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A brief global review on the species of cucurbit powdery mildew fungi and new records in Taiwan

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Abstract: In spite of the economic importance of powdery mildew on cucurbits, literature and databases about the fungal species reveal different species numbers and names, often indicating only two species, with inconsistent host specificities for different members of *Cucurbitaceae*. Revision of the available literature indicates the presence of at least six species of powdery mildews on *Cucurbitaceae* with different focuses on hosts and geographic distribution. *Golovinomyces orontii* is recorded from *Cucurbitaceae* in Taiwan for the first time based on internal transcribed spacer sequences and the morphology of the anamorph found on wild plants of *Coccinia grandis*. In the literature; although, winter melon (*Benincasa hispida*) is considered resistant against *Podosphaera xanthii*, we confirmed a previously doubtful record on this host in Taiwan. Our new records of *G. orontii* in wild cucurbits and of *P. xanthii* for winter melon indicate a higher potential threat against cultivated cucurbits than previously estimated.

Key words: *Erysiphaceae*, *Erysiphales*, plant pathogenic fungi, quarantine

INTRODUCTION

Fungi belonging to *Erysiphales* cause major losses in cucurbit production worldwide. In spite of their economic importance, the number and identity of fungal species and their host ranges within the *Cucurbitaceae* are still confusing. Several *Erysiphales* species records listed in the literature and online databases (Farr & Rossman 2018, GenBank https://www. ncbi.nlm.nih.gov/) are doubtful or based on outdated species concepts. Five species of *Erysiphales* on cucurbits were accepted by Braun & Cook (2012). Other recent publications might be misleading by focusing only on *Golovinomyces orontii* (Castagne) V.P. Heluta and *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff as if only these two species were present on cucurbits. By progressing study of powdery mildew, further changes of species names and new cucurbit hosts may be expected.

In Taiwan, hitherto only *P. xanthii* has been known for cucurbits, namely *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumis sativus* L., *Cucurbita* spp. (*C. maxima* Duchesne, *C. moschata* Duchesne, *C. pepo* L.), *Lagenaria siceraria* (Molina) Standl., *Momordica charantia* L., *Solena amplexicaulis* (Lam.) Gandhi, and *Zehneria mucronata* (Blume) Miq. (Hsu et al. 2002, Kuo 1989, Liu & Kirschner 2015). Records of *P. xanthii* (as *Sphaerotheca fuliginea*) on *Benincasa hispida* (Thunb.) Cogn. in Taiwan and other countries listed from a nonconfirmed source (Amano 1986) in Farr & Rossman (2018) lack documentation and were not included in Kuo (1989) and Hsu et al. (2002). This fungus-host combination has not been found in an extensive screening in Thailand, although *Podosphaera* specimens were found in five other genera of *Cucurbitaceae* (Meeboon et al. 2016). Wax gourd (*B. hispida*) is widely cultivated in tropical and subtropical Asia. In Taiwan, different cultigens are distinguished depending on the size of fruits. A variety with relatively small fruits, considered by some authors as a distinct variety (*B. hispida* var. *chieh-qua* F.C. How), was introduced from southern mainland China to Taiwan in 1947 (Hsueh 2000). The invasive wild cucurbit *Coccinia grandis* (L.) Voigt was first recorded from Taiwan in 1997 and spread to roadsides and similar places particularly in eastern Taiwan (Chen 2008). Demonstrating new host records of powdery mildews from Taiwan serves as an example of the lack of data about powdery mildews on cucurbit hosts.

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MATERIALS AND METHODS

Shoots and leaves of cucurbits with powdery mildew symptoms were collected in the field or in a garden shop and processed for microscopy and DNA extraction in the fresh stage. For microscopy, fresh specimens were mounted in 10% KOH (Kirschner & Chen 2008); sizes were given as extremes (given without brackets) or, as mean value ± standard deviation of n measurements with extreme values in brackets. DNA was isolated from freshly collected conidia, amplified, sequenced, and edited as in Wei & Kirschner (2017). Sequences of the internal transcribed spacer of the nuclear rRNA genes (ITS) were deposited at GenBank (see below). Among the results obtained with BLAST searches with these sequences at GenBank (https://blast.ncbi.nlm.nih.gov), only published records with species names fitting to the concepts of Braun & Cook (2012) were considered for comparison. Dried specimens were deposited in the fungal collection of the National Museum of Natural Science, Taichung, Taiwan (TNM, http://collection.nmns.edu.tw/scripts/fungi.dll).

RESULTS

Golovinomyces orontii (Castagne) V.P. Heluta, Ukr. bot. Zh. 45(5): 63 (1988), Fig. 1

Mycelium amphigenous, effuse or in irregular patches, white. Hyphae smooth and hyaline, ca. 3 µm wide. Hyphal appressoria nipple-shaped to slightly broadened. Conidiophore stipes erect, arising both from the middle of mother cell or toward one end of the mother cell, up to about 170 µm long. Foot-cell straight to slightly curved, (28−)34−53(−57) × (9−)10−12(−13) µm (n=20), followed by 0−3 shorter cells, developing conidia not very swollen. Conidia in chains, cylindrical or oblong, 28−35 × 12−18 µm, covered with scattered minute cylindrical scales. Conidia with terminal or occasionally lateral germ tube, about as long as the conidia or shorter, conidial appressoria not seen. Teleomorph not developed.

Specimen examined. On leaves of a wild mature plant of *Coccinia grandis*, TAIWAN, Taitung City, Donghe Township, 09.02. 2017, leg. *R. Kirschne*r, det. *T.-Y. Wu* WTY07 (TNM F31684), ITS MH465241.

DNA-based identification. Comparing the BLAST search results among sequences exceeding a length of 570 bp, the ITS sequence (GenBank accession number MH465241) of our specimen and those of *G. orontii* (GenBank numbers AB430813−AB430818, Takamatsu et al. 2009) showed a similarity of 100% (571/571 bp). Sequences of other, closely related species such as *G. fischeri* (S. Blumer) U. Braun & R.T.A. Cook and *G. sordidus* (L. Junell) V.P. Heluta had a maximum similarity of 99% (with 3 or more different positions). There were no sequences labeled as *G. cucurbitacearum* in GenBank.

Takamatsu et al. (2013) in their ITS analysis found three clades comprising sequences of *G. orontii*, with specimens from cucurbit hosts included in each of these three clades. BLAST searches with our ITS sequence for sequences of *G. orontii* from other cucurbits in GenBank had 99−100% similarity, i.e. from *Cucumis sativus* yielded 1 to 4 different bp (548/549−550 bp, AB427187, AF229017) and from *Cucurbita* spp. 0 (549/549 or 558/558 bp, AB077696, AB077670, respectively) to 4 different bp (542/546 bp, AF229016).

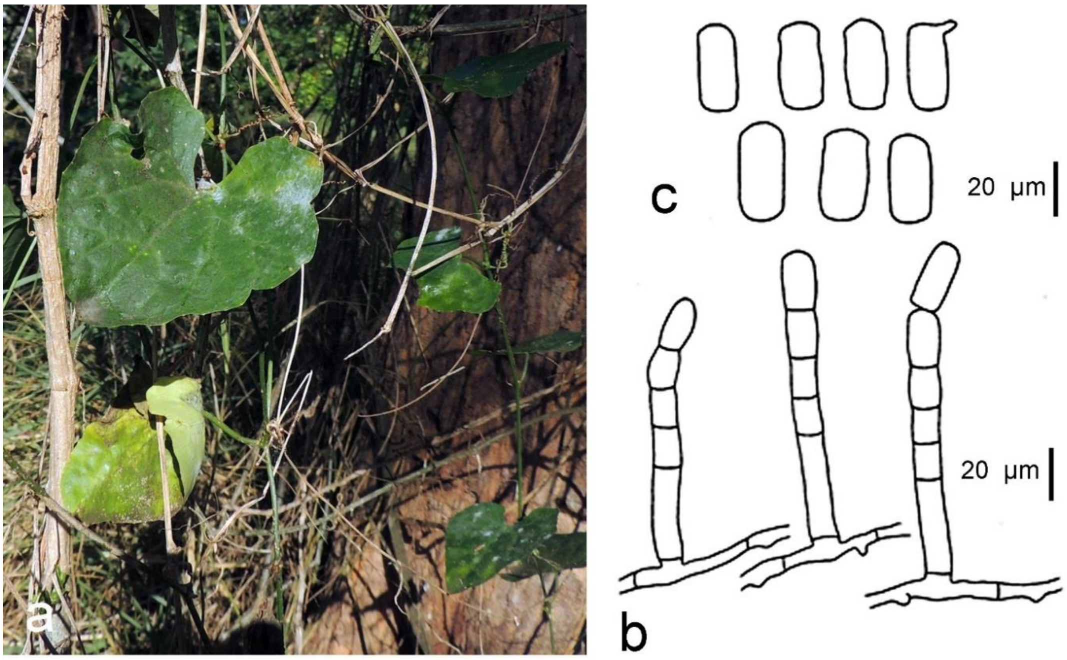

Fig. 1. *Golovinomyces orontii* on *Coccinia grandis* (WTY07). **a.** Powdery mildew symptoms on the host. **b–c.** Microscopic characteristics. **b.** Conidiophores arising from hyphae with nipple–shaped appressoria. **c.** Conidia (Drawing: Y.-T. Wu).

The identity of 100% of our sequence with those of *G. orontii* from *Galium* spp. (AB430817, AB430818) indicates that they together with the above mentioned 100% identical sequences of *G. orontii* on cucurbits (AB077670, AB077696) all belong to *G. orontii* sensu stricto or lineage XI in Takamatsu et al. (2013) and to group 1 within this lineage.

Podosphaera xanthii **(Castagne) U. Braun & Shishkoff, in Braun & Takamatsu, Schlechtendalia 4: 31 (2000) Fig. 2**

Mycelium amphigenous, effuse or in irregular patches, white. Hyphae smooth to verruculose, hyaline, 4−8 µm wide. Hyphal appressoria nipple-shaped. Conidiophore stipes erect, arising both from the middle of mother cell or more commonly toward one end of the mother cell, sometimes two stipes arising from the same mother cell, ca. 70−230 µm long, 14−16 µm wide at the uppermost cell. Foot-cell straight, (26−)30−60(−73) × 9−11(−12) µm (n=30), followed by 0−8 shorter cells. These cells and conidia with fibrosin bodies. Conidia in chains, cylindrical or oblong, (25−)26.5−32(−35) × (14−)15.5 × 18(−19) µm (n=30), smooth. Conidial germ tubes or appressoria not seen. Teleomorph not developed.

Specimens examined. On leaves of a potted young plant of *Benincasa hispida* var. *chieh-qua*, TAIWAN, Taoyuan City, Zhongli District, gardener shop, 28 April 2018, leg. & det. *R. Kirschner* 4632 (TNM F31685), ITS MH465242; on leaves of a potted young plant of a cultigen of *B. h.* var. *hispida* with medium-sized fruits, same place, 5 May 2018, leg. & det. *R. Kirschner* 4633 (TNM F31686), ITS MH465243.

DNA-based identification. Comparing the BLAST search results among sequences being at least 550 bp long, the ITS sequences (GenBank MH465242 and MH465243) of our specimens and those of *P. xanthii* had 99% similarity to 100% similarity (e.g. KM505135, Liu & Kirschner 2015), whereas the similarity with the closely related *P. erigerontis-canadensis* (Lév.) U. Braun & T.Z. Liu was 98% (570/579 bp; KY660809, KY661091, and KY661075, Ellingham 2016).

DISCUSSION

According to the most comprehensive taxonomic treatment of *Erysiphales* (Braun & Cook 2012), powdery mildew symptoms on cucurbits are induced by five species belonging to different genera.

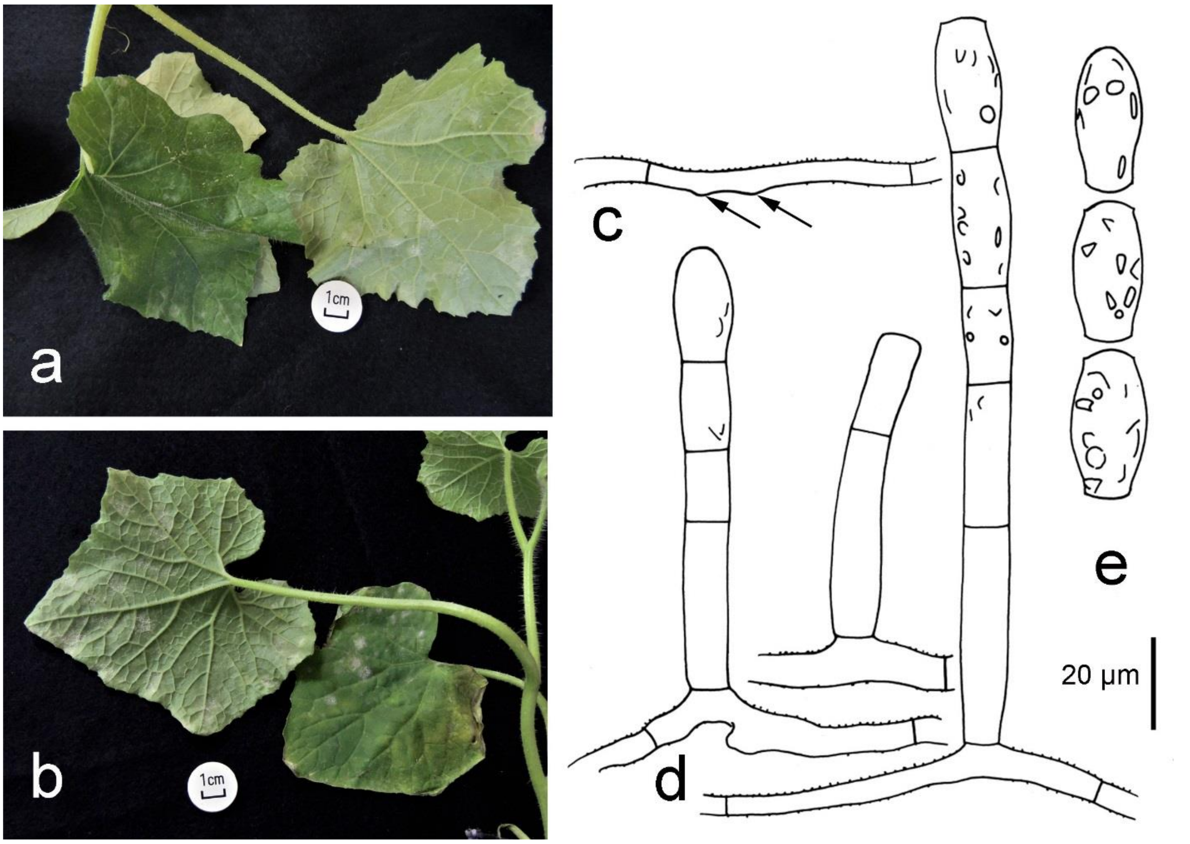

Fig. 2. *Podosphaera xanthii* on *Benincasa hispida*. **a.** Powdery mildew symptoms on *B. hispida* var. *chieh-qua* (R. Kirschner 4632). **b.** Powdery mildew symptoms on *B. hispida* var. *hispida* (R. Kirschner 4633). **c–e.** Microscopic characteristics (R. Kirschner 4632). **c.** Hypha with nipple–shaped appressoria (arrows). **d.** Conidiophores with fibrosin bodies in the apical cells. **e.** Conidia with fibrosin bodies (Drawing: R. Kirschner).

Three species with catenate conidia can occur on both sides of the leaf, *Golovinomyces cucurbitacearum* (R.Y. Zheng & G.Q. Chen) Vakal. & Kliron., *G. orontii* (Castagne) V.P. Heluta (syn. *Erysiphe cichoracearum* s.l.) and *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff ("*P. fusca*", "*Sphaerotheca fuliginea*") (Braun & Cook 2012). These fungi cannot be distinguished by their symptoms, but by light microscopy: fibrosin bodies are present in *P. xanthii* only; curved conidiophore bases occur only in *G. orontii*. *Leveillula taurica* (Lév.) G. Arnaud which penetrates through stomata tends to be limited to the abaxial side of the leaf and forms solitary conidia (Braun & Cook 2012). *Erysiphe actinostemmatis* U. Braun is comparatively rare and limited to *Actinostemma* and *Schizopepon* species in China and Japan (Braun & Cook 2012). It can grow on both sides of the leaf and forms solitary conidia.

A further species of *Leveillula* being different from *L. taurica*, but remaining unnamed, was found on *Cucurbita* sp. in Iran (Khodaparast et al. 2016) as well as an unnamed species of *Erysiphe* on *Luffa acutangula* in Thailand (Meeboon & Takamatsu 2016) indicate the presence of further taxonomically unresolved species of powdery mildews on *Cucurbitaceae*. In the future, we, therefore, have to expect reports of more than five species of *Erysiphales* on cucurbits.

Leveillula sp. is molecularly characterized (Khodaparast et al. 2016) and *Erysiphe* sp. morphologically (Meeboon & Takamatsu 2016). GenBank contains DNA sequences labeled as *G. orontii*, *L. taurica*, and *P. xanthii*, but lack those labeled as *E. actinostemmatis* or *G. cucurbitacearum*. Particularly the morphological distinction between *G. orontii* and *G. cucurbitacearum* should be supported by molecular analyses, as soon as ITS sequences of the latter are available. The relationships between the three clades of *G. orontii* among each other as well as with other species of *Golovinomyces* could not be resolved with ITS sequences (Takamatsu et al. 2013). More collections with detailed morphological characterization and ITS sequence data are urgently needed in order to clarify the species boundaries of certain powdery mildew fungi on cucurbits. Particularly for resolving the relationships within genera, other gene regions than ITS should be applied.

Two *Golovinomyces* species have been recorded on *Coccinia* hosts (Meeboon et al. 2017). In *G. cucurbitacearum* the foot cell of the conidiophore is always straight, whereas in *G. orontii* it is often slightly curved (Braun & Cook 2012). In Taiwan, *G. orontii* is a new record for *Cucurbitaceae*.

Winter melon (*B. hispida*) is considered resistant against *P. xanthii* and susceptible to *G. orontii* (Rabelo et al. 2017), although some unconfirmed records from Amano (1986) still persist (Braun & Cook 2012, Farr & Rossman 2018). Since these records are mere names in a list, they are unreproducible and have not been taken up in lists of phytopathogenic fungi of Taiwan (Hsu et al. 2002, Kuo 1989). Our findings documented with specimens, morphology and DNA data, however, confirm the record for Taiwan (Amano 1986 in Farr & Rossman 2018) for both varieties of *B. hispida*.

Correct information about the identity of pathogens and their hosts may have an impact on quarantine and other plant protection measures. Most cucurbit species show susceptibility or resistance only on the level of cultivars, which may again differ between species and "races" of the fungi (Lebeda et al. 2010, Luitel et al. 2016, Rabelo et al. 2017). Whereas powdery mildew caused by *G. orontii* is prevalent in Central Europe, *P. xanthii* is more prevalent in other regions, e.g. North America (Lebeda et al. 2010) and South America (Rabelo et al. 2017). For several countries, including like up to recently also Taiwan, *P. xanthii* is the single known powdery mildew species on cucurbits (Pirondi et al. 2015). From our finding, we may predict that additional species will also be recorded for those countries.

Several new host species, genera and families are published for *P. xanthii* (Meeboon et al. 2016) every year. *Leveillula taurica* is known on hosts of different families but tends to be more common in tropical and subtropical regions (Braun & Cook 2012), whereas for *G. orontii* which is already known for about 40 host families, occasional new records are limited to the level of host species. Since fungicides used against cucurbit powdery mildew are mainly based on the single-site mode of action, i.e. one step of the fungal metabolic pathways, fungal resistance may be gained by mutation more easily than against fungicides with multiple effects on the fungal metabolism (McGrath 2001). Again, because of its wide geographical distribution, such resistance may be more likely to spread in populations of fungi with broad host ranges such as *P. xanthii* and *G. orontii* than in *Erysiphales* with narrow host ranges.

Podosphaera xanthii is considered to be more widespread in tropical areas, whereas *G. orontii* is more prevalent in temperate regions (Rabelo et al. 2017). When both species occur in the same area, *G. orontii* may be predominant until summer and then be replaced by *P. xanthii* on the same cucurbit host. This biogeographic and bioclimatic pattern may be explained by the lower temperature optimum of *G. orontii* (Rur et al. 2017). It may be predicted that globalization and global warming may have an impact on the spread and prevalence of the two most common fungal agents of cucurbit powdery mildew. Since compared to many other fungi, *Erysiphales* are very detailed recorded in many countries, they may serve as a model for responses of pathogens to climate change (Glawe 2009).

For sustainable plant protection as well as developing and promoting cucurbit cultivars with resistance against powdery mildew, it is, therefore, important to distinguish clearly between the fungal species (not only *G. orontii* and *P. xanthii*) which can serve as causative agents and to monitor their presence permanently.

Golovinomyces orontii has been recorded on *C. grandis* in India and Thailand (Mulpuri et al. 2016, Meeboon et al. 2017). This host is also susceptible to *Erysiphe actinostemmatis* U. Braun and *Podosphaera* sp. (most likely *P. xanthii*) (Braun & Cook 2012, Meeboon et al. 2016). Our finding of *G. orontii* on wild *C. grandis* indicates the threat by spread from this weed to cultivated cucurbits such as *B. hispida* and *Luffa* species which are widely cultivated throughout Taiwan and known to be susceptible hosts in other countries (Braun & Cook 2012, Farr & Rossman 2018).

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REFERENCES

Amano K. 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Sci. Soc. Press, Tokyo, 741 pages (n.v., from Farr & Rossman 2018).

Braun U, Cook RTA. 2012. Taxonomic manual of the Erysiphales (powdery mildews). CBS Biodiversity Series No. 11. CBS–KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Chen S-H. 2008. Naturalized plants of eastern Taiwan. National Hualien University of Education, Hualien, Taiwan.

Ellingham O. 2016. Powdery mildew survey 2016. http://blogs.reading.ac.uk/crg/powdery-mildew-survey-2016

Farr DF, Rossman AY. 2018. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved January 21, 2018, from https://nt.ars-grin.gov/fungaldatabases/.

Glawe DA. 2008. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. Annu. Rev. Phytopathol. 46: 27–51.

Hsu ST, Chang TT, Chang CA, Tsai JL, Tsay TT. 2002. List of Plant Diseases in Taiwan, 4th edn. Taiwan Phytopathological Society, Taichung, Taiwan.

Hsueh C-X. 2000. The encyclopedia of vegetables and fruits in Taiwan. Vol. 1. Taiwan Pulo Company, Yunlin City, Taiwan.

Khodaparast SA, Takamatsu S, Shadlou A, Damadi M, Pirnia M, Jahani M. 2015. Notes on the genus Leveillula (Erysiphaceae): a new unrecorded species and notes on Leveillula infecting Ficus, Cucurbita and Tropaeolum in Iran. Phytotaxa 260: 267–275.

Kirschner R, Chen CJ. 2008. The Ovulariopsis anamorph of Phyllactinia bauhiniae var. cassiae: first record outside India and morphological characterization. Sydowia 60: 57–67.

Kuo KC. 1998. Checklist of Erysiphaceae from Taiwan. Fung. Sci. 13: 39–59.

Lebeda A, McGrath MT, Sedlakova B. 2010. Fungicide resistance in cucurbit powdery mildew fungi. In Carisse O.: Fungicides, 221–246. InTech, Rijeka, Croatia.

Liu WA, Kirschner R. 2015. First report of powdery mildew caused by Podosphaera xanthii on wild bitter gourd in Taiwan. Plant Dis. 99: 726.

Luitel BP, Kim SG, Sung JS, Hur O-S, Yoon MS, Rhee J-H, Baek H-J, Ryu K-Y, Ko H-C. 2016. Screening of pumpkin (Cucurbita spp.) germplasm for resistance to powdery mildew at various stages of seedlings growth. Res. Plant Dis. 22: 133–144.

McGrath MT. 2001. Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. Plant Dis. 85: 236–245.

Meeboon J, Hidayat I, Takamatsu S. 2016. Notes on powdery mildews (Erysiphales) in Thailand I. Podosphaera sect. Sphaerotheca. Plant Pathology & Quarantine 6: 142–174.

Meeboon J, Takamatsu S. 2016. Notes on powdery mildews (Erysiphales) in Thailand II. Erysiphe species on Adoxaceae Anacardiaceae, Apocynaceae, Araliaceae, Aristolochiaceae, Bixaceae, Brassicaceae, Cleomaceae, Convolvulaceae, Cucurbitaceae and Euphorbiacea (sic!). Trop. plant pathol. 41: 357–369.

Meeboon J, Kokaew J, Takamatsu S. 2017. Notes on powdery mildews (Erysiphales) in Thailand V. Golovinomyces. Trop. plant pathol. 43: 202–217.

Mulpuri S, Soni PK, Gonela SK. 2016. Morphological and molecular characterization of powdery mildew on sunflower (Helianthus annuus L.), alternate hosts and weeds commonly found in and around sunflower fields in India. Phytoparasitica 44: 353–367.

Pirondi A, Pérez-García A, Battistini G, Muzzi E, Brunelli A, Collina M. 2015. Seasonal variations in the occurrence of Golovinomyces orontii and Podosphaera xanthii, causal agents of cucurbit powdery mildew in Northern Italy. Ann. Appl. Biol. 167: 298–313.

Rabelo HO, Santos LS, Diniz GMM, Marin MV, Braz LT, McCreight JD. 2017. Cucurbits powdery mildew race identity and reaction of melon genotypes. Pesq. Agropec. Trop., Goiânia 47: 440–447.

Rur M, Rämert B, Hökeberg M, Vetukuri RR, Grenville-Briggs L, Liljeroth E. 2018. Screening of alternative products for integrated pest management of cucurbit powdery mildew in Sweden. Eur. J. Plant Pathol. 150: 127–138.

Takamatsu S, Heluta V, Havrylenko M, Divarangkoon R, 2009. Four powdery mildew species with catenate conidia infect Galium: molecular and morphological evidence. Mycol. Res. 113: 117–129.

Takamatsu S, Matsuda S, Grigaliunaite B. 2013. Comprehensive phylogenetic analysis of the genus Golovinomyces (Ascomycota: Erysiphales) reveals close evolutionary relationships with its host plants. Mycologia 105: 1135–1152. Wei I-C, Kirschner R. 2017. Two fungicolous anamorphic species of Hypomyces s. lat. from Taiwan. Fung. Sci. 32: 15–25.

بازبینی کوتاه جهانی گونه های سفیدک پودری گیاهان جالیز (cucurbits) و گزارش های جدیدی برای تایوان

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چکیده: علیرغم اهمیت اقتصادی سفیدک پودری روی گیاهان جالیز، در نوشته ها و بانک های اطلاعاتی مربوط به گونه های قارچی تعداد و اسامی مختلفی از این قارچ ها ذکر شده و اغلب فقط به دو گونه اشاره دارند که دارای اختصاصیت میزبانی بالایی با اعضای مختلف گیاهان تیره *Cucurbitaceae* هستند. بررسی نوشته های موجود بیانگر حضور حداقل شش گونه سفیدک پودری روی گیاهان تیره *Cucurbitaceae* با درنظر گرفتن میزبان ها و گسترش جغرافیایی است. گونه *Glovinomyces orontii* از روی تیره *Cucurbitaceae* برای اولین بار براساس ویژگی های مبتنی بر توالی ITS و ریخت شناختی آنامورف یافت شده روی گیاهان وحشی گونه *Coccinia grandis* گزارش می شود. گرچه در منابع، خربزه زمستانی (*Benincasa hispida*) میزبان مقاوم در مقابل *Podosphaera xanthii* محسوب می گردد، ما یک گزارش مشکوک قبلی روی این میزبان در تایوان را تایید می کنیم. گزارش جدید ما از گونه *G. orontii* از روی گیاهان جالیز و گونه *P. xanthii* از گیاه خربزه زمستانی نشان دهنده خطر بالقوه بالاتری برای گیاهان جالیز در مقایسه با تخمین های قبلی است.

کلمات کلیدی: *Erysiphaceae*، *Erysiphales*، قارچهای بیمارگر گیاهی، قرنطینه

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