Gilmaniella subornata, a new species for the funga of Iran

Amir Amirivafa: MSc Student, Department of Plant Protection, College of Agriculture, Razi University, Kermanshah, Iran

Samad Jamali⊠**:** Associate Prof., Department of Plant Protection, College of Agriculture, Razi University, Kermanshah, Iran (jamali454@yahoo.com)

Abstract

A fungal isolate of *Gilmaniella subornata* (Ascomycota, *genera incertae sedis*) was recovered from the crowns of pistachio trees (*Pistacia vera*) in orchards of Kermanshah (Iran). The species is characterized by septate aerial hyphae, hyaline to pale yellowish-brown conidiophores, holoblastic conidia that are globose to subglobose (9–11 µm in diam.), and a single germ pore (0.8 µm wide). Molecular analysis of ITS and beta-tubulin gene sequences confirmed the identification. This paper provides morphological description, molecular data, and distribution of the species. This represents the first report of *G. subornata* in Iran and its first global association with pistachio trees. The species shows potential applications in industry and bioremediation due to its thermotolerance and ability to degrade organic pollutants.

Keywords: Beta-tubulin, endophytic fungus, gene sequences, molecular analysis, pathogenicity, *Pistachia vera*

Gilmaniella subornata، گونه جدیدی برای قارچ‌های ایران

امیر امیری‌وفا: دانشجوی کارشناسی ارشد بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه رازی، کرمانشاه، ایران

صمد جمالی⊠: دانشیار گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه رازی، کرمانشاه، ایران (jamali454@yahoo.com)

خلاصه

جنس قارچی *Gilmaniella* Barron (از آسکومیکوتا و فاقد جایگاه مشخص در آرایه‌های پایین‌تر) که در سال ۱۹۶۴ توسط بارُن پایه‌گذاری شد، شامل نه گونه ثبت شده در پایگاه‌های Index Fungorum و MycoBank است. در دی ماه ۱۴۰۱، جدایه‌ای از این جنس از طوقه درخت پسته (*Pistacia vera* L.) با علایم زوال در یکی از باغ‌های کرمانشاه جداسازی شد. این جدایه با ویژگی‌های ریخت‌شناختی نظیر ریسه‌های هوایی شفاف تا قهوه‌ای کم‌رنگ با عرض ۱/۵ تا ۵ میکرومتر و کنیدیوم‌های کروی تا نیمه‌کروی با قطر ۹ تا ۱۱ میکرومتر، به عنوان *G. subornata* Morinaga, Minoura & Udagawa شناسایی شد. این شناسایی با مقایسه با منابع تخصصی (Morinaga *et al.* 1978, Umali *et al.* 1998) و تحلیل توالی‌های ژنی ITS (شماره دسترسی PV162860) در بانک ژن تایید شد که شباهت ۹۹ درصدی با سویه‌ای از چین (MK419028) نشان داد. همچنین، توالی ژن بتا-توبولین این جدایه با شماره دسترسی PV477267 در GenBank ثبت شد که برای این گونه، نخستین ثبت توالی از این ناحیه محسوب می‌شود. مایه‌زنی این جدایه روی نهال‌های دوساله پسته هیچ‌گونه علایم بیماری ایجاد نکرد که حاکی از نقش احتمالی آن به عنوان اندوفیت است. نمونه مرجع در مجموعه قارچ‌های مؤسسه تحقیقات گیاه‌پزشکی کشور (تهران) تحت شماره IRAN 5348C نگهداری می‌شود. این نخستین گزارش از حضور *G. subornata* برای قارچ‌های ایران و ارتباط آن با درختان پسته در سطح جهانی است، درحالی‌که پیش‌تر تنها *G. humicola* Barron از *Hordeum vulgare* L. در ایران گزارش شده بود (Ershad *et al.* 2018). این یافته‌ها نقش بوم‌شناختی متنوع و پتانسیل کاربردی این قارچ را برجسته می‌کند.

واژه‌های کلیدی: بتاتوبولین، بیماری‌زایی، توالی‌های ژنی، قارچ اندوفیت، *Pistachia vera*

Introduction

The genus *Gilmaniella* Barron (Ascomycota, *genera incertae sedis*), established by Barron in 1964, comprises nine species listed in the Index Fungorum and MycoBank databases [*G. bambusae* Umali, Goh & Hyde (1998); *G. humicola* Barron (1964); *G. indica* Dubey, Rai, Shrivast. & Verma (2011); *G. macrospora* Moustafa (1975); *G. multiporosa* Moustafa & Ezz-Eldin (1989); *G. nyukfahii* Goh, Lee & Teo (2013); *G. punctiformis* Sivanesan & Sutton (1985); *G. subornata* Morinaga, Minoura & Udagawa (1978); and *G. thermophile* Qureshi & Mirza (1983)]. These species are primarily known for their saprobic lifestyles, often colonizing decaying plant material, soil, and other organic substrates in diverse ecological niches (Kirk *et al*. 2008). *Gilmaniella* species are of particular interest due to their ecological adaptability and potential biotechnological applications. Many species within this genus exhibit traits such as thermotolerance, halotolerance, and the ability to degrade complex organic compounds, making them candidates for industrial and environmental applications (Mouchacca 2007). For instance, their enzymatic capabilities have been explored for the production of heat-resistant enzymes, which are valuable in bioprocessing industries, and their pollutant-degrading abilities suggest potential roles in bioremediation of contaminated soils and water (Prenafeta-Boldú *et al*. 2019). In the context of plant-associated fungi, *Gilmaniella* species have been reported as saprobes, endophytes, and occasionally as opportunistic pathogens, depending on environmental conditions and host susceptibility (Verma *et al*. 2022). Endophytic fungi, in particular, play crucial roles in plant health, including enhancing stress tolerance, nutrient acquisition, and protection against pathogens (Rodriguez *et al*. 2009). However, the association of *Gilmaniella* species with economically important crops (e.g., pistachio), remains poorly documented.

In Iran, the fungal diversity associated with agricultural crops is still underexplored, with only a few *Gilmaniella* species, such as *G. humicola* Barron, previously reported in association with barley (*Hordeum vulgare* L.) (Ershad *et al.* 2018). The discovery of new fungal species or novel host associations can provide valuable insights into fungal ecology, host specificity, and potential applications in agriculture and biotechnology.

Materials and Methods

In December 2022, declining pistachio trees (*Pistacia vera* L.) exhibited cankers on their crowns in orchards of Kermanshah (Iran) were sampled. Wood pieces (3–6 mm) were cut off from the margin between healthy and dead tissue at the canker sites; surface sterilized by sodium hypochlorite (0.5% active chlorine) for 1.5–2 min., rinsed thrice in sterile distilled water, and blotted dry with sterile paper towel. Wood pieces were then plated on potato-dextrose-agar (PDA) supplemented with 25 µg/mL chloramphenicol and 100 mg/L streptomycin sulfate as bacterial inhibitors, and incubated at 25 °C for five days. Single conidial cultures of ten representative isolates of *Gilmaniella*, selected for molecular studies, were grown on PDA at 25 °C in dark for five days. Total genomic DNA was extracted using the DNA extraction Kit (DenaZist Asia Co.). The ITS-1/ITS-4 and βt2a/βt2b primer pairs were used to amplify the ITS regions and β-tubulin gene, respectively.

Results and Discussion

An isolate of *Gilmaniella* was recovered from the crowns of declining pistachio trees in orchards of Kermanshah (Iran). The isolate exhibits septate aerial hyphae, hyaline to pale yellowish-brown or pale brown, smooth to verrucose, measuring 1.5–5 µm wide (Fig. 1). Conidiophores arise laterally from superficial hyphae, are light brown to brown, thin, smooth, and branched. Conidiogenous cells are hyaline to light brown, smooth, cylindrical, often inflated, and measure 5–25 × 4–6 µm (Fig. 2 a-b). Conidia were holoblastic, produced singly or in clusters, hyaline to dark olivaceous-brown or dark brown, with smooth to predominantly verrucose or spinulose surfaces, globose to subglobose, 9–11 µm in diam., with a single germ pore approximately 0.8 µm wide (Fig. 2 c-d). These characteristics, supported by comparisons with specialized literature (Moustafa & Ezz-Eldin 1989, Umali *et al*. 1998), confirmed the isolate as *G. subornata*. Molecular analysis of ITS and beta-tubulin gene sequences (GenBank accession numbers PV162860 and PV477267, respectively) supports this identification. BLAST analysis of the ITS sequence revealed 99% nucleotide identity with *G. subornata* (MK419028) from China. The beta-tubulin sequence represents the first submission for *G. subornata* in GenBank, as no prior data exist for *Gilmaniella* species. Inoculation of two-year-old pistachio seedlings produced no disease symptoms, suggesting an endophytic role. A voucher specimen is deposited in the Fungal Reference Collection of the Iranian Research Institute of Plant Protection (Tehran, Iran) under No. IRAN 5348C.

Gilmaniella subornata typically inhabits salt-marsh soils and decomposes organic matter, including plant debris, in high-salinity, moderate-temperature environments (Mouchacca 2007). It also colonizes herbaceous plant material, such as leaves and stems, highlighting its versatility as a saprobe and potential endophyte (Chen *et al*. 2020, Verma *et al*. 2022). Its thermotolerance supports bioconversion at elevated temperatures, offering potential for industrial applications, particularly in producing heat-resistant enzymes and metabolites, while its ability to degrade organic pollutants positions it as a candidate for bioremediation (Mouchacca 2007). It has been previously reported from China, Japan, Slovakia, and the United States (Chen *et al.* 2020). This study marks the first record of *G. subornata* for the funga of Iran and its novel association with *P. vera* globally. In contrast, *G. humicola* is the only *Gilmaniella* species previously documented in Iran, associated with *H. vulgare* L. (Ershad *et al*. 2018). This finding expands the known distribution and host range of *G. subornata*, highlighting its ecological and biotechnological significance.

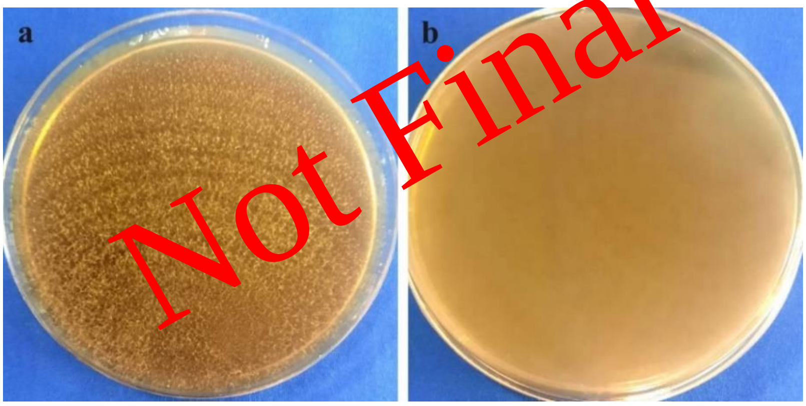

Fig. 1. *Gilmaniella subornata* colony on PDA after seven days: a. Obverse, b. Reverse.

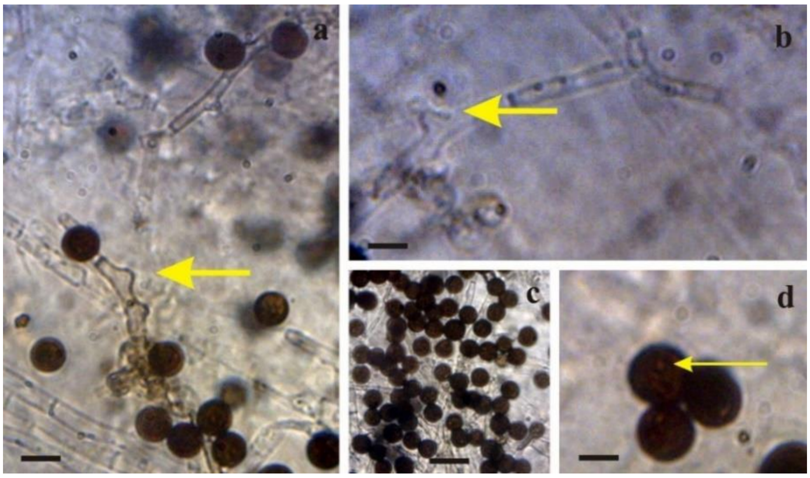

Fig. 2. *Gilmaniella subornata*: a-b. Conidiogenous cells, c. Conidia, d. Conidia with germ pores (arrow) [Bars = 15 µm (a), 10 µm (b, d), 20 µm (c)].

References

Chen, J., Akutse, K.S., Saqib, H.S.A., Wu, X., Yang, F., Xia, X., Wang, L., Goettel, M.S., You, M. & Gurr, G.M. 2020. Fungal endophyte communities of crucifer crops are seasonally dynamic and structured by plant identity, plant tissue and environmental factors. Frontiers in Microbiology 11: 1519. DOI: 10.3389/fmicb.2020.01519.

Ershad, D., Asef, M.R., Bakhshi, M., Javadi, A., Zangeneh, S., Asgari, B., Aliabadi, F. & Mehrabi, M. 2018. Genera of Fungi and Fungal Analogues of Iran. Ministry of Jihad-e-Agriculture. Agricultural Research, Education and Extension Organization, Tehran, Iran, 977 p. (In Persian).

Mouchacca, J. 2007. Heat tolerant fungi and applied research: addition to the previously treated group of strictly thermotolerant species. World Journal of Microbiology and Biotechnology 23: 1755–1770. DOI: 10.1007/s11274-007-9426-3.

Moustafa, A.F. & Ezz-Eldin, E.K. 1989. *Gilmaniella multiporosa*, a new dematiaceous hyphomycete from Egyptian soils. Mycological Research 92(4): 502–505. DOI: 10.1016/S0953-7562(89)80200-X.

Prenafeta-Boldú, F.X., de Hoog, G.S. & Summerbell, R.C. 2019. Fungal communities in hydrocarbon degradation. Pp. 233–253. *In*: Fungi in Extreme Environments: Ecological Role and Biotechnological Significance. Springer, Cham.

Rodriguez, R.J., White Jr, J.F., Arnold, A.E. & Redman, A.R.A. 2009. Fungal endophytes: diversity and functional roles. New Phytologist 182(2): 314–330. DOI: 10.1111/j.1469-8137.2009.02773.x.

Umali, T.E., Goh, T.K. & Hyde, K.D. 1998. A new species of *Gilmaniella* from Hong Kong. Mycological Research 102(4): 435–438. DOI: 10.1017/S0953756297004966.

Verma, A., Shameem, N., Jatav, H.S., Sathyanarayana, E., Parray, J.A., Poczai, P. & Sayyed, R.Z. 2022. Fungal endophytes to combat biotic and abiotic stresses for climate smart and sustainable agriculture. Frontiers in Plant Science 13: 953836. DOI: 10.3389/fpls.2022.953836.