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Aspergillus salwaensis, a first global record species of section *Circumdati* from South Africa since its original description

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Abstract: During a scientific journey to attend the 2nd conference organized by Pan African Medical Mycology Society (PAMMS) during 6-8 May, 2007 in Cape Town, South Africa, a soil sample collected from Cape Town was brought and mycologically analyzed. An interesting isolate of *Aspergillus* was obtained at 25°C. It was identified using phenotypic features as belonging to section *Circumdati* and was deposited in Assiut University Mycological Centre culture collection as AUMC 4505. Recently, genotypic characterization carried out for many suspected strains deposited at AUMC revealed the high similarity (99%) of this isolate to *Aspergillus salwaensis*. To the best of our knowledge, this is the first global record since its original description by Visagie *et al.* (2014). The ITS gene sequences of the current strain was deposited in the National Centre of Biotechnology Information (NCBI) and given GenBank no. MF737084.

Keywords: *Aspergillus*, Cape Town, phenotypic features, ITS sequencing.

Introduction

Aspergillus section *Circumdati* (Gams *et al.* 1985; *Aspergillus ochraceus* species group according to Raper & Fennell 1965) includes species with rough walled stipes, biseriate conidial heads in shades of yellow to ochre and sclerotia that do not turn black. An early study of the Japanese yellow Aspergilli (Nehira 1949) was followed by the taxonomic treatment of *Aspergillus ochraceus* group by Raper & Fennell (1965) that included nine species (*A. ochraceus, A. fresenii, A. alliaceus, A. sclerotiorum, A. auricomus, A. petrakii, A. melleus, A. elegans* and *A. ostianus*). Christensen & Raper (1970, 1982) described *A. robustus, A. campestris* and *A. bridgeri* as members of this group, while Samson (1979) added four more species: *A. dimorphicus, A. insulicola, A. lanosus* and *A. ochraceoroseus*. A sexually reproducing ochratoxin producing species with a *Neopetromyces* teleomorph, *A. muricatus*, was also found to belong to this section (Varga *et al.* 2000a, Frisvad & Samson 2000). Phylogenetic analysis of sequences of parts of the ribosomal RNA gene cluster indicated that *A. campestris* and *A. lanosus* belong to *Aspergillus* sections *Candidi* and *Flavi*, respectively, while *A. dimorphicus* and *A. sepultus* are members of section *Cremei* (Peterson 1995, Varga *et al.* 2000a). Two teleomorphic species previously assigned to this section, *Petromyces alliaceus* and *P. albertensis* were found to belong to *Aspergillus* section *Flavi* (Varga *et al.* 2000b). Later another new species, *A. persii*, was assigned to this section (Zotti & Corte 2002). Recently, seven new species were described in section *Circumdati*: *A. cretensis, A. flocculosus, A. neobridgeri, A. pseudoelegans, A. roseoglobulosus, A. steynii* and *A. westerdijkiae* (Frisvad *et al.* 2004). More recently, Visagie *et al.* (2014) provide a monographic treatment on the section using a polyphasic approach to species delimitation, including morphological characters, extrolite data and partial calmodulin, β-tubulin and ITS sequences. In their monograph, 27 species are accepted, introducing seven new species: *A. occultus, A. pallidofulvus, A. pulvericola, A. salwaensis, A. sesamicola, A. subramanianii* and *A. westlandensis*. They also correctly applied the name *A. fresenii* (≡ *A. sulphureus* (nom. illeg.)) and provided a guide for the identification of these 27 species. Based on their study, the previously described *A. onikii* and *A. petrakii* were found to be conspecific with *A. ochraceus*, whilst *A. flocculosus* is tentatively synonymized with *A. ochraceopetaliformis*, despite extrolite differences between the two species.

Some species of *Aspergillus* section *Circumdati* are economically important; for example, *A. ochraceus* or *A. sclerotiorum* are used for biochemical transformation of steroids, alkaloids or phenazines (Chen *et al.* 1994), *A. sclerotiorum* and *A. melleus*, are important sources of proteolytic enzymes (Luisetti *et al.* 1991) and several exometabolites (Matsukuma *et al.* 1992). Sclerotia of several species contain anti-insect compounds (Whyte *et al.* 1996, Ooike *et al.* 1997). Species of this section are also known for the production of aspergamides, notoamides, norgeamides, stephacidins, avrainvillamides, among which are several promising anti-cancer compounds (Finefield *et al.* 2011). *Aspergillus westerdijkiae, A. melleus, A. ochraceus, A. steynii* and *A. subramanianii* were commonly reported from a wide range of habitats such as soil, agricultural and stored foods (Kozakiewicz 1989, Frisvad *et al.* 2004, Morello *et al.* 2007, Noonim *et al.* 2008, Gil-Serna *et al.* 2009a,

2011). *Aspergillus ochraceus* and *A. sclerotiorum* have also been identified as human pathogens causing allergic bronchopulmonary aspergillosis (Novey & Wells 1978) and otomycosis (Harima *et al.* 2004). Several species are involved in nail infections, including *A. sclerotiorum*, *A. ochraceus* and *A. melleus* (Zotti *et al.* 2011). *Aspergillus persii* in Italy (Zotti & Corte 2002, Zotti *et al.* 2010) and *A. ochraceopetaliformis* in Germany (Brasch *et al.* 2009) have also been reported to cause onychomycosis.

Several species are able to produce mycotoxins harmful for animals and humans including ochratoxins, penicillic acid, xanthomegnins and viomellein (Lai *et al.* 1970, Ciegler 1972, Hesseltine *et al.* 1972, Durley *et al.* 1975, Varga *et al.* 1996, Frisvad *et al.* 2004, Davolos & Pietrangeli 2014). Based on the extrolite data, 13 species of section *Circumdati* produce large amounts of ochratoxin A, while 7 additional species produce ochratoxin A inconsistently and/or in trace amounts, but the most important species regarding potetial ochratoxin A contamination in agricultural products are *A. ochraceus*, *A. steynii* and *A. westerdijkiae* (Visagie *et al.* 2014).

Various molecular methods have been used for genotypic studies of aspergilli (Rinyu *et al.* 2000, Varga *et al.* 2000a). The internal transcribed spacer (ITS) region, located between the 18S and 28S rRNA genes, is an area of particular importance in discriminating between closely related species or at intraspecific level, because it has areas both of high conservation and high variability. ITS has been used to identify *Aspergillus* species (Henry *et al.* 2000).

An interesting isolate of *Asperillus* was isolated at 25°C from a soil sample obtained from South Africa in 2007. The macro- and micro-morphological characteristics of the isolate proved to be related to section *Circumdati*.

Materials and Methods

Strain examined: the strain examined was isolated on agar plates at 25°C from a soil sample obtained from Cape Town, South Africa during attendance of PAMMS conference in 2007. It was isolated by Zeinab Soliman and Margrete William in the laboratory of Assiut University Mycological Centre (AUMC), Assiut, Egypt. It was identified morphologically as belonging to *Aspergillus* section *Circumdati* and deposited at Assiut Universiy Mycoloical Centre Culure Collection as AUMC 4505.

Morphology: For macro-morphological observations, the strain was grown in the dark on the Czapek´s yeast autolysate agar, CYA (Samson & Pitt 1985) and creatine sucrose agar, CREA (Frisvad 1985). Three replicate-plates of 3-pointed inoculations of both media were incubated at 25ºC for 7 days. Growth rates were recorded as colony diameter in mm. Growth and acid production on CREA were also recorded by visible colour change of medium from purple to yellow. Colony colors were identified according to Kornerup & Wanscher (1978). For micro-morphological observations, microscopic wet mounts were made in lactophenol cotton blue from CYA colonies after 7 days. Microscopic measurements (in µm) of stipes (conidiophores), vesicles, supporting cells (metulae), conidiogenous cells (phialides) and conidia were determined. Surface texture and colour of the stipe and conidia were also recorded.

Molecular identification of the fungal strain

The fungus was grown on CYA and incubated at 25 °C for 7 days. A small amount of fungal biomass was scraped off and suspended in 100 µl of sterile distilled water and boiled at 100 °C for 15 minutes and stored at - 70 °C following the manufacturer's protocol (SolGent Company, Daejeon, South Korea). The sample was directly sent to Korea for DNA extraction and sequencing. Fungal DNA was extracted and isolated using SolGent purification beads at this company. Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using the universal primers ITS1 (5'- TCC GTA GGT GAA CCT GCG G -3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). The PCR reaction mixture was prepared using SolGent EF-Taq. The PCR product was then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) and was sequenced in sense and antisense direction (Refer to Moubasher *et al.* 2016, 2017). Contig was created from the sequence data using the CLCBio Main Workbench program. The sequence was further analysed using BLAST from the National Center of Biotechnology Information (NCBI) website. Nucleotide sequence of the target strain together with those retrieved from the GenBank database were subjected to the Clustal W analysis using MegAlign software version 5.05 (DNASTAR Inc., Madison, Wisconsin, USA) for the phylogenetic analysis (Thompson *et al.* 1994).

Results and Discussion

Brief description

***Aspergillus salwaensis* Visagie, Houbraken, Fotedar, Frisvad & Samson 2014**

Colony diameter after 7 d at 25° C on CYA 52-54 mm, on CREA 35-55 mm.

Colony characters: CYA 25 °C, 7 d: Colony surface floccose to somewhat velutinous; mycelial areas white; sporulation light yellow to yellow (3A5 – 6); sclerotia abundant, white; soluble pigment yellowish

orange; reverse light yellow to greyish yellow (4A4 – B6); acid not produced on CREA 25 °C (Fig. 1).

Microscopic features: Conidial heads radiate; conidiophores biseriate; stipes hyaline to yellow, rough walled, 800 × 12.5 µm; vesicles globose to flattened at apex, 37– 45 µm wide; metulae, 10 x 5 µm covering 100 % of head; phialides ampulliform, 10 – 15 × 5 µm; conidia globose, smooth, 2.5 × 2.5 µm; sclerotia white, 175 – 625 µm (Fig. 2).

Distinguishing characters: *Aspergillus salwaensis* is characterized by fast growth on CYA at 25°C and often has conidiophores with vesicles that are flattened at the apex.

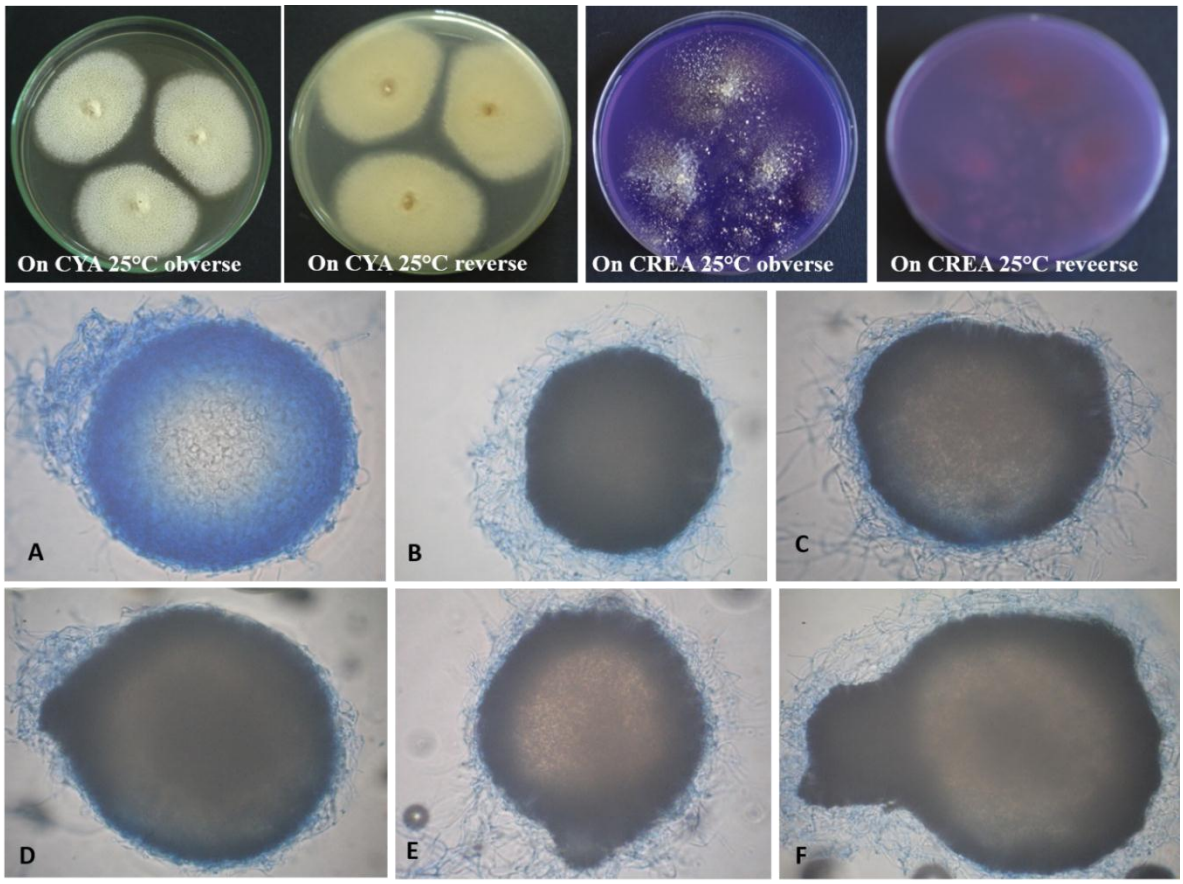

Figure 1: *Aspergillus salwaensis* AUMC 4505 (GenBank number MF737084); growth on Czapek yeast agar (CYA) and creatine sucrose agar, and Sclerotia with different views (A-F).

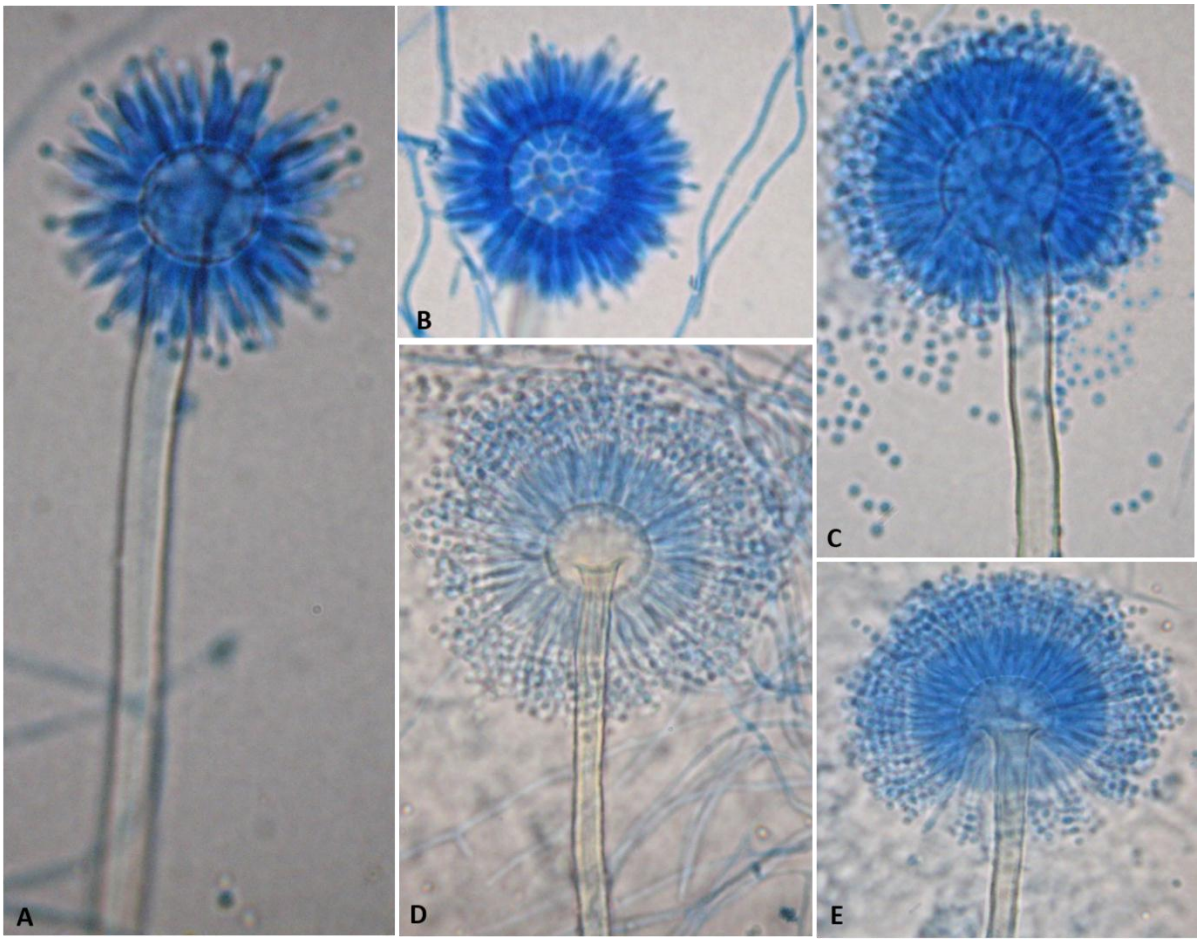

Figure 2: *Aspergillus salwaensis* AUMC 4505**;** conidiophores, radiate conidial heads and conidia (A-E).

Molecular identification

ITS sequence of the present strain (AUMC 4505) showed 99% similarity with the type strain of *A. salwaensis* (GenBank accession numbers NR_135455= CBS 138172^{T}) with only 3 gaps out of 589 nucleotides (0%), however the similarity of ITS sequence with other aspergilli within section *Circumdati* was still high in some species such as *A. bridgeri, A. subramanianii* and *A. sclerotiorum* (98.45%), *A. roseoglobulosus* (98.43%), *A. sesamicola* (98.27%) and *A. persii* (98.24%), with 5-8 gaps (0-1%), but lower in others ranging from 93 to < 98% with gaps ranging from 5-30 (1-4 %) (Table 1, Figure 3).

In accordance with the current results, Visagie *et al.* (2014) noted that *A. salwaensis* has identical ITS sequences with *A. bridgeri, A. subramanianii, A. persii,* and *A. sclerotiorum,* but its BenA and CaM sequences are unique, meaning that an additional identification marker is necessary. BenA and CaM are good alternatives and CaM overall performs well for identification of *Aspergillus* strains (Peterson 2008, Jurjevic *et al.* 2012, Visagie *et al.* 2014). Additionally, in their study only 18 of the 27 species of section Circumdati were identified using ITS, leaving nine species (including the current species) that were not identified (Visagie *et al.* 2014).

Conclusion: This species was first described in Qatar, Salwa beach, from soil collected in 2013, and isolated by J. Houbraken (The type strain was designated as QCC F001/14 = CBS 138172 = DTO 297-B3). To the best of our knowledge, the isolation of *Aspergillus salwaensis* from South Africa (Africa) is being recorded for the first time worldwide since its original description in Qatar (Asia) by Visagie *et al.* (2014) which may indicate its probable wide distribution.

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Table 1: The closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences with the newly recorded species *A. salwaensis* strain AUMC 4505 (accession GenBank number MF737084, length of base pairs = 616).

Closest Genbank match # ITS		Length (pb)	Sequencing similarity (%)	Gaps	Species	Reference
Culture Collection Code	Accession no.					
CBS 138172^T	NR_135455	586	583/589(98.98%)	3/589(0%)	*A. salwaensis*	Visagie *et al.* 2014
NRRL 13000^T	NR_135386	620	573/582(98.45%)	7/582(1%)	*A. bridgeri*	Peterson 2008
NRRL 6161^T	NR_135385	618	572/581(98.45%)	7/581(1%)	*A. subramanianii*	Peterson 2008
NRRL 415^T	NR_131294	618	572/581(98.45%)	7/581(1%)	*A. sclerotiorum*	Peterson 2008
NRRL 13078^T	NR_135387	618	566/582(97.25%)	9/582(1%)	*A. neobridgeri*	Peterson 2008
ATCC MYA-4773^T	NR_111684	607	582/610(95.41%)	15/610(2%)	*A. affinis*	NCBI websie
CBS 137321^T	NR_135451	574	567/590(96.10%)	17/590(2%)	*A. westlandensis*	Visagie *et al.* 2014
CBS 112812^T	NR_077197	600	581/612(94.94%)	15/612(2%)	*A. steynii*	Gil-Serna *et al.* 2009a
NRRL 391^T	NR_135388	607	555/581(95.93%)	18/581(3%)	*A. auricomus*	Peterson 2008
CBS 102.14^T	NR_077196	589	570/603(94.53%)	17/603(2%)	*A. elegans*	Gil-Serna *et al.* 2009a
CBS 137327^T	NR_135453	579	560/591(94.75%)	14/591(2%)	*A. pulvericola*	Visagie *et al.* 2014
CBS 112800^T	NR_137503	507	503/511(98.43%)	5/511(0%)	*A. roseoglobulosus*	Frisvad *et al.* 2004
NRRL 6138^T	NR_135391	625	555/588(94.39%)	14/588(2%)	*A. insulicola*	Peterson 2008
CBS 112795^T	NR_135421	502	501/510(98.24%)	8/510(1%)	*A. persii*	NCBI website
NRRL 4752^T	NR_135390	615	550/583(94.34%)	14/583(2%)	*A. ochraceopetaliformis*	Peterson 2008
ATCC 16887^T	NR_077151	612	571/613(93.15%)	30/613(4%)	*A. ostianus*	Haugland *et al.* 2004
NRRL 35674^T	NR_135392	623	554/591(93.74%)	22/591(3%)	*P. muricatus*	Peterson 2008
CBS 137330^T	NR_135454	594	560/601(93.18%)	19/601(3%)	*A. occultus*	Visagie *et al.* 2014
CBS 137324^T	NR_135452	562	453/461(98.27%)	5/461(1%)	*A. sesamicola*	Visagie *et al.* 2014
NRRL 398^T	NR_077150	613	455/465(97.85%)	5/465(1%)	*A. ochraceus*	Haugland *et al.* 2004
NRRL 4789^T	NR_137468	596	455/466(97.64%)	8/466(1%)	*A. pallidofulvus*	Peterson 2008
NRRL 3174^T	NR_135389	596	453/466(97.21%)	8/466(1%)	*A. westerdijkiae*	Peterson 2008
CBS 117.55^T	NR_103606	591	552/612(90.20%)	22/612(3%)	*A. heteromorphus*	Meijer *et al.* 2011

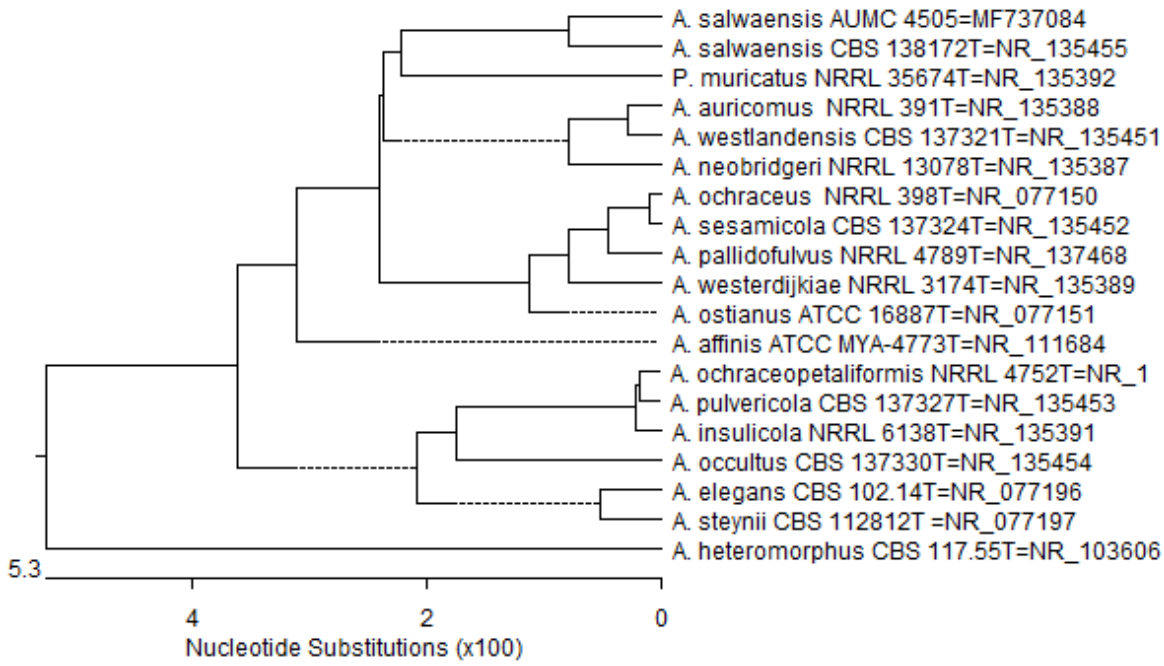

Figure 3: Phylogenetic tree based on DNA sequence data of ITS region of *Aspergillus salwaensis* AUMC 4505, compared with 17 reference strains in Genbank of closely related species belonging to *Aspergillus* Section *Circumdati* and *A. heteromorphus* as an outgroup.

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