Trop. plant pathol. (2018) 43:202–217
https://doi.org/10.1007/s40858-017-0201-1

ORIGINAL ARTICLE

Notes on powdery mildews (Erysiphales) in Thailand V. *Golovinomyces*

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Received: 7 July 2017 / Accepted: 24 October 2017 / Published online: 10 November 2017

Abstract Records of *Golovinomyces* species new to Thailand are described on the hosts *Ageratum conyzoides*, *Bidens pilosa*, *Dahlia pinnata*, *D.* × *hortensis*, *Helianthus annuus*, *Lactuca indica, Laggera crispata*, *Sonchus oleraceus* (Asteraceae), *Lygisma inflexum* (Asclepiadaceae), *Myosotis scopioides* (Boraginaceae), *Coccinia indica*, *Coccinia grandis* (Cucurbitaceae), *Vigna umbellata* (Fabaceae), *Torenia fournieri* (Linderniaceae), *Plantago major* (Plantaginaceae) and *Verbena* × *hybrida* (Verbenaceae). The identifications of the particular *Golovinomyces* species have been performed by means of morphological examinations supplemented by molecular sequence analyses. On the basis of molecular analyses, the powdery mildew on *Ocimum tenuiflorum* (Lamiaceae) proved to represent a species of its own, which is referred to as *Golovinomyces ocimi* comb. nov. The application of *Oidium ocimi*, the basionym of this combination, is determined by lecto- and epitypification. *Lygisma inflexum*, *Laggera crispata* and *Vigna umbellata* are new host records for *Golovinomyces* worldwide.

Keywords Anamorph · Biodiversity · *Erysiphaceae* · Molecular phylogeny · Tropics

Section Editor: Meike Piepenbring

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Introduction

Golovinomyces (U. Braun) Heluta [type species: *Erysiphe cichoracearum* DC. (≡ *Golovinomyces cichoracearum* (DC.) Heluta)] is a genus of powdery mildew having polyascal ascoma (chasmothecia) with mycelioid appendages and catenescent conidia without fibrosin bodies (Braun and Takamatsu 2000; Matsuda and Takamatsu 2003). This genus is divided into two sections, sect. *Golovinomyces* and sect. *Depressi* (U. Braun) U. Braun. *Golovinomyces* is a large genus comprising 45 species (Braun and Cook 2012) with an almost worldwide distribution. The host range of this genus is mostly restricted to herbaceous plants, including up to 2283 species from 58 families such as the Asteraceae, Boraginaceae, Scrophulariaceae, and Cucurbitaceae (Amano 1986). The taxonomic history of the genus was described in Braun and Cook (2012) in detail. Investigations of powdery mildews in Southeast Asia in the past 14 years, in particular in Thailand, have contributed to the discovery of new taxa and new records mainly belonging to the tribe Erysipheae (for example, Meeboon et al. 2016; Meeboon and Takamatsu 2016, 2017a, b, c, d). In this paper, three hosts of powdery mildews are reported that are new worldwide, one new combination is introduced, and 11 host-fungus combinations of the powdery mildew species (*Golovinomyces ambrosiae*, *G. cynoglossi*, *G. orontii*, *G. sonchicola*, and *G. sordidus*) new for Thailand are listed. The identifications of the species concerned were verified by phylogenetic methods, i.e. molecular sequence analyses.

Materials and methods

Morphological examination

Morphological examinations were carried out as outlined in Meeboon and Takamatsu (2015a). All the specimens were

examined using a light microscope with phase contrast 10×, 20× and 40× objectives. Herbarium samples were rehydrated before examination by boiling a small piece of infected leaf with the fungal mycelium downwards in a drop of lactic acid on a slide (Shin and La 1993). After boiling, the rehydrated mycelium was scraped off and studied in lactic acid using a light microscope. Thirty conidia and conidiophores were measured for each specimen. Herbarium specimens were deposited at Mie University Mycological Herbarium (TSU-MUMH), Japan.

The nucleotide sequences of the rDNA internal transcribed spacer (ITS) region including 5.8S rDNA were determined in accordance with Meeboon and Takamatsu (2015b). Representative new sequences determined in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC306656–LC306669. These sequences were aligned with closely related sequences of the Erysiphaceae using MUSCLE (Edgar 2004) implemented in MEGA version 6 (Tamura et al. 2013). Alignment was further manually refined using MEGA, and deposited in TreeBASE (http://www.treebase.org/) under the accession number S21253. Phylogenetic trees were obtained from the datasets by using the maximum parsimony (MP) method implemented in PAUP* 4.0b10 (Swofford 2002) according to the procedures of Meeboon and Takamatsu (2016).

Molecular phylogeny

Whole cell DNA was extracted from mycelia using the chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The respective primer pairs of PM5/ITS4 and ITS5/PM6 (Takamatsu and Kano 2001) were used to amplify ITS fragment 1 and fragment 2, respectively. PCR reaction was conducted using KOD FX NeoDNA polymerase (Toyobo, Japan) according to the manufacturer's protocol. The PCR product was sent to SolGent Co. Ltd. (Daejeon, South Korea) for sequencing using primer pair of ITS1 and ITS4 (White et al. 1990).

New representative sequences determined in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC163909, LC163911, LC163913, LC163917 and LC163922. Sequences generated in this study were aligned with other sequences of *Golovinomyces* retrieved from DNA databases (DDBJ, EMBL, NCBI) using MUSCLE (Multiple Sequence Comparison by Log Expectation) (Edgar 2004) implemented in MEGA 6 (Tamura et al. 2013). The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number S20344.

Phylogenetic trees were obtained from the data set using the maximum parsimony (MP) method performed in PAUP* 4.0b10 (Swofford 2002) with the heuristic search option using the tree bisection reconstruction (TBR) algorithm. This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. Tree scores, including tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC), were calculated. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analysis (Felsenstein 1985) using 1000 replications with the stepwise addition option set to simple and a maximum tree number of 100.

Results

Phylogenetic analyses

Fourteen ITS sequences of *Golovinomyces* species determined in this study were aligned with other *Golovinomyces* sequences retrieved from DNA databases. *Arthrocladiella mougeotii* (Lév.) Vassilkov (AB329690) was used as outgroup taxon. Of the 510 total characters, 376 were constant, 46 were variable but parsimony-uninformative, and 88 were parsimony-informative. The MP analysis produced about 115K equally parsimonious trees with 284 steps. Topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. A typical tree is shown in Fig. 1.

Taxonomy

Golovinomyces ocimi (S. Naray. & K. Ramakr.) Meeboon & S. Takam., comb. nov. Fig. 2.

MycoBank no.: MB821878

Basionym: *Oidium ocimi* S. Naray. & K. Ramakr., Madras Univ. J. 37–38: 87, 1967.

Lectotype (designated here, MycoBank, (MBT377568): in Narayanaswami & Ramakrishnan (Narayanaswami and Ramakrishnan 1967: 87). Epitype (designated here, MycoBank, MBT377567): on *Ocimum tenuiflorum* L. (Lamiaceae), THAILAND, Chiang Rai, Wiangpapao, 15 December 2015, J. Meeboon (TNS-F-80794), TSU-MUMH6621 (isoepitype).

Gene sequence (ex epitype): LC306657 (ITS).

Mycelium amphigenous, effuse or in irregular patches, almost persistent or evanescent. Hyphae hyaline, walls thin, smooth or almost so, 4–9 μm wide. Hyphal appressoria nipple-shaped, sometimes poorly developed. Conidiophores terminal on the surface of mother cells, 70–173 μm long, often increasing from base to top, erect, straight or curved at the base. Foot cells 27–42 × 7–11 μm, basal septum of the foot cell mostly raised, 5–15 μm above the junction with the mother cell, foot cell followed by 1–3 shorter cells, forming

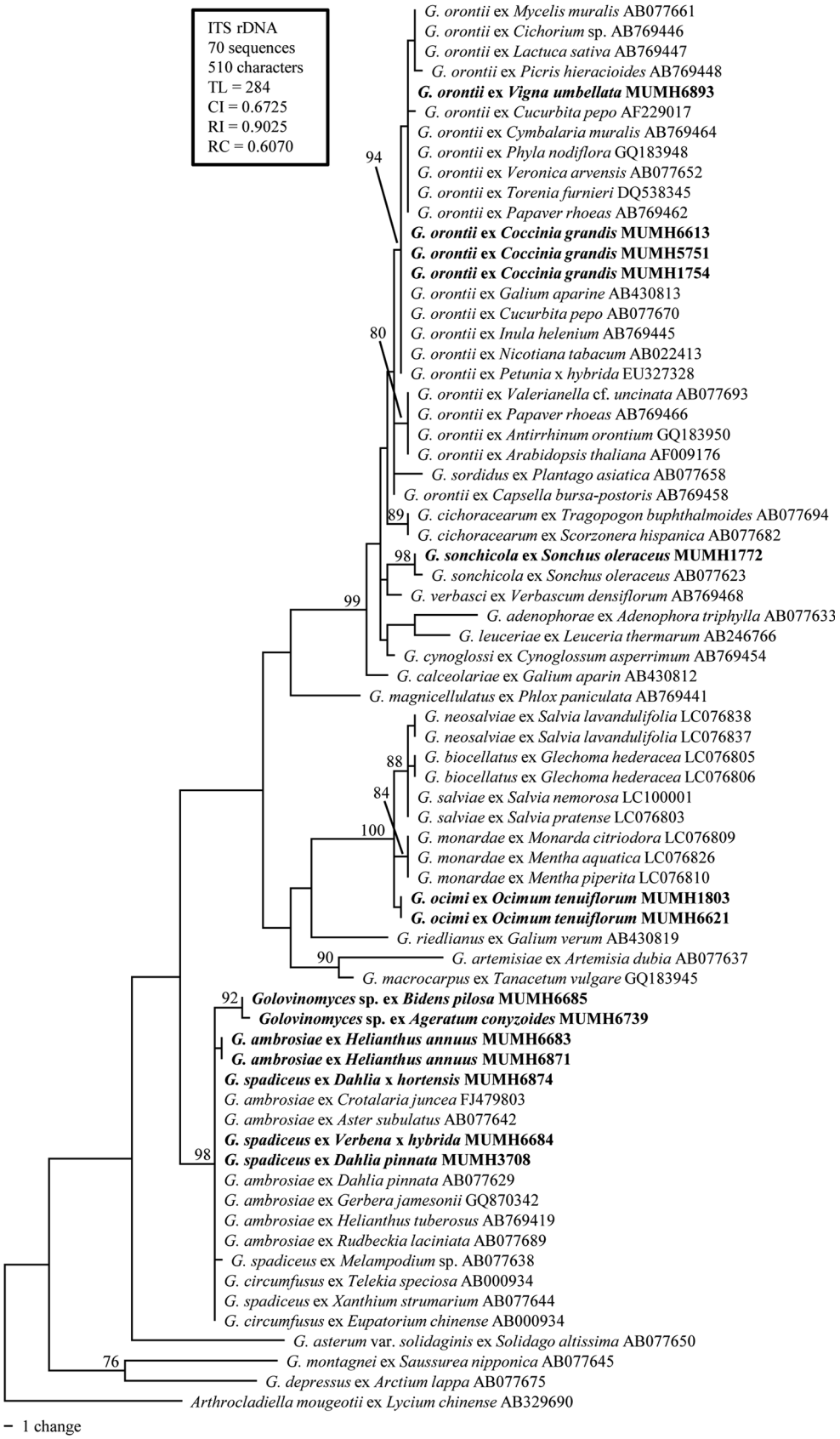

Fig. 1 Phylogenetic analysis of the rDNA ITS1-5.8S-ITS2 for 63 sequences from *Golovinomyces* spp. BS (>70%) values by the maximum parsimony (MP) are shown on the branches. Newly determined sequences were shown as boldface

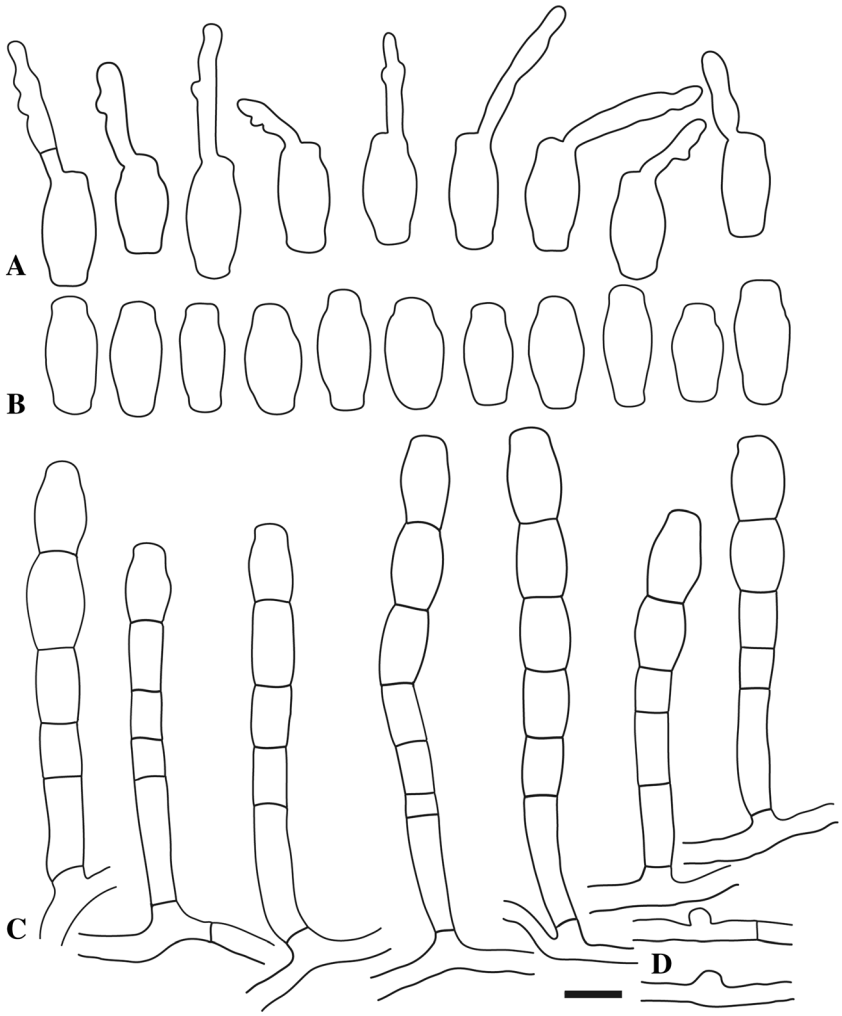

Fig. 2 *Golovinomyces ocimi* on *Ocimum tenuiflorum* (MUMH6621). **a**. Germinating conidia. **b**. Conidia. **c**. Conidiophores. **d**. Appressoria. Bar = 20 μm

catenescent conidia. Conidia doliiform, 32–40 × 15–20 μm, germ tubes subapically inserted, non to one-septate, mostly short to moderately long, terminating simply or in a club-shaped appressorium.

Further collections examined – on *Ocimum tenuiflorum* L. (Lamiaceae), THAILAND, Chiang Mai, Su Thep, 29 December 2007, J. Meeboon, MUMH1803; Chiang Rai, Wiangpapao, 25 December 2016, J. Meeboon, MUMH6892; Chiang Rai, Mae Suai, 29 December 2016, J. Meeboon & S. Takamatsu, MUMH6903, MUMH6942.

Notes – *Ocimum tenuiflorum*, commonly known as holy basil, is an aromatic plant which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. It is used in Thai cuisine as Thai holy basil (kaphrao). *Golovinomyces biocellatus* (Ehrenb.) Heluta was reported to occur on many host species of Lamiaceae, including *Ocimum* spp., in Europe, Asia, North and South America (Braun and Cook 2012). Narayanaswami and Ramakrishnan (1967) described *Oidium ocimi* on *O. tenuiflorum* (= *O. sanctum*) from India. The original description is rather brief and too insufficient to determine the taxonomic position of this asexual powdery mildew. Therefore, Braun (1987) listed *O. ocimi* under "Anamorphs of uncertain position". Bappammal et al. (1995) collected additional Indian specimens of *O. ocimi* and provided a more detailed description and illustration, suggesting a close affinity of this species to *Golovinomyces biocellatus*. This illustration was again published in Hosagoudar and Agarwal (2009). Furthermore, Amano (1986) listed *O. tenuiflorum* (= *O. sanctum*) as host species of *Erysiphe biocellata* from India. Therefore, Braun and Cook (2012) reduced *O. ocimi* to synonymy with *G. biocellatus*. Identification and application of the name *O. ocimi* require a clarification of this species via lecto- and epitypification. Type material of this species could not be traced and is probably not preserved. Bappammal et al. (1995) also failed to locate and examine type material. Therefore, the original illustration published in Narayanaswami and Ramakrishnan (1967), which is part of the original material in the sense of the ICN, has to be taken into consideration for lectotypification (see Art. 9.2, 9.3). An epitype (new material with corresponding sequence data) is proposed to allow a phylogenetic analysis of *O. ocimi* which

is essential for a taxonomic reassessment of this species. The *G. biocellatus* complex has recently been divided into four species (Scholler et al. 2016), viz, *G. biocellatus*, *G. monardae*, *G. neosalviae*, and *G. salviae*. The present collections from Thailand on *Ocimum tenuiflorum* are morphologically similar to these *Golovinomyces* species but differ in having relatively short conidiophores foot cells, 27–42 μm long, vs. 40–100(−140) μm long in *G. monardae*, 55–100(−130) μm long in *G. biocellatus*, 30–100(−120) μm long in *G. salviae*, 45–75(−115) μm long in *G. neosalviae*. Furthermore, two sequences retrieved from powdery mildew on *O. tenuiflorum* formed a distinct monophyletic clade. This clade grouped with the clades of *G. biocellatus*, *G. monardae*, *G. neosalviae*, and *G. salviae* with 100% BS value. The number of nucleotide differences of *Golovinomyces* on *O. tenuiflorum* was 3/486 characters (99.3% similarity, not including gaps) from *G. biocellatus* on *Glechoma hederacea* (LC076805), 3/485 characters (99.3 % similarity) from *G. monardae* on *Monarda citriodora* (LC076809), 4/485 characters (99.1 % similarity) from *G. neosalviae* on *Salvia lavandulifolia* (LC076838), and 4/486 characters (99.1 % similarity) from *G. salviae* on *Salvia nemorosa* (LC100001). These results suggest that the powdery mildew on *O. tenuiflorum* represents a species of its own morphologically and phylogenetically different from the allied *Golovinomyces* species on *Glechoma*, *Mentha*, *Origanum*, *Rosmarinus*, *Salvia* and *Thymus*, supported by the phylogenetic-taxonomic affinity and position of the host plant within the *Lamiaceae* [subfam. *Nepethoideae* tribe *Ocimeae*] (Walker et al. 2004). ICN (International Code of Nomenclature for algae, fungi, and plants), Art. 59.1, is applicable to this fungus. Thus, although its sexual morph is still unknown, it is assignable to *Golovinomyces*, which has priority over the anamorph-typified genus *Euoidium* that is now a heterotypic synonym of *Golovinomyces* (Rossman et al. 2016), and the name *Oidium ocimi* is used for this species (see above).

The *Golovinomyces ambrosiae* clade (III sensu Takamatsu et al. 2013)

This genetic assemblage is heterogeneous and unresolved. It comprises at least two morphologically distinguishable taxa that have been described as *Golovinomyces ambrosiae*, confined to *Ambrosia*, *Helianthus*, *Iva*, and *Rudbeckia* spp. as well as *Zinnia angustifolia*, all belonging to the composite tribe *Heliantheae* (Braun and Cook 2012). This species is characterized by having broadly ellipsoid-ovoid, doliiform to limoniform conidia, 25–45 × 15–27 μm when fresh, with a length/width ratio < 2, 1.3–1.9, mosty 1.4–1.6, and dimorphic germ tubes with a high percentage of longitubus pattern within the Euoidium type. The other taxon involved in this clade has

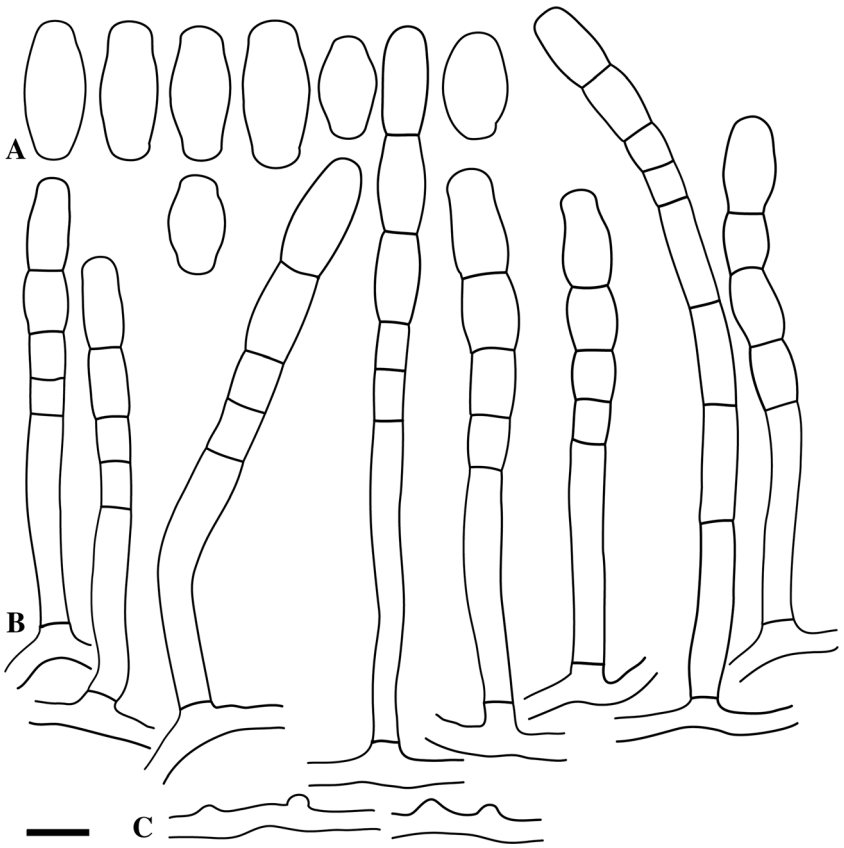

Fig. 3 *Golovinomyces ambrosiae* on *Helianthus annuus* (MUMH6871). **a**. Conidia. **b**. Conidiophores. **c**. Appressoria. Bar = 25 μm

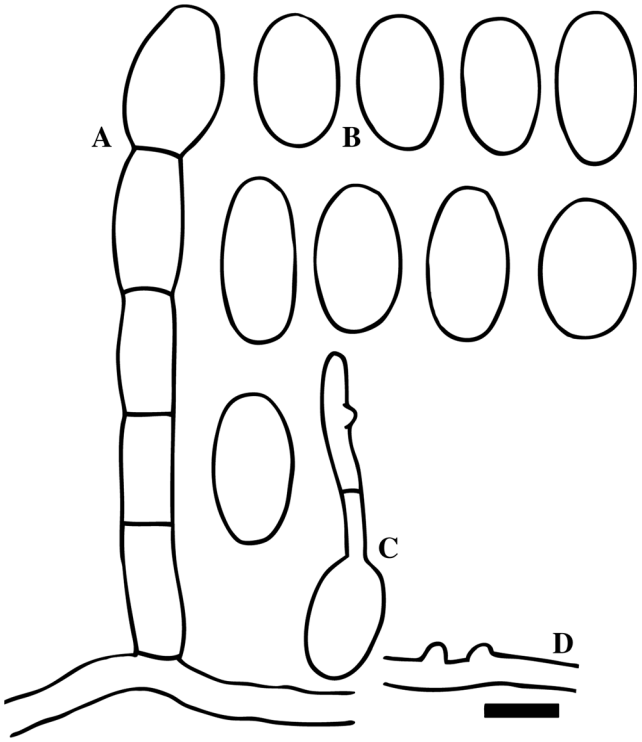

Fig. 4 *Golovinomyces cynoglossi* on *Myosotis scopioides* (MUMH1825). **a** Conidiophore. **b** Conidia. **c** Germinating conidium. **d** Appressoria. Bar = 20 μm

been referred to as *Golovinomyces spadiceus*, which differs from *G. ambrosiae* in having narrower conidia, 25–40 × 14–20 μm, with a length/width ratio of 1.5–2 and a *Euoidium* type of the conidial germination, lacking longitubus pattern germ tubes. *G. spadiceus* occurs on many hosts belonging to tribe *Heliantheae*, including species of the genera *Coreopsis*, *Dahlia*, *Xanthium*, and *Zinnia*, but is undoubtedly plurivorous with various hosts belonging to genera of other composite tribes and possibly even to some non-composite hosts. The situation is further complicated by the fact that some of the hosts involved in this complex are colonized by several *Golovinomyces* species, e.g., *Dahlia* spp. and *Helianthus* spp. are also hosts of *G. orontii* and *Zinnia* spp. are known to be hosts of *G. ambrosiae* as well as *G. spadiceus* (Braun and Cook 2012). It cannot be excluded that even *Helianthus* spp. might be hosts of *G. spadiceus*. Lineage III in Takamatsu et al. (2013) is probably a complex of several species. ITS sequences are not sufficient for a reliable resolution and differentiation on species level. Furthermore, the application of the species names involved can only be considered to be tentative since *Erysiphe ambrosiae* and *E. spadicea* were described from North America on *Ambrosia* sp. and *Xanthium* sp., respectively, but reference sequences and epitypes based on North American samples are not yet available. The application of the name *G. circumfusus* (Schltdl.) U. Braun to a

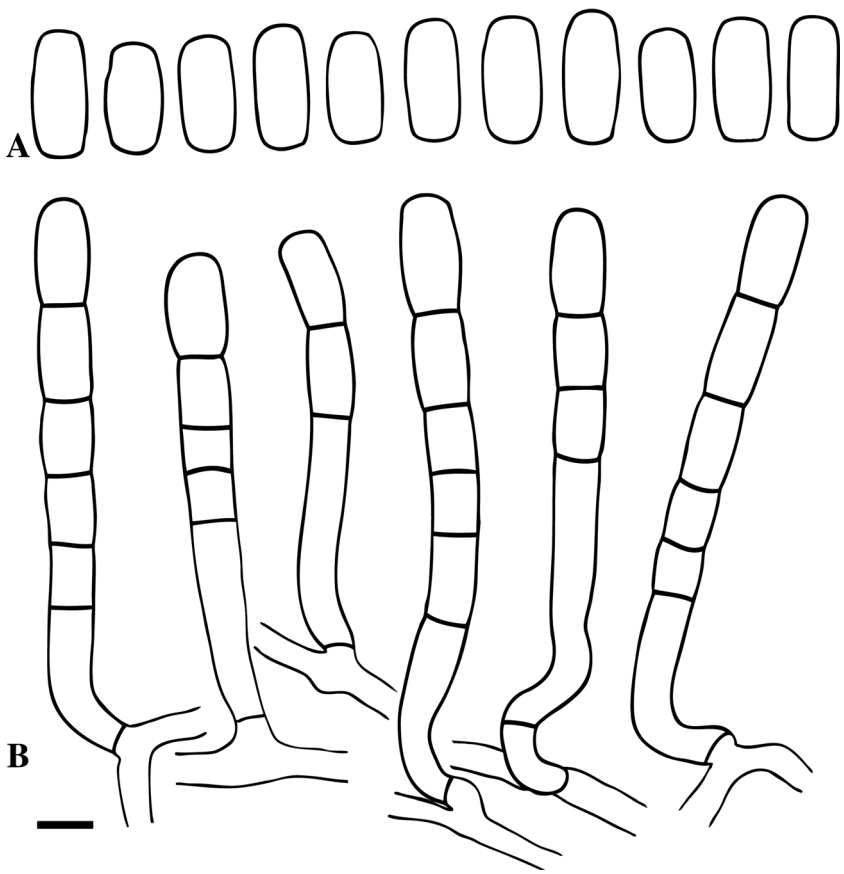

Fig. 5 *Golovinomyces orontii* on *Coccinia grandis* (MUMH6613). **a** Conidia. **b** Conidiophores. Bar = 20 μm

sequence retrieved from a Japanese sample on *Eupatorium chinense* in lineage III in Takamatsu et al. (2013) is also unclear and unproven since reference sequences obtained on the basis of European collections on *Eupatorium cannabinum* (type of *Erysiphe circumfusa*) are also still lacking. Up until a comprehensive genetic re-examination of the whole *G. ambrosiae* complex based on additional markers and epitypifications of the taxa involved, collections with sequences pertaining to lineage III can currently only tentatively be assigned to *G. ambrosiae* and *G. spadiceus* just based on morphology, as for instance done by Dugan (2013) in the case of *Golovinomyces* on *Coreopsis*. In this sense, *Golovinomyces* specimens recently collected in Thailand on *Dahlia pinnata*, *D.* × *hortensis*, and *Laggera crispata* can be assigned to *G. spadiceus*, and collections on *Helianthus annuus* to *G. ambrosiae*. *Golovinomyces* on *Verbena* × *hybrida* can only tentatively be assigned to *G. spadiceus* (conidiophores and conidia of the sample from Thailand are barely distinguishable from *G. verbenae*, but comparative sequence data retrieved from North American collection on *Verbena* are not yet available, i.e. the phylogenetic position of the latter species is still unknown). The identity of *Golovionomyces* on *Ageratum conyzoides* and *Bidens pilosa* in Thailand is unclear, since they are clearly different from *G. ambrosiae* and probably also distinct from *G. spadiceus*, and can currently only be classified as *Golovinomyces* sp. Further research is necessary. For the fungus on *Ageratum conyzoides* (*Eupatorieae*), the name *Euoidium agerati* (J.M. Yen) U. Braun & R.T.A. Cook, described on this host from Taiwan, is available (Braun and Cook 2012).

Golovinomyces ambrosiae (Schwein.) U. Braun & R.T.A. Cook, in Cook & Braun, Mycol. Res. 113(5): 628, 2009

Mycelium amphigenous, mainly epiphyllous, effuse or forming patches, thin, white, persistent or almost so; hyphae hyaline, walls thin, smooth, 4–8 μm wide. Hyphal appressoria nipple-shaped, solitary or in opposite pairs. Conidiophores arising centrally or towards one end of hyphal mother cells and from their upper surface, erect, straight, 177–298 μm long. Foot cells cylindrical, straight in the base, 71–129 ×

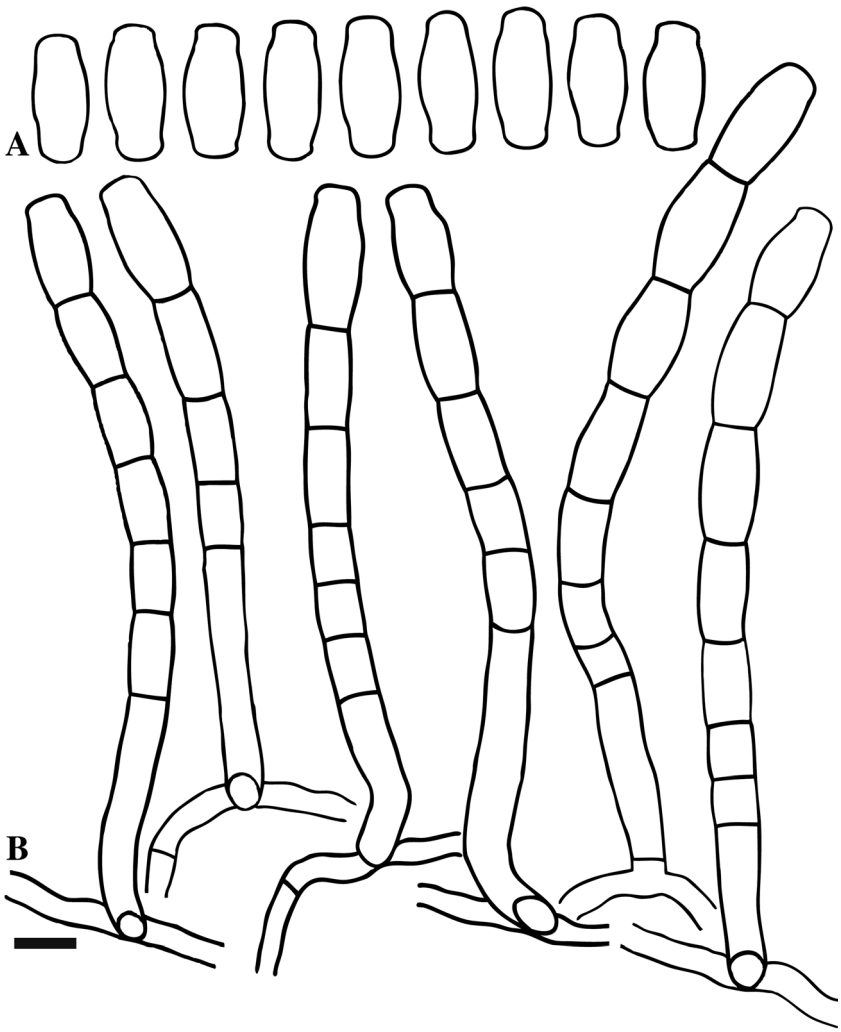

Fig. 6 *Golovinomyces orontii* on *Lactuca indica* (MUMH6936). **a** Conidia. **b** Conidiophores. Bar = 15 μm

11–18 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia broadly ellipsoid-ovoid, doliiform to somewhat limoniform, 39–60 × 23–27 μm, germ tubes terminal to subterminal, tips not swollen or only slightly swollen (Fig. 3).

Material examined – On *Helianthus annuus* L. (Asteraceae), Chiang Rai, Mae Suai, 30 December 2016, J. Meeboon & S. Takamatsu, MUMH6871; Chiang Mai, Mae Rim, Mon Jam, 19 January 2016, J. Meeboon, MUMH6683.

Notes – *Golovinomyces ambrosiae* and *G. orontii* have been recorded on *Helianthus* spp. (Braun and Cook 2012). These two species differ from each other by the shape of foot cells; *G. ambrosiae* has straight in the base of foot cells, *G. orontii* has curved foot cells. The morphological characteristics of the current specimen are more similar to *G. ambrosiae* than to *G. orontii* due to having straight foot cells and broad conidia, 25–40 × (10–)15–23(–25) μm in *G. orontii* vs. 25–45 × 15–27 μm in *G. ambrosiae* (Braun and Cook 2012). This is the first report of *G. ambrosiae* on *Helianthus annuus* from Thailand.

Golovinomyces cynoglossi (Wallr.) Heluta, Ukrayins'k. Bot. Zhurn. 45(5): 62, 1988

Mycelium amphigenous, dense, effuse or in patches, evanescent to almost persistent. Hyphae hyaline, thin-walled, smooth, 3.5–7 μm wide. Hyphal appressoria nipple-shaped. Conidiophores erect, arising from upper surface of hyphal mother cells, 90–160(–200) μm long. Foot cells straight, cylindrical, 30–110 × 10–13 μm, followed by 1–2(–3) shorter cells, forming catenescent conidia; conidia ellipsoid-ovoid, 33–45 × 18–25 μm, germ tubes arising from an end, moderately long, apex often with somewhat swollen appressorium as in *Euoidium* type (Fig. 4).

Material examined – on *Myosotis scopioides* L. (Boraginaceae), THAILAND, Chiang Mai, Su Thep, 5 January 2005, J. Meeboon, MUMH1825.

Notes – Braun and Cook (2012) described *G. cynoglossi* on many host species of various host genera of Boraginaceae including *Myosotis* throughout Europe, Asia, North and South Africa and North America. The asexual morph found on *Myosotis scopioides* agrees completely with *G. cynoglossi* and is the first record of powdery mildew on *M. scopioides* in Thailand.

Golovinomyces orontii (Castagne) Heluta, Ukrayins'k. Bot. Zhurn. 45(5): 63, 1988

Mycelium amphigenous, mainly epiphyllous, effuse or in patches, evanescent or persistent, white; hyphae slightly flexuous, branched at right angles. Hyphal appressoria nipple-shaped, often poorly developed. Conidiophores erect, arising laterally or from the upper surface of hyphal mother cells and almost centrally or towards one end of the cell. Foot cells straight or curved in the basal half, followed by 2–3 shorter cells, forming catenescent conidia. Conidia ellipsoid, doliiform, subcylindrical, germ tubes arising from an end, occasionally from a side, straight, bent, rarely forked, apically often with a somewhat swollen appressorium, *Euoidium* type.

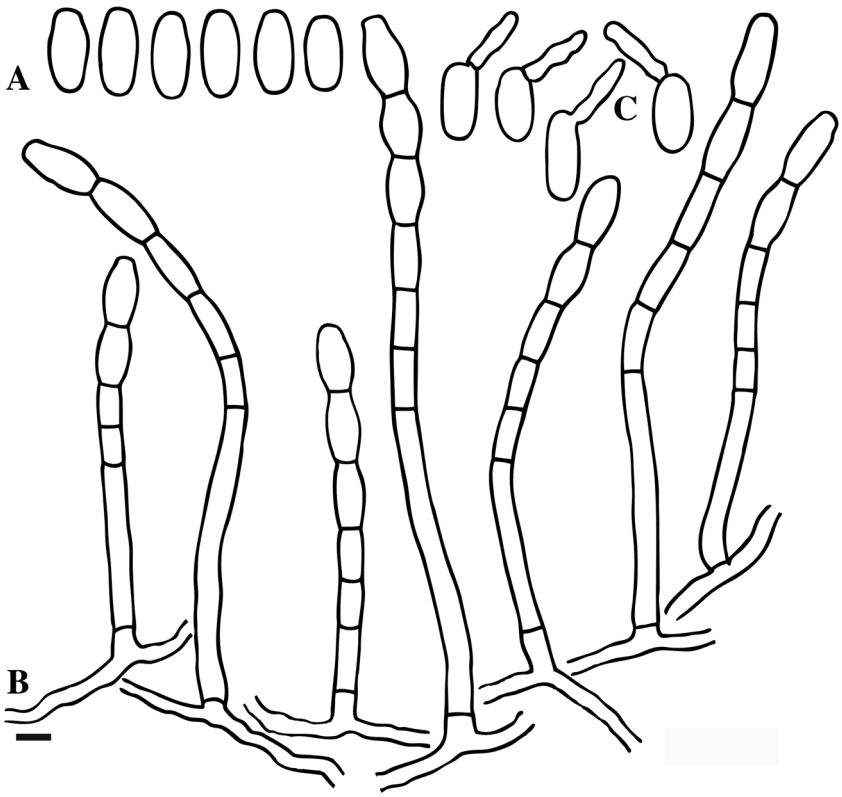

Fig. 7 *Golovinomyces orontii* on *Torenia fournieri* (MUMH6881). **a** Conidiophores. **b** Conidia. **c** Germinating conidia. Bar = 15 μm

Golovinomyces orontii* ex *Coccinia grandis

Conidiophores erect, arising laterally or the upper surface of hyphal mother cells, 130–200 μm long. Foot cells curved, rarely straight, 60–87 × 13–16 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia subcylindrical, 36–44 × 18–20 μm (Fig. 5).

Golovinomyces orontii* ex *Lactuca indica

Conidiophores erect, usually arising laterally from the hyphal mother cell but occasionally from its upper surface, 100–210 μm long. Foot cells straight to curved at the base, 36–61 × 5–10 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia doliiform to subcylindrical, 28–32 × 12–14 μm (Fig. 6).

Golovinomyces orontii* ex *Torenia fournieri

Conidiophores erect, arising from the upper surface of hyphal mother cells, 157–290 μm long. Foot cells straight or curved, 26–124 × 9.5–13 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia ellipsoid, doliiform to subcylindrical, 30–39 × 15–20 μm (Fig. 7).

Golovinomyces orontii* ex *Vigna umbellata

Conidiophores erect, arising from the upper surface of hyphal mother cells, 115–235 μm long. Foot cells straight or curved, 32–57 × 13–15 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia doliiform to subcylindrical, 38–50 × 20–23 μm (Fig. 8).

Materials examined – on *Coccinia grandis* (L.) Voigt (Cucurbitaceae), THAILAND, Chiang Rai, 5 January 2016,

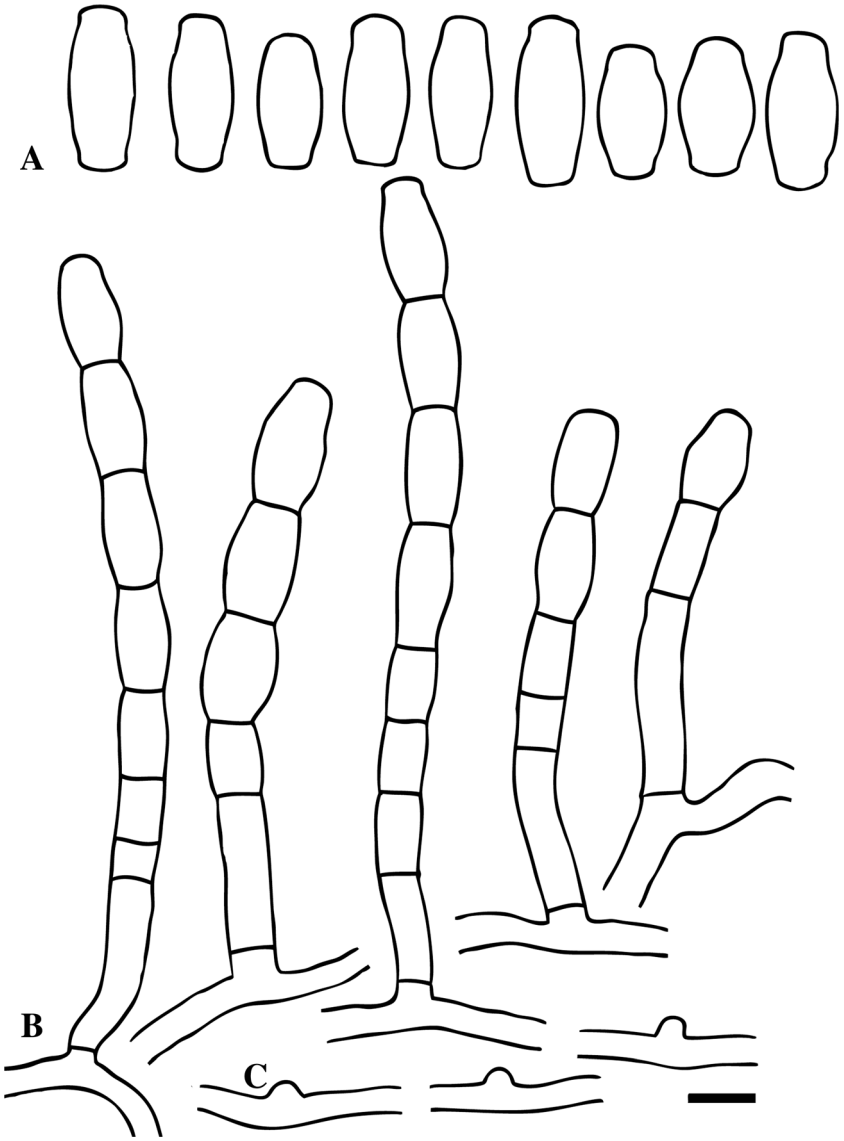

Fig. 8 *Golovinomyces orontii* on *Vigna umbellata* (MUMH6893). **a** Conidia. **b** Conidiophores. **c** Appressoria. Bar = 20 μm

J. Meeboon, MUMH6613; *Lactuca indica* L. (Asteraceae), THAILAND, Chiang Rai, Mae Suai, 29 December 2016, J. Meeboon & S. Takamatsu, MUMH6936; *Torenia fournieri* Linden ex Fourn. (Linderniaceae), Chiang Rai, Wiangpapao, 5 January 2016, J. Meeboon, MUMH6881, *Vigna umbellata* (Thunb.) Ohwi & H.Ohashi (Fabaceae), Chiang Rai, Mae Suai, 29 December 2016, J. Meeboon, MUMH6893.

Notes – *Golovinomyces cucurbitacearum* (R.Y. Zheng & G.Q. Chen) Vakal. & Kliron. is listed as powdery mildew on *Coccinia* spp. (Braun and Cook 2012). The asexual morph of the present sample is in good agreement with *G. orontii*, and the identity of this collection is confirmed by means of molecular sequence analyses (see Fig. 1). The fungus on *Coccinia* differs from *G. cucurbitacearum* in having longer foot cells and broader conidia. *Golovinomyces cichoracearum*, *G. orontii*, *Leveillula lactucarum*, *L. lactucae-serriolae*, and *Podosphaera xanthii* have been recorded on *Lactuca* spp. worldwide (Matsuda and Takamatsu 2003; Braun and Cook

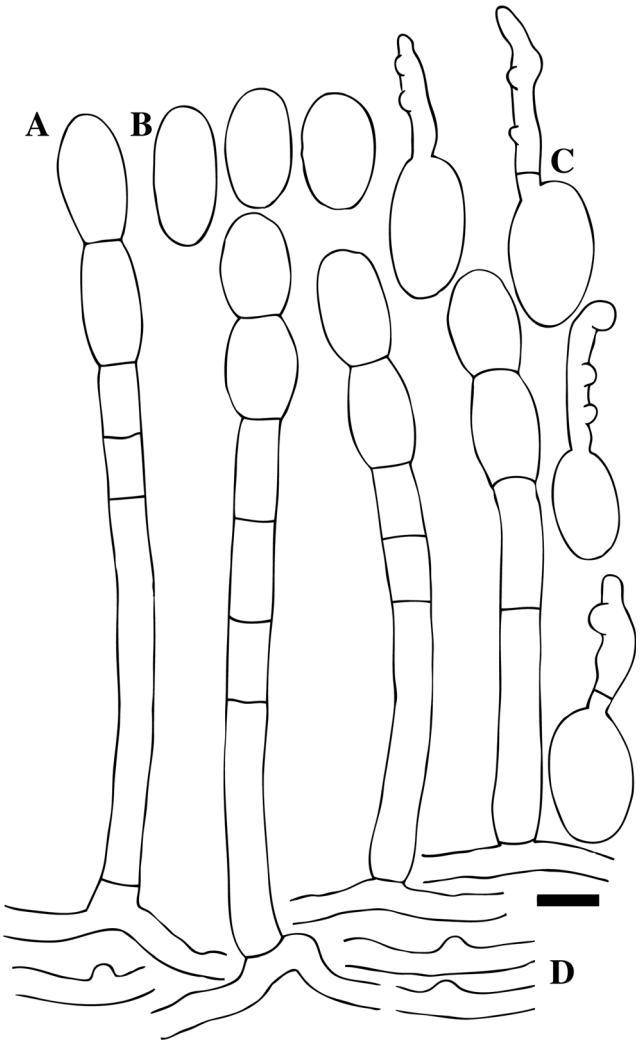

Fig. 9 *Golovinomyces spadiceus* on *Dahlia pinnata* (MUMH3708). **a** Conidiophores. **b** Conidia. **c** Germinating conidia. Bar = 15 μm

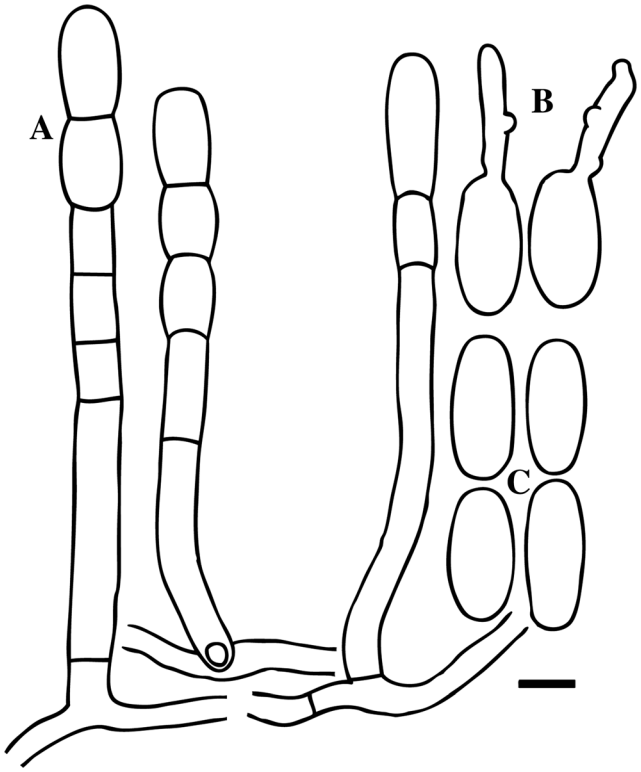

Fig. 10 *Golovinomyces spadiceus* on *Laggera crispata* (MUMH1748). **a** Conidiophores. **b** Germ tubes. **c** Germinating conidia. Bar = 15 μm

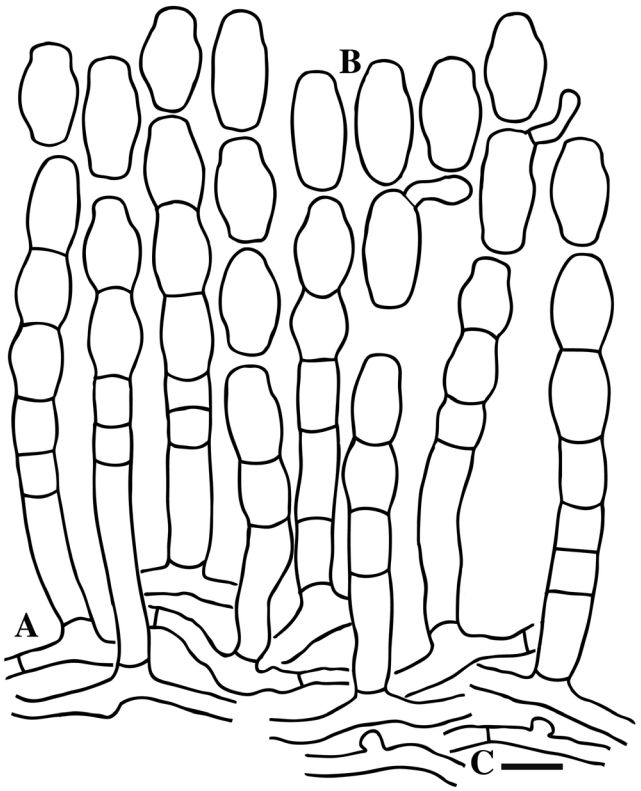

Fig. 11 *Golovinomyces spadiceus* on *Verbena hybrida* (MUMH6684). **a** Conidia. **b** Conidiophores. **c** Appressoria. Bar = 20 μm

2012; Takamatsu et al. 2013; Park et al. 2015; Cho et al. 2016). The asexual morph of the powdery mildew found in Thailand on *L. indica* having conidiophores arising laterally and from the upper surface of hyphae. However, some previous reports mentioned that the conidiophores of *G. orontii* obtained from *Lactuca* spp. are only arising laterally, thus producing curved foot cells (Braun and Cook 2012; Park et al. 2015; Cho et al. 2016). In addition, the identification as *G. orontii* has been confirmed by means of molecular sequence analyses (see Fig. 1). Vági et al. (2007) reported *Golovinomyces* sp. on *Torenia fournieri* from Hungary. The morphology of the specimen on *Torenia fournieri* from Thailand is similar to the fungus collected in Hungary, and a sequence retrieved from this collection clusters within *G. orontii* group 3 according to Takamatsu et al. (2013). *Vigna umbellata* was previously unknown as host of *Golovinomyces* spp. The sequences of the powdery mildews from *C. grandis* and *V. umbellata* belong to the big *G. orontii* complex and form a clade of its own with 94% BS support (Fig. 1). These hosts are new to Thailand.

Golovinomyces spadiceus **(Berk. & M.A. Curtis) U. Braun ex *Dahlia pinnata* and *Dahlia* × *hortensis***

Mycelium amphigenous, forming white patches, confluent, sometimes covering entire leaves, persistent or almost so. Hyphae 4–8 μm wide, thin-walled, smooth, hyaline. Hyphal appressoria solitary, nipple-shaped, 3–8 μm diam. Conidiophores erect, arising from upper surface of hyphal mother cell and usually towards one end of it, 125–188 μm long. Foot cells cylindrical, 52–98 × 9–13 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia ellipsoid-ovoid, 27–33 × 15–25 μm. Conidial germination of the *Euoidium* type (Fig. 9).

Materials examined – On *Dahlia pinnata* Cav. (Asteraceae), Chiang Mai, Su Thep, 21 January 2005, J. Meeboon, MUMH3708; *Dahlia* × *hortensis* Guillaumin, Chiang Rai, Mae Suai, 27 December 2016, J. Meeboon, MUMH6874.

Notes – Braun and Cook (2012) described the morphological characteristics of *G. spadiceus*, which overlapped with our own specimens. The molecular phylogenetic analysis based on the ITS rDNA sequence of this specimen showed that the present fungus nested in the *G. ambrosiae* clade (III sensu Takamatsu et al. 2013) with 98% BS (Fig. 1). This is the first report of *G. spadiceus* on *Dahlia pinnata* and *Dahlia* × *hortensis* from Thailand.

Golovinomyces spadiceus (Berk. & M.A. Curtis) U. Braun ex *Laggera crispata*

Mycelium amphigenous, effuse or in thin, irregular patches, white, persistent. Hyphae sparingly branched,

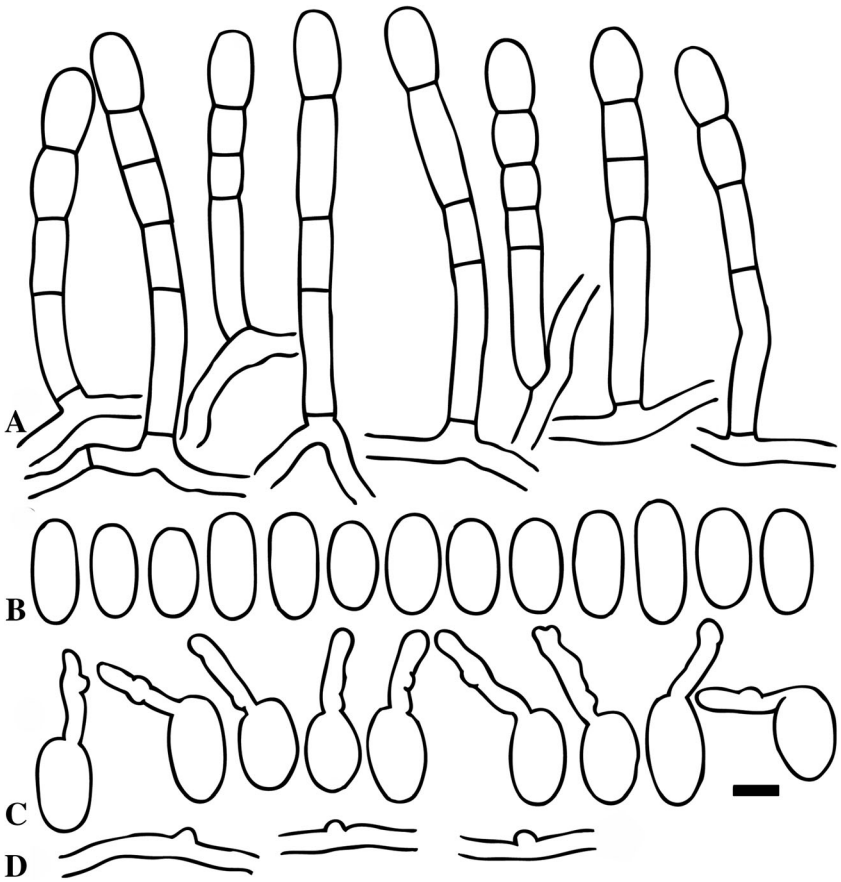

Fig. 12 *Golovinomyces sonchicola* on *Sonchus oleraceus* (MUMH1772). **a** Conidiophores. **b** Conidia. **c** Germinating conidia. **d** Appressoria. Bar = 18 μm

straight to moderately sinuous, 4–8 μm wide, hyaline, thin-walled, smooth. Hyphal appressoria almost indistinct to nipple-shaped, usually solitary, 2–7 μm diam. Conidiophores 156–187 μm long, arising laterally or from the upper surface of hyphal mother cells, and positioned almost centrally or towards one end of the cells, slightly curved at the base. Foot cells subcylindrical, 61–112 × 10–13.5 μm, followed by 0–3 shorter cells, forming catenescent conidia. Conidia ellipsoid-obovoid, often constricted at the ends, 35–41 × 16–21 μm. Conidial germination of the *Euoidium* type (Fig. 10).

Material examined – On *Laggera crispata* (Vahl) Hepper & J.R.I. Wood (= *L. pterodonta* (DC.) Sch.Bip. ex Oliv.) (Asteraceae, Inuleae), Chiang Mai, Mae Rim, 18 January 2005, J. Meeboon, MUMH1748.

Notes – This is the first report of powdery mildew on *L. crispata*. The morphological characteristics were typical of the asexual morph of the genus *Golovinomyces*, Foot cells of current material are longer than previous data, 30–80 × 9–15 μm (Braun and Cook 2012), and conidia are longer, 25–40 × 14–20 μm (Braun and Cook 2012). Based on these morphological characteristics, we identify the powdery mildew on *L. crispata* as *G. spadiceus*.

Golovinomyces spadiceus (Berk. & M.A. Curtis) U. Braun ex *Verbena* × *hybrida*

Mycelium amphigenous, effuse or in thin, irregular patches, white, persistent. Hyphae sparingly branched, straight to moderately sinuous, 4–8 μm wide, hyaline, thin-walled, smooth. Hyphal appressoria almost indistinct to nipple-shaped, usually solitary, 2–7 μm diam. Conidiophores 81–182 μm long, arising from the upper surface of hyphal mother cells, and positioned almost centrally or towards one end of the cells, straight to slightly curved at the base. Foot cells subcylindrical, 32–66 × 10–15 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia doliiform-limoniform, 38–46 × 20–26 μm. Conidial germination of the *Euoidium* type (Fig. 11).

Material examined – On *Verbena* × *hybrida* Groenland & Rümpler (Verbenaceae), Chiang Mai, Mae Rim, 19 January 2016, J. Meeboon, MUMH6684.

Notes – *Golovinomyces verbenae* (Schwein.) Heluta and *G. orontii* have been recorded on *Verbena* spp. (Amano 1986; Braun and Cook 2012). The current specimen was confirmed to be *G. spadiceus* by the size of foot cells and conidial shape. This is the first report of *G. spadiceus* on *V.* × *hybrida* from Thailand.

Golovinomyces sonchicola U. Braun & R.T.A. Cook, in Cook & Braun, Mycol. Res. 113(5): 629, 2009

Mycelium amphigenous, mainly epiphyllous, effuse or forming white patches, thin. Hyphae straight to sinuous, hyaline, thin-walled, smooth or almost so, 4–7 μm wide. Hyphal appressoria nipple-shaped. Conidiophores arising from the hyphal mother cell and towards one end of the cell, often close to a septum, rarely in the middle, 112–160 μm long. Foot cells curved, 38–70 × 12–16 μm, slightly constricted at the basal septum, followed by 1–3 shorter cells, forming catenescent conidia. Conidia ellipsoid-obovoid, 34–48 × 18–22 μm, germ tubes terminal or almost so, short to moderately long, often with a slightly swollen appressorium at the tip, *Euoidium* type (Fig. 12).

Material examined – on *Sonchus oleraceus* L. (Asteraceae), THAILAND, Chiang Mai, Su Thep, 5 January 2005, J. Meeboon, MUMH1772.

Notes – Shin (2000) described the asexual morphs of powdery mildews on *Sonchus asper*, *S. brachyotus*, and *S. oleraceus* collected in Korea. The ITS sequence of the powdery mildew on *S. oleraceus* was compared with the nucleotide sequences obtained from DNA databases. This fungus has the highest sequence similarity with *G. sonchicola* on *S. oleaceus* collected in Japan (99.8%). The present fungus formed a distinct clade with *G. sonchicola* on *S. oleraceus* (AB077623) collected in Japan with strong bootstrap support (98%) (Fig. 1). Based on the morphological and molecular characteristics, the powdery mildew on *S. oleaceus* is

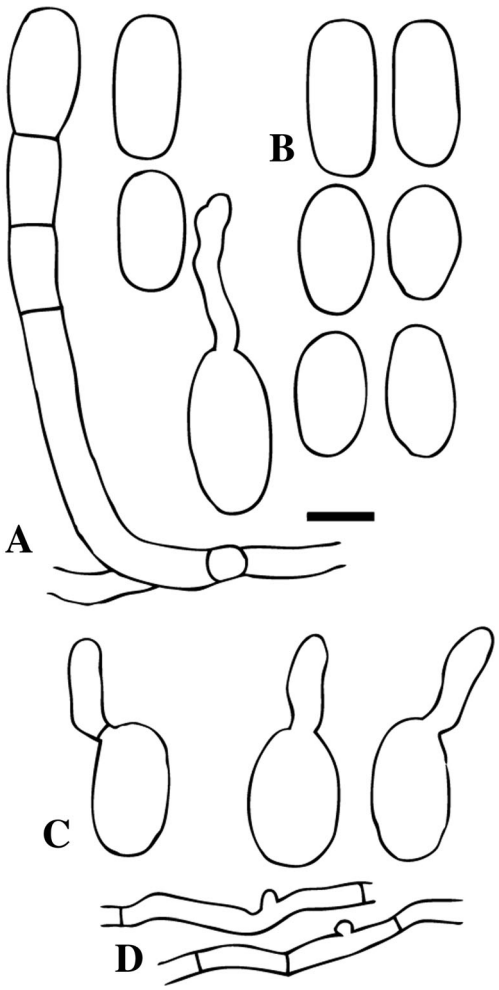

Fig. 13 *Golovinomyces sordidus* on *Plantago major* (MUMH6633). **a** Conidiophore. **b** Conidia. **c** Germinating conidia. **d** Appressoria. Bar = 20 μm

identified as *G. sonchicola*. This is the first report of *G. sonchicola* on *S. oleraceus* from Thailand.

Golovinomyces sordidus (L. Junell) Heluta, Ukrayins'k. Bot. Zhurn. 45(5): 63, 1988

Mycelium amphigenous, in irregular patches, almost persistent or evanescent. Hyphae hyaline, walls thin, smooth or almost so, 5–8 μm wide. Hyphal appressoria nipple-shaped or occasionally slightly lobed, sometimes poorly developed. Conidiophores arising more or less laterally from the hyphal mother cell and towards one end of the cell, often close to a septum, 98–240 μm long. Foot cells almost cylindrical, 40–100 × 11–14 μm, followed by 3–4 shorter cells, forming catenescent conidia. Conidia ellipsoid-ovoid to subcylindrical, 30–40 × 20–24 μm, germ tubes terminal or almost so, short to moderately long, ending in an unlobed, somewhat swollen appressorium, *Euoidium* type (Fig. 13).

Material examined – on *Plantago major* L. (Plantaginaceae), THAILAND, Chiang Mai, Inthanon National Park, 11 December 2014, MUMH6633.

Notes – The asexual morph of *G. sordidus* is the only *Euoidium* species occurring on *Plantago* spp. (Braun and Cook 2012). Although there are only minor differences in the sizes of conidiophores and foot cells, this fungus was identified as *G. sordidus*. This is the first report of *G. sordidus* on *P. major* from Thailand.

Golovinomyces sp. ex *Ageratum conyzoides*

Mycelium amphigenous, mainly epiphyllous, effuse or forming white patches, thin. Hyphae straight to sinuous, hyaline, thin-walled, smooth or almost so, 3–7 μm wide. Hyphal appressoria nipple-shaped. Conidiophores 110–237 μm long, erect, arising from upper surface of hyphal mother cell and

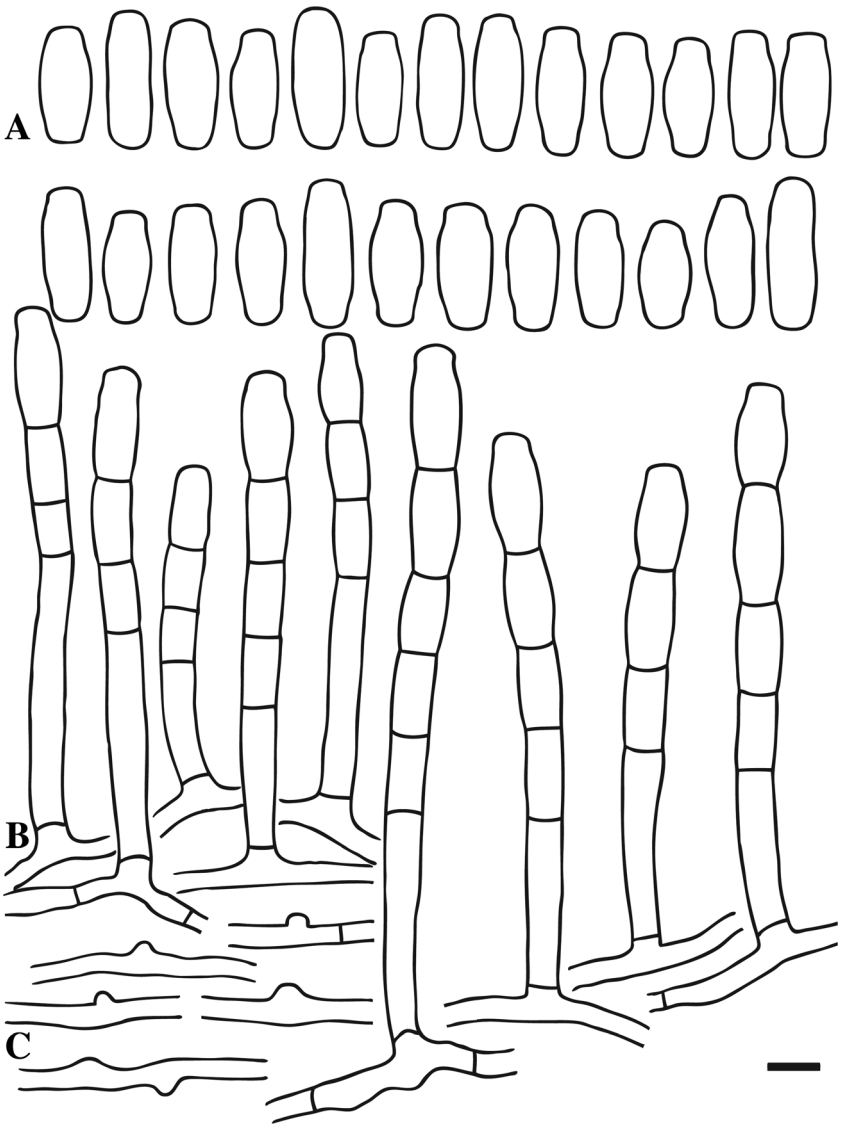

Fig. 14 *Golovinomyces* sp. on *Ageratum conyzoides* (MUMH6739). **a** Conidia. **b** Conidiophores. **c** Appressoria. Bar = 20 μm

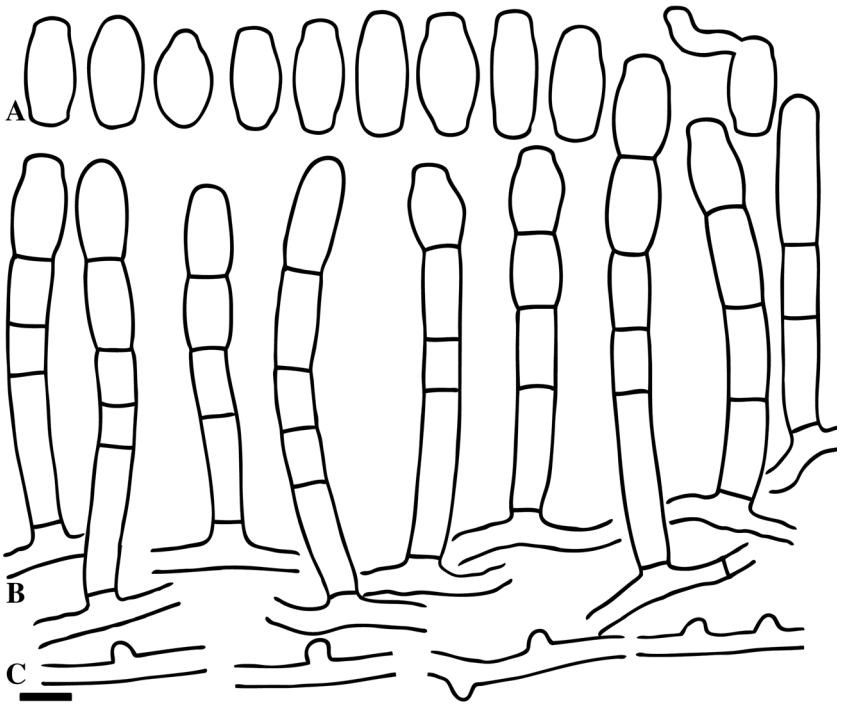

Fig. 15 *Golovinomyces* sp. on *Bidens pilosa* (MUMH6685). **a** Conidia. **b** Conidiophores. **c** Appressoria. Bar = 20 μm

usually towards one end of it. Foot cells cylindrical, 42–95 × 12–16 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia doliiform to subcylindrical 38–53 × 17–20 μm. Conidial germination of the *Euoidium* type (Fig. 14).

Material examined – On *Ageratum conyzoides* L. (Asteraceae, Eupatorieae), THAILAND, Lampang, Wang Nue, 13 January 2016, J. Meeboon, MUMH6739.

Notes – *Ageratum conyzoides* is known as host of *G. cichoracearum* s. lat. (Amano 1986). The foot cells and conidia of this specimen are longer than in *G. cichoracearum* s. str. (30–)40–80 μm long vs. 42–95 μm long and 25–42 × 14–23 μm vs. 38–53 × 17–20 μm, respectively (Braun and Cook 2012). The sequence of the powdery mildew on *A. conyzoides* clustered with those on *Bidens pilosa* in the clade of *G. ambrosiae* (III sensu Takamatsu et al. 2013) with 92% BS and the morphological characteristics are clearly different from *G. ambrosiae*, foot cells are shorter, 35–80 × 9–15 μm and conidia are broader, 25–45 × 15–27 μm (Braun and Cook 2012). Identification of *Golovinomyces* on *A. conyzoides* using solely based on the ITS rDNA sequences is insufficient. This is the first report of *Golovinomyces* sp. on *A. conyzoides* from Thailand.

Golovinomyces sp. ex *Bidens pilosa*

Mycelium amphigenous, mainly epiphyllous, effuse or forming white patches, thin. Hyphae straight to sinuous, hyaline, thin-walled, smooth or almost so, 4–6 μm wide. Hyphal appressoria nipple-shaped. Conidiophores arising from upper surface of hyphal mother cell and usually towards one end of it, 95–190 μm long. Foot cells 41–68 × 10–16 μm, slightly

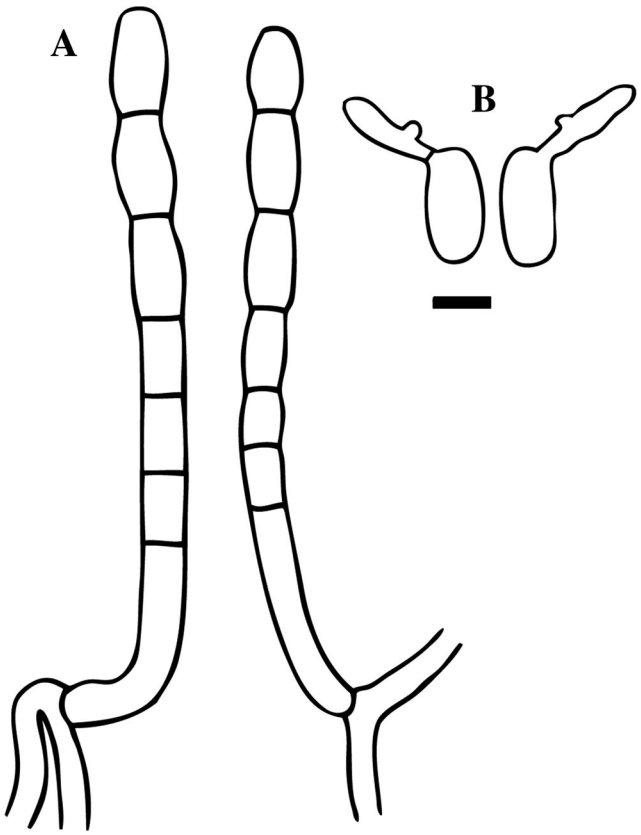

Fig. 16 *Golovinomyces* sp. on *Lygisma inflexum* (MUMH1784). **a** Conidiophores. **b** Conidia with germ tubes. Bar = 20 um

constricted at the basal septum, followed by 1–3 shorter cells, forming catenescent conidia. Conidia doliiform 34–50 × 20–25 μm. Conidial germination of the *Euoidium* type (Fig. 15).

Material examined – On *Bidens pilosa* L. Chiang Rai, Wiangpapao, 3 January 2016, J. Meeboon, MUMH6685

Notes – *Bidens pilosa* is known as host of *G. cichoracearum* s. lat. (Amano 1986). This is the first report of *Golovinomyces* sp. on *B. pilosa* from Thailand.

Golovinomyces sp. ex *Lygisma inflexum*

Mycelium amphigenous, forming patches or effuse, often confluent, persistent, particularly on the upper leaf surface, often evanescent on the lower surface, white or dingy greyish white. Hyphae straight to sinuous-geniculate, walls thin, smooth or almost so, hyaline, 3–8 μm wide. Hyphal appressoria almost indistinct to nipple-shape. Conidiophores arising from upper surface and usually towards one end of hyphal mother cells, 186–262 μm long. Foot cells straight to curved, cylindrical, 76–82 × 12–16 μm, followed by 1–3 shorter cells, forming catenescent conidia, often in long chains. Conidia ellipsoid-cylindrical, 35–43.5 × 20–21 μm, germ tubes short to moderately long, *Euoidium* type (Fig. 16).

Material examined – on *Lygisma inflexum* (Costantin) Kerr (Asclepiadaceae), THAILAND, Chiang Mai, Su Thep, 5 January 2005, J. Meeboon, MUMH1784.

Notes – *Golovinomyces cichoracearum* s. lat. has been recorded on the asclepidaceous hosts *Asclepias syriaca* L. and *A. tuberosa* L. and *G. orontii* on *A. incarnata* L. and *Hoya carnosa* (L.) R.Br. (Farr and Rossman 2017), but *Lygisma inflexum* has not yet been listed as host of any *Golovinomyces* species (Braun and Cook 2012). This is the first record of powdery mildews on *L. inflexum*. Although DNA isolation of this specimen failed, based on conidial chains with sinuous edge lines and conidia without fibrosin bodies, this powdery mildew is assigned to the genus *Golovinomyces*.

Acknowledgements This work was financially supported in part by a Grant-in-Aid for Scientific Research (No. 16K07613 and 16F16097) from the Japan Society for the Promotion of Science to ST; and The JSPS postdoctoral fellowship to JM (P16097).

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