

# Rust (*Puccinia psidii*) recorded in Indonesia poses a threat to forests and forestry in South-East Asia

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**Abstract** Over the past decade, *Puccinia psidii*, which causes rust on species of Myrtaceae, has spread rapidly to new areas and is now widespread. Quarantine has done little to prevent its movement through the Americas, the Pacific, Africa, and in this report, South-East Asia. *Puccinia psidii* is reported for the first time from Indonesia on two genera of Myrtaceae, namely *Eucalyptus* and *Melaleuca*. Its identity was confirmed using morphology, a molecular barcode comparison to an epitype specimen, and with a molecular phylogenetic approach. Comparison of seven microsatellite markers indicates that the rust genotype in Indonesia and Australia is identical. The potential impacts of *P. psidii* in South-East Asia to the natural environment and plantation forestry are discussed in light of this first report from the region.

**Keywords** *Melaleuca* · Myrtaceae · Myrtle rust · Pucciniales · Quarantine · Sphaerophragmiaceae · Uredinales

## Introduction

*Puccinia psidii* (Pucciniales, Pucciniomycotina) causes rust on plants in the Myrtaceae and is a serious threat to *Eucalyptus* plantations and native ecosystems with myrtaceous species (Glen et al. 2007; Coutinho et al. 1998). The pathogen has a wide host range and is reported from approximately 450 species in 73 genera (Giblin and Carnegie 2014). In this regard, it is unusual in being one of only a few rust fungi that occur on multiple host genera. It was first described on *Psidium guajava* from Brazil, and was believed to have undergone a host shift to species of *Eucalyptus* and *Syzygium jambos* (Castro et al. 1983). However, the populations of *P. psidii* on *Eucalyptus* and *Psidium* diverged more than 1000 years ago, and a host shift most likely did not occur after the introduction of *Eucalyptus* to Brazil (Graça et al. 2013). The genotype of *P. psidii* on *Eucalyptus* and *S. jambos* has an as yet unexplained origin (Graça et al. 2013).

*Puccinia psidii* is known from three life-cycle stages, urediospores, teliospores and basidiospores. The mitotic, uredinal stage produces masses of yellow urediniospores that defoliate juvenile trees, cause severe stem and foliage blight, and can affect developing fruits and inflorescences (Pegg et al. 2014b). The symptoms produced by the uredinia of *P. psidii* are characteristic and useful to distinguish it from other rusts on Myrtaceae, such as *Phakopsora myrtacearum* (Maier et al. 2015). A study on the lifecycle of *P. psidii* could not conclusively determine the function of the meiotic, telial stage, and indicated that populations of *P. psidii* are clonal and spread by uredinia (Morin et al. 2014).

*Puccinia psidii* spread through South America (Telechea et al. 2003), the Caribbean (Coutinho et al. 1998), North America (Marlatt and Kimbrough 1979), Hawaii (Uchida et al. 2006), Japan (Kawanishi et al. 2009), Australia (initially

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reported as *Uredo rangelii* (Carnegie et al. 2010), China (Zhuang and Wei 2011), South Africa (Roux et al. 2013) and New Caledonia (Giblin 2013). In July 2015, symptoms of rust similar to that caused by *P. psidii* were observed on *Eucalyptus pellita* and *Melaleuca leucadendra* in north and south Sumatra, Indonesia. Morphology, and a molecular barcoding and phylogenetic approach with markers from ribosomal DNA (rDNA) were used to identify the cause of rust on these myrtaceous hosts in Indonesia.

## Materials and methods

### Specimen collection and morphology

Rust specimens were obtained from plants in two regions of Sumatra, Indonesia. Leaves were pressed, dried and lodged in the herbarium of the South African National Fungus Collection (PREM) at the Agricultural Research Council (ARC) in Pretoria.

Rust spores were scraped from leaf material, mounted in 85 % lactic acid and gently heated to boiling. Preparations were examined with a Zeiss microscope and photographed with a Zeiss camera.

### DNA extraction, PCR and sequencing

Uredinia were removed from plant material and DNA was extracted with the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories Inc., Solana Beach, CA, USA). The internal transcribed spacer region (ITS) of ribosomal DNA (rDNA) was amplified with primers ITS1F (Gardes and Bruns 1993)/ITS4rust (Beenken et al. 2012). The ITS2-Large Subunit (LSU) region of rDNA was amplified with Rust2inv (Aime 2006)/LR6 (Vilgalys and Hester 1990). PCRs were performed with FastStart Taq (Roche Diagnostics Corporation, Indianapolis, USA) according to the manufacturer's instructions. The PCRs were performed with the following annealing temperatures: ITS at 55 °C and LSU at 62 °C. PCR products were cleaned by ethanol precipitation and sequenced in both directions using an ABI PRISM Dye-Terminator Cycle Sequencing Kit (Applied Biosystems) on an automated ABI 3130xl sequencer at the DNA Sequencing Facility of the Faculty of Natural and Agricultural Sciences (NAS), University of Pretoria. Sequences were assembled using the CLC Main Workbench (Qiagen).

### Phylogenetic analyses

A phylogenetic species concept was tested for the rust collected in Indonesia. *Puccinia psidii*, rusts on Myrtaceae, and members of the closely related Sphaerophragmiaceae

(Beenken and Wood 2015; Maier et al. 2015) were included in the analyses (Table 1). The LSU region of all taxa was aligned with the MAFFT algorithm (Katoh et al. 2009) in SATE (Liu et al. 2012), and run using two phylogenetic criteria, Bayesian inference and maximum likelihood. GTRGAMMA with an estimate of invariable sites was used as the model of evolution in both criteria. MrBayes was used

**Table 1** Taxon names and LSU GenBank numbers for isolates used in the phylogenetic analyses

Taxon	GenBank number	Reference
<i>Allodus podophylli</i>	JQ423285	Minnis et al. (2012)
<i>Caecoma torreyae</i>	AF522183	Szaro and Bruns unpublished
<i>Coleosporium plumeriae</i>	KM249866	McTaggart et al. (2014b)
<i>Cronartium ribicola</i>	DQ354560	Aime (2006)
<i>Dasyscypha echinata</i>	JF263462	Beenken et al. (2012)
<i>Dasyscypha gregaria</i>	JF263474	Beenken et al. (2012)
<i>Dasyscypha guianensis</i>	JF263479	Beenken et al. (2012)
<i>Dasyscypha winteri</i>	JF263492	Beenken et al. (2012)
<i>Endoraecium phyllodiorum</i>	KJ862324	McTaggart et al. (2015a)
<i>Endoraecium tierneyi</i>	KJ862335	McTaggart et al. (2015a)
<i>Hamaspora acutissima</i>	KT199398	McTaggart et al. (2015c)
<i>Hemileia vastatrix</i>	DQ354566	Aime (2006)
<i>Kernkampella breyniae</i>	KJ862346	McTaggart et al. (2015a)
<i>Maravalia cryptostegiae</i>	KT199401	McTaggart et al. (2015c)
<i>Masseëlla capparis</i>	JX136798	Liberato et al. (2014)
<i>Phakopsora annonae-sylvaticae</i>	KF528998	Beenken (2014)
<i>Phakopsora cherimoliae</i>	KF528012	Beenken (2014)
<i>Phakopsora myrtacearum</i>	KP729473	Maier et al. (2015)
<i>Phakopsora pistila</i>	KF528028	Beenken (2014)
<i>Phakopsora rolliniae</i>	KF528036	Beenken (2014)
<i>Phragmidium mexicanum</i>	DQ354553	Aime (2006)
<i>Prosopodium lippiae</i>	DQ354555	Aime (2006)
<i>Prosopodium tuberculatum</i>	KJ396195	Pegg et al. (2014b)
<i>Puccinia graminis</i>	KM249852	McTaggart et al. (2014b)
<i>Puccinia lagenophorae</i>	KF690700	McTaggart et al. (2014a)
<i>Puccinia psidii</i>	KT590038	This study
<i>Puccinia psidii</i>	KM282154	Machado et al. (2015b)
<i>Puccinia psidii</i>	KF318453	Pegg et al. (2014b)
<i>Puccinia psidii</i>	KF318443	Pegg et al. (2014b)
<i>Puccinia psidii</i>	KF318447	Pegg et al. (2014b)
<i>Puccinia stylidii</i>	KJ622215	McTaggart et al. (2014a)
<i>Puccinia ursinae</i>	KF690705	McTaggart et al. (2014a)
<i>Ravenelia neocaledoniensis</i>	KJ862348	McTaggart et al. (2015a)
<i>Sphaerophragmium</i> sp.	KJ862350	McTaggart et al. (2015a)
<i>Sphenorchidium polyalthiae</i>	JF263493	Beenken et al. (2012)
<i>Thekopsora minima</i>	KC763340	McTaggart et al. (2013)
<i>Uredinopsis pteridis</i>	KM249869	McTaggart et al. (2014b)
<i>Uromycladium acaciae</i>	KR612235	McTaggart et al. (2015b)
<i>Uromycladium simplex</i>	KJ632990	Doungsa-ard et al. (2015)

to conduct a Markov Chain Monte Carlo (MCMC) search with Bayesian inference (Ronquist and Huelsenbeck 2003). Maximum likelihood (ML) was implemented as a search criterion in RAxML (Stamatakis 2014).

### Microsatellite analysis

The genotypes of the three collections of *P. psidii* from Indonesia were compared with the genotype of Australian isolates using seven microsatellite markers designed by Zhong et al. (2008) and modified by Graça et al. (2013). These markers (PpSS012, PpSS014, PpSS018, PpSS022, PpSS102, PpSS161 and PpSS195) were labelled with either NED™, FAM™, PET® or VIC™ fluorescent dye on the forward primer. PCR mixtures included 1× PCR Fast Taq Buffer with MgCl<sub>2</sub> (Sigma-Aldrich, St. Louis, Missouri, USA), 200 μM dNTPs, 0.1 μM primers, 1 unit Fast Taq DNA polymerase (Sigma-Aldrich), and DNA template in 12.5 μL reaction volumes.

PCR products were amplified with the following conditions: one cycle at 95 °C for 5 min, followed by three cycles at 95 °C for 30s, 52–56 °C (depending on the locus) for 30s, 72 °C for 80s, 35 cycles at 94 °C for 15 s, 52–56 °C (depending on the microsatellite) for 15 s and 45 s at 72 °C. Genescan was performed using an Applied Biosystems PRISM 3500xl (Life Technologies) at the Sequencing Facility of the Faculty of Natural and Agricultural Science, University of Pretoria. Positive and negative controls were included for each of the markers. Each allele was scored using Genemapper® Software 4.4 (Applied Biosystems, Waltham, Massachusetts, USA).

## Results

### Morphology

The urediniospores and teliospores from one specimen on *Eucalyptus* and two specimens on *Melaleuca* in Indonesia matched the morphology of *P. psidii* reported in Australia (available at: <http://collections.daff.qld.gov.au/web/key/>

[rustfungi/Media/Html/pucciniapsidii.html](http://collections.daff.qld.gov.au/web/key/rustfungi/Media/Html/pucciniapsidii.html); (Shivas et al. 2014). Uredinia occurred on leaves and petioles, single or gregarious, amphigenous on young shoots and leaves, erumpent, round, up to 0.5 mm, bright yellow to yellowish brown (Fig. 1a). Urediniospores were globose to ovoid, pyriform, yellowish brown, 13–20 × 12–16 μm; wall 1.5–2.0 μm thick, finely echinulate, germ pore absent or inconspicuous (Fig. 1b, c). Telia occurred on leaves, up to 0.5 mm diam., abaxial, erumpent, pulvinate, yellowish brown to brown. Teliospores were cylindrical or ellipsoidal, apex rounded, pale yellowish brown, 22–38 × 14–18 μm; wall 1–2 μm thick, smooth, 2–3 celled, short remnant of pedicel attached up to 15 μm long, phragmobasidium up to 60 μm long (Fig. 1d, e).

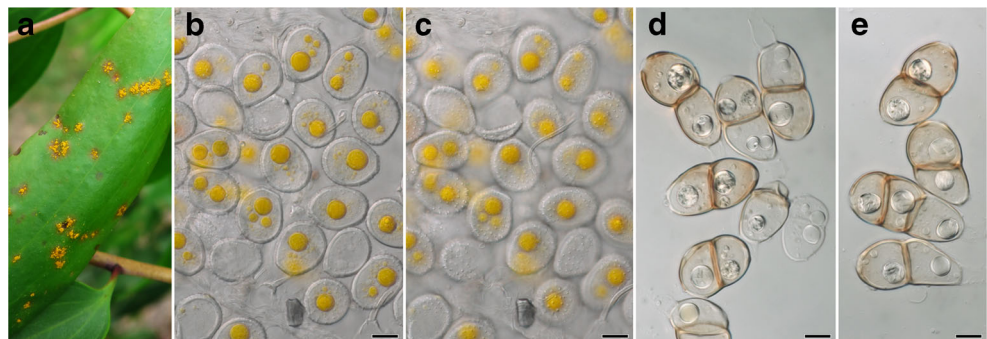
Specimens examined: INDONESIA, North Sumatra, Porsea, on *Eucalyptus grandis* × *E. pellita*, clone collected by M.J. Wingfield, 10 July 2015, PREM 61284 (GenBank ITS: KT590039); on *Melaleuca leucadendra*, collected by M.J. Wingfield, 01 July 2015, PREM 61283 (GenBank ITS: KT590038); Sumatra, Pelalawan, on *M. leucadendra*, collected by M.J. Wingfield, 10 July 2015, PREM 61282 (GenBank ITS-LSU: KT590038).

### Sequencing and phylogenetic analyses

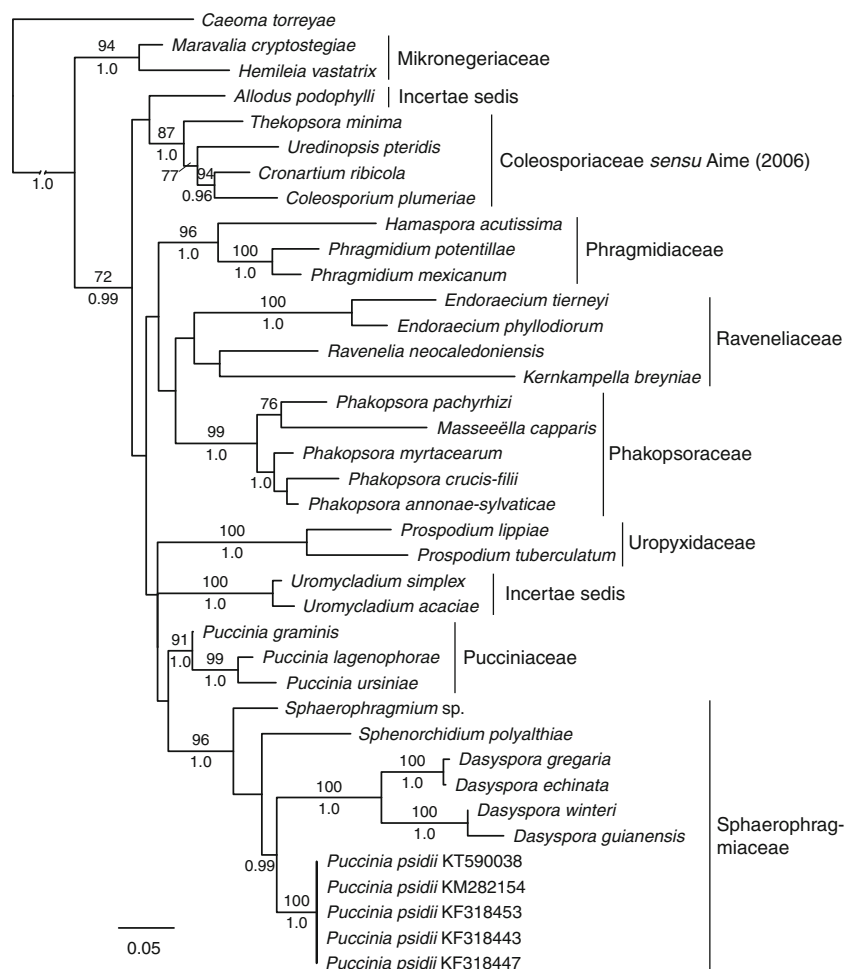
The ITS regions of the specimens from Indonesia on *Eucalyptus* (KT590039) and *Melaleuca* (KT590038 and KT590037) had 563/567 identities to the epitope of *P. psidii* (KM282154) (Machado et al. 2015b) in a BLASTn search on GenBank. Three of the mismatched bases were degenerate bases caused by intra-individual single nucleotide polymorphisms, and one was caused by a nucleotide mismatch. The LSU region had high identity to *P. psidii* (KM282159 1098/1098 identities and KF318436 1013/1013 identities) in a BLASTn search.

All isolates of *P. psidii*, which included one specimen from Indonesia, were recovered in a monophyletic group under both phylogenetic criteria (Fig. 2). *Puccinia psidii* was recovered as sister to species of *Dasyscypha* in the Sphaerophragmiaceae sensu McTaggart et al. (2015c).

**Fig. 1** *Puccinia psidii* on *Melaleuca leucadendra* (PREM 61282). **a** host symptoms **b** equatorial plane of urediniospores. **c** surface of urediniospores. **d–e**. teliospores. Scale bars = 10 μm



**Fig. 2** Phylogram obtained from a maximum likelihood search in RAxML with a dataset of the large subunit region of ribosomal DNA. Bootstrap values ( $\geq 70\%$ ) from 1000 replicates above nodes. Posterior probabilities ( $\geq 0.95$ ) summarized from 30,004 trees in a Bayesian search in MrBayes below nodes. GTRGAMMA with an estimate of invariable sites was the model of evolution for both phylogenetic criteria. Taxon name and GenBank numbers listed in Table 1



## Microsatellite analysis

The alleles present in the Indonesian isolates were identical to those from Australia (Table 2). A single genotype of *P. psidii* occurs in Indonesia and Australia, based on the currently available specimens.

## Discussion

The present study provides the first report of *P. psidii* in Indonesia and the first report of this pathogen from South-East Asia. An unidentified species of *Puccinia* has previously been recorded on hybrids of *Eucalyptus* in Indonesia

**Table 2** Allelic sizes of *Puccinia psidii* isolates from Indonesia and Australia. Microsatellite markers were used from the study by Graça et al. (2013)

Specimen number	Host	Country	PpSS012	PpSS014	PpSS018	PpSS022	PpSS0102	PpSS0161	PpSS0195
PREM 61282	<i>Melaleuca leucadendra</i>	Indonesia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
PREM 61283	<i>Melaleuca leucadendra</i>	Indonesia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
PREM 61284	<i>Eucalyptus grandis</i> × <i>E. pellita</i>	Indonesia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
BRIP 63350	<i>Melaleuca viminalis</i>	Australia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
BRIP 63351	<i>Gossia inophloia</i>	Australia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
BRIP 63352	<i>Backhousia citriodora</i>	Australia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
BRIP 63353	<i>Rhodamnia rubescens</i>	Australia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214
BRIP 57793	<i>Rhodamnia sessiflora</i>	Australia	230, 236	207, 211	170, 172	158, 160	140	276, 290	214



(Hardiyanto and Tridasa 2000). However, no specimens were lodged linked to that report and neither was a description of the rust provided. There have been no subsequent reports of *P. psidii* from Indonesia and it is not possible to confirm whether it was there prior to the present study.

Indonesia, Malaysia, Thailand and Vietnam have *Eucalyptus* plantations on a combined area of approximately 1,020,000 ha (Harwood and Nambiar 2014). The discovery of *P. psidii* in Indonesia and its importance as a *Eucalyptus* pathogen is likely to have implications for forest industries in the region. The pathogen also poses a potential threat to native species of Myrtaceae, which are represented by approximately 30 genera in Indonesia (Craven et al. 2003).

*Puccinia psidii* has spread globally during the past decade. The pathogen reached northern Asia (Kawanishi et al. 2009) and Australia (Carnegie et al. 2010) relatively recently. Countries in South-East Asia have native and introduced species of Myrtaceae suitable for infection by *P. psidii*, and its discovery in Indonesia is perhaps not surprising. It is unknown how long *P. psidii* has been present in Sumatra but it was undetected in active surveys in the region during the past 20 years (Wingfield, unpublished). This suggests that its establishment in Sumatra is relatively recent. Thus far, *P. psidii* has been found only on *Eucalyptus* and a native species of Myrtaceae, *M. leucadendra*. It is likely to infect many native and non-native Myrtaceae in Indonesia in the future.

The present study recovered *P. psidii* sister to *Dasyscypha* within the Sphaerophragmiaceae, sister to the Pucciniaceae, a relationship that was previously shown by Maier et al. (2015) and Beenken and Wood (2015). Puccinioid spores are a homoplasious character within the Pucciniales (Beenken and Wood 2015). The generic placement of *P. psidii* will require the resolution of other puccinioid genera within the Sphaerophragmiaceae and Uropyxidaceae sensu Cummins and Hiratsuka (2003).

The genotype of *P. psidii* in Indonesia and Australia is identical based on the seven microsatellite markers used in this study. Australia may be a potential source of the rust that has now reached Indonesia. This genotype of *P. psidii* on *Eucalyptus* has an unknown origin, but is similar to the rust in California, China, Hawaii and New Caledonia (Graça et al. 2013; Machado et al. 2015a). Previous studies have indicated that the origin of *P. psidii* may be from a location with shared plants in the Annonaceae and Myrtaceae (Maier et al. 2015).

Tropical Asia is one of the centers of diversity for species of Myrtaceae with over 800 described species of *Syzygium* (Govaerts et al. 2008). One of these species, *S. jambos*, is among the most susceptible plants to infection by *P. psidii*. While commercial interests such as those relating to *Eucalyptus* propagation are of concern, the greatest threat of *P. psidii* will likely reside in the damage caused to the native environment. This is consistent with observations in Australia

where the rust has brought 12 species of native Myrtaceae to the brink of extinction (Pegg et al. 2014b), and up to 50 % mortality of susceptible trees in natural ecosystems (Carnegie et al. 2015).

Pathogens of native Myrtaceae in Asia are already recognized as threats to *Eucalyptus* propagation in the region. For example, various species of the Chryphonectriaceae found on native *Syzygium* spp. in China were shown to be potential, important pathogens of *Eucalyptus* spp. grown in plantations (Chen et al. 2010). The extensive and growing *Eucalyptus* plantation industry in Indonesia and South-East Asia is likely to be affected by *P. psidii* in the future. However, there are many opportunities to manage the negative impacts of this and other pathogens in plantations. In this regard, breeding, selection and the propagation of resistant genotypes will be amongst the most effective strategies (Pegg et al. 2014a; Ribeiro and Pommer 2004; Silva et al. 2013; Wingfield et al. 2013; Wingfield et al. 2008).

The rapid, global spread of *P. psidii* is of concern and illustrates the ease with which tree pests and pathogens move (Wingfield et al. 2015). Pathways of spread differ depending on the ecology of the organisms involved. In the case of *P. psidii*, local spread is likely to occur rapidly via wind-borne spores. Long-distance spread has most likely occurred via transport of living plant tissue. Species of Myrtaceae susceptible to *P. psidii* produce edible fruits, which could be infected and moved to new areas. However, horticultural trade in living plant material including eucalypts and other Myrtaceae, represents one of the most important potential pathways of introduction into new areas. Great care should thus be taken to discourage exchange of potentially hazardous plant material between countries and continents.

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