# First report of the wattle rust pathogen, *Uromycladium acaciae* (Raveneliaceae, Pucciniales) in Ethiopia

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**Abstract**

Australian *Acacia* species are amongst the most important trees planted for wood and pulp production in several African countries, including Ethiopia. In 2020, symptoms of a serious shoot and leaf rust disease were observed on black wattle (*Acacia mearnsii* De Wild.) trees across the three main wattle growing regions of Ethiopia. The aim of this study was to describe the disease and identify its causal agent based on morphological characteristics as well as DNA sequence data for the ITS and LSU regions of ribosomal RNA. Here we report for the first time, the presence of the wattle rust pathogen, *Uromycladium acaciae,* in Ethiopia.

**Keywords:** *Acacia mearnsii*, East Africa, fungal pathogens, plantation forestry, rust disease

## Introduction

Plantation forestry utilising fast-growing tree species commenced in Ethiopia in the 1890s when *Eucalyptus* was first introduced into the country (Persson, 1995). Currently, Ethiopia has approximately 972,000 ha of planted forests of which the majority (78%) are managed by smallholder farmers (Lemenih and Kassa, 2014). These plantations are mainly based on non-native *Eucalyptus*, *Cupressus*, *Pinus* and *Acacia* species (Bekele, 2011; Lemenih and Kassa, 2014).

Similar to many species of *Eucalyptus*, Australian *Acacia* species have been increasingly established in plantations outside their native range in the tropics and southern hemisphere. Species belong to the Botrycephalae subclade of *Acacia sensu stricto* (Miller *et al.*, 2013), i.e. black wattle (*A. mearnsii* De Wild.), silver wattle (*A. dealbata* Link) and green wattle (*A. decurrens* Willd.), are among the most extensively planted of these trees in many African countries (Moreno Chan *et al.*, 2015; Richardson *et al.*, 2015). These species are grown especially for tannin extraction, timber and pulp production, and rehabilitation of degraded land (Midgley and Turnbull, 2003; Moreno Chan *et al.*, 2015, Chanie and Abewa, 2021).

As is true for many other regions, early establishment of plantation forestry in Africa based on non-native *Acacia* has been highly successful, largely due to their separation from natural enemies (Wingfield *et al.*, 2011). However, these plantations are increasingly threatened by pests and pathogens including those that are accidentally introduced or others that are native and have adapted to utilise the non-native trees as hosts (Wingfield *et al.*, 2011). Important diseases of plantation-grown *Acacia* spp. in Africa include Phytophthora root rot (Zeijlemaker, 1971; Roux *et al.*, 1995, 2005; Roux and Wingfield, 1997, Bose *et al.*, 2019), stem canker caused by species of Botryosphaeriaceae (Roux *et al.*, 1995, Roux and Wingfield, 1997), wattle wilt caused by the native pathogen *Ceratocystis albifundus* (Wingfield *et al.*, 1996; Roux and Wingfield, 2009; Roux *et al.*, 2001, 2005), and the recently

reported wattle rust caused by the non-native pathogen *Uromycladium acaciae* (McTaggart *et al.*, 2015).

Complaints from wattle growers in Ethiopia of diseased and dying wattle were conveyed from 2020 and led to surveys of pests and diseases associated with *Acacia* plantations in Ethiopia in 2022. From these surveys, symptoms of a serious shoot and leaf rust disease on *A. mearnsii* trees across the three main wattle growing regions of Ethiopia were observed. The aim of this study was to describe the disease and identify its causal agent based on morphological characteristics as well as DNA sequence data.

## Materials and Methods

### *Surveys and sample collection*

Surveys were conducted at 18 sites across three wattle growing regions, namely Awi, Gamo and Gurage (Figure 1). The first survey in March 2022 was conducted in Awi, where ten sites across three districts (Ankesha Gagusa, Banja and Fageta Lekoma) were assessed. A second survey was conducted in July 2022 in Gurage and Gamo. In Gurage, six sites across three districts, namely Cheha, Eja and Gumer were assessed while in Gamo, two sites in Qogota district were surveyed. Location, GPS coordinates, elevation, and tree age were recorded for each site (Table 1).

Infected plant material was collected from 40 trees across 18 sites and spore samples were stored in cryogenic vials containing RNAlater™ Stabilization Solution (Thermo Fisher Scientific, Waltham, MA, USA) and silica gel and transferred to laboratory for processing. Dried specimens were lodged in the herbarium housed at H.G.W.J. Schweickerdt Herbarium, PRU(M), at the University of Pretoria, South Africa.

**Table 1.** Site information for the disease survey conducted in wattle plantations in Ethiopia.

| Site No. | Zone | District | Site name | Tree age (yr) | Geographic coordinates | | Elevation (m.a.s.l.) |
|---|---|---|---|---|---|---|---|
| | | | | | Latitude (N) | Longitude (E) | |
| 1 | Awi | Fageta Lekoma | Ashewa Tsebel 1 | 3 | 11.1513 | 36.8652 | 2353 |
| 2 | Awi | Fageta Lekoma | Gafera | 3 | 11.0663 | 36.8935 | 2538 |
| 3 | Awi | Fageta Lekoma | Adilta | 3 | 11.0473 | 36.9021 | 2378 |
| 4 | Awi | Banja | Zikena Gomerta 1 | 3 | 10.9589 | 36.8165 | 2506 |
| 5 | Awi | Banja | Gashena Akayta | 3 | 10.9689 | 36.9510 | 2617 |
| 6 | Awi | Banja | Kesachewsa | 3 | 10.9234 | 36.9554 | 2543 |
| 7 | Awi | Ankesha Gagusa | Dangula | 3 | 10.8693 | 36.9341 | 2435 |
| 8 | Awi | Ankesha Gagusa | Habiti | 3 | 10.8647 | 36.8954 | 2366 |
| 9 | Awi | Ankesha Gagusa | Amara Mender | 4 | 10.9138 | 36.8297 | 2402 |
| 10 | Awi | Ankesha Gagusa | Aneba | 3 | 10.8799 | 36.8776 | 2337 |
| 11 | Gurage | Cheha | Dakuna | 2 | 8.0786 | 37.9919 | 2412 |
| 12 | Gurage | Cheha | Moche | 4 | 8.0516 | 38.0179 | 2680 |
| 13 | Gurage | Gumer | Jomboro | 3 | 8.0185 | 38.0769 | 2821 |
| 14 | Gurage | Gumer | Gibcha | 3 | 8.1322 | 37.9952 | 2272 |
| 15 | Gurage | Eja | Megeja | 5 | 8.1319 | 38.0194 | 2320 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 16 | Gurage | Eja | Kotar Geta | 5 | 8.1242 | 38.0636 | 2593 |
| 17 | Gamo | Qogota | Gircha | 5 | 6.3021 | 37.5663 | 2988 |
| 18 | Gamo | Qogota | Tula | 5 | 6.3321 | 37.5791 | 2843 |

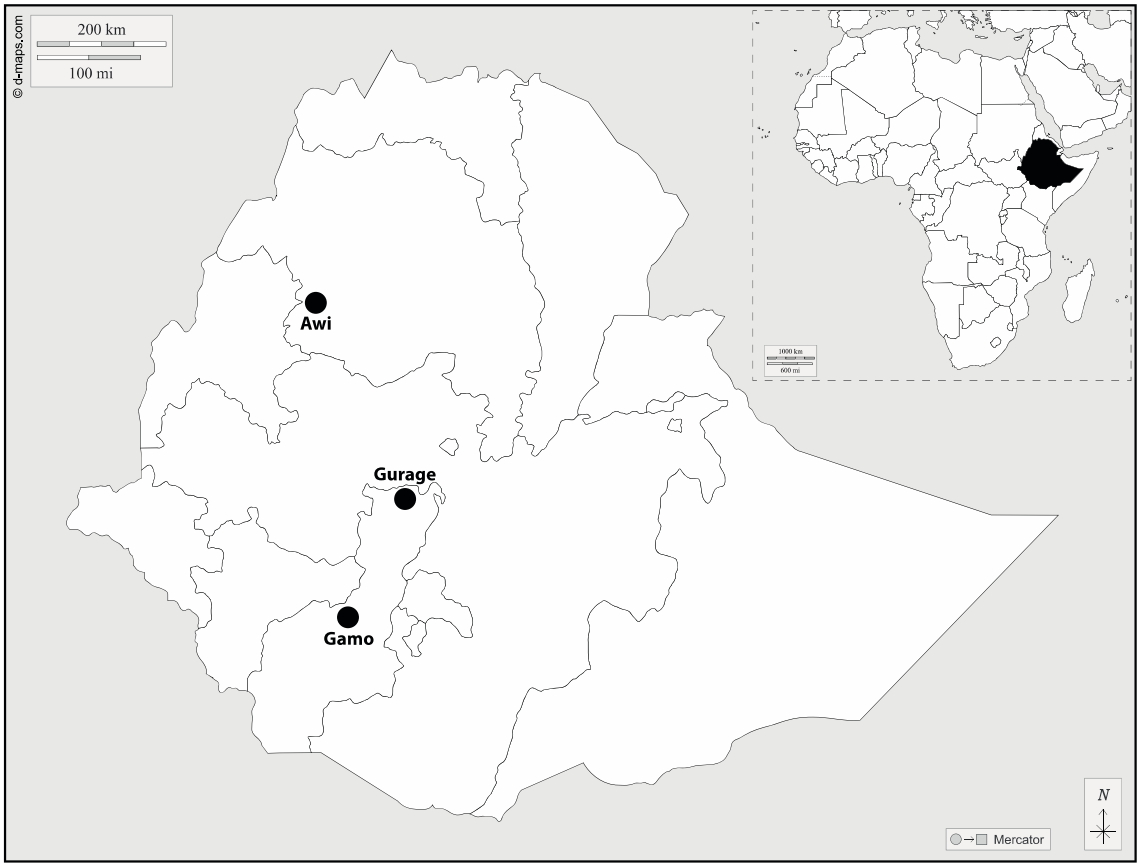


**Figure 1.** A map showing the three zones, namely Awi, Gurage and Gamo, where the survey and sampling was conducted.

***Pathogen identification***

*Morphology*

Infected plant materials were studied under a microscope (Nikon Eclipse Ni or SMZ18, Tokyo, Japan) to examine fungal structures. Spore masses were scraped from the plant material, mounted in water which was replaced with 85% lactic acid for further investigation. Images of the spore masses were captured with a camera (Nikon DS-Ri2, Japan) mounted on the microscope. The size and wall thickness of 50 spores were measured using an imaging software (NIS Elements, Nikon, Japan), and presented as mininum–maximum (average ± standard deviation).

*DNA extraction, PCR and sequencing*

Spore masses were scraped from the surface of infected plant material and DNA was extracted with the Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. The LSU region of rDNA was amplified with primers Rust2inv (Aime, 2006) and LR7 (Vilgalys and Hester, 1990) and the amplicons were nested using primers LR0R and LR6 (Vilgalys and Hester, 1990). The internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S rRNA region, were amplified using primers ITS1F and ITS4rust (Gardes and Bruns, 1993; Beenken *et al.*, 2012). The PCR reactions and conditions followed those used by Pham *et al.* (2019) and McTaggart *et al.* (2015). ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used to purify the amplicons. Cleaned amplified fragments were sequenced in both directions using an ABI PRISM™ 3100 DNA sequencer (Thermo Fisher Scientific, Waltham, MA, USA) at the Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Geneious Prime v. 2023.0.1 (https://www.geneious.com) was used to assemble and trim the raw sequences, which were deposited in GenBank (Table 2).

*Phylogenetic analyses*

Sequences obtained were subjected to BLASTn searches on the NCBI database (https://www.ncbi.nlm.nih.gov/). Subsequently, reference sequences for species closely related to those emerging from this study were downloaded from GenBank and subjected to phylogenetic analyses (Table 2). Alignments of all sequences were assembled using the online version of MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013), then confirmed manually in MEGA v. 7 (Kumar *et al.*, 2016). A concatenated data set comprising ITS and LSU sequences was used for phylogenetic analyses. Maximum likelihood (ML) analysis was conducted using RaxML v. 8.2.4 on the CIPRES Science Gateway v. 3.3 (Stamatakis, 2014) with default GTR substitution matrix and 1000 rapid bootstraps. Sequences for *Tranzschelia pruni-spinosae* (KR-M-0002755) were included as the outgroup.

**Table 2.** Collection details and GenBank accessions of specimens used in phylogenetic analyses.

| **Species** | **Specimens** [1] | **Host** | **Locality** | **GenBank accession number** | | **Reference** |
|---|---|---|---|---|---|---|
| | | | | **ITS** | **LSU** | |
| *Uromycladium acaciae* | PREM 61256 | *Acacia mearnsii* | KwaZulu-Natal, South Africa | KR612232 | KR612235 | McTaggart *et al.* (2015) |
| *U. acaciae* | PREM 61257 | *A. mearnsii* | KwaZulu-Natal, South Africa | KR612233 | KR612236 | McTaggart *et al.* (2015) |
| *U. acaciae* | PREM 61259 | *A. mearnsii* | Western Cape, South Africa | KR612234 | KR612241 | McTaggart *et al.* (2015) |
| *U. acaciae* | BRIP 59239 | *A. mearnsii* | Australia | KR994892 | KR994852 | McTaggart *et al.* (2015) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *U. acaciae* | BRIP 60092 | *A. terminalis* | Australia | KR994893 | KR994853 | McTaggart *et al.* (2015) |
| *U. acaciae* | **PRU(M) 4530** | *A. mearnsii* | Ashewa Tsebel, Fageta Lekoma, Awi, Ethiopia | OQ975026 | OQ970605 | This study |
| *U. acaciae* | **PRU(M) 4531** | *A. mearnsii* | Gafera, Fageta Lekoma, Awi, Ethiopia | OQ975027 | OQ970606 | This study |
| *U. acaciae* | **PRU(M) 4532** | *A. mearnsii* | Adilta, Fageta Lekoma, Awi, Ethiopia | OQ975028 | OQ970607 | This study |
| *U. acaciae* | **PRU(M) 4533** | *A. mearnsii* | Zikena Gomerta, Banja, Awi, Ethiopia | OQ975029 | OQ970608 | This study |
| *U. acaciae* | **PRU(M) 4534** | *A. mearnsii* | Gashena Akayta, Banja, Awi, Ethiopia | OQ975030 | OQ970609 | This study |
| *U. acaciae* | **PRU(M) 4535** | *A. mearnsii* | Kesachewsa, Banja, Awi, Ethiopia | OQ975031 | OQ970610 | This study |
| *U. acaciae* | **PRU(M) 4536** | *A. mearnsii* | Dangula, Ankesha Gasuga, Awi, Ethiopia | OQ975032 | OQ970611 | This study |

| | | | | | | |
|---|---|---|---|---|---|---|
| *U. acaciae* | **PRU(M) 45 37** | *A. mearnsii* | Habiti, Ankesha Gasuga, Awi, Ethiopia | OQ975033 | OQ970612 | This study |
| *U. acaciae* | **PRU(M) 45 38** | *A. mearnsii* | Amara Mender, Ankesha Gasuga, Awi, Ethiopia | OQ975034 | OQ970613 | This study |
| *U. acaciae* | **PRU(M) 45 39** | *A. mearnsii* | Aneba, Ankesha Gasuga, Awi, Ethiopia | OQ975035 | OQ970614 | This study |
| *U. acaciae* | **PRU(M) 45 40** | *A. mearnsii* | Dakuna, Cheha, Gurage, Ethiopia | OQ975036 | OQ970615 | This study |
| *U. acaciae* | **PRU(M) 45 41** | *A. mearnsii* | Moche, Cheha, Gurage, Ethiopia | OQ975037 | OQ970616 | This study |
| *U. acaciae* | **PRU(M) 45 42** | *A. mearnsii* | Jomboro, Gumer, Gurage, Ethiopia | OQ975038 | OQ970617 | This study |
| *U. acaciae* | **PRU(M) 45 43** | *A. mearnsii* | Gibcha, Gumer, Gurage, Ethiopia | OQ975039 | OQ970618 | This study |
| *U. acaciae* | **PRU(M) 45 44** | *A. mearnsii* | Megeja, Eja, Gurage, Ethiopia | OQ975040 | OQ970619 | This study |
| *U. acaciae* | **PRU(M) 45 45** | *A. mearnsii* | Kotar Geta, Eja, Gurage, Ethiopia | OQ975041 | OQ970620 | This study |
| *U. acaciae* | **PRU(M) 45 46** | *A. mearnsii* | Tula, Qogota, Gamo, Ethiopia | OQ975042 | OQ970621 | This study |

| | | | | | | |
|---|---|---|---|---|---|---|
| *U. acaciae* | **PRU(M) 4547** | *A. mearnsii* | Gircha, Qogota, Gamo, Ethiopia | OQ975043 | OQ970622 | This study |
| *U. falcatarium* | BRIP 57477 | *Falcataria moluccana* | Philippines | KJ632993 | KJ632973 | Doungsa-ard *et al.* (2015) |
| *U. falcatarium* | BRIP 57990 | *Falcataria moluccana* | Timor Leste | KJ632994 | KJ632974 | Doungsa-ard *et al.* (2015) |
| *U. fusisporum* | BRIP 57526 | *A. salicina* | Australia | KJ633009 | KJ632991 | Doungsa-ard *et al.* (2015) |
| *U. naracoortensis* | MEL 2357562 | *A. iteaphylla* | Australia | KR994920 | KR994880 | McTaggart *et al.* (2015) |
| *U. notabile* | BRIP 59234 | *A. dealbata* | Australia | KJ633011 | KJ632992 | Doungsa-ard *et al.* (2015) |
| *U. robinsonii* | BRIP 57538 | *A. melanoxylon* | Australia | KJ633012 | KJ632989 | Doungsa-ard *et al.* (2015) |
| *U. simplex* | BRIP 59214 | *A. pycnantha* | Australia | KJ633010 | KJ632990 | Doungsa-ard *et al.* (2015) |
| *U. tepperianum* | BRIP 57511 | *A. leiocalyx* | Australia | KJ633006 | KJ632982 | Doungsa-ard *et al.* (2015) |
| *U. tepperianum* | BRIP 56928 | *A. leiocalyx* | Australia | KJ633005 | KJ632981 | Doungsa-ard *et al.* (2015) |
| *Uromycladium* sp. aff. *maritimum* | BRIP 56556 | *A. thomsonii* | Australia | KR994918 | KR994878 | McTaggart *et al.* (2015) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Uromycladium* sp. aff. *maritimum* | BRIP 56551 | *A. thomsonii* | Australia | KR994917 | KR994877 | McTaggart *et al.* (2015) |
| *Tranzschelia pruni-spinosae* | KR-M 0002755 | *Anemone ranunculoides* | Germany | KX228769 | KX228774 | Scholler *et al.* (2018) |

Note: Specimens collected in this study are presented in **bold**.

[1.] **BRIP**: Queensland Plant Pathology Herbarium, Queensland, Australia; **KR-M**: Herbarium of the State Museum of Natural History Karlsruhe, Germany; **MEL**: National Herbarium of Victoria, Royal Botanic Gardens Victoria, Victoria, Australia; **PREM**: South African National Herbarium, Roodeplaat, Pretoria, South Africa; **PRU(M)**: H.G.W.J. Schweickerdt Herbarium, University of Pretoria, Pretoria, South Africa.

**Results**

Only teliospores were observed on the plants. Chocolate brown telia and powdery teliospore masses were found on the petioles, rachis, leaflets, seed pods and young stems of infected *A. mearnsii* (Figure 2A–F). When moistened, spore masses became slimy, coating the foliage of the infected plants to form a brown sticky crust resulting in the leaves or pods becoming matted (Figure 2C). Where trees were severely infected, gum exuded from the stems and branches and such infections usually led to stunted tree growth.

Teliospores were borne on a pedicel, two in each pedicel with a hyaline vesicle (Figure 2G–I). The teliospores were hyaline when young, becoming yellowish brown with age, broadly ellipsoidal to sub-globose, 22–27 × 18–23 (24.1±1.25 × 20.9±1.09) µm. They had minutely warted surface and a germ pore at apex, and their walls, slightly more thickened towards the apex, were 0.8–2.5 (1.7 ± 0.32) µm. Vesicles were borne below the septum on a pedicel.


**Figure 2.** *Uromycladium acaciae* on *Acacia mearnsii*. **A–C.** Trees in the field heavily Infected with the rust and moistened telia on seed pods showing slimy crust (C). **D–F**. Close-up of telia on rachis and pinnules (D, E: PRU(M) 4531. F: PRU(M) 4530). **G.** Teliospores (PRU(M) 4530). **H, I.** Two teliospores borne on a pedice, and inflated (v) and deflated vesicles (dv) underneath the septum (PRU(M) 4531). Scale bars: D–F = 2.5 mm; G = 25 µm; H, I = 10 µm.

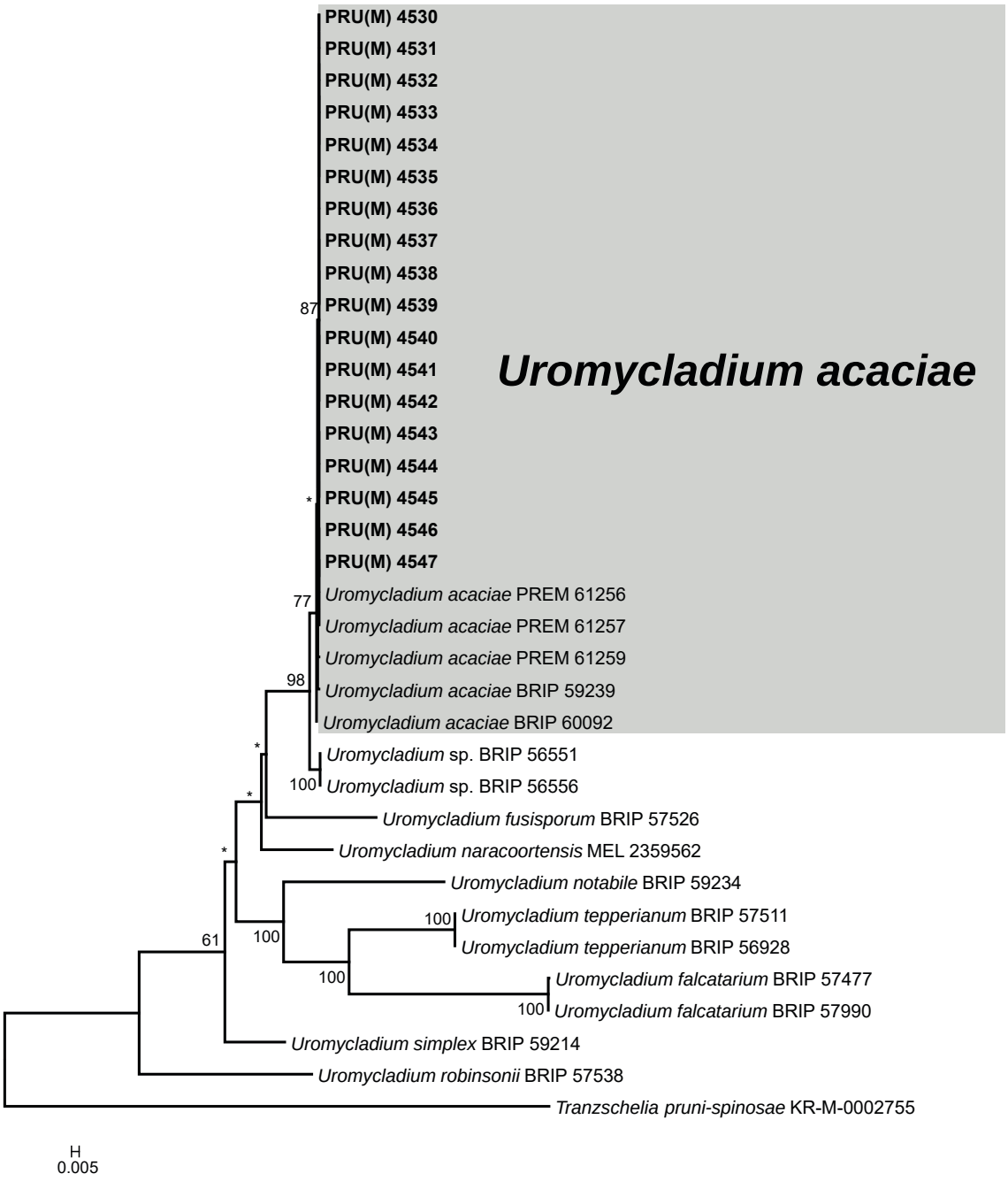


**Figure 3.** Phylogenetic tree based on maximum-likelihood (ML) analysis of ITS and LSU sequences for *Uromycladium* species. Specimens sequenced in this study are presented in **bold**. Bootstrap values (≥60%) for ML analyses are indicated at the nodes. *Tranzschelia pruni-spinosae* (KR-M-0002755) represents the outgroup taxon.

In total, 18 representative specimens, one from each site, were subjected to DNA-sequence analyses (Table 2). Amplicons of approximately 640 bp and 990 bp were generated for the ITS and LSU region, respectively. The combined dataset used in the phylogenetic analysis included 34 ingroup taxa and contained 1 790 characters including alignment gaps. The 18 specimens from Ethiopia had identical sequences and there were no differences in the ITS and LSU sequences between the Ethiopian collection and telial sequences of *Uromycladium acaciae* from *A. mearnsii* collected in South Africa (PREM 61256 and PREM 61257). These specimens differed from the uredinial sequences from Western Cape, South Africa (PREM 61259) by one bp in the ITS and LSU region. All Ethiopian specimens grouped together in a single monophyletic clade in the ML tree (Fig. 3) that included all representative specimens of *Uromycladium acaciae.* These specimens were thus identified as *U. acaciae*.

**Discussion**

This study provides the first report of the rust pathogen, *Uromycladium acaciae,* from Ethiopia where it is resulting in a serious disease problem. This is only the fourth country, besides New Zealand (Dick *et al.*, 2009), South Africa and Swaziland (McTaggart *et al.*, 2015), where the rust has been reported outside its native range in Australia. The disease was widespread, occurring in all the three surveyed zones of Ethiopia where *A. mearnsii* is planted, and has already emerged as a significant threat to the sustainability of these plantations.

An interesting outcome of the present study is the fact that only the telial stage of *U. acaciae* was found in Ethiopia. The first report of a rust on *A. mearnsii* in Africa was from the Western Cape Province of South Africa and based on uredinia of a *Uromycladium* sp., infecting only the pinnules and not causing any damage, which was identified as *U. alpinum* (Morris *et al.*, 1988). Later, McTaggart *et al.* (2015) reported the telial state of a rust causing serious damage to *A. mearnsii* in KwaZulu-Natal. They suggested that this species could be the uredinial rust species collected in the

1980s by Morris *et al.* (1988), and both the uredinial and telial states were components of the *U. acaciae* life cycle. However, Fraser *et al.* (2021a) suggested that telia and uredinia were part of separate life cycles on *A. mearnsii* in southern Africa and that further research was required to resolve the identity of these taxa. In this study, there was no evidence of a uredinial state, and it is likely that this state do not play a role in the life cycle of *U. acaciae* on *A. mearnsii* in Ethiopia. Despite the observed variations between the telial sequences for *U. acaciae* from Ethiopia and the uredinial sequences from Western Cape (PREM 61259), they formed a monophyletic group, implying a close relationship between them. In addition, it is interesting that no spermogonia were observed, as these were reported as commonly co-occuring with telia in South Africa (Fraser *et al.,* 2021a).

The origin of *U. acaciae* causing wattle rust in Ethiopia is unknown. However, one of the most typical avenues for non-native pests and pathogens to spread to new locations is via germplasm utilized to establish plantation programmes (Burgess and Wingfield, 2002; Wingfield *et al.*, 2008; Wingfield *et al.*, 2011). Due to the fact that most forest plantation trees grown in eastern Africa have been established from seeds or other planting stock commonly imported from South Africa or Australia, it is plausible that *U. acaciae* was introduced into this country with germplasm imported from one of those countries. Future studies at a population genetics level should be undertaken to resolve this question and thus to inform quarantine measures in order to reduce the chance of new pest or pathogen introductions.

As has been true in South Africa, rust on *A. mearnsii* caused by *U. acaciae* is a serious disease problem. Both short and long-term management approaches will need to be developed to manage the impact of the disease in Ethiopia. These could incoroporate chemical management (Little and Payn, 2016; Payn and Little, 2017), consideration of biological control tools (Fraser *et al.*, 2021b), understanding the mechanism underlying resistance (Moreno Chan and Isik, 2021) and screening for tolerant planting stock (Fraser *et al.*, 2019). In this regard, identifying the disease and its causal agent in the present study provides the first step towards the establishment of an effective management strategy for this pathogen in plantations and nurseries in Ethiopia.

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**Data availability statement**

The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/ and can be accessed with accession number listed in Table 2.

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