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First report of powdery mildew of rainforest spinach (*Elatostema reticulatum*), native to Australia, caused by *Podosphaera xanthii*

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Abstract
Powdery mildew-infected rainforest spinach (*Elatostema reticulatum*) plants were found in Queensland, Australia. Based on morphology and nrDNA ITS sequence analyses, the pathogen was identified as *Podosphaera xanthii*, a powdery mildew species with a worldwide distribution and a broad host range. This is the first record of *P. xanthii* infecting *E. reticulatum*, and the first powdery mildew infection recorded for this native Australian plant.

Keywords Bunya Mountains · Host range · Erysiphaceae · Native Australian plant

Rainforest spinach (*Elatostema reticulatum*) is an evergreen, herbaceous perennial plant native to eastern Australia. It is often found along rainforest creeks in Queensland and New South Wales. Unlike many other members of the nettle family (Urticaceae), *E. reticulatum* does not have stinging hairs, and the leaves and stems are sometimes harvested from the wild for local consumption (Fern 2020). In December 2019, powdery mildew infection was observed on the leaves of *E. reticulatum* populations grown in abundance along streams in Bunya Mountains National Park, Queensland, Australia. Whitish powdery mildew mycelium covered some parts of the abaxial leaf surfaces (Fig. 1a) in three sites, up to 1 km apart from each other. Infected leaves were collected from each site, kept separately to avoid cross-contaminations, and taken to the laboratory for further investigations. The sites were revisited in January and March 2020, and powdery mildew-infected plants observed each time at all sites. Powdery mildew-infected leaves were collected each time from each site to examine the pathogen species in the laboratory. A specimen collected in December 2019 was deposited at the Queensland Plant Pathology Herbarium (Brisbane, Australia) under accession number BRIP 70997.

The morphological characteristics of the pathogenic fungus were observed in the laboratory under a Nikon Eclipse Ni-U microscope (Nikon Co., Tokyo, Japan) with bright field and differential interference contrast (DIC) optics. To examine fresh conidia, microscope slides were gently touched to young powdery mildew colonies on *E. reticulatum* leaves collected from the field, then water was pipetted onto conidia on the slides, and covered with a cover slip before microscopic examination. To observe conidial germination patterns on a glass surface, fresh conidia were incubated for 48 h on microscope slides kept in 15 cm diameter plates, on glass rods placed on wet paper tissue. Plates were closed, sealed with Parafilm®, and kept at room temperature. Following incubation, a droplet of water was pipetted onto conidia on slides, covered with a cover slip, and slides were examined under the microscope. To examine hyphae and conidiophores, parts of fresh mycelia were removed from the leaf surface with 3–4 cm long pieces of clear cellotape. Cellotape pieces were placed with mycelia downwards in a droplet of water pipetted onto a microscope slide, and examined under the microscope.

Light microscopy revealed that hyphae were 4–9 µm wide, septate, with indistinct hyphal appressoria. Conidiophores consisted of a straight and cylindrical foot-cell, arising from the upper surface of the hyphal mother cells, and slightly constricted at the basal septum located at the branching point, 35–95 × 9–13 µm, followed by one to three shorter cells, and conidia produced in true chains (Fig. 1b). The edge lines of conidial chains were crenate as defined by Shin and La (1993). Conidia were ellipsoid-ovoid to doliiform, 27–47 × 13–23 µm (n = 30), with length/width ratios of 1.65–1.93, and contained crystalline cell inclusions known as fibrosin bodies (Braun and Cook 2012)

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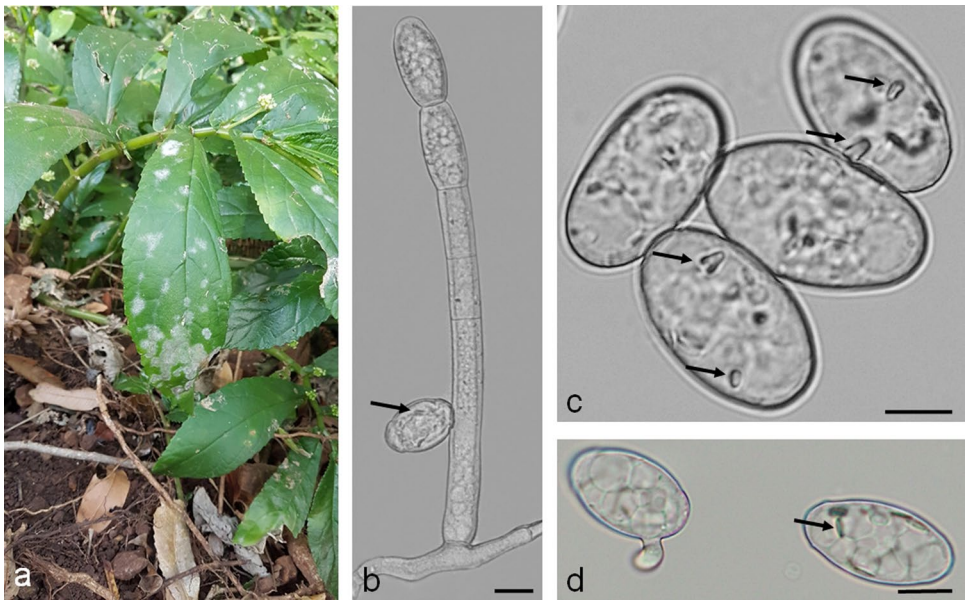

Fig. 1 Powdery mildew on rainforest spinach (*Elatostema reticulatum*). **a** Leaves infected with powdery mildew in Bunya Mountains National Park, Queensland, Australia. **b** A conidiophore of *Podosphaera xanthii* removed from an *E. reticulatum* leaf with cellotape, and mounted in water. **c** Conidia of *P. xanthii* mounted in water. **d** A germinating and a non-germinating conidium of *P. xanthii*. Arrows point to fibrosin bodies in conidia. Bars = 10 µm. Images were edited using Adobe Photoshop

(Fig. 1c). Following incubation for 48 h on glass slides, up to 27% of conidia produced short and simple or forked germ tubes arising laterally or sub-terminally, with simple apices (Fig. 1d). This conidial germination pattern was described as the brevitubus subtype of the Fibroidium type (Cook and Braun 2009). All these morphological characteristics were diagnostic for the powdery mildew species *P. xanthii* (Braun and Cook 2012).

To support the identification of the pathogen, total genomic DNA was extracted from fresh powdery mildew mycelial samples removed from the host plant surfaces of specimen BRIP 70997 with 1–1.5 cm^2 pieces of cellotape. Cellotape pieces with mycelia were placed in 1.5 ml eppendorf tubes and DNA was extracted using the Extract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) of the powdery mildew fungus was amplified from three DNA samples during a nested PCR protocol as described by Kiss et al. (2020). The first PCRs used the powdery mildew-specific primers PMITS1 and PMITS2 developed by Cunnington et al. (2003). The nested reactions were done with the universal fungal primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). PCR products of the nested reactions were purified and sequenced by Macrogen Inc. (Seoul, Korea) with primers ITS1-F and ITS4. Sequences were compiled from chromatograms following visual inspections for potential polymorphisms to identify any potential intra-sample variations in the nrDNA ITS sequences reported in some powdery mildews (Kovács et al. 2011). Consensus sequences were trimmed and assembled with Geneious Prime 2019.1.3 (Biomatters Ltd.), and a sequence deposited in NCBI GenBank under accession number MW282169. This was identical to 15 *P. xanthii* ITS sequences reported from mungbean (*Vigna radiata*), black gram (*V. mungo*), squash (*Cucurbita maxima*), watermelon (*Citrullus lanatus*) and *Cephalotus follicularis* in Australia (Cunnington et al. 2004, 2008; Kiss et al. 2020; Kelly et al. 2021), and also identical to over 100 other *P. xanthii* ITS sequences available in GenBank, and obtained from powdery mildews infecting diverse plant species overseas.

To reveal the phylogenetic relationship of *P. xanthii* infecting *E. reticulatum* with other *P. xanthii* specimens collected from diverse host plants, and other *Podosphaera* spp., the ITS sequence of BRIP 70997 was analysed together with reference sequences of *Podosphaera* spp. obtained from GenBank. Multiple sequence alignment was constructed using MAFFT v. 7.450 (Katoh and Standley 2013). Bayesian analysis was conducted in MrBayes v. 3.2.7 (Ronquist et al. 2012) based on the GTR+I+G nucleotide substitution model selected using MrModeltest v. 2.4 (Nylander 2004) and PAUP v. 4.0a169 (Swofford 2003). Two Markov Chain Monte Carlo (MCMC) chains were run, one tree was saved per 100 generations, and the run was ended when the standard deviation of split frequencies reached below 0.01. A second measure of branch support was obtained through Maximum Likelihood analysis of the same alignment using RAxML v. 8 (Stamatakis 2014) based on the GTR substitution model with gamma-distribution rate variation. The analysis confirmed that *P. xanthii* on *E. reticulatum* and other host plants are conspecific based on their ITS sequences (Fig. 2).

Podosphaera xanthii has an exceptionally wide host range, infecting cucurbits (Perez-Garcia et al. 2009; Polonio et al. 2020), legumes (Kelly et al. 2021), ornamentals (Kiss et al. 2008), and many other, only distantly related plants (Hirata et al. 2000; Braun and Cook 2012; Meeboon et al. 2016).

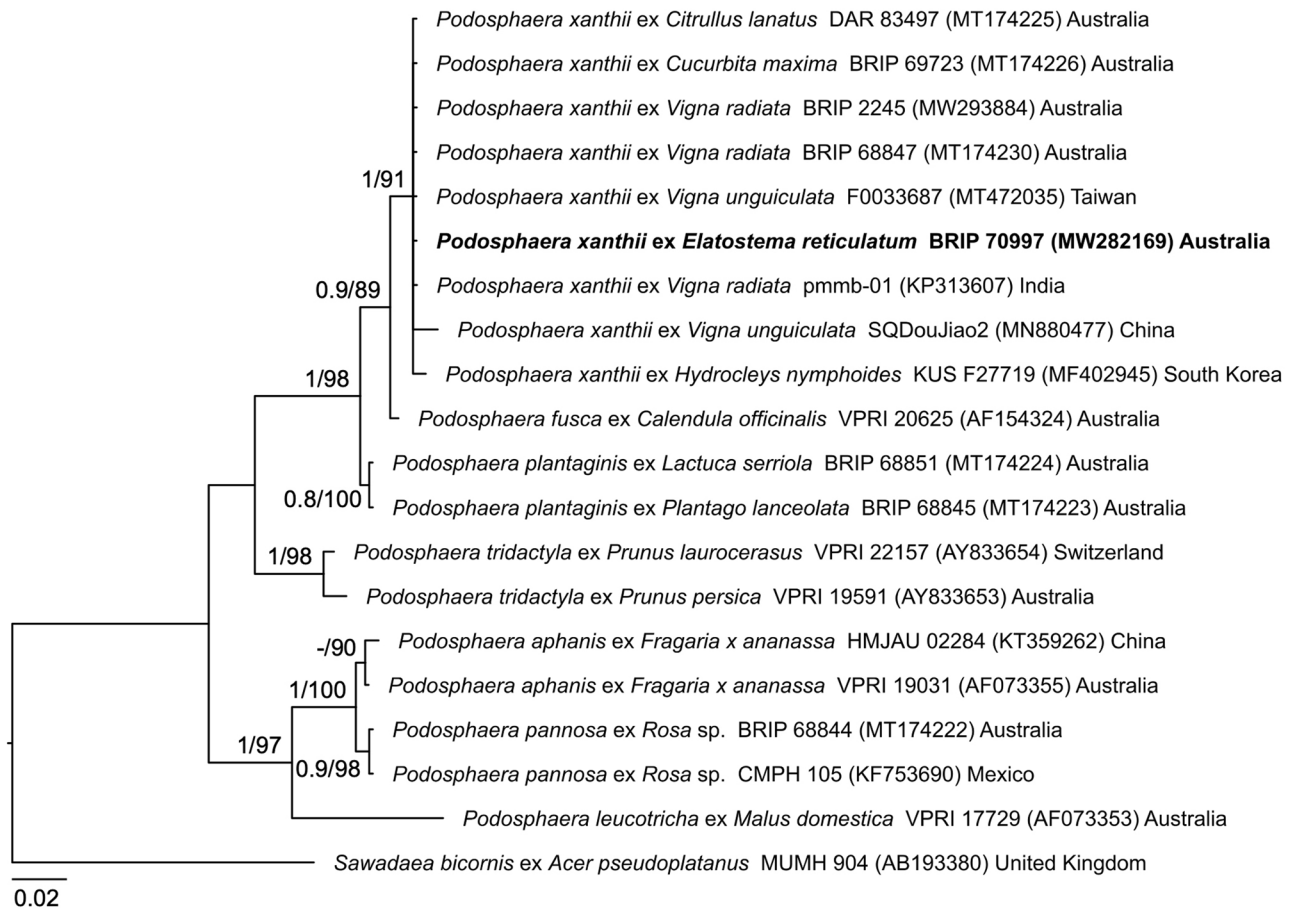

Fig. 2 Majority rule (50%) Bayesian phylogram based on the internal transcribed spacers of the nuclear ribosomal DNA and the intervening 5.8S region for *Podosphaera* species. Herbarium specimen or other accession numbers are shown for each entry, followed by GenBank accession numbers in parentheses, and countries of origin. The specimen sequenced in this study is in bold. Posterior Probability values > 0.80 and bootstrap support values > 70% are shown above or below branches. The tree is rooted to *Sawadaea bicornis* MUMH 904. The scale bar represents nucleotide substitutions per site

It was recorded on two Australian natives, *C. follicularis* (Cunnington et al. 2008) and *Trema tomentosa* (Kiss et al. 2020), as well. This is the first report of *P. xanthii* on a third Australian native plant, *E. reticulatum*. To our knowledge, there is only one single record of a powdery mildew on any *Elatostema* species globally: Amano (1986) listed a report of an unidentified *Oidium* sp. on *E. sessile* in Java, Indonesia. A recent analysis of all powdery mildews identified in Australia based on DNA barcodes indicated that all the species of this large group of plant pathogens, the Erysiphaceae, have been introduced to Australia since 1788, the beginning of the European colonisation of the continent, and powdery mildew infections of all native plants in Australia may have been the result of recent host range expansion events of some introduced, polyphagous species, such as *P. xanthii* (Kiss et al. 2020). Host range expansions have been recognised as important events during the evolution of some powdery mildews (Vági et al. 2007; Menardo et al. 2016; Frantzeskakis et al. 2019). It appears that the powdery mildew infection of *E. reticulatum* is a new example of a host range expansion of *P. xanthii*.

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Authors' contributions LK designed and coordinated the research, collected materials, and carried out the light microscopy, DNA extraction, and ITS sequencing works. NV analysed chromatograms, performed phylogenetic analyses, and wrote the respective parts of the paper. LK wrote the other parts. Both commented on earlier versions of the manuscript, and read and approved the final paper.

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