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SHORT COMMUNICATION

Golovinomyces longipes (Noordel. & Loer.) L. Kiss on *Matricaria chamomilla* L.: another host range expansion

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Abstract
Golovinomyces longipes is a widespread powdery mildew on *Solanaceae* (*Nicotiana*, *Petunia* and *Solanum* spp.). In the past, it has been reported only once on *Verbena* (*Verbenaceae*), a non-solanaceaous host. Recently, this powdery mildew has been found on the composite *Matricaria chamomilla*. The identification of the powdery mildew species on this unusual host has been proved by morphological studies and DNA sequence analysis. Both datasets coincide with the characteristic data for *G. longipes* on *Solanaceae*. First inoculation experiments with further composites resulted in an infection of *Brachyscome* hybrid 'Surdaisy'. To our knowledge, this is the first report of *G. lonigpes* on hosts belonging to the important family of *Asteraceae* and an additional proof of the broader host range of *G. longipes* beyond the *Solanaceae*.

Keywords *Erysiphaceae* · Powdery mildew · *Golovinomyces* · Chamomile · Asteraceous hosts

Introduction

Chamomile (*Matricaria chamomilla* L.) is a widespread, common annual composite, native in temperate Eurasia to Indo-China and introduced in multiple parts of the world, including North Africa, North and South America, West Indies, and Australia (http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:154715-2). Since ancient times, this plant has been used in folk medicine as well as in orthodox medicine, mainly against gastrointestinal problems and as remedy to treat irritations of the skin (Singh et al. 2011). Chamomile is susceptible to powdery mildew infections. Braun and Cook (2012) listed *M. chamomilla* as a host of *Golovinomyces macrocarpus* (Speer) U. Braun, *G. orontii* (Castagne) Heluta s. lat., and *Podosphaera erigerontis-canadensis* (Lév.) U. Braun & T. Z. Liu. Records of these species on chamomile are still in need of confirmation by means of sequence analyses, above all in the case of *G. orontii* s. lat., which has recently been divided into various species, including *G. bolayi* S. Takam. et al. and *G. tabaci* (Sawada) H. D. Shin et al. (Braun et al. 2019).

Golovinomyces longipes is a widespread powdery mildew that is known to specifically infect *Solanaceae* (*Nicotiana*, *Petunia* and *Solanum* spp.; Braun and Cook 2012). Only once it has been reported on *Verbena* (*Verbenaceae*), a non-solanaceaous host (Brielmaier-Liebetanz et al. (2015).

In 2020, severe spontaneous infections with powdery mildew on chamomile have been observed in a growth chamber (Julius Kühn-Institute, Institute for Plant Protection in Horticulture and Forests, Braunschweig, Germany). As the growth chamber was quite isolated from environmental conditions, it was more likely that infection was caused by *Golovinomyces longipes* that was abundant on petunia in this chamber than by any other powdery mildew species introduced from the outside. The aim of the study was to confirm the infection of chamomile by *G. longipes* and thus prove the host range expansion..

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Materials and methods

Collection and maintenance of the powdery mildew isolate

Golovinomyces longipes was originally isolated from *Petunia* plants in a garden. A single spore isolate was prepared and maintained on *Petunia* × hybrida 'Himmelsröschen' in a growth chamber at 20 ± 2 °C and 14 h light.

Ten-week old chamomile plants cv.'Mabamilla' were cultivated in the vicinity in order to test their susceptibility against *G. longipes*. The powdery mildew that spontaneously developed on the chamomile plants was easily transferable to further chamomile plants by dusting conidia onto the plants. It has been analysed by morphological and molecular biological means.

Morphological characterization

All morphological characteristics were examined using a light microscope (Axio Imager.A1 equipped with an AxioCam MRc5 camera, Zeiss, Germany) and differential interference contrast at magnifications 200×, 400×, and 1000×. Measurements were made and images were taken with the calibrated ZEN Blue Edition software rel. 3.1 (Zeiss, Germany). The images were processed using Adobe Photoshop CS4 software version 11.0 (Adobe Systems, USA).

Morphological characteristics covering size and shape of conidia ($n = 100$) and conidiophores ($n = 50$), position of the basal septum, shape and position of hyphal appressoria and presence or absence of fibrosin bodies were assessed. Conidial length and width were analysed following Frank et al. (1990) with modifications. Conidial germination patterns were determined following the method of Zaracovitis (1965) with slight modifications.

Molecular characterization

To confirm the identity of the powdery mildew from the chamomile donor plants total genomic DNA was extracted from visibly infected chamomile leaves including conidia and mycelia using the DNeasy plant mini kit (Qiagen GmbH, Germany) following the manufacturers´ instructions with modifications.

The 5´-end of the 28S rDNA and internal transcribed spacer (ITS) were amplified using the primers ITS5 and PM6 (Takamatsu and Kano 2001) for ITS fragment and PM5 (Takamatsu and Kano 2001) and NLP2 (Hirose et al. 2005) for 28S. The reaction mix contained MyFi Mix (Bioline), 0.2 µM of each primer and 4 µl template DNA in a total volume of 50 µl. PCR was performed in a Biometra Tone Cycler (Analytic Jena, Germany) with an initial denaturation step at 94 °C for 3 min following 40 cycles of 30 s at 94 °C for denaturation, 30 s at 50 °C for annealing, 45 s at 72 °C for extension and a final extension for 10 min at 72 °C. Amplicons were purified (MSB Spin PCRapace Kit, Stratec Biomedical AG, Germany) and sequenced for each primer pair twice in each direction (LGC Genomics GmbH, Germany). Contigs were generated and edited using CLC Main Workbench 20.0 (Qiagen Digital Insights, Germany) following the EPPO recommendations for sequence analysis (OEPP/EPPO 2021). The resulting 1287 bp consensus sequence was compared to sequences deposited in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the identity and deposited in GenBank as *Golovinomyces longipes* (Accession No. MW330009).

Pathogenicity tests

As this was the first observation of *G. longipes* on an asteraceous host, first inoculation experiments were conducted with *Brachyscome* hybrid 'Surdaisy', *Pallenis maritima* (syn. *Asteriscus maritimus*), *Argyranthemum frutescens, Bidens ferulifolia*, *Osteosperum* sp. to evaluate if further species of the *Asteraceae* were infected by *G. longipes*. At least 10 cuttings of each host plant were transferred into plastic boxes filled with tap water and covered with grids to enable petioles dip into water. Cuttings were inoculated by dusting of one to two days old conidia from heavily infected chamomile plants and incubated in a growth chamber as described before. The pathogenicity test was carried out twice.

Results

The morphology of the detected powdery mildew on chamomile corresponded well with *Golovinomyces longipes* on *Solanaceae* (Braun and Cook 2012). The observed asexual morph of *G. longipes* (≡ *Oidium longipes* Noordel. & Loer., *Euoidium longipes* (Noordel. & Loer.) U. Braun & R.T.A. Cook) is characterized as follows (Fig. 1a–k): White mycelium on leaves and stems, superficial, in patches or effuse, finally covering entire leaf segments or leaves. Hyphae branched, 5.7–8.6 µm wide, hyaline, septate, thin-walled, smooth. Hyphal appressoria rather scarce developed, solitary, nipple-shaped. Conidiophores solitary, on the upper surface of superficial hyphae, erect, 170–379 µm long, foot-cells straight, subcylindrical, 47.5–283 × 8.5–12.8 µm, followed by 1–4 shorter cells, basal septum at the junction with the mother hypha or

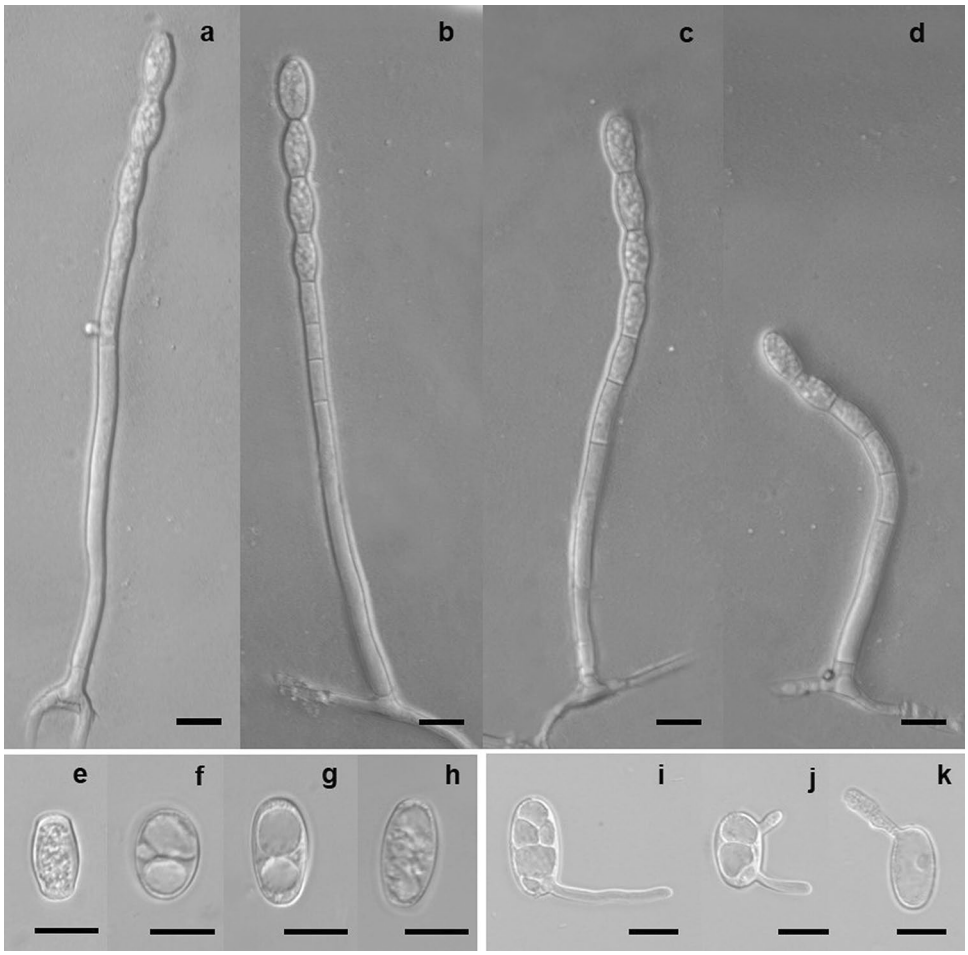

Fig1 *Golovinomyces longipes* on *Matricaria chamomilla*'Mabamilla': conidiophores **a**–**d**, conidia **e–h**, germination pattern **i–k**. The background was corrected with Adobe Photoshop to clarify the relevant conidiophores features. Bar = 20 µm

slightly elevated (–16.5 µm). Conidia catenescent, ellipsoid, subcylindrical, doliiform, (23–)24–35(–42) × (15–)17–21(–23) µm, on average 30 × 19 µm, length/width ratio 1.1–2.5 (on average 1.6), usually with a single germ tube, occasionally two germ tubes, subapical to lateral, aseptate or with a single septum, subcylindrical to club-shaped (voucher specimen: Germany, Lower Saxony, Braunschweig, Julius Kühn Institute, glasshouse, on *Matricaria chamomilla*, 2021, M. Götz, HAL 3367 F).

The sequence obtained for this isolate showed 99.92% identity to *G. longipes* (syn. *Euoidium longipes* [AB769440]) in GenBank and, therefore, confirmed this species as causative agent of powdery mildew on chamomile.

G. longipes was easily transferable from infected chamomile to healthy plants of different age. Symptoms appeared 10 to 14 days after inoculation and infection proceeded until the entire plant was heavily infected 28 days after inoculation (Fig. 2 a, b). The severity of infection differed from

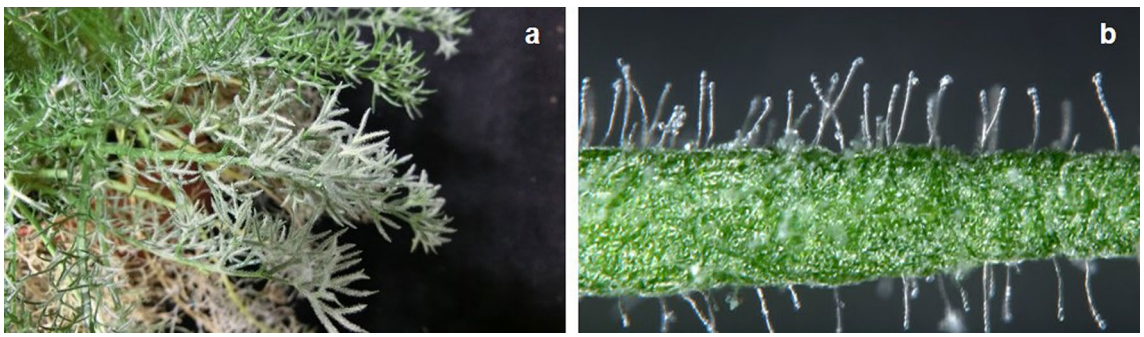

Fig2 *Golovinomyces longipes* on *Matricaria chamomilla*'Mabamilla' 28 days after inoculation **a**, infected leaf 28 after inoculation **b**

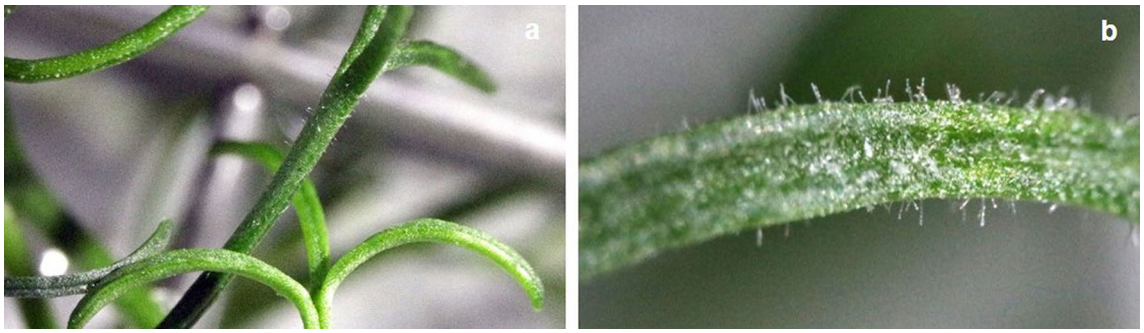

Fig3 *Golovinomyces longipes* on *Brachyscome* hybrid 'Surdaisy' 35 days after inoculation **a**, infected leaf 35 days after inoculation **b**

plant to plant. To assess if further *Asteraceae* could serve as hosts for *G. lonigpes* first inoculation experiments were performed. On *Brachyscome* hybrid 'Surdaisy' first pustules were observed ~ 21 days after inoculation. Disease severity developed with proceeding incubation time but never reached that observed for chamomile (Fig. 3 a, b). The other species tested were not infected.

Discussion

idium longipes was originally described to occur in the Netherlands on *Solanum melongena* L. (Noordeloos and Loerakker 1989). Braun and Cook (2012) reallocated this species to *Euoidium* Y.S. Paul & J.N. Kapoor, and, finally, Kiss (in Braun et al. 2019) introduced the name *Golovinomyces longipes*, based on results of phylogenetic analyses and the application of the rules of the current Code (ICNafp). *Euoidium* was originally introduced for asexual morphs of powdery mildews characterized by forming catenescent conidia, and *Oidium erysiphoides* Fr. was designated as the type species. Braun and Cook (2012) neotypified *O. erysiphoides* and applied *Euoidium* to asexual morphs of *Golovinomyces*.

Golovinomyces longipes is widespread in Europe on diverse hosts of the family *Solanaceae*, including *Nicotiana* sp., *Petunia* × *hybrida*, *Solanum lycopersicum*, and *S. melongena*, and known from Austria, Germany, Hungary, the Netherlands, Switzerland, and UK (Kiss and Bereczky 2011; Braun and Cook 2012; Braun and Brielmaier-Liebetanz 2013). Furthermore, Brielmaier-Liebetanz et al. (2015) reported *G. longipes* infections on *Calibrachoa* × *hybrida* under greenhouse conditions in Germany. This species is also known from North America, USA, New Jersey, on petunia (Kiss et al. 2008). Havrylenko and Takamatsu (2005) listed *O. longipes* from Argentina, and Toome et al. (2015) reported this species from New Zealand on *Solanum betaceum*. Members of the *Solanaceae* are undoubtedly principal hosts of *G. longipes*. However, Brielmaier-Liebetanz et al. (2015) reported the occurrence of *G. longipes* under greenhouse conditions on *Verbena* × *hybrida* (*Verbenaceae*) in Germany, which was the first record of this species on a non-solanaceous host. The present phylogenetically proven record of *G. longipes* on *Matricaria chamomilla* (*Asteraceae*) is the second unusual host expansion of this species to a host reaching beyond the *Solanaceae*. This sheds light on the potentially much wider host range of this species that might have been overlooked up to know. The actual host range could be evaluated by comprehensive surveys of representatives of the *Asteraceae* and further plant families and extensive pathogenicity tests. It might be of particular interest for growers who cultivate solanaceous plants in the vicinity of asteraceous ones and might affect the resulting strategies of plant protection measures that have to be taken if a powdery mildew infection is observed on hosts of the *Solanaceae*.

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Authors' contributions Monika Götz designed and performed the experiments and conducted the morphological and molecular characterization of the powdery mildew. Both authors undertook the data analysis and discussion. The first draft of the manuscript was written by Uwe Braun and complemented by Monika Götz. Both authors read and approved the final manuscript.

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Data availability A specimen voucher of Golovinomyces longipes on Matricaria chamomilla (HAL 3367 F) was deposited in the Herbarium of the Martin-Luther-University (Halle-Wittenberg, Germany). The sequence of Golovinomyces longipes C20-EM32 was submitted to Genbank (Accession number MW330009).

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval The experiments did not involve human participants and/or animals.

Consent to participate The two authors agreed to participate.

Consent for publication Both authors agreed to publish the manuscript as Short Communication in the Journal of Plant Diseases and Protection.

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