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Original Article

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

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Abstract

Australian *Acacia* species are among 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 DNA. 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 utilizing fast-growing tree species commenced in Ethiopia in the 1890s when *Eucalyptus* was first introduced into the country (Persson 1995). Currently, Ethiopia has ~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 (*Acacia mearnsii* De Wild.), silver wattle (*Acacia dealbata* Link) and green wattle (*Acacia 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 utilize 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 *et al.* 2001, 2005, Roux and Wingfield 2009), 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 (Fig. 1). The first survey in March 2022 was conducted in Awi, where 10 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

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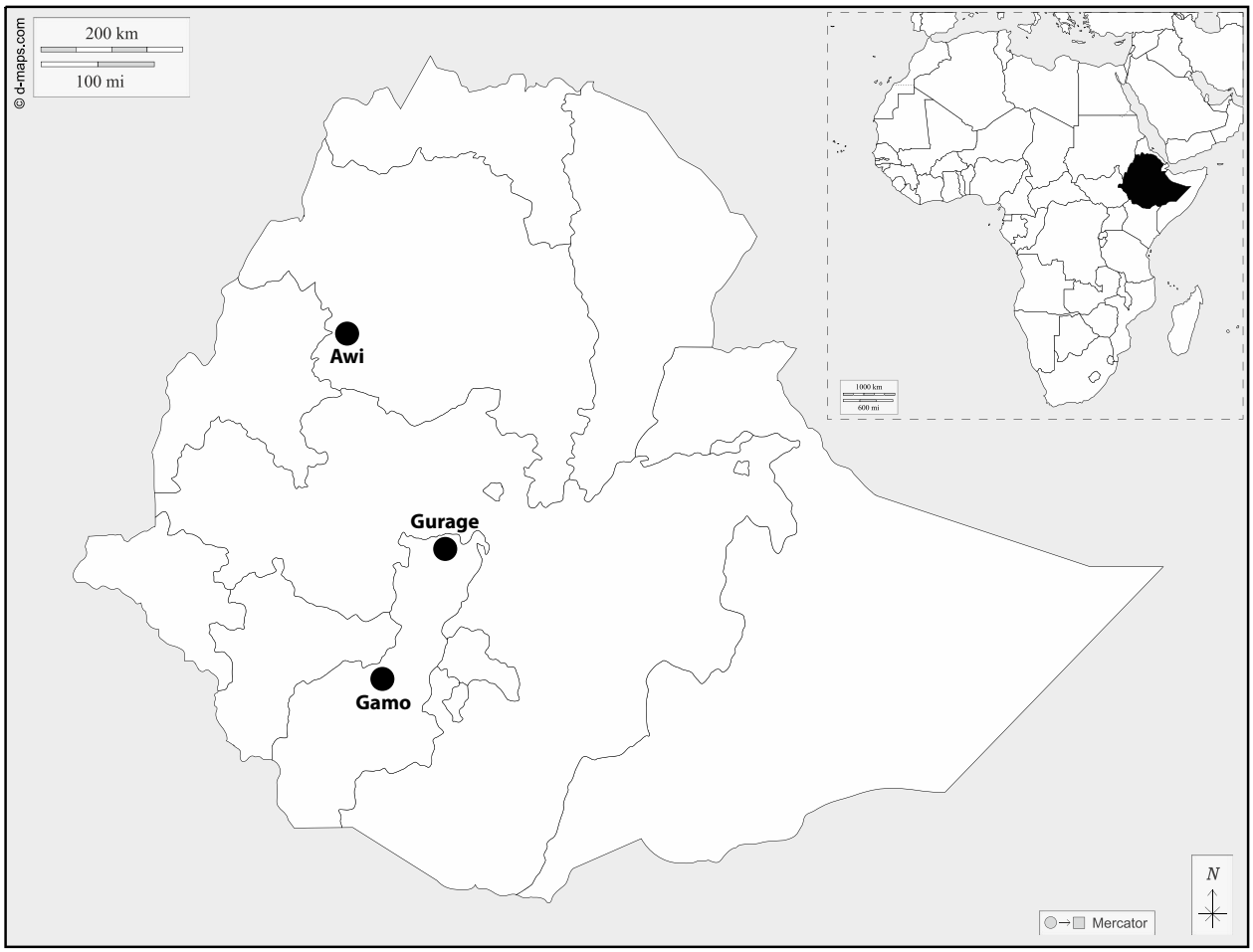

Figure 1. A map showing the three zones, namely Awi, Gurage, and Gamo, where the survey and sampling was conducted.

Gumer were assessed while in Gamo, two sites in Qogota district were surveyed. Location, Global Positioning System (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.

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, Polymerase Chain Reaction (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 nuclear large subunit (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 National Center for Biotechnology Information (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

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

Site No.	Zone	District	Site name	Tree age (y)	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

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.

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* (Fig. 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 (Fig. 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 (Fig. 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 toward the apex, were 0.8–2.5 (1.7 ± 0.32) μm. Vesicles were borne below the septum on a pedicel.

In total, 18 representative specimens, one from each site, were subjected to DNA-sequence analyses (Table 2). Amplicons of ~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 1790 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 *U. 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 *U. acaciae*. These specimens were thus identified as *U. acaciae*.

Discussion

This study provides the first report of the rust pathogen, *U. acaciae*, from Ethiopia where it is resulting in a serious disease problem. This is only the fourth country, besides New Zealand (Dick 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 *Uromycladium 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 programs (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

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

Species	Specimens[a]	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	*Acacia 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) 4537**	*A. mearnsii*	Habiti, Ankesha Gasuga, Awi, Ethiopia	OQ975033	OQ970612	This study
U. acaciae	**PRU(M) 4538**	*A. mearnsii*	Amara Mender, Ankesha Gasuga, Awi, Ethiopia	OQ975034	OQ970613	This study
U. acaciae	**PRU(M) 4539**	*A. mearnsii*	Aneba, Ankesha Gasuga, Awi, Ethiopia	OQ975035	OQ970614	This study
U. acaciae	**PRU(M) 4540**	*A. mearnsii*	Dakuna, Cheha, Gurage, Ethiopia	OQ975036	OQ970615	This study
U. acaciae	**PRU(M) 4541**	*A. mearnsii*	Moche, Cheha, Gurage, Ethiopia	OQ975037	OQ970616	This study
U. acaciae	**PRU(M) 4542**	*A. mearnsii*	Jomboro, Gumer, Gurage, Ethiopia	OQ975038	OQ970617	This study
U. acaciae	**PRU(M) 4543**	*A. mearnsii*	Gibcha, Gumer, Gurage, Ethiopia	OQ975039	OQ970618	This study
U. acaciae	**PRU(M) 4544**	*A. mearnsii*	Megeja, Eja, Gurage, Ethiopia	OQ975040	OQ970619	This study
U. acaciae	**PRU(M) 4545**	*A. mearnsii*	Kotar Geta, Eja, Gurage, Ethiopia	OQ975041	OQ970620	This study
U. acaciae	**PRU(M) 4546**	*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.* (2018)
U. falcatarium	BRIP 57990	*F. moluccana*	Timor Leste	KJ632994	KJ632974	Doungsa-ard *et al.* (2018)
U. fusisporum	BRIP 57526	*Acacia salicina*	Australia	KJ633009	KJ632991	Doungsa-ard *et al.* (2018)
U. naracoortensis	MEL 2357562	*Acacia iteaphylla*	Australia	KR994920	KR994880	McTaggart *et al.* (2015)
U. notabile	BRIP 59234	*Acacia dealbata*	Australia	KJ633011	KJ632992	Doungsa-ard *et al.* (2018)
U. robinsonii	BRIP 57538	*Acacia melanoxylon*	Australia	KJ633012	KJ632989	Doungsa-ard *et al.* (2018)
Uromycladium simplex	BRIP 59214	*Acacia pycnantha*	Australia	KJ633010	KJ632990	Doungsa-ard *et al.* (2018)
U. tepperianum	BRIP 57511	*Acacia leiocalyx*	Australia	KJ633006	KJ632982	Doungsa-ard *et al.* (2018)
U. tepperianum	BRIP 56928	*Acacia leiocalyx*	Australia	KJ633005	KJ632981	Doungsa-ard *et al.* (2018)
Uromycladium sp. aff. *maritimum*	BRIP 56556	*Acacia thomsonii*	Australia	KR994918	KR994878	McTaggart *et al.* (2015)
Uromycladium sp. aff. *maritimum*	BRIP 56551	*Acacia thomsonii*	Australia	KR994917	KR994877	McTaggart *et al.* (2015)
Tranzschelia pruni-spinosae	KR-M 0002755	*Anemone ranunculoides*	Germany	KX228769	KX228774	Scholler *et al.* (2014)

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

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.

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),

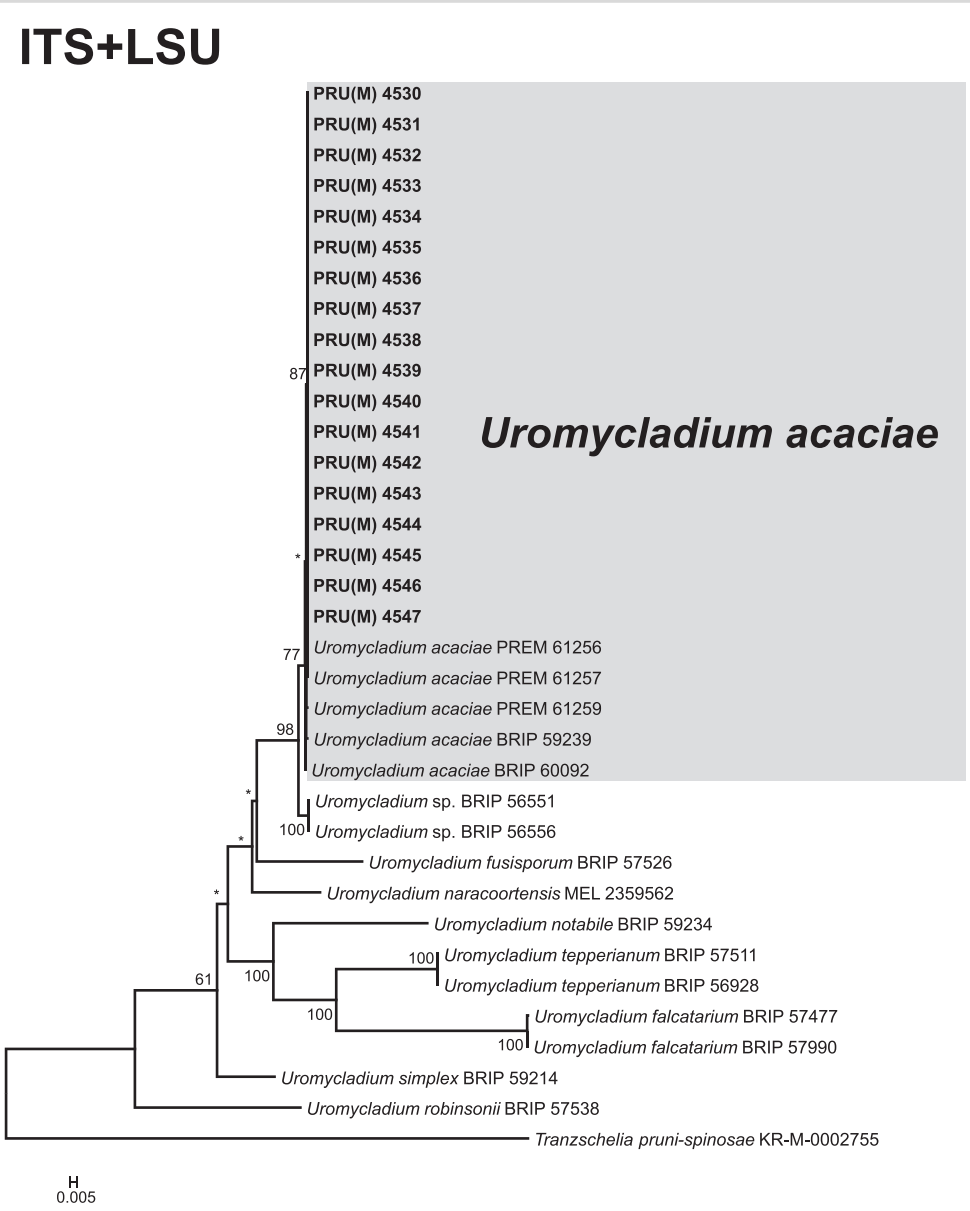

Figure 3. Phylogenetic tree based on ML analysis of ITS and LSU sequences for *Uromycladium* species. Specimens sequenced in this study are presented in **bold**. Bootstrap values (≥60%) for ML analyses are indicated at the nodes. *Tranzschelia pruni-spinosae* (KR-M-0002755) represents the outgroup taxon.

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 toward the establishment of an effective management strategy for this pathogen in plantations and nurseries in Ethiopia.

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Conflict of interest statement

None declared.

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Author contributions

Nam Q. Pham (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing—original draft, Writing—review & editing), Mike Wingfield (Conceptualization, Methodology, Project administration, Supervision, Validation, Writing—review & editing), S. Marincowitz (Data curation, Formal analysis, Methodology, Visualization, Writing—review & editing), Agena Tanga (Investigation, Writing—review & editing), Kumela Tiki (Investigation, Writing—review & editing), Weldesenbet Kassie (Investigation, Writing—review & editing), Brett Hurley (Funding acquisition, Investigation, Project administration, Resources, Writing—review & editing), Ilaria Germishuizen (Investigation, Writing—review & editing), Simon Lawson (Project administration, Writing—review & editing), Madaline Healey (Project administration, Writing—review & editing), and Mesfin Wondafrash (Conceptualization, Data

curation, Investigation, Methodology, Project administration, Writing—review & editing)

Data availability

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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