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First record of *Plasmopara sphagneticolae* (Peronosporales, Oomycota) in Taiwan

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ABSTRACT

Sphagneticola trilobata (Asteraceae) is one of the most common ornamental groundcover plants in public green spaces and also invasive in Taiwan. Downy mildew symptoms were found on this plant in Taipei City. Based on the host, morphology and ribosomal large subunit RNA gene (LSU) sequence analysis, the associated oomycete was identified as *Plasmopara sphagneticolae*. The most likely route of introduction of this oomycete together with its host from Hawaii is discussed.

Key words: Compositae, oomycete-host distribution, plant diseases, rDNA, *Wedelia*

Introduction

Sphagneticola trilobata (L.) Pruski (Asteraceae), also known under its older name *Wedelia trilobata*, originates from the Neotropics. In Taiwan it was introduced in the early 1980s from Hawaii and since then has become one of the most common ornamental groundcover plants in public green spaces but also one of the most invasive introduced plants (Hsueh and Yang 2014, Wu et al. 2004). In a public green space in Taipei City we found a plantation showing downy mildew symptoms on the leaves. Several downy mildew oomycetes (Oomycota) have been recorded from Asteraceae (Duarte et al. 2014). For these fungus-like eukaryotes, we occasionally use the traditional term “fungi” referring to the heterotrophic filamentous eukaryotic growth form and not to the systematic group “Fungi”.

Materials and Methods

Collection and morphology

Specimens were collected in a public green space in Da-an District in Taipei City. Fresh specimens were put into plastic bags and stored in the refrigerator for up to one week during the study. For photographs, dissecting and light microscopes with Olympus EP50 digital cameras were used. In order to give an impression of the sporangiophore branching which cannot be resolved with light microscopic photographs because of the three dimensions, sketches were done by hand on scaled paper. Illustrations were com-

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Fig. 1. Habitat of *Plasmopara sphagneticolae* and its host, *Sphagneticola trilobata* in Taipei City. A. Trapezoid green space, arrow indicates infected plantation of *Sph. trilobata*. Note "Taipei 101" in the background. B. Close-up from the location in A, lower leaves of *Sph. trilobata* with chlorosis and necrosis.

posed and processed with Photoshop software. For light microscopy, fresh sporangiophores were picked up from living leaves with tweezers and mounted in tap water or ca. 10% aqueous KOH. Measurements were done at 40× to 1000× magnification and presented as mean value ± standard deviation and extreme values in brackets or directly as extreme values. Dried specimens were deposited in the National Museum of Natural Science, Taichung, Taiwan (TNM).

Phylogenetic analysis

Fresh sporangiophores picked up from living leaves were also transferred to an Eppendorf cup with a drop of sterile water, ground with a power masher pestle and subjected to DNA extraction with EasyPure Genomic DNA Spin Kit/Plant (Bioman Scientific Co., Ltd., New Taipei City, Taiwan). The DNA extract was used for PCR with primers NL1 and NL4 and PCR conditions as given in Kurtzman and Robnett (1997) in order to amplify the partial ribosomal large subunit RNA gene (LSU). The PCR product was sequenced by Mission Biotech (Nangang). The forward and reverse sequences were assembled with CodonCode Aligner and the consensus sequence deposited in GenBank under MZ959822. A phylogenetic analysis was done with MEGAX by assembling an alignment based on most similar sequences received from BLAST searches in GenBank as well as from McTaggart et al. (2015) and using the default options of MUSCLE (Kumar et al. 2018). The sequence of *Plasmopara majewskii* Constant. & Thines was much shorter than

all other sequences and, therefore, excluded from the dataset. The alignment block was trimmed at the left and right ends without manual manipulation within the dataset. The phylogenetic relationships were inferred with Maximum Likelihood in MEGAX using the Hasegawa-Kishino-Yano model with Gamma distribution as best model (Hasegawa et al. 1985). The species names were adopted directly from GenBank without considering possible new synonymies or other name changes. The tree was rooted with *Phytophthora tabaci* Sawada (according to Index Fungorum a synonym of *Ph. nicotianae* Breda de Haan). In order to infer the relationship of another morphologically similar *Plasmopara* species on Asteraceae, *P. invertifolia* L.L. Duarte & R.W. Barreto, for which only partial cytochrome c oxidase subunit 2 gene (*cox2*) sequences are available, we used a *cox2* sequence of *P. invertifolia* for a BLAST search.

Results and Taxonomy

The morphology of the oomycete associated with the abaxial side of the leaf lesions conformed to Peronosporales, Oomycota (Figs. 1–3). According to the literature and databases, the single known downy mildew on *Sphagneticola* hosts is *Plasmopara sphagneticolae* (Farr and Rossman 2021). BLAST search with the 773 bp LSU sequence yielded two most similar sequences, both of *P. sphagneticola*e with 0 or 1 different base pair, namely from mainland China (773/773 bp, MW298155) and from the type locality in Australia (BRIP 61010, GenBank KM085176, McTaggart et al. 2015). The next similar sequences were 16 sequences from *P. halstedii* (Farl.) Berl. & De Toni, all exceeding 650 bp, with 9–12 different bp (98–99% identity). The phylogenetic analysis placed the Taiwanese material together with the two sequences of *P. sphagneticolae* in a strongly supported clade which was distinctly separated from a sister clade formed by sequences of *P. halstedii* (Fig. 4). In our BLAST search with one of the two *cox2* sequences of *P. invertifolia*, we found that it shows only up to 91% identity with any other oomycete, including *P. halstedii* and *P. sphagneticolae*.

Plasmopara sphagneticolae McTaggart & R.G. Shivas, in McTaggart, Shuey, McKenna, Davis & Shivas, Australas. Pl. Path. 44: 84. 2015.

Figs. 1–3

Associated with lesions of living leaves. Leaf spots amphigenous, angular and vein-limited, 2 mm diam., up to 20 × 10 mm, becoming confluent, pale yellow, yellow to brown. Mycelium internal, hyaline, smooth, branched, 4–14 µm wide, giving rise to ellipsoid intracellular haustoria, 8–10 × 6–8 µm. Sporangiophores hypophyllous, penetrating through stomata, forming white felt-like layer, hyaline, smooth, straight 325–725 µm long, composed of more than the lower half of an unbranched stipe, 225–475 µm long and 7–13 µm wide at the slightly bulbous base, branching mainly monopodial at 3–4 levels of up to four main branches and angles of 90° at the lower main branches and angles between 45 and 90° at the upper and secondary branches, occasionally 2 or 3 branches arising from the same node. Lower branches predominantly almost as long as the corresponding distal part of the main axis, lowermost branch longest, 180–275 µm long, next distal branch approx. as half as long as more proximal branch. Retraction septa (“callose

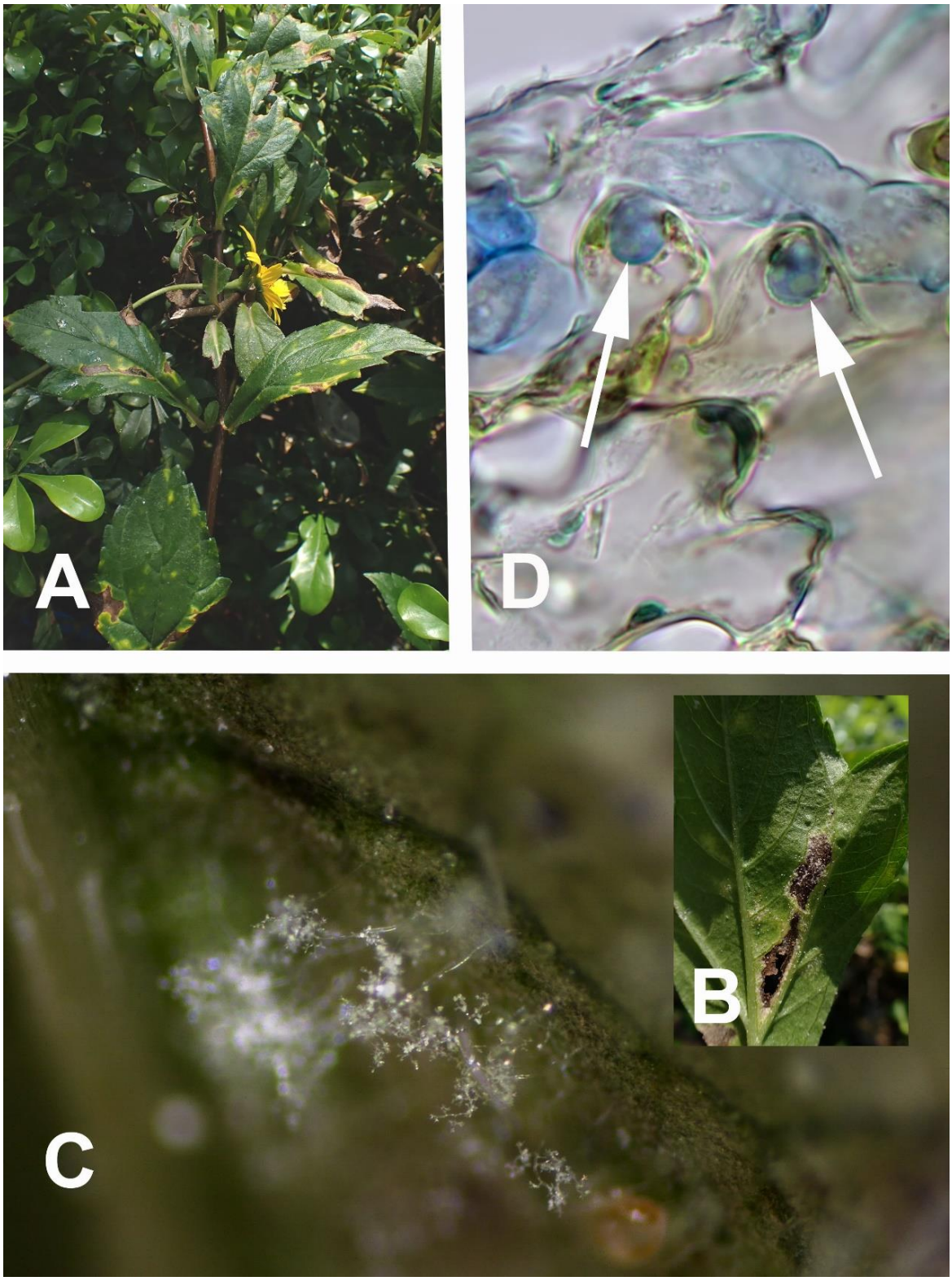

Fig. 2. Disease symptoms associated with *Plasmopara sphagneticolae*. A. Chlorosis and necrosis of leaves seen from upper side. B. Leaf lesions on abaxial leaf side with downy mildew symptom. C. Sporangiophores arising from abaxial side. D. Internal hypha (ca. 10 μm wide) inserting two ellipsoidal haustoria into mesophyll cells.

plugs") scattered in the stipe and branches, mostly 2–3 µm thick, sometimes irregular and thicker. Ultimate branchlets 1–3, straight or slightly curved, 4–22 µm long, 2–4 µm wide at base, narrowing to 1.5–2 µm at apex. Apex appearing truncate and slightly refractive after dehiscence of sporangium. Sporangia hyaline, smooth, subglobose, ellipsoidal to broadly ovoidal, slightly broader in the proximal half than in the distal, with apical papilla and 1.5–2 × 1 µm basal hilum when fully turgescent, (16–)18–22(–25) × (14–)15–18(–20) µm (n = 30). Oogonia not found.

Specimens examined. TAIWAN. Taipei City, Daan District, on living leaves of *Sphagneticola trilobata* with downy mildew symptoms, Wolong Street, public green, ca. 25.017834, 121.552391, ca. 10 m alt., 22 Aug 2021, *Kirschner R. 5316* (TNM), LSU sequence GenBank MZ959822; same place, 29 Aug 2021, *Kirschner R. 5316-B*.

Known hosts and distribution. *Lipochaeta integrifolia* (Nutt.) A. Gray, *Sphagneticola trilobata* (L.) Pruski (Asteraceae); Australia, Hawaii, mainland China? (unpublished sequences), Taiwan (new record).

Discussion

Species identification

Although appressorium morphology is a diagnostic characteristic for the genera of Peronosporales (Voglmayr et al. 2004), internal hyphae and appressoria were not mentioned in the previous publications. We found that these characteristics conformed to the concept of *Plasmopara* (Shin and Choi 2006, Voglmayr et al. 2004). Species of *Plasmopara* on Asteraceae in the past were considered as *P. halstedii* (Spring 2019). Although lengths of sporangiophores and sporangia are claimed as distinctive features for the species on Asteraceae (Duarte et al. 2014, McTaggart et al. 2015, Davis et al. 2020), the sizes strongly overlap (Duarte et al. 2014, Table 1). The sporangiophores of our specimen were up to 725 µm long, but only up to 500 µm in McTaggart et al. (2015); no sizes were given in Davis et al. (2020). The extreme values for the lengths of the sporangia in our measurements were identical with those in Davis et al. (2020) and for the widths with those of McTaggart et al. (2015). Sporangia were up to 2 µm longer in McTaggart et al. (2015) and up to 1 µm narrower in Davis et al. (2020). These data indicate that considerable differences of sporangiophore lengths and slight variation of sporangial sizes are not very significant. A better resolution of species was obtained with molecular data, but different DNA regions are preferred by different authors. Compared to true fungi, the ITS seems to be less commonly used in oomycetes so that ITS data lack for many species. *Plasmopara invertifolia* is a further recently described species on Asteraceae, which was considered as sister to *P. halstedii* (Duarte et al. 2014). *Plasmopara invertifolia* was not included in the phylogenetic analyses for comparison with *P. sphagneticolae* by McTaggart et al. (2015) and Davis et al. (2020), who considered *P. sphagneticola*e as sister to *P. halstedii*. Hitherto two *cox2* sequences from the original publication of *P. invertifolia* are the single available DNA data. In our BLAST search with one of these two sequences, it showed only up to 91% identity with any other oomycete, including *P. halstedii* and *P. sphagneticolae*. These latter two species in deed

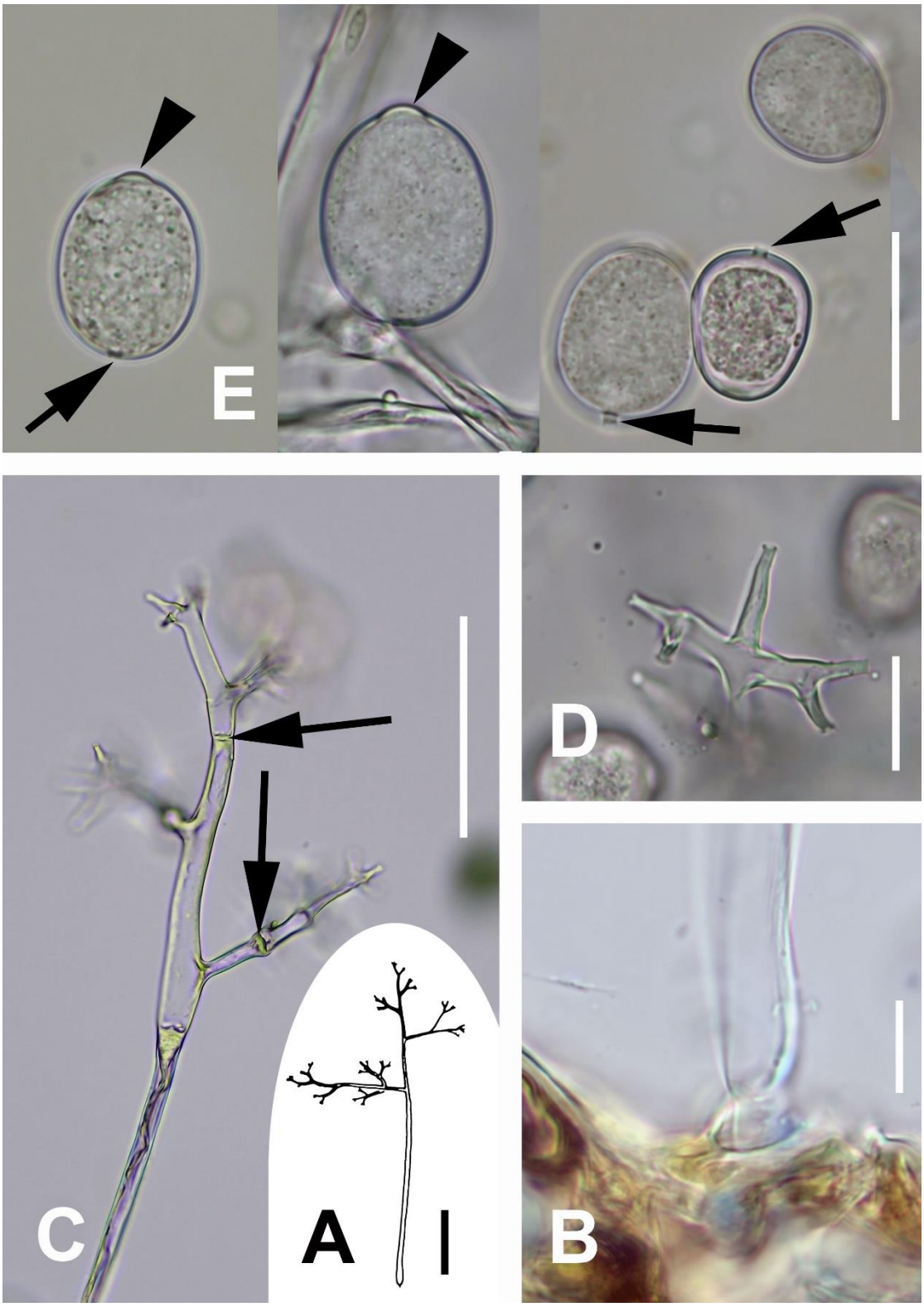

Fig. 3. A–E. Sporangiophores and sporangia of *Plasmopara sphagneticolae* seen with light microscopy. A. Sketch of sporangiophore. B. Base of sporangiophore arising from stoma in abaxial epidermis of diseased leaf. C. Upper branches of sporangiophore. Two retraction septa ("callose plugs") marked with arrows. D. Ultimate branchlets of sporangiophore. E. Sporangia. Basal hilum indicated with arrow, apical papilla with arrow head. Scale bars A = 100 µm, B = 10 µm, C = 50 µm, D, E = 20 µm.

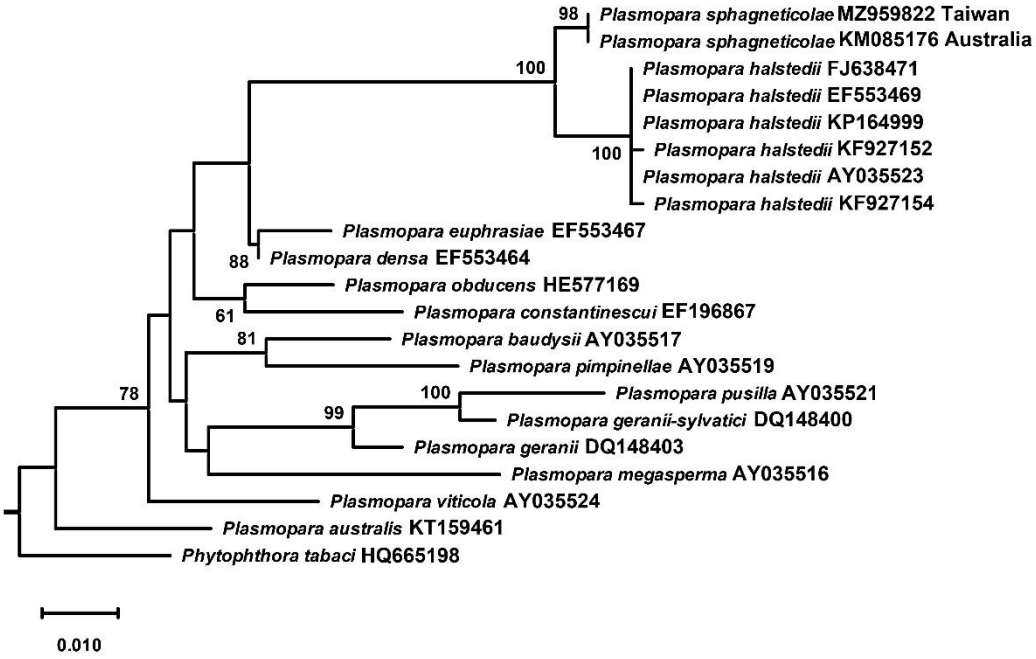

Fig. 4. Maximum likelihood phylogenetic analysis of LSU sequences of *Plasmopara* species. The tree was rooted with *Phytophthora tabaci*. GenBank numbers are given behind the species names. Bootstrap values of 1000 replicates lower than 50% not shown.

seem to have closer relationships to each other than to *P. invertifolia*.

Geographical and host distribution

It is most likely that the oomycete originated from the same area as the host plant in the Neotropics but remained unnoticed there before being first discovered outside its natural distribution. Records of the oomycete are published from Australia, where the host *Sph. trilobata* is not native, either, and from Hawaii on a native host, *Lipochaeta integrifolia* (Nutt.) A. Gray (Davis et al. 2020, McTaggart et al. 2015). Davis et al. (2020) suggested introduction of *P. sphagneticolae* to Hawaii through introduction of *Sph. trilobata*, where it had been introduced in the 1960s (Thaman 1999). The Hawaiian endemic genus *Lipochaeta* was considered hardly distinguishable from *Wedelia*-like taxa (Thaman 1999), which are now split into several genera with disputed boundaries (Orchard 2013). Perhaps the parasitic oomycete may be the better taxonomist? In Taiwan, the oomycete might have been introduced together with *Sph. trilobata* from Hawaii in the early 1980s. Our discovery of the oomycete implies that it has been overlooked in Taiwan for over thirty years. Our own recent first records of two fungi on this host in Taiwan, *Podosphaera xanthii* (Castagne) U. Braun & Shishkof and *Pseudocercospora wedeliae* (A.K. Kar & M. Mandal) Deighton (Kirschner 2014, Yeh et al. 2021), support the hypothesis that even many described fungal species remain unnoticed for many years in a given area. We roughly estimated periods between 30 and 300 years between

the introduction of a potential host plant and discovery of its associated introduced oomycete (Kirschner 2013, 2015a, b, Wang et al. 2020, Yeh et al. 2021). Knowledge of geographic distribution of fungi/oomycetes is still far behind of that of plants. *Sph. trilobata* spreads over short distances mainly by clonal reproduction; it grows and spreads very easily in its new environment and can be artificially also grown from seeds (Qi et al. 2014). We, therefore, assume that later introductions after the first one from Hawaii have not been necessary and after the 1980s not taken place for the market of ornamental plants. Oospores have not been found in *P. sphagneticolae*, whereas long-distance dispersal through oospore-contaminated sunflower seeds is a likely route in *P. halstedii* on sunflower (Spring 2019). The routes of global distribution of *P. halstedii* on sunflower from North America in the 20th century to other continents could be reconstructed based on numerous data worldwide (Spring 2019). For *P. sphagneticolae*, data of host and geographic distribution are yet too scarce for allowing sound conclusions about the origin and global spread. The lack of fundamental mycological data is a major obstacle for biogeography and risk assessment in quarantine and nature conservation.

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Plasmopara sphagneticolae 於臺灣的首次紀錄

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摘　　要

南美蚼蜞菊為一種常見於公共綠地的觀賞性地被植物之一，在臺灣同時也是一種外來入侵種。於臺北市，在此植物上發現了露菌病之病徵，而後基於宿主、形態學以及核糖體大亞基 RNA 基因 (LSU)序列分析，將該菌種鑑定為 *Plasmopara sphagneticolae*。本文亦針對此卵菌隨其宿主自夏威夷最可能的輸入途徑進行討論。

關鍵詞：菊科、卵菌-宿主分布、植物病、核糖體 DNA、蚼蜞菊屬