. With this paper we provide a check list for the already known pestalotioid fungi from Iran and characterize two pestalotioid species from Iran based on morphological and molecular data, which represent new records for Iran.

Materials and Methods

List of species

The list of pestalotioid fungi was compiled using reports available in the

literature. Most of the quoted works are the result of field research by Iranian mycologists, although a small number of reports have been documented by foreign investigators. The list in Table 1 includes pestalotioid species together with their host species from which they have been collected. The fungal nomenclature and taxonomy follows Index Fungorum (<http://www.indexfungorum.org/names/names.asp>) and MycoBank (<http://www.mycobank.org/>).

Additional fungal isolates were recovered from apparently healthy bamboo stems, and rock sample during 2010. Isolation was made from bamboo stems following routine plant pathology methods. For the rock sample, isolation was made using soil dilution technique on 2% malt extract agar (MEA, Fulka, Hamburg, Germany), supplemented with 2 ml of 20% lactic acid/liter. Single-spore cultures were deposited in the Culture Collection of Tabriz University (CCTU). Colony morphology including colour, shape, and growth rate was determined after 2 weeks of incubation on PDA at 25 °C in darkness.

Squash mounts and handmade sections

Table 1 Pestalotioid fungi known from Iran.

Species	Hosts	References
<i>Monochaetia</i>		
- <i>crataegi</i> (Ellis & Everh.) Sacc. & D. Sacc.	<i>Crataegus</i> sp.	Ershad 1995
- <i>concentrica</i> (Berk. & Broome) Sacc. & D. Sacc.	<i>Cydonia oblonga</i> Mill.	Ershad 1995
- <i>karstenni</i> (Sacc. & Syd.) Sutton	<i>Camellia</i> sp.	Fatehi & Mirhosseini-Moghadam 1993
<i>Pestalotiopsis</i>		
- <i>citri</i> (Mundk. & Khesw.) Ershad & Roohib.	<i>Citrus aurantium</i> L., <i>C. limettoides</i> Tanaka, <i>C. unshiu</i> Marcow	Roohibakhsh & Ershad 1997
- <i>funereooides</i> Steyaert	<i>Camellia sinensis</i> (L.) Kuntze, <i>Cedrus deodora</i> (Rob. ex Lambert) Don, <i>Cupressus arizonica</i> Greene, <i>C. sempervirens</i> L., <i>C. sempervirens</i> var. <i>horizontalis</i> (Mill.) Gord, <i>Picea abies</i> Degen, <i>Prunus</i> sp., <i>Sequoia sempervirens</i> Endl.	Fatehi & Mirhosseini-Moghadam 1993, Borhani & Moussazadeh 2004, Borhani et al. 2006, Ershad 1995, Khabiri 1952, Scharif & Ershad 1966
- <i>guepinii</i> (Desm.) Steyaert	<i>Cyperus rotundus</i> L., <i>Camellia sinensis</i> (L.) Kuntze, <i>Parrotia persica</i> L., <i>Peterocaria fraxinifolia</i> L., <i>Juglans regia</i> L.	Farzaneh et al. 2006, Aghajani et al. 2010 Fatehi & Mirhosseini-Moghadam 1993
- <i>longiseta</i> (Speg.) K. Dai & Ts. Kobay.	<i>Actinidia chinensis</i> Planch., <i>Camellia sinensis</i> (L.) Kuntze	Mousakhah et al. 2008, Fatehi & Mirhosseini-Moghadam 1993 Khodaparast et al. 1993
- <i>longisetula</i> Guba	<i>Fragaria ananassa</i> Duchense	Sharifi et al. 2008
- <i>macrospora</i> (Cesati) Steyaert	<i>Corylus avellana</i> L.	Taherzadeh et al. 1998
- <i>natrassii</i> Steyaert	<i>Camellia sinensis</i> (L.) Kuntze	Khodaparast and Hedjaroude 1994
- <i>neglecta</i> (Thümen) Steyaert	<i>Euonymus japonicas</i> L., Rock	Petrak 1956, Scharif & Ershad 1966, This study
- <i>smilacis</i> (Schweinitz) Sutton	<i>Smilax</i> sp.	Khodaparast & Hedjaroude 1995
- <i>theae</i> (Sawada) Steyaert	<i>Camellia sinensis</i> (L.) Kuntze	Esfandiari 1947, 1948, Scharif & Ershad 1966, Viennot-Bourgin 1976
- <i>uvicola</i> (Speg.) Bissett	<i>Vitis vinifera</i> L.	Ershad 1995
- sp.	<i>Rosa</i> sp.	Mirabolafathy & Ershad 2004
<i>Seimatosporium</i>		
- <i>fusisporum</i> H.J. Swart & D.A. Griffiths	<i>Amygdalus communis</i> L., <i>Malus pumila</i> L., <i>Pistacia vera</i> L., <i>Punica granatum</i> L., <i>Pyrus communis</i> L., <i>Rosa damascene</i> L., <i>Salix</i> sp., <i>Vitis vinifera</i> L.	Ershad 2009, Aminae & Ershad 2008
- <i>loniceriae</i> (Cooke) Shoemaker	<i>Vitis sylvestris</i> Gmel.	Gräfenhan 2006
- <i>lichenicola</i> (Corda) Shoemaker & Müll	<i>Eucalyptus</i> sp.	Aghapour et al. 2010
- sp.	<i>Vitis sylvestris</i> Gmel.	Grafenhan 2006
<i>Truncatella</i>		
- <i>angustata</i> (Pers.) Hughes	<i>Olea europaea</i> L.	Arzanlou et al. 2012

mounted in sterile distilled water or lactic acid were used for microscopic examinations. Dimensions of microscopic structures were calculated based on 30 measurements for conidial morphology (shape, colour, and cell number), size (length and width), and the presence and size of apical and basal appendages where possible. Photographs were

captured on an Olympus digital camera system DP21 (Olympus Corporation, Japan) attached to a BX 41 Olympus microscope.

DNA phylogeny

The isolates were grown on MEA for 10 days in dark and genomic DNA was extracted using the protocol of Moller et al.

(1992). The primers ITS1 and ITS4 (White et al. 1990) were used to amplify part of the nuclear rRNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The reaction mixture and PCR conditions followed Arzanlou & Khodaei (2012a,b) and Arzanlou et al. (2012). The reaction was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with cycling conditions consisting of 5 min at 96 °C for primary denaturation, followed by 40 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 60 s, with a final extension at 72 °C for 7 min. The obtained sequences were compared to the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search.

Results

A list containing four pestalotioid genera comprising 20 species is given in Table 1. The genus *Pestalotiopsis* with 12 species, which have been collected from 22 plant species, represents the highest number of pestalotioid fungi in Iran. For the genera *Monochaetia* and *Seimatosporium* only three species for each and for the genus *Truncatella* only a single species have been reported from Iran.

Bartalinia pondoensis Marincowitz, Gryzenhout & Wingfield, Mycotaxon 111: 312, 2010. Figs 1–6

Colonies on PDA fast growing, attaining a diam of 52 mm after 7 days in dark at room temperature, circular, with entire edge, olivaceous grey, with greyish white margin, covered with dense aerial mycelium. Mycelium immersed and superficial. Conidiomata pycnidial, immersed, globose, subglobose or ellipsoidal, 200–225 × 200–260 µm, ostiolate, unilocular, scattered over the whole colony surface. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, ampulliform to lageniform, hyaline, smooth, formed from the inner cells of the pycnidial wall, 4–7 µm long, Conidia fusiform, straight or slightly bent, predominantly 3–4-euseptate, with no constrictions at septa, 20–25

× 3–4 µm, with basal and apical appendages, the penultimate basal cell longer than the rest, hyaline or slightly pigmented, 2–4 µm long, 2 median cells cylindrical, thick-walled, pigmented, 16–19 µm long (the second cell from the base 8–11 µm long, the third cell from the base 5–9 µm long, apical cell conic, hyaline, 2–3 µm long, with a short tube (0.5–1 µm long) at the tip where branched appendages are attached; apical appendage with 2–3 branches attenuated toward tip, flexuous, (9–) 12–16 (–20) µm long; basal appendage single, filiform, exogenous, 2–6 µm long.

Material examined – IRAN, Bushehr Province, Kangan, Assaluyeh, on stems of *Bambusa* sp. (Poaceae), 10 June 2009. Living culture CCTU 459.

The ITS sequence data showed 100% homology with the sequence data for *Bartalinia pondoensis* in GenBank. The sequence is available in GenBank with the accession number JX854540.

Pestalotiopsis neglecta (Thümen) Steyaert, Transaction of the British Mycological Society 36: 83, 1953. Figs 7–11

Colonies on PDA, fast growing, attaining a diam of 85 mm after 7 days in dark at room temperature, circular, with entire edge, non-zonate. Conidiomata acervular, black, covered with black, slimy conidial masses protruding from the surface. Conidiophores hyaline and branched. Conidiogenous cells annellidic, hyaline and smooth. Conidia fusiform or narrow fusiform, straight or slightly curved with a tapering base, five-celled with three colored median cells, the upper two cells being brown to yellow brown and the lowest cell being olivaceous. Conidia always smooth, up to 27 µm long and 9 µm wide, with a rounded apical end. The apical and basal cells hyaline, cylindrical to conic; median cells dark brown, with the two upper ones sometimes darker. Three to four apical (usually three) appendages up to 27 µm long and one simple basal appendage 7 µm long.

Specimen examined – Iran, Guilan Province, Talesh, rock sample, July 2010. Living culture CCTU 12. The ITS sequence data was deposited in to GenBank with the accession number JX854541.



Figs 7–11 – *Pestalotiopsis neglecta*. **7** Colony morphology on PDA. **8** Conidia and conidiogenous cells. **9–11** Conidia. – Scale bars = 10 μ m.

Discussion

The short check list of pestalotioid fungi provided in this paper highlights the paucity of knowledge on the diversity of pestalotioid fungi in Iran. A search of MycoBank (September 2012; www.mycobank.org) revealed 258 names for *Pestalotiopsis*, 127 names for *Monochaetia*, 78 names for *Seimatosporium* and 23 names for *Truncatella*. The genus *Pestalotiopsis* is the most commonly encountered pestalotioid fungus in Iran with only 12 species occurring on 22 plant species. Many of the pestalotioid species represent important plant pathogens; while, some are well known for their secondary metabolites used in pharmaceutical industry (Aly et al. 2010, Xu et al. 2010). Hence, there is an urgent need to explore biodiversity of pestalotioid genera in Iran.

With this paper we have described *Bartalinia pondoensis* as a new record for Iran from a *Bambusa* sp. based on morphological and molecular data. The morphology of our isolates was in full agreement with the description provided by Marinowitz et al. (2010). We also characterized *Pestalotiopsis*

neglecta from a rock sample in northern Iran. The morphological and molecular data clearly fit with the description of *Pestalotiopsis neglecta*.

We hope that this work will stimulate other researchers to study the diversity of pestalotioid fungi in Iran.

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