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

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Abstract

Background: Previous phylogenetic analyses of species within the genus Golovinomyces (Ascomycota, Erysiphales), based on ITS and 28S rDNA sequence data, revealed a co-evolutionary relationship between powdery mildew species and hosts of certain tribes of the plant family Asteraceae. Golovinomyces growing on host plants belonging to the Heliantheae formed a single lineage, comprised of a morphologically differentiated complex of species, which included G. ambrosiae, G. circumfusus, and G. spadiceus. However, the lineage also encompassed sequences retrieved from Golovinomyces specimens on other Asteraceae tribes as well as other plant families, suggesting the involvement of a plurivorous species. A multilocus phylogenetic examination of this complex, using ITS, 28S, IGS (intergenic spacer), TUB2 (beta-tubulin), and CHS1 (chitin synthase I) sequence data was carried out to clarify the discrepancies between ITS and 28S rDNA sequence data and morphological differences. Furthermore, the circumscription of species and their host ranges were emended.

Results: The phylogenetic and morphological analyses conducted in this study revealed three distinct species named, viz., (1) G. ambrosiae emend. (including G. spadiceus), a plurivorous species that occurs on a multitude of hosts including, Ambrosia spp., multiple species of the Heliantheae and plant species of other tribes of Asteraceae including the Asian species of Eupatorium; (2) G. latisporus comb. nov. (≡ Oidium latisporum), the closely related, but morphologically distinct species confined to hosts of the Heliantheae genera Helianthus, Zinnia, and most likely Rudbeckia; and (3) G. circumfusus confined to Eupatorium cannabinum in Europe.

Conclusions: The present results provide strong evidence that the combination of multi-locus phylogeny and morphological analysis is an effective way to identify species in the genus Golovinomyces.

Keywords: Erysiphaceae, Powdery mildew, Heliantheae, 28S rDNA, ITS, Golovinomyces latisporus, IGS, TUB2, CHS1
Background

Powdery mildews are obligate biotrophic ascomycetes that occur on a wide range of dicotyledonous and monocotyledonous host plants. The family Erysiphaceae has a nearly worldwide distribution, with the exception of the Antarctic region, and currently comprises around 900 species in 18 genera [1–3]. Golovinomyces was originally introduced by Braun [4] as a section of the genus Erysiphe (s. lat.) and was later raised to genus rank by Heluta [5]. Braun [6] and Braun and Takamatsu [7] accepted Golovinomyces as a distinct genus and established the new tribe Golovinomyceae. Golovinomyces is characterized by having chasmothecia with mycelioid appendages, several, mostly 2-spored asci, an asexual morph with cateneshed conidia that lack fibrosin bodies, and mostly nipple-shaped apressoria. Golovinomyces currently encompasses 57 species and 5 varieties [1, 8–13]. Erysiphe cichoracearum [14] included nearly all of the species that are now assigned to Golovinomyces. Blumer [15, 16] split E. cichoracearum sensu Salmon [14] into several species but continued to maintain the species E. cichoracearum in a very broad sense (covering collections on Asteraceae and on hosts of multiple other plant families). Braun [17] confined E. cichoracearum to powdery mildews on hosts of Asteraceae and assigned specimens on hosts belonging to other plant families to Erysiphe orontii. Phylogenetic analyses of Golovinomyces, based on ITS and 28S rDNA sequence data [18], suggested the co-evolution between Golovinomyces species and certain tribes of Asteraceae. Based on these results, Braun and Cook [1] introduced a much narrower species concept for this genus, which included two morphologically differentiated species on hosts belonging to the Heliantheae, viz., G. ambrosiae and G. spadiceus. However, in more detailed phylogenetic analyses of ITS and 28S rDNA sequences, including Golovinomyces species on Asteraceae hosts, Takamatsu et al., [19] found that powdery mildews on hosts of the Heliantheae (previously referred to as G. ambrosiae and G. spadiceus), on hosts of an Asian species of Eupatorium (G. circumfusus s. lat.) and on a multitude of other hosts, including those on other plant families, formed a single large, unresolved clade (lineage III in Takamatsu et al., [19]). The taxonomic interpretation of these results posed a serious problem since G. ambrosiae and G. spadiceus, as circumscribed in Braun and Cook [1], are two morphologically differentiated species. Hence, the resolution within phylogenetic trees based only on ITS sequences was in this case insufficient to discriminate closely allied species. Therefore, most subsequent authors followed the taxonomic treatment in Braun and Cook [1] and recognized G. ambrosiae and G. spadiceus as separate species within lineage III, based on morphological differences [20–27]. The morphological differences used to differentiate the species include above all, much broader conidia and dimorphic germ tubes belonging to the longitudinal pattern within the Euoidium type of conidial germination in G. ambrosiae than in G. spadiceus [1]. Additional research has found that G. spadiceus to be extremely plurivorous, occurring on hosts of the Heliantheae and other tribes of Asteraceae, e.g., Aster and Chrysanthemum [19], Chrysogonum [28], as well hosts of various other plant families, including Abelsmoschus (Malvaceae) [29], Crotalaria (Fabaceae) [13], Persicaria (Polygonaceae) [11, 13, 30], Solanum (Solanaceae) [13], and Verbena (Verbenaceae) [13]. The taxonomic interpretation of the inclusion of a sequence obtained from a Japanese collection of powdery mildew on Eupatorium chinense in lineage III [19] caused an additional problem and raised the question whether the name G. circumfusus, originally described from Europe on Eupatorium cannabinum, is included in this species complex.

The purpose of the present study was to clarify and resolve the taxonomy of this Golovinomyces complex using a multilocus approach, based on ITS, 28S, IGS, and CHS1 DNA sequences. Multi-gene analyses are currently the method of choice to analyze phylogenetically and taxonomically difficult complexes of plant pathogenic fungi, including Colletotrichum spp. [31, 32]. However, there is minimal multilocus data for the powdery mildews currently available. Most of the research involves the intraspecific genetic diversity in species such as Blumeria graminis [33, 34], Erysiphe japonica [35], E. necator [36, 37], Podosphaera xanithi [38] and Golovinomyces orontii [39]. Recently, the geographic and temporal distributions of four genotypes found in E. gracilis var. gracilis were studied based on a combination of data from the ITS, 28S rDNA and IGS regions [40]. Comprehensive applications of multilocus approaches to solve complex taxonomic-phylogenetic problems connected with the species level classification of the powdery mildews are still lacking. The present study is the first to use a multilocus approach to solve species distinction issues within the Erysiphales. An additional issue regarding the taxonomic conclusions drawn from phylogenetic results is also addressed in this study. Older taxonomic names are often available, but the application and allocation of such names are usually problematic. Because species names are based on their type collections, epitypifications, with appropriate new material, and ex-type sequences tend to be the main method to overcome these obstacles and to determine the application of older names. During the current study, this issue was addressed using international collaboration.

Methods

Sampling
A total of 69 specimens belonging to Golovinomyces ambrosiae, G. circumfusus, and G. spadiceus were examined, including 39 samples collected in China in recent
years and 30 additional specimens from Germany, Japan, Russia, Switzerland, and the USA. Furthermore, eight specimens, consisting of three samples of *G. magnicellulatus*, three samples of *Neoërysiphe galeopsisis*, a sample of *Arthrocladiella mougeotii* and a sample of *Erysiphe kenjiana*, were used for phylogenetic analyses in this study. All of the plant materials used in this study were collected in the public gardens with Latin names or some are common ornamental plants which were identified by ourselves. Among the 69 specimens, ISC-F-0076752, ISC-F-0076753, and ISC-F-0076754 were deposited in the Herbarium of Iowa State University Fungi of Iowa, and the rest voucher specimens were deposited in the Herbarium of Mycology of Jilin Agricultural University. Names of the host plants, fungal species, locations and years of collection, voucher numbers and newly sequenced multi-gene accession numbers for the nucleotide sequence database (GenBank) in this study are given in Table 1.

**Morphological examinations**

For microscopic examinations, fresh samples were mounted in sterile water, and dried specimens, scraped from the leaf surface with a clean scalpel, were mounted in a drop of lactic acid on a microscope slide. Slides were examined using light microscopy with the total magnification at 200 and 400 (Zeiss Axio Scope A1, Germany). Fresh conidia were examined for the presence or absence of fibrosin bodies. A minimum of 30 measurements were made of asexual and sexual fungal structures. Germination of conidia was examined following the method of Hirata [41].

**Molecular techniques and phylogenetic analyses**

Whole-cell DNA was extracted from chasmothecia or conidia and mycelia by the Chelex-100 method [42, 43]. In the USA, whole-cell DNA was extracted from chasmothecia or conidia (for the herbarium specimens: ISC-F-0076752, ISC-F-0076753, and ISC-F-0076754) with the DNeasy plant mini kit (Qiagen, Hilden, Germany), 2 µL dNTP Mixture (10 mM total, 2.5 mM each), 1 µL each primer (20 ng/µL), 2 µL of total genomic DNA, 0.1 µL Taq polymerase (TaKaRa, Japan) (5 U/µL) and sterile ddH₂O up to a final volume of 25 µL. The PCR reactions were conducted under the following thermal cycling conditions: an initial denaturation step of 5 min at 95°C, 35 cycles of 1 min at 94°C, followed by 30 s at 52°C for annealing, and 2 min at 72°C for extension, and a final extension for 8 min at 72°C. A negative control that lacked template DNA was included in each set of reactions. PCR products were subjected to electrophoresis in a 1.2% agarose gel in 0.5× TBE buffer. The amplified DNA products were purified using Mag-MK PCR Products Purification Kit following the protocol of the manufacturer. Amplicons were sequenced in both directions with the same PCR primers using direct sequencing in a 3730xl DNA Analyzer (Applied Biosystems) by Sangon Biotech (Shanghai, China). The sequence reactions were conducted using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following instructions of the manufacturer.

The reaction components for the PCR conducted at the University of Washington were 5 µL AllTaq PCR Buffer (Qiagen, Germany), 0.5 µL dNTP mixture, 0.25 µL of each primer (100 uM), 2 µL of total genomic DNA, 0.5 µL, Taq Polymerase (Qiagen, Germany) and sterile ddH₂O up to a final volume of 25 µL. DNA was purified by isopropanol precipitation. These sequences [(The 28S rDNA sequence from ISC-F-0076754 and IGS sequences from ISC-F-0076752 and ISC-F-0076753) were manually trimmed using Geneious version 11.0.2 (https://www.geneious.com) and deposited in GenBank.

All other new sequences obtained in the present study were edited by DNAMAN version 6.0 and BioEdit Sequence Alignment Editor version 7.0, and then deposited in GenBank (Table 1). The ITS, 28S, IGS, TUB2 and CHS1 sequences were respectively aligned by ClastalW. Furthermore, a multilocus sequences alignment datasets file (ITS + 28S + IGS + TUB2 + CHS1) including 40 strains from Table 1 was also used for phylogenetic analyses. The six alignments were further refined manually in MEGA 7.0 [49] and deposited in TreeBASE (http://www.treebase.org/) under the Accession No. of S24404 (http://purl.org/phylo/treebase/phylo/treebase/study/TB2:S24404). Phylogenetic trees were obtained from the sequence
| Species                          | Host                  | Location                      | Year of collection | Voucher a GenBank accessions No. b |
|---------------------------------|-----------------------|-------------------------------|--------------------|-----------------------------------|
| Arthrocladiella mougeotii       | Lycium chinense       | Beijing, China                | 2018               | HMJAU-PM91837 MK452607 MK452680 – – – |
| Erysiphe kengiana               | Ulmus pumila          | Changchun, Jilin province, China | 2017               | HMJAU-PM91841 MK452611 MK452684 – MK452438 – |
| Golovinomyces ambrosiae         | Aster novi-belgii     | Changchun, Jilin province, China | 2017               | HMJAU-PM91804 MK452575 MK452648 MK452501 MK452460 MK452410 |
| G. ambrosiae                    | A. novi-belgii        | Changchun, Jilin province, China | 2018               | HMJAU-PM91805 MK452576 MK452649 MK452502 MK452461 MK452411 |
| G. ambrosiae                    | A. novi-belgii        | Dunhua, Jilin province, China  | 2018               | HMJAU-PM91806 MK452577 MK452650 MK452503 MK452462 MK452412 |
| G. ambrosiae                    | A. novi-belgii        | Dunhua, Jilin province, China  | 2018               | HMJAU-PM91807 MK452578 MK452651 MK452504 MK452463 MK452413 |
| G. ambrosiae                    | A. novi-belgii        | Changchun, Jilin province, China | 2017               | HMJAU-PM91808 MK452579 MK452652 MK452505 MK452464 MK452414 |
| G. ambrosiae                    | Ageratina ligustrina  | Sochi city, Krasnodar region, Russia | 2018               | ERY015 MK452643 MK452717 MK452570 – – |
| G. ambrosiae                    | Ambrosia artemisiifolia | Mudanjiang, Heilongjiang, China | 2017               | HMJAU-PM91809 MK452580 MK452653 MK452506 MK452465 MK452415 |
| G. ambrosiae                    | A. artemisiifolia     | Changchun, Jilin province, China | 2018               | HMJAU-PM91810 MK452581 MK452654 MK452507 MK452466 MK452416 |
| G. ambrosiae                    | A. artemisiifolia     | Tonghua, Jilin province, China  | 2018               | HMJAU-PM91811 MK452582 MK452655 MK452508 MK452467 MK452417 |
| G. ambrosiae                    | A. artemisiifolia     | Tonghua, Jilin province, China  | 2018               | HMJAU-PM91812 MK452583 MK452656 MK452509 – MK452418 |
| G. ambrosiae                    | A. artemisiifolia     | Guthrie County, Iowa, USA     | 1987               | ISC-F-0076752 – – MK452567 – – |
| G. ambrosiae                    | A. trifida            | Guthrie County, Iowa, USA     | 1987               | ISC-F-0076754 – – MK452715 – – – – |
| G. ambrosiae                    | A. trifida            | Guthrie County, Iowa, USA     | 1997               | ISC-F-0076753 – – – MK452568 – – |
| G. ambrosiae                    | A. trifida            | Siping, Jilin province, China  | 2018               | HMJAU-PM91813 MK452584 MK452657 MK452510 MK452468 MK452419 |
| G. ambrosiae                    | A. trifida            | Changchun, Jilin province, China | 2018               | HMJAU-PM91814 MK452585 MK452658 MK452511 MK452469 MK452420 |
| G. ambrosiae                    | A. trifida            | Anshan, Liaoning, China       | 2018               | HMJAU-PM91815 MK452586 MK452659 MK452512 MK452470 MK452421 |
| G. ambrosiae                    | A. trifida            | Shenyang, Liaoning, China     | 2018               | HMJAU-PM91816 MK452587 MK452660 MK452513 – MK452422 |
| G. ambrosiae                    | Dahlia pinnata        | Dandong, Liaoning, China      | 2012               | HMJAU-PM91817 MK452588 MK452661 MK452514 – MK452422 |
| G. ambrosiae                    | D. pinnata            | Changchun, Jilin province, China | 2017               | HMJAU-PM91818 MK452589 MK452662 MK452515 MK452471 MK452423 |
| G. ambrosiae                    | D. pinnata            | Changchun, Jilin province, China | 2017               | HMJAU-PM91819 MK452590 MK452663 MK452516 MK452472 MK452424 |
| G. ambrosiae                    | D. pinnata            | Changchun, Jilin province, China | 2018               | HMJAU-PM91820 MK452591 MK452664 MK452517 MK452473 MK452425 |
| G. ambrosiae                    | D. pinnata            | Siping, Jilin province, China  | 2018               | HMJAU-PM91821 MK452592 MK452665 MK452518 MK452474 MK452426 |
| G. ambrosiae                    | D. pinnata            | Panzhihua, Sichuan, China     | 2018               | HMJAU-PM91822 MK452593 MK452666 MK452519 MK452475 MK452427 |
| G. ambrosiae                    | Dahlia sp.            | Yolo Co. CA, USA              | 2018               | MVAP50000445 MK452632 MK452705 MK452557 – – |
| G. ambrosiae                    | Dahlia sp.            | Santa Barbara Co. CA,         | 2018               | LM0P03825217– MK452637 MK452710 MK452562 – MK452457 |
## Table 1 Information of powdery mildew vouchers studied in this paper (Continued)

| Species | Host | Location | Year of collection | Voucher a | GenBank accessions No. b |
|---------|------|----------|--------------------|-----------|-------------------------|
| G. ambrosiae | Dahlia sp. | Seattle, Washington, USA | 2018 | HMJAU-PM91854 | MK452641 MK452714 MK452566 – – |
| G. ambrosiae | Eupatorium japonicum | Aichi, Nagoya-shi, Japan | 2001 | MUMH4142 | MK452621 MK452604 MK452546 – – |
| G. ambrosiae | E. makinoi | Katashina-mura, Gunma, Japan | 2002 | MUMH4143 | MK452622 MK452695 MK452547 – – |
| G. ambrosiae | E. makinoi | Tochigi, Sano, Japan | 2002 | MUMH4424 | MK452623 MK452696 MK452548 – – |
| G. ambrosiae | E. makinoi | Okayama-shi, Okayama, Japan | 2003 | MUMH4794 | MK452625 MK452698 MK452550 – – |
| G. ambrosiae | E. makinoi | Shiga, Maibara, Japan | 2017 | MUMH7129 | MK452624 MK452697 MK452549 – – |
| G. ambrosiae | Z. elegans | Changchun, Jilin province, China | 2016 | HMJAU-PM91836 | MK452612 MK452685 MK452537 MK452487 MK452444 |
| G. ambrosiae | Z. elegans | Changchun, Jilin province, China | 2017 | HMJAU-PM91843 | MK452613 MK452686 MK452538 MK452488 MK452445 |
| G. ambrosiae | Z. elegans | Changchun, Jilin province, China | 2017 | HMJAU-PM91844 | MK452614 MK452687 MK452539 MK452489 MK452446 |
| G. ambrosiae | Z. elegans | Changchun, Jilin province, China | 2017 | HMJAU-PM91845 | MK452615 MK452688 MK452540 MK452490 MK452447 |
| G. ambrosiae | Z. elegans | Changchun, Jilin province, China | 2018 | HMJAU-PM91846 | MK452616 MK452689 MK452541 MK452491 MK452448 |
| G. ambrosiae | Z. elegans | Siping, Jilin province, China | 2018 | HMJAU-PM91847 | MK452617 MK452690 MK452542 MK452492 MK452449 |
| G. ambrosiae | Z. elegans | Tonghua, Jilin province, China | 2018 | HMJAU-PM91848 | MK452618 MK452691 MK452543 MK452493 MK452450 |
| G. ambrosiae | Z. elegans | Siping, Jilin province, China | 2018 | HMJAU-PM91849 | MK452619 MK452692 MK452544 MK452494 MK452451 |
| G. ambrosiae | Z. elegans | Santa Barbara Co., CA, USA | 2018 | LMOP06825217–3 | MK452633 MK452706 MK452558 – MK452456 |
| G. ambrosiae | Z. elegans | Yolo Co., CA, USA | 2018 | MVA050000452 | MK452634 MK452707 MK452559 – – |
| G. latisporus | Helianthus annuus | Changchun, Jilin province, China | 2017 | HMJAU-PM91830 | MK452601 MK452674 MK452527 MK452483 MK452435 |
| G. latisporus | H. annuus | Changchun, Jilin province, China | 2017 | HMJAU-PM91828 | MK452599 MK452672 MK452525 MK452481 MK452433 |
| G. latisporus | H. annuus | Yichun, Heilongjiang, China | 2017 | HMJAU-PM91829 | MK452600 MK452673 MK452526 MK452482 MK452434 |
| G. latisporus | H. annuus | Tonghua, Jilin province, China | 2018 | HMJAU-PM91831 | MK452602 MK452675 MK452528 MK452484 MK452436 |
| G. latisporus | H. annuus | Panzhihua, Sichuan, China | 2018 | HMJAU-PM91832 | MK452603 MK452676 MK452529 MK452485 MK452437 |
| G. latisporus | H. tuberosus | Chongqing, China | 2018 | HMJAU-PM91833 | MK452594 MK452667 MK452520 MK452476 MK452428 |
Table 1 Information of powdery mildew vouchers studied in this paper (Continued)

| Species          | Host                 | Location                        | Year of collection | Voucher   | GenBank accessions No. | IT5 | 2BS | IGS | TUB2 | CHS1 |
|------------------|----------------------|---------------------------------|--------------------|-----------|------------------------|-----|-----|-----|------|------|
| **G. latisporus**| *H. tuberosus*       | Shangqiu, Henan, China          | 2016               | HMJAU-PM91823 | MK452595 MK452668 MK452521 MK452477 MK452429 |
|                  |                      |                                 |                    |           |                        |     |     |     |      |      |
| **G. latisporus**| *H. tuberosus*       | Changchun, Jilin province, China| 2017               | HMJAU-PM91825 | MK452596 MK452669 MK452522 MK452478 MK452430 |
| **G. latisporus**| *H. tuberosus*       | Changchun, Jilin province, China| 2017               | HMJAU-PM91826 | MK452597 MK452670 MK452523 MK452479 MK452431 |
| **G. latisporus**| *H. tuberosus*       | Changchun, Jilin province, China| 2017               | HMJAU-PM91827 | MK452598 MK452671 MK452524 MK452480 MK452432 |
| **G. latisporus**| *H. tuberosus*       | Shakhty city, Rostov region, Russia| 2018         | ERY057     | MK452642 MK452716 MK452569 – – – |
| **G. latisporus**| *H. tuberosus*       | Shakhty city, Rostov region, Russia| 2018         | ERY061     | MK452644 MK452718 MK452571 – – – |
| **G. latisporus**| *H. tuberosus*       | Shakhty city, Rostov region, Russia| 2018         | ERY081     | MK452645 MK452719 MK452572 – – – |
| **G. latisporus**| *H. tuberosus*       | Shakhty city, Rostov region, Russia| 2018         | ERY094     | MK452646 MK452720 MK452573 – – – |
| **G. latisporus**| *H. tuberosus*       | Novoshakhthinsk city, Rostov region, Russia| 2018   | ERY152     | MK452647 MK452721 MK452574 – – – |
| **G. latisporus**| *H. annuus*          | Nyon, Vaud, Switzerland          | 2018               | HAL 3299 F | MK452627 MK452700 MK452552 MK452497 MK452454 |
| **G. latisporus**| *H. annuus*          | Solano Co. CA, USA               | 2018               | MVA500000419 | MK452635 MK452708 MK452560 MK452498 – – – |
| **G. latisporus**| *H. annuus*          | Santa Barbara Co. CA, USA        | 2018               | LM0P03825217-2 | MK452636 MK452709 MK452561 MK452499 – – – |
| **G. latisporus**| *H. annuus*          | Seattle Washington, USA          | 2018               | HMJAU-PM91853 | MK452640 MK452713 MK452565 – – – |
| **G. latisporus**| *H. mollis*          | Seattle Washington, USA          | 2018               | HMJAU-PM91851 | MK452638 MK452711 MK452563 – – – |
| **G. latisporus**| *Helianthus sp.*     | Seattle Washington, USA          | 2018               | HMJAU-PM91852 | MK452639 MK452712 MK452564 MK452500 – – – |
| **G. latisporus**| *Zinnia angustifolia*| Potsdam, Brandenburg, Germany    | 2008               | HAL 2338 F | MK452631 MK452704 MK452556 – – – |
| **G. latisporus**| *Z. elegans*         | Panzhihua, Sichuan, China        | 2018               | HMJAU-PM91850 | MK452620 MK452693 MK452545 MK452495 MK452452 |
| **G. magnicellulatus**| *Physalis alkekengi* | Yichun, Heilongjiang, China      | 2017               | HMJAU-PM91838 | MK452608 MK452681 MK452535 – – – MK452441 |
| **G. magnicellulatus**| *P. alkekengi*      | Changchun, Jilin province, China | 2017               | HMJAU-PM91839 | MK452609 MK452682 MK452536 – – – MK452442 |
| **G. magnicellulatus**| *P. alkekengi*      | Changchun, Jilin province, China | 2018               | HMJAU-PM91840 | MK452610 MK452683 MK452534 MK452486 MK452443 |
| **Neoërysiphe galeopsidis**| *Leonurus artemisia* | Beijing, China                  | 2018               | HMJAU-PM91833 | MK452604 MK452677 MK452530 – – – MK452438 |
| **N. galeopsidis**| *L. artemisia*       | Beijing, China                  | 2018               | HMJAU-PM91834 | MK452605 MK452678 MK452531 – – – MK452439 |
| **N. galeopsidis**| *L. artemisia*       | Changchun, Jilin province, China | 2017               | HMJAU-PM91835 | MK452606 MK452679 MK452532 – – – MK452440 |

*HMJAU Herbarium of Mycology of Jilin Agricultural University; HAL Herbarium of Halle University; GLM Herbarium of Senckenberg Museum für Naturkunde Görlitz; MUMH Mie University Mycological Herbarium; ERY herb. Bulgakov; LM and MVAP herb. S. Rooney Latham; ISC Iowa State University. The specimens GLM49501 (herbarium GLM, Görlitz, Germany), HAL 2338 F, HAL 3299 F, and HAL 3300 F (herbarium HAL, Halle [Saale], Germany) were supplied by Uwe Braun. The specimens MUMH4142, MUMH4143, MUMH4424, MUMH7129, MUMH4794, and HMJAU-PM91855 (herbarium MUMH, Mie, Japan) were provided by Susumu Takamatsu. The specimens MVAP500000419, MVAP50000445, MVAP500000452, LM0P03825217-1, LM0P03825217-2, and LM0P08825217-3 were supplied by Suzanne Latham-Rooney. The specimens HMJAU-PM91851, HMJAU-PM91852, HMJAU-PM91853, HMJAU-PM91854, ISC-F-0076752, ISC-F-0076753, and ISC-F-0076754 were supplied by Michael Bradshaw; and ERY015, ERY057, ERY061, ERY057, ERY081, ERY094 and ERY152 by Timur S. Bulgakov.

b “-” means failed to get sequence.
data using maximum parsimony (MP) in PAUP 4.0b [50]. The MP analyses were performed with 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 were tested with bootstrap (BS) analysis using 1000 replications. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. Five phylogenetic trees were generated based on the ITS, 28S, IGS, TUB2 and CHS1 nucleotide sequences.

Results
Phylogenetic analyses
Parsimoniuous trees were separately constructed based on sequences of five gene regions and their combination and the numerical data including the number of taxa and characters are shown in Table 3. The information of outgroup taxon for each phylogenetic tree was also included in Table 1. The phylogenetic trees based on the ITS and 28S rDNA sequences were topologically congruent and indicated that G. ambrosiae complex on many Asteraceae plants, including Eupatorium spp. from Japan, formed a single clade with 100 and 99% bootstrap support, respectively (see Additional files 1, 2: Figure S1, S2). Golovinomyces circumfusus on E. cannabinum from Germany did not form a monophyletic group with G. ambrosiae complex in all phylogenies (see Additional file 1–5: Figure S1–S5 and Fig. 1). The phylogenetic tree of IGS was similar to ITS tree, with the G. ambrosiae complex formed a single clade with 100% bootstrap support based on the individual genes (see Additional file 3: Figure S3). However, the isolates from Helianthus spp. and some Zinnia spp. differed by one base from isolates on other host genera, and forming a subclade with 64% bootstrap support (see Additional file 3: Figure S3 pink clade). The G. ambrosiae complex included two groups, one identified as G. ambrosiae emend. (see Additional file 3: Figure S3 green clade) and the other as G. latisporus comb. nov. (see Additional file 3: Figure S3 pink clade), based on the phylogenetic analysis of the IGS. The G. ambrosiae complex in TUB2 and CHS1 trees was divided into two subgroups, viz. G. ambrosiae emend., including G. spadiceus with 91 and 85% bootstrap support respectively (see Additional files 4, 5: Figure S4, S5 green clade), and G. latisporus comb. nov. with 70 and 78% bootstrap support respectively (see Additional files 4, 5: Figure S4, S5 pink clade). In the G. ambrosiae emend. Clade the sequences of CHS1 from isolates on Ambrosiae artemisiifolia and A. trifida differed by one base from isolates on other hosts. Golovinomyces ambrosiae emend. is a plurivorous species that occurs on a multitude of hosts including, Ambrosia spp., multiple species from the Heliantheae and plant species.

Table 2 Primer sets for multilocus sequence typing (MLST) analysis of Golovinomyces in this study

| DNA regions | Primer | Primer sequences (5′→3′) | Annealing temperature (°C) | Amplicon size (bp) | Reference |
|-------------|--------|--------------------------|---------------------------|-------------------|-----------|
| ITS         | ITSS ITS4 | GGAAGTAAGAAGTGAAACAGG TCCTCCGCTATTGATAT GC | 52                     | 600               | [44]      |
| 28S rDNA    | LSU1 LSU2 | ACCGGCCTGAACCTTAAAGCATA CCTTGCTCCGTGTCTCAAGA | 52                     | 500               | [45]      |
| IGS         | IGS-12a NS1R | AGTCTGTTGATTTGTCGAGCAAGCAGCATACG ACTAC | 52                     | 400               | [46]      |
| TUB2        | TubF1 TubR1 | AGGTTCACCTCCAGACTGG CCAGCAGCGAAGACGCATCCAT | 52                     | 450               | This study |
| CHS1        | gCS1a1 gCS1b | GGTGCATTCTCGGCGATATCG CTGTCACCCCTGTTGGTCCCAGAAG | 52                     | 1000              | This study |

Table 3 Information of the data matrices and the respective trees based on five individual gene regions

| DNA region | ITS | 28S | IGS | TUB2 | CHS1 | ITS+28S + IGS + TUB2 + CHS1 |
|------------|-----|-----|-----|------|------|----------------------------|
| Number of sequences | 74 | 75 | 74 | 44 | 49 | 40 |
| Number of characters | 509 | 639 | 393 | 432 | 968 | 2931 |
| Number of parsimony-uninformative characters | 50 | 26 | 1 | 112 | 22 | 182 |
| Number of parsimony-informative characters | 108 | 41 | 104 | 30 | 107 | 102 |
| Tree length | 228 | 87 | 133 | 164 | 154 | 305 |
| Consistency index (CI) | 0.8684 | 0.8621 | 0.8947 | 0.9512 | 0.9156 | 0.9902 |
| Retention index (RI) | 0.9242 | 0.9250 | 0.9595 | 0.9175 | 0.9698 | 0.9855 |
| Rescaled consistency index (RC) | 0.8026 | 0.7974 | 0.8585 | 0.8728 | 0.8879 | 0.9758 |
of other tribes of Asteraceae including the Asian species of *Eupatorium*. *Golovinomyces lattisporus* comb. nov. was confined to hosts of the Heliantheae genera *Helianthus* and *Zinnia*.

Furthermore, the bootstrap values of clades *G. ambrosiae* emend. and *G. lattisporus* comb. nov. (BS = 99 and 92% respectively) in combined analysis (see Fig. 1) were higher than in other trees that were constructed based on separate genes. *Golovinomyces circumfusus* on *E. cannabinum* from Europe, forming a single clade, represented a separate species based on the combined data analysis (see Fig. 1).

**Taxonomy**

*Golovinomyces ambrosiae* (Schwein.) U. Braun & R.T.A. Cook, in Cook & Braun, Mycol. Res. 113: 628 (2009). Figure 2.

≡ *Erysiphe ambrosiae* Schwein., Trans. Amer. Philos. Soc., N.S., 4: 270 (1834).
≡ *Erysiphe spadicea* Berk. & M.A. Curtis, Grevillea 4: 159 (1876).
≡ *Golovinomyces spadiceus* (Berk. & M.A. Curtis) U. Braun, in Braun & Cook, CBS Biodiversity Series 11: 329 (2012).
≡ *Erysiphe cichoracearum f. ambrosiae* Jacz., Karm. Opred. Grib., Vip. 2. Muchn.-rosj. Griby (Leningrad): 186 (1927).
≡ *Erysiphe cichoracearum f. xanthii* Jacz., Karm. Opred. Grib., Vip. 2. Muchn.-rosj. Griby (Leningrad): 212 (1927).
≡ *Oidium acanthospermi* Chidd., Lloydia 18: 46 (1955).
≡ *Acrosporium acanthospermi* (Chidd.) Subram., Hypomyces (New Delhi): 835 (1971).
= Oidium lagasceae Chidd., Lloydia 18: 47 (1955).
≡ Acrosporium lagasceae (Chidd.) Subram., Hyphomyces (New Delhi): 836 (1971).
= Oidium parthenii Satyapr. & Ushar., Curr. Sci. 50: 1081 (1981).
≡ E. cichoracearum var. transvaalensis G.J.M. Gorter & Eicker, S. Afr. J. Bot. 2(2): 130 (1983).
≡ Golovinomyces cichoracearum var. transvaalensis (G.J.M. Gorter & Eicker) U. Braun, Schlechtendalia 3: 51 (1999).
= Oidium blainvilleae Bappamm., Hosag. & Udaiyan, New Botanist 22: 117 (1995).
= Erysiphe cichoracearum auct. p.p.
= Golovinomyces cichoracearum auct. p.p.

Literature: Braun and Cook ([1], p. 330), Dugan [51], Takamatsu et al., [19], Khodaparast [52], Arzanlou et al., [53], Meeboon et al., ([11], p. 212), Moparthi et al., [29, 54], Trigano et al., [28], Braun et al., [13].

Illustrations: Bappammal et al., ([55], p. 110, Fig. 26, 115, Fig. 35), Nomura ([56], p. 182, Fig. 241), Braun and Cook ([1], p. 330, Fig. 359), Meeboon et al., ([11], p. 211, Figs. 9–11).

Exsiccatum: Seym. & Earle, Econ. Fungi 321.

Description: Mycelium amphigenous and caulicolous, white, in small to moderately large patches, confluent, sometimes covering entire leaves, persistent or almost so; hyphae 2–9 μm wide, thin-walled, smooth, hyaline, in old infections hyphae around ascomata sometimes turning brown; hyphal appressoria solitary, sometimes several per hyphal cell, nipple-shaped, occasionally slightly crenulate or irregular, 3–8 μm diam.; conidiophores erect, arising from the upper surface of the hyphal mother cell and usually towards one end of it; foot-cells cylindrical, straight, rarely slightly flexuous, 30–80 × 9–15 μm, followed by 1–3 shorter cells, forming catenulate conidia; conidia ellipsoid-ovoid, doliiform-
subcylindrical, 25–40 × 14–20(–24) μm, length/width ratio 1.5–2; conidial germination of the Euoidium type. Chasmothecia amphigenous, occasionally calyculous, scattered to gregarious, 80–140 μm diam., rarely larger; peridium cells irregularly shaped, polygonal to daedaleoid, 8–30 μm diam., walls of the cells up to 2 μm wide; appendages numerous, mostly arising from the lower half, mycelioid, usually unbranched, 0.2–1.5 times as long as the chasmothecial diam., mostly shorter than the diam., (3–)4–8(–10) μm wide, at first hyaline, later yellowish to brownish-green throughout or paler towards the tips, septate, walls thin, smooth or almost so; asci numerous, mostly (5–)8–15, obvoid-saccate, 40–70 × 25–35(–40) μm, almost sessile or short-stalked, wall thin, up to 1 μm thick, (2–3)-spored; ascospores broad ellipsoid-ovoid, 15–25(–28) × 10–15(–18) μm, colorless.

Material examined: additional collections for molecular analyses (see Table 1); USA, Pennsylvania, Lehigh & Northampton, Bethlehem, on leaves of <i>Ambrosia</i> sp. (Asteraceae), 1826, L. von Schweinitz, PH 62362, holotype of <i>E. ambrosiae</i>; USA, South Carolina, on leaves of <i>Xanthium</i> sp. (Asteraceae), ex herb. M.J. Berkeley, No. 2984, K(M) 164,976, holotype of <i>E. spadiceus</i>. JAPAN, Mie Pref., Tsu, on leaves of <i>Xanthium strumarium</i> (Asteraceae), 12 Nov. 1997, S. Takamatsu, TSU-MUMH 413 (reference material for <i>Erysiphe spadicea</i> with ex-reference material sequence – AB077644, see Braun et al. 2019). USA, Iowa, Guthrie County, Sheeder Prairie State Preserve, on leaves of <i>Ambrosia trifida</i>, 12 Aug. 1997, Lois H. Tiffany, ISC-F-007653, epitype of <i>Erysiphe ambrosiae</i> (designated here, MycoBank MBT385758).

Host range and distribution (see [1, 13]): widespread in Asia, Australia, Europe and North America, on species of numerous host genera belonging to the families Asteraceae (<i>Acanthospermum, Ambrosia, Aster, Blainvillea, Chrysogonum, Coreopsis, Dahlia, Eupatorium, Gerbera, Helianthus, Lagascea, Laggera, Leucanthemum, Mauanthemum [Chrysanthemum s. lat.], Melampodium, Parthenium, Telekia, Tithonia, Xanthium, Zinnia</i>), Fabaceae (<i>Crotalaria</i>), Malvaceae (<i>Abelmoschus</i>), Polygonaceae (<i>Persicaria</i>), Solanaceae (<i>Solanum</i>), and Verbenaceae (<i>Verbena</i>).

Notes: <i>Persicaria</i> species have recently been confirmed as hosts of <i>G. ambrosiae</i> by molecular sequence analyses (<i>P. alpina</i> [30], Azerbaijan; <i>P. decipiens</i> [13], Australia).}

**Golovinomyces circumfusus** (Schltdl.) U. Braun, in Braun & Cook, CBS Biodiversity Series 11: 309 (2012).

≡ <i>Erysibe circumfusca</i> (Schltdl.) Link, Sp. pl. 4, 6(1): 109, (1824).

≡ <i>Erysipe communis</i> f. <i>circumfusa</i> (Schltdl.) Fr., Syst. mycol. 3: 240 (1829).

≡ <i>E. communis</i> n. <i>corymbiferarum</i> Fr., Syst. mycol. 3: 241 (1829), p.p.

≡ <i>E. cichoracearum</i> f. <i>europarius</i> Dearn., in Rehm, Ascomyc., Fasc. 48, No. 1950 (1911) and Ann. Mycol. 9: 290 (1911).

≡ <i>E. cichoracearum</i> auct. p.p.

≡ <i>Golovinomyces cichoracearum</i> auct. p.p.

**Illustration:** Braun & Cook (2012, p. 309, Fig. 331).

**Literature:** Jaczewski ([57], p. 197).

**Ersicicatae:** Barthol., Fungi Columb. 2930, 4020, 4224, 4919. Rabenh., Klotzschii Herb. Viv. Mycol. 467. Rehm, Ascomyc. 1950. Syd., Mycoth. Germ. 1530.

**Description:** Mycelium amphigenous, but sometimes also calyculous, thin, white, effuse or in distinct patches, persistent on the upper leaf surface and on stems, less conspicuous and often evanescent on lower surface; hyphae branched mostly at right angles, hyaline, smooth or almost so, 3–9 μm wide; hyphal appressoria usually solitary, slightly to distinctly nipple-shaped, 3–7 μm diam.; conidiophores erect, solitary per hyphal mother cell, arising laterally or from the upper surface and usually towards one end of the mother cell, up to 160 μm long, foot-cells variable, straight to curved at the base or sinus, 30–110 × 9–14 μm, almost cylindrical to slightly increasing in width from base to top, occasionally slightly constricted at the 7–9 μm wide basal septum that is usually at the junction with the mother cell or occasionally raised by up to 5 μm, followed by 2–3 shorter cells, forming cateneshes conidia; primary conidia ellipsoid-ovoid, subcylindrical, limoniform, 25–40 × 12–20 μm, length/width ratio 1.3–2.6; germ tubes terminal or almost so, short to moderately long, slightly clavate, i.e. apex with slightly swollen appressorium, Euoidium type. Chasmothecia amphigenous and calyculous, scattered to gregarious, subglobose to somewhat depressed-globose, 85–140 μm diam., rarely larger; peridium cells irregularly polygonal, rounded to usually somewhat daedaleoid, 5–25(–30) μm diam., walls up to 2.5 μm thick; appendages numerous, equatorial and in the lower half, mycelioid, simple, rarely branched, (0.25–)0.5–2.5(–3.5) times as long as the chasmothecial diam., 3–8 μm wide, walls thin (up to 1 μm), smooth to faintly rough, on mature ascomata completely pale to medium dark brown throughout or somewhat paler towards the tip; asci numerous, usually 5–15, broad obvoid-saccate or almost globose, (40–)50–70(–80) × (20–)25–35(–40) μm, almost sessile to short-stalked, thin-walled, terminal oculus 8–15 μm diam., 2(–3)-spored; ascospores ellipsoid-ovoid, (15–)18–25 × 10–17 μm, colourless.
Material examined: all were collected on leaves of Eupatorium cannabinum, GERMANY, ex herb. Schlechtendal, without any further data, HAL 1423 F, lectotype [designated in Dörfelt & Ali (1987)]; Brandenburg, Landkreis Ostprignitz-Ruppin, Großzerlag, 22 Sep. 2006, H. Boyle, GLM-F74796; Brandenburg, Landkreis Ostprignitz-Ruppin, north-west of Rheinsberg, 24 Sep. 2006, H. Jage, GLM-F85832; Sachsen, Zittau, Westpark, 9 Aug. 2007, H. Boyle, GLM-F80897; Sachsen-Anhalt, Salzwedel, 19 Aug. 2000, H. Jage and H. Lehmann, GLM-F49501; Sachsen-Anhalt, Halle (Saale), Osendorfer See, 12 Nov. 2000, H. Jage (GLM-F47189); Sachsen-Anhalt, Salzwedel, Hoydersburg, 11 Aug. 2004, H. Jage, GLM-F65924. Germany, Brandenburg, Spreewald, Briensee, 8 Oct. 2016, V. Kummer, HAL 3300 F, epitype (designated here, MycoBank MBT385760).

Host range and distribution: on Eupatorium cannabinum (Asteraceae), Europe (Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, Lithuania, Poland, Romania, Russia, Slovakia, Sweden, Switzerland, UK) [58–61].

Notes: Braun and Cook [1] assigned Golovinomyces on host species belonging to Eupatorium s. lat. From the northern hemisphere, including Europe, North America and northern regions of Asia, to G. circumfusus. This species seems to be confined to its type host, E. cannabinum, as collections from Asian species of Eupatorium pertain to G. ambrosiae. The affinity and identity of North American collections on Eupatorium perfoliatum, Eutrochium maculatum (= Eupatorium maculatum), and Eutrochium purpureum (= Eupatorium purpureum) remain unclear since sequence data and results of detailed morphological examinations of the asexual morphs on these hosts are not yet available. Golovinomyces on these hosts is common in North America, including several collections distributed in exsiccatae (Barthol., Fungi Columb. 2930, 4020, 4224, 4919; Rehm, Ascomyc. 1950).

Golovinomyces latisporus (U. Braun) P.-L. Qiu & S.-Y. Liu, comb. nov. Figure 3.

MycoBank MB 829648.
Basionym: Oidium latisporum U. Braun, Zentralbl. Mikrobiol. 137: 315 (1982).
= Erysiphe cichoracearum f. helianthi Jacz., Karm. Opred. Grib., Vip. 2. Muchn.-rosj. Griby (Leningrad): 198 (1927).
= Erysiphe cichoracearum var. latispora U. Braun, Mycotaxon 18:117 (1983).
≡ Golovinomyces cichoracearum var. latisporus (U. Braun) U. Braun, Schlechtendalia 3: 51 (1999).

Fig. 3 Golovinomyces latisporus comb. nov. (HMJAU-PM91828 ex Helianthus annuus). a. Nipple-shaped hyphal appressorium. b–c. Conidiophores. d–f. Conidia. g–h. Conidial germination. i. Chasmothecium. j. Peridium cells of Chasmothecium. k–m. Asci with two or three ascospores. n. Ascospores. Scale bars = 20 µm
= E. cichoracearum auct. p.p.
= Golovinomyces cichoracearum auct. p.p.

Illustrations: Braun ([62], p. 316, fig. 1 [63], p. 118, fig. 6 [17]); p. 250, pl. 66, fig. A [58], p. 270, pl. 40, fig. A), Tanda et al., ([64], p. 254, figs. 1–2), Nomura ([56], p. 185, Fig. 249), Cook and Braun ([65], p. 627, Fig. 5), Chen et al., ([66], p. 4, fig. 1b).

Description: Mycelium amphiogenous, also on stems, effuse or forming parasites, thin, white, persistent or almost so; hyphae hyaline, walls thin, smooth, 3–8 μm wide; hyphal appressoria nipple-shaped, solitary or in opposite pairs, 4–8 μm diam.; conidiophores arising centrally or towards one end of hyphal mother cells and from their upper surface, erect, straight, foot-cells cylindrical, 35–80 × 9–15 μm, followed by 1–3 shorter cells, forming catenescen conidia; conidia broad ellipsoid-ovoid, doliiform to somewhat limoniform, 25–45 × 15–27 μm (when fresh), length/width ratio < 2 (1.3–1.9, mostly 1.4–1.6), germ tubes dimorphic, with terminal subterminal, occasionally lateral germination, on glass at 100% RH, long, fusiform or forming patches, thin, white, persistent or almost subrhomboideus, petiolaris, paecox subsp. hirtus, paecox subsp. runyonii, radula, rigidus, salicifolius, scaberrimus, schweinitzii, tuberosus), Rudbeckia (amplicalulis, bicolor, fulgida, hirta, lacinata, occidentalis, serotina, triloba), Zinnia (angustifolia, elegans) Asteraceae [Heliantheae]; Africa (South Africa, Tanzania), Asia (China, India, Israel, Japan, Korea, Nepal, Russia [Siberia, Far East], Turkey), Europe (Bulgaria, Germany, Greece, Hungary, Italy, Lithuania, Netherlands, Poland, Romania, Russia, Slovenia, Switzerland, Turkey, Ukraine, former Yugoslavia), North America (Canada, Mexico, USA), South America and West Indies (Argentina, Cuba, Bolivia, Brazil, Chile, Venezuela), Oceania (Fiji, Samoa), New Zealand (see [1, 58, 67], https://nt.arb-grin.gov/fungaldatabases/index.cfm).

Notes: Golovinomyces latisporus occurs on various Helianthus species almost worldwide. Zinnia angustifolia and Z. elegans are additional hosts proven by means of molecular methods. Golovinomyces collections found on various Rudbeckia spp. are assigned to G. latisporus with respect to the characters of the anamorph, although multilocus sequence analyses are still lacking. Taxonomy of a recently published record of “G. spadiceus” on Helianthus annuus in the United States [54] is unclear and urgently requires multilocus analyses for species identification. The identity of Golovinomyces on Iva spp. (axillaris, frutescens, xanthifolia) has not been sufficiently studied.

Discussion

The taxonomic history of the powdery mildews allied to Erysiphe cichoracearum dates back to de Candolle, in Lamarck and de Candolle [68]. He described E. cichoracearum on Scorzonera hispanica and Tragopogon porri folius. Salmon [14] widened the concept of E. cichoracearum considerably by assigning powdery mildew on numerous hosts of various plant families to this species, including Helianthus spp. In previous circumcriptions, E. cichoracearum was characterized by having ascomata with mycelium-like appendages, several usually 2-spored asci, and conidia formed in chains without fibrosin bodies [14–17]. Braun [62] described the asexual morph of powdery mildew found on Helianthus × laetiflorus in Germany as Oidium latisporum based on the differences in conidial characters (most notably broader...
conidia) from collections of *E. cichoracearum* on various other hosts. Later, Braun [63] introduced the name *E. cichoracearum* var. *latispora* based on holomorphic North American type material, and cited *G. ambrosiae* as a possible synonym. Heluta [69] reallocated *E. cichoracearum* to *Golovinomyces*. Braun and Cook [1] split *G. cichoracearum* into several species based on molecular analyses of this complex which suggested a co-evolutionary relationship between *Golovinomyces* species and tribes of Asteraceae [18].

*Golovinomyces* on hosts of the Heliantheae was divided into two species, *G. ambrosiae* and *G. spadiceus*, distinguished by clear morphological differences in their asexual morphs [1]. Type material of *E. ambrosiae* was examined, and this name was used for powdery mildew on *Ambrosia, Helianthus, Iva*, and *Rudbeckia* spp. *E. ambrosiae* was characterized by having broad ellipsoid-ovoid, doliiform to somewhat limoniform conidia, 25–45 × 15–27 μm (when fresh) with a length/width ratio < 2 (1.3–1.9, mostly 1.4–1.6), and dimorphic germ tubes that were long and filiform (longitubus pattern with the *Euoidium* conidial germination type) and consisted of a varying percentage of shorter germ tubes that were often swollen at the tip (ordinary *Euoidium* germ tubes) [1]. Whereas, the conidial shape and size, as well as the conidial germination pattern of *G. spadiceus* agrees with the common *Euoidium* type. These morphological differences were not reflected in a comprehensive phylogenetic analyses based on ITS and 28S rDNA powdery mildews previously referred to as *G. ambrosiae* and *G. spadiceus*. In the phylogenetic analyses, *G. ambrosiae* and *G. spadiceus* formed a single undifferentiated clade (lineage III in Takamatsu et al., [19]). Furthermore, this clade also encompassed sequences obtained from *Golovinomyces* on *Eupatorium chinense* in Japan [referred to as *G. circumfusus* based on the circumscription of this species in Braun and Cook [1] and the assumption that all *Golovinomyces* collections on various *Eupatorium* species in Asia, Europe and North America pertain to a single species] as well as sequences from *Golovinomyces* on numerous Asteraceae hosts from several tribes and even other families. The extensive host range exhibited by clade 3 suggests the involvement of a plurivorous species.

Sequences from the five gene regions could not be obtained for all samples used in this study. The phylogenetic affinity of *G. circumfusus* could be clarified by the inclusion of sequences obtained from powdery mildew on *E. cannabina* (type host) in Germany (type region). *G. circumfusus* on its type host does not cluster within the former “Heliantheae Clade” and is not closely allied with *G. ambrosiae* complex. It represents a well-supported species of its own, confined to *E. cannabina* in Europe. Blumer ([16], p. 188) summarized results of previous inoculation tests carried out by himself and other authors and classified *Erysiphe cichoracearum* s. lat. on *E. cannabina* as a biologically specialized form (f. sp. *eupatoriellii*), confined to this host. In order to stabilize the application of the old name *Erysiphe circumfusa*, described in the nineteenth century, an epitype has been designated. Powdery mildew on Asian *Eupatorium* spp. is not conspecific with *G. circumfusus* and pertains to a clade previously referred to as *G. spadiceus* [13]. This clade represents a plurivorous species on a wide range of hosts belonging to the Heliantheae and other tribes of Asteraceae as well as hosts of other plant families. However, the naming of this clade had to be corrected.

Sequences from *Golovinomyces* on *Ambrosia* spp. in Asia and North America do not cluster together with sequences obtained from *Golovinomyces* on *Helianthus* spp., but they pertain to the former plurivorous *G. spadiceus*. The morphological characters of the powdery mildew on *Ambrosia* also agree with that of *G. spadiceus* (the type material of *Erysiphe ambrosiae* contains chasmothecia, but the features of the asexual morph could not be properly examined). Hence, Braun [63] cited *E. ambrosiae* as a potential synonym of *E. cichoracearum* var. *latispora*. The application of the name *E. ambrosiae* in Braun and Cook [1], based on this questionable synonym, must be classified as a misinterpretation. These results have nomenclatural and taxonomic consequences, viz., the older name *Erysiphe ambrosiae*, which has priority over *G. spadiceus*, is now the correct name for this plurivorous species, and *G. spadiceus* and its synonyms must be reduced to synonymy with *G. ambrosiae*. Finally, *Golovinomyces* on *Helianthus* spp., morphologically distinguished from the former *G. spadiceus*, turned out be genetically different as well (although undoubtedly closely allied to the latter species).

Since *G. ambrosiae* now represents an older name for the species previously referred to as *G. spadiceus*, it is necessary to rename the species on *Helianthus*. Hence, *Oidium latisporum* (= *Erysiphe cichoracearum* var. *latispora*), the oldest valid name for this taxon at the species level, is used as the basionym for the combination *G. latisporus*. This species is common with a near global distribution, and also occurs on *Zinnia* [sequences retrieved from *Z. angustifolia* (HAL 2338 F) refer to a German collection from a botanical garden in which the *Zinnia* grew close to *Helianthus* plants infected by *G. latisporus*]. Sequences retrieved from *Z. elegans* (HMJAU-PM91850) refer to a collection from the Sichuan province of China where no *Helianthus* plants grew. The powdery mildew on *Rudbeckia* coincides morphologically with *G. latisporus*. However, currently only ITS and 28S sequences are available [19]. Future examinations based on IGS, *TUB2* and *CHSI* are necessary to confirm the identity. In any case, the example of *Zinnia* shows that host plants of other genera, such as
Helianthus or Iva, might also be infested by the two closely allied species, G. ambrosiae and G. latisporus. In order to answer this question, a combination of morphological examinations and phylogenetic analyses based on a multilocus approach are required in the future.

Conclusions

The phylogenetic analyses of multilocus sequence data, including ITS and 28S rDNA, IGS, TUB2, CHSI, and consideration of morphological characters enabled to resolve species delimitation in a heterogeneous complex within the genus Golovinomyces. The old names involved in this complex have been epitified, providing ex-epitype sequence data, and three species were distinguished in the complex named G. ambrosiae emend. (including G. spadiceus), G. latisporus comb. nov. (= Oidium latisporum), and G. circumfusus confined to Eupatorium cannabinum in Europe. This research illustrated that such approaches are suitable and promising in cases of phylogenetically closely allied assemblages of powdery mildew species in which ITS analyses do not yield sufficient resolution.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12866-020-01731-9.

Additional file 1: Figure S1. Phylogenetic analysis of the ITS region of the Golovinomyces ambrosiae complex and G. circumfusus. The tree was constructed based on 73 sequences from tribe Golovinomyceae. One sequence from Erysiphe kenjiana (accession number: MK452611) was used as an outgroup. Bootstrap values based on 1000 replications are indicated above the branches.

Additional file 2: Figure S2. Phylogenetic analysis of the 28S rDNA region of the Golovinomyces ambrosiae complex and G. circumfusus. The tree was constructed based on 74 sequences from tribe Golovinomyceae. One sequence from Erysiphe kenjiana (accession number: MK452684) was used as an outgroup. Bootstrap values based on 1000 replications are indicated above the branches.

Additional file 3: Figure S3. Phylogenetic analysis of the IGS region of the Golovinomyces ambrosiae complex and G. circumfusus. The tree was constructed based on 74 sequences from tribe Golovinomyceae. Three sequences from Neoeuryipsis galeopsidis (accession numbers: MK452530, MK452531, MK452532) were used as an outgroup. Bootstrap values based on 1000 replications are indicated above the branches.

Additional file 4: Figure S4. Phylogenetic analysis of Golovinomyces ambrosiae complex and G. circumfusus based on the TUB2 region. The tree was constructed based on 43 sequences from tribe Golovinomyceae and one sequence from Erysiphe kenjiana (accession number: MK452458) was used as an outgroup. Bootstrap values based on 1000 replications are indicated above/below the branches.

Additional file 5: Figure S5. Phylogenetic analysis of the CHSI region of the Golovinomyces ambrosiae complex and G. circumfusus. The tree was constructed based on 49 sequences from tribe Golovinomyceae. Three sequences from Neoeuryipsis galeopsidis (accession numbers: MK452438, MK452439, MK452440) was used as an outgroup. Bootstrap values based on 1000 replications are indicated above/below the branches.

Abbreviations

auct.: Auctorum (in the sense of other authors); comb. nov.: Combinatio nova (new combination); diam.: Diameter; e.g.: For example; emend.: Emendation; f.: Forma specialis; Figs.: Figures; No.: Number; P.: Page; p.p.: Pro parte (partly); s.: Sensu; s. lat.: Sensu latu (in the broad sense); spp.: Species plural; var.: Variety; viz.: Namely

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Authors’ contributions

In this research, UB, YL, SYL, and PLQ mainly provided the idea and the design the experiments; MB, SR-L, ST, TS B collected and examined the samples from abroad; PLQ, SRT and JF were in charge of the experiments. TA, DN J LLW were in charge of the collections from China. UB, SYL and PLQ mainly prepared the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The molecular data in the manuscript can be found in the GenBank database after publishing, and the materials can be found in the Herbaria shown in Table 1.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The manuscript does not infringe any personal or other copyright or property rights. The authors declare that they have no competing interests.

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