Uromycladium acaciae, the cause of a sudden, severe disease epidemic on *Acacia mearnsii* in South Africa

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Abstract A severe rust disease has caused extensive damage to plantation grown *Acacia mearnsii* trees in the KwaZulu-Natal Province of South Africa since 2013. The symptoms are characterized by leaf spots, petiole and rachis deformation, defoliation, gummosis, stunting of affected trees and die-back of seedlings. The cause of this new disease was identified using a combined morphological and DNA sequence approach. Based on morphology, the rust fungus was identified as a species of *Uromycladium*. It formed powdery, brown telia on petioles, stems, leaves, seedpods and trunks of affected trees. The teliospores were two per pedicel and either lacked or had a collapsed sterile vesicle. Sequence data and morphology showed that the collections from South Africa were conspecific, however telia were not produced in all provinces. *Uromycladium acaciae* is the most suitable name for this rust fungus, based on morphology and phylogenetic analyses of the internal transcribed spacer and large subunit regions of ribosomal DNA. The rust was first identified as *U. alpinum* in 1988, from minor symptoms on the leaflets caused by its uredinial stage on *A. mearnsii* in South Africa. It has now become a threat to plantations of *A. mearnsii,* with an altered life cycle and increased disease severity.

Keywords Botrycephaleae · Emerging disease · Microcyclic rust · Plantation forestry · Pucciniales · Taxonomy · Uredinales

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Introduction

Australian species of *Acacia s. str.* (Fabaceae, subfamily Mimosoideae; from here referred to as *Acacia*) in South Africa are either considered weeds, such as *A. dealbata* and *A. saligna*, or grown commercially for the production of timber for pulp, and bark for tannins, glues and other products (Midgley and Turnbull 2003; Dobson and Feely 2002). Species of *Acacia* used by the plantation forestry industry in South Africa include *A. decurrens* and *A. mearnsii*, which are planted on over 100,000 ha, mostly in the KwaZulu-Natal, Mpumalanga and Limpopo Provinces (South African Forestry and Forest Product Industry Facts 1980 – 2011 2012). Under the current system of classification, these trees fall within the Botrycephalae sublade of the plurinerved, uninerved and Botrycephalae (p.u.b.) group of *Acacia* (Murphy et al. 2010).

Two genera of rust fungi (Pucciniales), namely *Endoraecium* Hodges & D.E. Gardner and *Uromycladium* McAlpine, are known from species of *Acacia. Endoraecium* is thought to have co-evolved with species of *Acacia* in Australia (McTaggart et al. 2015). *Uromycladium* was established by McAlpine (1905) for rust fungi on *Acacia,* with branched pedicels that bear 1–3 teliospores, with or without a vesicle. He considered the arrangement and number of teliospores and vesicles on the pedicel as a valuable

taxonomic character. McAlpine (1905) described five new species of *Uromycladium* and transferred *Uredo notabilis* Ludw. and *Uromyces tepperianus* Sacc. to *Uromycladium*. Sydow and Sydow (1915) subsequently transferred *Uredo acaciae* Cooke, which was described on an Australian species of *Acacia* in New Zealand, to *Uromycladium acaciae*. They considered *U. bisporum* McAlpine, described from *A. dealbata*, a synonym of *U. acaciae*. Three additional species of *Uromycladium* have been combined or described, namely *U. fusisporum* (Cooke & Massee) Savile (Savile 1971), *U. naracoortensis* Berndt (Berndt 2010) and *U. falcatarium* Doungsa-ard, McTaggart & R.G. Shivas (Doungsa-ard et al. 2015). The most recent descriptions of new species of *Uromycladium* have included the use of DNA sequence data, host and lifecycle.

Berndt (2010) regarded the absence of uredinia (microcyclic lifecycle) as the main characteristic to distinguish *U. naracoortensis* from taxa with morphologically similar teliospores, such as *U. alpinum* McAlpine and *U. maritimum* McAlpine. Doungsa-ard et al. (2015) used a combined morphological and molecular approach to determine *U. tepperianum* was a species complex, and *U. falcatarium* represented a host jump from *Acacia* to *Falcataria*. There are currently ten accepted species of *Uromycladium*.

Two species of *Uromycladium* have been reported on *Acacia* from South Africa. *Uromycladium tepperianum* (Sacc.) McAlpine was introduced into the country as a biological control agent for weedy *A. saligna,* which became invasive after its introduction to the Western Cape Province (Morris 1987). *Uromycladium tepperianum* specifically caused severe galls on the stems and leaves of *A. saligna* and not native trees in South Africa formerly classified as *Acacia* (Morris 1997; Wood and Morris 2007). The second known species from South Africa, *U. alpinum*, was reported from *A. mearnsii* in the eastern provinces of South Africa, and Swaziland (Morris et al. 1988). It was known only from its uredinial stage, and was reported to cause minor leaf spot symptoms on these trees (Morris et al. 1988).

Uromycladium alpinum forms bullate sori on both surfaces of infected leaves, and uredinia and telia are formed in the same sorus (McAlpine 1905). It has two teliospores per pedicel with a vesicle beneath the septum. Three additional species of *Uromycladium* were described with two teliospores per pedicel. *Uromycladium acaciae* (on *A. dealbata*), which does not have a vesicle, and *U. maritimum* (on *A. longifolia,* Juliflorae p.p.) and *U. naracoortensis* (on *A. iteaphylla*, Botrycephalae), which both have a vesicle. These three species of *Uromycladium* have not been reported outside of Australia and New Zealand.

A severe outbreak of an unknown rust fungus on *A. mearnsii* in the KwaZulu-Natal Province was first detected from South Africa in 2013 and has caused damage to these trees for the past 3 years. The rust causes a gummosis of the bark on trunks and stems, matted leaves, pinnule and petiole malformation and severely stunted growth of saplings and young trees. Based on initial microscopic investigation of the teliospores, the rust was identified as a species of *Uromycladium*. Surveys were conducted to determine the extent of the disease and samples collected to identify the pathogen to species level. A combined morphological and DNA sequence approach was used to determine the cause of the new rust epidemic on *A. mearnsii* in South Africa.

Materials and methods

Disease symptoms and specimen collection

The distribution and impact of the rust disease on *A. mearnsii* was determined from plantations, woodlots and jungle stands throughout South Africa, including the KwaZulu-Natal, Limpopo, Mpumalanga and Western Cape Provinces, as well as neighboring Swaziland, in 2014 and 2015. Where encountered, *A. decurrens* trees were inspected for the presence of rust as it often occurs within and adjacent to *A. mearnsii* trees. Fresh infections of rust were pressed, dried and lodged in the herbarium of the South African National Fungus Collection (PREM) in Pretoria (Agricultural Research Council).

Morphology

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. Dried herbarium material from the original collection of *U. alpinum* on *A. mearnsii* by Morris et al. (1988), was obtained from PREM for comparative purposes (PREM 48897).

DNA extraction, amplification and sequencing

DNA was extracted from freshly collected rust specimens. Uredinia or telia were selectively 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)/LR7 (Vilgalys and Hester 1990) and nested with LROR/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, initial LSU at 57 °C, and nested

LSU at 62 °C. PCR products were cleaned by an 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, University of Pretoria. Sequences were assembled using the CLC Main Workbench (Qiagen) or Sequencher 4.8 (GeneCodes).

Phylogenetic analyses

The ITS and LSU sequences of 28 isolates of *Uromycladium* were aligned with the MAFFT algorithm (Katoh et al. 2009) in SATé (Liu et al. 2012). The data set included eight of the ten described species of *Uromycladium* (Table 1). Reference sequences for *U. alpinum* and *U. maritimum* were not available. *Ravenelia neocaledoniensis* and *Tranzschelia discolor* were included as outgroups in the phylogenetic analyses, based on the relationship shown by Doungsa-ard et al. (2015).

Maximum likelihood was implemented as a search criterion in RAxML version 8 (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 and Huelsenbeck 2003). Four runs, each consisting of four chains, were implemented for 10 million generations. 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/). A default burnin of 25 % was used by MrBayes and 30,004 trees were summarized to construct a consensus tree.

Results

Disease symptoms

Signs and symptoms of rust were observed on *A. mearnsii* in all surveyed areas, including Swaziland. In most areas the symptoms associated with the rust were mild, characterized by the presence of chlorotic lesions on leaflets that corresponded to abaxial uredinia, or brown, powdery lesions on leaflets that had adaxial or abaxial telia. However, in the KwaZulu-Natal Midlands, a severe rust epidemic on *A. mearnsii* was present. Symptoms associated with the epidemic included die-back of young shoots and the leader stems of young saplings, gummosis on trunks and stems (Fig. 1a, b), masses of powdery brown telia on pinnules, rachi, seed capsules and buds (Fig. 1h, i), malformation of rachi, leaf drop, and production of exudate that matted leaves and contained masses of teliospores (Fig. 1b). These severe symptoms were associated with the telial stage of the rust. Spermogonia were present on the adaxial surface of leaflets in severe infections, but were not associated with the symptoms (Fig. 1c, d).

Rust was observed on *A. decurrens* where these trees were found growing in association with *A. mearnsii* trees. Severe symptoms were not observed on *A. decurrens*, and mostly only single powdery patches of telia that caused matted leaflets were observed after careful examination. Infection was absent on the stems and trunks, and gummosis did not occur.

Morphology

The rust specimens collected on *A. mearnsii* in South Africa and Swaziland had similar morphology of their urediniospores (Fig. 1f, g), irrespective of geographic location. The morphology of urediniospores for specimens from KwaZulu-Natal and Mpumalanga was identical, but differed slightly to specimens from the Western Cape (Table 2). The urediniospores from the Cape specimens were wider (21–26 μm) than those from KwaZulu-Natal and Mpumalanga (16–23 μm). We examined 15 urediniospores from the first report of *U. alpinum* on *A. mearnsii* (PREM 48897) for comparison. These were (26–)29–40×20–24 μm, wall 1.5–3 μm, sometimes with a thickened apex up to 6 μm. The original description had thinner spores 24–40×14–20 μm, wall 2 μm at sides, up to 4 μm thick at the apex (Morris et al. 1988).

Telia occurred in brown, powdery patches on the petioles, leaflets, stems, fruits and trunks, separate to the uredinia. The teliospores were two per pedicel and usually lacked or had a collapsed sterile vesicle (Fig. 1j, k). Vesicles were rarely present in the examined specimens. The teliospores were globose to ellipsoid and measured 18–23×15–20 μm.

Phylogenetic analyses

Sequence data were obtained from nine rust specimens from *A. mearnsii* and one specimen from *A. decurrens* (Table 1). A BLASTn search of the ITS rDNA sequences had highest identities to *Uromycladium fusisporum* (KJ633009, 82 % identical, 507/619 identities) and *U. simplex* (KJ33010, 87 % identical, 360/416 identities). A BLASTn search of the LSU rDNA sequences had highest identities to *U. simplex* (KJ632990 99 % identical, 1017/1032 identities), and *U. simplex* (KJ862351 98 % identical, 1017/1044 identities).

The topologies recovered by RAxML (Fig. 2) and MrBayes were identical, except that MrBayes recovered *U. simplex* in a polytomy, rather than sister to the three-spored species of *Uromycladium* including *U. falcatarium*, *U. notabile* and *U. tepperianum*. *Uromycladium* was

Table 1 Isolates used in phylogenetic analyses. GenBank numbers obtained from this study in bold

Species	Voucher number	Host	Location	GenBank number	
				ITS	LSU
Uromycladium acaciae	PREM 61252	*Acacia mearnsii*	Pietermaritzburg, KwaZulu-Natal, South Africa	NA	**KR612237**
U. acaciae	PREM 61254	*A. mearnsii*	Pietermaritzburg, KwaZulu-Natal, South Africa	NA	**KR612239**
U. acaciae	PREM 61256	*A. mearnsii*	Dalton, KwaZulu-Natal, South Africa	**KR612232**	**KR612235**
U. acaciae	PREM 61257	*A. mearnsii*	Dalton, KwaZulu-Natal, South Africa	**KR612233**	**KR612236**
U. acaciae	PREM 61258	*A. mearnsii*	Gordonsbaai, Western Cape, South Africa	NA	**KR612240**
U. acaciae	PREM 61259	*A. mearnsii*	Gordonsbaai, Western Cape, South Africa	**KR612234**	**KR612241**
U. acaciae	PREM 61260	*A. mearnsii*	Gordonsbaai, Western Cape, South Africa	NA	**KR612242**
U. acaciae	PREM 61261	*A. mearnsii*	Gordonsbaai, Western Cape, South Africa	NA	**KR612243**
U. acaciae	PREM 61253	*A. decurrens*	Pietermaritzburg, KwaZulu-Natal, South Africa	NA	**KR612238**
U. acaciae	BRIP 59239	*A. mearnsii*	Australia	KR994892[a]	KR994852[a]
U. acaciae	BRIP 60092	*A. terminalis*	Australia	KR994893[a]	KR994853[a]
U. falcatarium	BRIP 57477	*Falcataria moluccana*	Philippines	KJ632993[b]	KJ632973[b]
U. falcatarium	BRIP 57990	*Falcataria moluccana*	Timor Leste	KJ632994[b]	KJ632974[b]
U. fusisporum	BRIP 27608	*A. salicina*	Australia	NA	DQ323921[c]
U. fusisporum	BRIP 57526	*A. salicina*	Australia	KJ633009[b]	KJ632991[b]
U. naracoortensis	MEL 2357562	*A. iteaphylla*	Australia	KR994920[a]	KR994880[a]
U. notabile	BRIP 59234	*A. dealbata*	Australia	KJ633011[b]	KJ632992[b]
U. robinsonii	BRIP 57538	*A. melanoxylon*	Australia	KJ633012[b]	KJ632989[b]
U. simplex	BRIP 59214	*A. pycnantha*	Australia	KJ633010[b]	KJ632990[b]
U. tepperianum	BRIP 57511	*A. leiocalyx*	Australia	KJ633006[b]	KJ632982[b]
U. tepperianum	BRIP 56928	*A. leiocalyx*	Australia	KJ633005[b]	KJ632981[b]
U. tepperianum	BRIP 57819	*A. acuminata*	Australia	KJ633001[b]	KJ632978[b]
U. tepperianum	BRIP 57756	*A. acuminata*	Australia	KJ633000[b]	KJ632977[b]
U. tepperianum	BRIP 56962	*A. saligna*	Australia	KJ632996[b]	KJ632985[b]
U. tepperianum	BRIP 57860	*A. saligna*	Australia	KJ632995[b]	KJ632988[b]
Uromycladium sp. aff. *maritimum*	BRIP 56556	*A. thomsonii*	Australia	KR994918[a]	KR994878[a]
Uromycladium sp. aff. *maritimum*	BRIP 56551	*A. thomsonii*	Australia	KR994917[a]	KR994877[a]
Ravenelia neocaledoniensis	BRIP 56908	*Vachellia farnesiana*	Australia	NA	KJ862348[d]
Tranzschelia discolor	BRIP 57662	*Prunus persica*	Australia	NA	KR994891[a]

[a] Doungsa-ard unpublished

[b] Doungsa-ard et al. (2015)

[c] Scholler and Aime (2006)

[d] McTaggart et al. (2015)

recovered as a monophyletic group. The specimens on *A. mearnsii* and *A. decurrens* from South Africa occurred in a monophyletic group with two specimens of *Uromycladium* with uredinia on *A. mearnsii* and *A. terminalis* from Australia. The isolates from KwaZulu-Natal and Mpumalanga were identical in the LSU region, and differed from Western Cape isolates by one base pair. The ITS region was identical for the isolates sequenced from KwaZulu-Natal and the Western Cape.

Taxonomy

Based on DNA sequence data and morphology we identified the rust on *A. mearnsii* and *A. decurrens* in South Africa as *U. acaciae*. *Uromycladium alpinum*, the name previously applied to the same fungus collected in the Western Cape, is most likely absent from South Africa. The morphology of the teliospores and host range of the rust on *A. mearnsii* matched *U. acaciae* rather than

Fig. 1 *Uromycladium acaciae* on *Acacia mearnsii*. **a**–**b** Field symptoms; **c**–**d** Spermogonia; **e** Uredinia; **f**–**g** Urediniospores; **h**–**i** Telia; **j**–**k** Teliospores. Scale bars=10 μm

U. alpinum because a vesicle was usually either collapsed or absent on the teliospores (Fig. 1j, k), the telia formed separately to the uredinia, and the host resides in sub-clade Botrycephalae rather than Juliflorae. Formation of telia in powdery patches on leaflets, petioles and stems matched the descriptions of *U. acaciae* by McAlpine (1905) (as *U. bisporum*) and Cunningham (1931). *Uromycladium acaciae* was first described from New Zealand as *Uredo acaciae* from its telial stage (Cooke 1890). Spermogonia and uredinia

Table 2 Urediniospore measurements of *U. acaciae* from different South African locations

Location/Specimen	Length	Width	Wall	Apex	Germ pores
Cape Province PREM 48897 (Morris et al. 1988)	(26–)29–40 μm (original: 24–40 μm)	20–24 μm (original: 14–20 μm)	1.5–3.0 μm (original: 2 μm)	3–6 μm (original: 3–4 μm)	4–5
Cape Province: PREM 61258, 61262	32–49 μm	21–26 μm	2 μm	Up to 4 μm	4–5
KwaZulu-Natal, Mpumalanga: PREM 61256	(27–)32–48 μm	(16–)17–23 μm	2 μm	Up to 6.5 μm	4–5

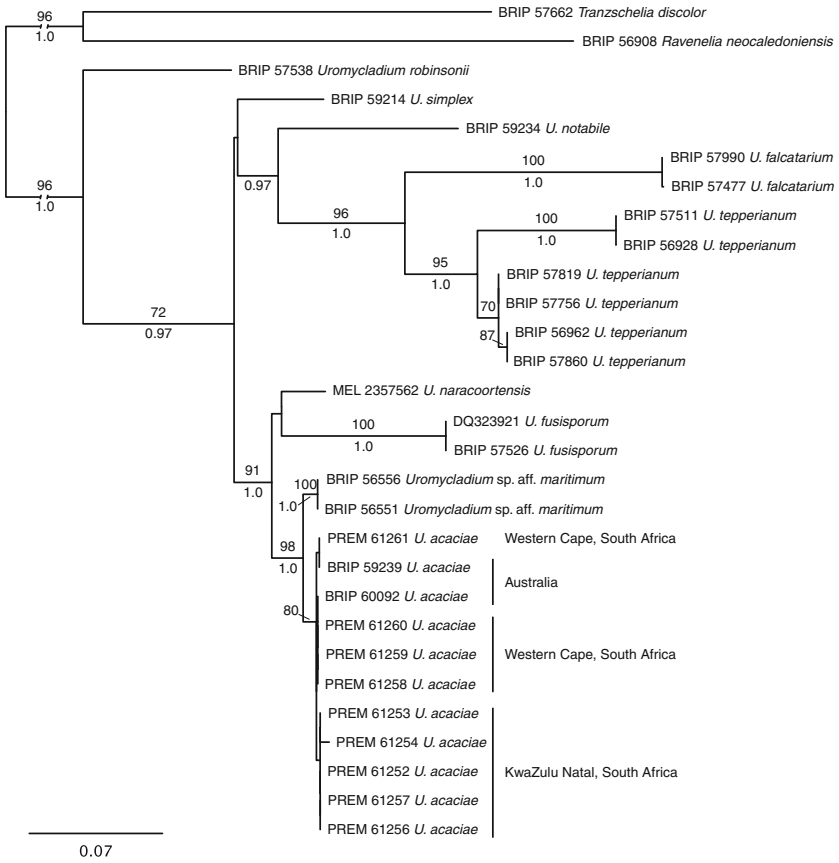

Fig. 2 Phylogram obtained from a maximum likelihood search in RAxML on the ITS and LSU regions of rDNA. Bootstrap values (≥70 %) from 1000 ML replicates above nodes. Posterior probability values (≥0.95) summarized from 30,004 trees in a Bayesian search below nodes

were previously unknown for *U. acaciae* and a description of these spore stages is included below.

Uromycladium acaciae (Cooke) P. Syd. & Syd. emend. McTaggart & Roux (Fig. 1)

Basionym *Uredo acaciae* Cooke, Grevillea 19(no. 89): 3 (1890)

= *Uromylcadium bisporum* McAlpine, Annls mycol. 3(4): 307 (1905) (synonymy by Sydow and Sydow (1915))

Spermogonia on leaflets or stems, adaxial, erumpent, hemispherical, 65–130 μm diam.

Spermatia globose or ellipsoidal, hyaline, 2.5–5.0×2.0–4.0 μm.

Uredinia on leaflets, abaxial, erumpent, covered by peridium made of host tissue, pulvinate, up to 0.5 mm, dark brown.

Urediniospores clavate or cylindrical or narrowly ellipsoidal, brown, (26–)33–49×(16–)17–26 μm; wall 1.5–3.0 μm thick, up to 6.5 μm thick at apex, verrucose, with 4–5 equatorial germ pores.

Telia separate to uredinia, sometimes surrounding spermogonia, on petioles, leaflets, stems, fruits and trunk, pale brown, forming powdery patches.

Teliospores formed in pairs, globose, subglobose or broadly ellipsoid, pale brown, 18–23×15–20 μm; wall 1.5–2.0 μm thick, smooth, pedicel persistent, usually no vesicle or a collapsed vesicle present on mature teliospores.

Type on *Acacia dealbata*

Epitype (designated here): PREM 61256 on *A. mearnsii* ITS: KR612232, LSU: KR612235

Hosts: *A. dealbata, A. decurrens, A. mearnsii*, *A. terminalis* (subclade Botrycephalae). These taxa are closely related and split from a recent common ancestor less than 5 million years ago (Miller et al. 2013).

Specimens examined: SOUTH AFRICA, KwaZulu-Natal, Hilton, on *Acacia mearnsii*, 29 Sept. 2014, *J. Roux & A.R. McTaggart*, PREM 61252, GenBank LSU: KR612237; KwaZulu-Natal, Hilton, on *A. decurrens*, 29 Sept. 2014, *J. Roux & A.R. McTaggart*, PREM 61253, GenBank LSU: KR612238; KwaZulu-Natal, Hilton, on *A. mearnsii*, 29 Sept. 2014, *J. Roux & A.R. McTaggart*, PREM 61254, GenBank LSU: KR612239; KwaZulu-Natal, Hilton, on *A. mearnsii*, 29 Sept. 2014, *J. Roux & A.R. McTaggart*, PREM 61255; KwaZulu-Natal, Dalton, on *A. mearnsii*, 30 Sept. 2014, *J. Roux & A.R. McTaggart*, PREM 61256, GenBank ITS: KR612232, LSU: KR612235; KwaZulu-Natal, Dalton, on *A. mearnsii*, 30 Sept. 2014, *J. Roux & A.R. McTaggart*, PREM 61257, GenBank ITS: KR612233, LSU: KR612236; Western Cape, Gordons Bay, on *A. mearnsii*, 07 Nov. 2014, *J. Roux*, PREM 61258, GenBank LSU: KR612240; Western Cape, Gordons Bay, on *A. mearnsii*, 07 Nov. 2014, *J. Roux*, PREM 61259, GenBank

ITS: KR612234, LSU: KR612241; Western Cape, Gordons Bay, on *A. mearnsii*, 07 Nov. 2014, *J. Roux*, PREM 61260, GenBank LSU: KR612242; Western Cape, Gordons Bay, on *A. mearnsii*, 07 Nov. 2014, *J. Roux*, PREM 61261, GenBank LSU: KR612243; Western Cape, Cape Town, on *A. mearnsii*, Nov. 2014, *M.J. Wingfield*, PREM 61262; SWAZILAND, Nisela Nature Reserve, on *A. mearnsii*, 14 Feb. 2015, *J. Roux*, *L.S. Shuey*, *A.R. McTaggart*, PREM 61263; AUSTRALIA, Tasmania, Coles Bay, Coles Bay Road, (−42.1078, 148.2556), on *A. terminalis*, 21 Dec. 2013, *A.R. McTaggart*, *L.S. Shuey*, *M.D.E. & R.G.* Shivas, BRIP 60092, GenBank ITS: KJ632993, LSU: KJ632973; Victoria, Ingliston, Werribee Gorge, Ironbank Road, (−37.6728, 144.3636), on *A. mearnsii*, 11 May 2013, *C. Doungsa-ard*, *W. Khemmuk & A.D.W. Geering*, BRIP 59239, GenBank ITS: KR994893, LSU: KR994853.

Key to species of *Uromycladium* with two teliospores per pedicel

1. Telia formed as powdery patches separate to uredinia, vesicle usually absent or collapsed beneath teliospores
U. acaciae
1. Telia formed in compact sorus, vesicle present beneath teliospores
2.
2. Urediniospores unthickened at apex, on *Acacia dallachiana*
U. alpinum
2. Urediniospores thickened at apex, on *A. longifolia*
U. maritimum
2. Urediniospores unknown, on *A. iteaphylla*
U. naracoortensis

Discussion

Pests and diseases have gradually increased on *A. mearnsii* since its introduction to South Africa in the 1860s (Wingfield et al. 2011). The most recent new disease report in South Africa on *A. mearnsii* was of *Ceratocystis albifundus* (as *C. fimbriata*) (Morris et al. 1993; Roux and Wingfield 2013). In late 2013, commercial farmers in the KwaZulu-Natal Province discovered a previously unknown disease symptom on *A. mearnsii*. By 2014 this disease had escalated to an epidemic of significant concern in the region. The cause of this rust disease was identified as *Uromycladium acaciae,* based on morphology and DNA sequence data. It has likely been present in South Africa since 1988.

Uromycladium acaciae on *A. mearnsii* identified in this study was obtained from trees in four Provinces of South Africa, as well as from neighboring Swaziland. In the Western Cape, the rust caused isolated leaf spots associated with uredinia. Telia were found in all other provinces and Swaziland. However, the severe epidemic only occurred in the KwaZulu-Natal Midlands.

The first report of rust on *A. mearnsii* in South Africa was of *U. alpinum,* identified based on the morphology of the uredinial stage (Morris et al. 1988). Morris et al. (1988) compared the rust from South Africa to three rusts on *A. mearnsii* held in Australian herbaria, namely *U. alpinum*, *U. notabile* (Ludw.) McAlpine and *Endoraecium digitatum* (G. Winter) M. Scholler & Aime. They regarded reports of *U. acaciae* on *A. mearnsii* (Gibson 1975) as unsubstantiated because no records existed in Australian herbaria.

Uromycladium acaciae is likely the rust reported on *A. mearnsii* in South Africa by Morris et al. (1988). We examined an original collection reported by Morris et al. (1988) and obtained fresh collections of a uredinial rust from the same locality. *Uromycladium acaciae* spread in South Africa by its urediniospores, which cause minor symptoms, and were distributed from the Western Cape to Swaziland (Morris et al. 1988), and in all surveyed areas of this study. *Uromycladium alpinum* was also reported as the cause of a uredinial rust on *A. mearnsii* in Brazil (Santos and Ferreira 2002). The Brazilian report may also refer to *U. acaciae*, but this has not been investigated in the current study.

There was intraspecific variation of one base pair in the LSU region of rDNA between *U. acaciae* specimens from KwaZulu-Natal and Mpumalanga with the Western Cape. A phylogenetic approach to species delimitation with the ITS and LSU regions of rDNA showed that these collections were conspecific. Intraspecific sequence variation of rDNA has been observed in other species of rust on *Acacia*, such as *Endoraecium auriculiforme* McTaggart & R.G. Shivas and *E. irroratum* McTaggart & R.G. Shivas (McTaggart et al. 2015). Further studies will determine if there are differences in the populations of *U. acaciae* between the provinces of South Africa.

The severity of the symptoms caused by *U. acaciae* are unusual, given that the fungus appears to have been present in South Africa for many years without causing serious damage. The telial stage of this rust has caused defoliation, gummosis, seedling death, stunting, and dieback of the leader stems on *A. mearnsii* since 2013 in the KwaZulu-Natal Midlands. Specimens of *U. acaciae* collected from the Western Cape and Limpopo Provinces, however, did not produce the telial stage. Future studies will investigate the environmental conditions in which telia are produced and the role they play in the life cycle of *U. acaciae*.

Uromycladium acaciae was considered to be microcyclic, known only from its telial stage (McAlpine 1906). The spermogonia and uredinia, which were previously unknown, are described here from South Africa and several questions are raised by their discovery. For example, it is unknown whether

these stages were overlooked, attributed to another species of rust, or not formed in Australia.

Rust fungi have the largest genomes of all fungi (Tavares et al. 2014). This large genome size is in part explained by transcriptome analyses that showed unique genes are expressed by different stages of the life cycle (Hacquard et al. 2013; Xu et al. 2011). Microcyclic rust fungi can lose their aecial and uredinial stages in conditions favourable for continuous growth and reproduction (Ono 2002); the unique genes associated with these stages may remain in the genome, but are not expressed. If spermogonia and uredinia of *U. acaciae* were not produced when it was first described, this could be an example of a rust thought to be microcyclic and capable of producing 'lost' stages given favourable environmental stimuli.

The uredinia of *U. acaciae* form separately from the powdery telia, and consequently, uredinia may have been overlooked, or not attributed to *U. acaciae* when they were found. The urediniospores of *U. acaciae* are now known to occur in Australia from specimens included in this study; for example uredinia are present on BRIP 60092 collected on *A. terminalis*. It is possible that collections of *U. acaciae* on *A. mearnsii*, with only the uredinial stage present, were identified as *U. alpinum*. This was the most accurately applied name, as urediniospores were not described for *U. acaciae*. The host range of *U. alpinum* requires further study to determine whether it is restricted to Juliflorae or whether it also occurs on hosts in Botrycephalae, as was described by McAlpine (1905).

Absence of a lifecycle stage in rust fungi does not definitively imply it does not exist. The presence or absence of spermogonia or uredinia was used to delimit species of *Uromycladium*, such as *U. naracoortensis* from *U. maritimum* (Berndt 2010). Other studies have indicated that the presence or absence of a lifecycle stage is homoplasious (McTaggart et al. 2014). In light of this study, we suggest this is not a reliable taxonomic character for *Uromycladium*. Rather that morphology, host range and phylogenetic concordance should define taxonomy.

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