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Short paper

A new and interesting record of genus *Gymnoascus* Baran (Ascomycota) from Iran

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Abstract: A specimen of an ascomycetous fungus collected during a field trip was identified as *Gymnoascus* Baran. Soil samples collected from 0-20 cm depth were studied for isolation of fungi using a soil dilution plate method. Based on the morphological and molecular characters the specimens were identified as *Gymnoascus reesii*. Genomic DNA was extracted and a nuclear rDNA region, containing the internal transcribed spacers 1, 2 and 5.8S gene of rDNA (ITS) were amplified and PCR products were sequenced. Amplicon was purified, sequenced and submitted to the GenBank (Acc. No. JQ387570-71). The resulting sequence (600 bp) was submitted to a BLAST search to find most similar sequences in GenBank. The search results showed highest similarity of Iranian isolates to other isolates of *G. reesii* from GenBank. In the light of literature on ascomycetous fungi, *Gymnoascus* Baran is a new record for Iran mycoflora. The specimens are kept in fungal collection of the Department of Plant Protection, Razi University, Kermanshah, Iran.

Keywords: *Gymnoascus*, Internal transcribed spacers of rDNA (ITS), Ascomycetes, Iran mycobiota

Introduction

The family *Gymnoascaceae* has not been yet an object of taxonomic investigation in Iran. The *Gymnoascaceae* are characterised by usually brightly coloured ascomata, sometimes without a well differentiated peridium, or with a gymnothecial peridium of interwoven hyphae forming a complete or incomplete, appendiculate or non-appendiculate reticulum, inside which an interascal tissue is absent, and the pseudoprototunicate, usually irregularly disposed,asci release ascospores by deliquescence. Anamorphs are absent or simply arthroconidial (Cannon and Kirk 2007). Unlike *Onygenaceae*, members of *Gymnoascaceae* are not keratinolytic (Scott and Untereiner 2004), but some are keratinophilic, some are mildly cellulolytic, others possibly chitinolytic (Lumley and Currah 1995).

Materials and Methods

Isolation

During 2011-2012, the presence and frequency of ascomycetous fungi was studied in various fields in Fars province. In each location, samples were collected from 0-20 cm depth and passed through 2mm, 40 and 60 mesh sieves. Using the soil plate method, 10 g of soil samples were placed in 90 ml of 0.1% water-agar containing 100 ppm NPX, mixed and serially diluted to 10^{-2} to 10^{-5} then 1 ml of each solution was flooded on potato dextrose agar (PDA) and malt extract agar

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(MEA) by an L shape rod. These media were amended with rose bengal (45 µg/ml) and chloramphenicol (25 µg/ml). Plates were incubated at 25-27 ºC for 3 to 5 days to allow the fungi to grow. Plates were observed daily for the appearance of fungal colonies. Individual isolates were sub-cultured in new petri dishes containing MEA and incubated at 25 ºC in the dark. The macroscopic features of the colonies and details of microscopic structures were observed using light microscope.

DNA extraction

For DNA extraction, isolates were grown on PDA for 10 to 15 days at 25 °C in the dark. Fungal mycelium from pure cultures were scraped and mechanically disrupted by grinding to a fine powder in nitrogen using a mortar and pestle. Total genomic DNA was extracted using a genomic DNA purification Kit (Fermentas, UK) according to the manufacturer´s instruction. The resulting DNA extracts were quantified by a NanoDrop spectrophotometer (NanoDrop Technologies, USA). DNA samples were kept at -20 ºC until they were used for PCR amplification.

DNA amplification

The ITS regions of nuclear rDNA were amplified with the universal ITS1 (5´-TCC GTA GGT GAA CCT GCG G-3´) and ITS4 (5´-TCC TCC GCT TAT TGA TAT G-3´) primers (White *et al.,* 1990) on a CORBETT RESEARCH model CG1-96 thermocycler. For ITS amplifications the samples were prepared as follows: a reaction tube contained, 12.5 µl of a diluted DNA sample (1: 10 or 1:100 dilutions of the original extract), 2.5 µl of 10 × PCR buffer, 20 pmoles of each primer, 1.25 nmoles of each deoxynucleotide, 1.5 mM of $MgCl_2$ and 0.5 U of *Taq* polymerase (CinnaGen, Iran) in a reaction volume of 25 µl. The thermocycle were carried out by the following program: an initial denaturation step at 94 ºC for 3 min; then 30 cycles, consisting of denaturation (30 s at 94 ºC), annealing (30 s at 50 ºC), and extension (2 min at 72 ºC); and a final extension step of 10 min was allowed at 72 ºC before cooling or removing the tubes. Amplified fragments were visualized under UV light after electrophoresis on 1% agarose gels stained with ethidium bromide and run in 1 × TBE buffer. Controls with no DNA were included in every set of amplification to check the DNA contamination in reagents and reaction buffers.

Sequencing of the amplified ITS regions

The amplification products of all specimens were purified using GeneJET PCR purification Kit (Fermentas, UK) to remove excess primers and nucleotides. Sequencing reactions were performed on purified PCR products in forward and reverse orientation using the primers used for amplification (ITS1 or ITS4). The sequence was determined with an ABI prism 377 DNA sequencer according to the manufacturer´s instruction. All DNA sequences of the ITS regions deposited at the National Center for Biotechnology Information GenBank (NCBI, http://www.ncbi.nlm.nih.gov/Entrez) (Bethesda, MD, USA).

Results and Discussion

One hundred fungal isolates were recovered from soil samples. The most common fungi isolated from most soil samples were *Aspergillus* and *Penicillium* species. Other fungi, were less frequent. All species of *Aspergillus* and *Penicillium*, plus other soil fungi (*Cladosporium, Rhizopus, Fusarium, Rhizoctonia*) were easily isolated on PDA and MEA media. *G. reesii* was identified on PDA by the production of orange colonies 3 days after inoculation.

Gymnoascus reesii

Colonies on PDA attained a diameter of 5 cm within seven days. Peridial hyphae smooth, thick-walled, red, septate, anastomosed and intertwined, bearing short, simple or branched, straight or curved appendages. Cleistothecia (gymnothecia) spherical, orange-red, 170-450 µm in diam were observed after two days. Asci hyaline, subglobose, 8-12 µm, 8-spored, wall evanescent. Ascospores smooth, light-yellow, globose and 2.5-4 µm in diam (Fig. 1).

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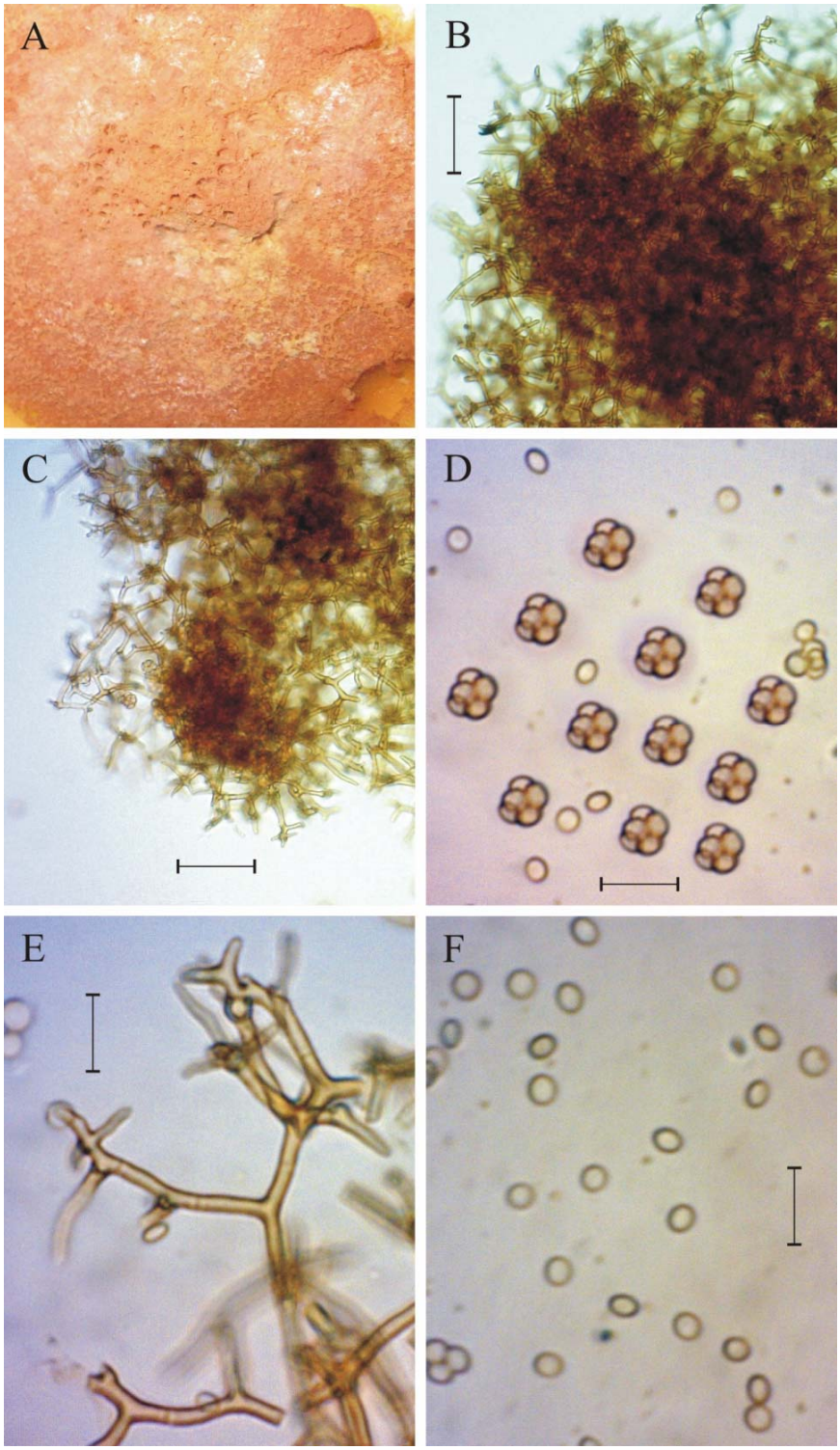

Figure 1 *Gymnoascus reesii*. A) Colony on PDA medium, B and C) Ascoma (gymnothecium), D) Asci, E) Appendages, F) Ascospores. Bars = 100 µm (Fig B); 62 µm (Fig C); 16.7 µm (Figs D, E and F).

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All *Gymnoascus* isolates previously identified based on morphological and cultural characters, were amplified using the primers pair ITS1 and ITS4. A amplicon of about 600 bp was obtained for all of the *Gymnoascus* isolates. Through Blast search in GenBank all isolates were identified as *Gymnoascus reesii.* All DNA sequences of *Gymnoascus* isolates showed 100% homology with valid sequences of this species that were previously identified and deposited in GenBank. Accession numbers of isolates that were submitted to GenBank are JQ387570 and JQ387571. In the light of the literature on ascomycetous fungi (Ershad, 2009), *Gymnoascus* Baran is a new genus record for Iran mycoflora. The specimens are kept in fungal collection of the Department of Plant Protection, Razi University, Kermanshah, Iran. According to previous reports in other countries, this species is suitable for biological control of plant pathogens (Liu *et al.,* 2011).

References

Cannon, P. F. and Kirk, P. M. 2007. Fungal Families of the World. CABI, United Kingdom.

Doveri, F. 2011. Additions to "Fungi Fimicoli Italici": An update on the occurrence of coprophilous Basidiomycetes and Ascomycetes in Italy with new records and descriptions. Mycosphere, 2 (4): 331-427.

Ershad, D. 2009. Fungi of Iran. Ministry of Jihad-e-Agriculture. 531 p.

Liu, J., Wang, L., Qiu, J., Jiang, L., Yan, J., Liu, T. and Duan, Y. 2011. Nematicidal activity of *Gymnoascus reesii* against *Meloidogyne incognita*. African Journal of Microbiology Research Vol. 5 (18), pp. 2715-2719.

Lumley, T. C. and Currah, R. S. 1995. A preliminary account of some unusual onygenalean fungi from decayed wood. Reports of Tottori Mycological Institute 33, 7-13.

Scott, J. A. and Untereiner, W. A. 2004. Determination of keratin degradation by fungi using keratin azure. Medical Mycology 42, 239-246.

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, In: Gelfand, M. A., Sninsky, D. H. and White, T. J. (Eds). PCR Protocols. A Guide to Methods and Applications. Academic Press, SanDiego, CA, pp. 315-322.

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معرفی یک جنس آسکومیست (*Gymnoascus* Baran) برای فلور قارچی ایران

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چکیده: طی سال‌های ۱۳۹۱- ۱۳۹۰ حضور قارچ‌های آسکومیست در مزارع مختلف استان فارس مورد بررسی قرار گرفت. نمونه‌های خاک از عمق ۲۰-۰ سانتی‌متری جمع‌آوری شد. جدایه‌های قارچی به روش تهیه رقت از خاک جداسازی شدند. در بین گونه‌های معمول خاک سه جدایه با پرگنه نارنجی رنگ وجود داشتند که جالب توجه بود. قطر پرگنه روی محیط سیب‌زمینی دکستروز آگار بعد از هفت روز به پنج سانتی‌متر رسید. آسکوکارپ کروی، به رنگ قرمز نارنجی و به قطر ۴۵۰-۱۷۰ میکرومتر بود. پریدیوم صاف، با دیواره ضخیم، قرمز رنگ، دیواره‌دار، درهم پیچیده و دارای زواید کوتاه ساده یا منشعب بود. آسک‌ها به‌رنگ روشن، تقریباً کروی، هشت اسپوری و به قطر ۱۲-۸ میکرومتر بودند. آسکوسپورها به‌رنگ زرد روشن، کروی و به قطر ۴-۲/۵ میکرومتر بودند. براساس مشخصات مورفولوژیک فوق، این قارچ *Gymnoascus reesii* تشخیص داده شد (Doveri, 2011). دی ان ای ژنومی قارچ استخراج و ناحیه توالی‌های جداکننده‌ی نسخه‌برداری شده‌ی داخلی ۱، ۲ و ژن ۵/۸ اس‌دی‌ان‌ای ریبوزومی جدایه‌های مذکور با استفاده از واکنش زنجیره‌ای پلی‌مراز تکثیر و قطعات تکثیر یافته، تعیین توالی گردیدند. پس از توالی‌یابی این ناحیه و ذخیره در بانک ژن (Acc. No. JQ387570, 71)، نسبت به ارزیابی همولوژی این توالی ۶۰۰ جفت بازی با توالی‌های موجود در بانک ژن به‌کمک ابزار جستجوی BLAST اقدام گردید. جدایه‌های حاصل از این بررسی با دیگر جدایه‌های موجود در بانک ژن درجه بسیار بالای همولوژی نشان دادند. این جنس به‌همراه گونه مذکور برای اولین بار از ایران گزارش می‌شود. نمونه‌های سند در بخش گیاه‌پزشکی دانشکده کشاورزی دانشگاه رازی نگهداری شدند.

واژگان کلیدی: فلورقارچ‌های ایران، Gymnoascus

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