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ORIGINAL ARTICLE

# Taxonomy and phylogenetic position of *Phyllactinia takamatsui*, a newly described powdery mildew on cotoneaster, based on molecular and morphological data

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**Abstract** The newly recognised powdery mildew species *Phyllactinia takamatsui* on *Cotoneaster nummularius* (Rosaceae) is described and illustrated. This species, collected in Kerman Province, Iran, is well characterised by its conidial morphology and rDNA ITS sequences clearly different from allied species. Conidia are broadly ellipsoid to subcylindrical, i.e. they are not clavate-spathuliform as in most *Phyllactinia* species. The rDNA ITS sequence analysis showed that this species is closely allied to other species described on hosts belonging to Rosaceae, such as *Ph. mali* and *Ph. pyri-serotinae*. The ITS sequence of *P. takamatsui* was 92 to 94 % similar to that of the closest known relatives. The new species is described in detail, illustrated and compared with other similar taxa.

**Keywords** Biodiversity · Erysiphales · *Phyllactinia mali* · rDNA · Rosaceae

## Introduction

Of the 16 genera of Erysiphaceae (Braun and Cook 2012), three genera, viz. *Leveillula*, *Phyllactinia* and *Pleochaeta*, are endophytic or semi-endophytic fungi and possess asexual morphs different from other powdery mildew genera. These differences led to their assignment to a separate tribe within the Erysiphaceae (Phyllactinieae). Species of the genera *Leveillula* and *Phyllactinia* are morphologically rather uniform. Above all, the sexual morphs (chasmothecia) are often barely distinguishable. However, recent molecular analyses (Takamatsu et al. 2008; Khodaparast et al. 2001, 2007, 2012; Voytyuk et al. 2009) indicate considerable levels of genetic diversity within morphologically rather uniform groups of taxa. According to Takamatsu et al. (2008), several collections of *Ph. guttata* s. lat. usually form single clades based on their host plant families or genera. According to these results, Takamatsu et al. (2008) suggested that such clades should be described as separate species. However, the taxonomy of *Phyllactinia* at the species level is usually hampered by the lack of morphological characters to differentiate allied taxa. In autumn 2015, a powdery mildew was observed on leaves of cotoneaster (*Cotoneaster nummularius* Fisch. & C.A. Mey.) at Riseh village in the vicinity of Shahr-e Babak in Kerman Province, Iran. Examination of the specimen revealed that the fungus is an as yet undescribed species. In this paper, the new species is compared with other *Phyllactinia* species described on species of the Rosaceae. A key to *Phyllactinia* species on rosaceous host plants is provided.

## Materials and methods

### Morphological examination

To examine the sexual morph of the cotoneaster *Phyllactinia*, chasmothecia were scraped off the leaf surfaces with a dissection needle and mounted in lactic acid (50 %). To observe the hyphae, conidiophores and conidia, clear adhesive tapes mounted in a solution consisting of equal amounts of glycerol

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and lactic acid was used to strip off these structures from the leaf surfaces. An Olympus light microscope equipped with a Sony camera was used for microscopic observations. All measurements were based on at least 15 to 25 observations. Usually, more than one photo was taken and selected photos of each structure were mounted in a single photo plate using Photoshop (version CS3).

## DNA sequencing and data analysis

Total DNA was isolated from fungal specimens by the Chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). A region spanning ITS1, 5.8S and ITS2 of rDNA was amplified as described by Khodaparast et al. (2012) using the primers ITS1 and ITS4 (White et al. 1990). The nucleotide sequences of the polymerase chain reaction (PCR) products were obtained using direct sequencing in an ABI 3730xl sequencer (Applied Biosystems, USA). The ITS sequence determined in this study was deposited in GenBank under accession number KU695459. Sequences were compared with the sequences available in the NCBI GenBank nucleotide database using a BLASTN search method. Several sequences from GenBank were selected for phylogenetic analyses. Phylogenetic trees were obtained using the minimum-evolution (ME) method in MEGA version 6 (Tamura et al. 2013). In the ME method, the evolutionary distances were computed using the

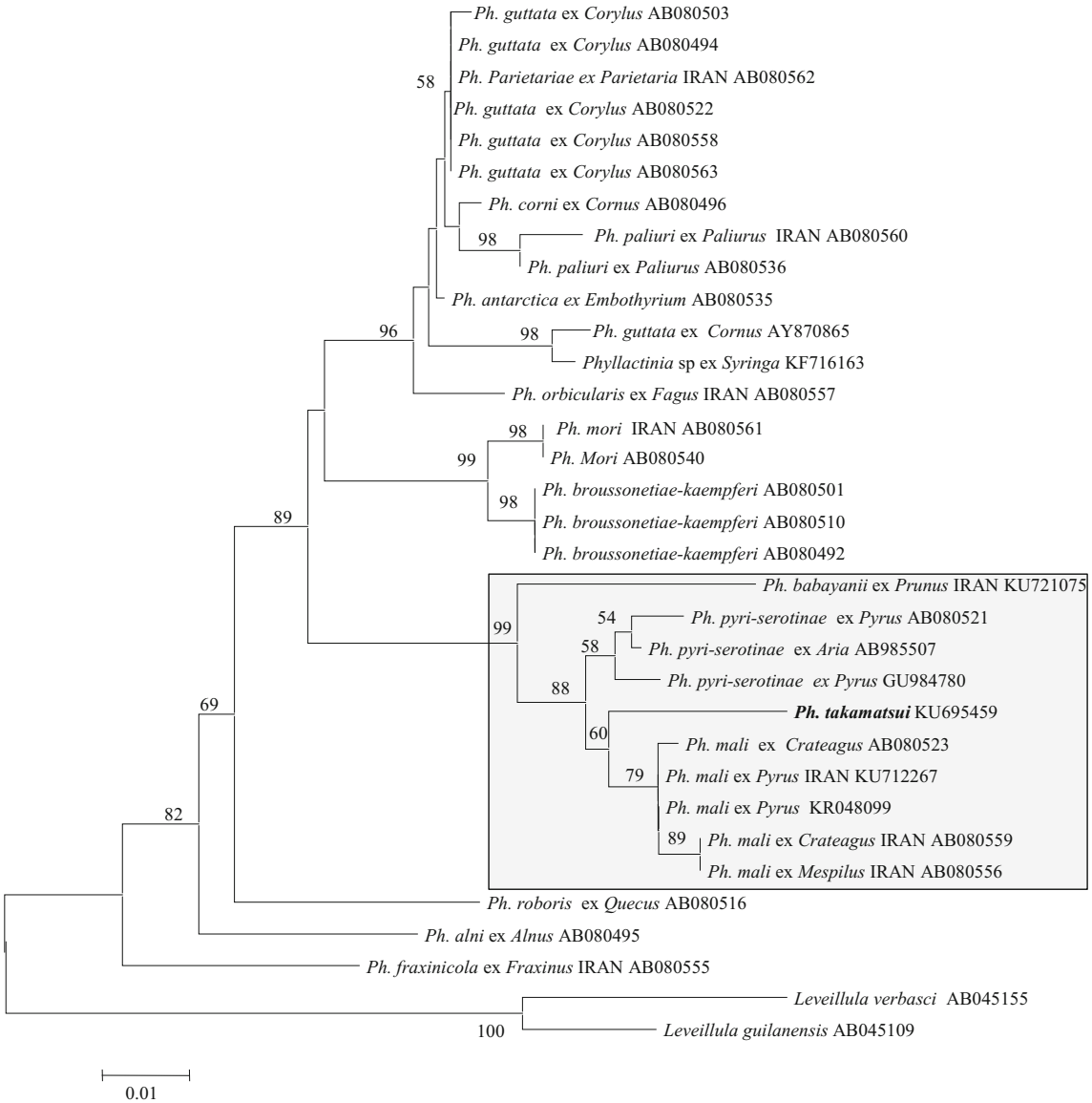


**Fig. 1** A minimum-evolution (ME) tree (length = 0.43390760) based on ITS data for 31 *Phyllactinia* sequences and two outgroup taxa. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The numbers on the branches represent branch support using 1000 bootstrap replications (bootstrap values below 50 % are not shown). Evolutionary analyses were conducted in MEGA version 6. Species on Rosaceae are shown in the shaded box

maximum composite likelihood method. The ME tree was searched using the close-neighbour-interchange (CNI) algorithm at a search level of 1. The neighbour-joining algorithm was used to generate the initial tree. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). All nucleotide substitutions were equally weighted and unordered. The analysis involved 33 nucleotide sequences. There were a total of 428 positions in the final dataset. The strength of the internal branches from the resulting trees was statistically tested by bootstrap analysis with 1000 replicates (Felsenstein 1985). The sequences of two *Leveillula* species were used as outgroup taxa.

## Results and discussion

According to morphological characteristics, the examined cotoneaster powdery mildew belongs to the genus *Phyllactinia*. This species is the first powdery mildew on cotoneaster in Iran and only the third pathogenic fungus recorded on this plant in Iran. Two species of the rust genus *Gymnosporangium* have already been reported on cotoneaster in this country (Ershad 2009).

The genus *Phyllactinia* is well characterised by its conidia that are usually clavate, angular-rhombiform (phyllactinioid type), although during the last two decades, several species with morphologically different conidia have been described, such as *Ph. adesmiae* (Havrylenko 1995), *Ph. chubutiana* (Havrylenko et al. 2006) and *Ph. sebastianiae* (Cabrera and Vobis 2011). The new species on cotoneaster has characteristic conidia, which resemble, at least in part, those of some *Leveillula* species. Moreover, the phylogenetic analysis showed that this taxon is closely allied to *Ph. mali* and *Ph. pyri-serotinae*, two other species reported on Rosaceae (Fig. 1), but clearly distinguished by more than 20 substitutions, in contrast to *Ph. pyri-serotinae*, a taxon that has been recorded on cotoneaster (Braun and Cook 2012). The record of *Ph. pyri-serotinae* on cotoneaster refers to *Ph. guttata* var. *rosacearum* Y.S. Paul & V.K. Thakur (nom. inval), described on *Cotoneaster bacillaris* from northern India. Braun and Paul (2009) re-examined type material and reduced this variety to synonymy with *P. pyri-serotinae*. However, conidiophores and conidia were not found in the Indian type material, i.e. the taxonomic decision was based on chasmothecia only, which more or less coincide with the material from Iran. Hence, it cannot be excluded that this Indian fungus on cotoneaster is conspecific with the fungus from Iran, but new

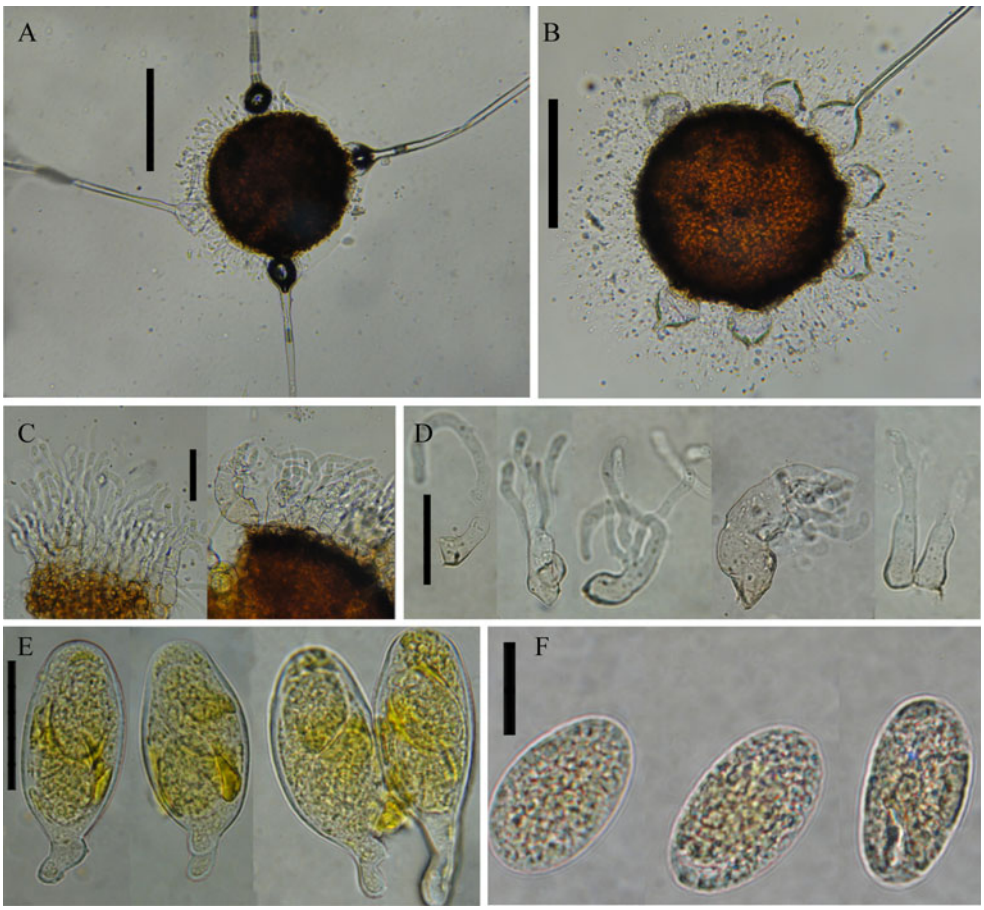


**Fig. 2** *Phyllactinia takamatsui* (**a**, **b**), ascoma (**c**), parts of ascomata with penicillate cells (**d**), penicillate cells (**e**), asci (**f**) ascospores. Scale bars: **a**, **b** = 200 μm, **c**–**e** = 50 μm, **f** = 20 μm

Indian collections of the asexual morph and, ideally, a molecular confirmation are needed. In any case, based on the morphological peculiarities and clearly different ITS data, the Iranian cotoneaster *Phyllactinia* can be described as a new species.

***Phyllactinia takamatsui*** **Khodap. & A. H. Mohammadi, sp. nov.**

MycoBank no.: 815766

Etym.: The new species is named in honour of Professor Susumu Takamatsu (Mie University, Tsu, Japan), a leading personality in the study of powdery mildew fungi.

**Mycelium** internal and external, thinly covering the lower surface of the leaves. **Hyphae** somewhat wavy, 4–6 μm wide, septate, thin-walled, smooth, a few nipple-shaped to oblong hyphal appressoria observed. **Conidiophores** formed singly on hyphal mother cells, with a long straight foot cell, about 100–177 × 5–7.5 μm, followed by 2–3 shorter cells, producing conidia singly. **Conidia** broadly ellipsoid, sublanceolate, sometimes slightly clavate to subcylindrical but not phyllactinioid, i.e. not strictly clavate-spathuliform, 48–60(−68) × 16–25 μm. **Chasmothecia** scattered, (225–)260–340 μm in diameter, blackish brown, wall-cells obscure, irregularly polygonal, 5–20 μm diameter. **Appendages** 4–8, arising around the equatorial zone of the chasmothecia, acicular with a bulbous base, 1.3–2.3 times as long as the chasmothecial diameter, bulbous base ca. 50–80 μm diam. **Penicillate cells** crowded on the upper part of the ascoma, stems 1–2-celled, 25–50 × 15–20(−28) μm, irregular to cylindrical, often divided into two branches at the upper portion, filaments up to 125 μm long and up to 10 μm wide. **Asci** numerous, ellipsoid, clavate, 77–100 × 37–45 μm, stalked, 2-spored, **ascospores** hyaline, ellipsoid, ovoid, 38–45 × 20–25 μm (Figs. 2 and 3).

**Holotype:** Iran, Kerman Province, Shahr-e Babak, Riseh village, on *Cotoneaster nummularius* Fisch. & C.A. Mey., 27.10.2015, A.H. Mohammadi (GUM 1364).

*Phyllactinia takamatsui* is well characterised by having broadly ellipsoid to lanceolate conidia, which distinguish this species from other *Phyllactinia* species on rosaceous hosts, such as *Ph. mali* (Duby) U. Braun, *Ph. pyri-serotinae* Sawada, *Ph. pyri-communis* Puzari & Sarbhoy, *Ph. babayanii* Simonyan and *Ph. holodisci* U. Braun, which all have clavate or angular-rhombiform conidia. During recent years, some *Phyllactinia* species with *Leveillula*-like conidia have been described, which are, however, characterised by dimorphic conidia, i.e. lanceolate to ellipsoid primary and cylindrical to clavate secondary conidia (Braun and Cook 2012).

Key to *Phyllactinia* species on Rosaceae (species based on Braun and Cook 2012, including the new species)

1 Asci with 2–6 spores……..….....*Ph. pyri-communis*

1* Asci 2-spored…….....…….…..2

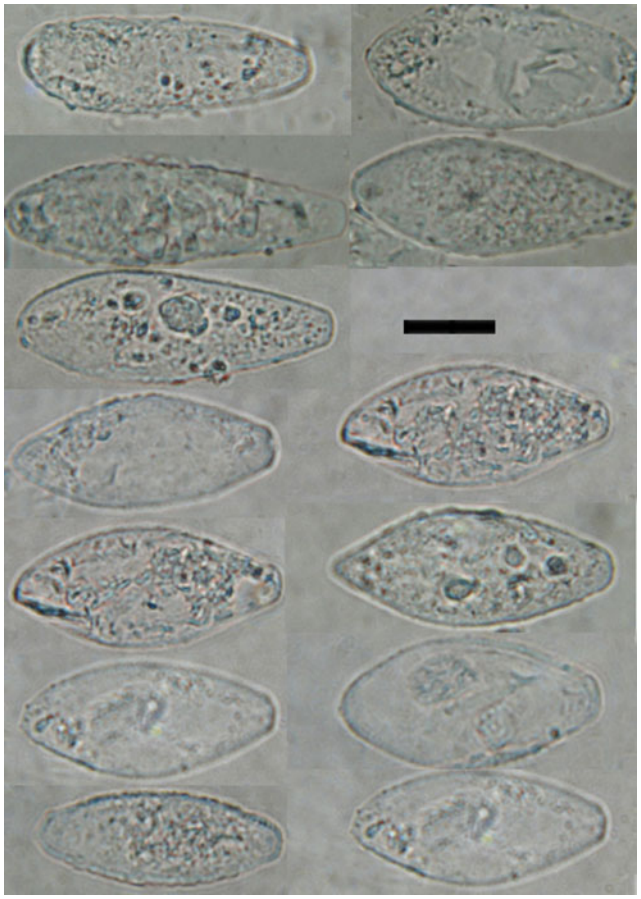

**Fig. 3** *Phyllactinia takamatsui*, conidia, scale bar = 20 μm

2 Conidia more or less ellipsoid, subcylindrical, sublanceolate, sometimes slightly clavate; filaments of the penicillate cells up to 125 μm long and up to 10 μm wide…….....……..….*Ph. takamatsui*

2* Conidia usually clavate or angular-rhombiform; filaments shorter…….....…3

3 Chasmothecia about (135–)140–185(−220) μm in diameter…….….....*Ph. mali*

3* Chasmothecia larger, about 170–350 μm in diameter…….…….....…4

4 Chasmothecia about 170–280 μm in diameter; bulbous basal swelling 30–55 μm in diameter……..*Ph. pyri-serotinae*

4* Chasmothecia larger 225–350; bulbous basal swelling 30–80 μm in diameter…….…..5

5 Bulbous basal swelling of the appendages hyaline; filaments of the penicillate cells as long as the stem or longer, apex up to 6 μm wide; on *Prunus* spp..…....…….…….*Ph. babayanii*

5* Bulbous basal swelling of the appendages hyaline to pigmented; filaments of the penicillate cells as long as stem or shorter, apex up to 10 μm wide; on *Holodiscus discolor*….....……..….....*Ph. holodisci*

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## References

Braun U, Cook RTA (2012) Taxonomic manual of the *Erysiphales* (powdery mildews). CBS Biodivers Ser 11:1–707

Braun U, Paul YS (2009) The Indian Erysiphaceae revisited. Nova Hedwigia 89(3–4):371–395

Cabrera MG, Vobis G (2011) *Phyllactinia sebastianiae* sp. nov. on *Sebastiania brasiliensis*. Mycotaxon 115(1):53–63

Ershad D (2009) Fungi of Iran. Iranian Research Institute of Plant Protection, Tehran

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

Havrylenko M (1995) New records of Erysiphaceae from North-Patagonia (Argentina). Nova Hedwigia 61(3–4):447–455

Havrylenko M, Takamatsu S, Divarangkoon R, Braun U (2006) *Phyllactinia chubutiana*: a new species of erysiphales from Patagonia (Argentina). Mycoscience 47(5):237–241

Hirata T, Takamatsu S (1996) Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37:265–270

Khodaparast SA, Takamatsu S, Hedjaroude GA (2001) Phylogenetic structure of the genus *Leveillula* (Erysiphales: Erysiphaceae) inferred from the nucleotide sequences of the rDNA ITS region with special reference to the *L. taurica* species complex. Mycol Res 105: 909–918

Khodaparast SA, Niinomi S, Takamatsu S (2007) Molecular and morphological characterization of *Leveillula* (Ascomycota: Erysiphales) on monocotyledonous plants. Mycol Res 111(6):673–679

Khodaparast SA, Takamatsu S, Harada M, Abbasi M, Samadi S (2012) Additional rDNA ITS sequences and its phylogenetic consequences for the genus *Leveillula* with emphasis on conidium morphology. Mycol Prog 11:741–752

Takamatsu S, Inagaki M, Niinomi S, Khodaparast SA, Shin HD, Grigaliunaite B, Havrylenko M (2008) Comprehensive molecular phylogenetic analysis and evolution of the genus *Phyllactinia* (Ascomycota: Erysiphales) and its allied genera. Mycol Res 112(3):299–315

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Voytyuk SO, Heluta VP, Wasser SP, Nevo E, Takamatsu S (2009) Biodiversity of the powdery mildew fungi (Erysiphales, Ascomycota) of Israel. Koeltz Scientific Books

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322