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Ageratum houstonianum and *Rudbeckia hirta*, new hosts for the powdery mildews *Golovinomyces asterum* and *Golovinomyces ambrosiae*

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Abstract
Powdery mildew fungi infect a variety of hosts such as many ornamental, agricultural and amenity plants. Powdery mildews reduce beauty and marketability of these plants. During identification of powdery mildew sampled from ornamental plants in Iran (2016–2019), two host-pathogen relationships were recorded for the first time. These were *Golovinomyces asterum* on *Ageratum houstonianum* and *Golovinomyces ambrosiae* on *Rudbeckia hirta.*

Keywords Erysiphales · Fungi · Pathogenicity · Plant pathogen · Powdery mildew

Recent phylogenetic studies have divided powdery mildew fungi (Erysiphales, Erysiphaceae) into five major clades (Mori et al. 2000). Braun and Cook (2012) recognized five tribes for the Erysiphaceae so that each tribe is almost equivalent to the phylogenetic clade based on Mori et al. (2000). The ectophyte parasite *Golovinomyces* is largest genus of the tribe *Golovinomyceteae* (Braun and Takamatsu 2000; Braun and Cook 2012) with specific sexual and asexual characteristics such as ascoma with numerous asci, mycelioid appendages, doliiform, limoniform to ellipsoid catenescent conidia, nipple shaped appressoria, erect and unbranched conidiophores and usually *Euoidium* type germ tubes (Braun and Cook 2012). *Golovinomyces* has nearly 50 species, commonly distributed throughout the world with wide host ranges of herbaceous plants (Braun and Cook 2012; Braun et al. 2019). Many ornamental plants have been recorded as host for this genus. Blue weed (*Ageratum houstonianum*, Asteraceae) and black-eyed Susan (*Rudbeckia hirta*, Asteraceae) are annual plants that are often grown as ornamentals for their flowers. Powdery mildew (Ascomycota: Erysiphales) on these plant causes great reduction of their beauty. In this paper we report the infection of these two ornamental plant species of Asteraceae by *Golovinomyces* spp.

During the regular mycological collection from 2016 to 2019, samples of ornamental plants infected with powdery mildew were collected from the Isfahan province of Iran and transferred to the laboratory of plant pathology.

Morphological characteristics of the fungus, host plant species and other information related to each species were investigated. Different structures of the fungus were examined under an optical microscope. To examine asexual forms, related structures were peeled of the leaf surface using clear adhesive tape and mounted in a 50% lactic acid solution. Measurements were based on data from 30 samples for each structure. Images were provided with a digital camera (Sony, DSH-HX) attached to an Olympus BH2 microscope. The images were assembled and edited using Photoshop (Adobe Photoshop CS). Exact identification and confirmation of taxa were done based on morphology using Braun (1987, 1995); Braun and Takamatsu (2000); Cook and Braun (2009); Braun and Cook (2012) and Qiu et al. (2020).

Recently, sequences of rDNA ITS regions of powdery mildews have been widely used to better understand powdery mildew systematics (Cunnington et al. 2003). Hence, we sequenced rDNA ITS for our collections. Total DNA was extracted from conidia and mycelia by the Hot Shot method (Montero-Pau et al. 2008). Universal primer set ITS1/ITS4 (White et al. 1990) was used to amplify the ITS region. The

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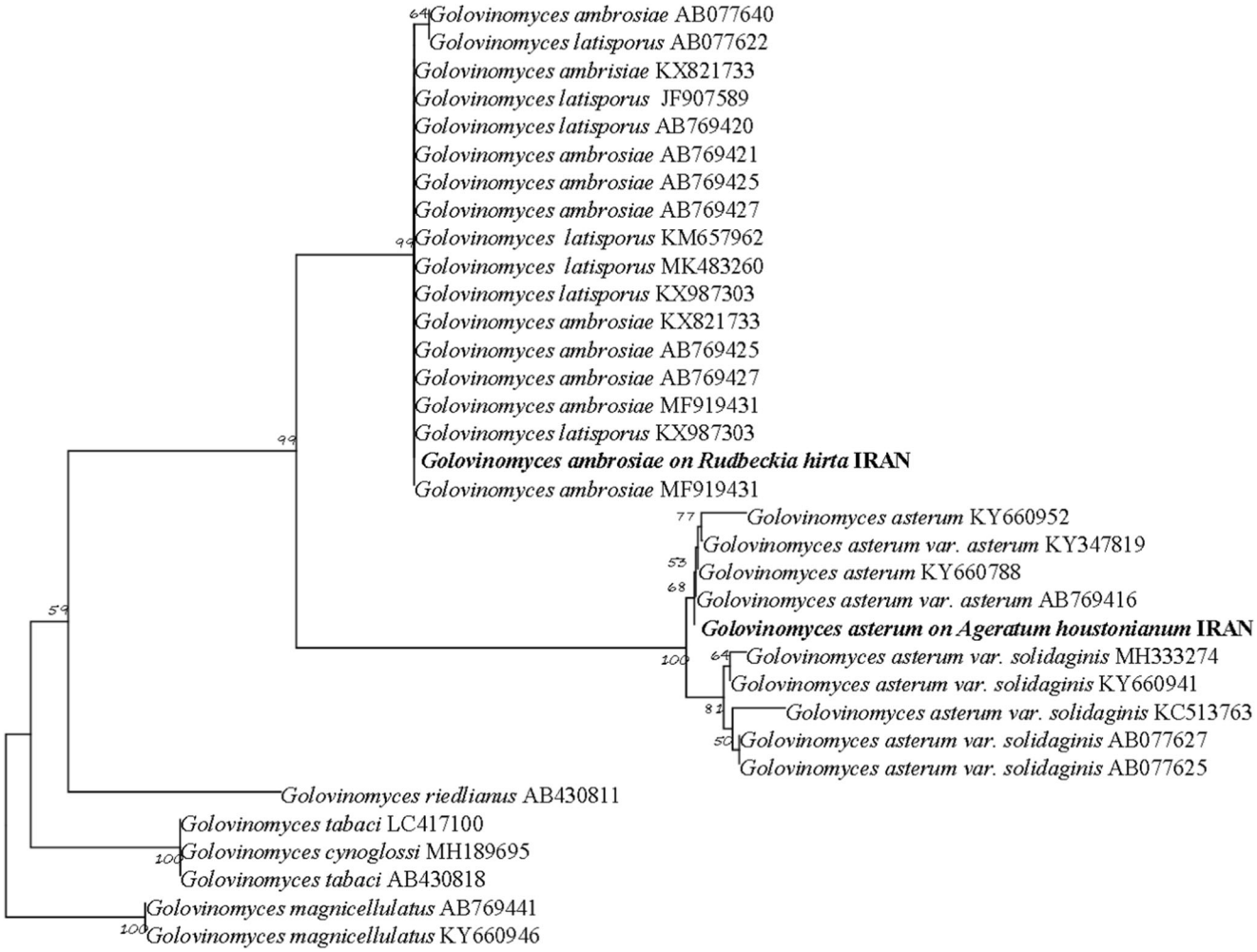

Fig. 1 Phylogenetic analysis of the rDNA ITS regions for 34 sequences of *Golovinomyces* by Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Numbers next to the branches shows the bootstraps values ≥50%. The evolutionary distances were computed using the Maximum Composite Likelihood method

PCR was performed in a Thermal Cycler in a total volume of 25 μl. The PCR mixtures contained 12.5 μl master mix (RNA Biotech company, Iran), 1 μl of each primer (0.4 pmol/ μl), 3 μl DNA template and 7.5 μl of double-distilled water. The PCR amplicons were electrophoresed on 1.5% agarose gels in TAE buffer to check the quality. The nucleotide sequences of the polymerase chain reaction (PCR) products were obtained using direct sequencing and amplified fragments were sent to a commercial sequencing provider (Tao Yang, Beijing, China). Sequences obtained were analyzed and edited using MEGA7.0 (Kumar et al. 2016) and subsequently were compared to the sequences available in the NCBI GenBank nucleotide database using the BLASTN search method. These sequences were aligned with other sequences retrieved from DNA databases using MUSCLE in MEGA 7 (Edgar 2004; Kumar et al. 2016). Phylogenetic trees were obtained using the Minimum-Evolution (ME) (Rzhetsky and Nei 1992) method in MEGA 7.0 (Kumar et al. 2016). In ME method, the evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980).

The ME tree was searched using the close-neighbour-interchange (CNI) algorithm at a search level of 1 (Nei and Kumar 2000). The neighbor-joining algorithm was used to generate the initial tree. All ambiguous positions were removed for each sequence pair. The strength of the internal branches from the resulting trees was statistically tested by bootstrap analysis with 1000 replicates (Felsenstein 1985). The ITS sequences determined in this study were deposited in GenBank (*A. houstonianum* with accession No. MN684151 and *R. hirta* with accession No. MN684152).

After BLASTN search our sequences showed high similarity with several reliable sequences available in GenBank. Using the output alignment of ITS generated by Mega 7, a rooted tree was obtained based on Minimum Evolution method (Fig. 1). Further considerations about each taxon are presented under species description.

Fig. 2 Powdery mildew infection on *Ageratum houstonianum* (blueweed)

In this study, several powdery mildew samples were examined. Two of these were new records and are described here.

Golovinomyces asterum U. Braun, Taxonomic Manual of the Erysiphales (Powdery Mildews): 304 (2012)

Material examined: on leaves of *Ageratum houstonianum* (Asteraceae), Iran, Isfahan province, Isfahan, K. Sharifi, 13 Jun. 2019 and 1 Sep. 2019.

Most *Ageratum houstonianum* (blueweed) grown in the garden showed powdery mildew infections. Symptoms appeared as irregular patches on both side of leaves and sometimes extended to whole leaves and other parts such as buds (Fig. 2).

Mycelium white, amphigenous, usually dense and occasionally scattered, hyphae 2–8 µm, hyphal appressoria solitary, nipple shaped, conidiophores erect, 60–200 × 7.5–14.5 µm, foot cell usually distinctly curved at the base, occasionally straight and cylindrical, 42.5–95 × 7.5–14.5 µm, followed by 1–3 shorter cells, conidia ellipsoid-ovoid, doliiform, subcylindrical, 25–40 × 12.5–17.5 µm. Germ tubes terminal, almost short and clavate, *Euoidium* type (Fig. 3). A specimen was deposited in the Fungarium of the University of Guilan under the accession number GUM 1589.

According to the literature, *Oidium* sp. on *Ageratum houstonianum* from Venezuela (Urtiaga 1986) and *Euoidium agerati* from *Ageratum conyzoides* (Braun and Cook 2012) have been recorded. *Golovinomyces asterum* is distinct from *Euoidium agerati*, in which the foot cells are usually distinctly curved at the base but occasionally straight, cylindrical. Phylogenetically it clustered with same species sequences with 99% similarity such as AB769416 (562/565, Takamatsu et al. 2013) and KY660788 99% (560/564, Ellingham et al. 2019). Braun and Cook (2012) have detected three morphological varieties of this species. From morphological characteristics (curved conidiophore foot cell and narrow conidia), we suggest

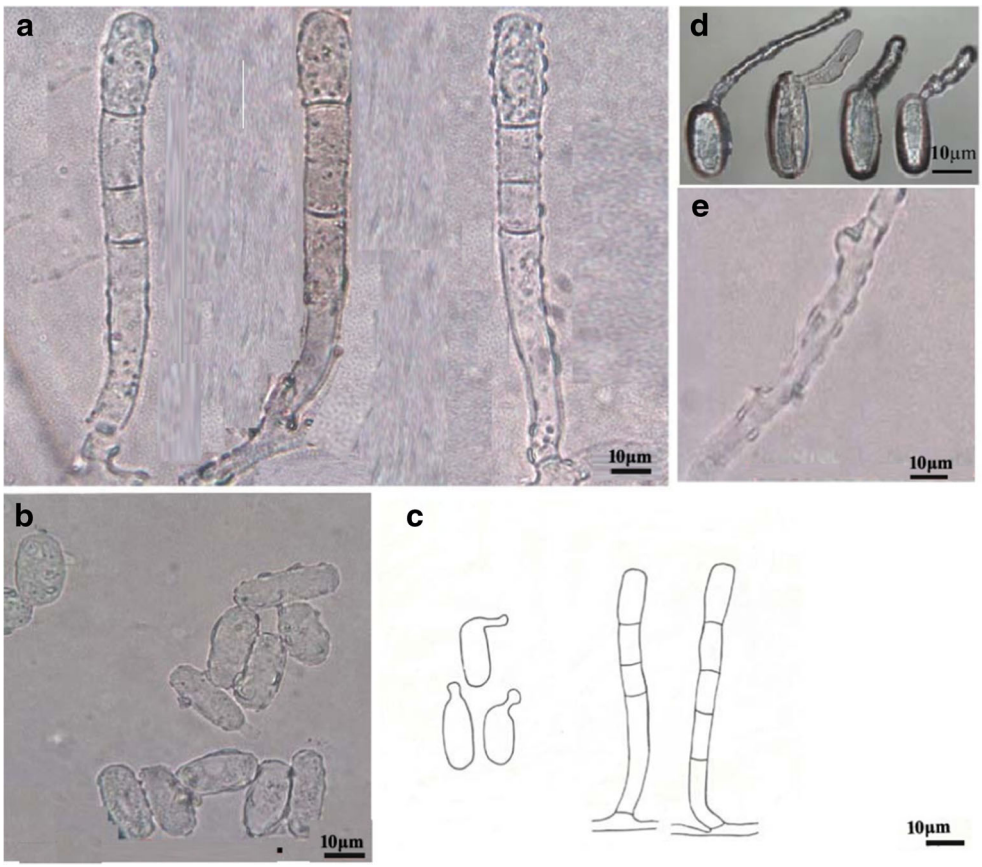

Fig. 3 *Golovinomyces asterum* **a** Conidiophores, **b** Conidia, **c** Drawing conidia germination & conidiophore, **d** Conidia germination, **e** Appresorium; Scale bar = 10 µm

Fig. 4 Powdery mildew infection on *Rudbeckia hirta* (black-eyed Susan)

our collection belongs to *G. asterum* var. *morocszkovskii*, However, we could not find ITS sequences for this variety in GenBank to fulfill molecular verification.

Golovinomyces ambrosiae (Schwein.) U. Braun & R.T.A. Cook, in Cook & Braun, Mycol. Res. 113: 628 (2009)

Material examined: on leaves of *Rudbeckia hirta* (Asteraceae), Iran, Isfahan province, Isfahan, K. Sharifi, 18 Aug. 2017.

Most *Rudbeckia hirta* (black-eyed Susan) grown in the garden showed powdery mildew infections. Symptoms appeared as white mycelium which extended to whole leaves and other parts such as stems (Fig. 4).

Mycelium amphigenous, white, sometimes covering entire leaves, hyphae 2–9 µm, hyphal appressoria solitary and nipple shaped, conidiophores erect, 55–140 × 9–15 µm, foot cell cylindrical, 30–75 × 9–15 µm, followed by 1–3 shorter cells, conidia catenescent, ellipsoid-ovoid, doliiform-subcylindrical, 22.5–39.5 × 12.5–19 µm, length/width ratio 1.5–2, conidial germination *Euoidium* type (Fig. 5). A specimen was deposited in Fungarium of University of Guilan under the accession number GUM 1590.

We identified our fungus as *G. spadiceus* based on descriptions in Braun and Cook (2012). However, Qiu et al. (2020), have shown that the older names *G. ambrosiae* has priority over *G. spadiceus* and *G. ambrosiae* is now the correct names for *G. spadiceus* sensu Braun and Cook 2012. Moreover, they have used the basionym *Oidium latisporum* (= *Erysiphe cichoracearum* var. *latispora*) for their new combination, *Golovinomyces latisporus* (U. Braun) P.-L. Qiu & S.- Y. Liu, to rename *G. ambrosiae* sensu Braun and Cook 2012.

According to Braun and Cook (2012), *G. ambrosiae* (now *Golovinomyces latisporus*) has been recorded on *Rudbeckia* species. Molecular evidence suggests that *Golovinomyces latisporus* and *G. ambrosiae* (*G. spadiceus* based on Braun

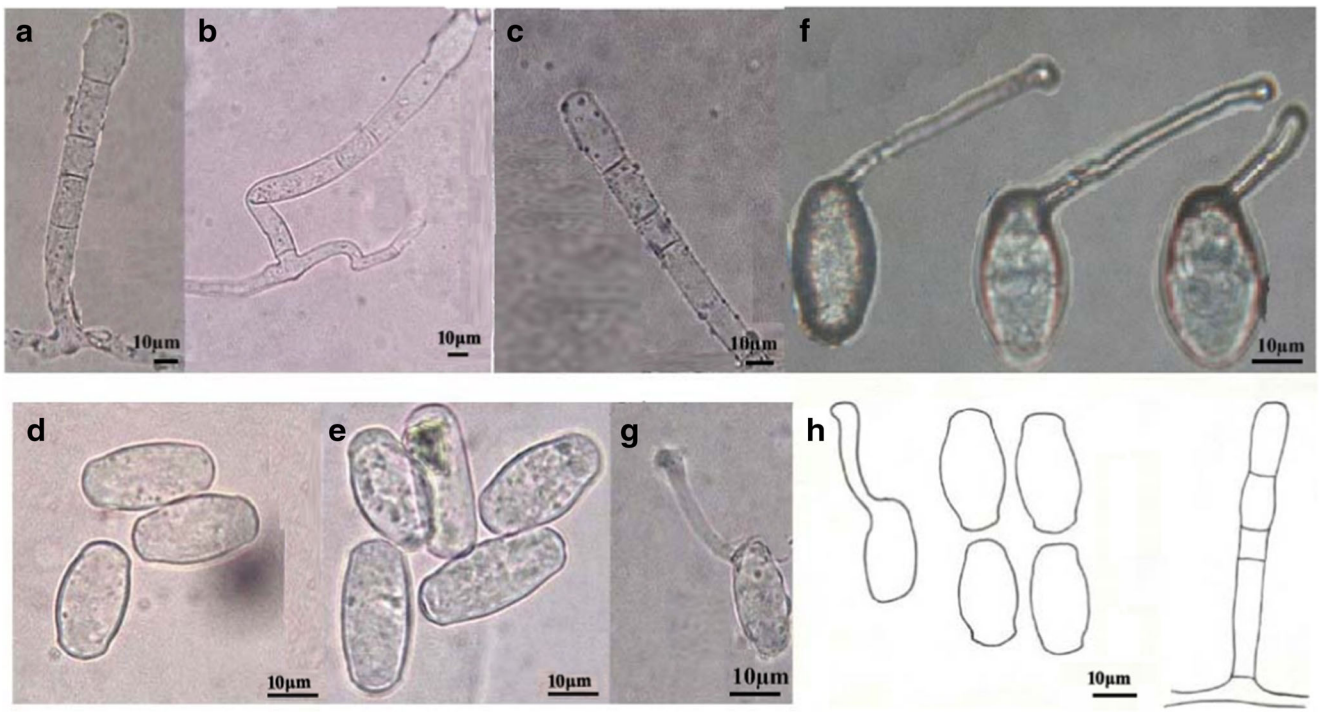

Fig. 5 *Golovinomyces ambrosiae* **a**, **b**, **c**: Conidiophores, **d**, **e** Conidia, **f**, **g** Conidia germination, **h** Drawing conidia, conidium germination & conidiophore; Scale bar = 10 µm

and Cook 2012) belong to a single clade (Takamatsu et al. 2013 and this study). However, these two species are distinguished by conidium size and germination type (Braun et al. 2019). In *G. latisporus* conidia are wider than *G. ambrosiae*, such that length/width ratio in *G. latisporus* is <2 (mostly 1.4–1.6) but in *G. ambrosiae* it is 1.5–2 μm.

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