

## ***Phakopsora myrtacearum* sp. nov., a newly described rust (Pucciniales) on eucalypts in eastern and southern Africa**

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Outbreaks of a rust disease in eucalypt forestry plantations and nurseries in Kenya, Mozambique and South Africa occurred between 2009 and 2014. The pathogen was identified using morphology and molecular phylogenetic analyses as an undescribed species in the Phakopsoraceae. A systematic study, based on nuclear ribosomal DNA, showed that it is a species of *Phakopsora*, herein named *Phakopsora myrtacearum* sp. nov. This new species of rust is the second validly described species on *Eucalyptus*, along with *Puccinia psidii*. *Phakopsora myrtacearum* is distinguished from *P. psidii* by leaf symptoms, morphology of the urediniospores and distinct phylogenetic placement. *Phakopsora myrtacearum* has been found on three species of *Eucalyptus* in Kenya, Mozambique and South Africa, and it may have future negative implications for commercial forestry in these areas.

**Keywords:** *Batistopsora*, host jumps, ITS rDNA, LSU rDNA, Myrtaceae, Uredinales

### **Introduction**

Eucalypts, which include the species-rich genera *Eucalyptus*, *Angophora* and *Corymbia* (Myrtaceae, Myrtales), have become a prominent source of timber and fuel in Africa since their introduction in the late 19th century (Dessie & Erkossa, 2011). Plantations of *Eucalyptus* occupy over 600 000 hectares in South Africa (Forestry South Africa, 2012). The sustainability of these plantations is threatened by numerous fungal diseases (Wingfield *et al.*, 2008).

Five rust fungi (Pucciniales) have been reported to infect eucalypts (*Eucalyptus sensu lato*): *Melampsora eucalypti* Rabh., *M. eucalypti* Avern., *Melampsora* sp., *Puccinia psidii* Winter, and an undescribed uredinial rust. *Melampsora eucalypti* Rabh. was described from *E. globulus* in India, but a morphological description of the spore stages was not provided (Rabenhorst, 1881). Simpson *et al.* (2006) considered this rust *nomen dubium*, as fungal material could not be found on the type specimen in earlier studies. Another rust, *M. eucalypti* Avern. (not *M. eucalypti* Rabh.), was listed as a parasite of *E. pilularis* in Brazil (Saccá, 1912). This may represent the first report of *P. psidii* on a eucalypt in South America; however, no species description was provided and no type was lodged (Joffly, 1944). Neither *M. eucalypti* Rabh., nor *M. eucalypti* Avern. are validly published names. A rust fungus identified from urediniospores and teliospores as *Melampsora* sp. on *Corymbia citriodora*

(as *E. citriodora*) in India, was deposited in Kew as IMI 165554 (Upadhyay & Bordoloi, 1975). This specimen was discarded from Kew, as its identity could not be confirmed, probably due to lack of any fungal structures (T. S. Caine, CABI Europe-UK, personal communication). Knipscheer & Crous (1990) reported an undescribed, uredinial rust fungus on the leaves of *E. nitens* in South Africa. The report was accompanied by scanning electron micrograph images of the urediniospores. Thus, *Puccinia psidii* is the only taxonomically legitimate species described from *Eucalyptus*.

*Puccinia psidii* was first described from *Psidium guajava* in Brazil, and is considered native to South America (Coutinho *et al.*, 1998; Glen *et al.*, 2007). It underwent a host shift to plantation-grown species of *Eucalyptus* in the early 1900s, and caused subsequent disease outbreaks on these trees in the 1970s (Ferreira, 1981). *Puccinia psidii* spread beyond its native range to the Caribbean (McLachlan, 1936), the United States (Marlatt & Kimbrough, 1979) and out of the Americas to Hawaii (Uchida *et al.*, 2006), Japan (Kawanishi *et al.*, 2009), Australia (Carnegie *et al.*, 2010), China (Zhuang & Wei, 2011), South Africa (Roux *et al.*, 2013) and New Caledonia (Giblin, 2013). Its host range has simultaneously expanded to include more than 160 species in 38 genera of Myrtaceae (Pegg *et al.*, 2014). *Puccinia psidii* causes leaf and shoot-dieback, and significant damage to eucalypts in nurseries (Coutinho *et al.*, 1998; Glen *et al.*, 2007), posing a global threat to the forestry industry. The recent introduction of *P. psidii* to Australia now threatens several endangered plant species such as *Baccharis oligantha*, *Gossia gonoclada*, *Rhodamnia angustifolia* and *R. maideniana* (Pegg *et al.*, 2014).

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More than 40 species of rust fungi have been described on hosts in the Myrtaceae, a family known to include over 5600 plant species. Most descriptions of these fungi are considered synonymous with *P. psidii* (Walker, 1983; Hennen *et al.*, 2005; Simpson *et al.*, 2006). Simpson *et al.* (2006) accepted eight taxa, and of these, five species are accepted under the new Code, namely, *Phakopsora juelii* Syd. & P. Syd., *Puccinia cygnorum* R.G. Shivas & J. Walker, *P. psidii* Winter (syn. *U. rangalii* J.A. Simpson, K. Thomas & C.A. Gruginovic), *Uredo seclusa* H.S. Jackson & Holway, and *Uredo xanthostemonis* J. Walker, (syn. *Physopella xanthostemonis* (J. Walker) J.A. Simpson, K. Thomas & C.A. Gruginovic).

Uredinia of an unknown rust fungus were found on leaves of three *Eucalyptus* species in parts of Mozambique (M. J. Wingfield and J. Roux, unpublished data), Kenya and South Africa (J. Roux, unpublished data) between 2009 and 2014. The symptoms associated with these collections included leaf spots that coalesced to form necrotic areas on young and old leaves. This leaf rust is now common in the Mpumalanga Province of South Africa. The aim of this study was to identify the rust on *Eucalyptus* based on a combination of morphological and molecular approaches.

## Materials and methods

### Disease and isolates

Leaves showing symptoms and structures caused by rust fungi were obtained from species of *Eucalyptus* in Kenya, Mozambique and South Africa. Plant material was dried and deposited in the herbarium of the South African National Fungus Collection (PREM 61155, 61156, 61157, 61158), and in the Fungus Collection at the Staatliches Museum für Naturkunde Karlsruhe, Germany (KR-M-0051790).

### DNA extraction, PCR and sequencing

DNA was isolated from dried, infected host leaves, or directly from spores, using the Plant DNeasy Mini kit (QIAGEN). The internal transcribed spacer (ITS) region of rDNA was amplified by PCR with the primer pair ITS1F and ITS4rust (Gardes & Bruns, 1993; Beenken *et al.*, 2012) as described by Beenken *et al.* (2012). The 5' end of the nuclear large subunit (LSU) rDNA gene was amplified with the primer pair LR0R and LR6 (Vilgalys & Hester, 1990) as described by Maier *et al.* (2003). A nested PCR was performed to increase product for direct sequencing using the primer pair NL1 and NL4 (Moncalvo *et al.*, 1995). PCR products were purified with either a QIAquick PCR purification kit (QIAGEN) or the DNA Clean and Concentrator kit (Zymo Research), and then 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, University of Pretoria. Sequences were assembled using CLC MAINWORKBENCH (QIAGEN) or SEQUENCHER v. 4.8 (GeneCodes).

## Phylogenetic analyses

The LSU rDNA sequences of 44 species of rust fungi (Table 1) were aligned in MAFFT (Katoh *et al.*, 2009) (<http://www.ebi.ac.uk/Tools/msa/mafft/>), then analysed with maximum likelihood and Bayesian inference as phylogenetic criteria. Maximum likelihood (ML) was implemented as a search criterion in RAXML (Stamatakis, 2014). GTRGAMMA was specified as the model of evolution for nucleotide sequence data. The RAXML analyses were run with a rapid bootstrap analysis (command `-f a`) using a random starting tree and 1000 maximum likelihood bootstrap replicates. A Markov chain Monte Carlo (MCMC) search in a Bayesian analysis was conducted with MRBAYES (Ronquist & Huelsenbeck, 2003). Four runs, each consisting of four chains, were implemented for 5 000 000 generations with a defined starting tree from the ML analysis. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 500 generations and trees were saved every 1000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander *et al.*, 2008) (available at: [ceb.csit.fsu.edu/awty/](http://ceb.csit.fsu.edu/awty/)) and 18 000 trees were summarized to form a consensus tree. The ML and Bayesian analyses were run three times to test accuracy.

## Light and scanning electron microscopy (SEM)

Infected plant tissues and urediniospores taken from various uredinia on different eucalypt leaves were studied. Free hand sections and scrape mounts of spores were prepared in lactic acid that was gently heated until boiling point and were observed using Zeiss and Olympus light microscopes. Spore measurements were based on 30 spores obtained from a single uredinium. For SEM, spores were stuck onto double-sided adhesive tape, sputter coated with gold and examined with a DSM 950 electron microscope (Zeiss).

## Results

### Disease symptoms and distribution

The unknown rust was observed in several localities in Kenya, Mozambique and South Africa. In Kenya, the rust was collected from nurseries in Maragua and Nairobi in the Central Province. In Mozambique, symptoms were observed as far north as Lichinga in the Niassa Province, near Nampula in the Nampula Province, and near the city of Maputo in the Province of Maputo. In South Africa, the rust was found in KwaZulu-Natal, Limpopo and Mpumalanga Provinces. Affected trees included *E. cloeziana*, *E. grandis* and *E. nitens*. Although leaves were often covered in rust pustules (Fig. 1a,b), no signs of defoliation or shoot dieback were observed.

Uredinia were minute and restricted to leaves, causing locally defined lesions. Host reactions were strong and leaf tissue was commonly necrotic in the areas where uredinia were densely distributed (Fig. 1b). Infected leaves showed strong discolouration (purple, red and black) around tiny, pale yellow to light brown uredinia.

Table 1 Taxa included in the phylogenetic analysis

Taxon	Voucher	Host	Country	GenBank	
				LSU	ITS
<i>Allodus podophylli</i>	BPI 844307	<i>Podophyllum peltatum</i>	USA	JQ423258	NA
<i>Blastospora smilacis</i>	PUR N270	<i>Smilax sieboldii</i>	Japan	DQ354568	NA
<i>Caeoma torreyae</i>	Not provided	Not provided	Not provided	AF522183	NA
<i>Cerotelium fici</i>	BRIP 58068	<i>Ficus</i> sp.	Australia	<b>KP753385</b>	NA
<i>Coleosporium plumeriae</i>	BRIP 55387	<i>Plumeria rubra</i>	Australia	KM249866	NA
<i>Cronartium ribicola</i>	BPI 871660	<i>Ribes</i> sp.	USA	DQ354560	NA
<i>Dasyscypha gregaria</i>	ZT Myc 3393	<i>Xylopiya cayennensis</i>	French Guiana	JF263474	NA
<i>Dasyscypha winteri</i>	S F30078	<i>Xylopiya sericea</i>	Brazil	JF263492	NA
<i>Desmella aneimiae</i>	BRIP 60995	<i>Nephrolepis hirsutula</i>	Australia	KM249867	NA
<i>Diorchidium polyalthiae</i>	ZT HeRB 251	<i>Polyalthia longifolia</i>	India	JF263493	NA
<i>Helicobasidium purpureum</i>	CBS324-47	Not provided	Not provided	AY885168	NA
<i>Hemileia vastatrix</i>	BPI 843642	<i>Coffea arabica</i>	Mexico	DQ354566	NA
<i>Kuehneola uredinis</i>	BPI 871104	<i>Rubus argutus</i>	USA	DQ354551	NA
<i>Masseella capparidis</i>	BRIP 56844	<i>Flueggea virosa</i>	Australia	JX136798	NA
<i>Milesina philippinensis</i>	BRIP 58421	<i>Nephrolepis</i> sp.	Philippines	KM249868	NA
<i>Naohidemycetes vaccinii</i>	BPI 871754	<i>Vaccinium ovatum</i>	USA	DQ354563	NA
<i>Phakopsora annonae-sylvaticae</i>	PUR 87311	<i>Annona sylvatica</i>	Brazil	KF528008	NA
<i>Phakopsora cherimoliae</i>	PUR 89695	<i>Annona cherimola</i> × <i>A. squamosa</i>	USA	KF528012	NA
<i>Phakopsora cingens</i>	BRIP 55628	<i>Bridelia tomentosa</i>	Australia	<b>KP729474</b>	NA
<i>Phakopsora crucis-filii</i>	PUR 87629	<i>Annona tomentosa</i>	Brazil	KF528023	NA
<i>Phakopsora myrtacearum</i>	KR-M-0043209	<i>Eucalyptus grandis</i>	Mozambique	<b>KP729471</b>	NA
<i>Phakopsora myrtacearum</i>	PREM 61156	<i>Eucalyptus</i> sp.	Kenya	<b>KP729472</b>	NA
<i>Phakopsora myrtacearum</i>	PREM 61155	<i>Eucalyptus grandis</i>	Kenya	<b>KP729473</b>	<b>KP729468</b>
<i>Phakopsora myrtacearum</i>	PREM 61157	<i>Eucalyptus cloeziana</i>	South Africa	NA	<b>KP729470</b>
<i>Phakopsora myrtacearum</i>	PREM 61158	<i>Eucalyptus nitens</i>	South Africa	NA	<b>KP729469</b>
<i>Phakopsora pachyrhizi</i>	BRIP 56941	<i>Macroptilium atropurpureum</i>	Australia	<b>KP729475</b>	NA
<i>Phakopsora pistila</i>	BPI 863563	<i>Annona sericea</i>	Guyana	KF528028	NA
<i>Phakopsora rolliniae</i>	NY 3237	<i>Annona exsucca</i>	Trinidad and Tobago	KF528036	NA
<i>Phakopsora tecta</i>	BRIP 56943	<i>Commelina</i> sp.	Australia	<b>KP729476</b>	NA
<i>Phragmidium mexicanum</i>	BPI 843392	<i>Duchesnea</i> sp.	USA	DQ354553	NA
<i>Porotenus biporus</i>	ZT Myc 3414	<i>Memora flavida</i>	French Guiana	JF263494	NA
<i>Prospodium lippiae</i>	BPI 843901	<i>Aloysia polystachya</i>	Argentina	DQ354555	NA
<i>Prospodium tuberculatum</i>	BRIP 57630	<i>Lantana camara</i>	Australia	KJ396195	NA
<i>Puccinia graminis</i>	BRIP 60137	<i>Glyceria</i> sp.	Australia	KM249852	NA
<i>Puccinia lagenophorae</i>	BRIP 57774	<i>Schoenia cassiniana</i>	Australia	KF690700	NA
<i>Puccinia psidii</i>	BRIP 58517	<i>Myrtus communis</i>	Australia	KF318447	NA
<i>Puccinia stylidii</i>	BRIP 60107	<i>Stylidium armeria</i>	Australia	KJ622215	NA
<i>Sphaerophragmium</i> sp.	BRIP 56910	<i>Albizia</i> sp.	Australia	KJ862350	NA
<i>Thekopsora minima</i>	BRIP 57654	<i>Vaccinium corymbosum</i>	Australia	KC763340	NA
<i>Trachyspora intrusa</i>	BPI 843828	<i>Alchemilla vulgaris</i>	Switzerland	DQ354550	NA
<i>Uredinopsis pteridis</i>	BRIP 60091	<i>Pteridium esculentum</i>	Australia	KM249869	NA
<i>Uredo geitonoplesii</i>	BRIP 57603	<i>Geitonoplesium cymosum</i>	Australia	KM249860	NA
<i>Uromyces appendiculatus</i>	BRIP 60020	<i>Phaseolus vulgaris</i>	Australia	KM249870	NA
<i>Uromyces viciae-fabae</i>	BRIP 59246	<i>Vicia faba</i>	Australia	KM249865	NA
<i>Uromycladium simplex</i>	BRIP 59214	<i>Acacia pycnantha</i>	Australia	KJ632990	NA

Sequences obtained for this study are in bold font.

These areas of discolouration were much larger than the uredinia themselves.

### Phylogenetic analyses

The ITS and LSU regions were sequenced for three isolates. These isolates were identical in the ITS region, and differed by a base-pair indel in the LSU region.

A BLASTN search of the ITS rDNA sequences had highest identities to *Phakopsora annonae-sylvaticae* (KF528008 85% identical, 539/633 identities), *Ph. cheri-*

*moliae* (KF528011 83% identical, 454/550 identities) and *Ph. (as Batistopsora) pistila* (KF528029 82% identical, 433/527 identities). A BLASTN search of the LSU rDNA sequences had highest identities to *Ph. annonae-sylvaticae* (KF528008 98% identical, 955/977 identities), *Ph. (as Batistopsora) pistila* (KF528029 97% identical, 944/977 identities) and *Ph. (as Batistopsora) crucis-filii* (KF528024 97% identical, 944/977 identities). In comparison, BLASTN revealed only 90% identity with a sequence of *P. psidii* (KF318430, 958/1070 identities) and 3% gaps (36/1070).

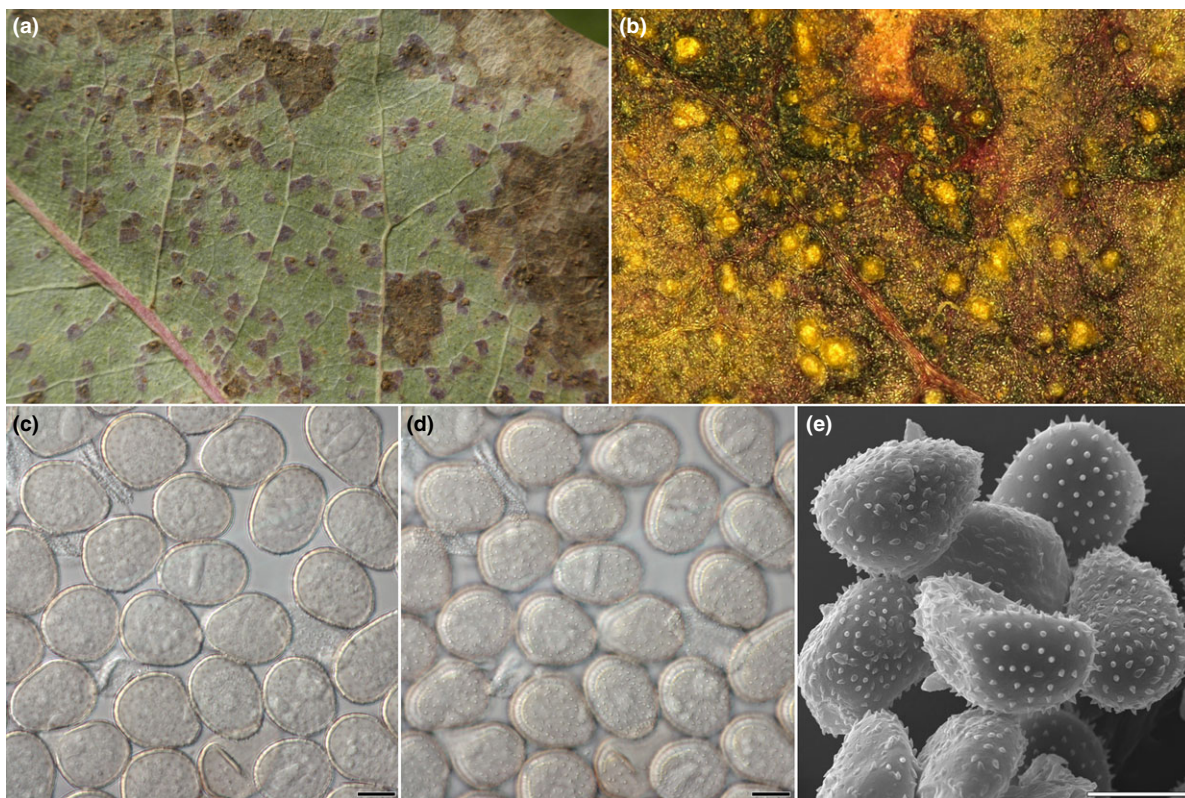


Figure 1 *Phakopsora myrtacearum* on *Eucalyptus nitens* PREM 61158. (a,b) Uredinia and lesions on abaxial leaf surface; (c,d) urediniospores, equatorial and surface views, respectively; (e) urediniospores under scanning electron microscope. Scale bars c–e = 10  $\mu$ m.

The recovered topologies were almost identical in separate searches with ML (Fig. 2) and Bayesian inference (not shown) as phylogenetic criteria. The topology from Bayesian inference differed in that the major rust families, Raveneliaceae, Phakopsoraceae, Phragmidiaceae and Pucciniaceae, were recovered in a polytomy in relation to each other. The uredinial rust on *Eucalyptus* was recovered with strong support (100% bootstrap, 1.0 posterior probability) as sister to the clade containing species of *Phakopsora* on hosts in the Annonaceae. These taxa were sister to other members of the Phakopsoraceae, including species of *Cerotelium*, *Masseella* and *Phakopsora*. The Phakopsoraceae *sensu stricto* was strongly supported (100% bootstrap, 1.0 posterior probability). *Puccinia psidii* was recovered with strong support (99% bootstrap, 1.0 posterior probability) in a clade with species of *Dasyscypha* and *Diorchidium polyalthiae*, all three from Annonaceae.

#### Morphological description and taxonomy

The rust fungus from Kenya, Mozambique and South Africa is distinct to other rust fungi in the Phakopsoraceae on Myrtaceae or other hosts (see below). Based on morphology and the results of the systematic analyses, it is described here as a new species of *Phakopsora*.

#### *Phakopsora myrtacearum* McTaggart, Maier, J. Roux, M. J. Wingfield sp. nov.

Mycobank: MB811580; Figure 1

Spermatogonia, aecia and telia unknown. Uredinia amphigenous, the majority hypophyllous, 0.1–0.5(–1) mm in diameter, sometimes on vein-limited, raised lesions, approx. 0.3–1.0 mm, scattered or aggregated in groups, sub-epidermal in origin, erumpent, surrounded by torn epidermis, pale brown, paraphyses absent. Urediniospores obovoid, mostly pyriform to ellipsoid, (20–)23–26(–30)  $\times$  14–17(–20)  $\mu$ m (average size: 24.5  $\times$  15.8  $\mu$ m), walls  $\pm$  uniformly 2–3  $\mu$ m thick, subhyaline to pale brown. Spore walls finely and densely echinulate, germ pores inconspicuous.

On the leaves of *Eucalyptus cloeziana*, *E. grandis*, *E. nitens*.

Etymology: Name reflects the host family, Myrtaceae.

*Specimens examined*: KENYA, Maragua, Central Province, on *Eucalyptus grandis*, 13 Jul 2013, J. Roux, PREM 61155 Holotype, GenBank ITS KP729468, LSU KP729473; Nairobi, TBPT Nursery, on *Eucalyptus* sp., Jul 2013, J. Roux PREM 61156, GenBank LSU KP729472. MOZAMBIQUE, Lichinga, on *E. grandis*, June 2009, M. J. Wingfield, UP 217, KR-M-0043209, GenBank LSU KP729471. SOUTH AFRICA, Limpopo Province, on *E. cloeziana*, 11 Sept 2014, J. Roux, PREM

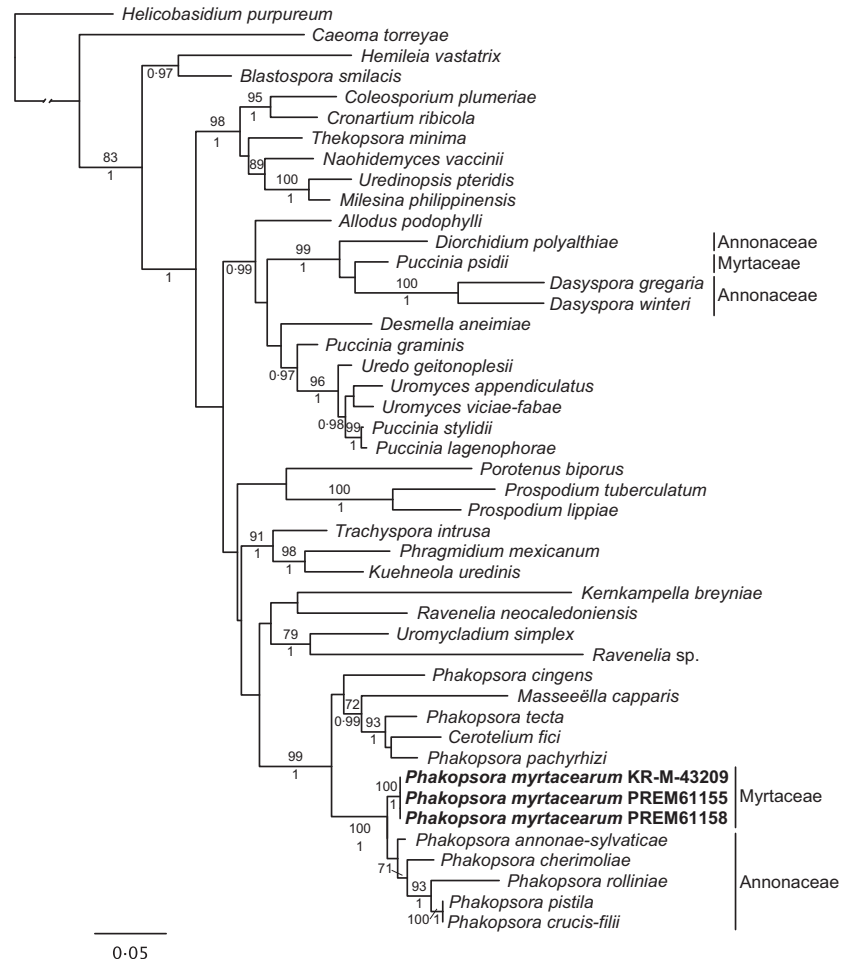


Figure 2 Phylogram obtained from a maximum likelihood search in RAxML on the LSU region of rDNA. Bootstrap support values ( $\geq 70\%$ ) from 1000 replicates in a maximum likelihood search above nodes; posterior probabilities ( $\geq 0.95$ ) summarized from 18 000 trees below nodes. The scale bar represents the proportion of nucleotide substitutions per site. The new species is in bold font.

61157, GenBank ITS KP729470; Lothair, on *E. nitens*, J. Roux, PREM 61158, GenBank ITS KP729469.

The following key can be used to distinguish the two validly described rust fungi on eucalypts:

- 1 Uredinia forming bright yellow-orange pustules on leaves, petioles, shoots, flowers or fruits. Urediniospores mainly globose to subpyriform,  $15\text{--}26 \times 14\text{--}22 \mu\text{m}$ .  
*Puccinia psidii*
- 2 Uredinia pale yellow to light brown, on discoloured areas on leaves. Urediniospores mainly pyriform to ellipsoid  $20\text{--}30 \times 14\text{--}20 \mu\text{m}$ .  
*Phakopsora myrtacearum*

*Phakopsora myrtacearum* can be differentiated from other phakopsoroid rusts on Myrtaceae, namely *Ph. juelii* and *Uredo xanthostemonis*, as it lacks paraphyses. The urediniospores of *Ph. juelii* are characteristically asymmetric-reniform [ $(14\text{--})18\text{--}23 \times (12\text{--})14\text{--}18 \mu\text{m}$ ] and the spore wall is  $0.5\text{--}1.0 \mu\text{m}$  thick (Hennen *et al.*, 2005), compared to *Ph. myrtacearum*, which has obovoid to ellipsoid urediniospores [ $(20\text{--})23\text{--}26\text{--}(30) \times 14\text{--}17\text{--}(20)$ ] with a wall  $2\text{--}3 \mu\text{m}$  thick. *Uredo xanthostemonis*, which may belong to *Phakopsora*, is known only on *Xanthostemon* from Australia. It resembles the host

symptoms of *Ph. myrtacearum*, and has similar sized urediniospores  $17\text{--}28 \times 15\text{--}20 \mu\text{m}$ , with a wall  $1.0\text{--}1.5 \mu\text{m}$  thick (Walker, 1983). *Uredo seclusa* has similar symptoms and urediniospores to *Ph. myrtacearum*. It is known only from the type collection in Brazil on an undetermined species of Myrtaceae (Jackson, 1931), and has somewhat larger urediniospores ( $24\text{--}32 \mu\text{m}$ ) with  $1.0 \mu\text{m}$  thick spore walls (Hennen *et al.*, 2005).

## Discussion

*Phakopsora myrtacearum* and *P. psidii* are the only taxonomically valid rust species described from eucalypts. These two species are distinguished by host symptoms, spore morphology and DNA sequence data.

Rust fungi can display a wide host range. The hosts of *Ph. myrtacearum* are exotic trees in southern Africa, which indicates the rust may originate from an incursion of an exotic pathogen or a host shift of a native rust to *Eucalyptus*. In this context, *Ph. myrtacearum* may represent a host extension to *Eucalyptus* of a validly described species of rust. *Phakopsora myrtacearum* was compared to three phakopsoroid species of rust known to infect myrtaceous plants in Australia and South America, namely *Ph. juelii*, *Uredo xanthostemonis*, and

*U. seclusa*, which are likely sources for a host shift in the Myrtaceae. The South American and Australian rust species on Myrtaceae were not regarded as the source of *Ph. myrtacearum*, which is morphologically distinct as it lacks paraphyses around its uredinia.

The disease symptoms, host and morphology of the urediniospores of an unidentified rust reported on *E. nitens* from KwaZulu-Natal in South Africa by Knipscheer & Crous (1990) are congruent with *Ph. myrtacearum*. The urediniospores in that collection were shorter and wider ( $15\text{--}25 \times 10\text{--}25 \mu\text{m}$ ) than described for *Ph. myrtacearum* ( $20\text{--}30 \times 14\text{--}20 \mu\text{m}$ ), which may reflect variation within the species, or be caused by the use of different mountants. There are no voucher specimens from the study by Knipscheer & Crous (1990), in the National Collection of Fungi, Pretoria (PREM), but all evidence indicates they observed *Ph. myrtacearum*.

The phylogenetic hypotheses from the present study recovered *Ph. myrtacearum* in the Phakopsoraceae. *Phakopsora myrtacearum* was sister to species of *Phakopsora* parasitizing Annonaceae and two species of rusts on Annonaceae that were treated in the genus *Batistopsora* (Dianese *et al.*, 1993). Beenken (2014) examined the species of *Phakopsora* and *Batistopsora* on species of *Annona*. In that study, *Batistopsora* and *Phakopsora* were considered congeneric based on molecular phylogenetic analyses of three gene regions. Minor differences in spermogonial morphology and the presence or absence of paraphyses were regarded insufficient characters to delimit the genera (compare Cummins & Hiratsuka, 2003; Berndt *et al.*, 2008). The two species within *Batistopsora* were transferred to *Phakopsora* (Beenken, 2014). The two genera *Cerotelium* and *Masseëlla* included in the present phylogenetic study were recovered in a clade with species of *Phakopsora*, which was paraphyletic with respect to these genera.

The two rusts on Myrtaceae included in the present phylogenetic analyses, *Ph. myrtacearum* and *P. psidii*, occurred in different phylogenetic lineages and had a sister relationship with species of rust fungi on Annonaceae from Central and South America. It is noteworthy that the examined rusts on Myrtaceae share a most recent common ancestor with rusts on Annonaceae. The analyses supported previous studies that *P. psidii* does not belong to the Pucciniaceae *sensu stricto* (van der Merwe *et al.*, 2008; Pegg *et al.*, 2014). The future generic and familial placement for *P. psidii* will depend on resolution of other genera and families in the Pucciniales to ensure that polyphyletic groups are not described.

Rust fungi are among the most serious pathogens on *Eucalyptus* (Coutinho *et al.*, 1998; Glen *et al.*, 2007). Species of rust specific to eucalypts were unknown in the native range of these trees (Walker, 1983), and only two rust fungi, *P. cygnorum* and *U. xanthostemonis*, were known from Myrtaceae in Australia before the appearance of *P. psidii*. Prior to this study there were five rust species known from over 5600 Myrtaceae species, and four of these rusts infect only one host species each in

four different genera. *Puccinia psidii* is now known from over 165 host species distributed over 38 genera in all subfamilies of Myrtaceae in Australia (Pegg *et al.*, 2014). *Phakopsora myrtacearum* is currently known from three species of *Eucalyptus* in southern and eastern Africa. *Puccinia psidii* underwent a rapid host expansion on Myrtaceae and it remains to be seen if *Ph. myrtacearum* will have a similar wide host range. Although the disease impact of *Ph. myrtacearum* is not as severe as that of *P. psidii*, it presents an additional threat to eucalypts and species of *Eucalyptus* used globally by plantation forestry industries.

The widespread appearance of *Ph. myrtacearum* in southern and eastern Africa is of concern to the eucalypt forestry industries in the region. This pathogen has been found on at least three different species of *Eucalyptus* during the last 5 years. There are two possible explanations for its occurrence in Africa; it is either a non-native species introduced once or multiple times, for example on germplasm of *Eucalyptus*, or it is a native rust that has undergone a host shift to *Eucalyptus*. If it is an introduced pathogen, there may be consequences for native plant species. The distribution of *Ph. myrtacearum* is best explained by wind dispersal of urediniospores to adjacent *Eucalyptus* plantations. As it is already widely distributed in Africa, control will be difficult. *Phakopsora myrtacearum* should be considered an important quarantine pathogen that may cause damage to eucalypt forestry and potentially to other species of Myrtaceae. It is essential to assess its host range and determine its potential economic and ecological impact.

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