Study on coprophilous fungi: new records for Iran mycobiota

Received: 26.04.2016 / Accepted: 23.10.2016

Youbert Ghosta✉: Associate Prof. in Plant Pathology, Department of Plant Protection, Urmia University, Urmia, Iran (y.ghoosta@urmia.ac.ir)

Alireza Poursafar: Researcher, Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Jafar Fathi Qarachal: Researcher, Department of Plant Protection, Urmia University, Urmia, Iran

Abstract

In a study on coprophilous fungi, different samples including cow, sheep and horse dung and mouse feces were collected from different locations in West and East Azarbaijan provinces (NW Iran). Isolation of the fungi was done based on moist chamber culture method. Purification of the isolated fungi was done by single spore culture method. Several fungal taxa were obtained. Identification of the isolates at species level was done based on morphological characteristics and data obtained from internal transcribed spacer (ITS) regions of ribosomal DNA sequences. In this paper, five taxa *viz. Arthrobotrys conoides*, *Botryosporium longibrachiatum*, *Cephaliophora irregularis*, *Oedocephalum glomerulosum*, and *Podospora pauciseta*, all of them belong to *Ascomycota*, are reported and described. All these taxa are new records for Iran mycobiota.

Keywords: Ascomycota, biodiversity, dung fungi, internal transcribed spacer, moist chamber

مطالعه قارچ‌های کوپروفیل: ثبت‌های جدید برای بیوتای قارچی ایران

دریافت: ۱۳۹۵/۰۲/۰۷ / پذیرش: ۱۳۹۵/۰۸/۰۲

یوبرت قوستا✉: دانشیار بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشگاه ارومیه، ارومیه، ایران (y.ghoosta@urmia.ac.ir)

علیرضا پورصفر: کارشناس ارشد بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران

جعفر فتحی قره‌چال: کارشناس ارشد بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

خلاصه

در مطالعه قارچ‌های کوپروفیل (سرگین‌دوست)، نمونه‌های مختلف از پهن گاو، گوسفند، اسب و فضله‌های موش، از مناطق مختلف در استان‌های آذربایجان غربی و شرقی جمع‌آوری شدند. جداسازی قارچها براساس روش کشت در محفظه مرطوب انجام گرفت. خالص‌سازی جدایه‌های قارچها براساس روش تک‌هاگ کردن انجام گرفت. چندین آرایه قارچی مختلف در این مطالعه به دست آمد. عمل شناسایی جدایه‌ها در سطح گونه براساس ویژگی‌های ریخت‌شناختی و اطلاعات به دست آمده از توالی‌یابی ناحیه آی.تی.اس. از دی.ان.ای ریبوزومی هسته‌ای انجام گرفت. در این مقاله، پنج آرایه شامل *Arthrobotrys conoides*, *Botryosporium longibrachiatum*, *Cephaliophora irregularis*, *Oedocephalum glomerulosum* و *Podospora pauciseta* به عنوان آرایه‌های جدید برای بیوتای قارچی ایران گزارش و توصیف می‌شوند.

واژه‌های کلیدی: آسکومیکوتا، بخش رونویسی شونده داخلی، تنوع زیستی، قارچ‌های پهن‌دوست، محفظه مرطوب

Introduction

Coprophilous fungi encompass a diverse ecological group of fungi which are adapted to live in animal feces and play an important role in the decomposition and recycling of nutrients, the carbon flow and ecosystem energetic (Angel & Wicklow 1975, Richardson 2001, Melo *et al*. 2016). These fungi have developed certain features to adapt themselves to the dung environment (Misra *et al*. 2014), are able to withstand and in most cases, dependent on passes through the digestive tract of the animals to facilitate spore germination (Richardson 2003, 2008). Representatives of nearly all taxonomic groups of fungi could be found in animal dung (Crous *et al*. 2009) and it seems that they are specialized to some extent on particular types of dung (Krug *et al*. 2004). Dung contains a large quantity of readily available nutrients such as carbohydrates, high nitrogen content, vitamins and growth factors (Webster 1970). Some physicochemical factors such as temperature and moisture content, pH, stage of decay and type of animal have profound influence on dung mycobiota. Many coprophilous micro-organisms produce important chemicals that may inhibit competing and invading organisms or stimulate fungal growth and are considered to have promising biological activity (Singh & Webster 1973, Wicklow 1988, Gloer 1997, Lehr *et al*. 2006). Based on available literature, most described dung fungi were attained from dung of various animals in temperate regions of the world. Diversity, distribution and different capabilities of coprophilous fungi have been reported in several studies (Nyberg & Persson 2002, Bell 2005, Piasai & Manoch 2009, Mungai *et al.* 2011, Prydiuk 2011). In a study on coprophilous fungi from 32 herbivore dung samples in Iceland, 81 species of coprophilous fungi were identified (Richardson 2004). In a latter addition to the coprophilous fungi of Iceland, 19 new species were recorded (Richardson 2011). In a study on the fungi from dung of four domesticated wild herbivores, 52 species were identified (Gupta 2010). Study on coprophilous ascomycetes from northern Thailand showed high diversity of these fungi and species from 11 genera in five orders were identified (Mungai *et al*. 2011). According to Misra *et al*. (2014), 52 genera of Zygomycota (s.l.), 169 of Ascomycota, and 33 of *Basidiomycota* have been associated with at least one type of dung as a substrate. Research on coprophilous fungi in Iran is still rare and the biodiversity of dung fungi was remained unexplored. The main objective of this study is to isolate and identify fungi found on various dung types in different locations of West and East Azarbaijan provinces (NW Iran).

Materials and Methods

- Dung samples

Samples of cow, horse and sheep dung and mouse feces which were appeared to be relatively recent were collected from different parts of West and East Azarbaijan provinces (NW Iran). Each sample was placed in a clean paper bag, labeled and transferred to the laboratory. The samples were air dried and stored at room temperature in sealed paper bags for further examinations.

- Fungal isolation

Fungi isolation was done based on moist chamber method. Each dung sample was placed in a sterile Petri plate (10 cm diameter) containing a moistened sterile filter paper. Care was taken to ensure that the samples were not too wet. Petri plates were incubated at room temperature (20–25º C) and under ambient light for two months. Plates were examined periodically with a dissecting microscope for fungal growth and sporulation or fruit body formation. Where needed, a small amount of sterile distilled water was added to moisten the filter paper. The growing fungi were directly isolated using a fine sterile needle to pick up the fungal spores from the dung samples and were transferred onto the PDA (potato dextrose agar, Merck, Germany) medium. Pure cultures of the growing fungi were obtained by single spore method (Crous *et al.* 2009). Purified fungi were transferred into slants of PCA (potato carrot agar) and PDA culture media containing a piece of filter paper for storage. In cases that fruit bodies were formed, these fungal structures were removed individually and

mounted in pure Lactic acid or sterile distilled water for examination at higher magnifications. Representative isolates were deposited in the Fungal Reference Collection of the Ministry of Jihad-e-Agriculture ("IRAN") located at Iranian Research Institute of Plant Protection, Tehran, and the Fungal Culture Collection of the Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran (UU).

- Identification of fungi

Morphological characteristics were assessed for 7- and 14-day-old cultures on PCA, PDA and MEA based on identification manuals (Haard 1968, Mirza & Cain 1969, Stalpers 1974, Richardson & Watling 1997, Bell 2005, Doveri 2008). Colony colors were described based on Rayners mycological color chart (Rayner 1970). Fifty measurements of each characteristic taxonomic structure were made. Identification was made based on macro- and micro-morphological characteristics and sequences obtained from ITS region of the nuclear ribosomal DNA.

- DNA extraction, PCR amplification and sequencing

Isolates were grown on PDB (potato dextrose broth) at 22–25° C for seven days. DNA was extracted from harvested mycelia based on Zhong and Steffenson method (Zhong & Steffenson 2001). The internal transcribed spacer (ITS) region of nuclear rDNA was amplified using primers ITS5 and ITS4 (White *et al*. 1990). PCR conditions were as follows: an initial 4 min heating step at 95° C; followed by 35 cycles of denaturation at 95° C for 30s, annealing at 56° C for 30s and extension at 72° C for 60s; and a final extension at 72° C for 6 min. The PCR products were checked on 1 % agarose gel stained with ethidium bromide. Amplified products were sequenced by Macrogen Inc. (Seoul, Korea) using the same primer set as for PCR amplification.

- Phylogenetic analysis

Sequence files were evaluated by Chromas 2.4 (Technelysium Pty Ltd., South Brisbane, Australia). New sequences were deposited in GenBank and accession numbers are provided (Table 1). Blast searches for retrieving sequences with high similarity to our sequences were performed using the BLASTn algorithm from GenBank (http://www.ncbi.nlm.nih.gov/) and incorporated to the analysis (Table 1). The multiple sequence alignment was done with the program MAFFT V. 7.304 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & Standley 2013), and was adjusted manually. For phylogenetic analysis, distance matrix was calculated using Kimura two parameter method (Kimura 1980), and analyzed with the Neighbor Joining method (Saitou & Nei 1987) in MEGA 6 (Tamura *et al*. 2013). The reliability of inferred phylogenetic tree was evaluated by bootstrap values with 1000 replicates (Felsenstein 1985). *Ermothecium gossypii* (GenBank accession number: AY046216) was used as outgroup.

Results

A total of 60 dung samples were processed. A great diversity of fungal forms was seen on the surfaces of the samples and was isolated as pure cultures. In this paper, five taxa *viz. Arthrobotrys conoides* Drechsler, *Botryosporium longibrachiatum* (Oudem.) Maire, *Cephaliophora irregularis* Thaxt., *Oedocephalum glomerulosum* (Bull.) Sacc., and *Podospora pauciseta* (Ces.) Traverso are introduced and described alphabetically as new taxa to Iran mycobiota. Morphological characteristics of all identified species were in full agreement with the descriptions provided in different literature.

- Molecular studies

PCR amplifications of ITS-rDNA in representative isolates produced a fragment with about 550–600 bp length. Blast search of the obtained sequences in GenBank nucleotide databases showed high similarity with representatives of identified species. In two cases, *Cephaliophora irregularis* and *Oedocephalum glomerulosum*, there was not any sequence of the ITS region of rDNA in GenBank database and the obtained sequences in this study are the first deposited ones. The sequence alignment comprised 24 taxa (Table 1) including our isolates and bootstrap values greater than 50% are shown on the upper branches (Fig. 1).

Table 1. Species used for phylogenetic analyses and their GenBank accession numbers

Taxon	Isolate/Strain	GenBank accession No.
Arthrobotrys conoides	**IRAN 2606C**	KX683419
A. conoides	NCIM 1245	KU218466
A. conoides	670	AY773455
A. elegans	CBS 319.94	KT215212
A. eudermata	CBS 377.97	KT215215
A. musiformis	ATCC 96675	EF445993
A. oligospora	ATCC 96709	EF445989
A. vermicola	CBS 513.66	GU178821
Botryosporium longibrachiatum	**IRAN 2605C**	KX683418
B. longibrachiatum	JBARES2013	KF372591
B. longibrachiatum	-	JX666334
Cephaliophora irregularis	**IRAN 2607C**	KX683420
C. tropica	KW 216	KX364741
Eremothecium gossypii	-	AY046216
Oedocephalum glomerulosum	**IRAN 2608C**	KX683422
O. adhaerens	-	FJ695215
O. nayoroense	NBRC 32546	LC146751
Podospora pauciseta	**IRAN 2604C**	KX683421
P. pauciseta *	-	EF197072
P. austroamericana	CBS 724.68	GQ922535
P. bullata	CBS 1155.76	DQ166960
P. glutinans	CBS 134.83	AY615207
P. minicauda	CBS 227.87	GQ922539
P. setosa	CBS 1183.91	GU391421

* This sequence appears in GenBank as *Podospora anserina*, but it has renamed as *P. pauciseta* in Index Fungorum and Mycobank by Kirk (2015). Newly obtained sequences for this study are in bold face.

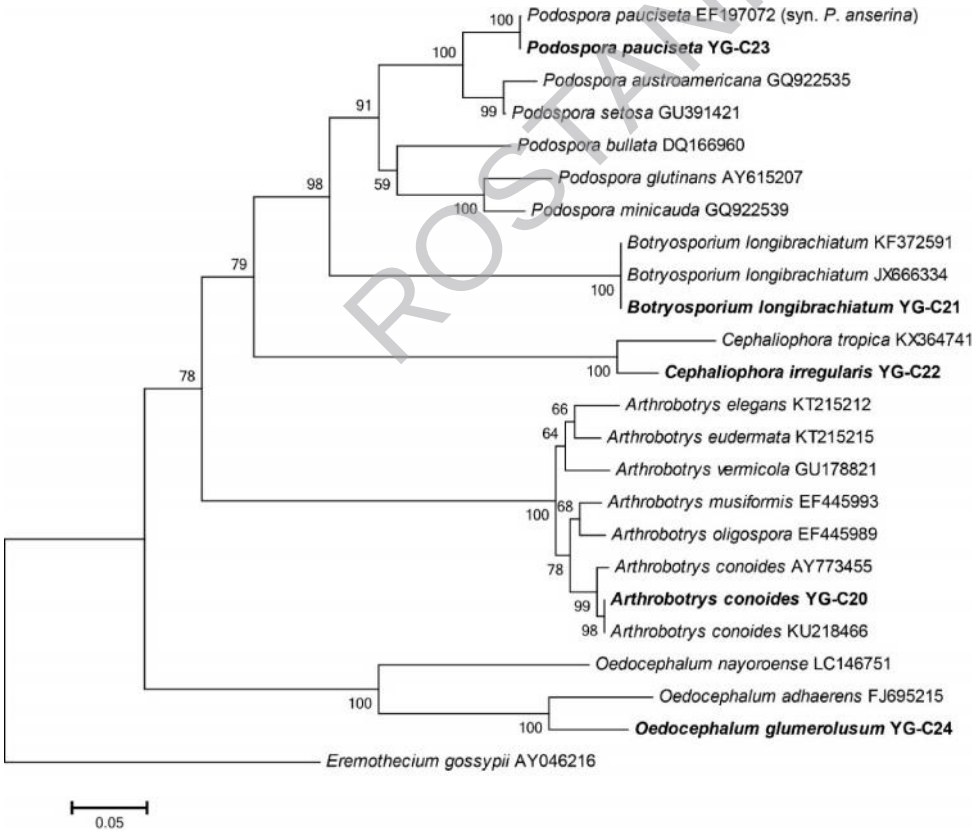

Fig. 1. Phylogenetic tree inferred from ITS-rDNA region of 24 sequences data set. Topology was inferred based on Neighbor-Joining analysis. The numbers on each branch represent the percentage of NJ bootstrap supports. *Ermothecium gossypii* (AY046216) was used as outgroup.

1. *Arthrobotrys conoides* Drechsler, Mycologia 29(4): 476 (1937)

Colonies white to pale pink on MEA, without aerial hyphae, fast-growing, covering a 9- cm diameter Petri plate in about 5 days. Mycelia composed of hyaline, septate and branched hyphae. Conidiophores hyaline, unbranched, erect, proliferating repeatedly, bearing 7–12 or more conidia in a tight capitate head, up to 400 µm in length. Conidia borne singly on denticles, hyaline, uniseptate, slightly constricted at the septum, with a broad distal end cell and an apiculate proximal cell, obconical, 20–35 × 8–13 µm (Fig. 2A-D).

Materials examined: Iran: East Azarbaijan, Malekan, Gharachal village, on sheep dung, 14 June 2015, J. Fathi, YG-C20 (IRAN 2606C); East Azarbaijan, Marand, on cow dung, 7 July 2014, Y. Ghosta, UU-86; West Azarbaijan, Miandoab, on sheep dung, 11 Aug. 2014, Y. Ghosta, UU-38.

Notes: This species is rather common on dung and has been reported from leaf duff, decaying wood and leave in soils especially nematode infested soils which preys nematodes using adhesive nets (Haard 1968, Hay 1995, Domsch *et al*. 2007). It is morphologically similar to *Arthrobotrys superba* and *A. oligospora*, but it can be distinguished from the former by unequal partitioning and larger size of conidia and from the latter by the shape of conidia (slender obconical versus plump and obovoid in *A. oligospora*) as well as ITS rDNA sequences (Cook & Godfrey 1964, Haard 1968, Falbo *et al*. 2013, Yu *et al*. 2014). Three taxa *viz. A. cladodes* Drechsler var. *macrolides* Drechsler, *A. oligospora* Fresen var. *oligospora*, and *Arthrobotrys* sp. were previously reported in Iran from compost, soil and wood, respectively (Ershad 2009) and this is a new species to Iran mycobiota.

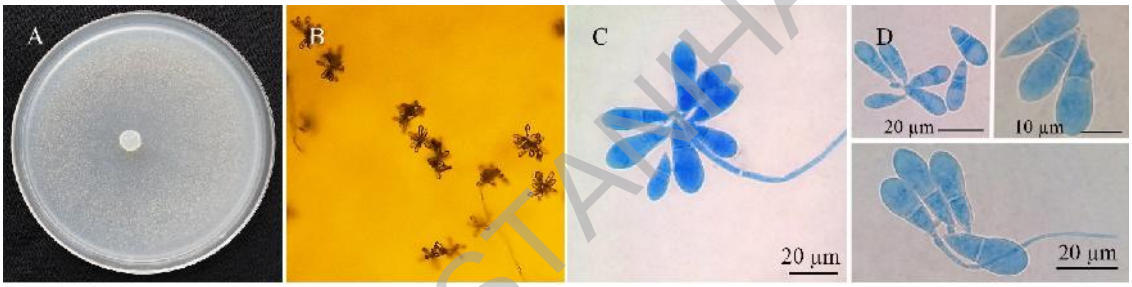

Fig. 2. *Arthrobotrys conoides*: A. Colony on MEA, B. Sporulation pattern on MEA, C. Apical part of conidiophore bearing several conidia, D. Conidia.

2. *Botryosporium longibrachiatum* (Oudem.) Maire, Annls mycol. 1(4): 341 (1903)

Colonies white, fluffy on PDA, fast-growing, covering a 9- cm diameter Petri plate in about 5 days. Mycelium composed of hyaline, septate and branched hyphae 3–6 µm diameters. Conidiophores are composed of an erect cylindrical main stalk, 6–15 µm wide, with lateral fertile branches each terminating in a swollen biconic vesicle, 10–11 µm diameters from which conidiogenous ampullae arise. Branch plus vesicle 60–120 long and branch 4–5 µm wide. Each vesicle bears 3–5 ampullae. Ampullae 2–5 lobed and attached at a very narrow point to the vesicle with a basal septum. Conidia are synchronous blastoconidia which are formed at the tips of small denticles. Conidia ellipsoid, hyaline, smooth, 6–8 × 2.5–3.5 µm (Fig. 3A-C).

Materials examined: Iran: East Azarbaijan, Maragheh, Malekan village, on sheep dung, 28 June 2015, J. Fathi, YG-C21 (IRAN 2605C); East Azarbaijan, Malekan, Gharachal village, on sheep dung, 14 June 2015, J. Fathi, YG-A165; West Azarbaijan, Salmas, Habashi village, on sheep dung, 8 Sept. 2013, Y. Ghosta, UU-113.

Notes: This species is similar to *Botryosporium pulchrum*, but in *B. longibrachiatum*, the conidiophore is unbranched, whereas dichotomously-branched conidiophores are characteristics of *B. pulchrum*. It is reported from dead stems and leaves of different plant species and as barn mould of burley tobacco, black stem on sweet basil and statice (Anderson & Welacky 1983, Tribe & Weber 2001, Park *et al*. 2013, Choi *et al*. 2014).

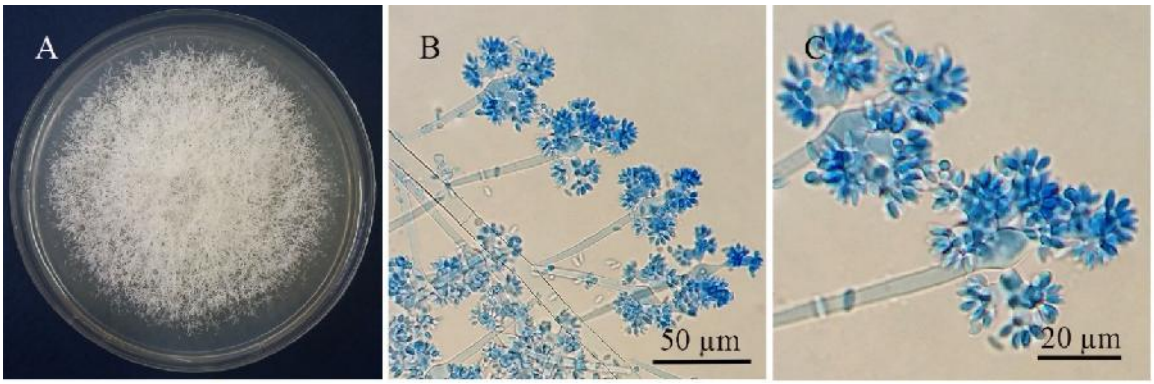

Fig. 3. *Botryosporium longibrachiatum*: A. Colony on PDA, B. Conidiophore main stalk with lateral fertile branches, C. Fertile part of conidiophore with biconic vesicles, conidiogenous ampullae and conidia.

3. *Cephaliophora irregularis* Thaxt., Bot. Gaz. 35: 158 (1903)

Colonies salmon to pale brown on MEA, fast-growing, cover a 9- cm diameter Petri plate in about four days. Sporulation is abundant. Conidiophores straight or flexuous, unbranched, clavate, with terminal swollen conidiogenous cell (ampullae), (11–)15–27 × (12–)15–40(–45) µm, colourless to pale brown. Conidia solitary, formed synchronously over the surface of swollen polyblastic conidiogenous cell (botryoblastosporous), broadly clavate, smooth, hyaline to pale brown, 2-celled with extremely large apical and small basal cell, 25–37 × 16–22(–26) µm and with a protuberant hilum (Fig. 4A-D). Materials examined: Iran: West Azarbaijan, Urmia, Nazloo village, on mouse feces, 16 June 2015, J. Fathi, YG-C22 (IRAN 2607C); West Azarbaijan, Urmia, Vazirabad village, on cow dung, 19 July 2013, Y. Ghosta, UU-106; West Azarbaijan, Khoy, Firouragh, on cow dung, 24 June 2014, Y. Ghosta, UU-18.

Notes: The genus *Cephaliophora* has been originally erected to include two species: *C. tropica* Thaxter, the type species and *C. irregularis* Thaxter, both were found on animal dung (Thaxter 1903). *Cephaliophora irregularis* was subsequently reported from soil, *Jatropha* seeds, cocoa beans, wood and other substrata (Ellis 1971, Srivastava *et al*. 2011, Kaushal & Singh 2012). Six species are recognized in this genus (Oct. 2016; www.indexfungorum.org). Another species, *C. tropica* has been reported previously from soil in Iran (Pordel *et al*. 2015) and this is the second species of that genus reported here for Iran mycobiota.

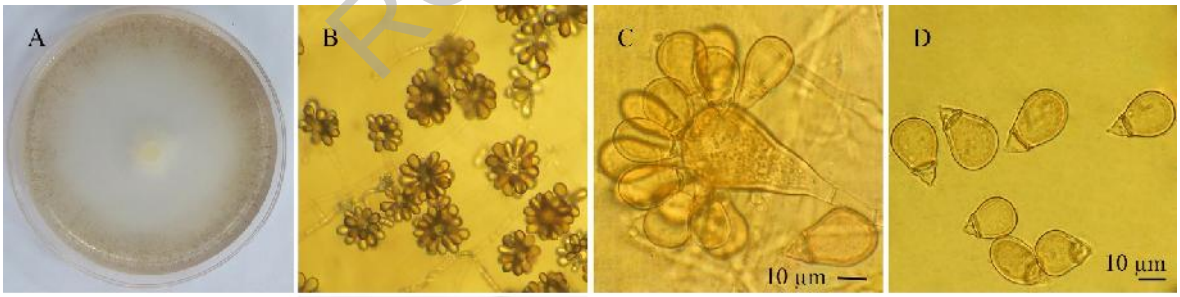

Fig. 4. *Cephaliophora irregularis*: A. Colony on MEA, B. Sporulation pattern, C. Terminal part of conidiophore showing ampullae with conidia, D. Conidia.

4. *Oedocephalum glomerulosum* (Bull.) Sacc., Syll. fung. (Abellini) 4: 47 (1886)

Colonies white on MEA and PDA, yellow to pale orange on PCA, moderately fast-growing, covering a 9- cm diameter Petri plate in about seven days, without spore production in PDA and MEA media in which, the fungus grows as submerged hyaline hyphae with chains of intercalary ellipsoidal thin-walled cells. Abundant sporulation was obtained on PCA medium under continuous fluorescent light at 23–25º C. Conidiophores erect, solitary, unbranched, septate, hyaline, 10 × 300 µm, with an apical globose to subglobose conidiogenous vesicle delimited by a septum, 20–30 × 18–35 µm, covered with minute denticles over the entire surface.

Conidiogenesis holoblastic synchronous. Conidia hyaline, aseptate, smooth, obovoid to broadly ellipsoid, 10–16 × 19–26 µm (Fig. 5A-H).

Materials examined: Iran: East Azarbaijan, Malekan, Gharachal village, on sheep dung, 16 July 2015, J. Fathi, YG-C24 (IRAN 2608C); West Azarbaijan, Mahabad, on sheep dung, 7 Nov. 2014, Y. Ghosta, UC-14; West Azarbaijan, Urmia, Aliabad village, on cow dung, 18 July 2015, A. Poursafar, YG-M15.

Notes: In a revision of the genus *Oedocephalum*, the only imperfect states of ascomycetes were retained in this genus and similar states of basidiomycetes were excluded (Stalpers 1974). Three sections were distinguished in this genus and *O. glomerulosum* was placed in the section *Glomerulosa*. Perfect states which were only known from *O. glomerulosum*, was named as *Iodophanus testaceus* (Moug.) Korf. *Oedocephalum glomerulosum* is closely related to *O. macrosporum* Penz. & Sacc., and *O. nicotianae* Oudem., but could be distinguished in the dimensions of conidia and conidiophores (Stalpers 1974). This species was reported from dung of various animals, compost and decaying vegetable matter and old paper (Cinto *et al*. 2007, Watling & Richardson 2010, Seifert *et al*. 2011).

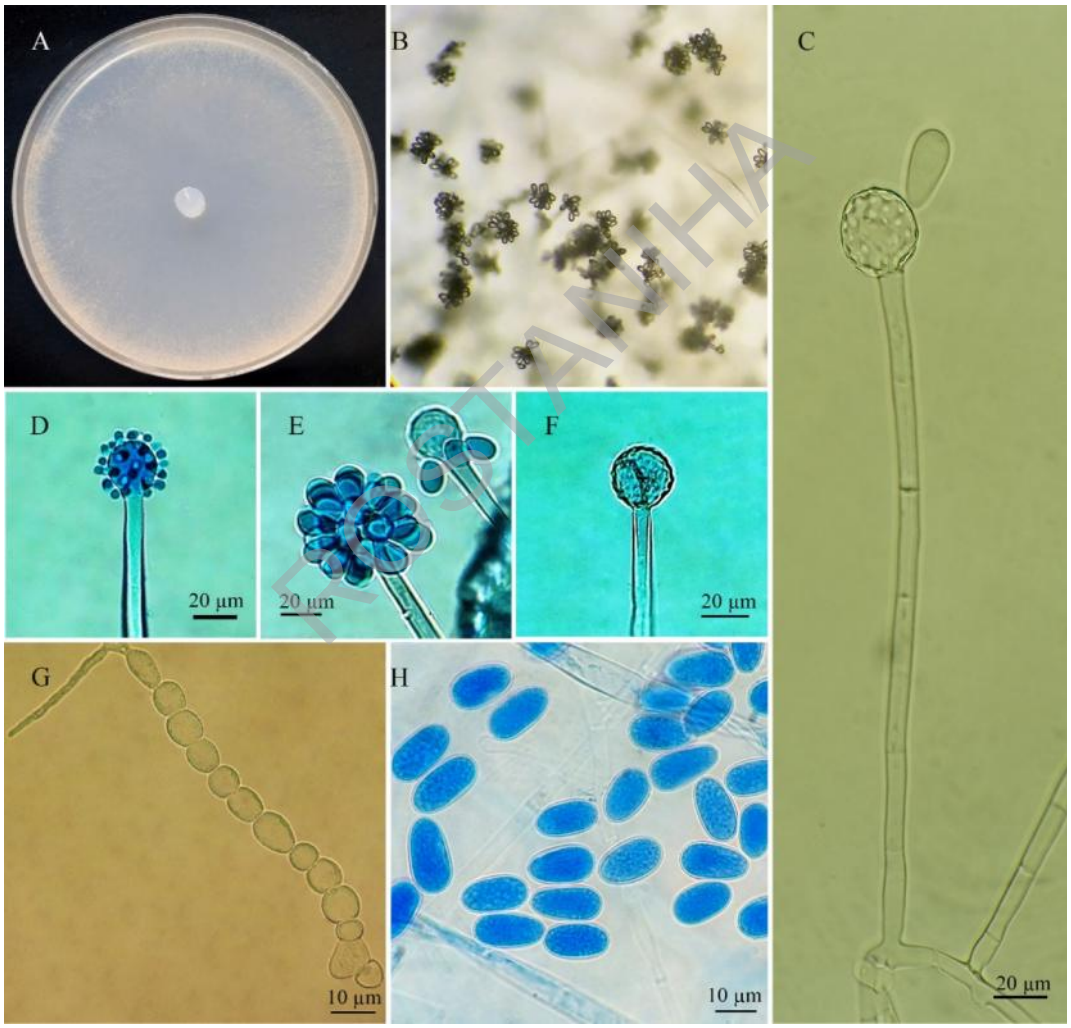

Fig. 5. *Oedocephalum glomerulosum*: A-B. Colony and sporulation pattern on PCA, C. Conidiophore with apical vesicle, D. Initials of conidia, E. Mature conidia on vesicle, F. Warted vesicle, G. Submerged hyphae in MEA and PDA media, H. Conidia.

5. *Podospora pauciseta* (Ces.) Traverso, Fl. ital. crypt., Fungi 2(2): 431 (1907)

Colonies on PCA pink to pale yellow at center and dark brown at margins, reaches 7- cm diameter after seven days under fluorescent 8:16 h light/dark cycle at 23–25º C. Perithecia solitary or clustered, obpyriform, mostly superficial, sometimes partially or completely immersed, 465–675 × 325–450 µm, peridium membraneous, semi-transparent, olivaceous brown, black near the neck, opaque, with tuft of stiff hairs on one side. Paraphyses interspersed with and longer than the asci, Asci clavate, unitunicate, 4-spored, in basal fascicle, sometimes with a medial constriction, with narrowed truncated apex and a short stipe, 110–170 × 18–22 µm. Ascospores hyaline when immature, then dark brown to black with age, ellipsoid, umbonate at the apex and truncate at the base, smooth, 32–35(–40) × 17–20 µm, pedicel cylindrical, hyaline (Fig. 6A-F).

Materials examined: Iran: West Azarbaijan, Urmia, Takaloo Village, on cow dung, 29 June 2015, A. Poursafar, YG-C23 (IRAN 2604C); West Azarbaijan, Urmia, Nazloo village, on cow dung, 12 May 2015, A. Poursafar, YG-C45; West Azarbaijan, Urmia, Vazirabad village, on cow dung, 10 Sept. 2014, Y. Ghosta, YG-C63.

Notes: *Podospora pauciseta* is a common and widespread species of *Podospora* and is also known as *P. anserina* (Rabenh.) Niessl (Mirza & Cain 1969, Chang & Wang 2005, Doveri 2008, Mungai *et al*. 2012). It is similar to *P. comata* Milovtzova, and *P. australis* (Speg.) Niessl, but could be distinguished based on spore size, shape and characteristics of the caudae (Mirza & Cain 1969, Bell 2005, Melo *et al*. 2015).

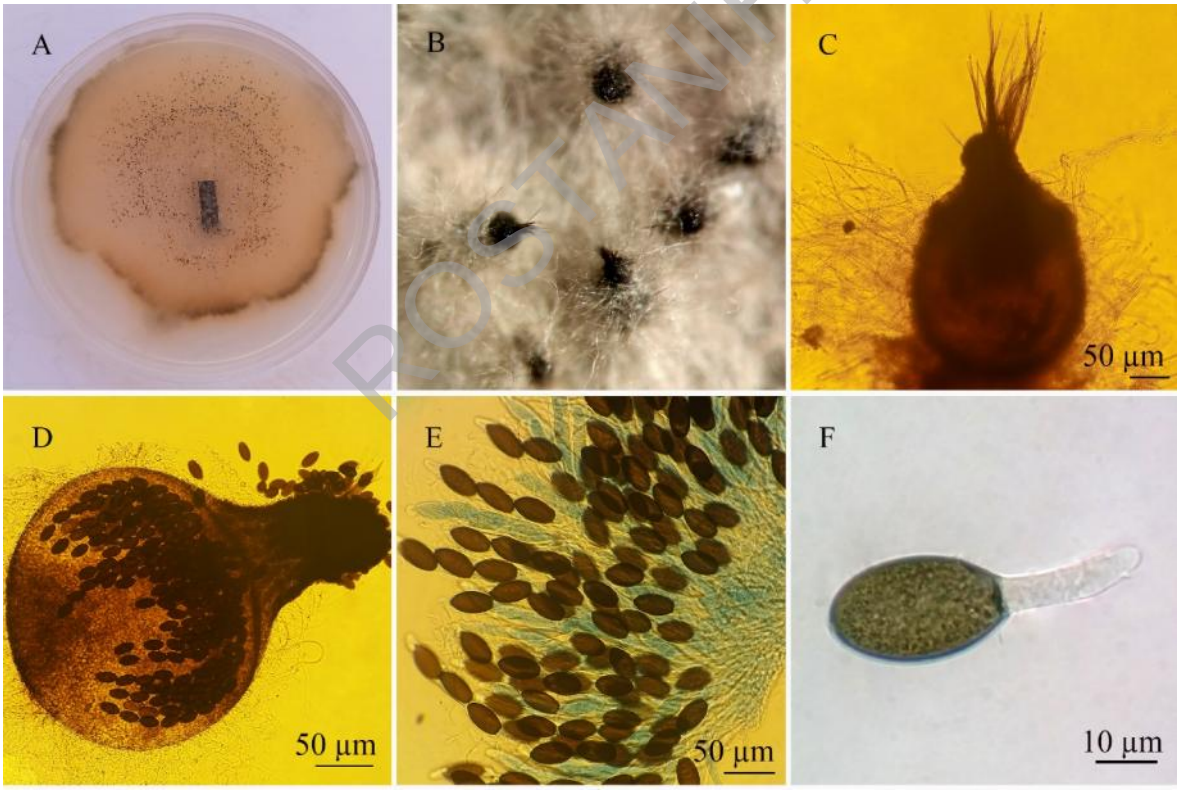

Fig. 6. *Podospora pauciseta*: A. Colony on PCA, B. Perithecia on agar surface, C. Perithecium with a tuft of stiff hairs on one side, D. Perithecium with transparent wall and darker neck, E. Asci and ascospores, F. Ascospore.

Discussion

Fungi as an underdescribed, poorly-documented phylum of Eukaryotes have immense ecological and economic impacts. So far, only 135000 species of fungi have been described and their actual diversity has been explored (Hibbett *et al*. 2016). Among different ecological groups of the fungi, coprophilous fungi, those that germinate, grow and sporulate on dung, are of great diversity and importance. They are very important in ecosystems by breaking down the substrates and recycling of micronutrients, antagonistic interactions with other fungi and micro-organisms and production of diffusible antibiotics that restrict the growth of fast-growing fungi. Estimation about species richness and endemism for coprophilous fungi is really difficult (Krug *et al*. 2004). Some taxa are cosmopolitan, but others appear to be rare and restricted to specific areas. Species frequency and richness of coprophilous fungi are correlated with geographic origin, collecting date and intensity, type of the dung and the expertise of collector (Richardson 2001, Krug *et al*. 2004, Piasai & Manoch 2009). In a study, 112 taxa of coprophilous *Sphaeriales* were recorded from Ontario, Canada (Cain 1934). The occurrence of coprophilous fungi on samples of four different dung types was studied (Caretta *et al*. 1998) and representatives of 40 fungal genera and 59 species were recorded. Based on their subjective impression, occurrence of some fungi were dependent on their distribution on plants and herbages eaten by animals. In Oman, Elshafie (2005) reported 45 species of coprophilous fungi belonging to 25 genera among which 21 species were new to the Arabian Peninsula. In a study on compost samples collected from sheep farms in Mazandran province (N Iran), *Arthrobotrys cladodes* var. *macrolides* was isolated from 3% of the samples (Eslami *et al*. 2005) and the isolates had nematophagous activities. During the studies on coprophilous ascomycetes in Thailand, dung samples from both domesticated and wild animals were assessed (Jeamjitt *et al*. 2007). In total, 12 genera and 15 species were identified. *Chaetomium globosum*, *Sordaria fimicola* and *Sporomiella minima* were the most common species. Seven species were new records for Thailand. Also, nine species were found only from one type of dung and the moist chamber method yielded the highest number of coprophilous ascomycetes. In a study, the distribution and occurrence of coprophilous ascomycetes on dung was investigated (Mungai *et al*. 2011). Species from eleven genera in five orders were identified. Cattle dung had the highest and chicken dung had the least fungal species. During a survey of coprophilous *Agaricales* in Brazil, 12 species of dung inhabiting mushrooms were identified (Melo *et al*. 2016). Eight species were new records for Brazil. Ascomycetous coprophilous fungi from feces of bird species were studied (Torbati *et al*. 2016) and from seven identified species, two were new records for Iran mycobiota. In all above-mentioned studies, the dung mycobiota was greatly different.

In the present study, the biodiversity of the fungi isolated from different dung and from different locations were significantly different from those of the literatures. In this paper, five taxa which are new records to Iran mycobiota from dung substrate are reported. The results of this study, therefore, points out that, dung is a favorable substrate to explore the fungal biodiversity in Iran requiring more work in different locations and with different climates to achieve this.

References

Anderson, T.R. & Welacky, T.W. 1983. Bran mold of burley tobacco caused by *Botryosporium longibrachiatum*. Plant Disease 67: 1158–1159.

Angel, K. & Wicklow, D.T. 1975. Relationships between coprophilous fungi and fecal substrates in a Colorado grassland. Mycologia 67: 63–74.

Bell, A. 2005. An Illustrated Guide to the Coprophilous Ascomycetes of Australia. CBS Biodiversity Series 3. 173 pp.

Cain, R.F. 1934. Studies of coprophilous *Sphaeriales* of Ontario. University of Toronto Studies, Biological Series 38: 1–126.

Caretta, G., Piontelli, E., Savino, E. & Bulgheroni, A. 1998. Some coprophilous fungi from Kenya. Mycopathologia 142: 125–134.

Chang, J.-H & Wang, Y.-Z. 2005. A new species of *Podospora* from Taiwan. Botanical Bulletin of Academia Sinica 46: 169–173.

Choi, I.Y., Kim, B.S., Park, J.H., Cho, S.E. & Shin, H.D. 2014. First report of black stem caused by *Botryosporium longibrachiatum* on statice in Korea. Plant Disease 98: 1431.

Cooke, R.C. & Godfrey, B.E.S. 1964. A key of nematode-destroying fungi. Transactions of the British Mycological Society 47: 61–74.

Crous, P.W., Verkley, G.J.M., Groenewald, J.Z. & Samson, R.A. 2009. Fungal biodiversity. CBS laboratory manual series, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. 269 pp.

Domsch, K.H., Gams, W. & Anderson, T.-H. 2007. Compendium of Soil Fungi. 2nd ed. IHW-Verlag, Eching. 672 pp.

Doveri, F. 2008. A bibliography of *Podospora* and *Schizothecium*, a key to the species, and a description of *Podospora dasypogon* newly recorded from Italy. Pagine di Micologia 29: 61–159.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute. Kew, UK. 608 pp.

Elshafie, A.E. 2005. Coprophilous mycobiota of Oman. Mycotaxon 93: 355–357.

Ershad, D. 2009. Fungi of Iran. Iranian Research Institute of Plant Protection, Tehran, Iran. 531 pp.

Eslami, A., Ranjbar-Bahadori, S., Zare, R. & Razzaghi-Abyaneh, M. 2005. The predatory capability of *Arthrobotrys cladodes* var. *macrolides* in the control of *Haemonchus contortus* infective larvae. Veterinary Parasitology 130: 263–266.

Falbo, M.K., Soccol, V.T., Sandini, I.E., Vicente, V.A., Robl, D. & Soccol, C.R. 2013. Isolation and characterization of the nematophagous fungus *Arthrobotrys conoides*. Parasitology Research 112: 177–185.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Cinto, I.E., Dokmetzian, D.A. & Ranalli, M.E. 2007. *Iodophanus carneus* and *I. testaceus* (Ascomycota-*Pezizales*): independent taxonomic identity or synonymy? A study of their morphology and isozymes. Boletín de la Sociedad Argentina de Botánica 42(3–4): 181–187.

Gloer, J.B. 1997. Applications of fungal ecology in the search for new bioactive natural products. Pp. 249–268. *In*: The Mycota. Vol. IV. Environmental and Microbial Relationships (Wicklow, D.T. & Söderström, B.E., eds). Springer-Verlag, Berlin, Germany.

Gupta, A.K. 2010. Studies on fungi from the dung of some herbivores inmates of Prince of Wales Zoological Garden, Lucknow. Ph.D. thesis, University of Lucknow, Lucknow, India.

Haard, K. 1968. Taxonomic studies on the genus *Arthrobotrys* Corda. Mycologia 60: 1140–1159.

Hay, F.S. 1995. Unusual germination of spores of *Arthrobotrys conoides* and *A. cladodes*. Mycological Research 99: 981–982.

Hibbett, D., Abarenkov, K., Kõljalg, U., Öpik, M., Chai, B., Cole, J.R., Wang, Q., Crous, P.W., Robert, V.A.R.G., Helgason, T., Herr, J.R., Kirk, P., Lueschow, S., O'Donnell, K., Nilsson, H., Oono, R., Schoch, C.L., Smiyth, C., Walker, D.M., Porras-Alfaro, A., Taylor, J.W. & Geiser, D.M. 2016. Sequence-based classification and identification of Fungi. Mycologia 108: 1049–1068.

Jeamjitt, O., Manoch, L., Visarathanonth, N., Chamswarng, C., Watling, R., Sharples, G.P. & Kijjoa, A. 2007. Coprophilous ascomycetes in Thailand. Mycotaxon 100: 115–136.

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7:

improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.

Kaushal, A. & Singh, R. 2012. *Cephaliophora*: reported first time from surrounding area of water bodies of Bhopal (M.P.), India. Science Secure Journal of Biotechnology 1: 36–38.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Krug, J.C., Benny, G.L. & Keller, H.W. 2004. Coprophilous fungi. pp. 467–499. *In*: Biodiversity of fungi. Inventory and monitoring methods (Mueller, G.M., Bills, G.F. & Foster, M.S., eds). Elsevier, Academic Press, Amsterdam, Boston. 777 pp.

Lehr, N-A., Meffert, A., Antelo, L., Sterner, O., Anke, H. & Weber, R.W.S. 2006. Antiamoebins, myrocin B and the basis of antifungal antibiosis in the coprophilous fungus *Stilbella erythrocephala* (syn. *S. fimetaria*). FEMS in Microbiology and Ecology 55: 105–112.

Melo, R.F.R., Chikowski, R.D.S., Miller, A.N. & Mala, L.C. 2016. Coprophilous *Agaricales* (*Agaricomycetes*, *Basidiomycota*) from Brazil. Phytotaxa 266: 001–014.

Melo, R.F.R., Miller, A.N. & Maia, L.C. 2015. The genus *Podospora* (*Lasiosphaeriaceae*, *Sordariales*) in Brazil. Mycosphere 6(2): 201–215.

Mirza, J.H. & Cain, R.F. 1969. Revision of the genus *Podospora*. Canadian Journal of Botany 49: 1999–2048.

Misra, J.K., Pandey, S., Gupta, A.K. & Deshmukh, S.K. 2014. Coprophilous fungi-a review and selected bibliography. pp. 170–200. *In*: Fungi from different substrates (Misra, J.K., Tewari, J.P., Deshmukh, S.K. & Vágvölgyi, C., eds). CRC Press, Boca Raton, FL.

Mungai, P.G., Chukeatirote, E., Njogu, J.G. & Hyde, K.D. 2012. Studies of coprophilous ascomycetes in Kenya. *Podospora* species from wildlife dung. Mycosphere 3: 978–995.

Mungai P.G., Hyde, K.D., Cai, L., Njogu, J. & Chukeatirote, K. 2011. Coprophilous ascomycetes of northern Thailand. Current Research in Environmental and Applied Mycology 1: 135–159.

Nyberg, A. & Persson, I.L. 2002. Habitat differences of coprophilous fungi on moose dung. Mycological Research 106: 1360–1366.

Park, J.H., Park, M.J., Han, K.S. & Shin, H.D. 2013. First report of black stem caused by *Botryosporium longibrachiatum* on sweet basil in Korea. Plant Disease 97: 425.

Piasai, O. & Manoch, L. 2009. Coprophilous ascomycetes from Phu Luang wildlife sanctuary and Khao Yai national park in Thailand. Kasetsart Journal (Natural Science) 43: 34–40.

Prydiuk, M.P. 2011. New records of dung inhabiting *Coprinus* species in Ukraine II. Section *Coprinus*. Czech Mycology 63: 13–32.

Pordel, A., Ahmadpour, A., Behnia, M. & Javan-Nikkhah, M. 2015. New records of Hyphomycetes fungi from Iran. Mycologia Iranica 2: 69–74.

Rayner, R.W. 1970. A Mycological Colour Chart. Kew: Commonwealth Mycological Institute.

Richardson, M.J. 2001. Diversity and occurrence of coprophilous fungi. Mycological Research 105: 387–402.

Richardson, M.J. 2003. Coprophilous fungi. Field Mycology 4: 41–43.

Richardson, M.J. 2004. Coprophilous fungi from Iceland. Acta Botanica Islandica 14: 77–102.

Richardson, M.J. 2008. Records of coprophilous fungi from the Lesser Antilles and Puerto Rico. Caribbean Journal of Science 44: 206–214.

Richardson, M.J. & Watling, R. 1997. Keys to Fungi on Dung. 2nd ed. British Mycological Society, Stourbridge, United Kingdom. 68 pp.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Seifert, K., Morgan-Jones, G., Gams, W. & Kendrick, B. 2011. The Genera of Hyphomycetes. CBS Biodiversity Series no. 9: 1–997. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.

Singh, N. & Webster, J. 1973. Antagonism between *Stilbella erythrocephala* and other coprophilous fungi. Transactions of the British Mycological Society 61: 487–495.

Srivastava, S., Sinha, A. & Srivastava, C.P. 2011. Screening of seed-borne mycoflora of *Jatropha curcas* L. Research Journal of Seed Science 4: 94–105.

Stalpers, J.A. 1974. Revision of the genus *Oedocephalum* (Fungi Imperfecti). Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C 77: 383–401.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Thaxter, R. 1903. New or peculiar North American Hyphomycetes III. Botanical Gazette (Chicago) 35: 153–159.

Torbati, M., Arzanlou, M. & Bakhshi, M. 2016. Morphological and molecular identification of ascomycetous coprophilous fungi occurring on feces of some bird species. Current Research in Environmental and Applied Mycology 6: 210–217.

Tribe, H.T. & Weber, R.W.S. 2001. Dead basil stem - a possible ecological niche for the hoar-frost fungus *Botrysporium longibrachiatum*. Mycologist 15: 158–161.

Watling, R. & Richardson, M.J. 2010. Coprophilous fungi of the Falkland Islands. Edinburgh Journal of Botany 67: 399–423.

Webster, J. 1970. Coprophilous fungi: Presidential address. Transactions of the British Mycological Society 54: 161–180.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322. *In*: PCR protocols: a guide to methods and applications (Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J., eds). Academic Press, New York.

Wicklow, D.T. 1988. Metabolites in the coevolution of fungal chemical defense systems. Pp. 174–201. *In*: Coevolution of Fungi with Plants and Animals (Pirozynski, K.A. & Hawksworth, D., eds). Academic Press, New York.

Yu, Z.-F., Mo, M.-H., Zhang, Y. & Zhang, K.-Q. 2014. Taxonomy of nematode trapping fungi from *Orbiliaceae*, Ascomycota. Pp. 41–210. *In*: Nematode Trapping Fungi (Zhang, K.-Q. & Hyde, K.D., eds). Springer Science+Business Media.

Zhong, S. & Steffenson, B.J. 2001. Virulence and molecular diversity in *Cochliobolus sativus*. Phytopathology 91: 469–476.