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New Host Records of the Genus *Golovinomyces* (*Erysiphales, Ascomycota*)

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Samples of *Polygonum alpinum* and *Alcea rosea* infected by powdery mildews collected from Azerbaijan were analyzed by using morphological and molecular methods. 28S rRNA gene including D1/D2 domains and ITS1/5.8S/ITS2 regions were determined for each specimen. Consequently, *Golovinomyces spadiceus* on *P. alpinum* and *G. magnicellulatus* on *A. rosea* were identified. Comprehensive morphological descriptions, illustrations of species and the results of molecular-phylogenetic analysis were given in the present article.

Keywords: *Erysiphaceae, molecular analysis, new host plant, powdery mildew fungi, taxonomy*

INTRODUCTION

Powdery mildew fungi (*Erysiphaceae*) are economically important plant pathogens of vascular plants, including many cultivated plants. This group of fungi is characterized by their obligate biotrophic nature. Powdery mildews have long been considered as strictly host specific, in which host range of single powdery mildew species is restricted to a single plant family, or narrow range of genera or species (Matsuda, Takamatsu, 2003; Glawe, 2008; Braun, Cook, 2012). This assumption was supported with molecular analyses in many cases. But there are some exceptions, in which isolates having identical or highly similar DNA sequences are found from many distantly related host plants. For example, *Golovinomyces orontii* (Castagne) Heluta, *Leveillula taurica* (Lév.) G. Arnaud and *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff. are strictly herb parasitic fungi and have wide range of host plants beyond family level. In comparison with tree-parasitic powdery mildews having older origin and narrow range of hosts, herb parasitic powdery mildews have recent origin and considered that they are continuing their host expansion (Khodaparast et al., 2001; Matsuda, Takamatsu, 2003; Takamatsu et al., 2013). Exclusively tree-parasitic powdery mildew fungus, *Phyllactinia guttata* (Wallr: Fr.) Lév. represents as a species complex composed of numerous species. Some tree parasitic species, such as *Erysiphe alphitoides* (Griff. & Maubl.) U. Braun & S. Takam., *E. platani* (Howe) U. Braun & S. Takam., and *E. quercicola* S. Takam. & U. Braun are also considered to be actively expanding their host ranges to numerous unrelated host genera and families other than their original hosts (Kirschner, Liu, 2014; Takamatsu et al., 2015; Siahaan et al., 2016). Recently, 11 tropical tree genera were listed as hosts for *E. quercicola* (Limkainsang et al., 2006). Sequences identical to *E. alphitoides* on herbaceous *Oenothera* spp. were determined by molecular analysis, which was the first record of this fungus on herbaceous plants (Bereczky et al., 2015). *E. platani* expanded its host range to Simaroubaceae (*Ailanthus altissima*), beyond its original host Platanaceae family (Beenken, 2017).

Studies of powdery mildews in Azerbaijan began in mid of the last century, when numerous taxa from Erysiphales were recorded during exploring of mycobiota in this country (Мехтиева, 1956; Ключева, 1965; Ахундов, Агаева, 1978; Ахундов, 1979). However, taxonomic literature has significantly changed during last 20 years. Comprehensive investigation is required to clarify and renovate the knowledge about this group of fungi by application of modern morphological and molecular methods with additional collections.

During our study, we found powdery mildews on *Polygonum alpinum* All. (Polygonaceae) and *Alcea rosea* L. (Malvaceae) collected in 2015–2016 from Baku, Azerbaijan. Both host species are herbaceous plants, of which *Alcea* L. species are grown as an ornamental plant. Based on the morphological examinations, catenate conidia and nipple shaped appressoria were found, suggesting that the fungi from our specimens belong to the genus *Golovinomyces*. Powdery mildews from this genus infect up to 2283 plant species from 58 families worldwide and have never been recorded on the Polygonaceae family (Amano, 1986). Two *Golovinomyces* species, *G. americanus* and *G. orontii* were recorded on some plant species from Malvaceae family, of which *G. americanus* is known to be distributed only in North America (Braun, Cook, 2012). *G. spadiceus* on *P. alpinum*

and *G. magnicellulatus* on *A. rosea* were identified based on molecular and morphological examinations of our specimens. Thus, morphological description, illustration of species and results of molecular-phylogenetic analysis were given in the present study.

MATERIALS AND METHODS

Samples used in this study were deposited in Mycological Herbarium of Mie University (TSUMUMH, Tsu, Japan) and Mycological Herbarium of Institute of Botany, Azerbaijan National Academy of Sciences (BAK, Mycological Herbarium, Baku, Azerbaijan). Collection date, location, host plant species and accession numbers of the nucleotide sequences were given.

Morphological examination. For observation of asexual morph on the dried herbarium samples, lactic acid was used according to the procedure described by Shin & La (1993). Little piece of infected leaf was mounted in lactic acid on microscopic slide and gently boiled, then asexual structures were scrapped off from the leaf surface and observed under the optical microscope (Axio Imager, Carl Zeiss, Göttingen, Germany) with phase contrast using 10×, 20× and 40× objectives. Thirty conidia and conidiophores were measured for each specimen. The original size of conidia reconstructed with Bulmer's factor (Braun, Cook, 2012). Drawings were carried out by freehand using scale bar.

Molecular and phylogenetic analysis. DNA was extracted from mycelia by the chelex method (Hirata, Takamatsu, 1996). The ITS1/5.8S/ITS2 regions and 5′-end of the 28S rDNA gene (including D1 and D2 domains) were amplified separately by single polymerase chain reaction (PCR) (Meeboon, Takamatsu, 2015). PCR reaction was conducted with TaKaRa PCR thermal cycler Dice (TaKaRa, Tokyo, Japan). A negative control lacking DNA template was included for reactions. Primer sets ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′)/ PM6G (5′-CGAGCCCCAACACCAA-3′), and PM5G (5′-GACCCTCCACCCGTGT-3′)/ NLP2 (5′-GGTCCCAACAGCTATGCTCT-3′) were used for amplification. PM6G and PM5G are specific primers for *Golovinomyces* species. The PCR products were subjected to electrophoresis in 1.5% agarose gel in TBE buffer, then stained with Midori Green Advance DNA stain (Nippon Genetics Europe GmbH, Germany) and visualized under UV light. DNA samples were sent to SolGent (Daejeon, South Korea) for sequencing.

Two newly obtained sequences determined in this study were deposited to DNA Data Base of Japan (DDBJ) under the accession numbers LC331790 and LC331791. These sequences were aligned with other closely related sequences of the Erysiphales retrieved from GenBank using MUSCLE implemented in MEGA 7 (Edgar, 2004; Kumar et al., 2016). Phylogenetic tree was constructed using maximum parsimony (MP) method implemented in PAUP 4.0a157 (Swafford, 2002). MP analysis was run using heuristic search option with tree bisection reconnection (TBR) algorithm with 100 random sequence additions to find global optimum tree. The strength of internal branches in resulting tree was tested with bootstrap (BS) analysis using 1000 replications with step-wise addition option set as simple (Felsentein, 1985). BS values of 70% or higher were given on the representative branch (Figure 1). Tree scores, including tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

RESULTS AND DISCUSSION

Phylogenetic analysis. Two sequences of ITS regions and 28S rRNA gene were determined from specimens on *P. alpinum* and *A. rosea* and aligned with closely related 21 sequences from the genus *Golovinomyces*. *Arthrocladiella mougeottii* (Lév.) Vassilkov (AB329690) was used as outgroup (Takamatsu et al., 2013). Totally the data matrix consisted of 24 sequences and 1272 characters, of which 1134 (89.5%) of them were constant and 58 (4.6%) characters were variable and parsimony-uninformative. Only 80 (6.3%) characters were informative for parsimony analysis. Subsequently, 518 equally parsimonious trees with 207 steps were constructed by the MP analysis. The best tree was chosen by Kishino-Hasegawa (Kishino, Hasegawa, 1989) and Shimodaira-Hasegawa (Shimodaira, Hasegawa, 1999) topology tests and a tree with the highest likelihood value was shown in Figure 1.

Taxonomy. Conidia producing in chains (catenate), *Euoidium* type of conidial germination and nipple shaped appressoria indicated that the fungi from our specimens belong to the genus *Golovinomyces*. Only morphology of asexual structures is not enough to identify powdery mildews in species level. Therefore, identification of species was done based on combined results of molecular and morphological analysis.

Golovinomyces spadiceus (Berk. & M.A. Curtis) U.Braun, Braun & Cook 329, 2012. (**Fig. 2, 3**).

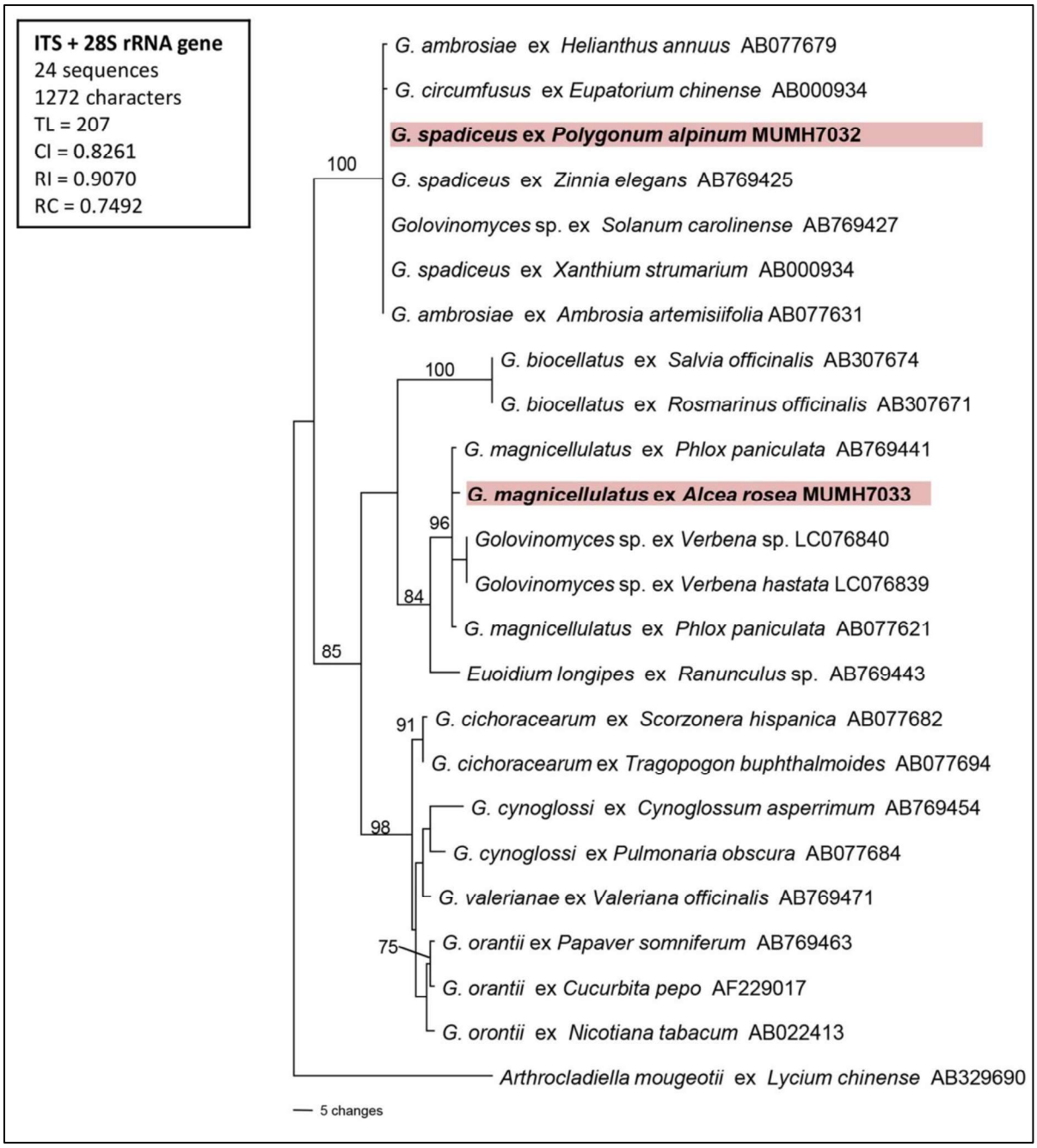

Figure 1. Phylogenetic tree of the genus *Golovinomyces* inferred from the ITS + 28S rRNA gene sequences constructed by maximum parsimony (MP) method. Bootstrap (BS) values (≥70%) were given on the branches. Sequences determined in this study were highlighted.

Description: Mycelium amphigenous, in dense, effuse or in irregular patches, sometimes covers entire leaf surface, white to grayish, persistent. Hyphae 3–7 µm diam., thin-walled, hyaline, smooth, hyphal appressoria nipple-shaped, solitary. Conidiophores arising from the upper surface of mother cells and towards one end of cell, up to 135 µm long. Foot-cells cylindrical, straight, 20–50 × 9–12 µm, followed by 1–3 shorter cells. Conidia formed in chains, ellipsoid, obovoid, doliiform, 26–41 × 16–23 µm, length/width ratio 1.4–2.0. Conidial germination is in the *Euoidoim* type, germ tubes arising lateral or slightly medium, with swollen appressorium on end.

Material examined: On *Polygonum alpinum* All. (Polygonaceae), Lokbatan, Baku, 08 Sept. 2015, leg. L.V. Abasova, BAK Mycological Herbarium No 10057, TSU-MUMH 7032, DDBJ ID number: LC331790 (ITS and 28S rRNA gene).

Remarks: *Leveillula taurica* and *Erysiphe polygoni* DC. have been described on *Polygonum*

species worldwide (Braun, Cook, 2012). Determined nucleotide sequences of the 28S rRNA gene and ITS regions from our specimen were completely identical to the sequences of *Golovinomyces spadiceus* (AB769425) on *Zinnia elegans* in GenBank. Three *Golovinomyces* species, *G. ambrosiae*, *G. spadiceus* and *G. circumfusus* listed in manual (Braun, Cook, 2012), mainly occur on the plants from the tribe Heliantheae of Asteraceae. Takamatsu et al. (2013) reported that isolates of these three species are homogenous and placed in the same clade and differentiation of these species on genetic level is difficult. So, it is not reliable to identify species only based on molecular results, because there are some allied taxa with closely related nucleotide sequence data (Takamatsu et al., 2013). In the present phylogenetic analysis (Figure 1), the sequence from our specimen placed in the clade with *G. ambrosiae*, *G. circumfusus*, and *G. spadiceus*, supported by 100% BS value in MP analysis. We checked the nucleotide similarity among these species. *G. ambrosiae* and *G. circumfusus* were 99.9% similar (only one base differences per each species) to our specimen in the ITS region. But there are two substitutions difference between *G. ambrosiae* and our specimen. However, some morphological differences among these species were reported. *G. circumfusus* has longer (30–110 µm) and straight to curved foot-cells, whereas foot-cells in *G. spadiceus* (30–80 µm) are shorter and straight. Limoniform shape of conidia was recorded in both, *G. circumfusus* and *G. ambrosiae*, but not in *G. spadiceus*. Length/width ratio in *G. ambrosiae* (1.4–1.6) is smaller than *G. spadiceus* (1.5–2), but in *G. circumfusus* (1.3–2.6) is bigger (Braun, Cook, 2012). Thus, our specimen was identified as *G. spadiceus* based on morphological and molecular data. This is the first record of the genus *Golovinomyces* on Polygonaceae family in the world. *P. alpinum* is a new host record for *G. spadiceus*.

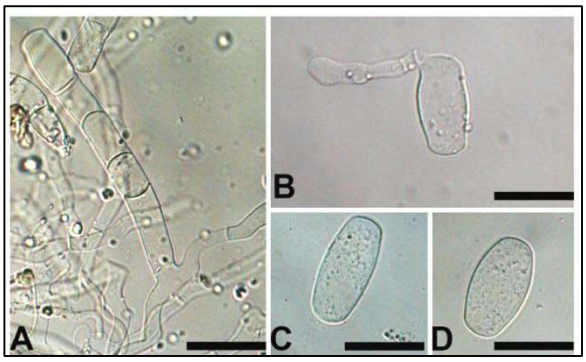

Figure 2. *Golovinomyces spadiceus* on *Polygonum alpinum* (MUMH 7032; MH No 10057). A. Conidiophore; B. Germinated Conidia; C, D. Conidia; **Scale bar = 30 µm.**

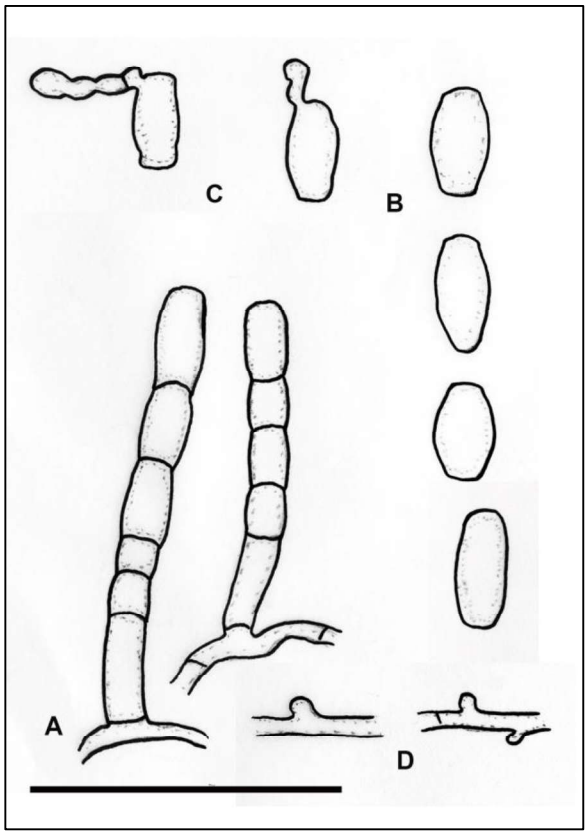

Figure 3. Asexual morphs of *Golovinomyces spadiceus* on *Polygonum alpinum* (MUMH 7032; MH No 10057). A. Conidiophores; B. Conidia; C. Germinated conidia; D. Hyphal appressoria; **Scale bar = 100 µm.**

Golovinomyces magnicellulatus (U. Braun) Heluta, Ukrayins'k Bot. Zhurn. 45 (5): 63, 1988 (**Fig. 4, 5**).

Description: Mycelium amphigenous, thin, effuse, covers entire leaf surface, grayish, evanescent to persistent. Hyphae thin-walled, hyaline, smooth, 4–7 µm diam., hyphal appressoria nipple-shaped, solitary. Conidiophores arising from upper surface of mother cells, towards one end of cell, 140–270 µm long. Foot-cells cylindrical, straight, (45–)58–130 × 9–12 µm, followed by 1–3 shorter cells, thin from the base, becoming wide above. Conidia formed in chains, ellipsoid, cylindrical to doliiform, (29–)31–37 × 16–19 µm, length/width ratio (1.3–)1.7–2.2, conidial germ tubes arising terminal, short and with swollen appressorium, *Euoidium* type.

Material examined: On *Alcea rosea* L. (Malvaceae), Botanical garden, Baku, 12 May 2016, leg. L.V. Abasova, BAK Mycological Herbarium No 10058, TSU-MUMH 7033, DDBJ ID number: LC331791 (ITS and 28S rRNA gene).

Remarks: *Alcea* species are biennial or perennial herbs and cultivated as an ornamental plant. *Leveillula contractirostris* Heluta & Simonyan was reported as powdery mildew pathogen on *Alcea*

species (Braun, Cook, 2012). However, molecular and morphological results indicated that our specimen belongs to the genus *Golovinomyces*.

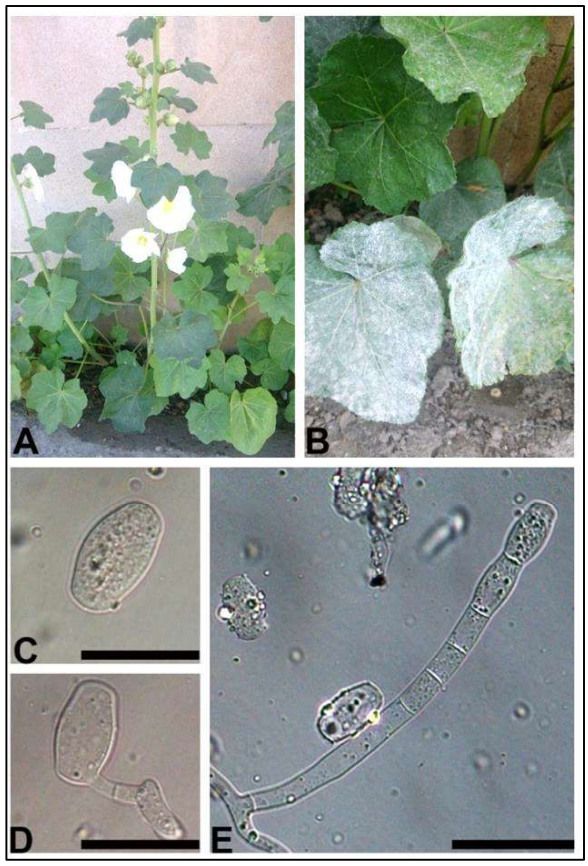

Figure 4. *Golovinomyces magnicellulatus* on *Alcea rosea* (MUMH 7033; MH No 10058). A. Plant; B. Disease symptoms; C. Conidia; D. Germinated conidia; E. Conidiophore; **Scale bar = 50 µm.**

Sequences of ITS and 28S rRNA gene were 99% identical to the sequences of *G. magnicellulatus* (AB077621; AB769442) on *Phlox paniculata* and *G. orontii* (AB307670) on *Verbena hortensis* in GenBank. Nucleotide sequence similarity between *G. magnicellulatus* and our specimen was 99% (4 substitutions difference) in ITS region and 100% identical in 28S rRNA gene. Two varieties of *G. magnicellulatus* are known, and both of them were recorded on *Phlox* and *Polemonium* spp. (*Polemoniaceae*). Morphological differences between the two varieties is in width of chasmothecial appendages and peridium cells. In the meantime, *G. orontii* and *G. americanus* are recorded on some species from Malvaceae family. *G. americanus* is a powdery mildew fungus endemic to North America, whereas *G. orontii* is distributed worldwide.

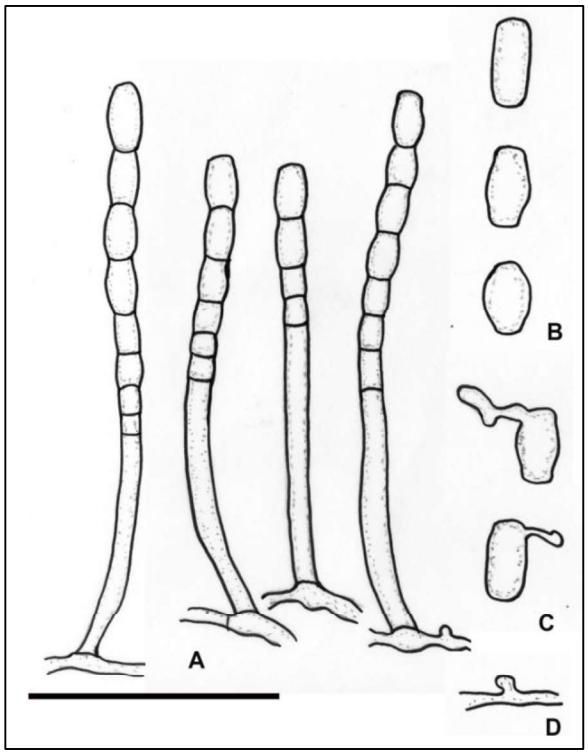

Figure 5. Asexual morphs of *Golovinomyces magnicellulatus* on *Alcea rosea* (MUMH 7033; MH No 10058). A. Conidiophores; B. Conidia; C. Germinated conidia; D. Hyphal appressoria; **Scale bar = 100 µm.**

Asexual morphs of our specimen are more similar to *G. magnicellulatus* than *G. orontii*. Curved foot-cells, twisted or forked germ tubes in *G. orontii* is distinguishable characters from our specimen (Braun, Cook, 2012). In the phylogenetic analysis (Figure 1) sequence obtained from our specimen is located within the sequences of *G. magnicellulatus* and *Golovinomyces* sp. on *Verbena* and supported by 96% BS value in MP analysis. Our specimen was identified as *G. magnicellulatus* according to the molecular and morphological data. However, we could not distinguish the variety, because our specimen was in asexual stage.

ACKNOWLEDGEMENT

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Golovinomyces (*Erysiphales, Ascomycota*) Cinsinə Aid Yeni Sahib Bitkilərə Dair Qeydlər

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Azərbaycandan toplanmış unlu şeh göbələkləri ilə yoluxmuş *Polygonum alpinum* və *Alcea rosea* nümunələri morfoloji və molekulyar metodlarla tədqiq edilmişdir. Hər bir nümunə üçün 28S rRNT-nin D1/D2 domenləri daxil olmaqla, ITS1/5.8S/ITS2 sahələri, müəyyən edilmişdir. Nəticədə, *P. alpinum* üzərində *Golovinomyces spadiceus* və *A. rosea* üzərində *G. magnicellulatus* növləri təyin olunmuşdur. Məqalədə bu növlərin ətraflı morfoloji təsviri, illustrasiyası və molekulyar-filogenetik analizlərinin nəticələri verilmişdir.

Açar sözlər: *Erysiphaceae, molekulyar analiz, yeni sahib bitki, unlu şeh göbələkləri, taksonomiya*

Новые Данные О Растениях-Хозяевах Рода *Golovinomyces* (*Erysiphales, Ascomycota*)

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Исследованы образцы с *Polygonum alpinum* и *Alcea rosea*, зараженные мучнисто-росяными грибами, собранные в Азербайджане с использованием морфологических и молекулярных методов. Для каждого образца, включая домены Д1/Д2 28S рРНК, были определены зоны ITS1/5.8S/ITS2. В результате на *P. alpinum* был идентифицирован вид *Golovinomyces spadiceus* и на *A. rosea* - *G. magnicellulatus.* В данной статье приведены подробное морфологическое описание, иллюстрация видов и результаты молекулярно-филогенетического анализа.

Ключевые слова: *Erysiphaceae, молекулярные анализы, новые растения-хозяева, мучнисто-росяные грибы, таксономия*