

***Sphaerulina quercicola*, a new species for the funga of Iran**

Received: 04.04.2023 ===== Revised: 10.05.2023 ===== Accepted: 13.05.2023

Mounes Bakhshi✉: Research Assistant Prof., Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran (mounesbakhshi@gmail.com)**Mansoureh Mirabolfathy**: Research Prof., Department of Plant Disease Research, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran**Rasoul Zare**: Research Prof., Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran**Abstract**

Sphaerulina is a genus of fungi which belongs to the family *Mycosphaerellaceae* in the order *Mycosphaerellales*. In a survey of leaf-inhabiting fungi associated with trees in Hyrcanian forests, several isolates of the genus *Sphaerulina* were recovered from leaf spot symptoms of oak trees. Integration of multi-locus phylogenetic analyses (LSU, ITS, *actA* and *tef1*) and morphological identification were applied to identify the isolates. The results revealed that, the isolates belong to a species of the genus *Sphaerulina*, namely, *S. quercicola*. To the best of author's knowledge, this is the first report of this species for the funga of Iran.

Keywords: *Fagaceae*, leaf spot, *Mycosphaerellaceae*, phylogeny, taxonomy***Sphaerulina quercicola* گونه جدیدی برای قارچ‌های ایران**

دریافت: ۱۴۰۲/۰۱/۱۵ ===== بازنگری: ۱۴۰۲/۰۲/۲۰ ===== پذیرش: ۱۴۰۲/۰۲/۲۳

مونس بخشی✉: استادیار پژوهش بخش تحقیقات رستنی‌ها، مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران (mounesbakhshi@gmail.com)**منصوره میرابوالفتحی**: استاد پژوهش بخش تحقیقات بیماری‌های گیاهی، مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران**رسول زارع**: استاد پژوهش بخش تحقیقات رستنی‌ها، مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران**خلاصه**

Sphaerulina یکی از جنس‌های قارچی متعلق به تیره *Mycosphaerellaceae* و راسته *Mycosphaerellales* می‌باشد که ۲۰۵ گونه از آن تاکنون در جهان تایید شده و در پایگاه میکوبانک به ثبت رسیده است. طی مطالعه گونه‌های قارچی درختان در جنگل‌های هیرکانی، علایم لکه‌برگی به صورت گسترده روی درختان بلوط در استان‌های مازندران و گلستان در اردیبهشت ۱۳۹۹ مشاهده شد. جدایه‌های قارچی به دست آمده از این علایم، پس از خالص‌سازی، با استفاده از تلفیق داده‌های ریخت‌شناختی و داده‌های مولکولی براساس ترکیب توالی نواحی ژنومی *actA*، *ITS*، *LSU* و *tef1* مطالعه و تحت عنوان گونه‌ای از جنس *Sphaerulina*، به نام *S. quercicola* شناسایی شدند. گونه حاضر روی محیط کشت SNA، کنیدیوماتای پیکنیدیومی یا آسروولی به رنگ قهوه‌ای، گرد تا کشیده، با توده کنیدیومی سفید شیری تا صورتی کم‌رنگ تولید نمود. یاخته‌های کنیدیوم‌زا مستقر در کنیدیوم‌برهای کوتاه ساده یا منشعب با ۳-۴ (۱-۳) بند عرضی، بی‌رنگ، استوانه‌ای یا تنگی شکل، هولوبلاستیک، با گسترش پی‌رُست (percurrent) با حلقه‌های نامشخص تا نسبتاً مشخص، گاهی با رشد سیمپودیال، به ابعاد (۳۰-۱۸) × ۷-۵/۵-۳/۴ میکرومتر بودند. کنیدیوم‌ها استوانه‌ای، مستقیم تا خمیده، با نوک گرد، ۰-۳ بندی، بی‌رنگ، به ابعاد (۴۵-۳۲) × (۲۵-۵) میکرومتر بودند. براساس اطلاعات موجود، این نخستین گزارش از این گونه برای قارچ‌های ایران است.

واژه‌های کلیدی: آرایه‌بندی، راشیان، فیلوژنی، لکه‌برگی، *Mycosphaerellaceae*

Introduction

Sphaerulina is a genus in the family *Mycosphaerellaceae* (Quaedvlieg *et al.* 2013). It was described based on the type species *Sphaerulina myriadea* (DC.) Sacc. by Saccardo in 1878. The phylogeny of this genus has been clarified by Quaedvlieg *et al.* (2013) and Verkley *et al.* (2013). A total of 205 legitimate *Sphaerulina* species are recorded in the MycoBank (<http://www.mycobank.org>, accessed Apr. 2023) database (Crous *et al.* 2004). Eight species of *Sphaerulina* including *S. aceris* (Lib.) Verkley, Quaedvl. & Crous, *S. berberidis* (Niessl) Quaedvl., Verkley & Crous, *S. cercidis* (Fr.) Quaedvlieg, Verkley & Crous, *S. cornicola* (DC.) U. Braun & Bensch, *S. frondicola* (Fr.) Verkley, Quaedvlieg & Crous, *S. westendorpii* (Westend.) Verkley, Quaedvlieg & Crous, *S. rumicis* (Arzanlou & M. Bakhshi) M. Bakhshi & Arzanlou, and *S. oxyacanthae* (Kunze & J.C. Schmidt) Quaedvl., Verkley & Crous have been reported from Iran (Mirzaei *et al.* 2021, Bakhshi *et al.* 2022, Ershad 2022).

Oaks (*Quercus* spp.) are important forest trees comprising many commercial taxa distributed in vast areas of Zagros, Arasbaran and Hyrcanian forests of Iran such as *Q. brantii* Lindl, *Q. castaneifolia* C.M.Mey., *Q. macranthera* Fisch. & C.A.Mey. ex Hohen, and *Q. petraea* (Matt.) Liebl. (Panahi *et al.* 2011, Sagheb Talebi *et al.* 2014). During the investigation of fungal species associated with leaf spot symptoms of trees in Hyrcanian forests, some dothideomycetous fungal strains were isolated from leaf spot symptoms of oak trees. The aim of this work was to identify these leaf-spotting fungi, in terms of DNA phylogeny and morphological characteristics.

Materials and Methods

The diseased leaf samples of oak trees (*Quercus* spp.) were collected in Hyrcanian forests in north of Iran. Single-conidial isolates were obtained from symptomatic fresh oak leaves following the method described by Bakhshi *et al.* (2021). Axenic cultures and dried plant specimens are maintained in the Culture Collection (IRANC), and the Fungarium (IRANF) of the Iranian

Research Institute of Plant Protection (Tehran, Iran), respectively.

Total genomic DNA was extracted from fungal colonies grown on MEA medium using the protocol of Möller *et al.* (1992). Amplification and sequencing of four nuclear loci, 28S nrRNA gene (LSU), internal transcribed spacer regions and intervening 5.8S nrRNA gene of the nrDNA operon (ITS), actin (*actA*) and translation elongation factor 1-alpha (*tef1*) were done with primers LR0R + LR5, V9G + ITS4, ACT-512F + ACT-783R, and EF1-728F + EF1-986R, respectively. The protocols and conditions for standard PCR amplification and sequencing of the loci were performed following Bakhshi & Braun (2022) for LSU and ITS and Bakhshi *et al.* (2019) for *actA* and *tef1*. The resulting sequences were subjected to a BLAST search at the NCBI's GenBank to find most similar sequences. The obtained sequences from GenBank together with the novel generated sequences during this study were aligned and subjected to multi-locus DNA sequence analyses using MrBayes Ver. 3.2.6 (Ronquist *et al.* 2012) as elucidated by Bakhshi *et al.* (2019). *Ramularia endophylla* Verkley & U. Braun (CBS 113265) (*Mycosphaerellaceae*) was used as the outgroup taxon.

Results and Discussion

During the study of foliicolous fungi in the Hyrcanian forests of Iran, severe leaf spot symptoms were observed on the leaf surface of oak trees apparently occur widely throughout these trees in Mazandaran and Golestan Provinces (northern Iran) in April-May 2020. The symptoms observed as scattered, definite, amphigenous, circular, subcircular, angular to irregular, pale brown to brown in the center leaf spots, with brown or black border giving an eyespot appearance to spots, generally with characteristic yellow halo, 1–3(–5) mm in diam., occasionally enlarged gradually and coalesced.

Based on the phylogenetic analyses, the Iranian isolates obtained from oak leaf spots clustered together with *Sphaerulina quercicola* reference cultures sequenced by Quaedvlieg *et al.* (2013) (Fig. 1). The fungus had the following morphological characteristics:

Sphaerulina quercicola (Desm.) Quaedvlieg, Verkley & Crous, *Studies in Mycology* 75: 347 (2013)

Description (*in vitro*): On SNA: Conidiomata pycnidial or acervuloid, pale to dark brown, lenticular to globose, releasing milky white to rosy buff conidial slime. Conidiogenous cells discrete or integrated in simple, short, (1–)3–4-septate conidiophores which may be branched at

the base, cylindrical or ampuliform, hyaline, holoblastic, proliferating sympodially or percurrently with more or less distinct annellations, 7–18(–30) × 3.5–4.5 μm. Conidia cylindrical, straight, flexuous or curved, broadly rounded at the apex, hyaline, (0–)1–3-septate, occasionally constricted around the septa, (25–)32–45 × 3–4(–5) μm (Fig. 2).

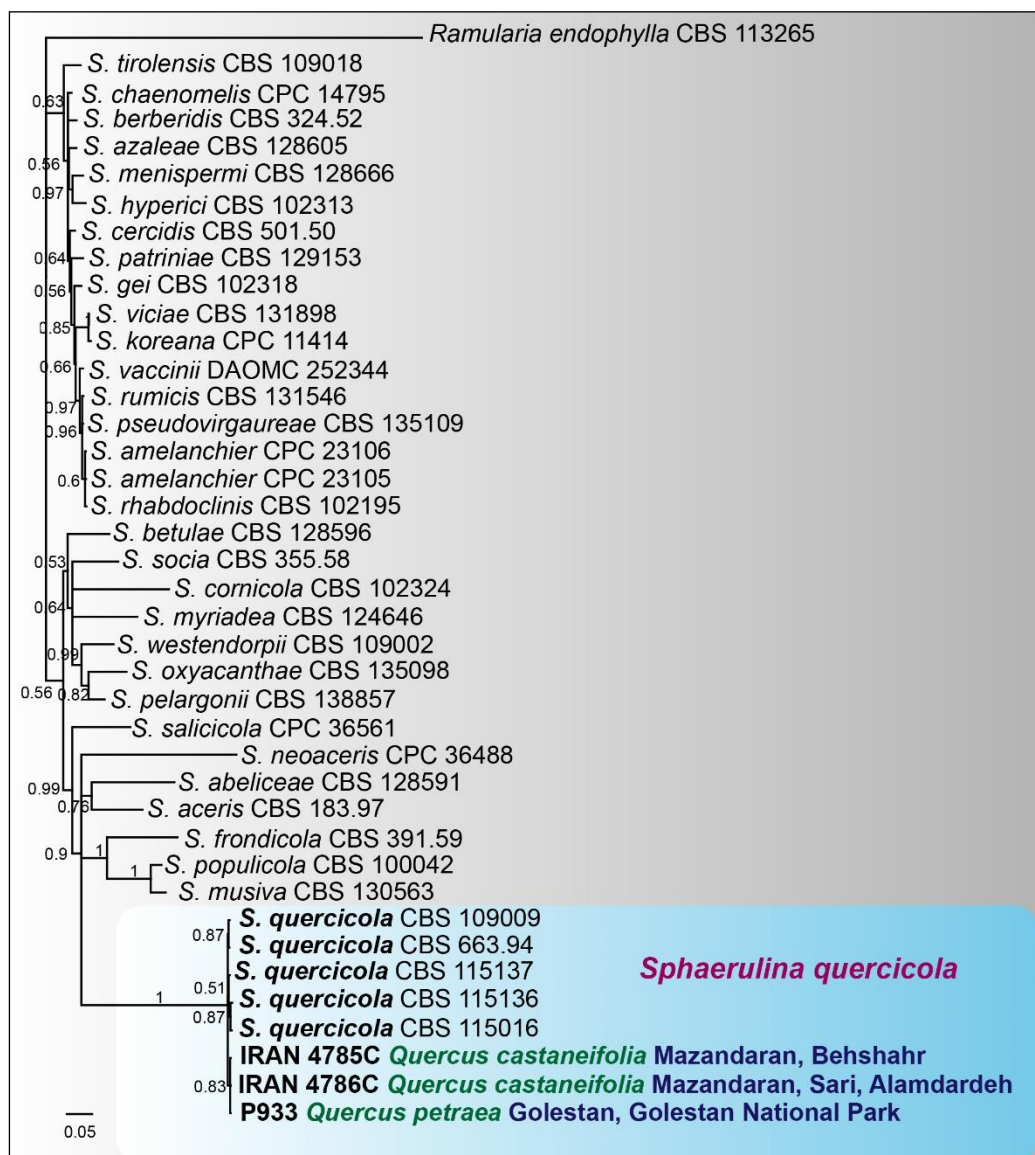


Fig. 1. Phylogenetic tree inferred by Bayesian analysis of the combined four-loci (LSU, ITS, *actA* and *tef1*) sequence alignment using MrBayes Ver. 3.2.6 for *Sphaerulina* species. The scale bar indicates 0.05 expected changes per site. The tree was rooted to *Ramularia endophylla* (CBS 113265).

Culture characteristics: (in the dark, at 25 °C after 20 days): Colonies on PDA restricted, surface folded, olivaceous grey to grey, irregularly pustulate, with an even to irregularly undulating margin, covered by dense to diffuse, finely felted, white aerial mycelium; reverse dark

grey, slow-growing, reaching 3–7 mm diam. Shape and size of the colonies on MEA are similar to those on PDA (Fig. 2).

Specimens examined: IRAN: Mazandaran Province, Behshahr, N 36° 39' 47", E 53° 32' 33", 300 m, on leaves

of *Q. castaneifolia* (*Fagaceae*), May 2020, M. Bakhshi, IRAN 4785C, IRAN 18261F (LSU = OQ625943, ITS = OQ625989, *actA* = OQ919480, *tefl* = OQ919485); Sari, Alamdardeh, N 36° 21' 20", E 53° 16' 12", 450 m, on leaves of *Q. castaneifolia*, April 2020, M. Bakhshi, IRAN 4786C, IRAN 18262F (LSU = OQ625944, ITS =

OQ625990, *actA* = OQ919481, *tefl* = OQ919486); Golestan, Golestan National Park, N 37° 21' 38", E 50° 00' 32", 900 m, on leaves of *Q. petraea*, May 2020, M. Mirabolfathy, P 933, IRAN 18263F (LSU = OQ625945, ITS = OQ625991, *actA* = OQ919482, *tefl* = OQ919487).

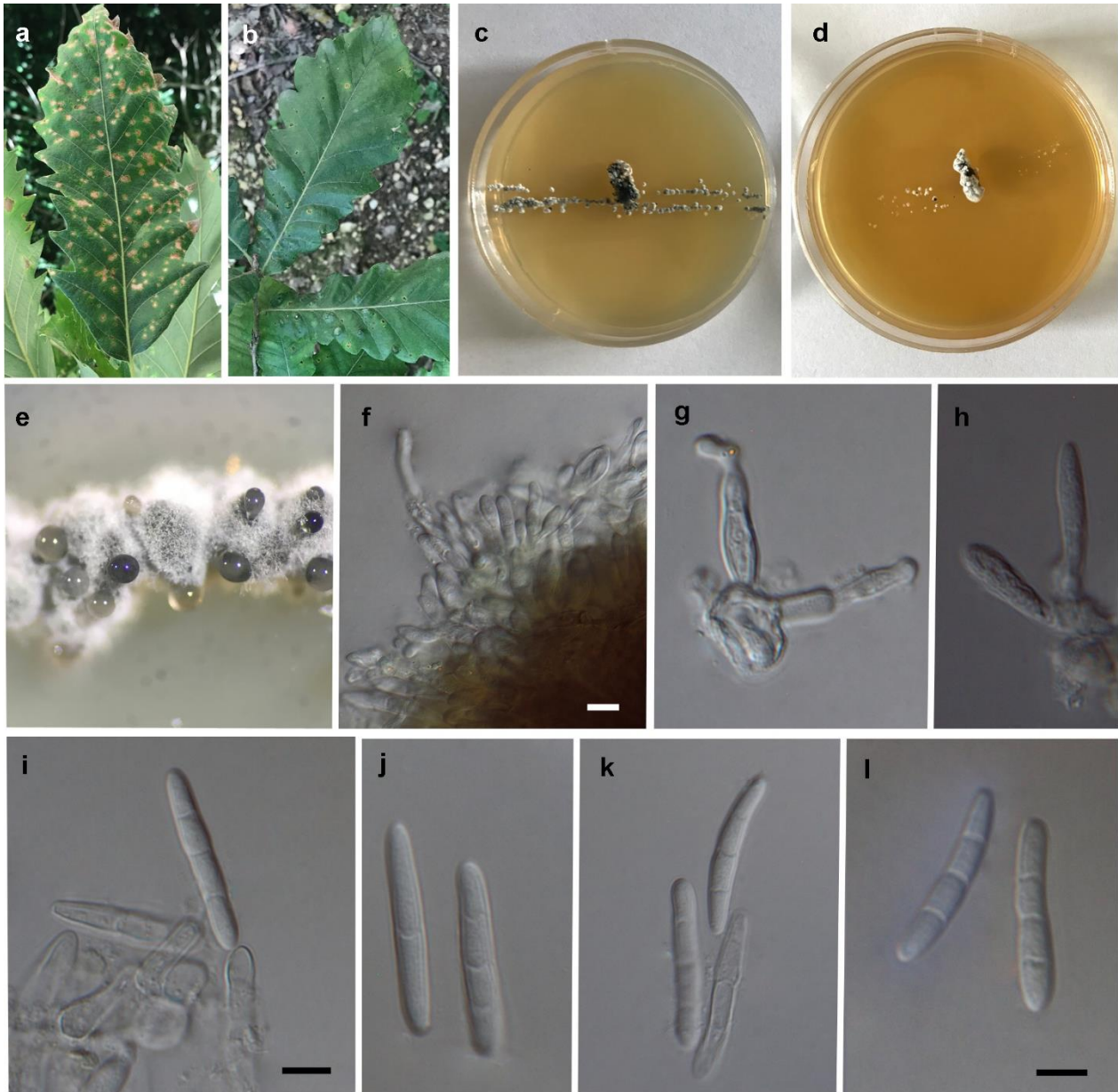


Fig. 2. *Sphaerulina quercicola*: a, b. Leaf spot symptoms on the host, c. Culture on PDA, d. Culture on MEA, e. Conidiomata forming in culture, f–l. Conidiophores, conidiogenous cells and conidia (Bars = 10 μ m).

References

- Bakhshi, M. & Braun, U. 2022. *Acericercospora hyrcanica* gen. et sp. nov. (Mycosphaerellaceae) and *Paramyco-centrospora acericola* gen. et sp. nov. (Dothidotthiaceae) on maple trees in Hyrcanian forests. *Mycological Progress* 21(8): 1–15.
- Bakhshi, M., Arzanlou, M., Zare, R., Groenewald, J.Z. & Crous, P.W. 2019. New species of *Septoria* associated with leaf spot diseases in Iran. *Mycologia* 111(6): 1056–1071.
- Bakhshi, M., Zare, R. & Ershad, D. 2022. A detailed account on the statistics of the Fungi and fungus-like taxa of Iran. *Mycologia Iranica* 9(2): 1–96.
- Bakhshi, M., Zare, R., Jafary, H., Arzanlou, M. & Rabbani-nasab, H. 2021. Phylogeny of three *Ramularia* species occurring on medicinal plants of the Lamiaceae. *Mycological Progress* 20(1): 27–38.
- Crous, P.W., Gams, W., Stalpers, J.A., Robert, V. & Stegehuis, G. 2004. MycoBank: an online initiative to launch mycology into the 21st. century. *Studies in Mycology* 50: 19–22.
- Ershad, D. 2022. *Fungi and Fungal Analogues of Iran*. Ministry of Agriculture, Agricultural Research, Education and Extension Organization, Iranian Research Institute of Plant Protection, Tehran, Iran. 695 pp.
- Mirzaei, M., Babai-Ahari, A., Narmani, A. & Arzanlou, M. 2021. Genetic diversity among *Sphaerulina frondicola* populations, the causal agent of poplar leaf spot disease in East Azarbaijan Province using RAPD and ISSR molecular markers. *Journal of Applied Research in Plant Protection* 9(4): 1–5 (In Persian).
- Möller, E.M., Bahnweg, G., Sandermann, H. & Geiger, H. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20: 6115–6116.
- Panahi, P., Jamzad, Z., Pourmajidian, M.R., Fallah, A. & Pourhashemi, M. 2011. A revision of chestnut-leaved oak (*Quercus castaneifolia* C.A.Mey.; Fagaceae) in Hyrcanian forests of Iran. *Caspian Journal of Environmental Sciences* 9(2): 145–158.
- Quaedvlieg, W., Verkley, G.J.M., Shin, H-D., Barreto, R.W., Alfenas, A.C., Swart, W.J., Groenewald, J.Z. & Crous, P.W. 2013. Sizing up *Septoria*. *Studies in Mycology* 75: 307–390.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Saccardo, P.A. 1878. *Fungi Veneti novi vel critici vel mycologiae Venetae addendi. Series IX. Michelia* 1(4): 361–445.
- Sagheb-Talebi, K., Sajedi, T. & Pourhashemi, M. 2014. *Forests of Iran: A Treasure from the Past, a Hope for the Future*. Springer, Dordrecht Heidelberg, New York, London.
- Verkley, G.J.M., Quaedvlieg, W., Shin, H-D. & Crous, P.W. 2013. A new approach to species delimitation in *Septoria*. *Studies in Mycology* 75: 213–305.