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Aspergillus osmophilus sp. nov., and a new teleomorph for *A. proliferans*

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ABSTRACT

A new species of *Aspergillus* and a new teleomorph for *A. proliferans*, both isolated from cereals in Iran, are described using morphological and molecular data. A combined sequence dataset of the ITS region, partial β-tubulin and partial calmodulin genes resolved the relationships of members of section *Aspergillus* largely in concordance with morphological traits of ascospores. *Aspergillus osmophilus* sp. nov. is differentiated from the closest species, *A. xerophilus* by possessing larger ascospores, conidia and associated fruiting bodies. Both species are strongly xerophilic and possess ascospores with lobate-reticulate convex surfaces. The newly discovered teleomorph for *A. proliferans* is characterized by delicately roughened ascospores with a shallow or distinct furrow and finely roughened to irregular equatorial crests.

1. Introduction

Aspergillus section *Aspergillus* (Gams et al. 1985; *A. glaucus* species group according to Raper and Fennell 1965) includes species with uniseriate, blue-green conidial heads and smooth-walled, yellow cleistothecia typically entangled by aerial hyphae encrusted with yellow, orange or orange-red granules. Members of section *Aspergillus* are generally xerophilic, requiring high concentrations of sugar or salt for optimal growth, and are widely distributed in nature. The growth attributes in combination with their ascospores that are able to survive at high temperatures, help explain why most species in this section are food and feed contaminants (Spittstoesser et al. 1989; Gock et al. 2003; Butinar et al. 2005; Samson and Varga 2010). Many species in section *Aspergillus* are also mycotoxin producers that may be harmful to animals and humans (Buchi et al. 1971; Nazar et al. 1987; Vesonder et al. 1988; Eicher and Ludwig 2002; Yildiz and Çoksöyer 2002).

The connection between the teleomorph genus *Eurotium* Link and *Aspergillus* P. Micheli ex Haller was primarily established by De Bary (1854). While Raper and Fennell (1965) maintained the teleomorphic species belonging to *A. glaucus* group in the anamorphic genus *Aspergillus*, Malloch and Cain (1972a, b) placed them under the teleomorphic genus *Eurotium* in the family Trichocomaceae. It was only Blaser (1976) who firmly established the connection of *Aspergillus glaucus* with *Eurotium herbariorum* (Weber ex F.H. Wigg.) Link by neotypification. However, in the last revision of the family

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Trichocomaceae s.l. (Houbraken and Samson 2011) based on sequence data of *RPB1*, *RPB2* (RNA polymerase II genes), *Tsr1* (putative ribosome biogenesis protein) and *Cct8* (putative chaperonin complex component TCP-1), the oldest family name Aspergillaceae was re-instated to include *Aspergillus* and its associated teleomorph genera, and Trichocomaceae was confined to species of *Talaromyces* C.R. Benj., *Thermomyces* Tsikl., *Sagenomella* W. Gams, *Rasamsonia* Houbraken & Frisvad and *Trichocoma* Jungh.

In the present study, in response to recent changes in the International Code of Nomenclature for Algae, Fungi and Plants (ICN) (Norvell 2011; McNeill et al. 2012), the principle "one fungus: one name" is applied and priority is given to the oldest genus name, *Aspergillus* (Hawksworth 2011; Hawksworth et al. 2011). A new species and a new teleomorph from section *Aspergillus* are described here based on morphological and molecular data.

2. Materials and methods

2.1. Strains, media and morphological observations

Fungal strains were isolated from *Hordeum vulgare* L. and *Triticum aestivum* L. during a survey of *Aspergillus* species associated with cereals in the northern and northwestern provinces of Iran, 2006–2011. The strains were recovered by a modified method of Raper and Fennell (1965) based on direct isolation of fungi from plant materials (Asgari and Zare 2011). Single-ascospore cultures were obtained according to O'Gorman et al. (2009).

Colony growth and characteristics were determined on Czapek agar (CZ), Malt Extract Agar (MEA; according to Blakeslee 1915), Malt yeast 40% sucrose agar (M40Y), CZ with 20% sucrose (CZ20S) and 70% sucrose (CZ70S) (Samson et al. 2004). The isolates were inoculated at three points on each medium (Klich 2002) and incubated at 25 °C in the dark. Growth rates were measured in duplicates at seven temperatures (10–40 °C with 5° intervals) after 7 days on M40Y. Colony growth characteristics were recorded on 14-day-old colonies.

For micro-morphological observations, microscopic mounts were made in lactic acid and a drop of alcohol was added to remove air bubbles and excess conidia. The terminology used for description of surface ornamentations of ascospores and conidia are those used by Kozakiewicz (1989). Average and standard deviations were calculated manually and using BioloMICS software (provided by Dr V. Robert, Bio-Aware, S.A., 2003, Version 1.0.2, Belgium). Photographs were taken using an Olympus (DP25) digital camera installed on a BX51 Olympus light microscope.

Scanning electron microscopy (SEM) was performed with a Zeiss ΣIGMA electron microscope. To prepare samples for SEM, mature cleistothecia were transferred to aluminum stubs with the use of double-sided adhesive tape. A small drop of 0.05% Tween 80 was added and the cleistothecia were crushed. The suspensions were air-dried and sputter-coated with gold.

Dried cultures of the types are preserved at the Mycological Herbarium of Iran, Iranian Research Institute of Plant Protection (IRAN…F), and the ex-type cultures are deposited at the Iranian Fungal Culture Collection (IRAN…C) at the same institute. Subcultures of the ex-type strain and others included in the molecular analyses are also preserved at Centraalbureau voor Schimmelcultures (CBS, Utrecht, the Netherlands).

2.2. Molecular studies

2.2.1. DNA extraction and amplification

DNA extraction was performed using a slightly modified method of Cenis (1992). DNAs were amplified for the ITS region of ribosomal DNA, partial β-tubulin and partial calmodulin genes. The ITS region (ITS1-5.8S-ITS2) was amplified using primers ITS1-F and ITS4 as described by White et al. (1990). Partial β-tubulin gene (*benA*) was amplified using primers Bt2a and Bt2b (Glass and Donaldson 1995). Amplification of parts of calmodulin (*CaM*) was performed using primers cmd5 and cmd6 (Hong et al. 2005) or CAL-228F and CAL-737R (Carbone and Kohn 1999).

The PCR products were purified using an AccuPrep® DNA cleaning kit (Cat. no. K-3034-1, Bioneer, Inc., USA). The purified DNA samples were then submitted to a capillary sequencing machine (ABI 3730 Capillary Electrophoresis Genetic Analyzer, University of California, Davis) for sequencing.

2.2.2. Phylogenetic analysis

The programs EditSeq and SeqMan, parts of the DNA*Lasergene (DNAstar, Madison, WI) software package, were used to assemble and edit the sequence files. The alignments were obtained using the Pairwise Alignment option in GeneDoc (Nicholas and Nicholas 1997). Sequences of the ITS region, *benA* and *CaM* were analyzed individually and in combination in MEGA5 (Tamura et al. 2011).

The evolutionary history was inferred using Distance (NJ) and Maximum Parsimony (MP) methods. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). The MP tree was obtained using the Close-Neighbor-Interchange algorithm of Nei and Kumar (2000) with search level 3 (Felsenstein 1985; Nei and Kumar 2000), in which the initial trees were obtained with random addition of sequences (100 replicates). The trees are drawn to scale, with branch lengths calculated using the average pathway method (Nei and Kumar 2000) and based on the number of changes over the whole sequence. All alignment gaps were treated as missing data. The partition homogeneity test (PHT) was applied from PAUP v4.0b10 (Swofford 2003). The sequences generated in this study were deposited at GenBank (Table 1), and the alignment was deposited in TreeBASE (S13852).

3. Results

In an investigation on *Aspergillus* species associated with cereals (wheat and barley) in the northern and northwestern provinces of Iran, a new species and a new teleomorph from section *Aspergillus* were discovered. To clarify the relationships