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Etiology

One Crop Disease, How Many Pathogens? *Podosphaera xanthii* and *Erysiphe vignae* sp. nov. Identified as the Two Species that Cause Powdery Mildew of Mungbean (*Vigna radiata*) and Black Gram (*V. mungo*) in Australia

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ABSTRACT

Powdery mildew is a significant threat to mungbean (*Vigna radiata*) and black gram (*V. mungo*) production across Australia and overseas. Although they have been present in Australia for at least six decades and are easily recognized in the field, the precise identification of the pathogens causing this disease has remained unclear. Our goal was to identify the powdery mildew species infecting mungbean, black gram, and wild mungbean (*V. radiata* ssp. *sublobata*) in Australia. The internal transcribed spacer (ITS) and large subunit sequences of the ribosomal DNA and/or morphology of 57 Australian specimens were examined. Mungbean and black gram were infected by two species: *Podosphaera xanthii* and a newly recognized taxon, *Erysiphe vignae* sp. nov. Wild mungbean was infected only with *P. xanthii*. Mungbean and black gram powdery mildew ITS sequences from China, India, and Taiwan revealed the presence of only *P. xanthii* on these crops despite controversial reports of an *Erysiphe* species on both crops in India. Sequence analyses indicated that the closest relative of *E. vignae* is *E. diffusa*, which infects soybean (*Glycine max*) and other plants. *E. vignae* did not infect soybean in cross-inoculation tests. In turn, *E. diffusa* from soybean infected black gram and provoked hypersensitive response in mungbean. The recognition of a second species, *E. vignae*, as another causal agent of mungbean and black gram powdery mildew in Australia may complicate plant breeding efforts and control of the disease with fungicide applications.

Keywords: *Erysiphe diffusa*, *Erysiphe polygoni*, *Erysiphe vignae*, etiology, fungal pathogens, new powdery mildew species, pathogen detection, *Podosphaera xanthii*, taxonomy, *Vigna mungo*, *Vigna radiata* (syn. *Phaseolus aureus*)

Mungbean or green gram (*Vigna radiata* [L.] Wilczek) is a legume crop grown most widely by smallholder farmers in South and Southeast Asia and expanding to Central Asia and Sub-Saharan Africa, with commercial production in Australia and Oklahoma in the United States. In Asia, it provides a vital source of inexpensive protein in cereal-based diets. Bean sprouts are also processed as a salad component throughout the world. Short crop duration, low water requirement, and the ability to fix atmospheric nitrogen are the major advantages of mungbean production. In some tropical and subtropical parts of Asia, mungbean has been grown for centuries. In Australia, mungbean production commenced as early as the 1930s, primarily as a forage or green manure crop to improve soil fertility in cereal-based farming systems (Chauhan and Williams 2018). In Queensland, Australia, its extensive production as a grain crop began in the early 1970s, when two new high-yielding and nonshattering cultivars, Berken and Celera, boosted the local industry (Bott and Kingston 1976; Lawn and Russell 1978). Today, mungbean is Australia's most widely grown summer legume crop, predominantly in Queensland and northern New South Wales, with a record crop of 180,000 tonnes produced in 2016 (Wells and Benjamin 2019).

Black gram or urdbean (*V. mungo* [L.] Hepper) is a close relative of mungbean that has also been grown in the same region in Australia in the past 50 years. In the 1970s, the black gram cultivar Regur was released for commercial production (Lawn and Russell 1978), then superseded by cultivar Onyx-AU, released in 2017. Black gram is a minor niche crop in Australia (Chauhan and Williams 2018).

Since the 1970s, powdery mildew infections have been identified as one of the most common fungal diseases of mungbean in Australia that appeared every season on the foliage (Bott and Kingston 1976),

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causing substantial leaf abscission in some fields (Lawn and Russell 1978). Powdery mildew has since been found in all areas of mungbean production across Australia (Cunnington et al. 2004) and Asia (Pandey et al. 2018). Field trials in southern Queensland, Australia demonstrated that the disease can reduce yields by >40% under conducive environmental conditions and if no management strategies are implemented (Kelly et al. 2017; Thompson 2016; Weir et al. 2017). All mungbean cultivars grown in Australia are susceptible to powdery mildew (AMA 2020). The most commonly grown cultivars, Jade-AU and Crystal, are considered moderately susceptible to the disease, whereas cultivar Opal-AU, released in 2020, has a rating of moderately resistant. The two black gram cultivars grown in Australia, Regur and Onyx-AU, are also susceptible to powdery mildew. Consequently, the management of powdery mildew in mungbean and black gram in Australia relies heavily on the application of fungicides.

The causal agent of the early mungbean powdery mildew epidemics in Queensland was identified at the time as *Sphaerotheca fuliginea* (Schltdl.) Pollacci (Bott and Kingston 1976). Currently, this species is known as *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, which has a global distribution (Braun and Cook 2012). *P. xanthii* has an exceptionally wide host range, infecting cucurbits (Hirata et al. 2000; Pérez-Garcia et al. 2009), ornamentals (Kiss et al. 2008), and many other, only distantly related plants, including native species in Australia (Kiss et al. 2020; Kiss and Vaghefi 2021). A few powdery mildew specimens from *Vigna* spp., collected in Queensland, Western Australia, and the Northern Territory, were also identified as *P. xanthii* based on morphology and nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences or ITS restriction fragment length polymorphism patterns (Cunnington et al. 2004; Kiss et al. 2020).

In Asia, several studies have reported that powdery mildew on mungbean and black gram was caused by another species, *Erysiphe polygoni* DC. (Jayasekhar and Ebenezar 2016; Kasettranan et al. 2010; Reddy 2009), although none of these works provided details of the methods used to identify the fungal species. It should be mentioned that the name *E. polygoni* was applied to many powdery mildews with similar morphology infecting a large number of plant species in different parts of the world (Braun and Cook 2012). Some of those powdery mildews are phylogenetically diverse (Takamatsu et al. 2015). Recently, a powdery mildew specimen from black gram in India was identified as *E. polygoni* based on its ITS sequence (Channaveerresh and Kulkarni 2017). *P. xanthii* was also reported on mungbean in India (Gautam and Avasthi 2018), and this is supported by two partial ITS sequences in National Center for Biotechnology Information GenBank (KP313607 and KP313608), from mungbean powdery mildew specimens collected in India in 2014. Most work on breeding for powdery mildew resistance in mungbean was done in Asia with *E. polygoni* used as the pathogen (Chankaew et al. 2013; Gawande and Patil 2003; Kasettranan et al. 2009; Sujatha and Kajjidoni 2013; Yundaeng et al. 2020). To date, *E. polygoni* has not been recorded on mungbean and black gram in Australia (Kiss et al. 2020). Because *E. polygoni* is not closely related phylogenetically to *P. xanthii* (Bradshaw and Tobin 2020), it is questionable whether powdery mildew resistance breeding results obtained with *E. polygoni* in Asia are transferable to the mungbean breeding program in Australia with respect to resistance to *P. xanthii*.

Soybean (*Glycine max* [L.] Merr.) is another important summer legume crop affected by powdery mildew throughout its production in eastern Australia. Based on morphological characteristics and ITS sequences of powdery mildew specimens collected from soybean in Queensland and New South Wales, the pathogen was identified as *E. diffusa* (Cooke & Peck) U.Braun & S.Takam. (Kiss et al. 2020; McTaggart et al. 2012). Outside Australia, morphological and ITS sequence analyses have identified two distinct species, *E. diffusa* and *E. glycines* F.L.Tai, as the causal agents of soybean powdery mildew (Baiswar et al. 2016; Takamatsu et al. 2002). Soybean, mungbean, and black gram are closely related (Chappill 1995), and the host ranges of the powdery mildew species reported from these fabaceous crops so far (i.e., *P. xanthii*, *E. diffusa*, *E. glycines*, and *E. polygoni*) are not well known. A hidden diversity of powdery mildew taxa on these crops may complicate breeding endeavors to identify resistant germplasm and develop powdery mildew-resistant cultivars. The efficacy of fungicide treatments and the longevity of their action are also important factors that may be affected by the presence of multiple powdery mildew taxa on the same crops if some develop fungicide resistance earlier than others.

This study reports the identification of powdery mildew species infecting mungbean, black gram, and wild mungbean in Australia. Wild mungbean (*V. radiata* ssp. *sublobata* [Roxb.] Verdc.), considered the progenitor of the cultigen *V. radiata* ssp. *radiata*, has a widespread natural distribution, including Southeast Asia, India, Africa, and Australia, and is used as a source of germplasm in crop improvement breeding programs (Takahashi et al. 2016). *V. radiata* ssp. *sublobata* was included in this study because some accessions are susceptible to powdery mildew (Lawn and Rebetzke 2006).

The objectives of this work were to examine as many fresh and herbarium powdery mildew specimens as possible collected from these plants across Australia; include mungbean and black gram powdery mildew specimens from China, India, and Taiwan in the study for comparative analyses; identify the species of powdery mildew based on nrDNA sequences and morphological characteristics; and conduct cross-inoculation tests to reveal the host ranges of the powdery mildew species infecting mungbean and black gram in Australia.

MATERIALS AND METHODS

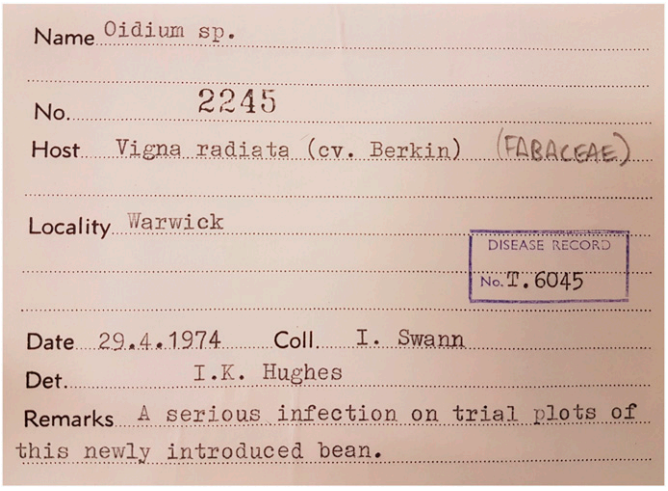
Powdery mildew specimens. Leaves, stems, and pods of mungbean, black gram, and wild mungbean plants with symptoms of powdery mildew were collected from a total of 34 field sites throughout Australia in 2017, 2018, 2019, and 2020. At each site, a minimum of six leaves, stems, or pods infected with powdery mildew were collected from each crop. Mungbean leaves naturally infected with powdery mildew during field and glasshouse experiments at the Department of Agriculture and Fisheries and University of Southern Queensland precincts in 2019 in Toowoomba were also collected and included in this study. Soybean leaves infected with powdery mildew were collected for comparative studies. Historical powdery mildew specimens from mungbean (Fig. 1) and wild mungbean, dating back as far as 1965 from Queensland, Northern Territory, and Western Australia, were borrowed from plant pathology herbaria to be examined in this work. After collection, all fresh powdery mildew samples were examined and photographed under a light microscope in the laboratory, and subsequently, the infected plant organs were dried and pressed as herbarium specimens and deposited at the Queensland Plant Pathology Herbarium (BRIP). Table 1 lists the sources and collection details of all Australian specimens included in this study. Mungbean and black gram powdery mildew specimens from China, India, and Taiwan, collected as part of this project or examined in earlier works, were also included in our study (Tables 1 and 2). Living isolates of mungbean and soybean powdery mildews were maintained on their host plants in isolation as described below.

Morphological studies. A Nikon Eclipse Ni-U (Tokyo, Japan) microscope with bright field and differential interference contrast optics was used to examine in each fresh and rehydrated powdery mildew specimen the shape and size of conidia, based on measurements of 30 conidia per specimen, presence or absence of fibrosin bodies in conidia, nature of conidiogenesis, characteristics of the conidiophores, position of conidial germ tubes, shape of conidial germ tube apices, and shape of hyphal appressoria. We rehydrated herbarium specimens by boiling small pieces of infected plant materials in lactic acid on a microscope slide, as described by Shin and La (1993). Powdery mildews belonging to the genus *Podosphaera* were easily distinguished from *Erysiphe* species because their conidiophores produce conidia in true chains, their hyphae

have simple or inconspicuous appressoria, fresh conidia always contain fibrosin bodies, and they produce germ tubes arising from the middle of their conidia, terminating in simple apices. Powdery mildews representing the genus *Erysiphe* have conidiophores that produce conidia singly or in pseudochains, hyphal appressoria are lobed or multilobed, fresh conidia do not contain fibrosin bodies, and their germ tubes emerge terminally or subterminally, ending in often lobed apices (Braun and Cook 2012).

Conidial germination patterns were observed in every specimen examined, including the rehydrated herbarium specimens, because there were germinating conidia in each material. To compare these diagnostic characteristics experimentally in the *Erysiphe* species infecting mungbean and soybean, fresh conidia of isolates BRIP 71005 from mungbean and BRIP 71011 from soybean were incubated on the following surfaces for 48 h at 22°C under continuous illumination: microscope slides (glass); sterile cellophane kept on 1.5% water agar, as described by Szentiványi and Kiss (2003); and healthy detached leaves of their hosts. One mungbean and one soybean leaf infected with young, sporulating powdery mildew colonies of isolates BRIP 71005 and BRIP 71011, respectively, were each gently touched to two microscope slides and two 1-cm^2 sterile cellophane pieces. Each was kept in a 6-cm-diameter plate with water agar. Two healthy, detached mungbean and soybean leaves were each kept in a 500-ml glass beaker, with their petioles wrapped in wet paper tissue, and the beakers were closed with transparent foil. Microscope slides bearing conidia were kept in 15-cm-diameter plates, on glass rods placed on wet paper tissue; plates were closed and sealed with Parafilm. After incubation for 48 h, a droplet of lactic acid was pipetted onto conidia on slides, covered with a cover slip, and slides were examined under a light microscope. Small pieces of cellophane bearing conidia were cut out and examined under the microscope to observe the germinated conidia. Conidia incubated on host plant leaves were removed with cellophane tape before being checked by microscopy. All germination tests were carried out twice.

Long-term maintenance of living powdery mildew isolates. Living isolates of *Erysiphe* sp. and *P. xanthii* collected from mungbean and *E. diffusa* collected from soybean were maintained on their potted host plants for use in cross-inoculation experiments. To produce these isolates, single leaves with young colonies of powdery mildew were collected and brought to the laboratory in isolation. After being identified microscopically, conidia from young colonies were used to inoculate potted mungbean plants of cultivar Jade-AU or soybean plants of cultivar Bunya. Inoculated plants were kept in isolation in BugDorm insect rearing cages with very fine mesh in different bays of the Department of Agriculture and Fisheries and the University of Southern Queensland glasshouse facilities in Toowoomba, under natural daily illumination. Temperature was kept between 18 and 26°C and relative humidity between 70 to 80% in all glasshouse bays. Plants were watered regularly, without opening the cages, and replaced every 3 to 5 weeks with newly grown and freshly inoculated plants to ensure the continuous maintenance of two isolates of an *Erysiphe* species and one isolate of *P. xanthii*, all collected from mungbean, and one isolate of *E. diffusa* collected from soybean. All isolates came from different locations. These were designated as BRIP 71005, BRIP 71598, BRIP 71599, and BRIP 71011, based on the accession numbers of their representative specimens deposited in Queensland Plant Pathology Herbarium (Table 1).


Name Oidium sp.

No. 2245

Host Vigna radiata (cv. Berkin) (FABACEAE)

Locality Warwick

DISEASE RECORD
No. T.6045

Date 29.4.1974 Coll. I. Swann

Det. I.K. Hughes

Remarks A serious infection on trial plots of this newly introduced bean.

Fig. 1. Label for specimen BRIP 2245: *Podosphaera xanthii* (*Oidium* sp.) on mungbean cultivar Berken (Birken) collected in Warwick, Queensland, Australia in 1974. This represents the oldest record of powdery mildew on mungbean in Australia that was molecularly verified in this study. The specimen was collected soon after the start of the large-scale commercial mungbean production in Queensland.

Cross-inoculation studies. Cross-inoculation experiments were undertaken to determine whether the *P. xanthii* and the two *Erysiphe* isolates collected from mungbean and the *E. diffusa* isolate collected from soybean were able to infect other closely related plant species in addition to infecting their own host plants. Young plants of mungbean cultivar Jade-AU, black gram cultivars Regur and Onyx-AU, cowpea (*Vigna unguiculata* [L.] Walp.) cultivar Caloona, and soybean cultivar Bunya were used as the test plants in the cross-inoculation experiments. Young plants of each variety were grown from seeds sown into 10-cm-diameter pots containing potting mix (Rocky Point Mulching). Each pot contained two or three plants of a single host species. Potted plants were grown together in single BugDorm insect rearing cages with very fine mesh for ≥2 weeks to ensure that they were free of powdery mildew before being used in the cross-inoculation experiments.

For each experiment, one of the following was used the source of powdery mildew inoculum: mungbean cultivar Jade-AU infected with *P. xanthii* isolate BRIP 71599, mungbean cultivar Jade-AU infected with either *Erysiphe* isolate BRIP 71005 or BRIP 71598, and soybean cultivar Bunya infected with *E. diffusa* isolate BRIP 71011. Each cross-inoculation experiment commenced 14 days after the first fully expanded leaves developed in the test plants. A pot of donor plants infected with one of the powdery mildew isolates was placed within the BugDorm insect rearing cage next to the disease-free test plants grown in another pot. The test plants were inoculated by gently touching their leaves against fresh powdery mildew colonies actively growing on the leaves of the infected donor plants. The inoculated test plants then remained close to the symptomatic plants in the cage (Fig. 2). A pot of non-inoculated, disease-free test plants, kept in another cage, was included in each experiment as the negative control. The positive control was a pot of disease-free donor plants, which received the same inoculum as the test plants and was kept in isolation in a separate cage. All plants were watered regularly, without the cage being opened, for ≥3 weeks. Each experiment was carried out twice in separate bays in two glasshouses.

Inoculated test plants were visually inspected daily for symptom development and classified as susceptible when powdery mildew colonies actively grew on the inoculated leaves. If found, the powdery mildews were identified based on morphology and, in the case of *Erysiphe* isolates, by sequencing of the ITS region at the end of the experiments.

DNA extractions, PCR amplifications, and sequencing. DNA was extracted from powdery mildew mycelia collected from fresh plants and a selection of historical herbarium specimens. Powdery mildew mycelia were removed from plant surfaces with 1 to 1.5-cm^2 pieces of cellophane tape. Total genomic DNA was extracted from cellophane tape pieces with powdery mildew samples with an Extract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. A

modified version of the nested PCR method developed by Cunnington et al. (2003), with primers PMITS1 and PMITS2, was used to amplify the ITS region from DNA samples as described by Kiss et al. (2020). PCR products were visualized by gel electrophoresis on a 1% wt/vol agarose gel containing 0.01% GelRed (Gene Target Solutions, Dural, Australia) in TAE (containing a mixture of Tris base, acetic acid, and EDTA) buffer under ultraviolet light. PCR products of the nested reactions were purified and sequenced by Macrogen Inc. (Seoul, Korea) with primers ITS1-F and ITS4.

The 5′-end of the nrDNA large subunit (LSU or 28S) gene, including the D1 and D2 regions, was amplified in representative *Erysiphe* specimens from mungbean and soybean during semi-nested PCRs and sequenced by Macrogen Inc. with primers T2, T4, and TW14, as described by Kiss et al. (2020).

TABLE 1. Powdery mildew specimens from mungbean, wild mungbean, black gram, and soybean collected and identified in this study

Powdery mildew species	Host plant species	Herbarium accession number	Place and date of collection	GenBank accession numbers of nrDNA internal transcribed spacer sequences (and 28S sequences where applicable)[a]
Podosphaera xanthii	*Vigna radiata*	BRIP 2235	Gayndah, QLD, May 1965	–
P. xanthii	*V. radiata*	BRIP 2245	Warwick, QLD, April 1974	MW293884
P. xanthii	*V. radiata*	DNAP 0970	Berrima, NT, October 1979	–
P. xanthii	*V. radiata*	DNAP 1559	Berrima, NT, May 1983	–
P. xanthii	*V. radiata*	BRIP 14043	Kununurra, WA, August 1983	–
P. xanthii	*V. radiata* ssp. *sublobata*	PERTH 741337	Kununurra, WA, 1988	–
P. xanthii	*V. radiata*	BRIP 50233	Kununurra, WA, March 1997	–
P. xanthii	*V. radiata*	BRIP 48240	Kingsthorpe, QLD, April 2006	–
P. xanthii	*V. radiata*	BRIP 48242	Toowoomba, QLD, June 2006	–
P. xanthii	*V. radiata*	BRIP 48238	Kingaroy, QLD, July 2006	–
P. xanthii	*V. radiata*	BRIP 49652	Biloela, QLD, April 2007	–
P. xanthii	*V. radiata*	BRIP 49629	Kingaroy, QLD, April 2007	–
P. xanthii	*V. radiata*	BRIP 51764	Cecil Plains, QLD, March 2008	–
P. xanthii	*V. radiata*	BRIP 51883	Emerald, QLD, March 2008	–
P. xanthii	*V. radiata*	BRIP 53340	Emerald, QLD, April 2010	–
P. xanthii[b]	*V. radiata*	BRIP 71599	Killarney, QLD, May 2017	MW293885
P. xanthii	*V. radiata*	HMJAU91765	Changchun, China, August 2017	MW293886
P. xanthii	*V. radiata*	BRIP 66746	Hyderabad, India, December 2017	MW293896
P. xanthii	*V. radiata* cultivar Celera II-AU	BRIP 70998	Kununurra, WA, August 2018	MW293887
P. xanthii	*V. radiata* cultivar Crystal	BRIP 70999	Kununurra, WA, August 2018	MW293888
P. xanthii	*V. radiata* cultivar Celera II-AU	BRIP 71001	Duaringa, QLD, April 2019	MW293889
P. xanthii	*V. mungo*	BRIP 71591	Brisbane, QLD, April 2019	–
P. xanthii	*V. mungo*	BRIP 71592	Brisbane, QLD, April 2019	–
P. xanthii	*V. mungo*	BRIP 71593	Brisbane, QLD, April 2019	–
P. xanthii	*V. mungo*	BRIP 71594	Brisbane, QLD, April 2019	–
P. xanthii	*V. radiata* cultivar Jade-AU	BRIP 71002	Emerald, QLD, April 2019	MW293890
P. xanthii	*V. radiata* ssp. *sublobata*	BRIP 71003	Capella, QLD, May 2019	MW293891
P. xanthii	*V. radiata* ssp. *sublobata*	BRIP 71004	Gindie, QLD, May 2019	MW293892
P. xanthii	*V. mungo* cultivar Onyx-AU	–	Cecil Plains, QLD, May 2019	MW291160
P. xanthii	*V. radiata*	BRIP 71008	Ayr, QLD, June 2019	MW293893
P. xanthii	*V. radiata*	BRIP 71596	Rolleston, QLD, April 2020	–
P. xanthii	*V. radiata* cultivar Jade-AU	BRIP 71583	Cecil Plains, QLD, April 2020	–
P. xanthii	*V. radiata* cultivar Opal-AU	BRIP 71578	Pampas, QLD, April 2020	–
Erysiphe vignae	*V. mungo* cultivar Regur	BRIP 68837	Toowoomba, QLD, April 2018	MT628284
E. vignae[b]	*V. radiata* cultivar Jade-AU	BRIP 71005	Toowoomba, QLD, March 2019[c]	MT628282 (MT628017)
E. vignae[b]	*V. radiata* cultivar Jade-AU	BRIP 71598	Toowoomba, QLD, April 2019[c]	MW293895 (MT628018)
E. vignae	*V. radiata* cultivar Jade-AU	BRIP 71006	Warwick, QLD, June 2019	MT628285
E. vignae	*V. radiata* cultivar Jade-AU	BRIP 71007	Warwick, QLD, June 2019	MW293894
E. vignae	*V. radiata*	BRIP 71010	Gatton, QLD, August 2019	MT628286
E. vignae	*V. radiata*	BRIP 71590	Toowoomba, QLD, November 2019	–
E. diffusa[b]	*Glycine max*	BRIP 71011	Gatton, QLD, August 2019[c]	MW009056 (MT628019)
E. diffusa	*G. max*	BRIP 71012	Gatton, QLD, August 2019	MW009057
E. diffusa	*G. max* cultivar Bunya	BRIP 71014	Toowoomba, QLD, August 2019	MW009059
E. diffusa	*G. max*	–	Gatton, QLD, October 2020	–
E. vignae and *P. xanthii*	*V. radiata*	BRIP 71595	Osborne, QLD, December 2019	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71584	Kincora, QLD, April 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71585	Cecil Plains, QLD, April 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71586	Cecil Plains, QLD, April 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71582	Clifton, QLD, April 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71581	Pampas, QLD, April 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71577	North Branch, QLD, April 2020	–
E. vignae and *P. xanthii*	*V. radiata*	BRIP 71589	Theodore, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71587	Pampas, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71597	Pampas, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Jade-AU	BRIP 71588	Pampas, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. radiata* cultivar Crystal	–	Formartin, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. mungo* cultivar Regur	–	Formartin, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. radiata*	BRIP 71579	Brisbane, QLD, May 2020	–
E. vignae and *P. xanthii*	*V. radiata*	BRIP 71580	Theodore, QLD, July 2020	–

[a] Specimens without nuclear ribosomal DNA (nrDNA) sequence data were identified based on morphology only. Dashes indicate missing data.
[b] Isolates maintained on their respective host plants in isolation during this study.
[c] Collected in glasshouse after spontaneous infections of experimental plants.

An approximately 1,000-bp fragment of the beta-tubulin (*TUB2*) gene was amplified in *P. xanthii* specimen BRIP 71599 with primers TubRT6F (Vela-Corcía et al. 2014) and Bt2b (Glass and Donaldson 1995). The PCRs were carried out in 20-µl final volumes, consisting of 10 µl of hot start PCR ReadyMix from an Extract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO), 1 µl of each primer at 10 µM, 6 µl of ultrapure water, and 2 µl of total genomic DNA. PCR conditions were as follows: 94°C for 10 min; 34 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C; and finally 10 min at 72°C. PCR products were purified and sequenced by Macrogen Inc. with the PCR primers.

Initial sequence analyses. Sequences were compiled from chromatograms after visual inspections for potential sequencing errors. To obtain reliable consensus sequences, single-nucleotide polymorphisms were accepted only if the base call quality was ≥30 and if the polymorphism occurred in more than one sequence (James et al. 2009). We produced consensus sequences by trimming and assembling the forward and reverse sequences in Geneious Prime 2019.1.3 (Biomatters Ltd.). The ITS, LSU, and *TUB2* consensus sequences determined in this study were deposited in GenBank.

Phylogenetic analyses. Phylogenetic analyses were conducted on three different datasets depending on the requirements of each analysis and availability of reference sequences in the National Center for Biotechnology Information GenBank. ITS sequences of *Erysiphe* and *Podosphaera* are too divergent to allow nonambiguous alignment of all sequences (Kiss et al. 2020); therefore, separate ITS phylogenies were constructed for *Erysiphe* and *Podosphaera* species. Because of the unavailability of LSU sequences for multiple *Erysiphe* species included in the ITS analysis, a third phylogeny including fewer taxa was constructed to evaluate the phylogenetic relationship of some selected *Erysiphe* species based on their LSU sequences.

Multiple sequence alignments were constructed in MAFFT version 7.388 (Katoh and Standley 2013), visually inspected for potential misalignments or ambiguously aligned regions, and trimmed to the length of the shortest sequence. Phylogenetic analyses were conducted via Bayesian inference and maximum likelihood (ML) approaches. For Bayesian inference, the Akaike information criterion estimated in MrModeltest version 2.3 (Nylander 2004) and PAUP version 4.0 (Swofford 2002) was used to determine the best-fit nucleotide substitution model for each alignment. MrBayes version 3.2.4 (Ronquist and Huelsenbeck 2003) was used to run two Markov chain Monte Carlo chains. One tree per 100 generations was saved, and the runs were ended when the standard deviation of split frequencies were <0.01. The 50% majority rule consensus tree was estimated after a 25% burn-in of the saved trees. For ML analysis, RAxML version 8.2.11 (Stamatakis 2014) was used with the GTRGAMMA model of nucleotide substitution and 1,000 bootstrap replicates. Trees were viewed in Figtree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Alignments and trees were deposited in TreeBASE (www.treebase.org/treebase-web/home.html).

The *Podosphaera* dataset consisted of 34 ITS sequences, including 13 sequences obtained in this study, 20 reference sequences of representative specimens obtained from GenBank (Table 2), and *Cystotheca wrightii* (AB000932) as the outgroup based on Takamatsu et al. (2000). This resulted in an alignment with a total length of 492 characters, including 359 identical and 133 variable sites. The *Erysiphe* ITS dataset consisted of 37 sequences, including 9 sequences obtained in this study, 27 reference sequences of representative specimens obtained from GenBank (Table 2), and *E. glycines* (AB078807) as the outgroup based on Takamatsu et al. (2002). This resulted in an alignment with a total length of 590 characters, including 410 identical and 180 variable sites. The *Erysiphe* LSU dataset consisted of 17 sequences, including three sequences obtained in this study, 13 reference sequences of representative specimens obtained from GenBank (Table 2), and *Salmonomyces javanicus* (MT133550) as the outgroup based on Kiss et al. (2020). This resulted in an alignment with a total length of 839 characters, including 760 identical and 79 variable sites.

Fig. 2. A cross-inoculation experiment in a BugDorm insect rearing cage. The source of inoculum was *Erysiphe vignae* isolate BRIP 71005 on mungbean cultivar Jade-AU plants. Soybean cultivar Bunya plants were tested for their susceptibility to the *E. vignae* isolate. No powdery mildew symptoms developed on the soybean plants during the 3-week-long experiment.

RESULTS

Identification of powdery mildew specimens based on morphology. Powdery mildews collected from mungbean, wild mungbean, and black gram in this study from 2017 to 2020 were identified as either *P. xanthii* or an *Erysiphe* species based on their morphological characteristics. Both powdery mildews caused heavy infections in the field, with colonies covering most of the leaves and stems, and by the end of the growing season sometimes also part of the pods (Figs. 3, 4, and 5). In 19 of the 34 crops sampled from 2017 to 2020, only one of the two powdery mildew species was found. In the remaining 15 crops, both species of powdery mildew were present (Table 1).

All powdery mildew specimens from mungbean and wild mungbean collected from 1965 to 2018, including all herbarium specimens available in Australian plant pathology herbaria, were identified as *P. xanthii* based on morphology (Table 1). The specimens collected from mungbean in China and India, HMJAU91765 and BRIP 66746, respectively, were also identified as *P. xanthii* based on morphology (Table 1). Seven specimens collected from mungbean and black gram between 2018 and 2019 were identified as an *Erysiphe* sp. based on morphological characteristics. All four powdery mildew specimens from soybean resembled *E. diffusa* based on their morphology.

Conidial germination patterns compared experimentally on glass, cellophane, and detached leaves differed markedly between the *Erysiphe* isolate BRIP 71005, collected from mungbean, and the *E. diffusa* isolate BRIP 71011, collected from soybean. After 48 h incubation on all the three surfaces in parallel, 88% of the *Erysiphe* conidia collected from mungbean developed germ tubes that were shorter than the length of conidia or as long as the conidium. These germ tubes emerged terminally or subterminally and ended in lobed or multilobed apices (Figs. 4F, and 6A and B). In contrast, 65% of the germ tubes that emerged from *E. diffusa* conidia on glass, cellophane, and host leaf surfaces were longer than the conidial length, sometimes three to five times longer, and ended in usually simple, sometimes swollen but not lobed apices (Fig. 6C and D), a pattern named as *longitubus* by Cook and Braun (2009) (Fig. 6D). The rest of the germinated conidia of *E. diffusa* produced germ tubes that were shorter than the conidial length and ended in lobed or multilobed

apices, similar to the most common germination patterns observed in *Erysiphe* isolate BRIP 71005 from mungbean.

Cross-inoculation studies. Table 3 displays the results of the cross-inoculation experiments. Both *Erysiphe* isolates and the *P. xanthii* isolate collected from mungbean infected black gram cultivars Regur and Onyx-AU plants in all cross-inoculation experiments. The infection process started with a hypersensitive response (HR) in black gram leaves, which was overcome by each powdery mildew

TABLE 2. Powdery mildew specimens used in phylogenetic analyses[a]

Powdery mildew species	Host plant species	Herbarium accession number	GenBank accession numbers of nuclear ribosomal DNA		Place and date of collection	Reference
			Internal transcribed spacer sequences	28S sequences		
Cystotheca wrightii	*Quercus glauca*	MUMH 137	AB000932	–	Japan	Takamatsu et al. 2000
Erysiphe alphitoides	*Quercus* sp.	VPRI 18763	AB292705	–	VIC, 1993	Takamatsu et al. 2007
E. aquilegiae	*Catharanthus roseus*	BRIP 46649	DQ335569	–	QLD, 2005	Liberato and Cunnington 2006
E. aquilegiae	*Aquilegia vulgaris*	MUMH 2456	LC010016	–	Argentina	Takamatsu et al. 2015
E. aquilegiae	*Ranunculus japonicus*	MUMH 0287	–	LC009942	Japan	Takamatsu et al. 2015
E. aquilegiae	*Clematis terniflora*	MUMH 0098	–	LC009920	Japan	Takamatsu et al. 2015
E. caricae-papayae	*Carica papaya*	MUMH 6941	–	LC228614	Thailand	Braun et al. 2017
E. chloranthi	*Chloranthus serratus*	MUMH 0202	–	LC009931	Japan	Takamatsu et al. 2015
E. cruciferarum	*Brassica juncea*	BRIP 69033	MT174187	–	QLD, 2017	Kiss et al. 2020
E. diffusa	*Glycine max*	BRIP 68997	MT174191	–	NSW, 2018	Kiss et al. 2020
E. diffusa	*G. max*	BRIP 62030	MT174190	–	QLD, 2014	Kiss et al. 2020
E. diffusa	*G. max*	BRIP 58458	MT174189	–	QLD, 2013	Kiss et al. 2020
E. diffusa	*G. max*	MUMH 1464	AB078811	–	USA	Takamatsu et al. 2002
E. diffusa	*G. clandestina*	BRIP 68827	MT174188	–	QLD, 2018	Kiss et al. 2020
E. euonymicola	*Euonymus japonicus*	BRIP 69034	MT174192	–	QLD, 2017	Kiss et al. 2020
E. glycines	*Amphicarpaea edgeworthii*	MUMH 56	–	LC028952	Japan	Takamatsu et al. 2015
E. glycines	*G. max*	MUMH 1462	AB078807	–	Japan	Takamatsu et al. 2002
E. heraclei	*Daucus carota*	BRIP 68829	MT174195	–	QLD, 2017	Kiss et al. 2020
E. izuensis	*Rhododendron indicum*	BRIP 68833	MT174197	–	QLD, 2018	Kiss et al. 2020
E. knautiae	*Knautia arvensis*	MUMH 2571	–	LC010042	Ukraine	Takamatsu et al. 2015
E. macleayae	*Macleaya cordata*	TPU-1873	–	LC010092	Japan	Takamatsu et al. 2015
E. medicaginis	*Medicago polymorpha*	BRIP 70957	MT160214	MT248412	QLD, 2019	Crous et al. 2020
E. medicaginis	*M. polymorpha*	BRIP 70958	MT160215	MT248413	QLD, 2019	Crous et al. 2020
E. pedaliacearum	*Sesamum indicum*	MUMH 411	–	LC342967	Japan	Shin et al. 2019
E. pisi	*Lathyrus latifolius*	UC 1512315	AF011306	–	USA	Saenz and Taylor 1999
E. pisi	*Pisum sativum*	VPRI 19688	AF073348	–	VIC, 1993	Cunnington et al. 2003
E. platani	*Platanus* × *hybrida*	BRIP 68800	MT174199	–	SA, 2018	Kiss et al. 2020
E. polygoni	*Polygonum aviculare*	MUMH 7036	–	LC328322	Azerbaijan	Bradshaw and Tobin 2020
E. polygoni	*Rumex crispus*	UC 1512308	AF011308	–	USA	Saenz and Taylor 1999
E. quercicola	*Quercus* sp.	VPRI 20422	AB295455	–	VIC, 1994	Takamatsu et al. 2007
Erysiphe sp.	*Phaseolus vulgaris*	–	AY739109	–	Brazil, 2004	Almeida et al. 2008
Erysiphe sp.	*Vigna unguiculata*	–	KY515231	–	Brazil, 2016	Unpublished
Erysiphe sp.	*P. vulgaris*	PMbean-1	KU320678	–	Spain, 2012	Unpublished
Erysiphe sp.	*G. max*	HD-3	MG171170	–	China, 2017	Unpublished
Erysiphe sp.	–	EG1	MT878222	–	–	Unpublished
E. syringae	*Syringa vulgaris*	VPRI 41368	FJ755790	–	VIC, 2008	Cunnington and Brett 2009
E. takamatsui	*Nelumbo nucifera*	MUMH 5659	–	AB916689	Japan	Meeboon and Takamatsu 2015
E. cf. *trifoliorum*	*P. sativum*	BRIP 68831	MT174202	–	QLD, 2017	Kiss et al. 2020
E. trifoliorum	*M. littoralis*	MUMH 7038	LC270860	–	Azerbaijan	Abasova et al. 2018
Podosphaera aphanis	*Fragaria* × *ananassa*	VPRI 19031	AF073355	–	VIC, 1993	Cunnington et al. 2003
P. fusca	*Calendula officinalis*	VPRI 20625	AF154324	–	VIC, 1995	Cunnington et al. 2003
P. leucotricha	*Malus domestica*	VPRI 17729	AF073353	–	NSW, 1991	Cunnington et al. 2003
P. pannosa	*Rosa* sp.	BRIP 68844	MT174222	–	QLD, 2017	Kiss et al. 2020
P. plantaginis	*Plantago lanceolata*	BRIP 68845	MT174223	–	QLD, 2018	Kiss et al. 2020
P. tridactyla	*Prunus persica*	VPRI 19591	AY833653	–	VIC, 1982	Cunnington et al. 2005
P. tridactyla	*Prunus* sp.	VPRI 19006	AF154321	–	VIC, 1993	Cunnington et al. 2003
P. xanthii	*V. unguiculata*	MUMH 340	AB040340	–	Japan	Hirata et al. 2000
P. xanthii	*V. unguiculata*	F0033687	MT472035	–	Taiwan, 2020	Unpublished
P. xanthii	*V. unguiculata*	SQDouJiao2	MN880477	–	China	Unpublished
P. xanthii	*V. unguiculata*	VPRI 18815	AY450960	–	NT, 1993	Cunnington et al. 2004
P. xanthii	*V. unguiculata* ssp. *sesquipedalis*	PM1	MH645799	–	India	Unpublished
P. xanthii	*V. radiata*	MGPM_3	MN833717	–	Taiwan, 2016	Unpublished
P. xanthii	*V. radiata*	pmmb-01	KP313607	–	India	Unpublished
P. xanthii	*V. radiata*	pmmb-02	KP313608	–	India	Unpublished
P. xanthii	*V. radiata*	BRIP 68847	MT174230	–	QLD, 2018	Kiss et al. 2020
P. xanthii	*V. radiata*	VPRI 19910	AY450961	–	QLD, 1994	Cunnington et al. 2004
P. xanthii	*Cucurbita maxima*	BRIP 69723	MT174226	–	NSW, 2017	Kiss et al. 2020
P. xanthii	*Trema tomentosa*	BRIP 70495	MT174229	–	QLD, 2019	Kiss et al. 2020
P. xanthii	*Glandularia aristigera*	BRIP 70491	MN190028	–	QLD, 2019	Crous et al. 2019
P. xanthii	*Dahlia pinnata*	BRIP 68846	MT174227	–	QLD, 2018	Kiss et al. 2020
Pseudoidium kalanchoes	*Kalanchoe blossfeldiana*	HAL 3298	–	MK411006	Germany, 2018	Crous et al. 2020
Salmonomyces javanicus	*Acalypha wilkesiana*	BRIP 68804	–	MT133550	QLD, 2018	Kiss et al. 2020

[a] Dashes indicate missing data.

isolate, and all developed heavily sporulating and expanding colonies on black gram leaves at later stages of development. Similarly, HR was sometimes even detected on the leaves of the original hosts of all three powdery mildew isolates (i.e., mungbean cultivar Jade-AU plants) soon after inoculations. However, these reactions did not cease the development of heavily sporulating powdery mildew colonies on cultivar Jade-AU at later stages of development.

The *Erysiphe* isolate BRIP 71005 collected from mungbean induced an HR in cowpea and no response in soybean. This isolate was not able to overcome the HR provoked in cowpea and develop sporulating colonies on its leaves. Inoculations of cowpea and soybean with the *P. xanthii* isolate also resulted in HRs, which were overcome by *P. xanthii*, and the isolate was able to develop scarce, poorly sporulating colonies on approximately 80% of the test cowpea and soybean plants. *E. diffusa* infected black gram and always produced an HR in mungbean (Fig. 7). *E. diffusa* was able to overcome the HR and establish small, sparse, and weakly sporulating colonies on mungbean leaves. ITS sequencing confirmed the identity of the *Erysiphe* isolates at the end of each cross-inoculation test because the ITS sequences of isolates BRIP 71005 and BRIP 71598 originating from mungbean differed in four conserved nucleotide positions from that of the *E. diffusa* isolate BRIP 71011 that originated from soybean.

Phylogenetic analyses of nrDNA sequences. The ITS sequences determined in this study in all *Podosphaera* specimens included in the DNA work were identical to several *P. xanthii* ITS sequences available in GenBank, including MT174226, MT174230, and MT472035, reported as *P. xanthii* from squash (*Cucurbita maxima* Duch. ex Lam.), mungbean, and cowpea, respectively (Fig. 8). Other ITS sequences available in GenBank for *P. xanthii* differed in one or more nucleotide positions, but all *P. xanthii* specimens included in our analysis grouped into a single clade with a Bayesian posterior probability (PP) of 1.0 and an ML bootstrap (BS) probability of 91% (Fig. 8).

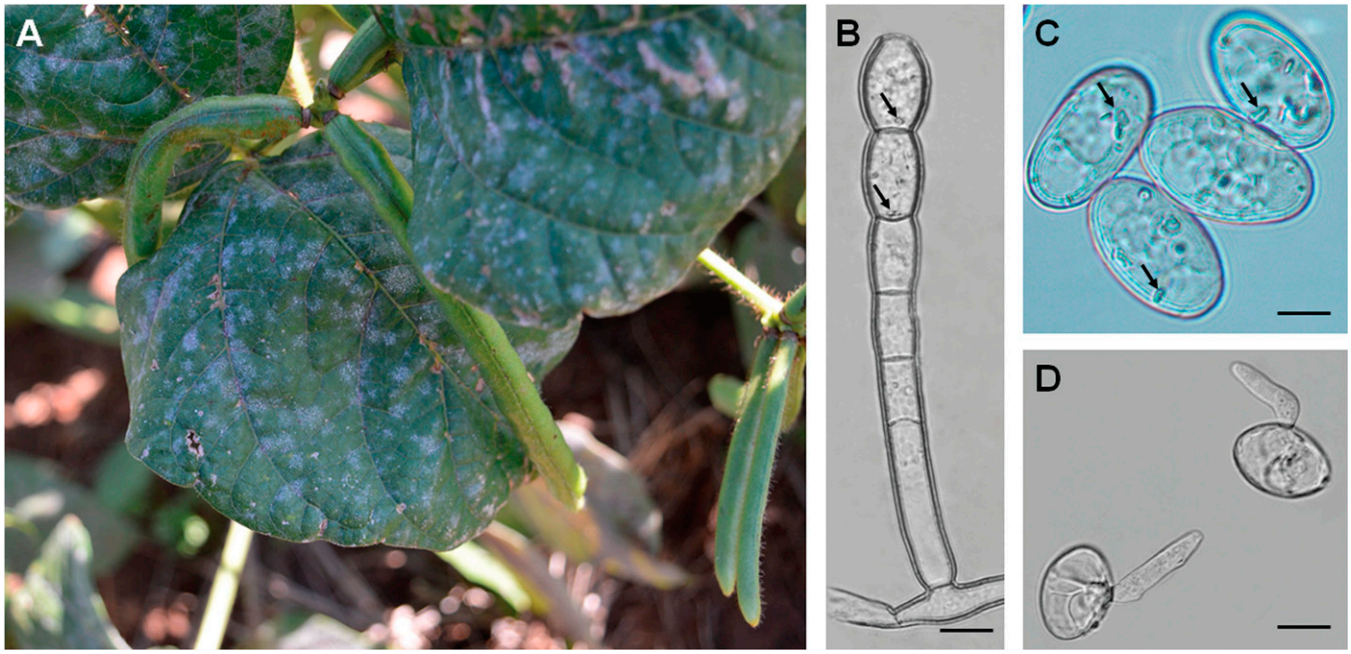


Fig. 3. Mungbean infected with *Podosphaera xanthii* in a commercial paddock near Killarney, Queensland, Australia in 2017 (specimen: BRIP 71599). **A,** Symptoms on leaves. **B,** A conidiophore mounted in water; arrows point to fibrosin bodies. Bar = 10 µm. **C,** Conidia in water. Arrows point to fibrosin bodies. Bar = 10 µm. **D,** Two conidia germinated on sterile cellophane in 48 h. Bar = 20 µm.

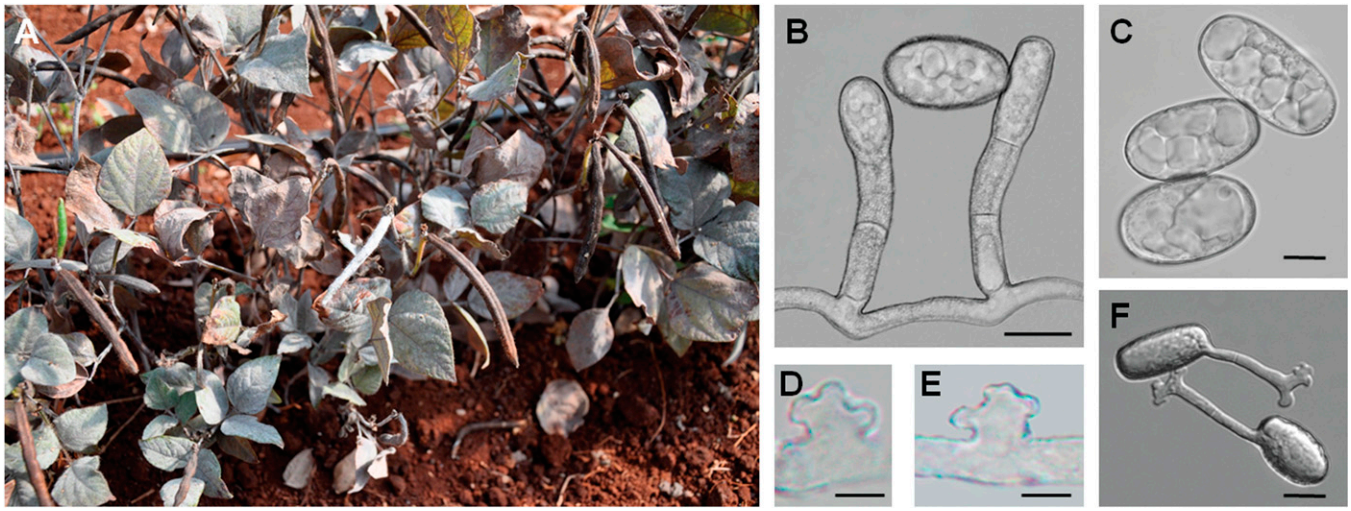


Fig. 4. Mungbean cultivar Jade-AU naturally infected with *Erysiphe vignae* in an experimental field site in Toowoomba, Queensland, Australia in 2019 (specimen: BRIP 71005). **A,** Heavily infected plants. Note symptoms on leaves, stems and pods. **B,** Two conidiophores and a detached conidium mounted in water. Bar = 20 µm. **C,** Conidia in water. Bar = 10 µm. **D and E,** Lobed hyphal appressoria. Bar = 5 µm. **F,** Two conidia germinated on glass in 48 h. Bar = 20 µm.

The ITS sequences determined in this study in all *Erysiphe* specimens from mungbean and black gram were identical. As of 20 October 2020, a BLAST search identified five other ITS sequences in GenBank that were identical to these sequences: KY515231 and AY739109 came from powdery mildews infecting cowpea and common bean (*Phaseolus vulgaris* L.), respectively, in Brazil; MG171170 was found in powdery mildew on soybean in China; KU320678 was found in powdery mildew infecting common bean in Spain; and MT878222 was found in a powdery mildew deposited as *E. diffusa* without further specifications of the host plant and geographic origin.

The ITS sequences of all *E. diffusa* specimens collected from soybean in this study were identical to those from soybean in the United States (AB078811) and Australia (MT174189, MT174190, and MT174191), and they differed in four conserved single-nucleotide polymorphisms from the ITS sequences of the *Erysiphe* specimens originating from mungbean and black gram.

The *Erysiphe* specimens from mungbean and black gram, together with the other five specimens with identical ITS sequences in GenBank, grouped together in a clade, with a Bayesian PP of 0.98 and an ML BS of 99%. This was the sister clade of the one consisting of eight *E. diffusa* sequences, with a Bayesian PP of 0.81 and ML BS of 97% (Fig. 9).

The LSU sequences of two *Erysiphe* specimens collected from mungbean in this study, BRIP 71005 and BRIP 71598, were identical and differed from the LSU sequence of *E. diffusa* BRIP 71011 by two conserved single-nucleotide polymorphisms. The LSU phylogeny (Fig. 10) supported the ITS analysis of the *Erysiphe* specimens (Fig. 9) and revealed that the *Erysiphe* sp. found on mungbean and black gram in Australia differs from *E. diffusa* on soybean.

***TUB2* gene sequence analysis.** The 981-bp-long *TUB2* sequences determined in a *P. xanthii* specimen from mungbean, BRIP 71599, and deposited in GenBank, were identical to the corresponding fragment of the *TUB2* gene available for *P. xanthii* in GenBank under accession no. KC333362, reported by Vela-Corcía et al. (2014). That study did not find any intraspecific variation in this *TUB2* fragment of 17 *P. xanthii* isolates originating from melon, zucchini, pumpkin, and cucumber (Vela-Corcía et al. 2014). Our results indicate that this part of the *TUB2* gene cannot be used to distinguish *P. xanthii* isolates infecting mungbean, a noncucurbitaceous host, from those infecting cucurbits. Therefore, the *TUB2* sequences determined in this work were not included in further analyses.

Taxonomy. Light microscopy studies and phylogenetic analyses of powdery mildew specimens collected from mungbean and black gram in Australia revealed the presence of two species on both crops, *P. xanthii* and an *Erysiphe* species, which differed from all known *Erysiphe* spp. based on its morphology and ITS and LSU sequences. Therefore, this species is described here as a new taxon, *Erysiphe vignae* L. Kelly, L. Kiss & Vaghefi, sp. nov.

Etymology. Name refers to the genus *Vigna*, from which this obligate biotrophic fungus was isolated.

Classification: Erysiphaceae, Erysiphales, Leotiomycetes. Mycelium on leaves, epiphytic, amphigenous, producing dense, white patches on the upper and the lower leaf surfaces, stems, and young pods. Hyphae hyaline, thin-walled, 4 to 7 µm wide; hyphal appressoria lobed or multilobed. Conidiophores erect, consisting of a foot-cell, straight or occasionally slightly curved, sinuous at the base, (18–)20–36(–39) × 6–9 µm, basal septum at the branching point, followed by (0–)1–2 cells, shorter than or up to the same length as the foot-cell. Conidia produced singly, mostly ellipsoid, ellipsoid-cylindrical, or doliiform, (24–)30–40(–44) × 12–20(–22) µm. Germ tubes terminal or subterminal, shorter or 1.2–3(–5) times longer than conidia (*longitubus* pattern when four to five times longer), terminating in lobed or multilobed, or sometimes simple or knob-like appressoria. Sexual morph not observed.

Fig. 5. Black gram cultivar Regur naturally infected with *Erysiphe vignae* in an experimental field site in Toowoomba, Queensland, Australia in 2018 (specimen: BRIP 68837). **A,** Heavily infected plants. **B,** A conidiophore rehydrated from the herbarium specimen in hot lactic acid. Bar = 10 µm. **C,** A germinated and a nongerminated conidium from the herbarium specimen after rehydration in hot lactic acid. Bar = 20 µm.

Typus. Australia, Queensland, Toowoomba, –27.534456, 151.928203, on leaves, pods, and stems of *V. radiata* cultivar Jade-AU (Fabaceae), 22 March 2019, *L. Kelly* (holotype BRIP 71005, ITS and LSU sequences GenBank MT628282 and MT628017, MycoBank MB 836888).

Additional material examined. Australia, Queensland, Toowoomba, –27.607731, 151.931467, on leaves of *V. radiata* cultivar Jade-AU, 3 April 2019, *L. Kiss*, BRIP 71598, ITS and LSU sequences GenBank MW293895 and MT628018; Queensland, Warwick, –28.208014, 152.1, on stems of *V. radiata*, June 2019, *R. Snyman*, BRIP 71006 and BRIP 71007, ITS sequence GenBank MT628285; Queensland, Toowoomba, –27.607731, 151.931467, on leaves of *V. mungo* cultivar Regur, April 2018, *L. Kelly*, BRIP 68837, ITS sequence GenBank MT628284; Queensland, Gatton, –27.551375, 152.336773, on leaves of *V. radiata*, 9 August 2019, *L. Kelly* and *L. Kiss*, BRIP 71010, ITS sequence GenBank MT628286; Queensland, Pampas, –27.85, 151.46, on leaves of *V. radiata*, 8 May 2020, *L. Kelly*, BRIP 71587, *P. xanthii* also present; Queensland, North Branch, –27.88, 151.61, on leaves, stems, and pods of *V. radiata* cultivar Jade-AU, 8 May 2020, *L. Kelly*, BRIP 71577, *P. xanthii* also present.

Notes. According to ITS sequence analyses, the closest relative of *E. vignae* is *E. diffusa* infecting soybean. The two species differ especially in their conidial germination patterns because *E. vignae* produces germ tubes that are mostly shorter than the conidial length and terminate in mostly lobed to multilobed appressoria, whereas the germ tubes of *E. diffusa* are usually much longer, sometimes three to five times longer, with often swollen but not lobed ends (*longitubus* pattern). Cross-inoculation tests done in a glasshouse showed that *E. vignae* did not infect soybean cultivar Bunya. *E. diffusa* was sometimes able to establish small and sparse colonies on mungbean cultivar Jade-AU that have only rarely sporulated and always provoked a hypersensitive response in mungbean leaves. To date, *E. vignae* has been identified only in Australia, but two specimens from Brazil with ITS sequences identical to *E. vignae* (GenBank accession numbers KY515231 and AY739109) may represent records of this species on cowpea (*V. unguiculata*) and common bean (*P. vulgaris*) in South America. The ITS sequence of *E. vignae* is also identical to three more sequences available in GenBank: MG171170, a soybean powdery mildew specimen from China; KU320678, a powdery mildew specimen from common bean collected in Spain; and MT878222, a powdery mildew deposited as *E. diffusa* without further specifications of the host plant and geographic origin. These DNA sequence data may indicate that *E. vignae* has a global distribution on diverse fabaceous hosts.

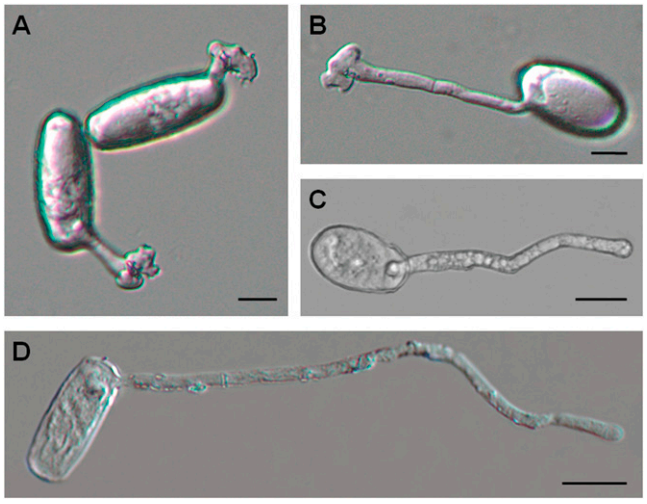


Fig. 6. Conidial germination patterns in *Erysiphe vignae* isolate BRIP 71005 from mungbean and *E. diffusa* isolate BRIP 71011 from soybean. **A and B,** Conidia of *E. vignae* germinated on glass in 48 h. Bar = 10 µm. **C and D,** Conidia of *E. diffusa* germinated on sterile cellophane in 48 h. Note the longitubus pattern in **D**. Bar = 20 µm.

DISCUSSION

To provide an accurate identification of the powdery mildew species infecting mungbean, black gram, and wild mungbean in Australia, we examined all specimens available in Australian plant pathology herbaria and collected in Queensland, Western Australia, and the Northern Territory from 1965 to 2018, and we identified every specimen as *P. xanthii* based on conidiophore morphology after

Fig. 7. Hypersensitive response on mungbean cultivar Jade-AU caused by *Erysiphe diffusa* isolate BRIP 71011 collected from soybean.

TABLE 3. Results of cross-inoculation experiments repeated two to four times in isolation in glasshouse experiments

Host plant	*Erysiphe vignae* isolate BRIP 71005	*E. vignae* isolate BRIP 71598	*E. diffusa* isolate BRIP 71011	*Podosphaera xanthii* isolate BRIP 71599
Vigna radiata cultivar Jade-AU	+	+	HR[a]	+
V. mungo cultivar Onyx-AU	+	ND[b]	+	+
V. mungo cultivar Regur	+	ND	+	ND
V. unguiculata cultivar Caloona	HR	ND	ND	+/–, HR
Glycine max cultivar Bunya	–	–	+	+/–, HR

[a] HR, hypersensitive response.
[b] ND, not determined.

rehydration and ITS sequences. *E. vignae* was first found on black gram in 2018 and then on black gram and mungbean in 2019 and 2020, sometimes on the same leaves and stems of both crops. It is likely that *E. vignae* was present in the field before 2018 but remained unnoticed. Our targeted surveys in 2020 revealed that both species, *P. xanthii* and *E. vignae*, were present throughout Queensland in both mungbean and black gram fields, often living simultaneously on the same leaves. Clearly, the number of mungbean and black gram powdery mildew specimens available in Australian herbaria is insufficient to conclude that *E. vignae* has only recently appeared in these crops. Legumes may host a number of cryptic powdery mildew species; for example, *E. medicaginis* L. Kiss, L. Kelly & Vaghefi has only recently been described from burr medic (*Medicago polymorpha* L.) despite its widespread occurrence in Australia and probably also elsewhere (Crous et al. 2020).

This study supported previous research that identified *P. xanthii* as the causal agent of powdery mildew in mungbean in Australia (Cunnington et al. 2004) and India (Gautam and Avasthi 2018) and provided further evidence of its occurrence on mungbean in China, Taiwan, and India. *P. xanthii* is known to have an extremely wide host range and has recently been identified on nine plant species in Australia, including natives (Kiss et al. 2020). Previous studies concluded that *P. xanthii* can be regarded as a species complex comprising multiple races that could each be specialized to distinct hosts or have broad host ranges (Braun et al. 2001; Hirata et al. 2000). Cucurbits are the best-known hosts of *P. xanthii* (Pérez-Garcia et al. 2009; Polonio et al. 2021; Vela-Corcía et al. 2014). More research is needed to determine whether multiple races of *P. xanthii* are present in Australia, each with limited host ranges, or whether *P. xanthii* infecting mungbean and black gram is also causing disease on cucurbits and other hosts. Gaining a greater understanding of the potential host range of *P. xanthii* and *E. vignae* in Australia will aid in the development of integrated disease management strategies for mungbean and black gram growers.

Outside Australia, *E. polygoni* has also been reported as one of the causal agents of mungbean powdery mildew (Kasettranan et al. 2009; Reddy 2009). Apparently, *E. polygoni* was the pathogen in most breeding works targeting powdery mildew resistance in mungbean in Asia (Chankaew et al. 2013; Gawande and Patil 2003; Kasettranan et al. 2009; Sujatha and Kajjidoni 2013; Yundaeng et al. 2020), although none of those breeding projects provided any conclusive morphological or molecular evidence to support the identity of the powdery mildew species. Recently, the causal agent of black gram powdery mildew in India was identified as *E. polygoni* based on its ITS sequence (Channaveerresh and Kulkarni 2017); however, the closest match for that ITS sequence is an entry for *Alternaria* in GenBank. Nevertheless, two true *Erysiphe* ITS sequences from India are available in GenBank: one from black gram powdery mildew (MN507405) and one from mungbean powdery mildew (MH208718), both deposited as *E. polygoni*. The

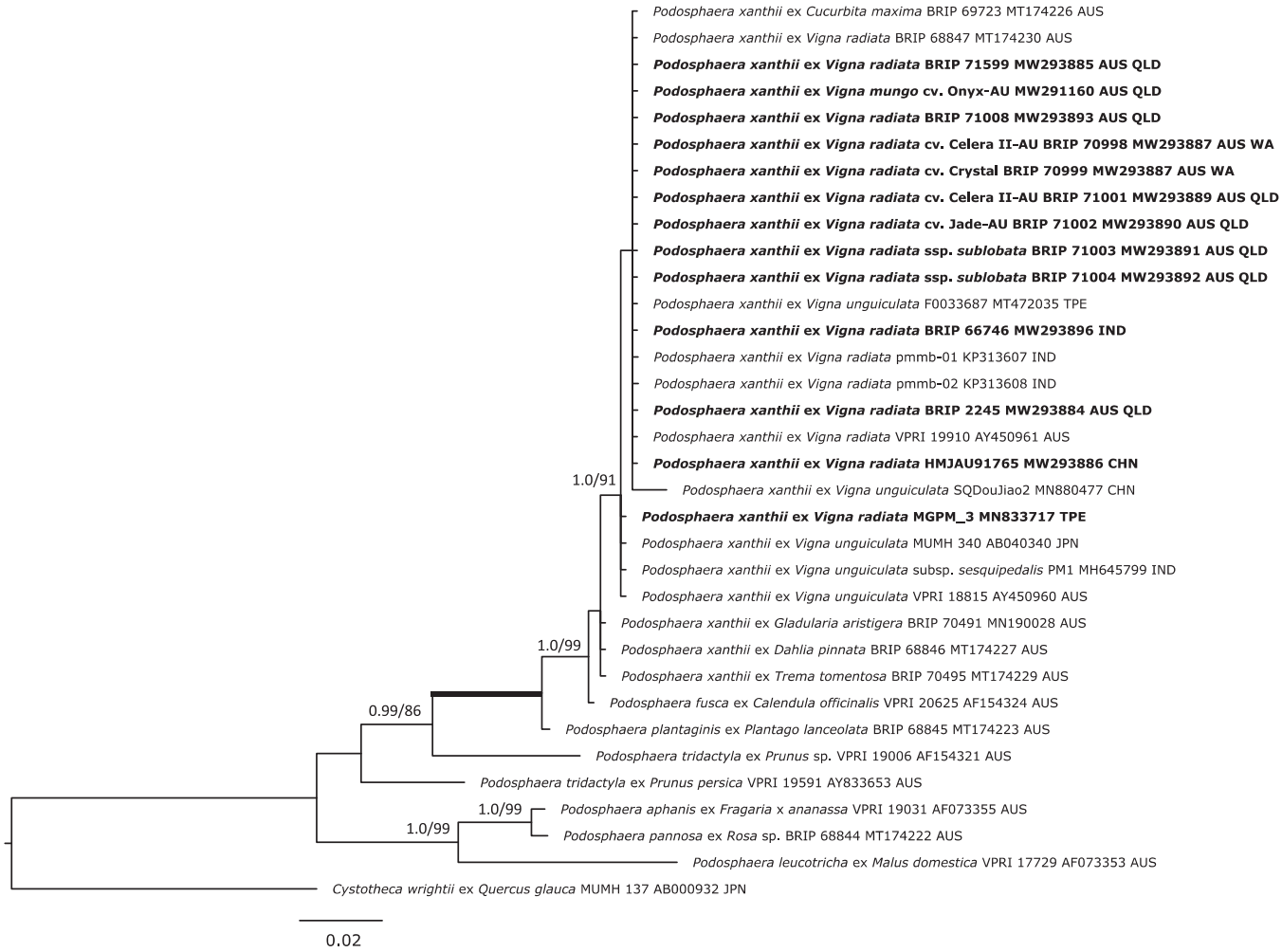


Fig. 8. Maximum likelihood phylogram based on the internal transcribed spacer (ITS) sequences of the nuclear ribosomal DNA of powdery mildew specimens belonging to the genus *Podosphaera*. The specimens collected in this study (Table 1) are in bold. All other specimens were obtained from GenBank (Table 2). The tree is rooted to the ITS sequence of *Cystotheca wrightii* MUMH 137. The herbarium accession number or strain identifier, GenBank accession number of the ITS sequence, and country and state codes are shown for each specimen where available. Maximum likelihood bootstrap values >80% and Bayesian posterior probability values >0.80 are shown above the branches. Thick branches represent maximum likelihood bootstrap values of 100% and Bayesian posterior probability of 1.00. The scale bar represents nucleotide substitutions per site.

two sequences are 99% similar to each other but differ in >100 nucleotide positions from the ITS sequence of an authentic *E. polygoni* specimen from *Polygonum aviculare*, MUMH 7045 (GenBank accession no. LC328323). These two sequences, MN507405 and MH208718, also exhibit approximately the same genetic distance from the ITS sequence of *E. vignae* and therefore were not included in our phylogenetic analyses and may indicate that a different *Erysiphe* sp. infects these pulse crops in India. *P. xanthii* has also been confirmed on mungbean in India by at least two ITS sequences available in GenBank, KP313607 and KP313608.

Powdery mildew is the only mungbean disease that occurs regularly and at high levels in all areas of production across Australia. When symptoms develop before flowering under conducive environmental conditions, the pathogens that cause powdery mildew are capable of reducing yields by >40% in susceptible cultivars (Kelly et al. 2017; Thompson 2016; Weir et al. 2017). The management of powdery mildew in mungbean in Australia has relied largely on the application of fungicides. Tebuconazole, a sterol demethylation inhibitor fungicide, has traditionally been used by mungbean growers to manage powdery mildew throughout Queensland. A second fungicide, Custodia (active ingredients 120 g/liter of azoxystrobin and 200 g/liter of tebuconazole) has been available to mungbean growers since 2016. Field trials across southern Queensland have demonstrated that the greatest yield benefit occurred when the fungicides were applied at the first sign of disease and then again 14 days later (Kelly et al. 2017; Thompson 2016; Weir et al. 2017), but despite this recommendation, many growers apply fungicides before the first sign of disease. Overreliance on these fungicides may lead to fungicide resistance developing in the pathogen population. Resistance to demethylation inhibitor fungicides has been detected in several fungi, including *P. xanthii* infecting cucurbits (López-Ruiz et al. 2010; Vielba-Fernández et al. 2020).

In this study, all powdery mildews were identified based on morphological characters and ITS and LSU sequences. Despite recent advancements in DNA barcode development for many fungi, ITS sequences are still the most reliable molecular tool for identification of powdery mildew species (Kiss et al. 2020). However, accurately identifying closely related powdery mildew species from ITS sequences alone can be difficult (Bradshaw and Tobin 2020; Ellingham et al. 2019; Jankovics et al. 2008). New DNA barcodes that may distinguish *P. xanthii* and *E. vignae* from other closely related *Podosphaera* and *Erysiphe* species, respectively, are clearly needed.

The main goal of the host range testing in this study was to clarify whether the *Erysiphe* isolates found on mungbean and black gram came from soybean and were, in fact, *E. diffusa*, the well-known soybean powdery mildew pathogen. Cross-inoculation tests

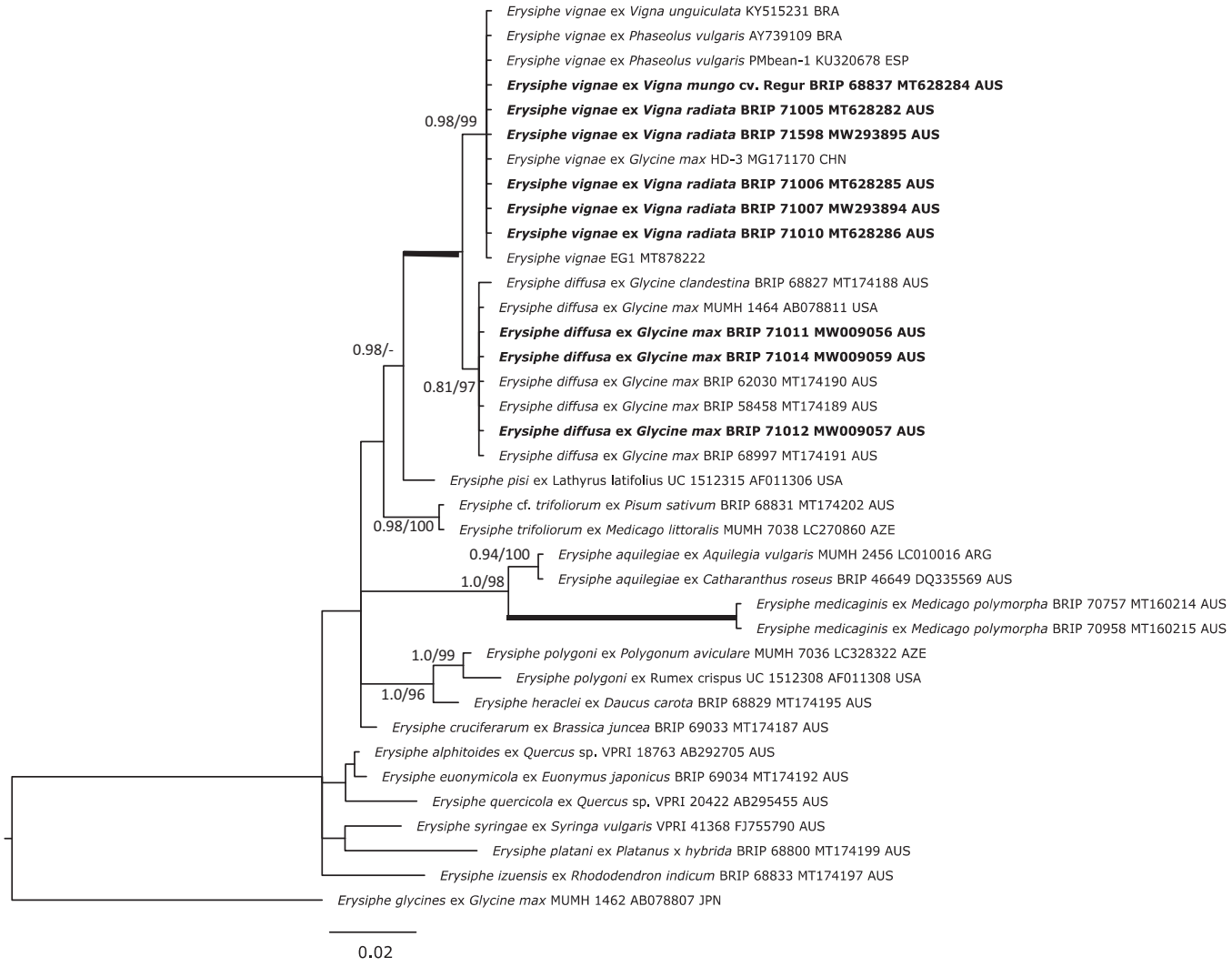


Fig. 9. Maximum likelihood phylogram based on the internal transcribed spacer (ITS) sequences of the nuclear ribosomal DNA of powdery mildew specimens belonging to the genus *Erysiphe*. The specimens collected in this study (Table 1) are in bold. All other specimens were obtained from GenBank (Table 2). The tree is rooted to the ITS sequence of *E. glycines* MUMH 1462. The herbarium accession number or strain identifier, GenBank accession number of the ITS sequence, and country and state codes are shown for each specimen where available. Maximum likelihood bootstrap values >80% and Bayesian posterior probability values >0.80 are shown above the branches. Thick branches represent maximum likelihood bootstrap values of 100% and Bayesian posterior probability of 1.00. The scale bar represents nucleotide substitutions per site.

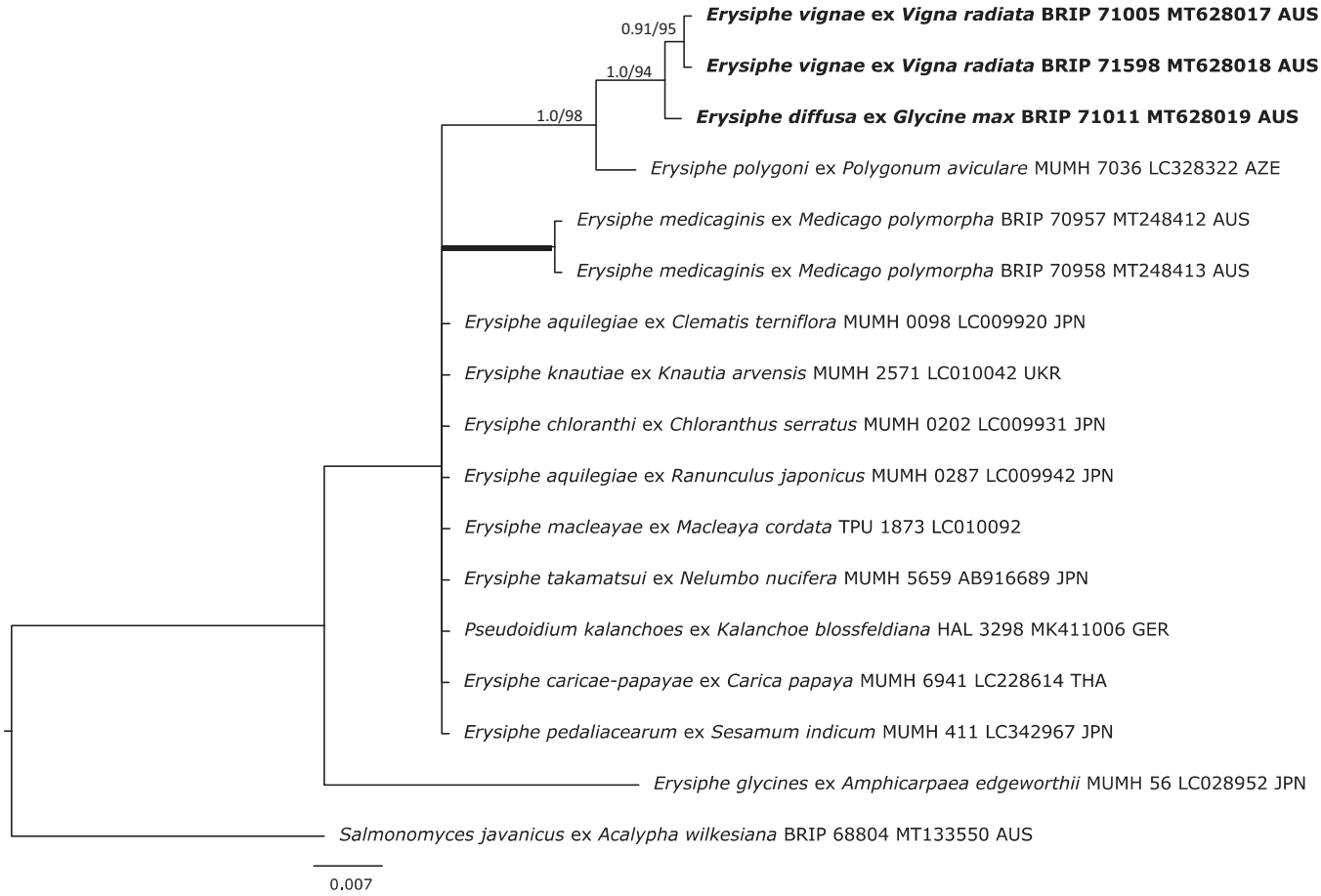


Fig. 10. Maximum likelihood phylogram based on the large subunit (LSU or 28S) sequences of the nuclear ribosomal DNA of powdery mildew specimens belonging to the genus *Erysiphe*. The specimens collected in this study (Table 1) are in bold. All other specimens were obtained from GenBank (Table 2). The tree is rooted to the ITS sequence of *Salmonomyces javanicus* BRIP 68804. The herbarium accession number or strain identifier, GenBank accession number of the ITS sequence, and country and state codes are shown for each specimen where available. Maximum likelihood bootstrap values >80% and Bayesian posterior probability values >0.80 are shown above the branches. Thick branches represent maximum likelihood bootstrap values of 100% and Bayesian posterior probability of 1.00. The scale bar represents nucleotide substitutions per site.

did not support this presumption because *E. vignae* was unable to infect soybean, whereas *E. diffusa* always provoked a hypersensitive response in mungbean leaves and could only rarely overcome that reaction to produce some sparse colonies, which rarely sporulated. On the other hand, *E. diffusa* was able to infect and sporulate on black gram. *E. vignae* and *E. diffusa* were always distinguished in our cross-inoculation tests based on the differences in their ITS and LSU sequences. Cowpea was included in the host range testing because a GenBank entry, KY515231, determined in a cowpea powdery mildew specimen in Brazil was identical to the ITS sequence of *E. vignae*. However, during the cross-inoculation tests *E. vignae* induced hypersensitive response in cowpea leaves without being able to establish sporulating colonies there.

A hypersensitive response occurs when plant cells located at the infection site rapidly die, and it is recognized as a host resistance mechanism (Lam et al. 2001). This reaction is thought to occur during incompatible host–pathogen interactions. Although *E. diffusa* was sometimes able to establish sparse, mostly nonsporulating colonies on some of the inoculated mungbean leaves, the experiments did not indicate that mungbean is its host. These results have also supported the delimitation of *E. vignae* from *E. diffusa*, in addition to the phylogenetic analyses of the ITS and LSU sequences and morphological differences, particularly differences in conidial germination patterns.

In Australia, the National Mungbean Improvement Program has recently released cultivar Opal-AU with a rating of moderately resistant. The mungbean cultivar Jade-AU, which has been the most widely grown cultivar, is rated as moderately susceptible. With the recent discovery of *E. vignae*, it is not clear whether this resistance rating is relevant to one or both pathogens. This warrants further investigation.

This study has revealed that both *P. xanthii* and the newly recognized *E. vignae* are responsible for causing powdery mildew in mungbean and black gram in Australia. *P. xanthii* was particularly common in mungbean and black gram paddocks in all years when specimens were collected. In this study, *P. xanthii* was also identified in mungbean specimens from China, Taiwan, and India. *E. polygoni*, reported as the causal agent of mungbean and black gram powdery mildew outside Australia, was not found on these *Vigna* spp. hosts during this study. ITS sequences available in GenBank indicate that *Erysiphe* isolates do infect mungbean and black gram in Asia, Spain, and Brazil; these may represent two species, one of which is likely to be *E. vignae*, at least in Spain and Brazil. In Australia, the disease resistance component of the mungbean breeding program may be complicated by the presence of two powdery mildew species in the field instead of just one. The efficacy of fungicide treatments could also be compromised if one pathogen species is more likely to develop fungicide resistance. This study demonstrates the complexity of crop disease diagnostics even in easily recognized foliar diseases of broad-acre crops.

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