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First report of *Pseudocercospora platanigena* in Australia

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Abstract
A distinct black leafspot with fungal growth was found on *Platanus orientalis* 'Digitata' (cut-leaf oriental plane tree) in northern Sydney, New South Wales, Australia, in February 2019. Morphological characteristics, internal transcribed spacer sequencing and phylogenetic analysis of the associated fungus identified it as *Pseudocercospora platanigena* (= *Stigmina platani*). This pathogen has previously been reported from *Pl. orientalis* (oriental plane tree) in Europe and Asia and more recently from *Pl. orientalis* 'Digitata' in New Zealand. This is the first report of *Ps. platanigena* in Australia. *Pseudocercospora platanigena* is now common on *Pl. orientalis* 'Digitata' throughout Sydney and has been found as far north as Dorrigo in northern New South Wales and west to the Blue Mountains. *Pseudocercospora platanigena* has not been observed on *Pl. orientalis* other than the cultivar 'Digitata', nor on the more commonly planted London plane tree (*Pl.* x *acerifolia*) in Australia. It has not been found in other states in Australia.

Keywords Biosecurity surveillance · Amenity trees · Invasive species · Forest pests

Plane trees (*Platanus* spp.) are among the most commonly planted amenity trees in Australia (Bennett 2020). For example, the City of Sydney has 4,000 plane tees (representing 12% of street and park trees in the local council area), the City of Adelaide has 1,200 plane trees (20% of total trees), the City of Melbourne has 5,700 plane trees (7.5% of total trees), and the City of Perth has 2,030 plane trees (13% of total trees). London plane trees (*Platanus* x *acerifolia*) are the most prevalent, constituting almost 90% of plane trees planted in Australian cities, with smaller plantings of oriental plane tree (*Pl. orientalis*), cut-leaf oriental plane tree (*Pl. orientalis* 'Digitata') and American sycamore (*Pl. occidentalis*). There is some ambiguity over the variants of *Pl. orientalis* planted in Australia (Spencer 1997), but two taxa are identified in Sydney, *Pl. orientalis* and *Pl. orientalis* 'Digitata' (Bennett 2020), with distinct leaf morphology separating them. On 1 February 2019, an arborist (KH) noticed unusual black leaf spots on a *Pl. orientalis* 'Digitata' tree in Wahroonga (northern Sydney), New South Wales, Australia, and notified the Department of Primary Industries. Previous arborist assessments of this tree indicated no evidence of unusual leaf damage in 2017 or 2018. On 4 February 2019, black leaf spots on a *Pl. orientalis* 'Digitata' tree on the central coast of New South Wales were reported to the Exotic Plant Pest Hotline by a member of the public.

Morphological characteristics of specimens were examined under a dissecting and a light microscope (Olympus BX53, DIC mounted in 3% KOH). Sprorodochia were amphigenous, generally less than 5 mm diam., singular, becoming confluent and forming extensive colonies when severe (Fig. 1). Lime-green to chlorotic leaf spots occurred on the adaxial surface, becoming necrotic light-brown leafspots with red-brown margins on both sides of the leaf (Fig. 1). Conidia were ellipsoidal to cylindrical, rounded at the apex, truncate at the base, mid- to dark-brown, commonly verrucose and 2–4 septate (usually 3), 14–28 × 7–11 µm (Fig. 1). This is consistent with the morphological description of *Pseudocercospora platanigena* (= *Stigmina platani*) by Smith and Smith (1941), Ellis (1971) and Crous and Corlett (1998).

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Fig. 1 *Pseudocercospora platanigena* on *Platanus orientalis* 'Digitata'. Typical leaf spots on (**A**) abaxial surface and (**B**) adaxial surface; (**C**) severe disease; (**D**) sporodochia on leaf (bar = 10 mm); (**E**) close-up of sporodochia (bar = 500 µm); (**F**) conidia (bar = 50 µm)

Genomic DNA was extracted from 5-day old mycelium grown on potato dextrose agar using the NucleoMag® Plant Kit (Macherey–Nagel GmbH & Co., Düren, Germany) according to the manufacturer's instructions. The nuclear ribosomal DNA internal transcribed spacer (ITS) 1 and 2 region was amplified using the primers ITS1 and ITS4 with conditions described in White et al. (1990). The software jModelTest (Posada 2008) determined that the General Time Reversible model with a gamma distribution was the most appropriate nucleotide substitution model. Randomized Axelerated Maximum Likelihood (RAxML) analysis was used to infer the phylogeny under maximum likelihood criterion for the ITS data set. Bayesian inference was used to generate posterior probabilities for consensus nodes with the Monte Carlo Markov Chain run with 5,000,000 generations, with a subsampling frequency of 1,000 generations and a burn-in length of 1,250 trees. The RAxML and Bayesian analyses were performed in Geneious Prime (Version

2021.0.3) (Kearse et al. 2012) with the RAxML (Version 4.0) and MRBAYES (Version 2.2.4) plug-ins. Resulting phylogenetic trees were visualised and edited using FigTree v1.4 (Rambaut 2012).

No major topological variations were detected between trees derived from the RAxML and Bayesian phylogenetic inferences (Fig. 2). The Australian isolate DAR84735 (GenBank OK422844) clustered with *Ps. platanigena* (Strain CBS125773) and *Ps. platanigena* (Strain CBS110755) from Taylor et al. (2003) and Vu et al. (2019), respectively. The lineage was sister to *Ps. pseudostigmina-platani* (Strain CBS131588), and the lineage was well supported with Bayesian posterior probabilities. The phylogenetic analyses confirm that the Australian DAR84735 isolate belongs to *Ps. platanigena*, which is the first identification of this species in Australia.

Surveillance of *Pl.* x *acerifolia* in Sydney from 2015 to 2018 as part of the Department of Primary Industries' forest biosecurity surveillance program (https://www.dpi.nsw.gov.au/forestry/science/forest-health) had not detected *Ps. platanigena*, but no surveys were conducted of *Pl. orientalis* 'Digitata' because of its rarity in target areas. Following the initial detection, surveillance to determine the distribution of *Ps. platanigena* was conducted from February to April 2019, primarily targeting areas with known concentrations of *Platanus* within the Sydney Basin, but also ad hoc surveillance in regional areas (Fig. 3). Trees were examined for symptoms of *Ps. platanigena* (i.e., black leaf spots), with binoculars where necessary, and suspect samples collected for morphological identification (only trees displaying symptoms were sampled). A biosecurity alert (Anon. 2019) was sent to the Botanic Gardens Biosecurity Network, local councils in Sydney and arborist networks (e.g., Institute of Australian Consulting Arborists) in February 2019 seeking reports of this new disease.

During February to April 2019, 52 *Pl. orientalis* 'Digitata' trees were surveyed in the Sydney Basin across 16 locations and 48 had *Ps. platanigena* (Fig. 3). Samples from all locations were examined morphologically to confirm field diagnosis. Observations during surveys indicated disease severity ranged from only a few leaves infected per tree through to severe leaf spots in more than half the crown causing leaf distortion and leading to premature defoliation. Of 76 *Pl.* x *acerifolia* trees and 32 *P. orientalis* trees surveyed during this period (February to April 2019), none were affected by *Ps. platanigena*. Ad hoc surveillance of *Pl. orientalis* 'Digitata' in regional areas discovered *Ps. platanigena* on five trees in Bellingen and one tree in Dorrigo on the north coast of New South Wales but not on more than 20 trees in Tamworth. A single positive report of *Ps. platanigena* on *Pl. orientalis* 'Digitata' was received via the biosecurity alert, in western Sydney, and only negative reports from *Pl. orientalis* 'Digitata' outside New South Wales (i.e., Canberra, Melbourne and Tasmania). *Pseudocercospora platanigena* was subsequently determined to be established and not technically feasible nor cost-beneficial to eradicate. Surveillance during 2020 and 2021 as part of the Department of Primary Industries' forest biosecurity surveillance

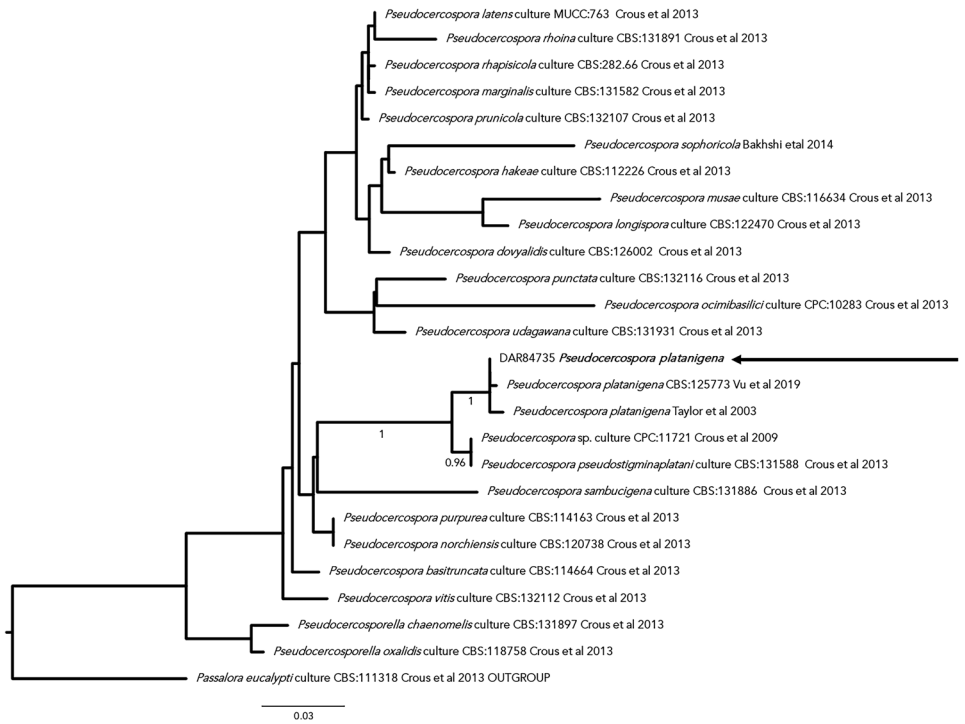

Fig. 2 RAxML tree depicting the phylogenetic relationship between the Australian isolate DAR84735 (indicated by arrow) and vouchered *Pseudocercospora* reference taxa for the ITS data set. Bayesian posterior probabilities are shown for nodes of interest

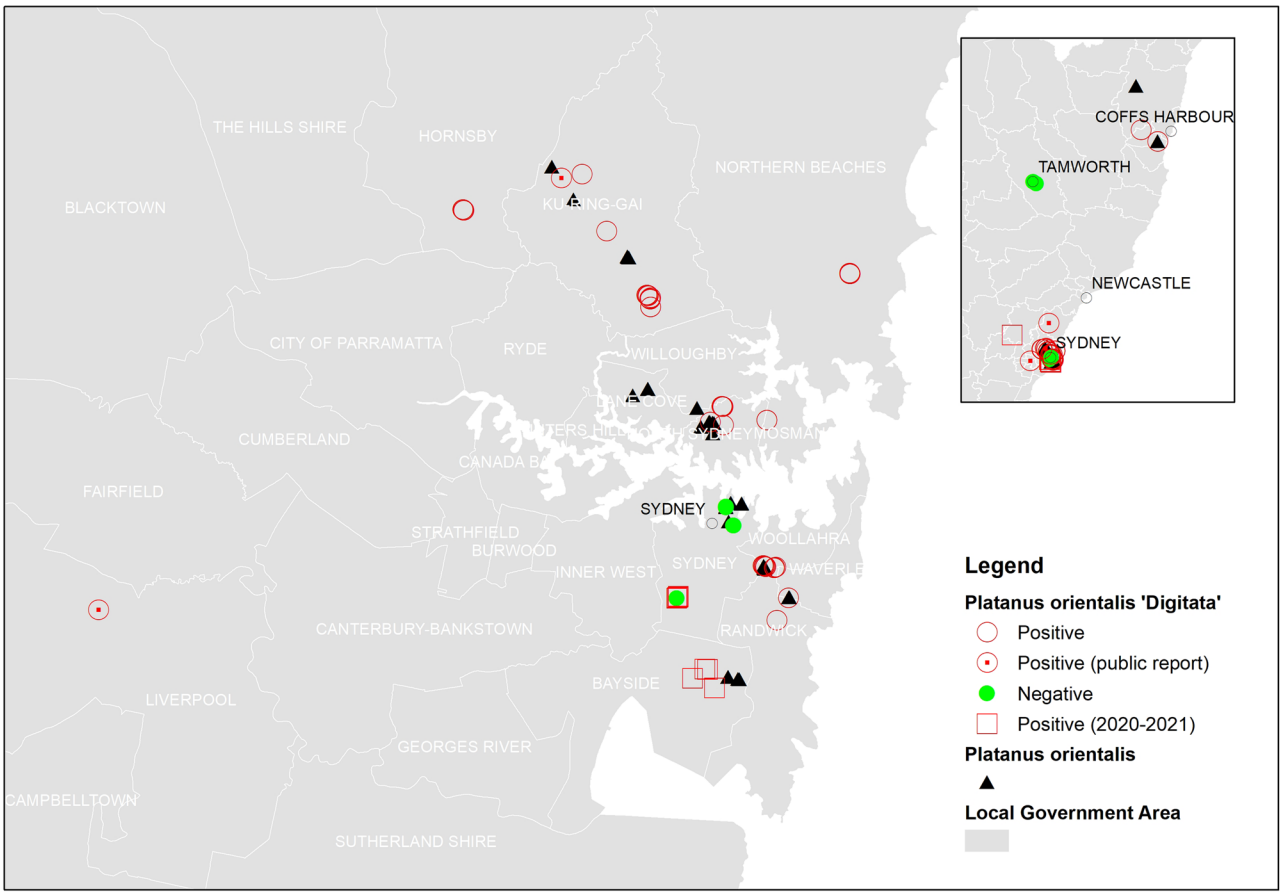

Fig. 3 Surveillance for *Pseudocercospora platanigena* in New South Wales: *Platanus orientalis* 'Digitata' with (red circle) and without (green circle) *Ps. platanigena* from 2019 surveillance; *Pl. orientalis* 'Digitata' with *Ps. platanigena* from 2020–2021 surveillance (red square); *P. orientalis* without *P. platanigena* (black triangle). [Map produced by M. Nagel, Department of Primary Industries]

program has continued to target *Platanus* spp. and revealed further *Pl. orientalis* 'Digitata' trees affected by *Ps. platanigena* within the Sydney Basin, and on 12 trees in a single location in the Blue Mountains (Fig. 3), but not on more than 670 *Pl.* x *acerifolia* nor 46 *P. orientalis* surveyed.

Three *Stigmina*-like species have been described from *Platanus*. Ellis (1971, 1976) and Crous and Corlett (1998) recognised *S. platani* and *S. platani-racemosae*, which can be distinguished on conidial morphology. Videira et al. (2017) provided a new name for *S. platani* — *Ps. platanigena* — as *Stigmina* is now recognised as a synonym of *Pseudocercospora* (Braun and Crous 2006). *Pseudocercospora pseudostigmina-platani* was described from *Pl. occidentalis* in South Korea (Crous et al. 2013). There has been no recent taxonomic revision of *S. platani-racemosae,* which is known from *Pl. racemosa* in the USA (Smith and Smith 1941; Ellis 1976). *Pseudocercospora platanigena* is known from *Platanus* in Europe, Asia and North America (Smith and Smith 1941; Ellis 1971; Farr and Rossman 2021), and more recently from New Zealand (Froud and Clover 2011). Most reports of this species are from *Pl. orientalis*. Images in Smith and Smith (1941) show *Ps. platanigena* on leaves of *Pl. orientalis* with strikingly similar morphology to *Pl. orientalis* 'Digitata' (i.e., leaves with extremely deep-cut and wide-spreading lobes with few teeth on their margins). Smith and Smith (1941) were able to infect *Pl. orientalis* with conidial suspensions of *Ps. platanigena* but not *Pl. racemosa*, *Pl. occidentalis* or *Pl.* x *acerifolia*. *Pseudocercospora platanigena* is known from *Pl. orientalis* 'Digitata' in New Zealand (Chris Inglis, pers. comm.); although listed as occurring on *Pl.* x *acerifolia* in the NZFUNGI2 Database (http://nzfungi2.landcareresearch.co.nz//), this was a misidentification of the host (B. Rogan, pers. comm.). *Stigmina platani-racemosae* is known from *Pl. racemosa* only from the USA (Smith and Smith 1941; Ellis 1976).

A review of exotic pests and diseases that have established on arborescent hosts in Australia since 1885 revealed 11 species on *Platanus* (Nahrung and Carnegie 2020). Sycamore lace bug (*Corythucha ciliata*) is common and damaging throughout New South Wales, having established in 2007 (Dominiak et al. 2019). Plane tree leaf scorch (*Apiognomonia errabunda*) and anthracnose (*Apiognomonia veneta*)

both established more than a century ago, are common in New South Wales and Victoria, and often confused with leaf scorch caused by summer drought (Marks et al. 1982; Newcastle City Council 2018). Platanus powdery mildew (*Erysiphe platani*) was first detected in 1977 (Marks et al. 1982) and can be severe in wet years such as was observed in 2020–2021 in Sydney (authors, pers. obs.). We predict that *Ps. platanigena* will be a significant issue for cut-leaf oriental plane trees in New South Wales, with repeated severe disease leading to dieback when associated with other stress events such as drought.

More than 70% of detections of exotic pests of arborescent hosts in Australia since 1996 occurred by general (passive) surveillance (Carnegie and Nahrung 2019), as occurred with *Ps. platanigena* reported here. While plane trees are targeted for specific (active) surveillance as part of the Department of Primary Industries' forest biosecurity surveillance program (Carnegie et al. 2018), prior to 2019 only *Pl.* x *acerifolia* was targeted for surveillance in high risk areas (e.g. Port Botany, Port Kembla), as it is a known host of nationally-listed exotic high priority pests. *Pseudocercospora platanigena* was detected in Australia by an arborist in an area not considered high risk for entry of exotic forest pests. This highlights the benefits of harnessing the public to assist in early detection of invasive pests (Carnegie and Nahrung 2019) and why governments need to expand links with citizen science networks.

Selected specimens examined: *Pseudocercospora platanigena*: on *Platanus orientalis* 'Digitata', Knox Grammar School, Wahroonga, NSW, 1 Feb. 2019, A.J. Carnegie & K. Hollstein (DAR84735); on *Pl. orientalis* 'Digitata', Wisemans Ferry Rd, Mangrove Mountain, 4 Feb. 2019, M. Steiner (DAR84708); on *Pl. orientalis* 'Digitata', Creswell O'Reilly Lookout, Pacific Highway, Pymble, NSW, A.J. Carnegie, 7 Feb. 2019 (DAR85406); on *Pl. orientalis* 'Digitata', Jarrett Park, Bellingen, NSW, A.J. Carnegie, 19 March 2019 (DAR85407); on *Pl. orientalis* 'Digitata', Mitchell Rd, Alexandria (Sydney), NSW, 26 April 2021, A.J. Carnegie (DAR85405); on *Pl. orientalis* 'Digitata', Bells Line of Road, Bilpin, NSW, A.J. Carnegie, 12 April 2021 (DAR85404).

Declarations

Conflict of interest The authors declare no conflict of interest.

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