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Research Papers

A cryptic powdery mildew (*Golovinomyces hieraciorum* sp. nov.) on *Hieracium* and *Pilosella* (*Compositae*)

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Summary. The *Golovinomyces cichoracearum* complex is morphologically highly variable and causes powdery mildew on a wide range of *Compositae*, including *Hieracium* spp. and *Lapsana communis*. A comprehensive phylogenetic analysis of *Golovinomyces*, published in 2013, revealed that *G. cichoracearum s. str.* was confined to *Scorzonera* and *Tragopogon* spp. as hosts (*Compositae*, tribe *Lactuceae*, subtribe *Scorzonerinae*). To clarify the phylogeny and taxonomy of *Golovinomyces* on *Hieracium* spp. (*Compositae*, tribe *Lactuceae*, subtribe *Hieraciinae*), which is common in Asia and Europe, morphology and phylogenetic analyses of ITS + 28S rDNA sequences were conducted for 57 powdery mildew specimens of *Hieracium* spp. and *Lapsana communis*. *Golovinomyces* on *Hieracium* spp. in Asia and Europe, previously referred to as *G. cichoracearum*, is a previously undescribed, cryptic species, which is described here as *Golovinomyces hieraciorum* sp. nov.. Since a sequence retrieved from a powdery mildew on *Lapsana communis* in Switzerland clustered in sister position to the *G. hieraciorum* clade, numerous German specimens identified as *G. cichoracearum* were included in the present examinations. All the re-examined collections of "*G. cichoracearum*" on *L. communis* were misidentified, and were shown to belong to *Neoërysiphe nevoi* or *Podosphaera ergerontis-canadensis*.

Keywords. *Golovinomyces cichoracearum*, *Erysiphaceae*, *Ascomycota*, taxonomy, phylogenetic analysis.

INTRODUCTION

Hieracium L. (*Compositae*, tribe *Lactuceae*; hawkweed) is a taxonomically challenging genus of herbaceous perennial herbs, with ligulate flowers and milky latex in stems and leaves. This genus comprises terrestrial perennial plants, native to Africa, Asia, Europe and North to South America, which are used as forage for livestock (Wilson *et al*., 2006). *Hieracium* comprises ca.

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770 sexually producing species, and ca. 5200 apomictic microspecies, according to The International *Compositae* Alliance (TICA) (CWG, 2021). *Hieracium* is divided into three subgenera, viz. *Pilosella*, *Hieracium*, and *Chionoracium* (Wilson *et al.*, 2006). Subgenus *Pilosella* is considered a separate genus, *Pilosella*, i.e., the former subgenus *Hieracium* is treated as *Hieracium s. str.*, and subgenus *Chionoracium* is assigned to the genus *Crepis* (Gaskin and Wilson, 2007). Members of the genera *Hieracium* and *Pilosella* are model plants that are used to study the mechanisms of apomixis (not involving the fusion of male and female gametes in reproduction) (Hand *et al.*, 2015; Płachno *et al.*, 2017; Chrtek *et al.*, 2020). Apomixis is a key evolutionary mechanism in certain angiosperms, providing reproductive assurance and isolation (Mráz and Zdvořák, 2019).

Hieracium spp. are susceptible to powdery mildews. Six species, viz. *Erysiphe lamprocarpa*, *Golovinomyces cichoracearum*, *Leveillula taurica*, *Podosphaera fusca, Po. fuliginea*, and *Po. macularis*, have been reported on *Hieracium* from many countries (Farr and Rossman, 2021). However, these names do not reflect the current taxonomy, i.e., they were based on outdated broad species concepts. *Golovinomyces cichoracearum* (basionym: *Erysiphe cichoracearum*) was originally described on *Scorzonera hispanica* and *Tragopogon porrifolius* (de Lamarck and de Candolle, 1805). Salmon (1900) widened the concept of *G. cichoracearum* by assigning powdery mildew on numerous hosts of various plant families, including *Boraginaceae* and *Plantaginaceae*, to this species. Braun (1987) confined *G. cichoracearum* to a wide range of *Compositae* hosts. Braun and Cook (2012) further reduced its circumscription to *Golovinomyces* on hosts in the subfamily *Cichorioideae* of *Compositae*, but emphasized that this circumscription still represented a heterogenic complex species. This is demonstrated by molecular sequence analyses (Matsuda and Takamatsu, 2003; Lebeda and Mieslerová, 2011), which supported this compound species comprising at least several *formae speciales* or possibly cryptic species.

The morphology of *G. cichoracearum s. lat.* is not uniform on various hosts, i.e., somewhat lobed hyphal appressoria and conidiophores with curved foot-cells are common on *Lactuca*, *Scorzonera*, and *Tragopogon*, whereas most appressoria are nipple-shaped and the foot-cells are mostly straight on *Hieracium* and *Picris* (Lebeda and Mieslerová, 2011; Braun and Cook, 2012). Takamatsu *et al.* (2013) showed in comprehensive phylogenetic analyses that *G. cichoracearum s. str.* is further confined to host species of the genera *Scorzonera* and *Tragopogon* (*Compositae*, *Cichorioideae*, tribe *Lactuceae*, subtribe *Scorzonerinae*), since the powdery mildews on hosts of these genera morphologically coincide and are a clade which is separate from all other clades within *Golovinomyces*.

Golovinomyces cichoracearum (or *E. cichoracearum*) on *Hieracium* (tribe *Lactuceae*, subtribe *Hieraciinae*) and *L. communis* (tribe *Lactuceae*, subtribe *Crepidinae*) is common throughout Europe, North America, and in parts of Asia (Zeller and Levy, 1995; Braun and Cook, 2012; Farr and Rossman, 2021). Takamatsu *et al.* (2013) showed a sequence retrieved from *Golovinomyces* on *L. communis* in Switzerland was genetically distinct from *G. cichoracearum s. str.*, and forms a separate lineage. Additional sequences and morphological observations are required to clarify the relationship between *G. cichoracearum s. str.*, *Golovinomyces* collections on *Hieracium*, and *Golovinomyces* collections on *L. communis*.

In 2014 and 2017, *Golovinomyces* sp. was found on *H. umbellatum* in Heihe City, Heilongjiang Province, China. To identify the powdery mildew species involved, morphological and molecular phylogenetic analyses (based on ITS and 28S rDNA sequences) were conducted. Morphologically, this fungus is close to *G. cichoracearum* as circumscribed in Braun and Cook (2012), but genetically it clusters far away from *G. cichoracearum s. str.* and forms a clade of its own in sister position to a sequence obtained from *Golovinomyces* on *L. communis* in Europe. Therefore, the present study aimed to: (i) clarify the identity of the causal agent of the *Hieracium* powdery mildew based on morphological re-examinations and molecular analyses, and determine if this pathogen is distributed and common in Asia and Europe; and (ii) identify the *Golovinomyces* on *L. communis* in Europe.

MATERIALS AND METHODS

Fungal material

A total of 57 specimens were examined from four countries. Twenty-eight specimens of *Hieracium* (including 14 species) with *Golovinomyces* were examined, including two samples collected in China, one specimen from Russia, one from Slovakia, and 24 additional collections from Germany. One specimen on *Pilosella procera* from China was included, and 27 *Lapsana communis* specimens from Germany were also examined.

Morphological examination

For morphological observations, dried samples from herbarium collections were mounted in lactic acid and examined using a light microscope (Zeiss Axio Scope A1, Germany). Dimensions of at least 30 different fungus anamorph and teleomorph structures were measured for each sample.

DNA extractions, PCR amplification and sequencing

Whole-cell DNA was extracted from chasmothecia or conidia and mycelia using the Chelex-100 method (Walsh *et al.*, 1991; Hirata and Takamatsu, 1996). The rDNA internal transcribed spacer (ITS) regions including the 5.8S rDNA were amplified with primers ITS5 and ITS4 (White *et al.*, 1990), or PM10 and PM2 (Bradshaw and Tobin, 2020). And the partial 28S rDNA, including D1 and D2 domains, was amplified with primers LSU1 and LSU2 (Scholin *et al.*, 1994; Mori *et al.*, 2000), or PM28F and PM28R (Bradshaw and Tobin, 2020). The DNA amplification and sequencing were carried out as outlined in Qiu *et al.* (2018).

Molecular phylogenetic analyses

The obtained sequences, including complete ITS and partial 28S rDNA, were deposited in GenBank. The combined dataset of ITS and 28S rDNA sequences was aligned with closely related sequences of the genus *Golovinomyces* (Table S1) (Kiss *et al.*, 2001; Matsuda and Takamatsu, 2003; Takamatsu *et al.*, 2006; 2008; 2009; 2013; Park *et al.*, 2010; Scholler *et al.*, 2016; Bradshaw *et al.*, 2017; Braun *et al.*, 2018; 2019; Meeboon *et al.*, 2018; Ellingham *et al.*, 2019; Qiu *et al.*, 2020). *Golovinomyces inulae* was selected as the outgroup taxon (Takamatsu *et al.*, 2013). Multiple sequence alignments were carried out using MUSCLE implemented in the MEGA X (Kumar *et al.*, 2018). Subsequently, the alignments were manually refined with MEGA X and deposited in TreeBASE (Piel *et al.*, 2009) under the accession number of S28413. Phylogenetic trees were obtained from the generated dataset using the maximum parsimony (MP), maximum likelihood (ML) and Bayesian Inference (BI) methods. MP analysis was implemented in PAUP* 4.0 (Swofford, 2002) with the heuristic search option using the "tree-bisection-reconnection" (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analysis using 1000 replications with the step-wise addition option set as simple and maximum tree number as 100 to save analysis time. Tree scores, including tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. ML analyses were carried out in raxmlGUI 2.0 beta (Edler *et al.*, 2021) under a GTRGAMMA model. The BS supports and trees were obtained by running rapid bootstrap analyses of 1000 replicates followed by a search for the tree with the greatest likelihood. Bootstrap supports below 75% were discarded for both analyses. In BI analyses, the best-fit substitution models for different datasets were estimated by MrModeltest ver. 2.3 based on the implementation of the Akaike information criterion (AIC) (Nylander, 2004). Four Markov Chain Monte Carlo (MCMC) were run from random starting trees for 2,000,000 generations, and trees were sampled every 100 generations by MrBayes v. 3.2.7 (Ronquist and Huelsenbeck, 2003). The runs ended when the standard deviation of split frequencies reached below 0.01. The first 25% of all generations were discarded as burn-in and a majority rule consensus tree of all remaining trees was calculated to determine the posterior probabilities for individual branches. The resulting trees were visualized using FigTree 1.3.1 (Rambaut, 2009).

RESULTS

Morphological description

Golovinomyces hieraciorum G.-X. Guan & S.-Y. Liu **sp. nov.** (Figure 1).

MycoBank Number: MB10001660

Etymology. Epithet derived from the name of the host genus, *Hieracium*, using the plural genitive, meaning "*Golovinomyces* of *Hieracium* spp."

Typification. on *Hieracium umbellatum*, China. Heilongjiang Province, Heihe City, Sunwu County, the south coast of Xunbiela River, 20 Sep. 2014, Feng-Yun Zhao, Jian Liu, Shu-Rong Tang, Peng-Lei Qiu (holotype, HMJAU-PM91858; isotype, HMAS249776); GenBank number: MZ420204 (ITS+28S). Paratype: Heilongjiang Province, Heihe City, Sunwu County, 2 Sep. 2017, Feng-Yun Zhao, Van-Ninh Nguyen, Jia-Ni Li, Jing-Sheng Lu (HMJAU-PM91859), GenBank number: MZ420205 (ITS+28S).

Description. Mycelium on leaves, amphigenous, and stems, forming irregular white patches or effuse, finally covering whole leaf surfaces, persistent. Hyphae straight to sinuous-geniculate, hyaline, thin-walled, septate, hyphal cells 60–90 µm long and 4–10 µm wide. Later in the season hyphae becoming pigmented, above and all around chasmothecia (secondary hyphae). Hyphal appressoria nipple-shaped, solitary or in opposite pairs, 4–8 µm diam. Conidiophores on the upper surfaces of hyphal mother cells, erect, position usually towards

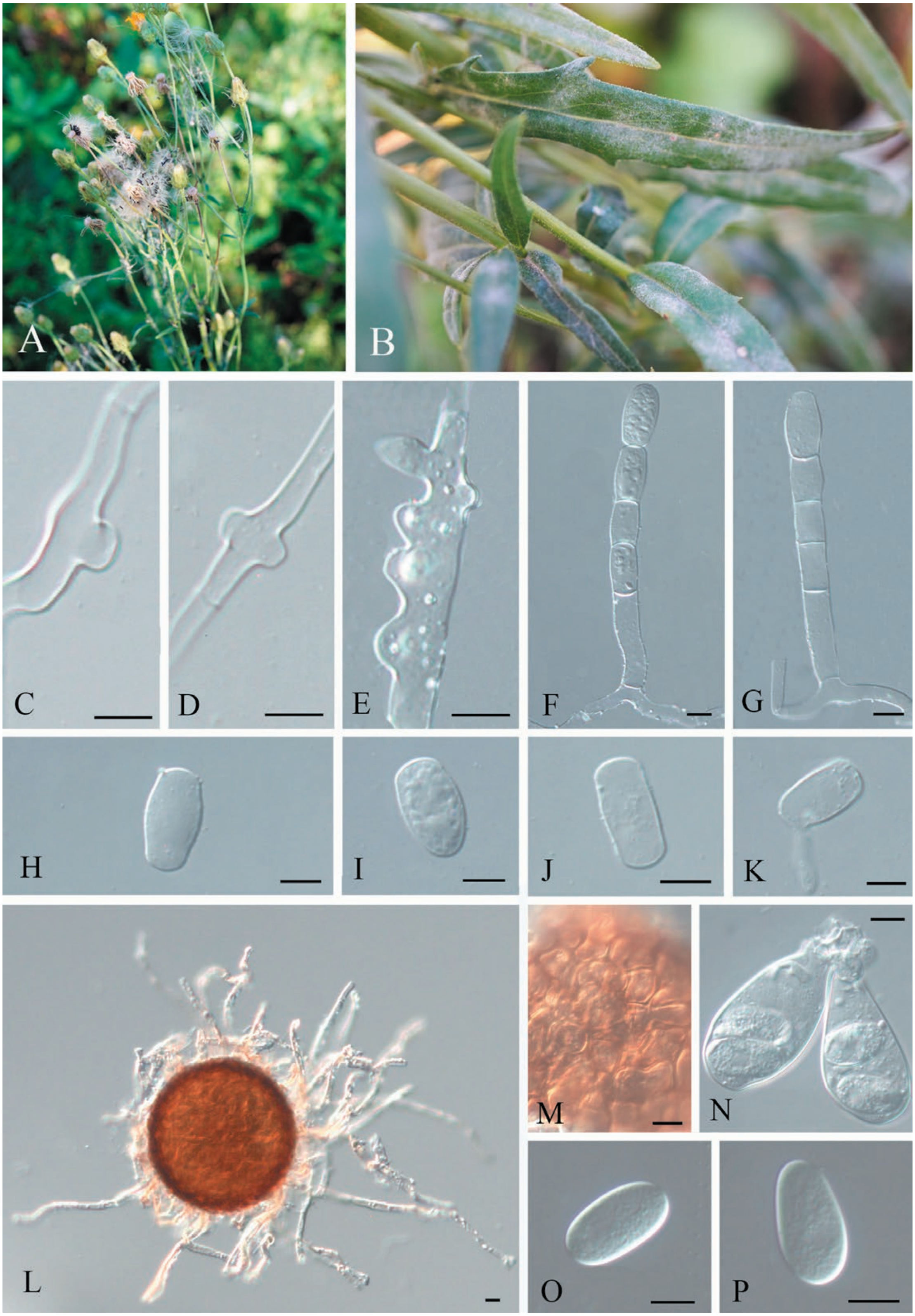

Figure 1. *Golovinomyces hieraciorum* on *Hieracium umbellatum* (HMJAU-PM91858). A and B, symptoms on host plants. C to E, hyphal appressoria. F and G, conidiophores. H to J, conidia. K, conidium with germ tube. L, chasmothecium. M, peridium cells. N, asci. O and P, ascospores. Bars = 10 µm.

one septum of each mother cell, rarely in the middle or almost so, (40–)47–90(–120) × 9–15 µm. Conidiophores foot-cells cylindrical or slightly increasing in width from bases to tops, straight, occasionally somewhat curved at the bases, with basal septae at the junctions (or almost so) with the hyphal mother cells, occasionally slightly elevated (5–10 µm), 22–80 × 8–15 µm, followed by 1–3 short cells. Conidia formation catenescent, outline of conidium chains distinctly sinuate. Conidia ellipsoid-ovoid to doliiform-cylindrical, 22–35(–40) × 11–20 µm, length/width ratio (1.3–)1.5–2.1(–2.7). Germ tubes club-shaped, produced in perihilar positions, short, without swollen tips. *Euoidium type*. Chasmothecia, amphigenous, scattered to gregarious, when mature often surrounded by secondary (pigmented) hyphae, 80–156 µm diam. Peridium cells with irregularly curved-sinuous walls, (7.4–)10–25 µm diam. Appendages numerous, in the lower half of the ascomata, sometimes also in the upper half, mycelioid, simple but rarely irregularly branched and interlaced with each other, 0.2–2.3 times as long as the chasmothecial diam., 3–9 µm wide, septate, walls thin, smooth or somewhat rough, at first hyaline, later brown, paler towards the apices or colourless near the apices, shorter appendages may be brown throughout (differentiation between appendages and secondary hyphae often difficult). Asci 6–16 per chasmothecium, clavate to saccate, (28–)45–75(–80) × (18–)20–40(–45) µm, length/width ratio (1.2–)1.5–2.5(–3.3), usually with numerous small to moderately large oil droplets, sessile to short-stalked, stalks 5–15 µm long, rarely longer, to 25 µm, walls thin, 1–2 µm, terminal oculi inconspicuous, 7–15 µm diam., 2-spored. Ascospores ellipsoid-ovoid, (11–)15–20(–31) × 11–18 µm, length/width ratio 1.0–1.9(–2.3), colourless.

Host range and distribution. *Compositae* (tribe *Lactuceae*, subtribe *Hieraciinae*): On *Hieracium* (*albiflorum*, *alpinum*, *amplexicaule*, *anchusoides*, *argillaceum*, *aurantiacum*, *auricula*, *belonodontum*, *bifidum*, *bombycinum*, *boreale*, *bupleuroides*, *caesium*, *caespitosum*, *canadense*, *carpathicum*, *carpaticum*, *cinerascens*, *cymosum*, *danubiale*, *dentatum*, *echioides*, *fallax*, *flagellare*, *florentinum*, ×*floribundum*, *gentile*, *glaucinum*, *glaucum*, *groenlandicum*, *hryniawiense*, *humile*, *incisum*, *integrifolium*, *jankae*, *juranum*, *kotschyanum*, *lachenalii*, *laevicaule*, *laevigatum*, *lanatum*, *lanceolatum*, *lehbertii*, *leucophaeum*, *longifolium*, *lycopifolium*, *murorum*, *oistophyllum*, *onosmoides*, *pallidum*, *paniculatum*, *pictum*, *pilosella*, *piloselloides*, *pleiotrichum*, *porrifolium*, *praealtum*, *praecox*, *pratense*, *prenanthoides*, *pulmonarioides*, *quercetorum*, *racemosum*, *rapunculoides*, *regelianum*, *robustum*, *rotundatum*, *rubrum*, *sabaudum*, *scabrum*, *schmidtii*, *semisylvaticum*, sp., *staticifolium*, *subinuloides*, *submurorum*, *sylvaticum*, *sylvularum*, *tenuiflorum*, *trachselianum*, *transylvanicum*, *tridentatum*, *tritum*, *umbellatum*, *vagum*, *villosum*, *virosum*, *viscosum*, *vulgatum*), and *Pilosella* (*aurantiaca*, *procera*). Asia (Armenia, China), Europe (Belarus, Czechoslovakia, Denmark, Estonia, Finland, France, Germany, Hungary, Iceland, Italy, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, UK, Ukraine, Yugoslavia), and North America (Amano, 1986; Braun, 1995; Farr and Rossman, 2021).

Additional material examined. on *H. amplexicaule*, Germany, Sachsen-Anhalt, Landkreis Jerichower Land, Burg, 10 June 1997, H. Jage (GLM-F-50151); on *H. bombycinum*, Germany, Sachsen, Dresden, Großer Garten, 27 Sep. 1994, F. Klenke (GLM-F-102977); on *H. fallax*, Germany, Sachsen-Anhalt, Landkreis Wittenberg, Kemberg, 25 Aug. 2009, H. Jage (GLM-F-97335); on *H. glaucinum*, Germany, Sachsen, Görlitz, 1 Nov. 2009, H. Boyle (GLM-F-99602); Germany, Sachsen-Anhalt, Landkreis Mansfeld-Südharz, between Questenberg and Hainrode, 15 May 2009, A. Hoch (GLM-F-97576); on *H. lachenalii*, Germany, Sachsen-Anhalt, Landkreis Anhalt-Bitterfeld, Gröbzig, 10 Oct. 2006, H. Zimmermann (GLM-F-95815); on *H. laevigatum*, Germany, Sachsen-Anhalt, Sandersdorf, Stakendorfer Busch, 13 Sep. 1977, U. Braun (HAL 946 F); Germany, Sachsen, landkreis Görlitz, Boxberg, OT Bärwalde, 26 Aug. 2004, H. Boyle (GLM-F-53711); Germany, Sachsen-Anhalt, Eisleben, Steinmetzgrund, 18 Oct. 2001, H. John (GLM-F-54365); Germany, Thüringen, Landkreis Gotha, Friedrichroda, 25 Sep. 2004, H. Jage & H. Boyle (GLM-F-64230); on *H. murorum*, Germany, Sachsen-Anhalt, Landkreis Wittenberg, north west of Oranienbaum, 20 May 2003, H. Jage (GLM-F-63253); Germany, Sachsen-Anhalt, Landkreis Mansfeld-Südharz, Grillenberg, 9 Nov. 2003, H. Jage (GLM-F-63260); on *H. porrifolium*, Slovakia, Žilinský kraj, Okres Bytča, Súľovské vrchy, 26 Jul. 2011, F. Klenke (GLM-F-105347); on *H. racemosum*, Germany, Nordrhein-Westfalen, Mönchengladbach, Schloß Dyk, 6 Sep. 2003, U. Raabe (GLM-F-63139); on *H. sabaudum*, Germany, Sachsen-Anhalt, Dübener Heide, Rotta, 09 Oct. 1976, U. Braun (HAL 953 F), GenBank number: MZ420213 (28S rDNA); Germany, Sachsen, Bautzen, 15 Oct. 2003, H. Boyle (GLM-F-51367); Germany, Sachsen, Landkreis Bautzen, Bischofwerda, 12 Jul. 2007, H. Boyle (GLM-F-81076); Germany, Sachsen, Görlitz, 2 Sep. 2003, H. Boyle (GLM-F- 51329); Germany, Sachsen, Görlitz, 9 Sep. 2003, S. Hoeflich & H. Boyle (GLM-F-51429); Germany, Sachsen, Landkreis Görlitz, Niesky, 16 Nov. 2006, H. Boyle (GLM-F-78755); Germany, Sachsen, Bautzen, 19 Sep. 2006, H. Boyle (GLM-F-74935); Germany, Sachsen, Landkreis Görlitz, Herrenhut, 28 June 2007, H. Boyle

(GLM-F-79431); Germany, Sachsen, Landkreis Görlitz, Seifhennersdorf, 28 June 2007, H. Boyle & G. Zschieschang (GLM-F-79445); Germany, Sachsen, Landkreis Bautzen, Großröhrsdorf, 23 Sep. 2009, H. Boyle (GLM-F-99681); *H. schmidtii*, Germany, Sachsen-Anhalt, Saalekreis, Gimritzer Porphyrlandschaft, 21 Nov. 2007, H. John (GLM-F-94444); on *Hieracium* sp., Germany, Thüringen, Greiz, Cossengrün, 5 Sep. 1999, H. Jage (GLM-F-47535); on *H. virosum*, Russia, Bashkortostan Tujmazinskij Rayon, south west of Tujmazy, Kandrikul Lake, 12 Jul. 1977, U. Braun (HAL 859 F), GenBank number: MZ420206 (ITS+28S); on *Pilosella procera*, China, Xinjiang, Altay, Fuhai, 6 Aug. 1975, Z.Y. Zhao (HMAS39956).

Notes. *Golovinomyces cichoracearum s. lat.* (syn. *Erysiphe cichoracearum*) on *Hieracium* and *Pilosella* spp. is common and widespread in Europe, North America and parts of Asia. *Golovinomyces cichoracearum s. str.* does not occur on *Hieracium* spp. Based on comprehensive phylogenetic examinations, Takamatsu *et al.* (2013) demonstrated that *G. cichoracearum s. str.* is confined to host species of *Scorzonera* and *Tragopogon*. A single reexamined collection on *H. sabaudum* (HAL 953 F) was a mixed infection with *G. hieraciorum* and *Neoërysiphe nevoi*. This is the first report of the *N. nevoi* on *Hieracium*. The asexual stage in this collection was *N. nevoi*, confirmed by ITS rDNA sequence data retrieved from mycelia and conidia, whereas the chasmothecia in this collection were of *G. hieraciorum*, which was also confirmed by ITS rDNA sequences data obtained from chasmothecia.

Golovinomyces cichoracearum on *L. communis* has been reported from numerous European countries (Amano, 1986; Braun, 1995; Farr and Rossman, 2021). In order to clarify the phylogenetic and taxonomic position of this taxon and to find appropriate material for additional sequence analyses, all German powdery mildew collections on *L. communis*, deposited at GLM and HAL, have been re-examined. All of these collections were shown to be either misidentified or were powdery mildew species other than *G. cichoracearum s. lat.*

The following re-examined collections, including some originally misidentified as *G. cichoracearum*, pertain to *Podosphaera ergerontis-canadensis* on *L. communis*. Germany, Hessen, Schwalm-Eder-Kreis, Bad Zwesten, 14 Jul. 2007, C. Klencke (GLM-F-104902). Germany, Sachsen, Landkreis Nordsachsen, Zschepplin, OT Krippehna, 29 Sep. 1997, H. Jage (GLM-F-48602). Germany, Sachsen, Landkreis Nordsachen, Laußig, OT Pressel, 22 Oct. 1998, H. Jage (GLM-F-50206). Germany, Sachsen, Oschatz, park, 8 Oct. 2006, H. Jage (GLM-F-86559). Germany, Sachsen, Landkreis Görlitz, Oberoderwitz, Kirchweg, 19 Jul. 2007, H. Boyle (GLM-F-80873). Germany, Sachsen, Landkreis Görlitz, Niesky, Stannewisch, 5 Jul. 2007, H. Boyle & S. Hoeflich (GLM-F-79525). Germany, Sachsen, Landkreis Görlitz, Boxberg, Uhyst, 20 Oct. 2005, H. Boyle (GLM-F-70244). Germany, Sachsen, Görlitz, 26 Aug. 2005, H. Boyle (GLM-F-70145). Germany, Sachsen, Zittau, Westpark, 9 Aug. 2007, H. Boyle (GLM-F-80893). Germany, Sachsen-Anhalt, Landkreis Börde, north east of Samswegen, Heidberg, 16 Oct. 2006, H. Jage (GLM-F-86402). Germany, Sachsen-Anhalt, Landkreis Wittenberg, Wörlitz, Wörlitzer Park, 16 Nov. 2000, H. Jage (GLM-F-47950). Germany, Sachsen-Anhalt, Burgenlandkreis, Burg Saaleck, 29 Sep. 2003, H. Jage (GLM-F-66171). Germany, Sachsen-Anhalt, Dessau, Kühnauer Park, 13 Sep. 2001, H. Jage (GLM-F-54331). Germany, Sachsen-Anhalt, Burgenlandkreis, Osterfeld, east of OT Waldau, 27 Aug. 2003, H. Jage (GLM-F-63279). Germany, Sachsen-Anhalt, Landkreis Wittenberg, Bleddin, west of Bleddiner Riß, 31 Oct. 2000, H. Jage (GLM-F-48009). Germany, Sachsen-Anhalt, Halle (Saale), Herrmann-Street, 1 Jun. 2020, U. Braun (HAL 3350 F). Other collections (only asexual morphs), mostly identified as *G. cichoracearum*, proved to be *N. nevoi* on *L. communis* [Germany, Mecklenburg-Vorpommern, Usedon, east of Ückeritz, 9 Aug. 1988, H.Jage (GLM-F-58821). Germany, Rheinland-Pfalz, Kreis Cochem, Zell, OT Tellig, 12 Jul. 1999, H. Jage (GLM-F-46819). Germany, Sachsen, Landkreis Nordsachsen, Laußig, OT Görschlitz, 22 June 1997, H. Jage (GLM-F-50126). Germany, Sachsen, Landkreis Nordsachen, Zscheppelin, OT Hohenprießnitz, 10 May 1998, H. Jage *et al.* (GLM-F-49966). Germany, Sachsen, Landkreis Görlitz, Bertsdorf, 31 May 2007, H. Boyle (GLM-F-79245). Germany, Sachsen, Landkreis Görlitz, Johnsdorf, Hänischmühe, 21 June 2007, H. Boyle & S. Hoeflich (GLM-F-79470). Germany, Sachsen-Anhalt, Landkreis Wittenberg, Kemberg, 3 Apr. 1999, H. Jage (GLM-F-46829). Germany, Sachsen-Anhalt, Salzlandkreis, Calbe, 5 May 2001, H. Jage (GLM-F-57042). Germany, Sachsen-Anhalt, Landkreis Börde, Oscherleben, OT Hadmersleben, 12 June 2002, H. Jage (GLM-F-57288). Germany, Sachsen-Anhalt, Halle (Saale), Gustav-Anlauf-Street, 2 Jun. 2020, U. Braun (HAL 3349 F). Germany, Thüringen, Ilm-Kreis, Arnstadt, 5 June 1997, H. Jage (GLM-F-50147)].

Therefore, reports of *G. cichoracearum s. lat.* (syn. *Erysiphe cichoracearum*) on *L. communis* from various countries [Europe (Belarus, England, France, Hungary, Italy, Netherlands, Norway, Poland, Romania, Sweden, Switzerland, Ukraine, United Kingdom, Yugoslavia), Asia (Armenia)] are doubtful, and need to be reviewed and confirmed.

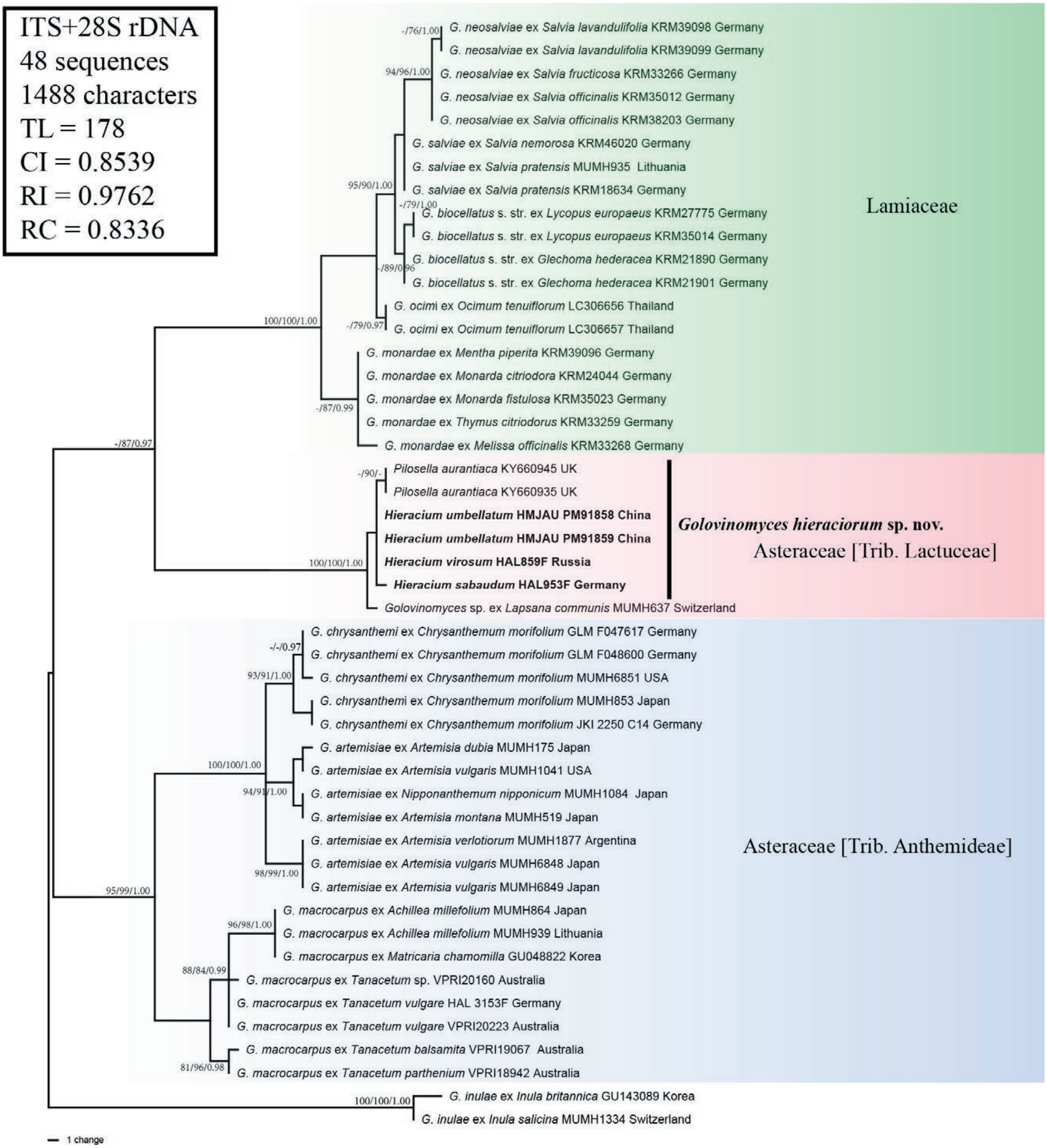

Figure 2. Phylogenetic analysis of the ITS and 28S rDNA regions. The bootstrap support values greater than 75% for maximum parsimony (MP) and maximum likelihood (ML) are displayed followed by posterior probabilities greater than 0.90 for Bayesian Inference (BI). The sequences determined in this study were shown in bold font and pink shade. *Golovinomyces inulae* was used as the outgroup taxon.

Phylogenetic analyses

ITS and 28S rDNA sequences were generated from four *Hieracium* spp. specimens (highlighted in bold font in Figure 2). Newly determined sequences were aligned with other closely related sequences that were retrieved from DNA databases, and were based on Scholler *et al.* (2016), Bradshaw *et al.* (2017), and Meeboon *et al.* (2018).

Golovinomyces inulae was used as the outgroup taxon. The combined dataset of ITS and 28S rDNA sequences consisted of 48 sequences including 1488 characters, of which 108 (7.3%) were parsimony informative and 14 (0.9%) were parsimony-uninformative. The maximum parsimony tree (TL = 178, CI = 0.8539, RI = 0.9762, RC = 0.8336) with the greatest likelihood value is shown in Figure 2. A phylogenetic tree generated from ML and BI analysis was almost identical to the MP tree, so this tree is not presented here.

The tree comprises three groups with high reliability, one comprising isolates from *Compositae* (tribe *Anthemideae*) (MP = 95%, ML = 99%, BI = 1.00), the second representing the *G. biocellatus* complex clade, comprising isolates from *Lamiaceae* with high reliability (MP =100%, ML = 100%, BI = 1.00), and the third was a cluster composed of isolates from *Compositae* (tribe *Lactuceae*) also with high reliability (MP =100%, ML = 100%, BI = 1.00). The second and third groups formed a big clade with high reliability (ML = 87%, BI = 0.97).

Sequences from *Hieracium* spp. and *Pilosella aurantiaca* as hosts are situated in lineages IX, comprising isolates from *Compositae* (tribe *Lactuceae*) with strong reliability (Figure S1). Two sequences from powdery mildew on *Pilosella aurantiaca* formed a subclade with strong BS supports (ML = 90%) (Figure 2).

DISCUSSION

Golovinomyces, with *Euoidium* as the synonymous anamorph genus, currently comprises 71 species and five varieties (Braun and Cook, 2012; Liu and Wen, 2013; Bradshaw *et al*., 2017; Meeboon *et al*., 2018; Braun *et al*., 2019; Qiu *et al*., 2020). Takamatsu *et al.* (2013) published comprehensive phylogenetic analyses of 33 *Golovinomyces* spp., which were split into 11 genetically distinct lineages. Sequences obtained from seven collections from hosts in tribe *Lactuceae* of the *Compositae* were in two genetically distinct lineages. One was composed of sequences from collections on *Scorzonera* and *Tragopogon* (tribe *Lactuceae*, subtribe *Scorzonerinae*), viz. *G. cichoracearum s. str.*. The second lineage consisted of a single sequence from a collection on *L. communis* (tribe *Lactuceae*, subtribe *Crepidinae*) from Switzerland, which can currently only be referred to as *Golovinomyces* sp. New sequences retrieved from *Golovinomyces* collections on *Hieracium* spp. and *Pilosella aurantiaca* (tribe *Lactuceae*, subtribe *Hieraciinae*) have been generated and added to the current phylogenetic ITS and 28S rDNA analyses (Figure S1, including 43 species of *Golovinomyces*), which significantly increased the *Golovinomyces* lineage. This lineage, with high reliability (MP = 100%, ML = 100%, BI = 1.00), is distant from all other clades within *Golovinomyces*, and remains divided into two clades, viz., the *Hieracium* clades (including *Pilosella aurantiaca*) and a *L. communis* clade. The present study supports previous presumptions of a close co-evolution between *Golovinomyces* species and particular host tribes and subtribes of the *Compositae* (Matsuda and Takamatsu, 2003; Takamatsu *et al*., 2013; Qiu *et al*., 2020).

The *Hieracium* clade, represents a new, hitherto undescribed species of *Golovinomyces*, previously hidden within *G. cichoracearum s. lat.*, which is described here as *G. hieraciorum*. The new species is confined to *Hieracium* and *Pilosella* spp. (tribe *Lactuceae*, subtribe *Hieraciinae*), which reflects the close co-evolution of *Golovinomyces* with host tribes and subtribes within the *Compositae*. *Pilosella* is phylogenetically close to *Hieracium* and was previously often treated as a subgenus of *Hieracium*. Fehrer *et al.* (2007) and Krak *et al.* (2013) conducted comprehensive phylogenetic analyses, indicating that *Hieracium* and *Pilosella* should be two distinct plant genera. The phylogenetically proven occurrence of *G. hieraciorum* on *Hieracium* and *Pilosella* spp. reflects the close affinity of these two host genera.

Golovinomyces on *Hieracium* and *Pilosella* spp. is widespread in Europe, North America, and parts of Asia. Besides *G. hieraciorum*, two additional powdery mildews may occur on *Hieracium* spp., viz. *Neoërysiphe nevoi* and *Podosphaera erigerontis-canadensis.* Mixed infections with these two pathogens have to be previously taken into consideration. One collection on *H. sabaudum* from Germany was such a mixed infection of *G. hieraciorum* and *N. nevoi.* This is the first report of *N. nevoi* on *Hieracium*. *Neoërysiphe nevoi* and *G. hieraciorum*, two powdery mildew species on *Compositae*, are easily confusable and not easily distinguishable based on morphology. However, the asci of *Neoërysiphe* spp. remain immature, and ascospores do not develop in 2–8-spored asci until after overwintering. In *Golovinomyces* spp., including *G. hieraciorum*, ascospores mature in the current season and usually develop in 2-spored asci. Furthermore, the hyphal appressoria in *N. nevoi* are lobed, in contrast to nipple-shaped hyphal appressoria on *G. hieraciorum*.

The *L. communis* clade within *Golovinomyces* posed a special problem. *Lapsana* belongs to subtribe *Crepidinae* of tribe *Lactuceae* and is now confined to a single species, *L. communis* (Pak and Bremer, 1995). Attempts failed to solve this problem by tracing additional collections for detailed morphological and phylogenetical examinations of this powdery mildew. Numerous collections on *L. communis* from the herbaria GLM and HAL, identified as *Erysiphe cichoracearum* or *G. cichoracearum*, have been re-examined, but all were misidentified. Most collections (without chasmothecia) were shown to belong to *N. nevoi* (hyphal appressoria lobed, foot-cells of the conidiophores characteristically short, 20–50 × 9–15 µm, basal septae of conidiophores foot-cells often conspicuously constricted, conidia with striate surfaces [as shown by scanning electron microscopy]). The occurrence of *N. nevoi* on *L. communis* has also been confirmed by sequence analyses and affirmed in the first report of this species on this host from Germany (Schmidt and Braun, 2020). There were even some collections with chasmothecia, originally identified as "*Golovinomyces cichoracearum*", which turned out to be *Po. erigerontis-canadensis* (chasmothecia with single asci). *Podosphaera erigerontis-canadensis* is already known on *L. communis* in Germany (reported in Jage *et al.* (2010), under the name *Po. fusca*). In summary, all collections on *L. communis* assigned to *G. cichoracearum* or *E. cichoracearum*, including those reported in Jage *et al.* (2010), were here shown to be misidentified. Therefore, the identity of the true *Golovinomyces* on *L. communis* could not be clarified in the course of the present study. Records of *G. cichoracearum* and *E. cichoracearum*, respectively, on *Lapsana* from various countries in Europe (Belarus, England, France, Hungary, Italy, Netherlands, Norway, Poland, Romania, Sweden, Switzerland, Ukraine, United Kingdom, former Yugoslavia) and Near East-Caucasus (Armenia) remain unclear and need to be verified.

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