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# *Golovinomyces* powdery mildews on Asteraceae in the Czech Republic

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**Abstract:** Powdery mildews on the Asteraceae family were surveyed during 2007–2015 in the Czech Republic with the aim to increase our knowledge about occurrence, morphological characteristics and host specificity of powdery mildews on this family. In total, 32 host species with symptoms of powdery mildew were collected, and the fungal species were identified based on microscopic observations. These showed great variability in their morphological characteristics. Our study confirmed the high host specificity of powdery mildew species to their original hosts. A deeper knowledge of the taxonomy of the Asteraceae has brought substantial changes in the delimitation of powdery mildew species. In particular, delimitation of the three varieties of *Golovinomyces asterum* was studied and discussed.

**Keywords:** *Aster*; *Symphyotrichum*; Erysiphaceae; anamorph and teleomorph stage; host specificity; ITS

Asteraceae (Compositae) represents the largest and most widespread family of flowering plants (Angiospermae). This family currently contains around 25 000–30 000 species, in over 1 600–2 000 genera (Funk et al. 2009). Traditionally, two subfamilies had been recognized, Asteroideae and Cichorioideae (Carlquist 1976; Wagenitz 1976). However, the latter was found paraphyletic, and thus has been divided into 11 subfamilies. It is apparent that the four subfamilies Asteroideae, Cichorioideae, Carduoideae, and Mutisioideae contain 99% of the species diversity of the entire family, in which the subfamily Asteroideae alone covers ca. 70% (Stevens 2001; Panero & Funk 2002; Funk et al. 2009).

Recent taxonomic studies have focused on many asteraceous plants; e.g., on details of the genus *Aster* (*Aster* spp.) including herbaceous annual and perennial plants from the subfamily Asteroideae, tribe Astereae. Recently, this genus was broken down into several small genera, uncovered by the use of molecular tools. One of these is the genus *Symphyotrichum*, which consists of 90 species, the majority of which are endemic to North America, although several occur in western India, Central and South America, as well as in eastern Eurasia. Many of these species have been introduced to Europe as garden specimens, primarily the New England aster, *Symphyotrichum novae-angliae* (Linnaeus) Nesom and the New York aster, *Symphyotrichum novi-belgii* (Linnaeus) Nesom (Morgan & Holland 2012).

Ornamental as well as wild asteraceous plants are often infected by powdery mildews, biotrophic asco-

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mycetes belonging to the order Erysiphales. In general, these fungi are easily recognized by white superficial mycelium growing primarily on leaves, stems, and less frequently on flowers and fruits of flowering plants (Angiospermae) (Glawe 2008). However, individual species can only be distinguished by detailed studies of microscopic characteristics, molecular data, and their host specificity. A very confusing issue is the fact that more than one powdery mildew species can parasitize a single host plant.

To better understand the situation as to why new powdery mildew species are still being described on hosts of the Asteraceae and other families, a brief insight into the history of the taxonomy of *Erysiphales* is needed. Most of the traditional taxonomy of powdery mildew was based on descriptions of the sexual stage (mainly appendages on chasmothecia, e.g. Jaczewski 1927; Blumer 1933; 1967; Salmon 1900, and also the older works of Braun 1987, 1995). Later, the works of Cook et al. (1997), Cook and Braun (2009), as well as the molecular works of Matsuda and Takamatsu (2003) and Takamatsu et al. (2008; 2010), showed that features of the asexual stage, e.g. conidial development and germination, are vitally important in the classification of genera of powdery mildews.

Considering the latest information summarized in the Taxonomic Manual of the *Erysiphales* (Braun & Cook 2012), it is clear that the number of described powdery mildew species has increased. In the beginning of the 20$^{th}$ century Salmon (1900) recognised 6 genera, 49 species, and 11 varieties of powdery mildew. The early work of Braun (1987) showed that many species, according to Salmon's classification, were too broadly defined. On the basis of many features (mainly the teleomorph but also the anamorph stage and host range), Braun (1987) distinguished 18 genera containing 515 accepted species; whilst the recent monograph by Braun and Cook (2012) reduced the genera to 15 but increased the accepted species to 873 (including 794 holomorphs, i.e. those with described asexual and sexual morphs).

For a long time *Golovinomyces cichoracearum* (S. Blumer) (previously named *Erysiphe cichoracearum*) was described as the predominant powdery mildew species infecting hosts of the family Asteraceae. This powdery mildew was considered to have a very wide host range, also infecting plants from the families Apocynaceae, Campanulaceae, Crassulaceae, Malvaceae, Papaveraceae, Solanaceae, Violaceae, etc. (Blumer 1933; Salmon 1900). Even in 1987 Braun stated that *G. cichoracearum* sensu lato was a complex of numerous formae speciales and cryptic species (Braun 1987). Hammett (1977) divided *G. cichoracearum* into the two groups – *G. cichoracearum* sensu stricto infecting only plants from the family Asteraceae, and *G. cichoracearum* s. lat. able to infect other hosts from various families. The second group was related to a plurivorous powdery mildew taxon previously referred to as *Erysiphe polyphaga* (Castagne) V.P. Heluta (nom. inval.). Braun (1995) gave this species its older valid name of *E. orontii* (now *G. orontii*). The first molecular study of *G. cichoracearum* was done by Zeller and Levy (1995) who analysed the diversity among field collections of *G. cichoracearum* from a variety of hosts, with RFLPs from a PCR amplified ribosomal DNA (rDNA) segment. The *G. cichoracearum* samples expressed six distinct RFLP haplotypes. Each haplotype was specific to either a single host or to a set of related host species.

Another interesting fact is that in his monograph Salmon (1900) only described *E.* (= *G.*) *cichoracearum* on Asteraceae. On the other hand, Braun (1995) also described other powdery mildew species on Asteraceae, e.g., *Leveillula* (*L. taurica*, *L. lactucarum* and *L. picridis* on the genera *Chondrilla*, *Lactuca* and *Picris* respectively), and also *Sphaerotheca*, as *Sphaerotheca fusca* (now *Podosphaera xanthii*) on *Dendranthema*. Additionally, some powdery mildews on Asteraceae forming conidia singly (*Pseudoidium* type) and chasmothecia with mycelioid appendages are still classified in the genus *Erysiphe,* specifically *E. mayorii* varieties *mayorii* and *cicerbitae* (on *Cirsium* and *Cicerbita*). Most other species previously described as *Erysiphe* on Asteraceae by Braun (1995) are of the *Euoidium* type (having conidiophores producing catenescent conidia maturing in chains), and many belonged to *Golovinomyces*, specifically *G. echinopis* (on *Echinops*), *G. helichrysi* (on *Helichrysum*), *G. depressus* (on *Arctium, Centaurea*), *G. artemisiae* (on *Artemisia*, rarely on *Achillea*), and *G. cichoracearum* with three varieties (*fischeri*, *latispora,* and *cichoracearum*) still occurring on a wide range of host species.

Later, Braun (1999) specified five varieties of *G. cichoracearum*, viz.: *cichoracearum*, *fischeri*, *latisporus*, *poonensis*, and *transvaalensis*, which have now been treated as separate species. *Golovinomyces cichoracearum* var. *latisporus* was renamed by Cook and Braun (2009) as *G. ambrosiae,* with a host range on *Ambrosia*, *Helianthus*, *Rudbeckia*, and *Zinnia*; and *G. c.* var. *fischeri* was renamed *Golovinomyces*

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*fischeri* infecting *Senecio* spp. (Cook & Braun 2009). *G. sonchicola* previously part of *G. cichoracearum* was revealed as a new species with a host range on *Sonchus* spp. (Cook & Braun 2009).

Braun and Cook (2012) defined *G. cichoracearum* s. str. as a species specialised on hosts of the family Asteraceae, subfamily Cichorioideae, and this was further supported by molecular sequence analysis (Matsuda & Takamatsu 2003). Nevertheless, this concept has also been reconsidered (Takamatsu et al. 2013). Braun and Cook (2012) recognised several *Golovinomyces* species infecting hosts of the Asteraceae family, starting with the formerly well-defined *G. ambrosiae*, *G. artemisiae, G. caulicola* (on Asteraceae/Cichorioideae) and *G. depressus,* all with wide host ranges, as well as those with more restricted ranges, i.e. *G. echinopis, G. leucheriae* (on Asteraceae/Carduoideae)*, G. pseudosepultus* (on Asteraceae/Astereae) and maintaining *G. sonchicola* as specific to *Sonchus* spp. This still left *G. orontii* with a very wide host range that included hosts of the Asteraceae family (e.g., *Chrysanthemum, Dahlia, Helianthus*). The same authors also introduced some new names for species within *G. cichoracearum* s. lat., as comb. nov., specifically *G. asterum* with three varieties: *asterum, moroczkovskii, solidaginis* (on *Aster, Solidago*)*,* as well as *G. circumfusus* (on Asteraceae/Eupatoriae), *G. franseriae* (on *Franseria*), *G. inulae* (on Asteraceae/Inuleae), *G. macrocarpus* (on Asteraceae/ Anthemideae), *G. montagnei* (on Asteraceae/Carduoideae), *G. poonaensis* (on *Goniocaulon*), *G. prenanthis* (on *Prenanthes*), *G. senecionis* (on Asteraceae/Senecioneae), and *G. spadiceus* (Asteraceae/Heliantheae). Later, *G. chrysanthemi* for powdery mildew on *Chrysanthemum* × *morifolium* was introduced by Bradshaw et al. (2017).

In the recent comprehensive study by Takamatsu et al. (2013), species delimitation within *Golovinomyces* based on molecular data (183 sequences) was re-evaluated. Of the 11 lineages recognized in this study, seven included the Asteraceae as the host family, when lineage XI consisted of isolates from a wide range of plant families involving 11 *Golovinomyces* species, situated at the most derived position in the tree. Powdery mildews on *Cichorium*, *Mycelis*, *Lactuca*, and *Taraxacum*, previously considered as *G. cichoracearum*, were now designated as *G. orontii* in this lineage. In a recent work of Braun et al. (2019), for powdery mildew infecting *Lactuca, Mycelis, Taraxacum* and others, a new name *Golovinomyces bolayi* sp. nov. was introduced. Anyway, the occurrence of co-speciation had clearly been suggested between *Golovinomyces* species and their asteraceous hosts in the early evolution of this genus (Takamatsu et al. 2013). Thus, the above-mentioned trend of more narrowly defined species has given rise to several new powdery mildew taxa. This was foretold by Braun (1995) who had stated that former *G. cichoracearum* s. str. was a mix of specialized forms and varieties.

Apart from some specialized studies focused on powdery mildew on *Lactuca* species (e.g., Lebeda & Mieslerová 2011; Lebeda et al. 2012, 2013; Mieslerová et al. 2013) and a survey of Asteraceae known in the Czech Republic summarised by Kubát et al. (2002), there was until now no detailed study of powdery mildews on Asteraceae in the Czech Republic. However, in the Slovak Republic (till end of 1992 part of former Czechoslovakia), a rather detailed survey of powdery mildews on Asteraceae was made by Paulech (1995). The aim of our recent work was to elucidate the complexity of powdery mildews infecting representatives of the family Asteraceae in the Czech Republic, with an emphasis on *Golovinomyces asterum*.

## MATERIAL AND METHODS

**Field survey.** Between 2007 and 2015 a survey of powdery mildews was done on medicinal, ornamental and wild plants in botanical gardens in the Czech Republic. For purposes of this manuscript, samples of those powdery mildews infecting the Asteraceae family were collected and microscopically analysed.

**Microscopic analysis of morphological characteristics.** Pieces (cca 20 × 20 mm) of severely infected leaves were used for evaluation by light microscopy (Olympus BX60, Japan). The pathogen was not separated from the host tissue and microscopy was done on leaf segments fixed in glacial acetic acid (acetic acid 99%; Lach-Ner, the Czech Republic), for 48 h and stored in glycerol (Glycerolum 85%; Tamda, the Czech Republic). Conidia and conidiophores, mostly on upper leaf surfaces, were examined microscopically after staining with cotton blue (methyl blue, aniline blue; Sigma-Aldrich, USA) (Lebeda & Reinink 1994). The presence of fibrosin bodies in the conidia was assessed by mounting fresh conidia in 3% KOH (potassium hydroxide 90%, Fichema, the Czech Republic) (Lebeda 1983). The sexual morph (chasmothecia) was inspected without any

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staining. In cases where dry leaf samples were analysed, a modified method of Shin (2000) was used; i.e., the heating of herbariumised tissues in fuchsine (Sigma-Aldrich, USA) in lactic acid (80%; Lach-Ner, the Czech Republic). For statistical analyses (means, standard deviations, and range), 30 measurements of each of the characteristics (when possible) were calculated using MS Excel (version 2010).

**Cross-inoculation tests**. Of the entire set of samples, four isolates were selected and maintained long-term on their original hosts by sequential re-inoculations: i.e., *Golovinomyces asterum* var. *asterum* isolate GC/AN/12 originating from *Symphyotrichum* (*Aster*) *novi-belgii* (in the Rosarium, Olomouc, the Czech Republic) was maintained on *S. novi-belgii* (genotype 84/98; seeds orig. Hortus Botanicus, Masaryk University, Brno, the Czech Republic), *G. cichoracearum* isolate GC/HA/12 originating from *Hieracium aurantiacum* (Paseka, the Czech Republic) on *H. aurantiacum* (genotype 89/377; seeds orig. Botanical Garden East Lansing, Michigan, USA), *G. bolayi* isolate GC/LS/11 originating from *Lactuca serriola* (Olomouc, the Czech Republic) on *L. serriola* (genotype LSE/57/15; seeds orig. Department of Botany, Faculty of Science, Palacký University, Olomouc, the Czech Republic), and *G. asterum* var. *solidaginis* isolate GC/SG/12 originating from *Solidago gigantea* (Rosarium, Olomouc, the Czech Republic) on *S. gigantea* (genotype 87/386; seeds orig. University of Wroclaw, Wroclaw, Poland). These fungal isolates were maintained under plastic covers (30 × 30 × 80 cm) in a glasshouse under a day/night temperature of 22/20 °C and ambient light conditions supplemented with 12 h artificial lighting (if needed). These isolates were re-inoculated onto new plants every 3–4 weeks.

For the cross-inoculation tests, leaf discs (14 mm in diameter) were cut from the true leaves of the three respective plant species from the family Asteraceae with a cork borer (the list of tested plant species with their places of origin is shown in Table 1). The leaf discs were oriented with the adaxial surface uppermost and placed in Petri dishes on wet cellulose cotton wool and filter paper. Leaves of the host plants covered by fresh (10–14 days old) sporulating mycelium were used as the source of inoculum. The upper surface of each leaf disc was inoculated by surface contact (dusting/tapping) with leaves bearing conidia of the powdery mildew species under test. After inoculation, the Petri dishes were incubated in a growth chamber at 15–18 °C, 12 h photoperiod, relative humidity 60–70% and light intensity of 100 μm/m$^2$ per second. From each of the three plants, five leaf discs were used for the tests. In addition, two leaf discs of the host species of each isolate served as susceptible controls.

The degree of susceptibility of the tested plant species to each powdery mildew isolate was evaluated macroscopically on the 7$^{th}$ and 14$^{th}$ day after inoculation (7 and 14 dpi). For assessment of the infection degree (ID), a 0–3 scale was used: 0 – without symptoms of pathogen development; 1 – mild development of mycelium without sporulation; 2 – intensive sporulation and well-developed mycelium covering < 50% of leaf disc area; 3 – intensive sporulation and well-developed mycelium covering 50–100% of the leaf disc area. The percentage of maximum infection degree (% max ID) was calculated for each interaction from data recorded on the last day of assessment (i.e., 14 dpi), according to the formula proposed by Towsend and Heuberger (1943):

$$\%\ \text{max ID} = \sum \frac{(n \times v) \times 100}{x \times N} \quad (1)$$

where: $n$ – number of leaf discs in each scale of infection degree (0–3); $v$ – infection degree; $x$ – number of infection degrees (i.e. 3); $N$ – total number of evaluated leaf discs.

The % max. ID was calculated for individual interactions and categorized as follows: R – resistant ≤ 30, MR – moderately resistant 31–60, and S – susceptible 61–100. The means were then calculated for each plant/powdery mildew interaction.

**Study of initial infection stages of powdery mildews.** The microscopic study of the initial infection stages of the four powdery mildew isolates was conducted on leaf discs of the following host plants: *S. novi-belgii*, *H. aurantiacum*, *L. serriola*, and *S. gigantea* as described above. Leaf discs were examined at 24, 48, and 72 h post inoculation (hpi), and at 9 dpi. They were prepared for microscopic examination as described above. Pathogen infection structures were stained with cotton blue (1%), and observed with a BX60 light microscope equipped with a DP70 CCD camera (Olympus, Japan). Five leaf discs from two to three plants were collected for each incubation period. Germination of conidia was assessed at 24 hpi as % of conidia producing true germ tubes per 100 conidia per leaf disc. The initial stages of infection were recognized as described by Cook et al. (2015) for *Erysiphe* spp., where 'secondary germ tubes' or colony forming hyphae

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Table 1. Results of cross-inoculation experiments of powdery mildew isolates originating from *Symphyotrichum* (*Aster*) *novi-belgii, Hieracium aurantiacum, Lactuca serriola* and *Solidago gigantea* (shaded cells indicate compatible reaction)

| Host genotype | Origin of seeds | Powdery mildew isolate (response category/% max ID) | | | |
|---|---|---|---|---|---|
| | | GC/AN/12[a] | GC/HA/12[b] | GC/LS/11[c] | GC/SG/12[d] |
| *Achillea millefolium* (49/66) | University of Salzburg, Salzburg, Austria | R[e] (0) | nt[f] | R[e] (0) | R[e] (0) |
| *Symphyotrichum* (*Aster*) *novi-belgii* (84/98) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | S[f] (62.9) | R[e] (0) | R (0) | R (0) |
| *Bidens tripatrita* (87/34) | University of Wroclaw, Wroclaw, Poland | nt | nt | R (3.7) | nt |
| *Calendula officinalis* (84/104) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (3.7) | R (0) | R (3.7) | R (0) |
| *Centaurea cyanus* (84/90) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | nt | nt | R (3.7) | nt |
| *Centaurea jacea* (40/839) | Kärtner Botanikzentrum, Klagenfurt, Austria | nt | nt | R (0) | nt |
| *Cichorium intybus* (40/56) | Kärtner Botanikzentrum, Klagenfurt, Austria | nt | nt | R (0) | nt |
| *Crepis biennis* (49/127) | University of Salzburg, Salzburg, Austria | R (0) | nt | R (3.7) | R (3.7) |
| *Eupatorium cannabinum* (84/18) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | nt | nt | R (0) | nt |
| *Galinsoga parviflora* (49/156) | University of Salzburg, Salzburg, Austria | R (0) | nt | R (0) | R (0) |
| *Hieracium aurantiacum* (89/377) | Botanical Garden East Lausing, Michigan, USA | R (0) | S (66.7) | R (0) | R (0) |
| *Hieracium murorum* (89/378) | Botanical Garden East Lausing, Michigan, USA | nt | nt | R (18.5) | nt |
| *Hieracium pilosella* (75/75) | Botanical Garden der Universität Osnabrück, Osnabrück, Germany | nt | nt | R (0) | nt |
| *Hypochaeris radicata* (84/74) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (0) | nt | R (0) | R (0) |
| *Chondrilla juncea* (84/115) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | nt | nt | R (3.7) | nt |
| *Inula helenium* (84/124) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (0) | nt | R (7.4) | R (3.7) |
| *Lactuca serriola* (LSE/57/15) | Department of Botany, Palacky University, Olomouc, the Czech Republic | R (0) | R (3.7) | S (74) | R (0) |
| *Leucanthemum vulgare* (84/498) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | nt | nt | R (0) | nt |
| *Mycelis muralis* (84/71) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (3.7) | nt | R (25.9) | R (0) |
| *Prenanthes purpurea* (40/854) | Kärtner Botanikzentrum, Klagenfurt, Austria | nt | nt | R (0) | nt |
| *Rudbeckia hirta* (84/710) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (0) | nt | R (0) | R (3.7) |
| *Senecio sylvaticus* (40/138) | Kärtner Botanikzentrum, Klagenfurt, Austria | nt | nt | R (0) | nt |
| *Senecio vulgaris* (84/70) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | nt | nt | R (3.7) | nt |
| *Solidago gigantea* (87/386) | University of Wroclaw, Wroclaw, Poland | R (0) | R (0) | R (0) | S (62.9) |
| *Solidago virgaurea* (84/166) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (0) | nt | R (11.1) | R (7.4) |
| *Tanacetum corymbosum* (10/84) | Hortus Botanicus Tallinnensis, Tallinn, Estonia | nt | nt | R (3.7) | nt |
| *Tanacetum vulgare* (84/171) | Hortus Botanicus, Masaryk University, Brno, the Czech Republic | R (0) | nt | R (0) | R (0) |
| *Tragopogon orientalis* (49/239) | University of Salzburg, Salzburg, Austria | nt | nt | R (14.8) | nt |

[a]*Golovinomyces asterum* var. *asterum* isolate originating from *Symphyotrichum* (*Aster*) *novi-belgii* (Olomouc, Czech Republic); [b]*Golovinomyces cichoracearum* isolate originating from *Hieracium aurantiacum* (Paseka, Czech Republic); [c]*Golovinomyces bolayi* isolate originating from *Lactuca serriola* (Olomouc, Czech Republic); [d]*Golovinomyces asterum* var. *solidaginis* isolate originating from *Solidago gigantea* (Rosarium, Olomouc, Czech Republic); R[e] – resistant; S[f] – susceptible; nt – not tested

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Table 2. Primers used for amplification of nuclear ribosomal DNA region (ITS1-5.8S-ITS2; ITS) and D1/D2 domains of the large subunit (28S) of nrDNA (the primers used for sequencing are highlighted with *)

| | | Primer name | Primer sequence | Reference |
|---|---|---|---|---|
| **ITS region (ITS1-5.8S-ITS2; ITS)** | | | | |
| 1st PCR | forward primer | PM-ITS1 | 5' - TCGGACTGGCCYAGGGAGA - 3' | Cunnington et al. (2003) |
| | reverse primer | PM-ITS2 | 5' - TCACTCGCCGTTACTGAGGT - 3' | Cunnington et al. (2003) |
| 2nd PCR | forward primer | ITS1F* | 5'- CTTGGTCATTTAGAGGAAGTAA - 3' | Gardes & Bruns (1993) |
| | reverse primer | ITS4* | 5' - TCCTCCGCTTATTGATATGC - 3' | White et al. (1990) |
| **D1/D2 domains of 28S LSU** | | | | |
| 1st PCR | forward primer | PM3 | 5' - GKGCTYTMCGCGTAGT - 3' | Takamatsu & Kano (2001) |
| | reverse primer | TW14 | 5' - GCTATCCTGAGGGAAACTTC - 3' | Mori et al. (2000) |
| 2nd PCR | forward primer | NL1* | 5' - AGTAACGGCGAGTGAAGCGG - 3' | Mori et al. (2000) |
| | reverse primer | TW14* | 5' - GCTATCCTGAGGGAAACTTC - 3' | Mori et al. (2000) |

(CFH) (Micali et al. 2008) were produced after the terminal appressorium of the true germ tube had successfully penetrated a host cell. The number of CFH was assessed on 20–30 germinated conidia on each leaf disc to give a minimum of 100 conidia for each accession and incubation period. The intensity of sporulation of powdery mildews on leaf discs was assessed at 9 dpi for each leaf disc, and this was expressed semi-quantitatively (0; < $10^1$; $10^1$–$10^2$; $10^2$–$10^3$; > $10^3$) (Mieslerová et al. 2004).

**Sequencing.** Molecular analyses were performed for powdery mildews infecting *Symphyotrichum novi-belgii*, *Solidago gigantea* and *Solidago* sp. Total DNA was extracted from fungal mycelium scraped from the leaves given specific herbarium vouchers [OL 34144 (*S. novi-belgii*), 37858 (*S. gigantea*), 37859 (*Solidago* spp.)] using the SDS extraction method (Edwards et al. 1991). Concentration of DNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA), and kept at –80 °C until used for further analysis. A part of the nuclear ribosomal DNA region (ITS1-5.8S-ITS2; ITS) and D1/D2 domains of the large subunit (28S) of nrDNA were amplified using nested PCR as described in Cunnington et al. (2003) and Takamatsu et al. (2013) (for details see Table 2). PCRs were conducted in a 20 µL reaction volume containing 1.1 µL of DNA (50 ng/µL), 0.8 µL of each primer (10 µM), 2 µL of 10× Reaction Buffer "B", 0.4 µL of 10 mM dNTP's, 0.08 µL of KAPA DNA Polymerase (Kapa Biosystems, USA), and 14.82 µL of PCR grade water. PCR was carried out in an Eppendorf Mastercycler (Eppendorf, Germany) using the following conditions: 5 min at 94 °C; 35 cycles of 45 s at 94 °C, 45 s at 60 °C for 1st PCR or 55 °C for 2nd PCR, 1 min at 72 °C, and a final extension (10 min at 72 °C). The PCR products were cleaned using a GenElute PCR Clean-Up Kit (Sigma-Aldrich, USA), and sequenced from both directions (Macrogene Europe, the Netherlands). Geneious 7.1.8 (Biomatters Ltd., New Zealand) was used for contig assembly from partial reads, as well as for the editing of base calls and concatenation of partial genomic regions. The resulting nucleotide sequences have been deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/; accession numbers: ITS – KY347819, MK953714, MK953715; 28S - MK955450, MK955451), and were used to search against the NCBI database using BLAST. Subsequently, all sequences having an identity value ≥ 99 % were compared in MEGA 6 software (version 6) (Tamura et al. 2013).

## RESULTS

**Field survey**. During the period 2007–2015, a total of 32 host species from the family Asteraceae with powdery mildew symptoms were collected (Figure 1). A list of host plants complemented with recently valid names of powdery mildews according to Takamatsu et al. (2013), Braun et al. (2019) and previously valid names according to Braun (1995) and Braun and Cook (2012) is shown in Table 3. All species had been previously recorded in Europe except *G. asterum* var. *asterum* so far only recorded in America and Asia.

From our field survey it is clear that the upper sides of the leaves were most frequently attacked; and also the stems and petioles to a lesser extent. No visible infection was recorded on the flowers or achenes. All plants were found to be more or less seriously at-

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Figure 1. Symptoms of powdery mildew infection on representatives of Asteraceae family: (A) *Achillea nobilis*, (B) *Arctium lappa*, (C) *Artemisia absinthium*, (D) *Symphyotrichum* (*Aster*) *novi-belgii*, (E) *Centaurea montana*, (F) *Cicerbita alpina*, (G) *Cirsium arvense*, (H) *Echinops sphaerocephalus*, (I) *Helianthus tuberosus*, (J) *Hieracium aurantiacum*, (K) *Lactuca serriola*, (L) *Prenanthes purpurea*, (M) *Senecio fuchsii*, (N) *Solidago hybrida* var. *nana*, (O) *Sonchus oleraceus*, (P) *Tanacetum corymbosum*, (R) *Taraxacum* sect. *Ruderalia*, (S) *Zinnia elegans*

tacked during the summer (July, August) until early autumn (September).

**Microscopic analysis of morphological characteristics.** Basic morphological characteristics of powdery mildews on hosts of the Asteraceae family are described in Table 4, and photos of the basic morphological structures are shown in Figures 2 and 4 and line drawings in Figure 3. Figure 3B of *G. asterum* var. *moroczkovskii* could be mistaken for a *Pseudoidium* type, i.e. a foot cell bearing three other cells followed by a swelling conidium. However, this non-catenescent pattern is sometimes seen alongside catenescent conidiophores in *Euoidium* species, see the drawing of the conidiophores of this particular species in Braun and Cook (2012).

From Table 4 it is obvious that some *Euoidium* anamorphs parasitizing the Asteraceae and now distinguished from *G. cichoracearum* have very different morphological features; e.g., *G. depressus* with very long conidiophores and foot-cells; *G. inulae*, *G. echinopis* with very long and slim conidia, the same as in *G. macrocarpus*. Some species were confirmed with a length/width ratio under 2; e.g., some samples of *G. ambrosiae, G. depressus,* and *G. fischeri*. However, morphological variability within the original *G. cichoracearum* and *G. orontii* was still found to be relatively large; as same as within *G. artemisiae*, viz. conidial width of the pathogen on *Artemisia absinthium* was clearly greater than its width on *A. dracunculus*. In our results even varieties of *G. asterum* showed some obvious differences, *e.g.* conidial width of var. *asterum* was smaller on *Symphyotrichum novae-angliae* than that of var. *solidaginis* on *S. canadensis* (their ranges did not overlap). This difference occurred between the two varieties that were most closely related to each other phylogenetically (Takamatsu et al. 2013).

Only the asexual morph was recorded in most samples, except for six samples containing the sexual morph. Chasmothecial diameters differed slightly; appendages were mycelioid, mostly unbranched and variable in number and length; the

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Table 3. List of representatives of Asteraceae family infected by powdery mildews collected during 2007–2015 in the Czech Republic

| Host species | Place of collection | Date of collection | Tax. name of powdery mildew according to Braun (1995) | Tax. name of powdery mildew according to Braun and Cook (2012)* |
|---|---|---|---|---|
| *Achillea nobilis* | Praha | 09/2011 | *E. cichoracearum* var. *cichoracearum* | *G. macrocarpus* |
| *Arctium lappa* | Vernířovice | 08/2008 | *E. depressa* | *G. depressus* |
| *Artemisia absinthium* | Praha | 09/2011 | *E. artemisiae* | *G. artemisiae* |
| *Artemisia dracunculus* | Brno | 09/2015 | *E. artemisiae* | *G. artemisiae* |
| *Artemisia vulgaris* | Olomouc | 10/2013 | *E. artemisiae* | *G. artemisiae* |
| *Symphyotrichum dumosum* (*Aster dumosus*) | Olomouc | 09/2013 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *moroczkowskii* |
| *Symphyotrichum* (*Aster*) *novi-belgii* | Olomouc | 09/2008 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *asterum* |
| *Symphyotrichum* (*Aster*) *novae-angliae* | Praha | 09/2011 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *moroczkowskii* |
| *Symphyotrichum dumosum* (*Aster dumosus*) | Praha | 09/2015 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *moroczkowskii* |
| *Calendula officinalis* | Olomouc | 09/2008 | *E. cichoracearum* var. *cichoracearum* and *Podosphaera xanthii* | Only *Podosphaera xanthii* |
| *Centaurea montana* | Olomouc, Prostějov | 05/2009, 08/2015 | *E. depressa* | *G. depressus* |
| *Cicerbita alpina* | Paseka | 08/2011 | *E. cichoracearum* var. *cichoracearum* | *G. cichoracearum* |
| *Cirsium arvense* | Olomouc | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *G. montagnei* |
| *Dahlia* spp. | Smržice | 08/2015 | *E. cichoracearum* var. *cichoracearum* | *G. orontii; G. spadiceus* |
| *Echinops* spp. | Jičín, Huslenky | 08/2015 | *E. echinopis* | *G. echinopis* |
| *Erigeron canadense* | Olomouc | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *Podosphaera erigerontis-canadensis* |
| *Helianthus tuberosus* | Olomouc, Brno | 08/2008 10/2010 | *E. cichoracearum* var. *latispora* | *G. ambrosiae (G. latisporus[c])* |
| *Helianthus giganteus* | Praha | 09/2011 | *E. cichoracearum* var. *latispora* | *G. ambrosiae (G. latisporus[c])* |
| *Hieracium aurantiacum* | Paseka | 08/2011 | *E. cichoracearum* var. *cichoracearum* | *G. cichoracearum* |
| *Inula magnifica* | Brno | 09/2015 | *E. cichoracearum* var. *cichoracearum* | *G. inulae* |
| *Lactuca serriola* | Olomouc | 2008, 2009 | *E. cichoracearum* var. *cichoracearum* | *G. orontii*[a], *G. bolayi*[b] |
| *Lapsana communis* | Karlov | 08/2015 | *E. cichoracearum* var. *cichoracearum* | *G. cichoracearum* and uncertain species[a] |
| *Mycelis muralis* | Vernířovice | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *G. orontii*[a], *Golovinomyces bolayi*[b] |
| *Prenanthes purpurea* | Vernířovice | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *G. prenanthis* |
| *Senecio ovatus* | Vernířovice | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *G. senecionis* or *G. fischeri* |
| *Solidago canadensis* | Olomouc | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *solidaginis* |
| *Solidago gigantea* | Olomouc | 08/2008 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *solidaginis* |
| *Solidago hybrida* var. *nana* | Praha | 09/2011 | *E. cichoracearum* var. *cichoracearum* | *G. asterum* var. *solidaginis* |
| *Sonchus oleraceus* | Olomouc | 08/2008; 10/2013 | *E. cichoracearum* var. *cichoracearum* | *G. sonchicola* |
| *Tanacetum corymbosum* | Protivanov | 08/2015 | *E. cichoracearum* var. *cichoracearum, E. orontii* | *G. macrocarpus* |
| *Taraxacum sect. Ruderalia* | Olomouc, Huslenky | 09/2013, 09/2015 | *E. cichoracearum* var. *cichoracearum* | *G. orontii*[a], *G. bolayi*[b] |
| *Zinnia elegans* | Olomouc | 09/2015 | *E. cichoracearum* var. *cichoracearum* | *G. spadiceus (G. ambrosiae[c])* |

[a]Takamatsu et al. (2013); [b]Braun et al. (2019); [c]Qiu et al. (2020, where species names differ); unchanged names are in grey cells; the only new record in the area of the Czech Republic is *Golovinomyces asterum* var. *asterum;* *unless otherwise indicated in footnotes

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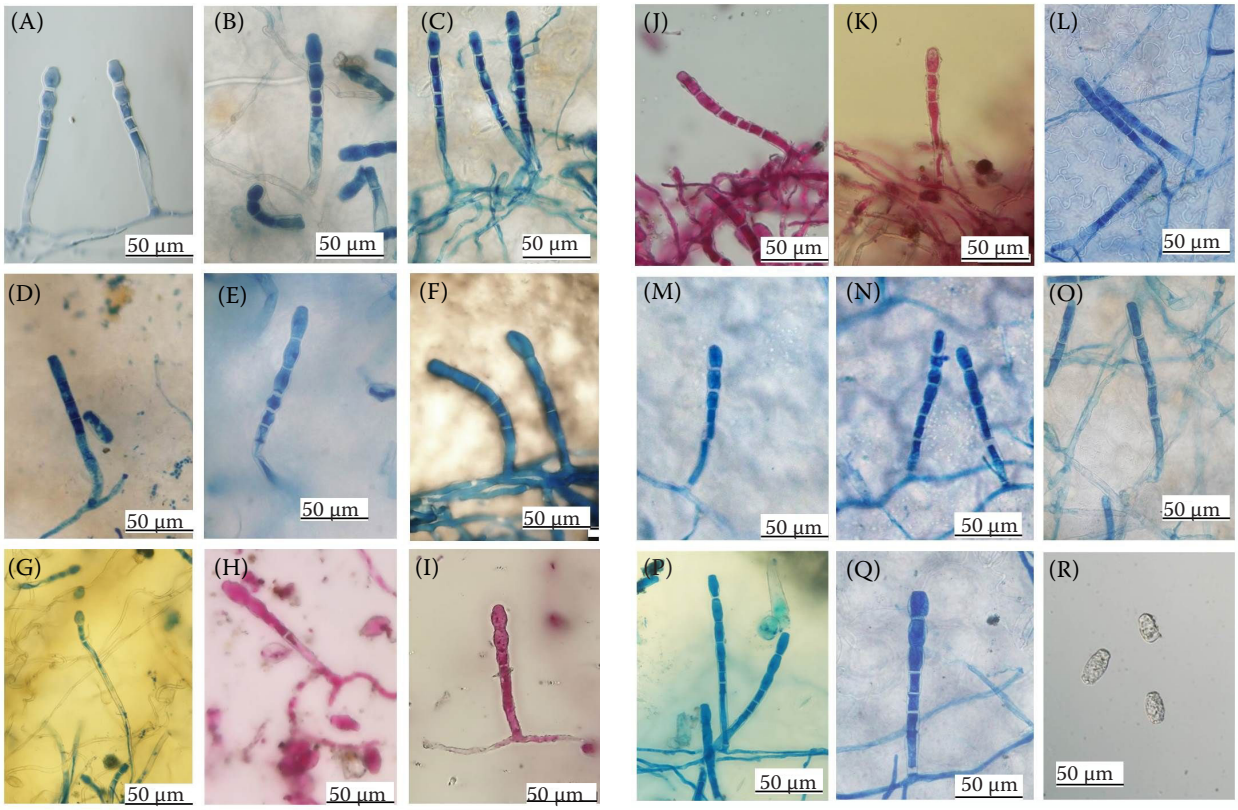


Figure 2. Conidiophores of *Golovinomyces* (and *Podosphaera*) species occurring on the Asteraceae: (A) *G. ambrosiae* (*G. latisporus*) (on *Helianthus tuberosus*); (B) *G. artemisiae* (on *Artemisia vulgaris*); (C) *G. asterum* var. *asterum* (on *Symphyotrichum novi-belgii*); (D) *G. asterum* var. *moroczkovskii* (on *Symphyotrichum novae-angliae*); (E) *G. asterum* var. *solidaginis* (on *Solidago gigantea*); (F) *G.* cf. *cichoracearum* (on *Hieracium aurantiacum*); (G) *G. depressus* (on *Arctium lappa*); (H) *G. echinopis* (on *Echinops* sp.); (I) *G. inulae* (on *Inula magnifica*); (J) *G. macrocarpus* (on *Tanacetum corymbosum*); (K) *G. montagnei* (on *Cirsium arvense*); (L) *G. bolayi* (on *Lactuca serriola*); (M) *G. prenanthis* (on *Prenanthes purpurea*); (N) *G. senecionis* (on *Senecio ovatus*); (O) *G. sonchicola* (on *Sonchus oleraceus*); (P) *G. spadiceus* (*G. ambrosiae*) (on *Zinnia elegans*); (Q–R) *Podosphaera xanthii* (on *Calendula officinalis*)

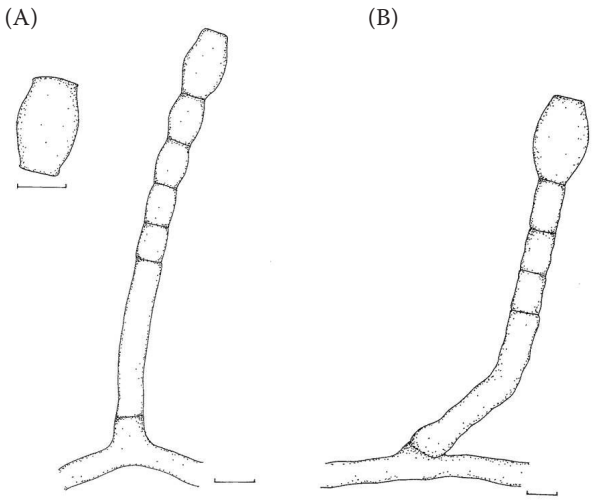


Figure 3. Comparison of foot-cell arrangements of (A) *G. asterum* var. *asterum* and (B) *G. asterum* var. *moroczkovskii* (bars represent 10 µm)

only markedly different species was *G. depressus*, with depressed chasmothecia (Figure 4B). Thus, the separation of species from the previously complex *G. cichoracearum* was hardly feasible, based on morphological characteristics alone. In many cases species delimitation was only based on small differences and characteristics which are difficult to distinguish especially when the sexual morph was not available.

**Cross inoculation tests.** The results of cross-inoculation tests of powdery mildew isolates originating from *L. serriola*, *S. gigantea*, *S. novii-belgii*, and *H. aurantiacum* showed that each powdery mildew isolate was very host specific, and was able to successfully infect only the plant species from which it was collected. Differences were obvious also from the macroscopic observations in the cross-inoculation tests (Table 1), and as they were from the microscopic observations (Table 5 and Figure 5). Powdery mildew

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Table 4. Morphological characteristics of asexual and sexual stages of powdery mildew samples collected on hosts of Asteraceae family [mean ± SD (min–max)]

| Putative *Golovinomyces* sp. | Host species | Collection place, date | Conidial length (µm) | Conidial width (µm) | L/W ratio | Foot-cell length (µm) | Conidiophores length (µm) | No. of distal cells | Chasmothecial diameter | No. of appendages | Length of appendages | Ascus dimensions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Golovinomyces cf. ambrosiae** | *Helianthus tuberosus* | Olomouc 08/2008 | – | – | – | – | – | – | 104.5 ± 6.9 (95.2–114.7) | 29.4 ± 6.3 (17–36) | 65.2 ± 20.5 (36.6–87.8) | – |
| *Golovinomyces cf. ambrosiae** | *Helianthus tuberosus* | Olomouc 09/2013 | 26.94 ± 2.06 (22.5–30) | 14.63 ± 1.51 (11.25–18.8) | 1.87 ± 0.28 (1.4–2.7) | 51.3 ± 10.8 (30–75) | 122.8 ± 22.2 (78.8–161.3) | 3.73 ± 0.87 (2–6) | – | – | – | – |
| *Golovinomyces cf. ambrosiae** | *Helianthus giganteus* | Praha 09/2011 | 30.25 ± 2.4 (27.5–37.5) | 14 ± 1.25 (12.5–15) | 2.18 ± 0.31 (1.82–3) | 50.75 ± 19.37 (17.5–87) | 124.67 ± 25.35 (82.5–175) | 3.63 ± 1 (1–6) | 122.19 ± 18.8 (80–150) | more than 30 | 30.8 ± 5.07 (26–37) | – |
| *Golovinomyces artemisiae* | *Artemisia absinthium* | Praha 09/2011 | 30.92 ± 2.22 (27.5–37.5) | 17.33 ± 1.46 (15–20) | 1.80 ± 0.21 (1.5–2.17) | 83.33 ± 27.16 (37.5–145) | 139.58 ± 31.16 (82.5–195) | 3.20 ± 0.55 (2–4) | – | – | – | – |
| *Golovinomyces artemisiae* | *Artemisia dracunculus* | Brno 09/2015 | 29.57 ± 1.59 (26.8–31.7) | 12.60 ± 0.78 (12.2–14.64) | 2.35 ± 0.16 (2–2.6) | 52.6 ± 10.8 (30.5–63.2) | 95.6 ± 15.26 (81.3–128.4) | 3.1 (2–4) | 152.20 ± 16.36 (129.32–180) | more than 20 | | 72.76 ± 4.6 × 30.14 ± 1.49 |
| *Golovinomyces asterum* var. *asterum* | *Symphyotrichum (Aster) novi-belgii* | Olomouc 09/2008 | 23.34 ± 3.31 (14.64–29.28) | 10.98 ± 1.37 (7.32–12.2) | 2.14 ± 0.28 (1.4–2.75) | 50.75 ±7.9 (36.6–73.2) straight foot-cell | 110.45 ± 15.94 (75.64–143.96) | 3.1 ± 0.94 (2–7) | – | – | – | – |
| *Golovinomyces asterum* var. *moroczkovskii* | *Symphyotrichum (Aster) novae-angliae* | Praha 09/2011 | 28.25 ± 2.14 (22.5–30) | 11.94 ± 1.67 (7.5–15) | 2.41 ± 0.35 (2–3.5) | 55.31 ± 7.25 (41.25 ± 71.3) curved foot-cell | 131.63 ± 26.17 (86.25–225) | 3.83 ± 1.09 (2–8) | – | – | – | – |
| *Golovinomyces asterum* var. *solidaginis* | *Solidago gigantea* | Olomouc 08/2008 | 26.75 ± 4.24 (14.64–34.16) | 12.4 ± 0.96 (9.76–14.64) | 2.14 ± 0.27 (1.5–2.6) | 33.18 ± 5.47 (24.4–43.92) | 120.61 ± 22.68 (61–161.04) | 4.86 ± 0.95 (2–6) | – | – | – | – |
| *Golovinomyces asterum* var. *solidaginis* | *Solidago canadensis* | Olomouc 08/2008 | 32.23 ± 2.93 (26.2–40.1) | 16.3 ± 1.48 (14–20) | 1.99 ± 0.28 (1.53–2.87) | 49.09 ± 14.6 (28.3–70.2) | 127.82 ± 32.64 (98–200) | 3.38 ± 0.48 (3–4) | | | | |
| *Golovinomyces cichoracearum sensu lato* | *Cicerbita alpina* | Paseka 08/2011 | 22.04 ± 2.98 (12.2–24.4) | 10.32 ± 1.36 (7.32–12.2) | 2.15 ± 0.29 (1.6–2.5) | 40.42 ± 12. 11 (24.4–73.2) | 119.23 ± 23.47 (78.08–175.7) | 4.66 ± 0.86 (2–6) | – | – | – | – |
| *Golovinomyces cichoracearum sensu lato* | *Hieracium aurantiacum* | Paseka 08/2011 | 24.17 ± 5.54 (12.2–34.16) | 9.54 ± 2.42 (4.88–12.2) | 2.38 ± 0.39 (1.66–3) | 28.61 ± 4.30 (21.96–39.04) | 52.79 ± 6.92 (41.48–63.44) | 2.6 (1–4) | – | – | – | – |
| *Golovinomyces depressus* | *Arctium lappa* | Vernířovice 08/2008 | 26.02 ± 2.46 (19.52–29.28) | 14.88 ± 1.82 (9.76–17.08) | 1.76 ± 0.19 (1.42–2.2) | 109.9 ± 30.5 (63.5–168.4) | 192.27 ± 30.97 (114-234) | 3.26 ± 0.67 (2–5) | – | – | – | – |
| *Golovinomyces depressus* | *Centaurea montana* | Prostějov 08/2015 | 27.5 ± 1.45 (24.4–30.5) | 14.03 ± 0.8 (12.2–14.64) | 1.96 ± 0.16 (1.75–2.5) | 34.64 ± 4.73 (29.28–41.48) | 92.72 ± 14.88 (85.4–115.01) | 2.4 ± 0.49 (2–3) | 100.44 ± 17.44 (82.96–134.2) | more than 25 | – | 47.9 ± 9.62 × 28.4 ± 4.6 |
| *Golovinomyces echinopis* | *Echinops* spp. | Jičín 08/2015 | 30.72 ± 1.22 (28.06–32.94) | 14.52 ± 0.98 (13.42–15.86) | 2.12 ± 0.15 (1.92–2.45) | 116.93 ± 39.82 (73.2–207.4) | 198.95 ± 51.62 (122–341.6) | 2.84 ± 0.53 (2–4) | – | – | – | – |
| *Golovinomyces inulae* | *Inula magnifica* | Brno 09/2015 | 30.98 ± 0.80 (29.28–31.72) | 13.72 ± 1.21 (12.2–15.86) | 2.27 ± 0.20 (1.84–2.5) | 44.43 ± 9.18 (29.28–64.63) | 99.91 ± 20.14 (73.2–141.5) | 2.84 ± 0.74 (2–4) | – | – | – | – |

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Table 4. to be continued

| Putative *Golovinomyces* sp. | Host species | Collection place, date | Conidial length (µm) | Conidial width (µm) | L/W ratio | Foot-cell length (µm) | Conidiophores length (µm) | No. of distal cells | Chasmothecial diameter | No. of appendages | Length of appendages | Ascus dimensions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Golovinomyces macrocarpus* | *Achillea nobilis* | Praha 09/2011 | 29.5 ± 2.56 (26.25–37.5) | 14.06 ± 1.69 (11.3–16.9) | 2.13 ±0.33 (1.56–2.67) | 50.88 ± 12.21 (37.5–82.25) | 132 ± 20.6 (97.5–176.25) | 4.23 ± 0.68 (3–6) | – | – | – | |
| *Golovinomyces macrocarpus* | *Tanacetum corymbosum* | Protivanov 08/2015 | 30.09 ± 1.61 (26.84–32.94) | 14.34 ± 0.74 (13.42–15.86) | 2.10 ± 0.15 (1.69–2.45) | 34.21 ± 9.54 (21.96–65.88) | 100.26 ± 15.20 (68.32–129.32) | 3.59 ± 0.57 (3–5) | – | – | – | – |
| *Golovinomyces montagnei* | *Cirsium arvense* | Olomouc 08/2008 | 24.4 ± 4.36 (17.08–36.6) | 10.98 ± 1.5 (7.32–12.2) | 2.31 ± 0.74 (1.6–4.66) | 44.32 ± 15.5 (26.84–85.4) | 103.94 ± 26.6 (48.8–101.04) | 3.2 ± 1.19 (1–5) | – | – | – | – |
| *Golovinomyces spadiceus* | *Dahlia* | Smržice 08/2015 | 30.79 ± 1.67 (29.28–34.16) | 16.84 ± 0.81 (15.86–18.3) | 1.83 ± 0.13 (1.6–2.15) | 52.28 ± 11.72 (29.28–73.2) | 137.45 ± 16.52 (109.8–170.8) | 3.85 ± 0.64 (3–5) | – | – | – | – |
| Golovinomyces bolayi[a] | *Lactuca serriola* | Olomouc 08/2008 | – | – | – | – | – | – | 112.4 ± 11.40 (92.72–134.2) | 10.7 ± 3.4 (7–16) | 88.5 ± 33.5 (46.3–139.1) | – |
| *Golovinomyces bolayi*[a] | *Lactuca serriola* | Olomouc 09/2009 | 26.96 ± 1.55 (23.18–29.28) | 10.57 ± 1.31 (8.54–13.42) | 2.58 ± 0.3 (2.09–3.28) | 31.46 ± 11.07 (17.08–78.08) | 100.85 ± 21.46 (61 –80.56) | 3.37 ± 0.80 (2–5) | | | – | – |
| *Golovinomyces bolayi*[a] | *Mycelis muralis* | Vernířovice 08/2008 | 24.4 ± 4.77 (17.08–36.6) | 10.77 ± 1.56 (7.32–12.2) | 2.35 ± 0.83 (1.6–5) | 41.27 ± 7.99 (24.4–48.8) | 114.68 ± 22.86 (61–148.8) | 3.66 ± 1.17 (1–5) | – | – | – | – |
| *Golovinomyces bolayi*[a] | *Taraxacum officinale* | Paseka 09/2011 | 20.08 ± 5.69 (12.2–39.04) | 10.89 ± 1.37 (7.32–12.2) | 1.86 ± 0.56 (1.2–3.2) | 30.41 ± 6.71 (17.08–48.8) | 79.95 ± 28.31 (41.48–134.2) | 3.23 ± 2.17 (1–8) | – | – | – | – |
| *Golovinomyces prenanthis* | *Prenanthes purpurea* | Vernířovice 08/2008 | – | – | – | – | – | – | 95.97 ± 14.07 (68.32–112.24) | – | – | – |
| *Golovinomyces senecionis* | *Senecio ovatus* | Vernířovice 08/2008 | 23.01 ± 2.15 (19.52–29.28) | 12.6 ± 1.1 (9.76–14.64) | 1.84 ± 0.25 (1.33–2.5) | 42.45 ± 9.9 (21.96–61) | 145.26 ± 25.59 (43.92–183) | 4.23 ± 1.2 (1–6) | – | – | – | – |
| *Golovinomyces sonchicola* | *Sonchus oleraceus* | Olomouc 08/2008 | 22.93 ± 2.40 (19.52–26.84) | 11.87 ± 0.82 (9.76–12.2) | 1.94 ± 0.26 (1.6–2.5) | 35.46 ± 9.14 (17.08–53.68) | 148.75 ± 27.62 (78.08–190.3) | 6.3 ± 1.5 (3–9) | – | – | – | – |
| *Golovinomyces spadiceus*** | *Zinnia elegans* | Olomouc 09/2008 | 29.64 ± 1.68 (26.84–34.16) | 12.75 ± 0.81 (10.98–14.64) | 2.33 ± 0.18 (2–2.8) | 64.9 ± 10.97 (48.8–92.72) | 137.49 ± 29.41 (95.16–195.2) | 3.2 ± 0.51 (2–4) | – | – | – | – |
| *Podosphaera xanthii* | *Calendula officinalis* | Olomouc 09/2008 | 20.74 ± 3.76 (12.2–26.84) | 11.87 ± 1.9 (7.32–17.08) | 1.76 ± 0.3 (1.2–2.5) | 28.95 ± 7.2 (17.08–43. 92) | 101.17 ± 37.04 (31.72–158.6) | 4.46 ± 2.04 (1–8) | – | – | – | – |
| *Podosphaera erigerontis-canadensis* | *Erigeron canadense* | Olomouc 08/2008 | 21.63 ± 3.31 (14.64–26.84) | 13.17 ± 2.23 (7.32–14.64) | 1.67 ± 0.25 (1.33–2.2) | 33.67 ±8.07 (19.52–51.24) | 135.4 ± 44.7 (58.56–212.3) | 5.86 ± 1.8 (3–9) | – | – | – | – |

Species names were inferred based on Braun and Cook (2012); apart from – [a]based on Braun et al. (2019); *according to Qiu et al. (2020) described as *G. latisporus*; **according to Qiu et al. (2020) described as *G ambrosiae*; L/W – the length/width ratio of conidia; isolates of the same species are in shaded-rows

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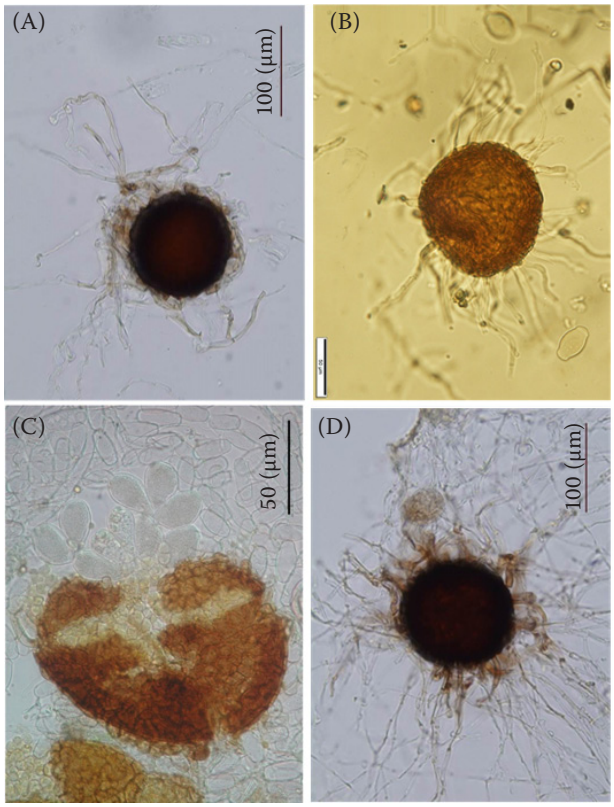


Figure 4. Chasmothecia of species of *Golovinomyces*: (A) *G. bolayi* (on *Lactuca serriola*); (B) *G. depressus* (on *Arctium lappa*); (C) *G.* cf. *orontii* (on *Cucurbita pepo*); (D) *G. sonchicola* (on *Sonchus oleraceus*)

isolates did not show any significant specificity of conidial germination to their hosts. The max ID ranged from 62.9 to 74% in host species from which the powdery mildew isolate originated; on other hosts it was 0 or just slightly above 0.

**Study of initial infection stages of powdery mildews.** Germination was measured 24 hpi (Table 5) and at each combination (accession/incubation period) some percentage of germinated conidia was observed. Although only *G. bolayi* originating from *L. serriola* (GC/LS/11) had a germination rate as high as 45–70%, those of other isolates were mostly under 30%. Undoubtedly the quality of the inoculum was more important for germination than the host genotype. The number of germ tubes originating from a germinating conidium, i.e. the true germ tube (Figure 6) plus 0–3 primary colony forming hyphae (sometimes termed 'secondary' germ tubes), of powdery mildew isolates from *S. novi-belgii*, *H. aurantiacum*, *L. serriola*, and *S. gigantea* on these plant species at 72 hpi is summarized in Figure 5. Conidia produced one or two colony forming hyphae in addition to the true germ tube only on the original host, while the development of powdery mildew isolates was stopped after the formation of the true germ tube on non-host plant species. Conidiophores were formed only in compatible interactions, where intense sporulation was observed.

**Molecular identification of *Golovinomyces asterum* on *Aster novi-belgii*.** The amplification of the ITS region resulted in a 609 bp long contig, which consisted of a part of the 18S ribosomal RNA gene (56 bp), complete ITS1-5.8S-ITS2 region (190, 154, and 164 bp, respectively), and a part of the 26S ribosomal RNA

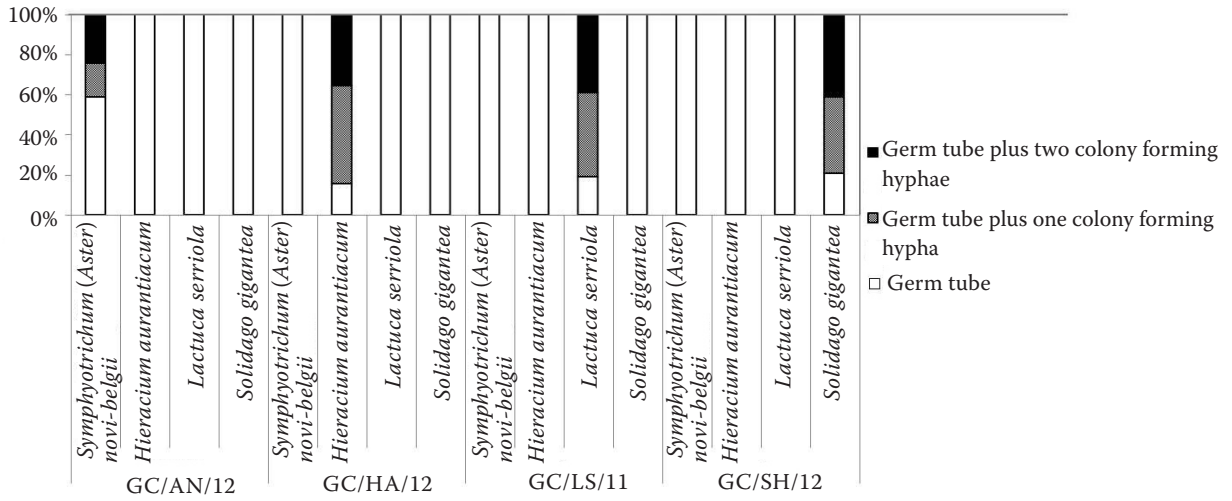


Figure 5. Percentages of conidia with just a germ tube or a germ tube plus one or two colony forming hyphae involving powdery mildew isolates originating from *Symphyotrichum* (*Aster*) *novi-belgii*, *Hieracium aurantiacum*, *Lactuca serriola* and *Solidago gigantea* on selected host species 72 hpi
Colony forming hyphae only develop when the isolates infect their original susceptible hosts

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Table 5. Germination of powdery mildew isolates originating from *Symphyotrichum* (Aster) *novi-belgii*, *Hieracium aurantiacum*, *Lactuca serriola* and *Solidago gigantea* on selected host species 24 h post inoculation (hpi)

| Host genotype | Germination (%) of powdery mildew isolate (mean ± SD) | | | |
|---|---|---|---|---|
| | GC/AN/12[a] | GC/HA/12[b] | GC/LS/11[c] | GC/SG/12[d] |
| *Symphyotrichum* (*Aster*) *novi-belgii* (84/98) | 30.6 ± 14.02 | 25.2 ± 11.26 | 58.2 ± 21.23 | 18.2 ± 7.4 |
| *Hieracium aurantiacum* (89/377) | 29.3 ± 13.2 | 34.81 ± 15.02 | 61.7 ± 15. 02 | 25.7 ± 16.57 |
| *Lactuca serriola* (LSE/57/15) | 22.2 ± 9.32 | 33.60 ± 12.43 | 70.8 ± 32.15 | 29.8 ± 11.02 |
| *Solidago gigantea* (87/386) | 23.5 ± 11.51 | 18.2 ± 6.51 | 45.46 ± 21.33 | 32.2 ± 15.43 |

[a]*Golovinomyces asterum* var. *asterum* isolate originating from *Symphyotrichum* (*Aster*) *novi-belgii* (Olomouc, Czech Republic); [b]*Golovinomyces cichoracearum* isolate originating from *Hieracium aurantiacum* (Paseka, Czech Republic): [c]*Golovinomyces bolayi* isolate originating from *Lactuca serriola* (Olomouc, Czech Republic); [d]*Golovinomyces asterum* var. *solidaginis* isolate originating from *Solidago gigantea* (Rosarium, Olomouc, Czech Republic)

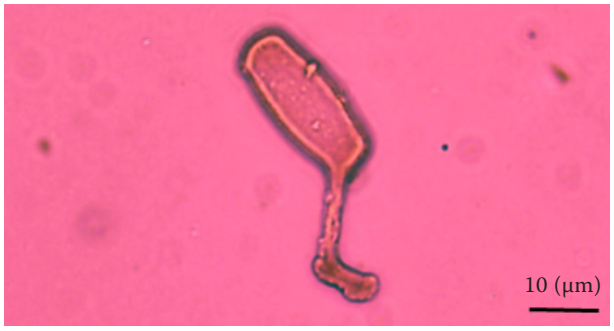


Figure 6. Germinating conidium of *G. asterum* var. *solidaginis* showing a true germ tube

gene (45 bp). We determined ITS sequences for one isolate from *G. a.* var. *asterum* and two isolates from *G. a.* var. *solidaginis*. These three sequences were identical to each other, as well as to the complete ITS region of *G. asterum* var. *asterum* (AB769416 ex *Aster* sp.), *G. cichoracearum* (GQ183937 ex *Aster subulatus* (*Symphyotrichum subulatum*); and to shorter sequences of *G. asterum* var. *asterum* (partial ITS1; AB077674 ex *A.* × *salignus*) retrieved from GenBank. But these sequences differed in two SNP positions and a single gap from *G. a.* var. *solidaginis* sequences retrieved from GenBank (AB077625 ex *S. altissima* and AB077627 ex *S. virgaurea* subsp. *asiatica*) (Figure 7).

Sequencing of D1/D2 domains of 28S rDNA region resulted in 868 bp long sequences for the two specimens extracted from *Solidago*. These two sequences (MK955450, MK955451) were identical to the 28S sequences of *G. a.* var. *solidaginis* AB769418 reported by Takamatsu et al. (2013).

## DISCUSSION

In recent years, studies of powdery mildew taxonomy have brought many changes to the traditional taxonomic system. First, much more attention is now given to the characteristics of the asexual morph, including conidial surface arrangement and germ tube shapes (Cook et al. 1997, 2015; Cook & Braun 2009), but also to the great intraspecific variability of DNA sequences within main species having wide host ranges (e.g., Matsuda & Takamatsu 2003; Takamatsu et al. 2008, 2010), leading to newly introduced species. This case study of powdery mildews on the Asteraceae family is one of the clearest examples.

*Golovinomyces* is a strictly herb-parasitic genus in the Erysiphaceae. The study of Matsuda and Takamatsu (2003) revealed the close co-evolutionary history of *Golovinomyces* species and their host plants (asteraceous hosts), which occurred in the early evolutionary stage of this genus. Results of the phylogenetic analyses, based on ITS and 28S rDNA sequences, revealed the presence of five major groups in *Golovinomyces*, each of which is represented by a single host tribe of the Asteraceae (Matsuda & Takamatsu 2003). The most extensive study of powdery mildews on hosts of the family Asteraceae was reported by Takamatsu et al. (2013), in which they analysed 183 nucleotide sequences of two nrDNA (ITS and 28S rDNA) regions of powdery mildews collected worldwide. Separation of certain species of powdery mildew from the traditional *G. cichoracearum* complex was confirmed; e.g., the separation of *G. montagnei*, *G. depressus*, and *G. echinopis* supported the species delimitation of Braun and Cook (2012). Similarly, well-resolved independent lineages were observed for *G. macrocarpus*, *G. artemisiae*, and *G. inulae*. Isolates of *G. asterum* formed an independent lineage as well, albeit with two sub-clades consisting of *G. asterum* var. *asterum* and *G. asterum* var. *solidaginis*. Whilst some species such as *G. ambrosiae, G. circumfusus* and *G. spadiceus* apparently fell into one indistinguishable clade, recent work based on a multi-locus

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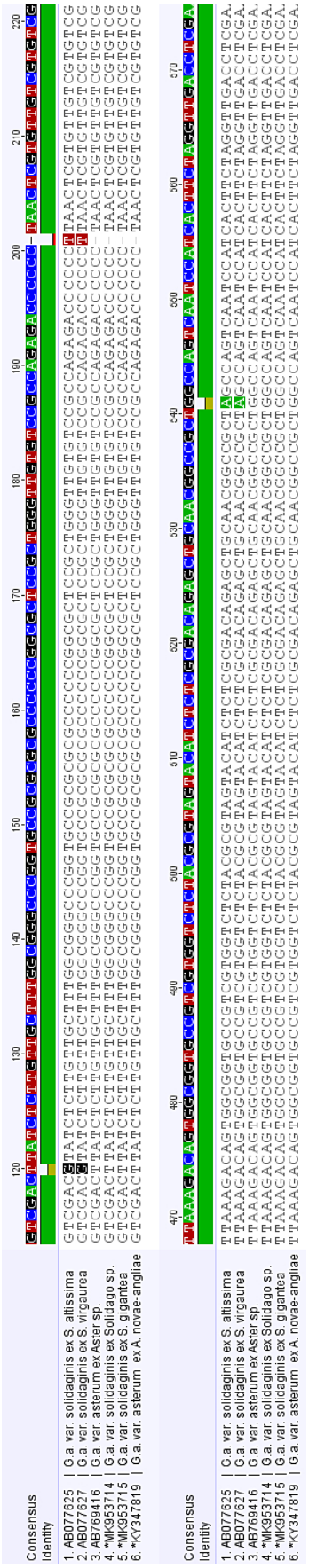

Figure 7. PrintScreen of the nucleotide alignment of two parts of the ITS region

For each isolate, GenBank accession number, taxonomical identification and host species is provided. Asterisks indicate sequences obtained in this study. Nucleotide substitutions are highlighted with colours

approach has found differences (Qiu et al. 2020). *G. cichoracearum* (s. str.) turned out to be confined to *Golovinomyces* on *Scorzonera* and *Tragopogon* spp. Collections on other hosts, assigned in Braun and Cook (2012) to *G. cichoracearum* clustered within the large *G. orontii* complex (Takamatsu et al. 2013).

Both the results of molecular analysis (Takamatsu et al. 2013) and the morphological studies of Braun (1995; 1999) and Braun and Cook (2012) revealed that the taxonomy within both *G. orontii* and *G. cichoracearum* is very complicated. From the ITS phylogeny by Takamatsu et al. (2013) the isolates designated as *G. cichoracearum* (mainly type isolate from *Scorzonera hispanica* and *Tragopogon)* as well as those designated *G. orontii* [infecting both representatives of tribe Cichorioideae (e.g., *Lactuca, Cichorium, Mycelis*)] belonged to a single lineage (IX). In addition, pathogens in groups 1, 2 and 3 in this lineage were associated with hosts from more than 17 different families. Later, Braun et al. (2019) described some new species, including *Golovinomyces bolayi*, *G. orontii* s. str., and *G. tabaci* – three species corresponding to groups 3, 2, 1 in the phylogenetic trees (previously assigned to *Golovinomyces orontii* s. lat.). It was introduced a new name, *G. bolayi* for powdery mildew on *Lactuca, Mycelis, Cichorium, Taraxacum* and as well as hosts from other plant families (e.g. Bignoniaceae, Brassicaceae, Campanulaceae, Crassulaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Linderniaceae, Papaveraceae, Plantaginaceae, Rosaceae, and Solanaceae). However, on the basis of morphological features, it is nearly impossible to distinguish *G. orontii* and/or *G. bolayi* from *G. cichoracearum*, especially since their chasmothecia are rarely formed. This fact corresponds with a lot of discrepancies in information about occurrence of these species in literature.

In the Taxonomic Manual of the Erysiphales (Braun & Cook 2012) 46 species of the genus *Golovinomyces* have been described, of which 27 species occur in Central Europe including 16 infecting the family Asteraceae, viz., *G. ambrosiae, G. artemisiae*, *G. asterum*, *G. cichoracearum*, *G. circumfusus*, *G. depressus*, *G. echinopis*, *G. fischeri*, *G. inulae*, *G. macrocarpus*, *G. montagnei*, *G. orontii* (now *G. bolayi*), *G. prenanthis*, *G. senecionis*, *G. sonchicola*, and *G. spadiceus*. In a survey within the Czech Republic the occurrence of 14 powdery mildew species was confirmed, but the lack of records of *G. circumfusus* and *G. fischeri* was probably due to the absence of host plants in the area we surveyed (certainly in the case of *G. circumfusus*).

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Our survey brought some knowledge as well as new questions. A complex situation occurs on the powdery mildews on *Senecio* spp. There are two powdery mildew species on *Senecio* in Europe. *G. fischeri* [mainly on annual host species of the genus *Senecio* (above all on *S. vulgaris*)] with larger chasmothecia, and conidia with a length/width ratio under 2. The second species, *G. senecionis*, is common in Europe on hosts of the *S. nemorensis* group. *G. fischeri* differs from *G. senecionis* in having larger chasmothecia, conidiophores with curved foot-cells, and rather short and broad conidia. In our collections, an isolate on *Senecio ovatus* was identified as *G. senecionis* according to the shape of the conidia and foot-cells, as was expected according to its host range.

In our collections, we found powdery mildew on *Prenanthes purpurea*, previously reported by Braun (1995) as *G. cichoracearum*, and later delimited by Braun and Cook (2012) as *G. prenanthis* (comb. nov.). Since Takamatsu et al. (2013) did not include isolates from *Prenanthes* spp. in their comprehensive molecular analysis, it could not be confirmed whether this was only a specialized biological form of *G. cichoracearum* or more likely of *G. orontii* that was confined to its host, or whether it was a separate species. Our cross-inoculation experiments showed that the powdery mildew isolate (*G. bolayi*) from *L. serriola* was not able to infect *Prenanthes* spp. (both host species are members of the subfamily/tribe Cichorioideae/Cichorieae). These problems should be solved by molecular methods.

An interesting situation arises with *G. asterum*. In Braun's European monograph (Braun 1995) *G. cichoracearum* var. *cichoracearum* was reported as infecting *Aster* (*Symphyotrichum*) spp. Recently the Aster powdery mildew was separated from *G. cichoracearum s.* lat. as *G. asterum* and confined to the tribe *Astereae* (Braun & Cook 2012). One of the main morphological features of *G. asterum* refers to the shorter chasmothecial appendages. Braun and Cook (2012) separated *G. asterum* on *Symphyotrichum* into two varieties; *asterum* and *moroczkovskii*, with the main morphological difference being curved foot-cells in *moroczkovskii* and straight foot-cells in *asterum* (Figure 3). The third variety of *G. asterum*, var. *solidaginis*, was described on *Solidago* by U. Braun (Braun & Cook 2012). They placed this variety in a separate clade from var. *asterum*. According to Braun and Cook (2012), *G. asterum* var. *asterum* is mainly distributed and very common in North America on *Symphyotrichum* spp. while *G. asterum* var. *moroczkovskii* is rare in USA, but widespread in Europe on tribe Astereae. The North American collection on *S. novi-belgii* was similar in morphological features to var. *moroczkovskii* by having curved to sinuous foot-cells. Indeed, Braun and Cook (2012) stated that all examined European collections on cultivated *Aster* (s. lat.) species of North American origin had distinctly curved foot-cells. However, they also stated that there were numerous records of powdery mildew (cf. *G. cichoracearum*) on other *Aster* (s. lat.) in Europe that had not yet been distinguished in this way. According to our observations in the Czech Republic, both morphological forms (var. *asterum* and var. *moroczkovskii*) were observed on *Symphyotrichum* spp. and var. *solidaginis* was confirmed as clearly distinguishable from var. *asterum*. Our cross-inoculation tests revealed that *G. asterum* isolates originating from *Solidago* spp. and from *Symphyotrichum* were highly specialized on their hosts; and stopped their development at the stage of the true germ tube when inoculated on different host genera. Precise molecular and biological data necessary to distinguish collections on *Aster* s. lat. and *Solidago* at the species level are missing. However, the possibility of *P. xanthii* occurring on *Symphyotrichum* spp. in our tests was excluded by a lack of fibrosin bodies as well as by molecular analysis.

It was also very important to determine whether some morphological characteristics; e.g., the length/width (L/W) ratio of conidia were useful for distinguishing individual species as previously expected. Earlier, Braun (1999) had divided the genus *Golovinomyces* into the two morphologically defined sections; sect. *Golovinomyces* and sect. *Depressae* with broader conidia (L/W ratio < 2) where e.g., *G. ambrosiae*, *G. artemisiae*, *G. depressus*, *G. echinopis*, and *G. fischeri* belonged. However, according to molecular analysis (Takamatsu et al. 2013) this classification did not reflect their phylogenetic relationships. This discrepancy may have been caused, in the earlier taxonomic systems, by an underestimation of the influence of the hosts on morphology of asexual states and an overestimation of the importance of some morphology of the sexual states, e.g., apices of chasmothecial appendages. However, recent multilocus molecular analyses showed that the L/W ratio was, after all, important to distinguish species as mentioned above (Takamatsu, pers. comm. 2019).

Our cross-inoculation tests revealed that each powdery mildew isolate tested was very specific to its host species. Only the isolate from *L. serriola* (*G. bolayi*) was able to infect some other plants of the

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subfamily Cichorioideae to some extent, but both isolates of *G. asterum* (var. *asterum* and var. *solidaginis*) were found to be highly specialized to their specific host. Another interesting finding is that powdery mildew development on non-host species stopped after production of the true germ tube (Figure 6), in each non-host interaction. In non-host interactions production of a true germ tube was not strongly affected by the host plant; however, after producing the germ tube, the conidia stopped their development, as previously recorded in other reports (Mieslerová et al. 2004; Cook et al. 2015). To confirm these results, a more comprehensive study involving greater numbers of powdery mildew isolates from a wider range of host species and more cross-inoculation experiments are needed. Moreover, the latter are rare in the literature, due to the difficulty in setting them up and the laborious long-term maintenance of isolates on their hosts. It should be pointed out that no significant influence of the hosts to germination rates was found (Table 5). This agrees with conidia of *Erysiphe* spp. showing similar germination rates on host surfaces, and indeed on any substrate (Cook & Braun 2009; Cook et al. 2015).

The main aims of our work were to study the huge variation in host specificity to powdery mildews, the problematic situation when the *G. cichoracearum* species complex was divided into many cryptic species that are so difficult to define. Our results confirmed the morphological variability and strong host specificity of *Golovinomyces* species illustrating the very intimate relationships between the asteraceous hosts and their pathogens. Thus, individual isolates within *Golovinomyces* spp. are very specific to their original host species, and hence essentially incapable of inducing infection on other asteraceous species.

We hope that the results summarized in this paper add some missing pieces to the puzzle, and help towards a better understanding of the complicated situation in the Asteraceae–*Golovinomyces* relationship.

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## REFERENCES

Blumer S. (1933): Die Erysiphaceen Mitteleuropas unter besonderer Berücksichtigung der Schweiz. Beitrage zur Kryptogamenflora der Schweiz, 7: 1–483.

Blumer S. (1967): Echte Mehltaupilze (Erysiphaceae). Stuttgart, Gustav Fischer.

Bradshaw M., Braun U., Götz M., Meeboon J., Takamatsu S. (2017): Powdery mildew on *Chrysanthemum* × *morifolium*: phylogeny and taxonomy in the context of *Golovinomyces* species on *Asteraceae* hosts. Mycologia, 109: 508–519.

Braun U. (1987): A monograph of the Erysiphales (powdery mildews). Beihefte zur Nova Hedwigia, 89: 1–700.

Braun U. (1995): The Powdery Mildews (Erysiphales) of Europe. Jena, G. Fischer Verlag.

Braun U. (1999): Some critical notes on the classification and generic concept of the Erysiphaceae. Schlechtendalia, 3: 49–55.

Braun U., Cook R.T.A. (2012): Taxonomic manual of the Erysiphales (powdery mildews). CBS Biodiversity Series, 11: 1–707.

Braun U., Shin H.D., Takamatsu S., Meeboon J., Kiss L., Lebeda A., Kitner M., Götz M. (2019): Phylogeny and taxonomy of *Golovinomyces orontii* revisited. Mycological Progress, 18: 335–357.

Carlquist S. (1976): Tribal interrelationships and phylogeny of the Asteraceae. Aliso, 8: 465–492.

Cook R.T.A., Braun U. (2009): Conidial germination patterns in powdery mildews. Mycological Research, 113: 616–636.

Cook R.T.A., Denton J.O., Denton G. (2015): Pathology of oak-wisteria powdery mildew. Fungal Biology, 119: 657–671.

Cook R.T.A., Inman A.J., Billings C. (1997): Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycological Research, 101: 975–1002.

Cunnington J.H., Takamatsu S., Lawrie A.C., Pascoe I.G. (2003): Molecular identification of anamorphic powdery mildews (Erysiphales). Australasian Plant Pathology, 32: 421–428.

Edwards K., Johnstone C., Thompson C. (1991): A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research, 19: 1349.

Funk V.A., Susanna A., Stuessy T.F., Bayer R.J. (2009): Systematics, Evolution and Biogeography of Compositae. Vienna, International Association for Plant Taxonomy (IAPT).

Gardes M., Bruns T.D. (1993): ITS primers with enhanced specificity for basidiomycetes — Application to identification of mycorhizae and rusts. Molecular Ecology, 2: 113–118.

Glawe D.A. (2008): The powdery mildews: A review of the world´s most familiar (yet poorly known) plant pathogens. Annual Review of Phytopathology, 46: 27–51.

Hammett K.R.W. (1977): Taxonomy of the Erysiphaceae in New Zealand. New Zealand Journal of Botany, 15: 687–711.

Jaczewski A. (1927): Karmannyj opredelitel' gribov, II. Mučnisto-rosjanye griby. Mikologicheskaya Laboratoriya Immeni Professora A.A. Jaczewskogo, Gasudarstvennogo Instituta Opytnoy Agronomii, Leningrad.

Kubát K., Štech M., Štěpánek J. (2002): Klíč ke květeně České republiky. Praha, Academia.

https://doi.org/10.17221/129/2019-PPS

Lebeda A. (1983): The genera and species spectrum of cucumber powdery mildew in Czechoslovakia. Journal of Phytopathology, 108: 71–79.

Lebeda A., Reinink K. (1994): Histological characterization of resistance in *Lactuca saligna* to lettuce downy mildew (*Bremia lactucae*). Physiological and Molecular Plant Pathology, 44: 125–139.

Lebeda A., Mieslerová B. (2011): Taxonomy, distribution and biology of lettuce powdery mildew (*Golovinomyces cichoracearum sensu stricto*). Plant Pathology, 60: 400–415.

Lebeda A., Mieslerová B., Petrželová I., Korbelová P., Česneková E. (2012): Patterns of virulence variation in the interaction between *Lactuca* spp. and lettuce powdery mildew (*Golovinomyces cichoracearum*). Fungal Ecology, 5: 670–682.

Lebeda A., Mieslerová B., Petrželová I., Korbelová P. (2013): Host specificity and virulence variation in populations of lettuce powdery mildew pathogen (*Golovinomyces cichoracearum s. str.*) from prickly lettuce (*Lactuca serriola*). Mycological Progress, 12: 533–545.

Lebeda A., Mieslerová B., Huszár J., Sedláková B. (2017): Padlí kulturních a planých rostlin. Olomouc, Agriprint.

Matsuda S., Takamatsu S. (2003): Evolution of host–parasite relationship of *Golovinomyces* (Ascomycete: Erysiphales) inferred from nuclear rDNA sequences. Molecular Phylogenetics and Evolution, 27: 314–327.

Micali C., Gollner K., Humphry M., Consonmi Ch., Panstruga R. (2008): The powdery mildew disease of Arabidopsis: A paradigm for the interaction between plants and biotrophic fungi. The Arabidopsis Book, 6: e0115. doi.org/10.1199/tab.0115

Mieslerová B., Lebeda A., Kennedy R. (2004): Variation in *Oidium neolycopersici* development on host and non-host plant species and their tissue defence response. Annals of Applied Biology, 144: 237–248.

Mieslerová B., Lebeda A., Petrželová I., Korbelová P. (2013): Incidence of lettuce downy mildew (*Bremia lactucae*) and powdery mildew (*Golovinomyces cichoracearum*) in natural populations of prickly lettuce (*Lactuca serriola*). Plant Protection Science, 49: S24–S32.

Morgan D.R., Holland B. (2012): Systematics of Symphyotrichinae (Asteraceae: Astereae): Disagreements between two nuclear regions suggest a complex evolutionary history. Systematic Botany, 37: 818–832.

Mori Y., Sato Y., Takamatsu S. (2000): Evolutionary analysis of the powdery mildew fungi using nucleotide sequences of the nuclear ribosomal DNA. Mycologia, 92: 74–93.

Panero J.L., Funk V.A. (2002): Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). Proceedings of the Biological Society of Washington, 115: 909–922.

Paulech C. (1995): Flóra Slovenska. X/1. Mycota (Huby), Ascomycetes (Vreckaté), Erysiphales (Múčnatkotvaré). Bratislava, Veda.

Qiu P-L., Liu S-Y., Bradshaw M., Rooney-Latham S., Takamatsu S., Bulgakov T.S., Tang S.-R., Feng J., Jin D.-N., Aroge T., Li Y., Wang L-L., Braun U. (2020): Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces* (Ascomycota, Erysiphales), including *G. ambrosiae, G. circumfusus* and *G. spadiceus*. BMC Microbiology, 20, 51 (2020). doi.org/10.1186/s12866-020-01731-9

Salmon E.S. (1900): A monograph of the Erysiphaceae. Torrey Botanical Club: Memoirs, 9: 1–292.

Shin H.D. (2000): Erysiphaceae of Korea. Suwon, Korea: National Institute Agricultural Science and Technology.

Stevens P.F. (2001): Angiosperm Phylogeny Website. Angiosperm Phylogeny Website, version 14.

Takamatsu S., Havrylenko M., Wolcan S.M., Matsuda S., Niinomi S. (2008): Molecular phylogeny and evolution of the genus *Neoerysiphe* (Erysiphaceae, Ascomycota). Mycological Research, 112: 639–649.

Takamatsu S., Kano Y. (2001): PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. Mycoscience, 42: 135–139.

Takamatsu S., Matsuda S., Grigaliunaite B. (2013): Comprehensive phylogenetic analysis of the genus *Golovinomyces* (Ascomycota: Erysiphales) reveals close evolutionary relationships with its host plants. Mycologia, 105: 1135–1152.

Takamatsu S., Niinomi S., Harada M., Havrylenko M. (2010): Molecular phylogenetic analyses reveal close evolutionary relationship between *Podosphaera* (Erysiphales: Erysiphaceae) and their rosaceous hosts. Persoonia, 24: 38–48.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30: 2725–2729.

Towsend G.R., Heuberger W. (1943): Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter, 27: 340–343.

Wagenitz G. (1976): Systematics and phylogeny of the Compositae (Asteraceae). Plant Systematics and Evolution, 125: 29–46.

White T.J., Bruns T., Lee S., Taylor J.W. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. San Diego, Academic Press.

Zeller K.A., Levy M. (1995): Intraspecies differentiation in the powdery mildew *Erysiphe cichoracearum* determined with rDNA RFLPs. Molecular Ecology, 4: 277–284.

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