



## Morphological and molecular characterization of *Podosphaera minor* on *Spiraea* in Iran

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**Abstract:** Powdery mildew is a common disease of *Spiraea* spp. worldwide; however, there are no reports of this destructive disease in *Spiraea* species from Iran. In recent years, severe powdery mildew symptoms have been observed in *Spiraea* spp. throughout the Guilan Province. In this study, we collected infected plant specimens and used morphological and molecular approaches to identify the powdery mildew fungus involved in this disease. We did not observe the teleomorphic state of the fungus in this area, and it appears that the fungus occurred only in the anamorphic state. At least six species of *Podosphaera* occur worldwide, and there is a considerable overlap between the anamorphic characteristics of some species. Hence, the ITS sequence was used to identify common powdery mildew species occurring in *Spiraea* in the Guilan Province. The results showed that the ITS sequence is useful for *Podosphaera* species in *Spiraea*. *Podosphaera minor* Howe. was identified as the causal agent of powdery mildew disease in this plant. To our knowledge, this is the first report of *Podosphaera minor* as a fungus in Iran.

**Keywords:** *Erysiphaceae*, ITS-rDNA, Plant disease, Powdery mildew.

### INTRODUCTION

*Spiraea* is a genus of flowering plants and includes about 80–100 species of shrubs in the family *Rosaceae* (<http://www.efloras.org/>). Many *Spiraea* species are ornamental and often cultivated in gardens, city landscapes, and parks because of their attractive white, pink, or purple flowers. Powdery mildew disease in *Spiraea* is a common problem worldwide (Braun & Cook 2012, Farr et al. 2023). At least six powdery mildew species attack *Spiraea* spp., belonging to the genus *Podosphaera* (Braun & Cook

2012, Farr & Rossman 2023). Although the disease has been reported on *Spiraea* spp. from all over the world, there is no report from Iran. In this paper, we describe *P. minor* on *Spiraea* sp. from Iran based on morphological and molecular studies using ITS-rDNA sequences.

### MATERIALS AND METHODS

For morphological examination, a small piece of mycelia (including conidia and conidiophores) was transferred onto a slide containing a drop of 1:1 glycerin: lactic acid using a clear adhesive tape, and slightly heated. The photos were taken using a compound Olympus BH2 microscope with a Sony digital camera (Olympus, Tokyo, Japan) attached. One of the specimens was subjected to sequence ITS-rDNA. Total DNA was extracted from mycelia using Thermolysis method (Zhang et al. 2010; Khodaparast et al. 2021). Internal transcribed spacer (ITS) regions were amplified by the PM10 (5'-GGCCGAAAGTTGTCCAAAC-3') and PM11 (5'-TACCGCTTCACTCGCCGTTA-3') primers (Bradshaw and Tobin 2020). PCR components and conditions were in accordance with Darsaraei et al. (2021). PCR products were subjected to electrophoresis in a 2% agarose gel in 1X TBE buffer and after confirmation of amplification, sent to Codon Genetic Group, Tehran, Iran to be sequenced using PM10 primer. The ITS sequence generated in this study was deposited in the GenBank under accession number OQ254755.

Sequences retrieved from GenBank, along with the newly generated sequence were aligned in MEGA 7 (Kumar et al. 2016). The maximum likelihood analysis was done using raxmlGUI (Silvestro & Michalak 2012), under a GTRGAMMA model. The Bootstrap (BS) supports and trees were then obtained by running rapid bootstrap analysis of 1000 pseudoreplicates, followed by a search for the tree with the highest likelihood.

### RESULTS AND DISCUSSION

*Podosphaera minor* Howe, Bull. Torrey bot. Club 5: 3 (1874)

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Specimens examined. Iran, Guilan Province, Rasht, on *Spiraea* sp., Oct. 2021, H. Darsaraei (GUM 1927); Chaboksar, 31 Oct. 2020, M.J. Pourmoghaddam (GUM 1929).

The disease develops on the plant during the summer and reaches its peak by early September. Infected bushes turn white color from a distance, and the infection occurs widely on the infected bushes and sometimes covers almost whole aerial parts under suitable conditions (Fig. 1). Morphological characteristics are as follows: The mycelium developed on both sides of the leaves, mostly epiphyllous, often covers the whole surface of the leaves. Hyphal cell width 3–5  $\mu\text{m}$ , hyphal appressoria little differentiated. Conidiophores erect, 65–200  $\mu\text{m}$ , foot-cells cylindrical, often with a constriction near the basal septum, occasionally elevated from mother cell, 40–80  $\times$  6–12  $\mu\text{m}$ , followed by 1–3 cells, the first cell occasionally about the same size or so and two other cells are shorter, producing catenescence conidia. Conidia ellipsoid-ovoid, sub-cylindrical, 26–37 (–45)  $\times$  10–15  $\mu\text{m}$ . Chasmothecium was not observed (Figs. 2 and 3). Specimens were deposited in the Fungarium of University of Guilan (GUM).

Six *Podosphaera* species have been recorded from this plant worldwide including: *P. clandestina* (Wallr.) Lév., *P. leucotricha* (Ellis & Everh.) E.S. Salmon, *P. minor* Howe, *P. spiraeicola* U. Braun, *P. spiraeae-douglasii* U. Braun, and *P. spiraeae* (Sawada) U. Braun & S. Takam. (Cook and Braun 2012, Farr & Rossman 2023). Among these species, *P. spiraeae* belongs to *P.* section *Sphaerotheca* and other species belong to *P.* section *Podosphaera*. The most recorded *Podosphaera* species on *Spiraea* spp. belong to *P. clandestina*, so that, it has been recorded on *Spiraea* spp. from Asia (Korea, Japan), Europe (Belgium, Estonia, Germany, Sweden, Romania), North America (Canada, United States) and South America (Argentina). *P. minor* shows little distribution and has been recorded from Asia (China, Korea, Japan, Far East), Siberia, the United Kingdom, and North America (Braun & Cook 2012, Farr & Rossman 2023). *P. clandestina* has a wider host range and occurs in several genera of *Rosaceae*, while *P. minor* is confined to species of the genus *Spiraea* (Braun & Cook 2012). These species are well characterized and differentiated using chasmothecial and asci features. For example, *P. minor* differs from *P. clandestina* and *P. spiraeicola* by having short and less branched appendages and asci with small or indistinct ascus oculi (Quoted from Braun & Cook 2012). Anamorphic characteristics are very overlapping. ITS sequence of our specimen is well characterized and differentiated from other reliable sequences of *Podosphaera* species on *Spiraea* spp. According to our phylogenetic analysis, our sequence clustered with a reliable sequence of *P. minor* (AB525941, Takamatsu et al. 2010) and showed 99.7% similarity (identity 581/583, no substitution, with two gaps) with this sequence. Hence, even though identification of some powdery mildew fungi

based solely on anamorphic state may be difficult, ITS sequencing is very useful and is a good complementary option to distinguish above-mentioned species on *Spiraea* especially when chasmothecium is unavailable. This is the first report of *P. minor* from Iran.



Fig. 1: Symptoms of powdery mildew caused by *Podosphaera minor* on *Spiraea* in Guilan province

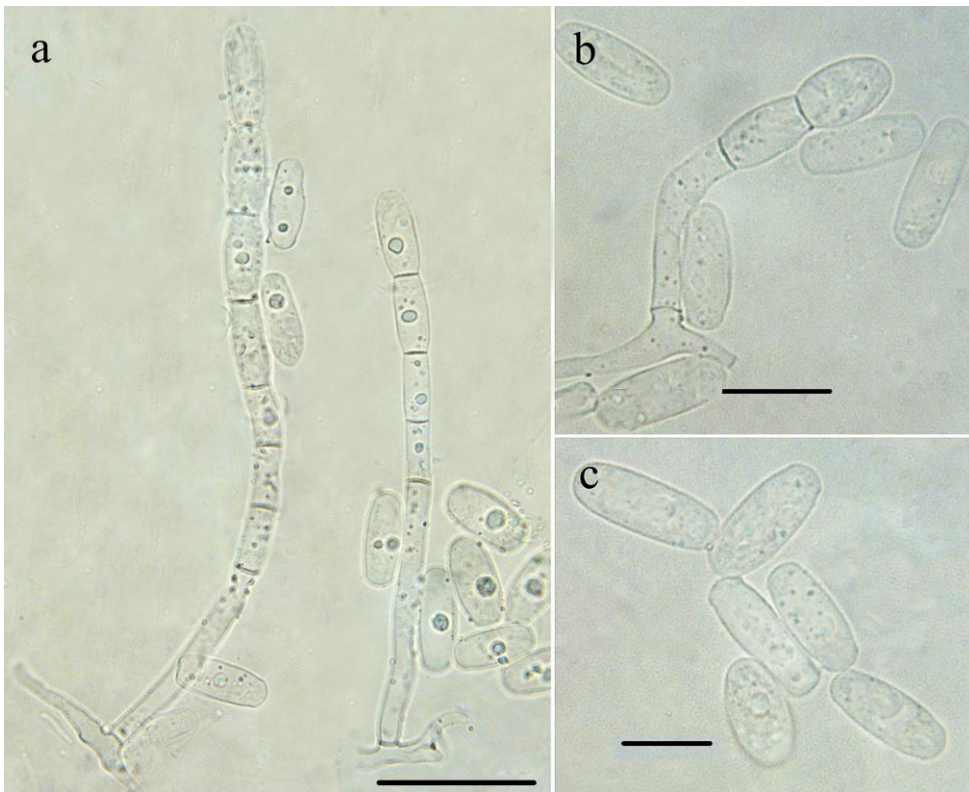


Fig. 2: *Podosphaera minor*. a, b. conidiophore and conidia; c. conidia; Scale bars: a = 50  $\mu$ m, b = 25  $\mu$ m, c = 20  $\mu$ m.

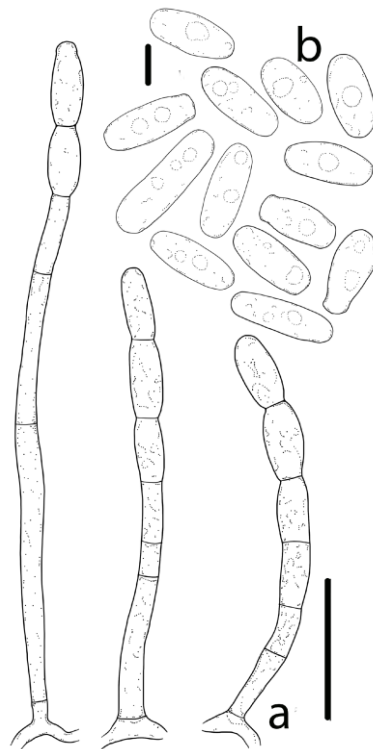


Fig. 3. An illustration of *Podosphaera minor*. a. conidiophores, b conidia; Scale bars: a = 50  $\mu$ m, b = 10  $\mu$ m.

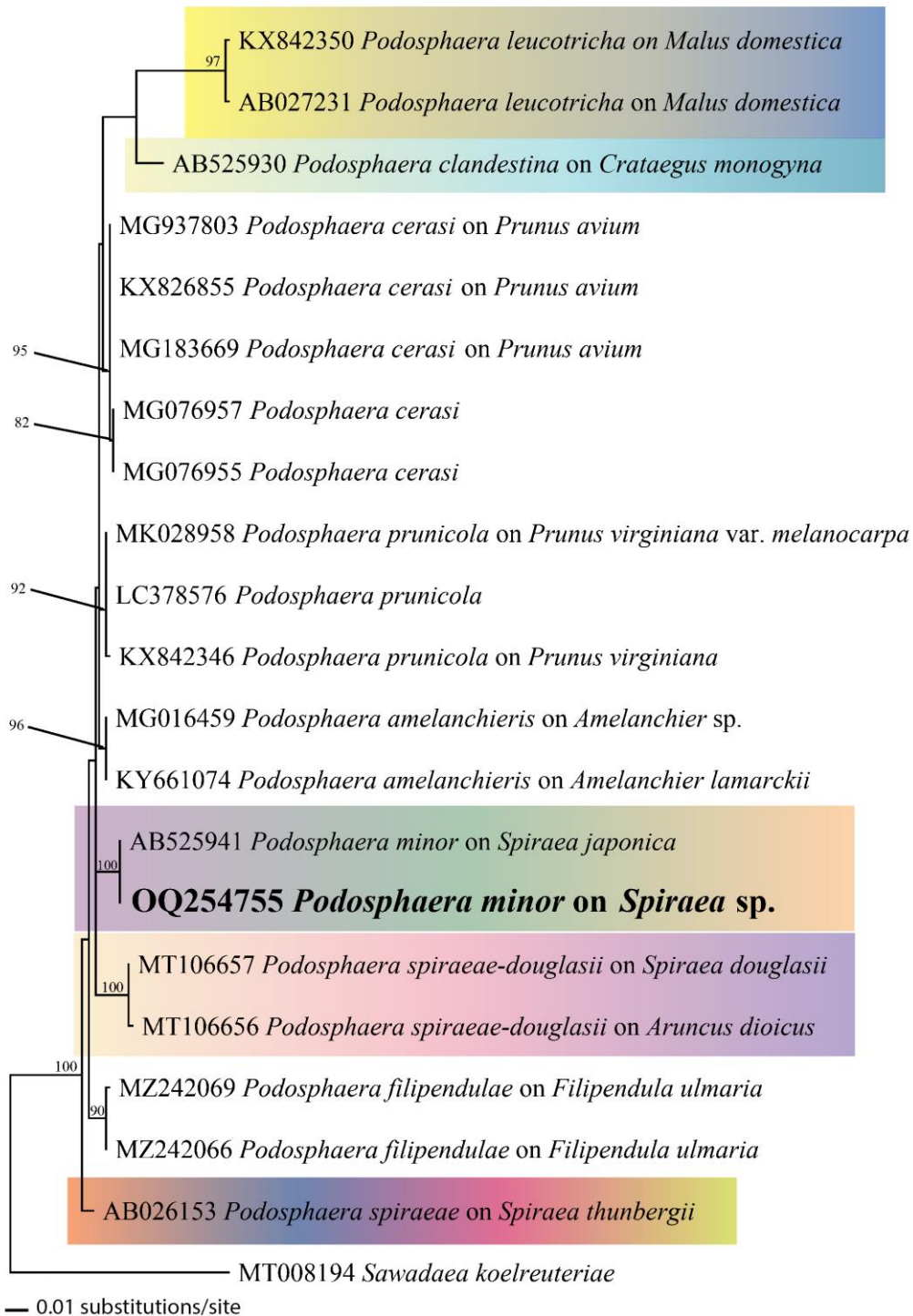


Fig. 4. Maximum-Likelihood (ML) consensus tree inferred from ITS ribosomal DNA sequences of some closely related *Podosphaera* species on *Rosaceae* including *Spiraea* and an outgroup (*Sawadaea koelreuteriae*). Numbers at the branches indicate bootstrap support above 70 %. The scale bar indicates expected changes per site. Species with color block have been reported on *Spiraea* spp.

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## بررسی ریخت‌شناختی و مولکولی *Podosphaera minor* روی اسپیره (*Spiraea sp.*) در ایران

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**چکیده:** سفیدک پودری یک بیماری معمول روی گونه‌های *Spiraea* در سراسر جهان است، با اینحال تاکنون، این بیماری مخرب روی این گیاه از ایران گزارش نشده است. طی سالهای اخیر، علایم شدید بیماری سفیدک پودری روی اسپیره در استان گیلان مشاهده شد. در این مطالعه تعدادی گیاه میزبان آلوده جمع آوری شدند و عامل بیماری بر اساس ویژگی‌های ریخت‌شناسی و مولکولی بررسی گردید. مرحله جنسی قارچ در این مطالعه یافت نشد و فقط مرحله غیرجنسی مشاهده شد. حداقل شش گونه *Podosphaera* روی اسپیره در جهان گزارش شده است و ویژگی‌های مربوط به شکل غیر جنسی بعضی گونه‌ها روی این گیاه با یکدیگر همپوشانی دارند، از اینرو از توالی ناحیه ITS برای تشخیص گونه شایع در استان گیلان استفاده شد. نتایج نشان داد که این ناحیه ژنی برای تشخیص گونه‌های *Podosphaera* روی اسپیره بسیار مفید است. در نتیجه گونه *Podosphaera minor* Howe به عنوان عامل سفیدک پودری اسپیره در استان گیلان تشخیص داده شد. این اولین گزارش این گونه از ایران است.

**کلمات کلیدی:** *Erysiphaceae*, ITS-rDNA, سفیدک پودری، بیماری گیاهی.