



The genus *Ravenelia* (Pucciniales) in South Africa

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Abstract

The genus *Ravenelia* represents the third largest genus of rust fungi and parasitizes a great number of leguminous shrubs and trees, mainly in the subtropics and tropics. Molecular phylogenetic analyses of this genus using nc 28S rDNA and *CO3* sequences are presented with a special focus on South African representatives of *Ravenelia*. Many of the specimens had been collected by us in recent years, mainly from acacia species of the genera *Vachellia* and *Senegalia*. Morphological characters were extensively studied using light microscopy and scanning electron microscopy. The analyses resolved several well-supported phylogenetic groups. By linking these groups to their morphology and life cycle characteristics, it was possible to interpret the outcomes in terms of their evolutionary ecology and biogeography. Several characters previously used to define subgeneric groups within *Ravenelia* were found to be misleading because of assumed convergent evolution. However, host associations, the ability to induce aecial galls as well as the development of two-layered probasidial cells emerged as useful criteria for inferring monophyletic groups. Six novel *Ravenelia* species were discovered and described. Furthermore, five species represent new reports for South Africa, species descriptions were emended for two taxa, and a new host report emerged for *R. inornata*.

Keywords Pucciniales · Raveneliaceae · *Ravenelia moloto* sp. nov. · *Ravenelia molopa* sp. nov. · *Ravenelia modjadji* sp. nov. · *Ravenelia doidgeae* sp. nov. · *Ravenelia spinifera* sp. nov. · *Ravenelia dumeti* sp. nov. · Phylogeny · Diversity study

Introduction

In 1853, Berkeley introduced the genus *Ravenelia* within the rust fungi (Pucciniales). The genus initially comprised two species: *R. glandulosa* Berk. & M.A. Curtis, which was transferred from

Sphaeria epiphylla Schwein. collected on *Tephrosia virginiana* (L.) Pers. in South Carolina and the newly described *R. indica* Berk. found on pods of an unidentified acacia. Later then Dietel correctly recombined the type species *R. glandulosa* to *R. epiphylla* (Schwein.) Dietel (Dietel 1894). In subsequent years, many additional *Ravenelia* species were found throughout the tropics and subtropics and today, some 200 species are described (Hernández and Hennen 2002, Cummins and Hiratsuka 2003). *Ravenelia* thus became the third most species-rich rust fungal genus after *Puccinia* and *Uromyces*.

All known species of *Ravenelia* are confined to a great diversity of Fabaceae residing in all three traditionally recognized subfamilies (Mimosoideae, Faboideae, Caesalpinioideae) (Hennen et al. 2005). The most prominent morphological features that are shared by all species of *Ravenelia* are the multicellular teliospores, which are borne on compound pedicels composed of two to several hyphae. These spores have an ellipsoidal, reniform, or almost hemispherical shape in side view and bear a variable number of pendent hygroscopic cysts. Other characters include spermogonia of type 5 and 7 (Cummins and Hiratsuka 2003) but these are commonly absent.

All species of *Ravenelia* are autoecious and their life cycles range from macro- and demi to hemi-, and more rarely to

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microcyclic (Cummins and Hiratsuka 2003). The aecial stage of several macrocyclic *Ravenelia* spp. can easily be recognized in the field by their ability to induce galls and witches' brooms in host tissues. Another special feature is the production of uredinoid aecia in numerous species of *Ravenelia* (Cummins 1959; Cummins and Hiratsuka 2003).

The morphological diversity and the variability of *Ravenelia* life cycles prompted mycologists early in the twentieth century to establish sections (Long 1903; Dietel 1906) or even to split this genus into several distinct genera (Sydow and Sydow 1915; Sydow 1921). The most sophisticated taxonomic system for *Ravenelia* was proposed by Sydow (1921) distinguishing eight genera based on teliospore traits in combination with observed life cycles. Details of the competing taxonomic systems are summarized in Table S1. However, a broad genus concept of *Ravenelia* comprising all these suggested genera or sections (within one genus) remains most widely accepted (compare Cummins and Hiratsuka 2003).

More than 500 rust species are known from South Africa (Crous et al. 2006), making this country relatively well explored for these fungi in comparison with other countries in Africa. Most contributions to the collection and description of rust fungi in South Africa are attributed to the investigations of Ethel M. Doidge during the first half of the twentieth century. In her last comprehensive species list of southern African rust fungi, she mentioned 24 *Ravenelia* species eight of which she described (Doidge 1927, 1939, 1950). The most recent species list was published by van Reenen (1995) but nearly exclusively relied on literature data provided by Doidge. Due to changes in political borders, two species each now only occur in Mozambique (*R. deformans* and *R. le-testui*) and Zimbabwe (*R. indigoferae* and *R. bottomleyae*) respectively, while *R. baumiana* was recorded only from Angola. Two rusts, *R. atrides* and *R. bottomleyae*, were transferred to the genera *Uredopeltis* (Wood 2007) and *Spumula* (Thirumalachar 1946), respectively. Wood (2006) recorded *R. ornata* for the first time in South Africa and Ebinghaus et al. (2018) described *R. xanthophloae* on the *Vachellia xanthophloea*. Thus, 19 *Ravenelia* species are currently known for South Africa.

During the course of recent surveys, aiming at re-collecting the majority of *Ravenelia* species from South Africa and especially at investigating all known *Acacia* s.l. for potential rust infections, we have collected numerous specimens from acacias and fabaceous plants. The overarching aims were to re-evaluate the species diversity and systematics of *Ravenelia* rusts in South Africa by using microscopic investigations and molecular phylogenetic techniques. For a better understanding of the phylogeny of the genus as a whole also species mainly from the Neotropics were investigated. The emerging phylogenetic clades were interpreted using aspects of biogeographical distributions, life cycle traits, and host associations as well as morphological data. Furthermore, in order to illustrate

conflicts when applying the taxonomic system for *Ravenelia* proposed by Sydow (1921), we mapped his suggested nomenclatural system to the phylogenetic reconstructions and discussed these outcomes.

Material and methods

Specimens examined

The specimens used for the molecular phylogenetic and morphological analyses were collected during several field surveys from 2004 to 2015 in South Africa. In addition, we considered 13 herbarium specimens from BPI originating from North and South America as well as DNA sequences downloaded from GenBank (see Table 1). Freshly collected material was immediately dried between paper sheets in a plant press and deposited after determination at the National Collections of Fungi (PREM) in Roodeplaat, South Africa, and the herbarium of the Natural History Museum in Karlsruhe (KR), Germany. In total, 91 specimens representing 44 *Ravenelia* species and three outgroup species were included in the molecular phylogenetic analyses and all of them were examined microscopically. For comparative purposes, additional 32 specimens comprising 15 type specimens deposited at PREM were examined only microscopically. All specimens investigated in this study are listed in Table 1.

Light- and electron microscopic investigations

The spores from plant material were scraped from the infected tissues using sterile insect needles and mounted in lactophenol solution on microscope slides. Light microscopic examinations were made using a Zeiss Axioplan light microscope (Carl Zeiss Microscopy, Jena, Germany) with a Color View microscope camera (Olympus Soft Imaging System, Münster, Germany) and a Zeiss Axio Imager M2 microscope with an Axiocam 506 camera (both Carl Zeiss Microscopy, Jena, Germany). Morphological characteristics were measured using Cell[^]D v. 3.1 imaging software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) and Zen2 Blue Edition v.2.3 (Carl Zeiss Microscopy GmbH, Jena, Germany). The specimens comprising *Ravenelia albizziicola* (PREM40295), *R. baumiana* (PREM50553, PREM29870, PREM6886), *R. elephantorrhizae* (PREM8955), *R. escharoides* (PREM534), *R. glabra* (PREM2375, PREM10698), *R. halsei* (PREM30117, PREM50751), *R. inornata* (PREM2368, PREM2541, PREM20734), *R. minima* (PREM30779, PREM10697), *R. modesta* (PREM34572, PREM30110), *R. natalensis* (PREM2514, PREM1935), *R. peglerae* (PREM2544, PREM5626, PREM2331), *R. pianaarii* (PREM5627, PREM6658), *R.*

Table 1 List of specimens used in this study

Fungus	Host	Location	Voucher	GenBank accession	
				LSU	CO3
<i>Ravenelia acaciae-arabicae</i>	<i>Vachellia nilotica</i>	South Africa, KwaZulu-Natal, Weenen Game Reserve	KR-M-0006425*	—	—
<i>Ravenelia acaciae-arabicae</i>	<i>Vachellia nilotica</i>	South Africa, KwaZulu-Natal, S 29°49' 29.1", E 30° 32' 08.5"	PREM61853	MN072675	—
<i>Ravenelia acaciae-arabicae</i>	<i>Vachellia nilotica</i>	South Africa, KwaZulu-Natal, S 30° 01' 07.2", E 30° 13' 58.7"	PREM61854	MN072674	MN095319
<i>Ravenelia acaciae-nigrescentis</i>	<i>Senegalia nigrescens</i>	South Africa, Mpumalanga, KNP, Orpen Rest Camp	KR-M-0006413	MN072686	MN095329
<i>Ravenelia acaciae-nigrescentis</i>	<i>Senegalia nigrescens</i>	South Africa, Mpumalanga, KNP, Orpen Rest Camp	KR-M-0006619	MN072685	—
<i>Ravenelia acaciae-pennatulae</i>	<i>Vachellia pennatula</i>	Mexico, Veracruz Province, N 19° 26' 44.1", W 96° 49' 51.9"	BPI864189	MN072655	MN095299
<i>Ravenelia acaciicola</i>	<i>Senegalia senegal</i> var. <i>leiorhachis</i>	South Africa, Zwemkloof, Motse-River, Sekhukhuneland	PREM61891	MN072689	—
<i>Ravenelia acaciicola</i>	<i>Senegalia senegal</i> var. <i>leiorhachis</i>	South Africa, Mpumalanga, Komatipoort	PREM60999	MN072671	—
<i>Ravenelia acaciicola</i>	<i>Senegalia senegal</i> var. <i>leiorhachis</i>	South Africa, Limpopo, Steelport, S 24° 44' 00.6", E 30° 13' 25.1"	PREM61861	MN072683	MN095326
<i>Ravenelia acaciicola</i>	<i>Senegalia senegal</i> var. <i>rostrata</i>	South Africa, KwaZulu-Natal, S 27° 29' 27.7", E 32° 11' 37.5"	PREM61847	MN072677	MN095320
<i>Ravenelia acaciicola</i>	<i>Senegalia senegal</i> var. <i>rostrata</i>	South Africa, KwaZulu-Natal, S 27° 40' 49.3", E 32° 25' 24.0"	PREM61849	MN072676	—
<i>Ravenelia albizziicola</i> *	<i>Albizia harveyi</i>	—	PREM40295**	—	—
<i>Ravenelia argentinica</i>	<i>Vachellia aroma</i>	Argentina, Jujuy Province, Parque Nacional Calilegua	BPI841267	MN072654	MN095296
<i>Ravenelia argentinica</i>	<i>Vachellia aroma</i>	Argentina, Salta Province, road to Parque del Rey	BPI841256	MN072653	MN095298
<i>Ravenelia baumiana</i> *	<i>Senna petersiana</i>	—	PREM50553*	—	—
<i>Ravenelia baumiana</i> *	<i>Senna delagoensis</i>	—	PREM29870*	—	—
<i>Ravenelia baumiana</i> *	<i>Senna singueana</i>	Angola	PREM6886*/**	—	—
<i>Ravenelia cebil</i>	<i>Anadenanthera</i> sp.	Argentina, Tucuman Province, San Pedro de Colalao	BPI841029	MN072649	—
<i>Ravenelia cohniana</i>	<i>Senegalia praecox</i>	Argentina, Catamarca Province, Rio Las Juntas	BPI841185	MG954487	—
<i>Ravenelia dichrostachydis</i>	<i>Dichrostachys cinerea</i>	South Africa, Mpumalanga, Nelspruit, SANBI	KR-M-0006448	MN072690	MN095332
<i>Ravenelia doidgeae</i>	<i>Senegalia polyacantha</i> subsp. <i>polyacantha</i>	South Africa, Mpumalanga, Nelspruit, Parking area of Botanical Garden	PREM60992**	MN072672	MN095316
<i>Ravenelia dumeti</i>	<i>Senegalia brevispica</i>	South Africa, Mpumalanga, S 25° 54' 29.8", E 31° 53' 48.5"	ME406**	MN072680	—
<i>Ravenelia dumeti</i>	<i>Senegalia brevispica</i>	South Africa, Mpumalanga, S 25° 54' 29.8", E 31° 53' 48.5"	PREM61877**	MN072681	MN095324
<i>Ravenelia echinata</i> var. <i>ectypa</i>	<i>Calliandra formosa</i>	Argentina, Tucuman Province, San Pedro de Colalao	BPI841034	DQ323925	—
<i>Ravenelia elephantorrhizae</i>	<i>Elephantorrhiza burkei</i>	South Africa, North-West Province, Mountain Sanctuary Park	KR-M-0006449	MN072702	MN095341
<i>Ravenelia elephantorrhizae</i> *	<i>Elephantorrhiza elephantina</i>	South Africa, Gauteng, Pretoria, Koedoespoort	PREM8955*/**	—	—
<i>Ravenelia escharoides</i>	<i>Senegalia burkei</i>	South Africa, Mpumalanga, Blyde River Canyon	KR-M-0043650	MG954480	MN095330
<i>Ravenelia escharoides</i>	<i>Senegalia burkei</i>	South Africa, Limpopo, John Reservoir	KR-M-0043652	MG954479	—
<i>Ravenelia escharoides</i>	<i>Senegalia burkei</i>	South Africa, Limpopo, Waterberge, Waterberg Hiking Trail	KR-M-0043651	MG954481	—

Table 1 (continued)

Fungus	Host	Location	Voucher	GenBank accession	
				LSU	CO3
<i>Ravenelia escharoides</i> *	<i>Senegalia burkei</i>	South Africa, Gauteng, Pretoria Pienaars River	PREM29918*	—	—
<i>Ravenelia escharoides</i> *	<i>Senegalia burkei</i>	South Africa, Gauteng, Pretoria, Near Hartebeestpoort Dam	PREM27586*	—	—
<i>Ravenelia escharoides</i> *	<i>Senegalia burkei</i>	South Africa, Limpopo, Pruizen (near Mokopane)	PREM534*/**	—	—
<i>Ravenelia evansii</i>	<i>Vachellia robusta</i> subsp. <i>robusta</i>	South Africa, KwaZulu-Natal, False Bay	PREM61209	MG945991	—
<i>Ravenelia evansii</i>	<i>Vachellia robusta</i> subsp. <i>robusta</i>	South Africa, North-West Province, Groot Marico, River Still Guest Farm	PREM61208	MG945992	—
<i>Ravenelia evansii</i>	<i>Vachellia davyi</i>	South Africa, Mpumalanga, R40 north of Nelspruit	PREM61224	MN275523	—
<i>Ravenelia evansii</i>	<i>Vachellia davyi</i>	South Africa, KwaZulu-Natal, S 27° 19' 27.2", E 31° 26' 39.6"	PREM61845	MG946000	MN095322
<i>Ravenelia evansii</i>	<i>Vachellia sieberiana</i> var. <i>woodii</i>	South Africa, Mpumalanga, S 25° 23' 41.8", E 31° 05' 08.0"	PREM61881	MG945987	—
<i>Ravenelia evansii</i>	<i>Vachellia hebeclada</i>	South Africa, North-West Province, Leeuwfontein Farm	PREM61227	MG946001	—
<i>Ravenelia evansii</i>	<i>Vachellia swazica</i>	South Africa, Mpumalanga, Marloth Park, S 25° 20' 44.4", E 31° 46' 26.1"	PREM61028	MG945996	—
<i>Ravenelia evansii</i>	<i>Vachellia exuvialis</i>	South Africa, Mpumalanga, S 24° 52' 52.6", E 31° 23' 40.3"	PREM61868	MG945995	—
<i>Ravenelia evansii</i>	<i>Vachellia borleae</i>	South Africa, KwaZulu-Natal, S 28° 41' 30.1", E 31° 43' 16.9"	ME384	MG946003	—
<i>Ravenelia evansii</i>	<i>Vachellia luederitzii</i> var. <i>retinens</i>	South Africa, KwaZulu-Natal, S 27° 19' 30' 57.3", E 32° 00' 39.1"	PREM61846	MG945993	MN095321
<i>Ravenelia glabra</i>	<i>Calpurnea aurea</i>	South Africa, KwaZulu-Natal, Camperdown Game Farm	KR-M-0006450	MN072691	MN095333
<i>Ravenelia glabra</i> *	<i>Calpurnea sylvatica</i>	South Africa, KwaZulu-Natal, Muden	PREM2375*/**	—	—
<i>Ravenelia glabra</i> *	<i>Calpurnea sylvatica</i>	—	PREM10698*/**	—	—
<i>Ravenelia halsei</i>	<i>Senegalia ataxacantha</i>	South Africa, Nelspruit, Botanical Garden	KR-M-0006620*	—	—
<i>Ravenelia halsei</i>	<i>Senegalia ataxacantha</i>	South Africa, KwaZulu-Natal, S 29° 39' 13.8", E 30° 26' 59.2"	KR-M-0006444*	—	—
<i>Ravenelia halsei</i>	<i>Senegalia ataxacantha</i>	South Africa, KwaZulu-Natal, close to Oribi Gorge	PREM61855	MG954484	—
<i>Ravenelia halsei</i>	<i>Senegalia ataxacantha</i>	South Africa, Mpumalanga, S 25° 02' 33.4", E 31° 06' 10.3"	PREM61867	MN072682	MN095325
<i>Ravenelia halsei</i> *	<i>Senegalia ataxacantha</i>	South Africa, Mpumalanga, 60 km south of Nelspruit	PREM50751	—	—
<i>Ravenelia halsei</i> *	<i>Senegalia ataxacantha</i>	South Africa, KwaZulu-Natal, Ndwedwe	PREM30117**	—	—
<i>Ravenelia havanensis</i>	<i>Enterolobium cyclocarpum</i>	Mexico, Chiapas, N15°34'39.6" W92°09'23.3"	BPI871922	MN275524	MN095301
<i>Ravenelia hermosa</i>	<i>Leucaena</i> sp.	Colombia	BPI1107966	MN072657	MN095304
<i>Ravenelia hernandezii</i>	<i>Senegalia tenuifolia</i>	Costa Rica, Guanacaste Province, N 10° 50' 42.1", W 85° 36' 27.0"	BPI872308	MG954488	MN095304
<i>Ravenelia hieronymi</i>	<i>Vachellia caven</i>	Argentina, Tucuman Province, S 27° 40' 38.0", W 65° 46' 49.4"	BPI841165	MN072651	—
<i>Ravenelia holwayi</i>	<i>Prosopis glandulosa</i>	USA, Texas, Del Rio. North Hill/20 Rico	BPI871145	MN072656	MN095300
<i>Ravenelia holwayi</i>	<i>Prosopis</i> sp.	USA, Texas, San Ygnacio, bird sanctuary along Rio Grande	KR-M-0006618	MN072692	—

Table 1 (continued)

Fungus	Host	Location	Voucher	GenBank accession	
				LSU	CO3
<i>Ravenelia</i> aff. <i>indigoferae</i>	<i>Indigofera</i> sp.	South Africa, KwaZulu-Natal, S 27° 28' 32.9", E 32° 35' 44.2"	PREM61061	MN072664	MN095308
<i>Ravenelia inornata</i>	<i>Vachellia natalitia</i>	South Africa, KwaZulu-Natal, St. Lucia	KR-M-0006613	MN072666	—
<i>Ravenelia inornata</i>	<i>Vachellia karroo</i>	South Africa, Western Cape, Entrance Montagu Nature Reserve	PREM60135	MN072684	MN095310
<i>Ravenelia inornata</i> *	<i>Vachellia karroo</i>	South Africa, Eastern Cape, Kentani	PREM2368*	—	—
<i>Ravenelia inornata</i> *	<i>Vachellia karroo</i>	South Africa, Eastern Cape, Kentani	PREM2541*	—	—
<i>Ravenelia inornata</i> *	<i>Vachellia karroo</i>	South Africa, Eastern Cape, Somerset East	PREM20734*/**	—	—
<i>Ravenelia macowaniana</i>	<i>Vachellia natalitia</i>	South Africa, Limpopo, Steelport, S 24° 41' 32.3", E 30° 12' 32.3"	PREM61862	MG946012	—
<i>Ravenelia macowaniana</i>	<i>Vachellia natalitia</i>	South Africa, Mpumalanga, Nelspruit	PREM61218	MG946011	—
<i>Ravenelia macowaniana</i>	<i>Vachellia karroo</i>	South Africa, Limpopo, Sekhukhuneland, Winterveld Mine	PREM61222	MG946007	MN095331
<i>Ravenelia macowaniana</i>	<i>Vachellia permixta</i>	South Africa, Limpopo, S 24° 08' 52.4", E 29° 02' 21.9"	PREM61875	MG946014	MN095327
<i>Ravenelia macrocarpa</i>	<i>Senna subulata</i>	Argentina, Catamarca Province, S 28° 03' 44.1", W 65° 35' 19.4"	BPI841195	DQ323926	—
<i>Ravenelia mainsiana</i>	<i>Mimosa</i> sp.	Mexico, Veracruz Province, N 19° 7' 30.3", W 96° 50' 07.1"	BPI871923	MN275525	MN095302
<i>Ravenelia mesilliana</i>	<i>Senna bicapsularis</i>	South Africa, Mpumalanga, S 25° 02' 38.1", E 031° 1' 18.8"	KR-M-0006424	MN072693	MN095334
<i>Ravenelia mimosae-sensitivae</i>	<i>Mimosa debilis</i>	Argentina, Salta Province, between Rosario de la Frontera and Horcones	BPI841052	MN072650	—
<i>Ravenelia minima</i> *	<i>Albizia gummifera</i>	South Africa, KwaZulu-Natal, Durban	PREM30779	—	—
<i>Ravenelia minima</i> *	<i>Albizia gummifera</i>	South Africa, KwaZulu-Natal	PREM10697	—	—
<i>Ravenelia modesta</i>	<i>Vachellia gerrardii</i>	South Africa, SANBI	PREM61884	MN072688	—
<i>Ravenelia modesta</i>	<i>Vachellia gerrardii</i>	South Africa, Mpumalanga, Barberton, S 25° 45' 37.0", E 31° 03' 10.4"	PREM61878	MN072678	—
<i>Ravenelia modesta</i>	<i>Vachellia rehmanniana</i>	South Africa, Limpopo, Louis Trichard	PREM60991*	—	—
<i>Ravenelia modesta</i>	<i>Vachellia rehmanniana</i>	South Africa, Limpopo, S 23° 57' 40.3", E 29° 29' 17.1"	PREM60785*	—	—
<i>Ravenelia modesta</i>	<i>Vachellia rehmanniana</i>	South Africa, Limpopo, S 23° 57' 40.3", E 29° 29' 17.1"	PREM60791	MN072660	—
<i>Ravenelia modesta</i>	<i>Vachellia luederitzii</i> var. <i>retinens</i>	South Africa, Gauteng	PREM60795	MN072662	MN095306
<i>Ravenelia modesta</i>	<i>Vachellia luederitzii</i> var. <i>retinens</i>	South Africa, Gauteng, 31 miles North of Pretoria	PREM34572*	—	—
<i>Ravenelia modesta</i>	<i>Vachellia hebeclada</i>	South Africa, Gauteng, Pienaars River	PREM30110*/**	—	—
<i>Ravenelia moloto</i>	<i>Senegalia erubescens</i>	South Africa, North-West Province, Groot Marico	KR-M-0006445**	MN072697	—
<i>Ravenelia moloto</i>	<i>Senegalia erubescens</i>	South Africa, North-West Province, close to Madikwe	PREM61896**	MN072700	MN095339
<i>Ravenelia moloto</i>	<i>Senegalia erubescens</i>	South Africa, North-West Province, close to Groot Marico	PREM61890**	MN072701	MN095340

Table 1 (continued)

Fungus	Host	Location	Voucher	GenBank accession	
				LSU	CO3
<i>Ravenelia molopa</i>	<i>Senegalia galpinii</i>	South Africa, Mpumalanga, Nelspruit, S 28° 29' 50.5", E 30° 59' 27.3"	PREM61879**	MN072679	MN095323
<i>Ravenelia modjadji</i>	<i>S. polyacantha</i> subsp. <i>campylacantha</i>	South Africa, Limpopo, Louis Trichard	PREM61023**	MN072667	MN095311
<i>Ravenelia modjadji</i>	<i>S. polyacantha</i> subsp. <i>campylacantha</i>	South Africa, Limpopo, Louis Trichard	KR-M-0006422**	MN072668	MN095312
<i>Ravenelia modjadji</i>	<i>S. polyacantha</i> subsp. <i>campylacantha</i>	South Africa, Limpopo, S 23° 37' 55.1", E 030° 12' 08.0"	PREM60788**	MN072661	MN095305
<i>Ravenelia natalensis</i>	<i>Vachellia karroo</i>	South Africa, KwaZulu-Natal, Winkelspruit	PREM2514*	—	—
<i>Ravenelia natalensis</i>	<i>Vachellia karroo</i>	South Africa, KwaZulu-Natal, Winkelspruit	PREM1935*/**	—	—
<i>Ravenelia neocaledoniensis</i>	<i>Vachellia farnesiana</i>	Australia, Northern Territory, Timber Creek	BRIP56907	KJ862347	—
<i>Ravenelia neocaledoniensis</i>	<i>Vachellia farnesiana</i>	Australia, Western Australia, Kununurra	BRIP56908	KJ862348	KJ862460
<i>Ravenelia ornata</i>	<i>Abrus laevigatus</i>	South Africa, Gauteng, Pretoria, Botanical Garden	KR-M-0006447	MN072687	—
<i>Ravenelia ornata</i>	<i>Abrus</i> sp.	South Africa, Mpumalanga, East of Nelspruit, Pullen Farm	KR-M-0006617	MN072673	MN095317
<i>Ravenelia peglerae</i> *	<i>Senegalia caffra</i>	South Africa, Eastern Cape, Kentani	PREM5626*	—	—
<i>Ravenelia peglerae</i> *	<i>Senegalia caffra</i>	South Africa, Eastern Cape, Kentani	PREM2331*/**	—	—
<i>Ravenelia peglerae</i> *	<i>Senegalia caffra</i>	South Africa, Eastern Cape, Butterworth	PREM2544*/**	—	—
<i>Ravenelia pienaarii</i> *	<i>Senegalia caffra</i>	South Africa, Gauteng, Garstfontein	PREM6658*/**	—	—
<i>Ravenelia pienaarii</i> *	<i>Senegalia caffra</i>	South Africa, Gauteng, Garstfontein	PREM5627*/**	—	—
<i>Ravenelia pienaarii</i>	<i>Senegalia caffra</i>	South Africa, Mpumalanga, S 25° 34' 24.2", E 31° 10' 51.7"	KR-M-0045114	MG954483	—
<i>Ravenelia pienaarii</i>	<i>Senegalia caffra</i>	South Africa, Gauteng, Ezemvelo Nature Reserve	KR-M-0006442	MN072699	MN095338
<i>Ravenelia pienaarii</i>	<i>Senegalia caffra</i>	South Africa, Gauteng, Ezemvelo Nature Reserve	PREM61892	MG954482	MN095337
<i>Ravenelia piepenbringiae</i>	<i>Senegalia hayesii</i>	Panama, Chiriquí Province, Los Algarrobos, N 8° 29' 45.31", W 82° 25' 56.24"	MP5157	MG954489	MN095328
<i>Ravenelia platensis</i>	<i>Erythrina crista-galli</i>	Argentina, Buenos Aires Province, La Plata	BPI841204	MN072652	MN095297
<i>Ravenelia pretoriensis</i>	<i>Vachellia natalitia</i>	South Africa, KwaZulu-Natal, Charters Creek	PREM61021	MN072665	MN095309
<i>Ravenelia pretoriensis</i> *	<i>Vachellia karroo</i>	South Africa, Western Cape, Jan Marais Nature Reserve	PREM60134*	—	—
<i>Ravenelia pretoriensis</i> *	<i>Acacia</i> sp.	South Africa, Gauteng, Garstfontein	PREM1376**/**	—	—
<i>Ravenelia pretoriensis</i>	<i>Vachellia karroo</i>	South Africa, Gauteng, Roodeplaat, ARC Experimental Farm	PREM60809	MN072659	—
<i>Ravenelia pretoriensis</i>	<i>Vachellia karroo</i>	South Africa, Gauteng, Roodeplaat, ARC Experimental Farm	KR-M-0006615*	—	—
<i>Ravenelia</i> sp.	<i>Vachellia collinsii</i>	Costa Rica, Guanacaste Area de Conservación Guanacaste	BPI872307	MN326775	—
<i>Ravenelia stictica</i>	<i>Mundulea sericea</i>	South Africa, Gauteng, Pretoria	PREM60784	MN072663	MN095307
<i>Ravenelia stictica</i>	<i>Mundulea sericea</i>	South Africa, Gauteng, Pretoria, hill opposite to Freedom Park	ME138	MN072658	—

Table 1 (continued)

Fungus	Host	Location	Voucher	GenBank accession	
				LSU	CO3
<i>Ravenelia stictica</i> *	<i>Mundulea sericea</i>	South Africa, Limpopo, Mokopane	PREM28255*	—	—
<i>Ravenelia spinifera</i>	<i>Senegalia mellifera</i>	South Africa, Gauteng, Ditholo Nature Reserve	KR-M-0006418**	MN072694	—
<i>Ravenelia spinifera</i>	<i>Senegalia mellifera</i>	South Africa, Gauteng, Ditholo Nature Reserve	KR-M-0006416**	MN072695	MN09533
<i>Ravenelia spinifera</i>	<i>Senegalia mellifera</i>	South Africa, Gauteng, Ditholo Nature Reserve	KR-M-0006417**	MN072696	MN095336
<i>Ravenelia spinifera</i>	<i>Senegalia mellifera</i>	South Africa, North-West Province, close to Madikwe	PREM61895**	MN072698	—
<i>Ravenelia tephrosiae</i>	<i>Tephrosia praecana</i>	South Africa, Mpumalanga, Komatipoort	PREM61003	MN072670	MN095315
<i>Ravenelia tephrosiae</i>	<i>Tephrosia macropoda</i>	South Africa, KwaZulu-Natal, Entumeni	PREM14190*	—	—
<i>Ravenelia tephrosiae</i>	<i>Tephrosia macropoda</i>	South Africa, KwaZulu-Natal, Inanda	PREM10700*	—	—
<i>Ravenelia transvaalensis</i>	<i>Senegalia mellifera</i>	South Africa, North-West Province, Pilanesberg	PREM61024	MN072669	MN095313
<i>Ravenelia transvaalensis</i>	<i>Senegalia mellifera</i>	South Africa, North-West Province; S 25° 29' 44.3", E 26° 23' 57.0"	PREM61858	MG954485	MN095318
<i>Ravenelia transvaalensis</i>	<i>Senegalia mellifera</i>	South Africa, Gauteng, Ditholo Nature Reserve	PREM61893	MG954486	—
<i>Ravenelia transvaalensis</i>	<i>Senegalia mellifera</i>	South Africa, Gauteng	PREM27832*	—	—
<i>Ravenelia xanthophloea</i>	<i>Vachellia xanthophloea</i>	South Africa, Mpumalanga, S 25° 26' 10.0", E 31° 57' 48.6"	PREM61000**	MG946016	MN095314
<i>Ravenelia xanthophloea</i>	<i>Vachellia xanthophloea</i>	South Africa, Mpumalanga, S 25° 46' 52.5", E 31° 03' 10.7"	PREM61215**	MG946017	—
<i>Ravenelia xanthophloea</i>	<i>Vachellia xanthophloea</i>	South Africa, KwaZulu-Natal, S 29° 38' 21.6", E 31° 05' 27.3"	PREM61213**	MG946015	—
<i>Endoraecium tierneyi</i>	<i>Acacia harpophylla</i>	Australia, Queensland, Tambo	BRIP27071	KJ862335	KJ862450
<i>Endoraecium tropicum</i>	<i>Acacia tropica</i>	Australia, Northern Territory, Gregory	BRIP56557	KJ862337	KJ862452
<i>Endoraecium violae-faustiae</i>	<i>Acacia difficilis</i>	Australia, Northern Territory, Humpty Doo	BRIP56545	KJ862344	—

Sequences in bold were obtained in this study

*Specimens that were investigated only morphologically

**Type specimens used in this study

pretoriensis (PREM1376, PREM60134), *R. stictica* (PREM28255), *R. tephrosiae* (PREM1419, PREM10700), and *R. transvaalensis* (PREM27832) were examined at the facilities of the ARC-Plant Protection Institute (ARC-PPRI), Roodeplaat, South Africa, using a Leica Dialux 22 EB microscope and a ColorView III CCD color camera, and measurements for these specimens were made using analysIS LS software (LS Research Software GmbH, Germany). Scanning electron microscopy (SEM) was done using a ZEISS Sigma VP scanning electron microscope. For this purpose, infected leaflets from the herbarium specimens were mounted on double-sided adhesive carbon tape on metal stubs and coated with gold in a sputter coater BAL-TEC SCD OSO (Capovani Brothers Inc., USA).

DNA extraction and PCR

The isolation of spores and DNA extraction procedures were carried out using the INNUPrep Plant DNA Kit (Analytik Jena, Jena, Germany) as described by Ebinghaus et al. (2018).

For PCR of the nc 28S rDNA (LSU), the Taq-DNA-Polymerase Mix (PeqLab, Erlangen, Germany) and the GoTaq G2 HotStart DNA Polymerase Kit (Promega, Mannheim, Germany) were used, whereas only the GoTaq G2 HotStart DNA Polymerase Kit was used for PCR of CO3. To obtain sequences of the LSU, the primer pairs LR0R (Moncalvo et al. 1995) and LR6 (Vilgalys and Hester 1990) and 5.8SrustF/D1D2RustR (Ebinghaus et al. 2018) were used with the following conditions: 3 min at 96 °C followed by

40 cycles of 30 s at 95 °C, 40 s at 49 °C, and 1 min at 72 °C, final elongation was for 7 min at 72 °C; for primers 5.8SrustF/D1D2rustF: 3 min at 96 °C followed by 40 cycles of 30 s at 96 °C, 45 s at 54 °C, and 1 min 20 s at 72 °C, final elongation was for 7 min at 72 °C. For amplification of *CO3* sequences, the primer pair CO3-R1 and CO3-F1 (Vialle et al. 2009) was used with the following PCR conditions: initial denaturation for 3 min at 95 °C followed by 40 cycles of 95 °C for 50 s, annealing at 45 °C for 60 s, and elongation at 72 °C for 60 s. Final elongation was for 7 min at 72 °C. The PCR products were purified using either Sephadex G-50 columns (Sigma-Aldrich, Steinheim, Germany) or ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific GmbH, Schwerte, Germany). When only weak bands could be observed on agarose gels, PCR products were purified and concentrated using the Zymo Research DNA Clean & Concentrator™-5 Kit (Zymo Research GmbH, Freiburg, Germany) following the manufacturer's protocol. DNA sequencing was carried out in both directions using the same primers as those used for PCR on a 3130XL Genetic Analyzer (Applied Biosystems) at the sequencing service of the Faculty of Chemistry and Biochemistry of the Ruhr University Bochum, Germany, or at GATC Biotech AG (Konstanz, Germany).

Phylogenetic analyses

Following successful sequencing, the sequences were screened against the NCBI GenBank using the BLASTn algorithm (Altschul et al. 1990) to check for erroneously amplified contaminations and to exclude them from further processing. Forward and reverse strands were then individually assembled and manually edited using Sequencher 5.0 software (Gene Codes Corp., Ann Arbor, MI, USA). A total of 91 DNA sequences were used to construct the alignments of the LSU and 49 sequences for the *CO3* sequence data using MAFFT v7.154b (Kato and Standley 2014) applying the L-INS-i strategy and edited manually. Missing data were coded as question marks in all alignments.

Maximum likelihood (ML) analyses were conducted in RAXMLGUI v.1.3 (Silvestro and Michalak 2012) using RaxML 8.0.26 (Stamatakis 2014) applying the general time reversible model of nucleotide substitution (Lanave et al. 1984) with gamma distributed substitution rates (GTR+G). The analyses were run with a rapid bootstrap analysis using 1000 bootstrap replicates. The ML analyses were first conducted for each dataset separately and topological congruence was checked visually. Because no conflict of supported phylogenetic groupings was observed, a concatenated alignment was constructed for the LSU and *CO3* sequence alignments and the subsequent phylogenetic analyses were inferred by applying the same methodology as for individual datasets.

Bayesian inference (BI) was performed with siMBA v.1.0 implemented in MrBayes 3.2.5 (Larget and Simon 1999;

Ronquist et al. 2012; Mishra and Thines 2014) applying the GTR+G substitution model. The Markov chain Monte Carlo search was run for five million generations with trees sampled every 500 generations. The burnin was set to 0.3. A Bayesian consensus tree was automatically calculated in siMBA and with posterior probabilities plotted on the tree. The phylogenetic trees of all different analyses were viewed and edited in FigTREE v1.4.0 (Rambaut 2009).

The taxonomic system proposed by Sydow (1921) for *Ravenelia* was applied. The respective generic names in addition to morphological and life cycle characteristics provided by literature were thus plotted on the phylogenetic reconstruction based on the LSU data.

Results

Molecular phylogeny

The LSU sequence data resulted in an alignment comprising 91 sequences of 1016 characters in total length with 436 variable positions of which 372 were parsimony informative, whereas the *CO3* alignment comprised 49 sequences with a total length of 605 characters of which 183 were variable and 144 parsimony informative. All alignments are deposited at TreeBase (TB2:S24974, TB2:S24975, TB2:S24976).

The phylogenetic reconstructions of the LSU and *CO3* sequence datasets resolved similar tree topologies. Slight differences can be observed in the topologies of both data sets, but only in the placement of weakly or unsupported groupings, e.g., clades II and IV (Fig. 1, 2 and 3, Fig. S1). No significantly different tree topologies were observed in ML and BI approaches for either dataset. We recognized seven clades (i.e., I–VII) that included at least one South African *Ravenelia* species (Fig. 1).

Taxonomy

The results of the present study, which includes molecular phylogenetic analyses and morphological investigations, led us to propose six taxonomic novelties described in the following section. In addition, four *Ravenelia* species are newly reported from South Africa, the species descriptions are emended for three rusts and a novel host report is included for one species.

Ravenelia moloto W. Maier, M. Ebinghaus, & Begerow sp. nov. (Fig. 4a–g)

Mycobank MB831070

Etymology: Name refers to Moloto, which is the common name of the host tree *Senegalia erubescens* in the local Setswana language.

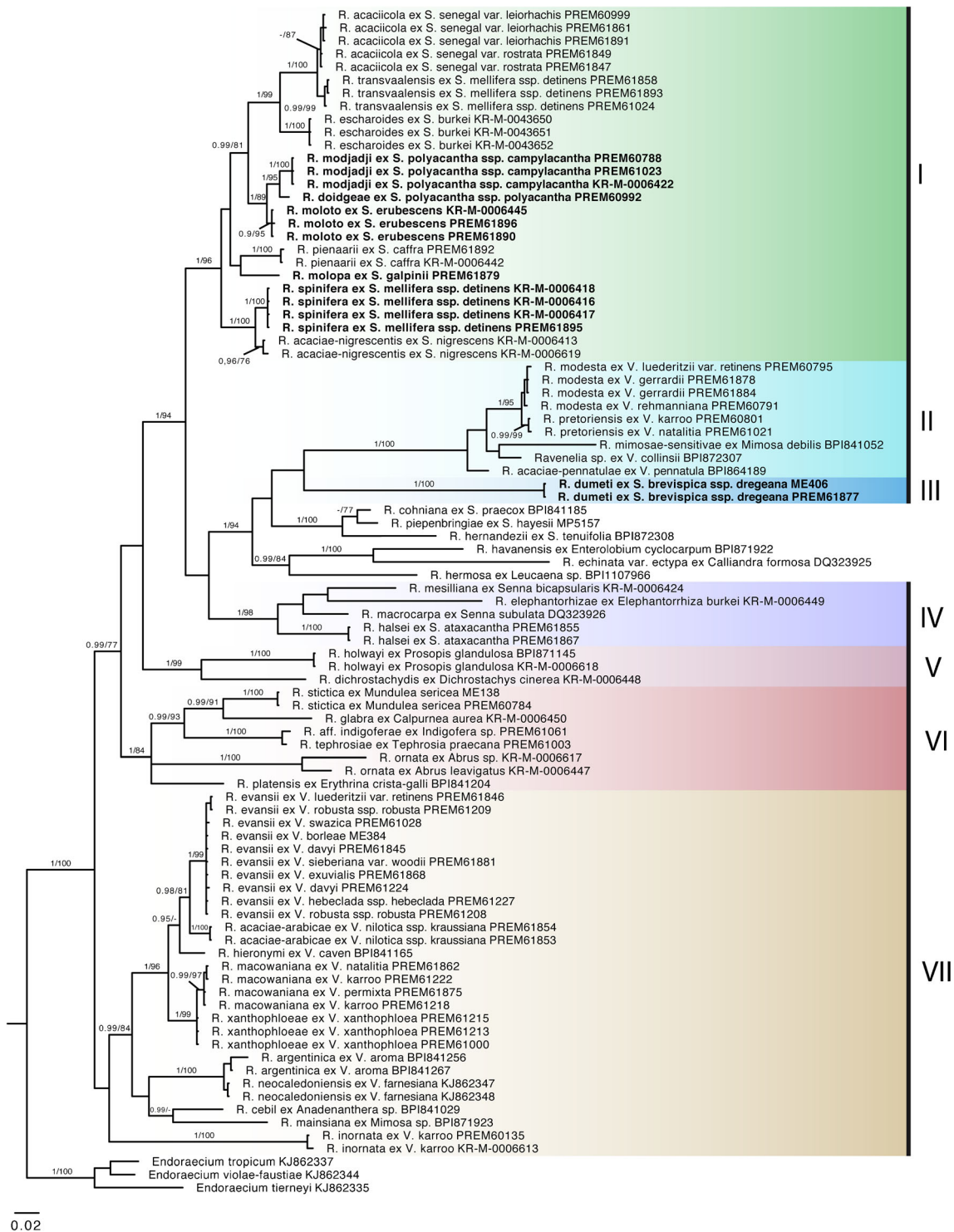


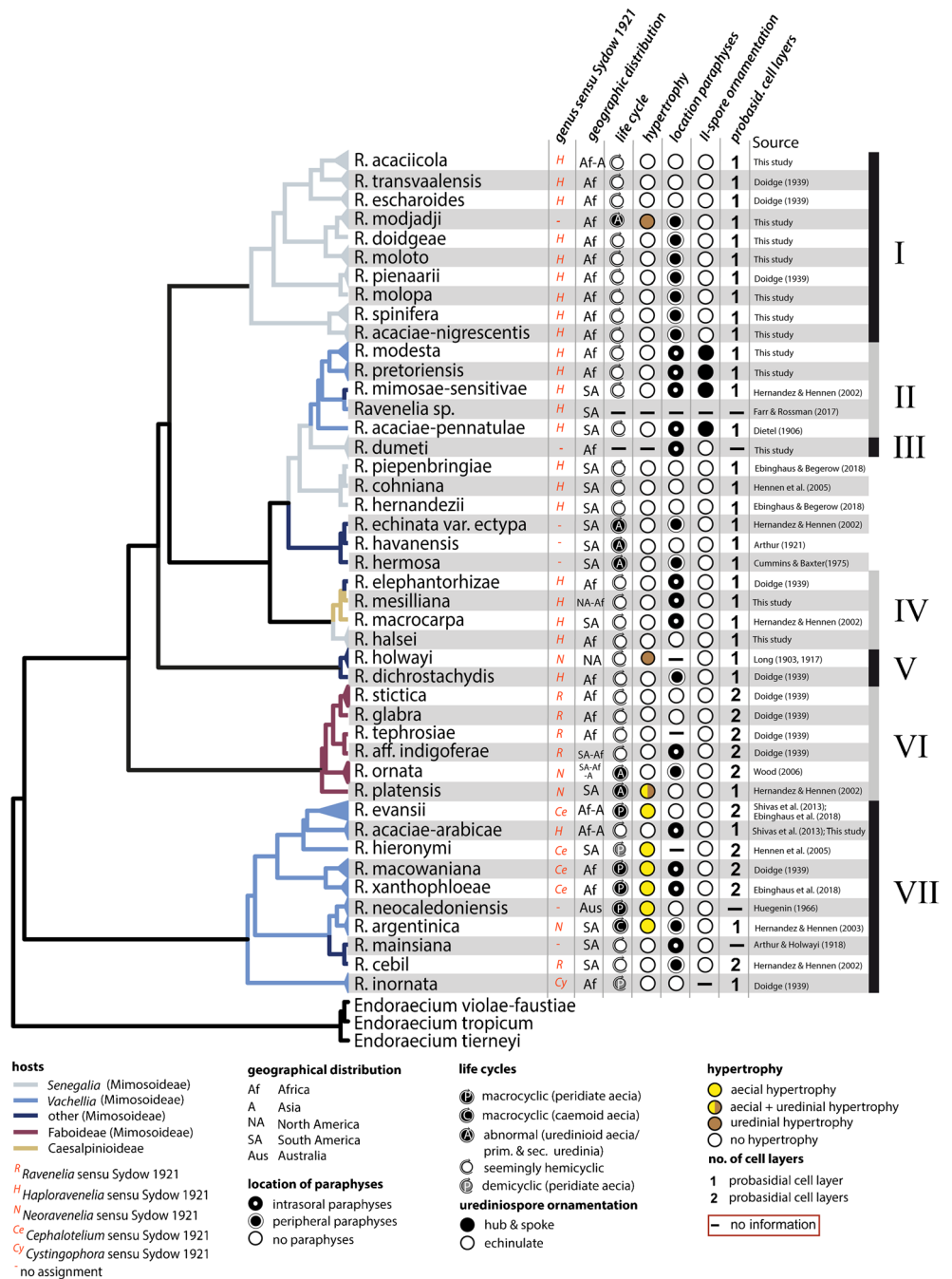
Fig. 1 Molecular phylogenetic reconstruction of the genus *Ravenelia* inferred from LSU sequences using BI. Posterior probabilities above 0.90 and ML-bootstrap support above 75 are shown. Highlighted in bold are those species that were described as novel taxa in this study

Type: South Africa, North-West Province, Groot Marico, on leaves of *S. erubescens* (Welw. ex Oliv.) Kyal. & Boatwr., 18 April 2009, W. Maier (WM3545), holotype KR-M-0006445.

Spermogonia and acelia not seen. Uredinia amphigenous but predominantly on the adaxial surface of the leaflets,

sometimes on pods, sori on leaflets scattered or in small groups, shape ranging from circular to elongated, (60)120–250(460) μm in diameter, up to 6 mm in diameter when occurring on pods where sori form concentric eventually confluent rings, subepidermal, erumpent; paraphyses

Fig. 2 Cladogram based on a phylogenetic reconstruction using BI showing character states linked to species. Terminal branches were collapsed



peripherically arranged within uredinia, cylindrical or sometimes clavate, often septate, (27)40–55(77) × 6–13 μm, cell wall 1–1.7 μm, transparent to light brown; urediniospores ovoidal to ellipsoidal, 12–16 × 21–28 μm, spore wall evenly 1.4–2.2 μm thick, echinulate, aculei approximately 1 μm in height, germ pores 5–6, equatorially arranged. Telia replacing uredinia, chestnut brown to dark brown; teliospores cinnamon brown to chestnut brown, circular to subcircular from above, (63)75–95(103) μm in diameter, upper side of the teliospores slightly convex to flattened, 5–7 probasidial cell

across, probasidial cells (20)24–28(34) × (12)15–20(26) μm, cell wall thickened at the top side of the spore and here seemingly bilaminate with an inconspicuously thin, or sometimes distinctly marked hyaline to pale brown outer layer and a chestnut brown inner layer, the inner layer (1)3–5(7) μm thick, each cell with 7–13 verrucose ornamentations, 1–3 μm in height; cysts pendent, globose, hyaline and smooth, in the same number as the probasidial cells, swelling in water but only slightly in lactophenol solution; pedicel multihyphal.

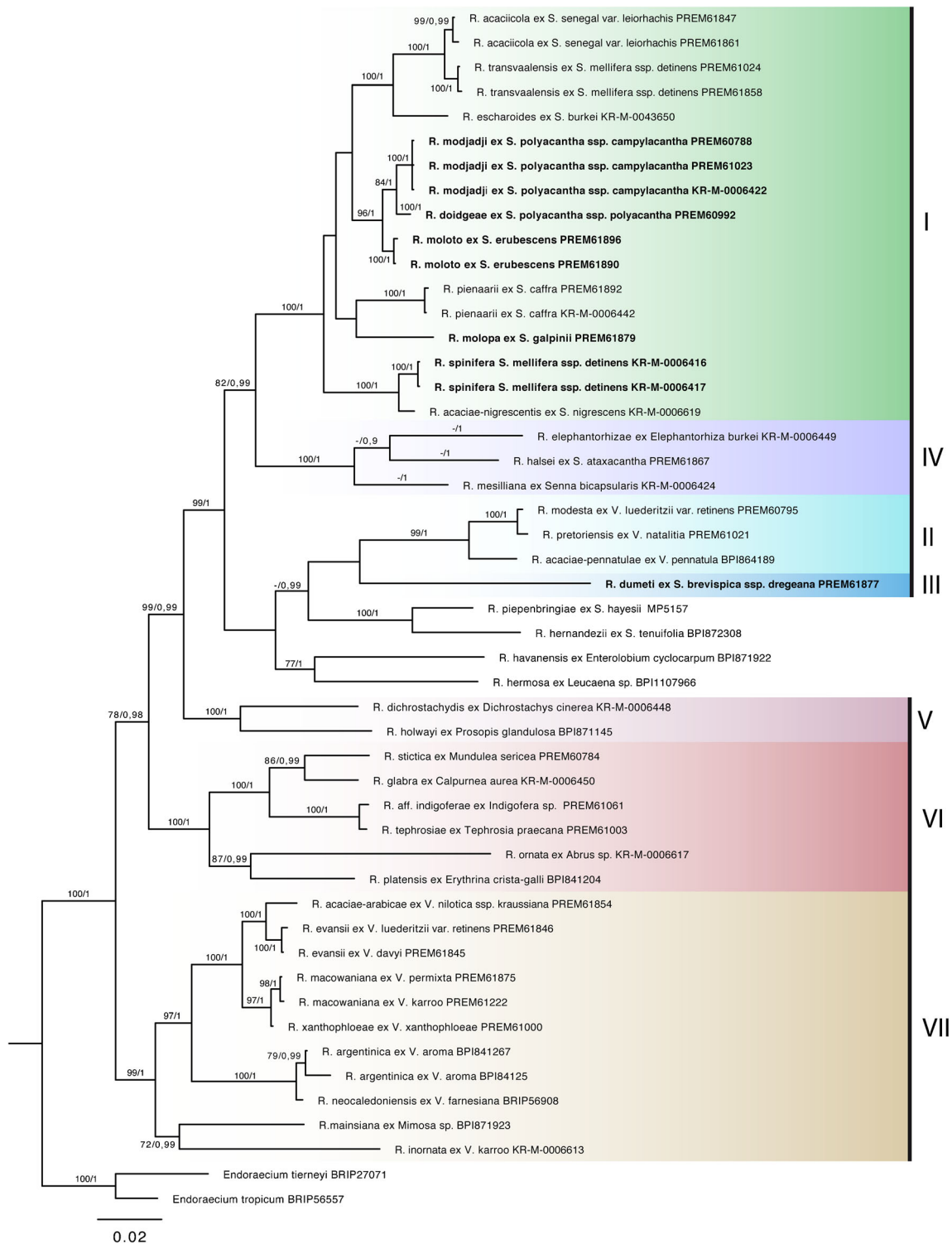
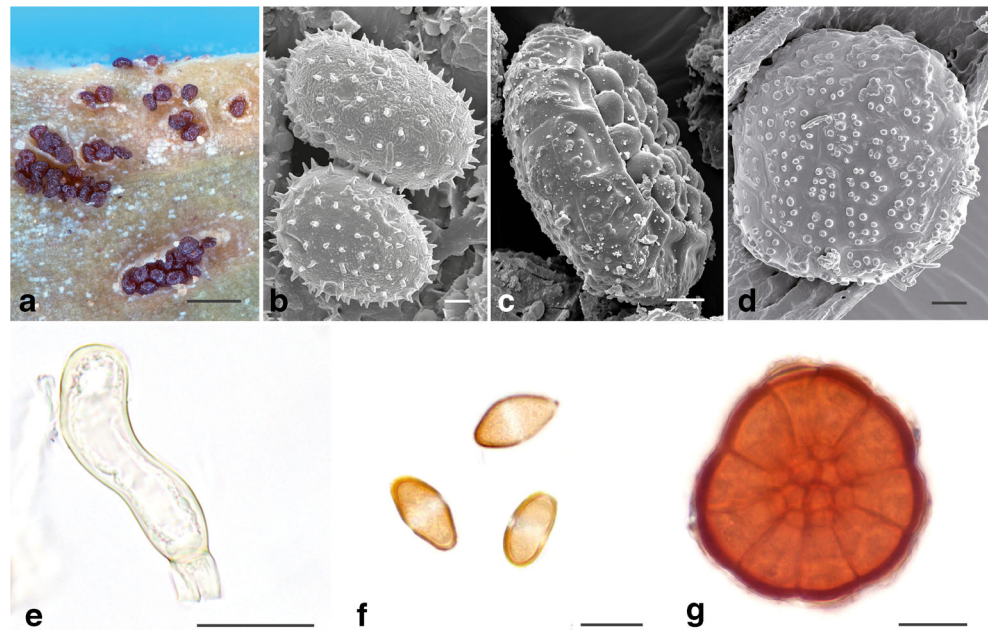


Fig. 3 Phylogenetic reconstruction based on a combined dataset of *CO3* and LSU sequence data. ML bootstrap values above 75 and *p* values above 0.95 are shown. Species, described as novel taxa in this study, are highlighted in bold

Additional specimens examined: South Africa, North-West Province, on leaves of *Senegalia erubescens*, 16 April 2009, W. Maier (WM3554), paratypes PREM61896, KR-M-0006443; close to Madikwe, 17 April 2009, W. Maier (WM3555), paratypes PREM61890, KR-M-0006415.

Notes: *Ravenelia moloto* was found to be closely related to *R. modjadji* on *S. polyacantha* subsp. *campylacantha* and to *R. doidgeae* found on *S. polyacantha* subsp. *polyacantha* but were clearly supported as distinct phylogenetic groups based on LSU and *CO3* sequence data.

Fig. 4 *Ravenelia moloto*. **a** Telia on leaflets of *Senegalia erubescens*. **b** SEM view of urediniospores showing germ pores. **c** Lateral SEM view of teliospore showing the arrangement of sterile cysts. **d** SEM view of verrucose teliospore. **e** Single-septated paraphysis seen in LM. **f** LM of urediniospores showing equatorially arranged germ pores. **g** Teliospore from top view by LM. Bars: **a** = 0.2 mm; **b** = 2 μ m; **c** and **d** = 10 μ m; **e**–**f** = 20 μ m



The close relationship between these species is reflected by their morphology that makes it difficult to distinguish between them. However, the teliospores of *R. moloto* tend to be smaller in diameter compared with those of *R. doidgeae* and its ornamentation often appears more pronounced. These two species show additional minor differences in urediniospore morphology as they often tend to be more globose in *R. doidgeae*. This rust has been found only on *S. erubescens*, a tree occurring in the dry savannah in the northeastern part of South Africa.

Ravenelia moloto might be restricted to this tree species in its natural environment.

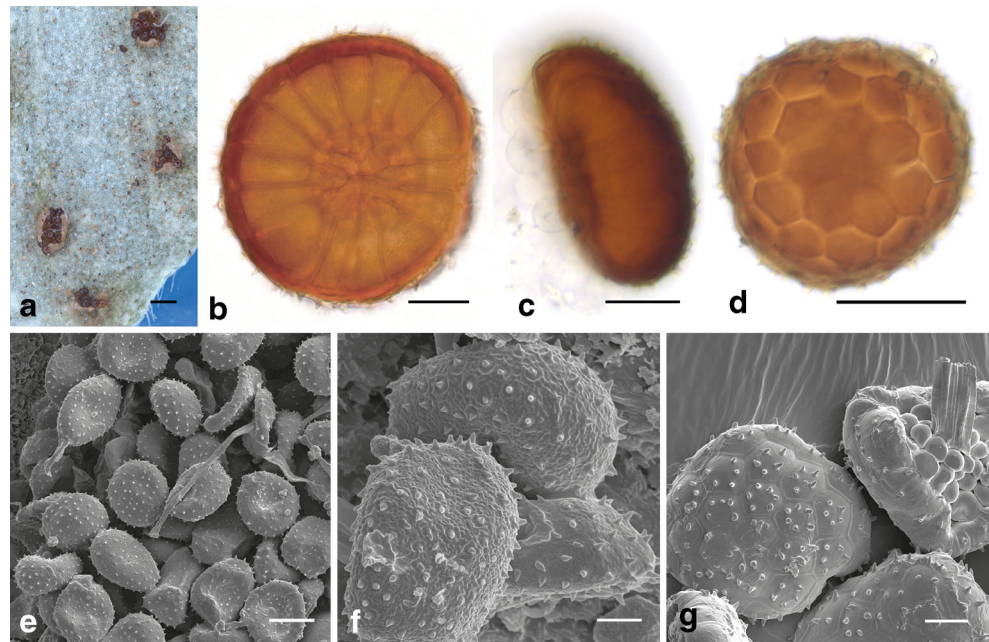
Ravenelia spinifera W. Maier, M. Ebinghaus, & Begerow sp. nov. (Fig. 5a–g)

Mycobank MB831071

Etymology: Name refers to the echinulate teliospores.

Type: South Africa, North-West Province, close to Madikwe, on leaves of *Senegalia mellifera* (Vahl) Seigel &

Fig. 5 *Ravenelia spinifera*. **a** Telia on adaxial leaflet surface of *S. mellifera*. **b** Bottomside of a teliospore seen in LM. **c** Lateral view of teliospore seen in LM showing the hyaline sterile cysts. **d** LM from topside showing the compound teliospore with single probasidial cells. **e–g** SEM view of urediniospores. **f** Teliospores seen by SEM. Bars: **a** = 0.2 mm; **b** = 20 μ m; **c** = 25 μ m; **d** = 20 μ m; **e** = 10 μ m; **f** = 3 μ m; **g** = 10 μ m



Ebinger subsp. *detinens* (Burch.) Kyal. & Boatwr., 16 April 2009, W. Maier (WM3548), holotype PREM61895, isotype KR-M-0006412.

Spermogonia and aecia not seen. Uredinia amphigenous on leaflets, scattered or in loose groups, circular to elongated, 0.1–0.6 mm, subepidermal, erumpent. Urediniospores ovoidal to ellipsoidal, ochraceous brown, (13)16–22 × (11)13–16 μm, spore wall evenly 1.5–2(2.5) μm thick, often with attached pedicel, echinulate aculei short, less than 1 μm, distances between aculei about 1.5–2 μm, germ pores (4)5–6, in equatorial position; only few paraphyses observed in light microscopy, peripherally arranged, cylindrical to spathuliform, up to 65 μm in length and 8 μm in width, cell wall 1–1.5 μm, hyaline to subhyaline; telia replacing the uredinia, color ranging from cinnamon brown to dark brown; teliospores circular to subcircular from above, upper side of teliospores cinnamon brown to chestnut brown, convex to almost hemispherical, (64)75–95(111) μm in diameter with 5–8 probasidial cells across, probasidial cells (21)25–28(34) × (12)15–19(24) μm, cell wall thickened at the top side and here seemingly bilaminar with a thin or inconspicuous hyaline to pale brown outer layer and a chestnut brown inner layer, the inner layer (2)4–5(7) μm thick, each probasidial cell bearing 4–7 spines, (2)3.5–4.5(6) μm long; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal, often persisting on detached spores.

Additional specimens examined: South Africa, Gauteng, Ditholo Nature Reserve, on leaves of *S. mellifera* subsp. *detinens*, 15 April 2009, W. Maier (WM3515), paratype KR-M-0006418; 15 April 2009, W. Maier (WM3516),

paratype KR-M-0006416; 15 April 2009, W. Maier (WM3517), paratype KR-M-0006417.

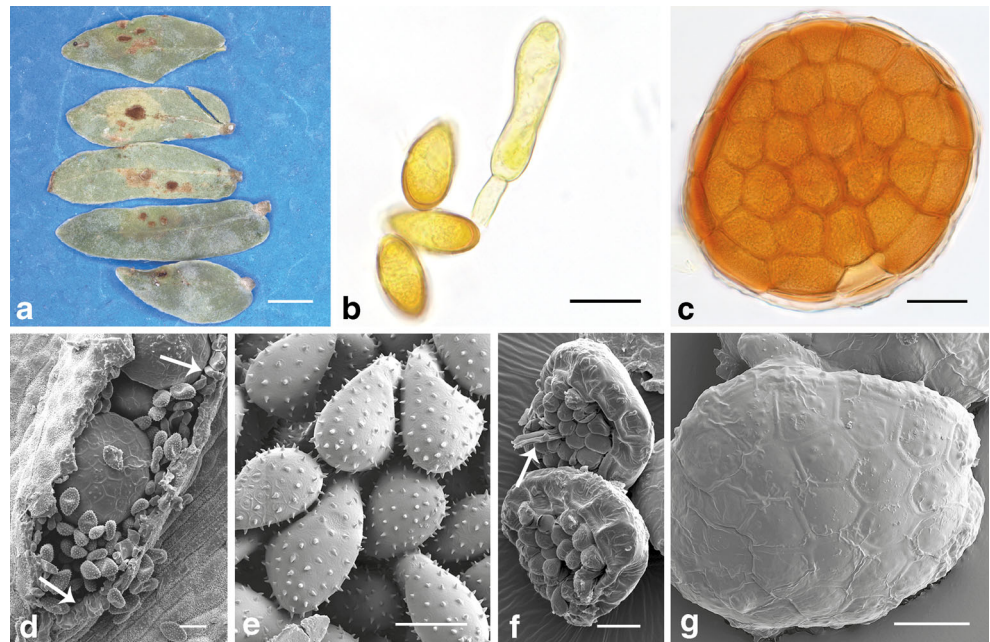
Notes: *Ravenelia spinifera* is one of three species including *R. transvaalensis* and *R. acaciae-melliferae* occurring on *Senegalia mellifera*. While *R. transvaalensis* is also known from South Africa, *Ravenelia acaciae-melliferae* has been reported only from Eritrea and Ethiopia (Farr and Rossman 2017). *Ravenelia spinifera* can easily be distinguished from *R. transvaalensis* by its teliospores that have well-developed spines while those of *R. transvaalensis* are smooth-walled. Remarkably, we found host individuals with infections caused by both *R. transvaalensis* and *R. spinifera* even on a single leaflet. The original description of *R. acaciae-melliferae* is very limited and it does not provide details of teliospore ornamentation (Baccarini 1917). We thus consider the teliospores of that species as smooth and it remains uncertain whether *R. acacia-melliferae* species should be reduced to synonymy with *R. transvaalensis*. *Ravenelia spinifera* further resembles *R. acaciae-nigrescentis* on *S. nigrescens* in overall morphology. Nonetheless, both species were resolved in two well-supported monophyla in phylogenetic reconstructions based on LSU and CO3 sequence data (Fig. 1 and 3). Additionally, *R. spinifera* appears to be restricted to *S. mellifera* subsp. *detinens* and the two species can thus also be distinguished by their host association.

Ravenelia molopa M. Ebginghaus, W. Maier, & Begerow sp. nov. (Fig. 6a–g)

Mycobank MB831072

Etymology: Name refers to the occurrence of this rust fungus on *Senegalia galpinii*, which is known as Molopa in the local Northern Sotho language.

Fig. 6 *Ravenelia molopa*. **a** Uredinia on leaflets of *S. galpinii*. **b** Urediniospores and a single-septated paraphysis seen in LM. **c** LM of a teliospore of *R. molopa*. **d** SEM view of a uredinium. Arrows indicate the peripherally arranged paraphyses. **e** Urediniospores seen in SEM. **f** Bottomside of teliospores seen in SEM showing the arrangement of sterile cysts. Arrow indicates the multihyphal pedicel. **g** Teliospore from top view seen in SEM. Small verrucose ornamentations are more pronounced at the margins of the teliospore. Bars: **a** = 1 mm; **b–d** = 20 μm; **e** = 10 μm; **f** and **g** = 20 μm



Type: South Africa, Mpumalanga, Nelspruit, S 28° 29' 50.5", E 30° 59' 27.3" on leaves of *Senegalia galpinii* (Burt Davy) Seigler & Ebinger, 16 February 2015, M. Ebinghaus (ME404), holotype PREM61879, isotype KR-M-0006614.

Spermogonia and aecia not seen. Uredinia amphigenous on leaflets, in small groups, subcircular to elongated, sometimes forming concentric rings, light brown, often surrounded by chlorotic areas, 0.1–0.6 mm in diameter, subepidermal, erumpent, peripheral paraphyses cylindrical and elongated to sometimes clavate, with a basal septum, 32–53 × 9–12 μm, cell wall thin and transparent 0.8–1.1(1.7) μm; urediniospores ovoidal to ellipsoidal, sometimes elongated, light brown (23)25–29(33) × 12–15 μm, spore wall laterally (1)1.3–1.6(2) μm thick, basally slightly thickened and apically often more pronounced thickened, echinulate, aculei approximately 1 μm in height, germ pores 4–5, equatorially arranged. Telia replacing the uredinia, orange brown to chestnut brown; teliospores orange brown to cinnamon brown, circular to subcircular from above, upper side of teliospores convex, (55)85–100(117) μm in diameter with 3–7 probasidial cells across, single probasidial cells (27)31–33(36) × (12)14–18(25) μm, cell wall thickened at the top side of the spore and here distinctly bilaminar with a hyaline brown outer layer and a cinnamon brown inner layer, 3.5–6.5 μm thick, the peripheral cells each with 5–9 small verrucae, 1–1.5(2.5) μm, but central cells often smooth; cysts pendent, globose, hyaline and smooth, in the same number as the probasidial cells, swelling in water but only slightly in lactophenol solution; pedicel multihyphal.

Notes: This rust was found only once on a single tree in Nelspruit, Mpumalanga, in a private garden. The tree was most probably planted as an ornamental as this region is

outside its natural distribution range that lies in the eastern part of the North-West Province, in western Limpopo and the northern parts of Gauteng and Mpumalanga (Coates Palgrave 2005; Smit 2008). The teliospores of *R. molopa* can easily be confused with those of *R. pienaarrii* that infects the widely distributed *S. caffra*. However, the two species can be distinguished by the peripherally arranged paraphyses in the uredinia of *R. molopa* (Fig. 6d), while *R. pienaarrii* is aparaphysate. These two rusts are also clearly separated by a significant genetic distance in molecular phylogenetic analyses of LSU and CO3 gene regions; however, the exact phylogenetic position of *R. molopa* within clade I could not be fully resolved (Figs. 1 and 3).

Ravenelia molopa can also be confused with *R. escharoides*, a rust species that infects *S. burkei*. However, the urediniospores and the teliospores of *R. molopa* are larger than those of *R. escharoides* and the sori of *R. escharoides* are aparaphysate.

Ravenelia doidgeae M. Ebinghaus, Begerow, & W. Maier sp. nov. (Fig. 7a–h)

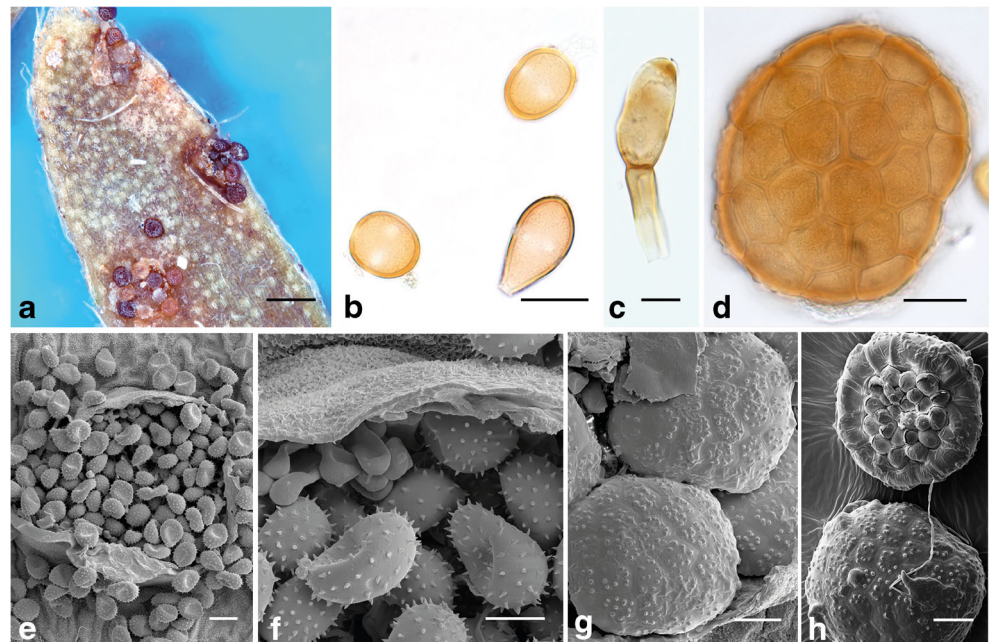
Mycobank MB831073

Etymology: Name honors the South African Mycologist Ethel M. Doidge for her fundamental contributions to the knowledge on South African *Ravenelia* species.

Type: South Africa, Mpumalanga, Nelspruit, car park at the back entrance of the botanical garden, on leaves of *Senegalia polyacantha* subsp. *polyacantha* (Willd.) Seigler & Ebinger, 10 April 2013, M. Ebinghaus (ME262), holotype PREM60992, isotype KR-M-0006612.

Spermogonia and aecia not seen. Uredinia amphigenous on leaflets, singly or in small groups, subcircular to elongated,

Fig. 7 *Ravenelia doidgeae*. **a** Telia on a leaflet of *S. polyacantha* subsp. *polyacantha*. **b** LM of urediniospores showing equatorially arranged germ pores. **c** Single-septated paraphysis seen in LM. **d** Teliospore of *R. doidgeae* seen in LM. **e** SEM view of an uredinium. **f** SEM view of an uredinium showing also peripherally arranged paraphyses and the torn epidermis. **g** Verrucose teliospores of *R. doidgeae* seen in SEM. **h** Bottomside of a teliospore seen in SEM showing sterile cysts. Bars: **a** = 0.2 mm; **b** = 20 μm; **c** = 10 μm; **d–e** = 20 μm; **f** = 10 μm; **g** = 20 μm



minute, 0.1–0.3 mm in diameter, light brown, subepidermal, erumpent; paraphyses peripheral, cylindrical to clavate, septated, with a distinctly thickened apical cell wall; ochraceous to light brown, total length 30–40 μm , upper cell 14–27 \times (5)8–10 μm , lateral cell wall 1–1.5 μm thick, at apex 1.5–3 μm ; urediniospores ovoidal to ellipsoidal, sometimes globose, light brown, (20)22–28(36) \times 16–19 μm , spore wall 1–1.5 μm thick, at the apex slightly thickened, echinulate, germ pores 4–7, equatorially arranged; telia replacing the uredinia, chestnut to dark brown. Teliospores circular to subcircular from above, upper side of teliospores convex, orange brown to chestnut brown, (80)87–95(104) μm in diameter with 5–8 probasidial cells across, single probasidial cells (21)24–28(33) \times (12)14–18(22) μm , cell wall thickened at the top side of the spore and here bilaminate with a hyaline to pale brown outer layer that often seemingly get detached from a chestnut brown inner layer, the inner layer (2)4–6 μm thick, each cell bearing 8–13 verrucose ornamentations, (1)1.5–2.5(3) μm in height; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal.

Notes: *Ravenelia doidgeae* was found only once and the teliospores are morphologically difficult to discriminate from those of *R. modjadji* that can frequently be found on *S. senegalia* subsp. *campylacantha*. But the urediniospores of this rust tend to be more distinctly ovoidal than those of *R. modjadji*, which are more ellipsoid. *Ravenelia doidgeae* further resembles *R. moloto* in overall morphology but that rust has been found only on *S. erubescens*. Despite their morphological resemblance, the phylogenetic analyses of the LSU region resolved *R. doidgeae* as a distinct lineage in a close sister relationship to *R. modjadji* and *R. moloto* (Fig. 1).

Ravenelia modjadji M. Ebinghaus, W. Maier, & Begerow sp. nov. (Fig. 8a–l)

Mycobank MB831074

Etymology: Name refers to the Rain Queen Modjadji of the Balobedu people that live in the region where the holotype specimen was collected.

Type: South Africa, Limpopo, near Modjadjiskloof, S 23° 38' 09.5", E 30° 11' 56.8", on malformed branches and leaves of *Senegalia polyacantha* (Willd.) Seigler & Ebinger subsp. *campylacantha* (Hochst. ex. A. Rich.) Kyal. & Boatwr., 21 February 2015, M. Ebinghaus (ME418), holotype PREM61860.

Spermatogonia not seen. Primary uredinia typically on young shoots but sometimes expanding to leaves, causing malformations (e.g., “witches brooms”), developing singly but quickly growing confluent and then covering extended areas of infected shoots; primary urediniospores densely packed in sori, ochraceous brown, ellipsoidal, sometimes ovoidal, often somewhat curved, (23)25–28(32) \times 10–12(14) μm , spore wall 1–1.5(2) μm thick, slightly thickened basally and at the apex,

germ pores 4–5, equatorially arranged; uredinia predominantly on the adaxial side of leaflets, singly or in small groups, subcircular to ellipsoidal, light brown, minute, 0.1–0.4 mm in diameter; paraphyses peripheral, cylindrical to slightly clavate, sometimes bi- or trifurcate, with a single septum, transparent to light brown, thickened apex often more intensively colored, total length 42–55 μm , the upper cell (14)20–28(45) \times 6–10(13) μm , cell wall distinctly thickened apically, 3–9 μm and 0.7–1.3 μm laterally; urediniospores light brown, ovoidal to ellipsoidal or of irregular shape, (21)24–28(30) \times (9)12–14(16) μm , spore wall evenly (1)1.3–1.5(2) μm thick, germ pores 4–6, equatorially arranged; telia replacing the uredinia, dark brown; teliospores subcircular from above, upper side of teliospores convex, orange brown to chestnut brown, (64)85–105(124) μm in diameter with 5–8 probasidial cells across, single probasidial cells (19)25–29(36) \times (12)15–19(30) μm , cell wall thickened at the top side of the spore and here bilaminate with a hyaline to pale brown outer layer that often seemingly get detached from a chestnut brown inner layer, the inner layer (2)4–6(8) μm thick, each cell with 8–14 verrucose ornamentations, 1–2(3) μm in height; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal.

Additional specimens examined: South Africa, Limpopo, Louis Trichard, on leaves of *Senegalia polyacantha* subsp. *campylacantha*, 4 April 2013, M. Ebinghaus (ME240), paratype KR-M-0006422; 4 April 2013, M. Ebinghaus (ME239), paratype PREM61023; S 23° 37' 55.1", E 30° 12' 08.0", June 2013, M. Ebinghaus (ME182), paratype PREM60788.

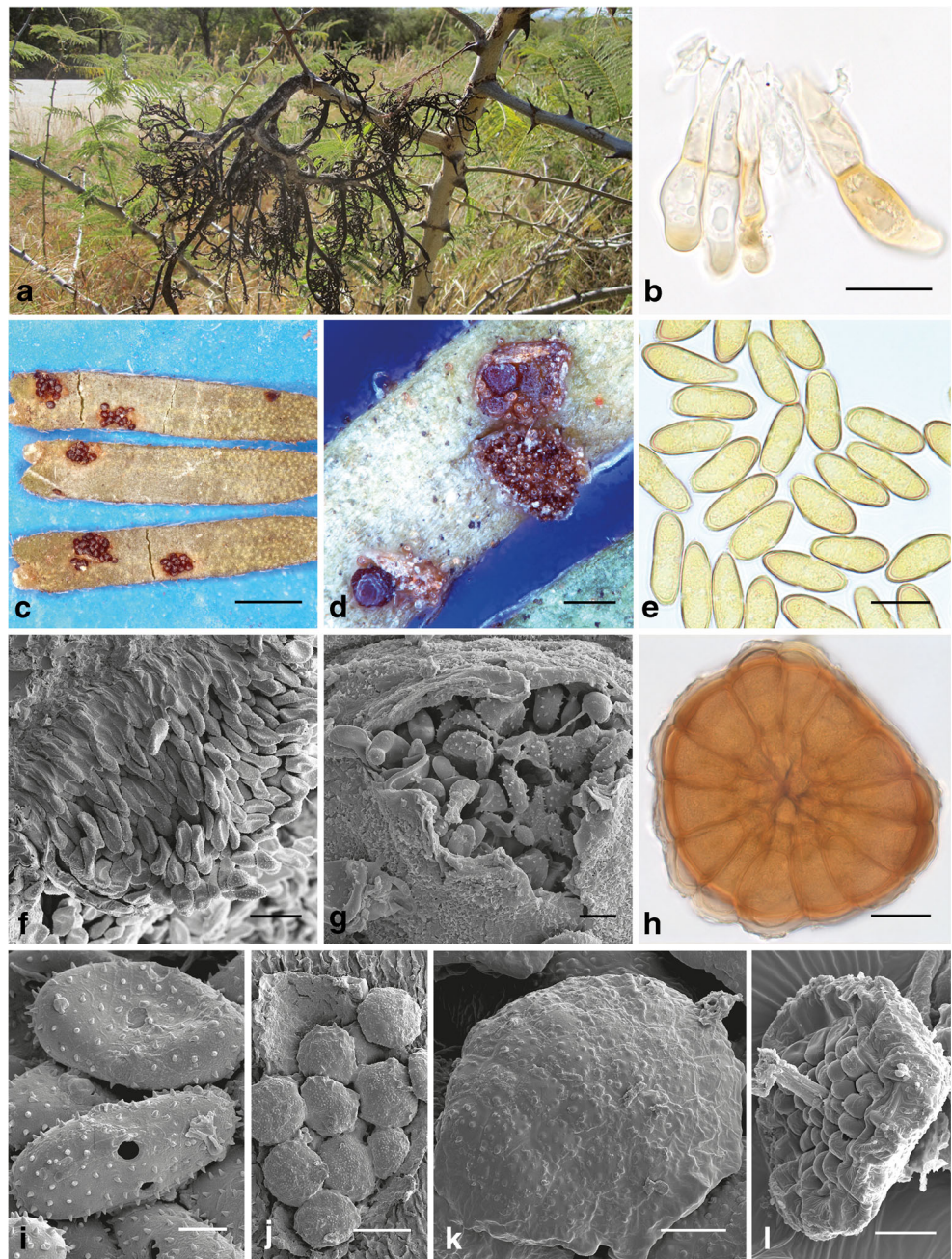
Notes: *Ravenelia modjadji* is the only species within a monophyletic lineage of seemingly hemicyclic *Senegalia* rusts (clade I), which produces primary and secondary uredinia, and the primary uredinia causing malformations in its host. Similar to the gall-forming *R. evansii* and *R. macowaniana*, this spore stage is spatially separate from the (secondary) uredinia and telia. Old malformed branches often become detached from the trees during heavy rainfall or strong wind and can then be found in large abundance below the trees. *R. modjadji* can thus be easily distinguished from its close relatives, *R. doidgeae* and *R. moloto* if the aecia are present. It can also be differentiated based on the morphology of the urediniospores, which are ellipsoid in this *Ravenelia* while the latter two species have more ovoidal urediniospores. *Ravenelia modjadji* however shares a similar teliospore morphology with its phylogenetic close relatives *R. doidgeae* and *R. moloto*.

Ravenelia dumeti M. Ebinghaus, W. Maier, & Begerow sp. nov. (Fig. 9a–h)

Mycobank MB831075

Etymology: The name is derived from the Latin word *dumetum* that describes a plant thicket and indicates the occurrence of the rust fungus in those habitats: the host

Fig. 8 *Ravenelia modjadji*. **a** Malformed branches (“witches brooms”) caused by the aecial state of *R. modjadji* on its host *S. polyacantha* subsp. *campylacantha*. **b** LM of septated paraphyses. **c** Telia on leaflets. **d** Uredinia intermixed with teliospores. **e** Aeciospores seen in LM. **f** SEM view of an aecium. **g** SEM view of a uredinium showing paraphyses. **h** Teliospore seen in LM. **i** SEM view of aeciospores showing equatorial germ pores. **j** SEM view of a subepidermally erumpent telium. **k** Teliospores with verrucose ornamentations seen in SEM. **l** Bottomside of teliospore seen in SEM. Scale bars: **b** = 20 μ m; **c** = 0.1 mm; **d** = 0.5 mm; **e** = 20 μ m; **f** = 30 μ m; **g** = 10 μ m; **h** = 20 μ m; **i** = 4 μ m; **j** = 60 μ m; **k** and **l** = 20 μ m



Senegalia brevispica forms dense and often impenetrable thickets at forest margins and along rivers in South Africa.

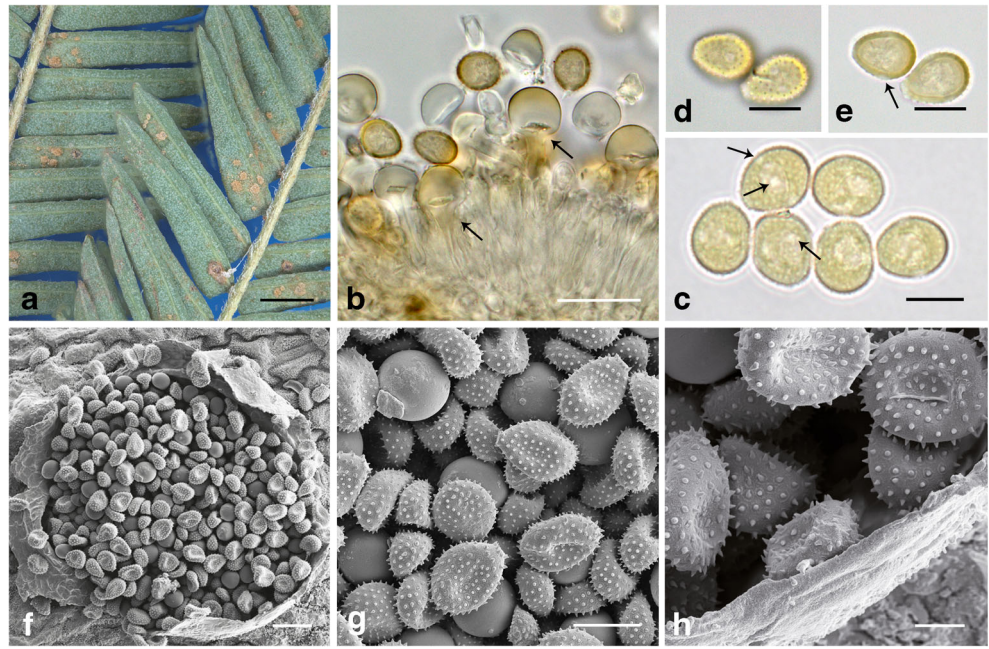
Type: South Africa, Mpumalanga, S 25° 54' 29.8", E 31° 53' 48.5", on leaves of *Senegalia brevispica* (Benth.) Kyal. & Boatwr., 16 Feb. 2015, M. Ebinghaus (ME407), holotype PREM61877.

Spermogonia and aecia not seen. Uredinia predominantly on the abaxial side of the leaflets but also adaxially, irregularly arranged in small groups, circular to sometimes elongated, subcuticular, erumpent, ochraceous brown, minute, (50)100–150(240) μ m; paraphyses intrasoral, distinctly capitate,

pedicel transparent and light brown at the thickened top, total length (22)24–29(35) μ m, capitulum 10–15 \times (9)11–15 μ m, pedicel 3–5 μ m in width, cell wall laterally 1.1–1.5–1.8 μ m but apically thickened, (2)5–9 μ m; urediniospores broadly ovoidal, light ochraceous brown, echinulate, 12–16 \times 9–12 μ m, spore wall evenly 1–1.5 μ m, germ pores 4–6, equatorially arranged.

Additional specimen examined: South Africa, Mpumalanga, S 25° 54' 29.8", E 31° 53' 48.5", on leaves of *Senegalia brevispica*, 16 Feb. 2015, M. Ebinghaus (ME406), paratype ME406.

Fig. 9 *Ravenelia dumeti*. **a** Small groups of hypophyllous uredia on *S. brevispica*. **b** Section of an uredinium showing capitate paraphyses seen in LM. **c–e** Urediniospores showing equatorial germ pores (arrows) seen in LM. **f** SEM view of an uredinium. **g** SEM view of urediniospores intermixed with intrasoral paraphyses. **h** Detailed view of urediniospores seen in SEM including the torn cuticle. Scale bars: **a** = 1 mm; **b** = 20 μ m; **c–e** = 10 μ m; **f** = 20 μ m; **g** = 10 μ m; **h** = 4 μ m



Notes: Only the uredinal stage is known for this species. Based on a close relationship to other *Ravenelia* species as well as its *Senegalia* host association, we have assigned this anamorphic rust to the genus *Ravenelia*. It is consequently the first rust fungus known to occur on *S. brevispica*. Unlike other *Senegalia* rusts in South Africa that occur in more open habitats, the host of this rust forms dense thickets along rivers and forest margins. This species clustered as a genetically distinct lineage when compared with other *Ravenelia* spp. infecting *Senegalia* hosts. The distinct phylogenetic position is also

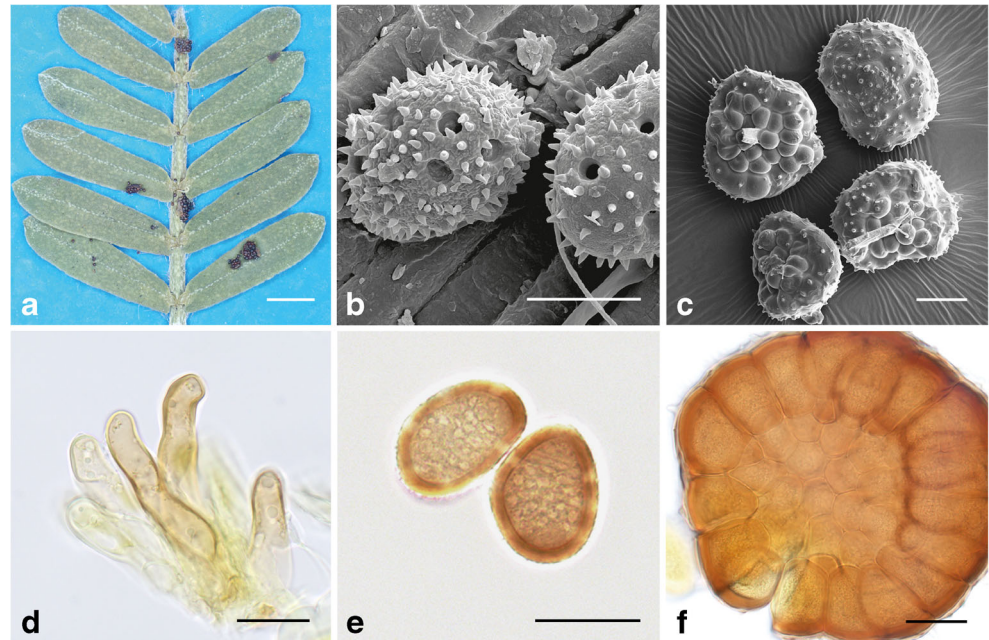
mirrored in its large and prominently capitate paraphyses that represent a rare feature in South African *Ravenelias*.

New records for South Africa

Ravenelia acaciae-arabicae Mundk. & Thirum., Myc. Papers 16:17. 1946. (Fig. 10a–f)

Specimens examined: South Africa, S 30° 01' 07.2", E 30° 13' 58.7", on leaves of *Vachellia nilotica* (L.) subsp. *kraussiana* (Benth.) P.J.H. Hurter & Mabb., 2 February

Fig. 10 *Ravenelia acaciae-arabicae*. **a** Infected leaflets of *V. nilotica* showing telia of *R. acaciae-arabicae*. **b** Urediniospores with germ pores seen in SEM. **c** SEM view of teliospores showing verrucosa ornamentation on the topside and the arrangement of cysts on the bottomside. **d** Cylindrical paraphyses of *R. acaciae-arabicae* seen in LM. **e** LM of urediniospores showing scattered germ pores. **f** LM picture of a teliospore from bottom view. Scale bars: **a** = 1 mm; **b** = 10 μ m; **c** = 30 μ m; **d–f** = 20 μ m



2015, M. Ebinghaus (ME374), PREM61854; KwaZulu-Natal, S 29° 49' 29.1", E 30° 32' 08.5", 4 February 2015, M. Ebinghaus (ME377), PREM61853; Weenen Nature Reserve, 13 March 2011, W. Maier (WM3674), KR-M-0006425; 13 March 2011, W. Maier (WM3675), KR-M-0006616.

Spermatogonia and aecia not seen. Uredinia on the adaxial side of the leaflets and on rhachis, sori on leaflets subcircular to elongated, 150–390 µm, sori on rhachis often larger, sometimes confluent, 790–1000 µm, subepidermal, erumpent; paraphyses numerous, mostly peripheral but a few intrasoral, cylindrical, 46–54 × 6–8 µm, light red-brown; urediniospores ovoidal to globose, ochraceous brown, 22–27 × 18–21 µm, spore wall evenly (2)2.5–3 µm thick, verrucose, germ pores 8–12, scattered; telia replacing the uredinia, chestnut brown to dark brown; teliospores circular to subcircular in diameter and almost hemispherical at the top, chestnut brown to light brown, (62)80–99(110) µm in diameter with 5–6 probasidial cells across, few probasidial cells two-layered, single probasidial cells (18)23–28 × (16)22–26(32) µm, cell wall thickened at the top side of the spore and here seemingly bilaminar with a thin hyaline to pale brown outer layer and a chestnut brown inner layer, the inner layer (3)4–5(6) µm, each cell with 6–9 blunt aculei, (1.5)3–5 µm in length; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal.

Notes: In 1946, this species was first described for India on *Acacia arabica* Willd. (= *Vachellia nilotica* P.J.H. Hurter & Mabb.) by Mundkur and Thirumalachar, and was recently more precisely reported on *V. nilotica* subsp. *indica* (Shivas et al. 2013). We report this rust for the first time on *V. nilotica*

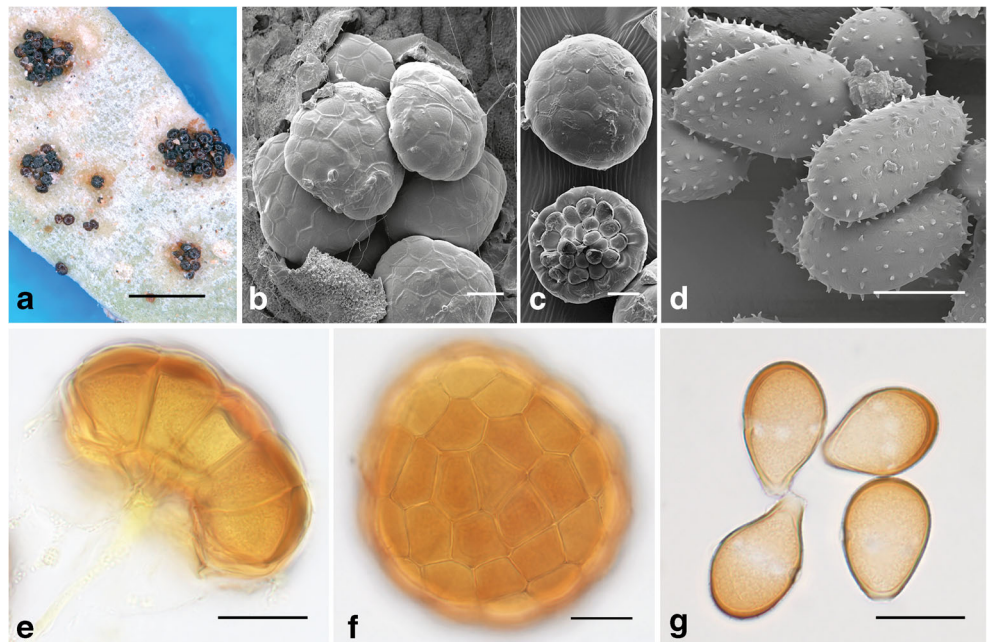
subsp. *kraussiana* that is widespread in southern Africa (Coates Palgrave 2005). *Ravenelia acacia-arabicae* is similar to *R. tandonii* that was described on *Senegalia catechu* (Sydow et al. 1937) but both species can be distinguished by predominantly hypophyllous uredinia in *R. acacia-arabicae* in contrast to the epiphyllous uredinia in *R. tandonii*. Furthermore, with 1–1.5 µm, the urediniospore wall of this rust is approximately half as thick as those of *R. acaciae-arabicae*, which is (2)2.5–3 µm thick. The urediniospores of the two rusts also differ in the number and arrangement of germ pores: 8–12 scattered germ pores in *R. acaciae-arabicae* compared with two rows of four germ pores in *R. tandonii*. The teliospores of *Ravenelia acaciae-arabicae* can be distinguished by having 6–9 blunt aculei per probasidial cell in contrast to 3–6 verrucose papillae in *R. tandonii*.

Phylogenetically, *R. acaciae-arabicae* is most closely related to *R. evansii* with which it shares major teliospore character traits such as size and its spinescent ornamentation. However, both species appear not to have a shared host range and can be thus easily distinguished based on their *Vachellia* hosts. Furthermore, *R. evansii* frequently causes aecial galls and malformations in infected host tissues while all collections of *R. acaciae-arabicae* in South Africa lack the aecial stage.

Ravenelia acaciicola (as *acacicola*) Sanwal, 1951. *Sydowia* 5: 414. (Fig. 11a–g)

Specimens examined: South Africa, KwaZulu-Natal, S 27° 40' 49.3", E 32° 25' 24.0", on leaves of *Senegalia senegal* (L.) Britton var. *rostrata* (Brenan) Kyal. & Boatwr., 11 February 2015, M. Ebinghaus (ME389), PREM61849; S 27° 29' 27.7",

Fig. 11 *Ravenelia acaciicola*. **a** Telia on a leaflet of *S. senegal* var. *leiorhachis*. **b** SEM view of telia showing smooth teliospores. **c** SEM view of teliospores showing topside and bottomside with sterile cysts. **d** SEM view of urediniospores of *R. acaciicola*. **e** LM of a teliospore in lateral view showing also hyaline cysts and the pedicel. **f** LM of a teliospore from top view. **g** LM of urediniospores with the equatorially arranged germ pores. Scale bars: **a** = 0.5 mm; **b** = 20 µm; **c** = 20 µm; **d** = 10 µm; **e**–**g** = 20 µm



E 32° 11' 37.5", on leaves of *S. senegal* var. *leiorhachis*, 12 February 2015, M. Ebinghaus (ME392), PREM61847; Mpumalanga, Komatipoort, 9 April 2013, M. Ebinghaus (ME251), KR-M0006610; 9 April 2013, M. Ebinghaus (ME252), PREM60999; Limpopo, Steelpoort, S 24° 44' 00.6", E 30° 13' 25.1", 19 February 2015, M. Ebinghaus (ME414), PREM61861.

Spermogonia and aecia not seen. Uredinia amphigenous on leaflets, circular to elongated, in small groups, brown, single sori minute, (120)180–300(400) μm , subepidermal, erumpent; urediniospores ovoidal, often tapered towards the basis, ochraceous brown, echinulate, (24)28–33(38) \times (13)15–19(21) μm , spore wall thin but slightly thickened towards the apex and at the basis, 1–2(2.5) μm , germ pores 5–7, equatorially arranged; paraphyses few, peripheral, capitate, about 45 μm in length, capitulum approximately 14 μm wide, light ochraceous brown; telia replacing the uredinia and therefore of same size and shape as the uredinia, dark brown to almost black; teliospores circular to subcircular in diameter, teliospores convex from above but with a concave bottomside, chestnut brown to light brown, (57)84–98(115) μm in diameter with 4–8 probasidial cells across, single probasidial cells (15)27–32(35) \times (13)16–19(28) μm , cell wall thickened at the top side of the spore and here bilaminate with a hyaline outer layer and a chestnut brown inner layer, the inner layer (2)3–6(8) μm , very rarely bearing single verrucose ornamentations of 1–2 μm in height; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal.

Notes: *Ravenelia acaciicola* was described by Sanwal (1951) in India on *Acacia senegal* Willd. (now *Senegalia senegal* (L.) Britton) without providing information regarding which of the four known subspecies of the tree was infected. Two varieties of *S. senegal* (var. *leiorhachis* and var. *rostrata*) occur in South Africa (Coates Palgrave 2005), both of which were found to be infected by *R. acaciicola*. Interestingly, we found some intraspecific genetic variability between specimens originating from the two different host varieties. Because these differences consisted of only one substitution each in the studied LSU and CO3 genes, we refrain from further splitting of *R. acaciicola* at this stage. However, it will be interesting to study additional specimens and gene regions for deeper insight.

The specimens examined in this study match the type descriptions given by Sanwal (1951) with respect to the size of the urediniospores as well as in the morphology of the teliospores. However, they differ in the number of germ pores in the urediniospores. Sanwal (1951) described four germ pores in an equatorial position, while 5–7 equatorial germ pores were observed in our collections. We also observed light brown and clavate paraphyses in a single specimen

(PREM61847) of *S. senegal* var. *leiorhachis* while Sanwal did not describe these structures. Considering the occurrence of this *Ravenelia* species on different subspecies of *S. senegal*, the observed morphological differences from type species could reflect phenotypic variability. Alternatively, cryptic species occur on the subspecies of the host tree. The mostly smooth teliospores of *Ravenelia acaciicola* resemble the closely related *R. transvaalensis* that infects *S. mellifera* in South Africa. But both rusts appeared to be host specific to *S. senegal* and *S. mellifera* subsp. *detinens*, respectively and were furthermore distinguished by phylogenetic analyses of the LSU and CO3 gene regions.

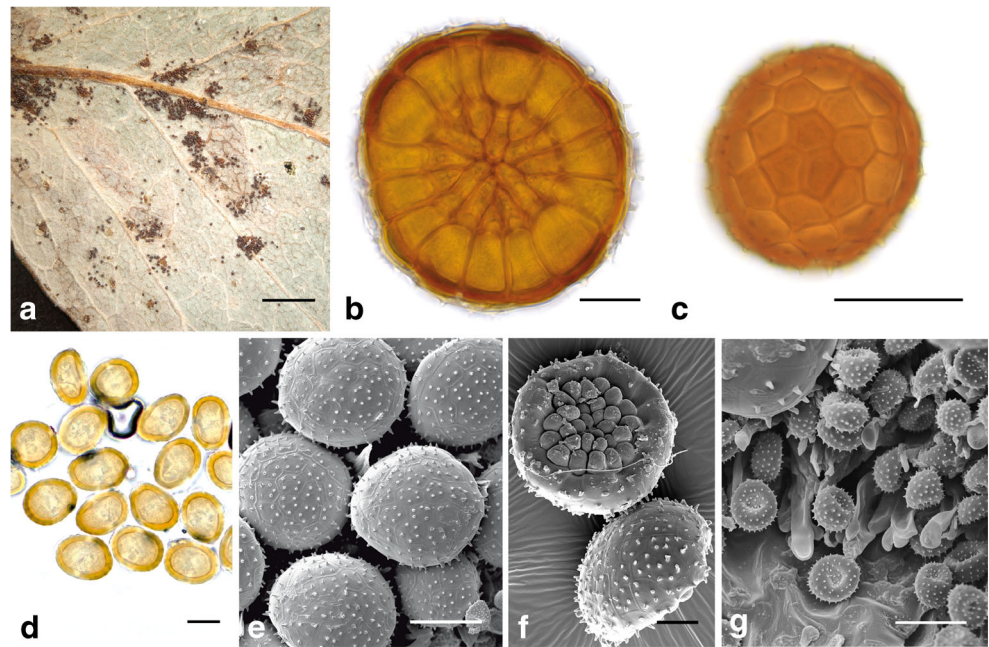
Ravenelia acaciae-nigrescentis Ritschel, Berndt, & Oberw., Mycol. Progr. 6: 137. 2007 (Fig. 12a–g)

Specimens examined: South Africa, Mpumalanga, KNP, Orpen Camp; on leaves of *Senegalia nigrescens* (Oliv.) P.J.H. Hurter, 17 May 2004, W. Maier (WM3282), KR-M-0006413; Mpumalanga, KNP, Orpen Camp; on leaves of *S. nigrescens*, 14 May 2004, W. Maier (WM3278), KR-M-0006619.

Spermogonia and aecia not seen. Uredinia minute, 180–540 μm , circular to elongated, subepidermal, erumpent; few peripheral paraphyses observed in light microscopy, cylindrical to spatuliform, hyaline to subhyaline, up to 70 μm in length and 6–8 μm in width, cell wall 1–1.5 μm ; urediniospores ochraceous brown, subglobose to ovoidal, sometimes ellipsoidal, (15)17–20(23) \times (11)13–15 μm , spore wall evenly 1.5–2 μm , echinulate, 5–6 equatorial germ pores; telia predominantly on the abaxial side of leaflets, subepidermal, erumpent, circular to elongated, chestnut brown to dark brown, 180–540 μm ; teliospores chestnut brown, circular to subcircular from above, upper side of teliospores convex, 85–107 μm in diameter, with 6–8 probasidial cells across, single probasidial cell 26–30 \times 12–19 μm , cell wall thickened at the top side of the spore and here bilaminate with a hyaline outer layer and a chestnut brown inner layer, the inner layer 3–6 μm , each probasidial cell bearing 4–6 spines, (1.8)4–6.2 μm in length; cysts pendent, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal.

Notes: The type specimen was collected in Namibia on *Acacia nigrescens* Oliv. (Ritschel et al. 2007) but its area of distribution is likely larger because the host tree is common throughout western and southern Africa (Coates Palgrave 2005). The original description lacks comments on the presence of paraphyses in *R. acacia-nigrescentis*. However, in the present study, a small number of peripherally arranged paraphyses were observed in light and scanning electron micrographs of the uredinia (Fig. 12g). This *Ravenelia* species resembles *R. spinifera* morphologically. The two species formed a highly supported monophyletic group representing two closely related but distinct sister species in our phylogenetic

Fig. 12 *Ravenelia acaciae-nigrescentis*. **a** Telia on the abaxial side of a leaflet of *S. nigrescens*. **b** LM of a teliospore from bottom view. **c** LM of a teliospore from top view. **d** LM of urediniospores. **e** Teliospores from top view seen in SEM. **f** SEM of teliospores seen in lateral view and from bottomside showing arrangement of cysts. **g** Urediniospores and paraphyses seen in SEM. Scale bars: **a** = 2 mm; **b**, **f**, and **g** = 20 μ m; **c** = 50 μ m; **d** = 10 μ m; **e** = 40 μ m



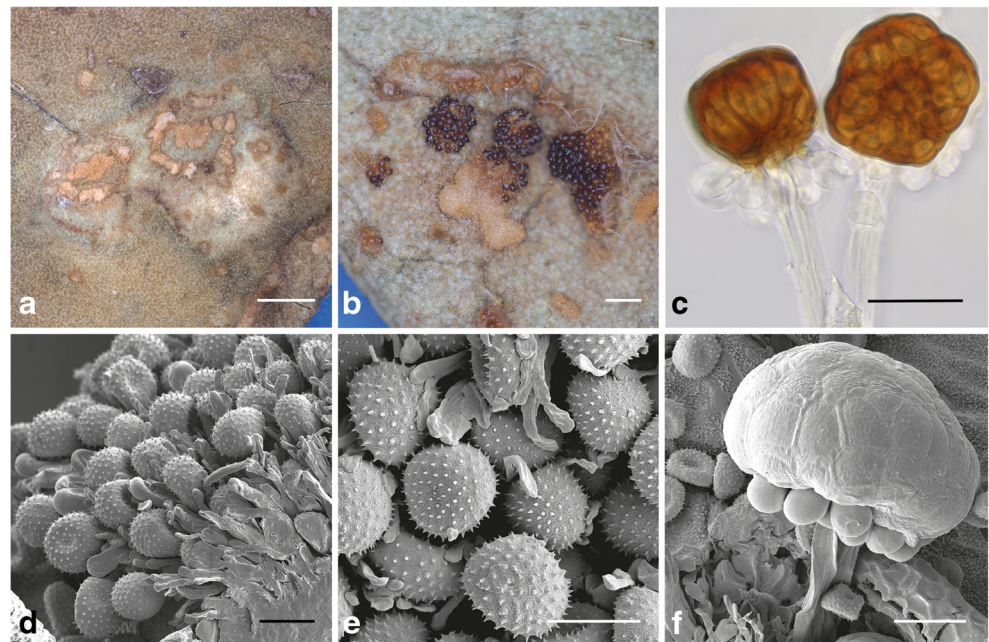
analyses (Figs. 1 and 3). *Ravenelia acaciae-nigrescentis* appeared host specific on *S. nigrescens* while *R. spinifera* was only found on *S. mellifera*.

Ravenelia mesilliana Ellis & Barthol., Bull. Torrey Bot. Club 25: 508. 1898. (Fig. 13a–f)

Specimen examined: South Africa, Mpumalanga, S 25° 0' 38.1", E 031° 16' 18.8", on leaves of *Senna bicapsularis* (L.) Roxb., 15 March 2006, W. Maier (WM3500), KR-M-0006424.

Spermatogonia and aecia not seen. Uredinia amphigenous on leaflets, roundish or of irregular shape, loosely in groups or singly, sometimes forming confluent concentric rings and then leading to chlorotic spots, very variable in size, (100)200–400(500) μ m, subcuticular, often surrounded by the torn remnants of the cuticle, light ochraceous brown; paraphyses numerous, intrasoral, size of paraphyses increasing from the center to the sorus margin, the central paraphyses very slender and cylindrical, about 25–35 μ m in length and 3 μ m wide, transparent; paraphyses towards the sorus margin clavate and

Fig. 13 *Ravenelia mesilliana*. **a** Uredinia forming concentric rings with chlorotic areas in its surrounding. **b** Uredinia intermixed with telia. **c** Teliospores in lateral view showing the multihyphal pedicel and hyaline cysts seen in LM. **d** SEM view of an uredinium with numerous intrasoral paraphyses. **e** Urediniospores and intrasoral paraphyses seen in SEM. **f** SEM view of the smooth teliospore showing pendent cysts and pedicel. Scale bars: **a** = 1 mm; **b** = 0.2 mm; **c** = 50 μ m; **d** = 3 μ m; **e–f** = 20 μ m



up to 40 µm in length and 5–10 µm in width; urediniospores globose to rarely ovoidal, light ochraceous brown, echinulate, 14–18 µm, spore wall evenly 1.5–2.5 µm, germ pores 9–12, scattered; telia replacing the uredinia and therefore of same size and shape as the uredinia but also on rhachis, dark brown to blackish, teliospores circular in diameter and hemispherical in lateral view, chestnut brown to dark brown, (66)70–85(102) µm in diameter with 5–7 probasidial cells across, single probasidial cells (24)28–34(41) × (12)15–19(25) µm, cell wall thickened at the top side of the spore and here with an inconspicuously thin outer hyaline to pale brown layer, (4)5–7(9) µm, cell wall laterally (1.5)2–3(3.5) µm thick, probasidial cells rarely bear a single short and hyaline papillum up to 3.5 µm in length; cysts pendent, globose, hyaline, smooth, easily detached, swelling in water but only slightly in lactophenol solution, number of cysts equal to number of probasidial cells. Pedicel sometimes light brown, up to 180 µm in length, multihyphal.

Notes: There are a small number of records of *Ravenelia arthuri* Long occurring on *Senna bicapsularis* (L.) Roxb. in the Caribbean and Venezuela (Lenné 1990; Boa and Lenné 1994). Arthur described this rust in 1904 as *R. portoricensis* based on urediniospores occurring on *Cassia emarginata* L. (= *Senna bicapsularis* (L.) Roxb). The rust was later reduced to synonymy with *R. arthuri* (Long 1906) who also described the telial stage of this rust. *Ravenelia mesilliana* resembles the latter species in teliospore morphology but teliospores of *R. arthuri* are distinctly larger (70–120 µm). Furthermore, the uredinia of *R. arthuri* were described as being subepidermal and mostly aparaphysate or having only few paraphyses. This is in contrast to *R. mesilliana* in which the sori originate subcuticularly and bear numerous intrasoral paraphyses of variable size and shape. *Ravenelia mesilliana* was originally described on *Senna bauhiniooides* (Gray) Irwin & Barneby. Consequently, *Senna bicapsularis* is a new host record for this rust fungus. *Ravenelia mesilliana* was most likely introduced into South Africa on *Senna bicapsularis*, which is an invasive species in this country (Foxcroft et al. 2003). Another rust, *R. baumiana* is known on *Senna bicapsularis* in Southern Africa (Angola) but it can be distinguished by its pronounced teliospore papillae (Doidge 1939), which were observed only rarely in *R. mesilliana* (Ellis and Everhart 1898, Baxter 1965).

Emended species descriptions

Ravenelia modesta Doidge, Bothalia 3: 504. 1939. emend. M. Ebinghaus, W. Maier & Begerow (Fig. 14a–g)

Types: South Africa, Gauteng, 31 miles North of Pretoria, on leaves of *V. gillettiae* Burt Davy (= *Vachellia luederitzii* var. *retinens* (Engl.) Kyal. & Boatwr.), 21 March 1945, A.O.D. Mogg, syntype PREM34572; Gauteng, Pretoria, Botanical Garden, on leaves of *V. luederitzii* var. *retinens*, 24 June 2012, M. Ebinghaus (ME190), syntype PREM60795.

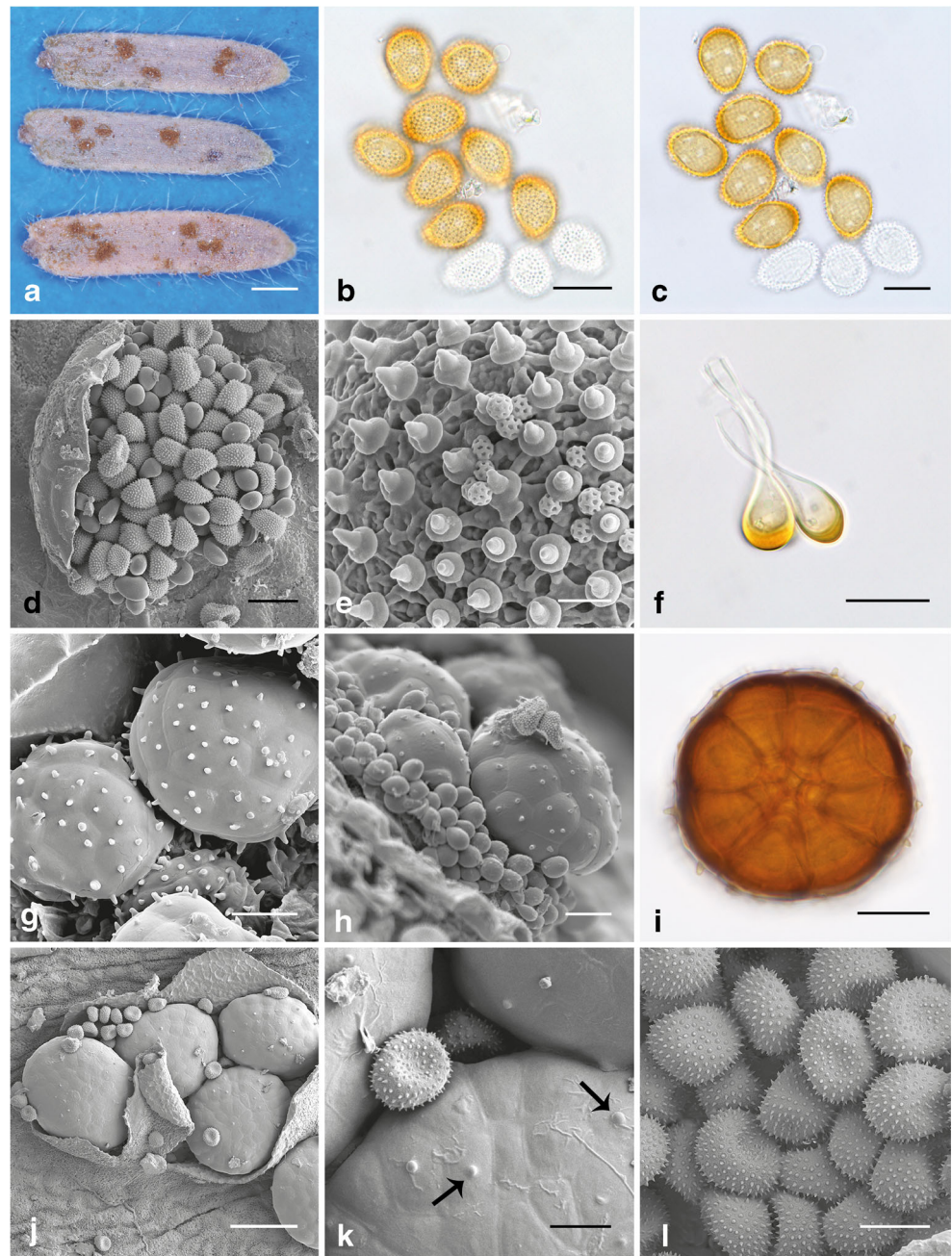
Spermogonia and aecia not seen. Uredinia amphigenous but predominantly hypophyllous, subcuticular, surrounded by the torn cuticle, subcircular, ochraceous brown, 0.1–0.2(0.3) mm; paraphyses intrasoral, capitate, ochraceous brown towards the thickened apex of the capitulum, total length (29)40–53 µm, capitulum (10)12–14(16) µm in width, pedicel (2)4–6(7) µm in width, cell wall 1–1.5(2) µm but apically thickened, 4–8 µm; urediniospores ovoidal to ellipsoidal, light to ochraceous brown (17)20–23(27) × (13)15–17(19) µm, spore wall slightly thickened towards the ends, (1)1.5–2(2.6) µm, verrucose-echinulate, projections composed of a discus-like basis and a verrucose to echinulate apex, each projection connected by a distinct ridge, germ pores 8–12, scattered or bizonate; telia replacing uredinia and therefore of same size and shape as the uredinia, dark brown, teliospores subcircular to circular from above and convex to hemispherical from side view, chestnut brown, (70)75–90(123) µm in diameter with (5)6–7(9) probasidial cells across, single probasidial cells (24)28–32(35) × (16)21–26(31) µm, spore wall thickened at the top side of the spore and here bilaminate with a hyaline outer layer and a chestnut brown inner layer, the inner layer (3)4–5(6.5) µm, each probasidial cell with 4–7 mostly blunt spines of (2)2.5–3.5(5) µm in length; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to number of the inner probasidial cells; pedicel multihyphal.

Additional specimens examined: South Africa, Limpopo, Polokwane Game Reserve; S 23° 57' 40.3", E 29° 29' 17.1", on leaves of *V. rehmanniana*, 9 June 2012, M. Ebinghaus (ME181), paratypes PREM60785, KR-M-0006423; S 23° 57' 40.3", E 29° 29' 17.1", 9 June 2012, M. Ebinghaus (ME180), paratype PREM60791; S 23° 57' 40.3", E 29° 29' 17.1", 9 June 2012, M. Ebinghaus (ME179), paratype PREM60792; Luis Trichard, 3 April 2013, M. Ebinghaus (ME233), paratypes PREM60991, KR-M-0006421; Mpumalanga, Nelspruit, Botanical Garden, on leaves of *V. gerrardii*, 21 June 2005, W. Maier (WM3425), paratypes PREM61884, K-M-0006426; Barberton, S 25° 45' 37.0", E 31° 03' 10.4", M. Ebinghaus (ME402), paratype PREM61878.

Notes: The type description of *R. modesta* (type: PREM30110, National Mycology Collections, Roodeplaat, South Africa) was based on telial rust infections found on *Acacia stolonifera* Burchell (Syn. for *Vachellia hebeclada* (D.C.) Kyal. & Boatwr.) (Doidge 1939). Our comparisons of teliospore morphology with the phylogenetically distinct *R. evansii* (Sydow and Sydow 1912), however, aroused suspicion of conspecificity as they were largely the same. This is further supported by the fact that both rusts occur on *V. hebeclada* as we showed recently (Ebinghaus et al. 2018).

In 1948, Doidge added a description of the aecial and uredinial stage of *R. modesta* based on a specimen (PREM34572) collected from *V. luederitzii* (Doidge 1948) and which is also

Fig. 14 *Ravenelia modesta* (a–g), *R. pretoriensis* (h–i), and *R. halsei* (j–l). **a** Uredinia on leaflets of *Vachellia rehmanniana*. **b–c** LM of urediniospores showing “hub and spoke” surface ornamentation (**b**) and scattered germ pores (**c**). **d** SEM of an uredinium of *R. modesta* with numerous intrasoral paraphyses. **e** SEM showing details of the “hub and spoke” surface ornamentation. Small spherical structures most likely represent brochosomes of insects. **f** Capitulate paraphyses of *R. modesta* seen in LM. **g** SEM view of a spinescent teliospore. **h** Telium showing paraphyses and a verrucose to spinescent teliospore seen in SEM. **i** Teliospore seen in LM. **j** SEM view of a telium with few scattered urediniospores. **k** SEM view of teliospores. Arrows indicate verrucae on single probasidial cells. **l** Urediniospores seen in SEM. Scale bars: **a** = 0.5 mm; **b–d** = 20 μ m; **e** = 1 μ m; **f–i** = 20 μ m; **j** = 50 μ m; **k–l** = 10 μ m



another common host of *R. evansii* (Ebinghaus et al. 2018). The urediniospores of this additional specimen of *R. modesta* were identical to the urediniospores of the specimens of *R. modesta* examined in our present study and these were clearly distinct from those of *R. evansii*. Based on our findings, we consequently consider the uredinial stage of this rust (PREM34572) as representing *R. modesta* Doidge.

However, it remains doubtful as to whether the aecial stage described by Doidge (1948) for this specimen is conspecific with the uredinia of *R. modesta* or whether the aecia actually represent *R. evansii*. We were unable to isolate DNA of sufficient quality from this specimen (PREM34572) and are thus

unable to unequivocally confirm the assumed conspecificity of both spore stages with *R. modesta*. Nonetheless, because both rusts are able to infect the same host species, we propose (i) that the original description Doidge provided in 1939 for the telial stage of *R. modesta* is not correct but describes the teliospores of *R. evansii* and that *V. hebeclada* does not represent a host for *R. modesta*. (ii) The “connection” of the aecial stage with *R. modesta* given in Doidge (1948) is doubtful and may represent *R. evansii* as well. *Ravenelia modesta* Doidge should thus be re-typified with specimen PREM34572 collected on *Vachellia luederitzii* var. *retinens* (Engl.) Kyal. & Boatwr. (syn. *Acacia gillettiae* Burt Davy) for the uredinial

stage and with PREM60795 on *V. luederitzii* var. *retinens* for the uredinial and telial stages. On the basis of our morphological as well as phylogenetic analyses we furthermore report *Vachellia gerrardii* and *V. rehmanniana* as new hosts for *R. modesta* and define the respective specimens as paratypes.

Ravenelia halsei Doidge, Bothalia 3: 504. 1939. emend. M. Ebinghaus, W. Maier, & Begerow (Fig. 14j–l)

Types: South Africa, KwaZulu-Natal, Ndwedwe, on leaves of *Senegalia ataxacantha*, 26 July 1938, R. H. Halse, holotype PREM30117.

Spermogonia and aecia not seen. Uredinia amphigenous but predominantly hypophyllous, subcuticular, erumpent, singly or in small groups, circular to elongated or of irregular shape, covered by the bursting brownish cuticle when young, light ochraceous brown when older, often very minute, (40)60–210 µm, paraphysate; urediniospores broadly ovoidal to globose, transparent to light ochraceous brown, densely echinulate, 14–17 × 12–14 µm, spore wall evenly 1.5–2 µm thick, germ pores 13–15, scattered; telia replacing uredinia and therefore of the same size and shape as uredinia, chestnut brown to dark brown; teliospores subcircular to circular in diameter and slightly convex at the upper side chestnut brown, 95–120 µm in diameter with 8–11 probasidial cells across, single probasidial cells 20–24(28) × (9)11–14(20) µm, cell wall thickened at the top side of the spore, (2)3–5(6) µm, few cells bearing a single short papillum of (1)2–2.5(3) µm in height; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyphal.

Additional specimens examined: South Africa, Mpumalanga, S 25° 02' 33.4", E31° 06' 10.3", on leaves of *S. ataxacantha*, 18 Feb. 2015, M. Ebinghaus (ME411), paratype PREM61867; Nelspruit, Botanical Garden, 22 June 2009, W. Maier (WM3424), paratype KR-M-0006620.

Notes: The type material used by Doidge for the type description of *R. halsei* (PREM30117) lacks the uredinial stage (Doidge 1939). Specimens collected in recent years showed uredinial infections (PREM61867, KR-M-0006620) and we have, therefore, presented detailed descriptions of the uredinial stage of *R. halsei*. Additionally, scanning electron microscopy (SEM) revealed the presence of verrucae on few teliospores of the investigated specimens that were described.

Ravenelia pretoriensis Syd & P. Syd, Annls Mycol. 10: 441. 1912. emend. M. Ebinghaus, Begerow, & W. Maier (Fig. 14h–i)

Types: South Africa, Gauteng, Garstfontein, on leaves of *Acacia horrida* Willd., 11 April 1911, E.M. Doidge, holotype PREM1376.

Spermogonia and aecia not seen. Uredinia amphigenous, subcuticular, circular to elongated, surrounded by the torn cuticle,

light brown, minute 70–250 µm; paraphyses intrasoral, capitate, only few appearing clavate, ochraceous brown towards the thickened apex of the capitulum, 34–53 µm in total length, capitulum (5)9–13 µm in width with a thickened apex of 4–9 µm, pedicel 3–5 µm in width, cell wall 1–2 µm thick; urediniospores ovoidal to ellipsoidal, light ochraceous brown, 19–23 × 14–17 µm, spore wall (1)1.5–2 µm and slightly thickened towards the apex, verrucose-echinulate, projections composed of a discus-like basis and a verrucose to echinulate apex, each projection connected by a distinct ridge, germ pores (8)10–12, scattered or bizonate; telia replacing uredinia and therefore of same size and shape as the uredinia, chestnut brown to dark brown, teliospores subcircular to circular from above, upper side of teliospores convex to hemispherical, chestnut brown to dark brown, (60)70–95(105) µm in diameter with 5–7 probasidial cells across, single probasidial cells (20)25–30(36) × (14)17–22(29) µm, cell wall thickened at the top side of the spore and here bilaminar with a hyaline outer layer and a chestnut brown to dark brown inner layer, the inner layer, (2)3–5(6) µm, each probasidial cell with 4–7 papillae of (1)2–3(4) µm in length; cysts pendent, globose, hyaline, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the inner probasidial cells; pedicel multihyphal.

Additional specimens examined: South Africa, Gauteng, ARC Roodeplaat, on leaves of *Vachellia karroo*, 22 May 2012, M. Ebinghaus (ME139), paratype PREM60809; 31 May 2012, M. Ebinghaus (ME153), paratype KR-M0006615; KwaZulu-Natal, Charters Creek, on leaves of *V. natalitia*, 19 March 2013, M. Ebinghaus (ME214), paratype PREM61021.

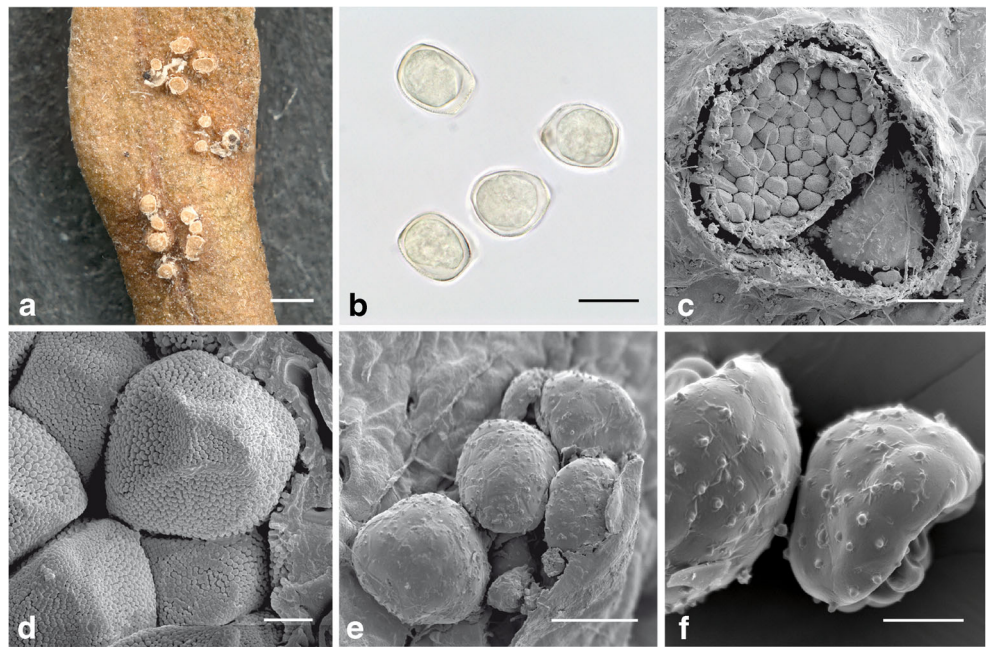
Notes: Sydow and Sydow (1912) described the uredinia as subepidermal but our microscopic examinations did not support this finding and we rather observed subcuticular sori. We further observed 10–12 scattered or bizonate germ pores in the urediniospores, while Sydow and Sydow (1912) noted the presence of 6–8 scattered germ pores. Arguably, the most important emendation concerns the surface structure of these spores that show a distinct “hub and spoke pattern” (Fig. 14b and e; see for definition also Gardner and Hodges 1985).

Ravenelia pretoriensis is morphologically similar to *R. modesta* and both species appeared to be closely related in our phylogenetic analyses based on fragments of the LSU and *CO3* gene regions (Figs. 1 and 3). However, *R. pretoriensis* appeared to be confined to *Vachellia karroo* and to the closely related *V. natalitia*, while *R. modesta* was never observed on these host trees. *Vachellia natalitia* is here reported as a new host species for this rust fungus.

New host report

Ravenelia inornata (Kalchbr.) Dietel, Hedwigia 33: 52 & 61. 1894 (Fig. 15a–f)

Fig. 15 *Ravenelia inornata*. **a** Aecidia on a leaflet of *Vachellia karroo*. **b** LM of aeciospores. **c** SEM of an aecium intermingled with a telium. **d** SEM of rectangular aeciospores showing verrucose ornamentations. **e** SEM of a subepidermal telium. **f** SEM of teliospores. Scale bars: **a** = 0.5 mm; **b** and **f** = 20 μ m; **c** and **e** = 50 μ m; **d** = 4 μ m



Type: South Africa, Eastern Cape, Somerset East, Jan 1881, on leaves of *Acacia karroo* Hayne (*Vachellia karroo* (Hayne) Banfi & Galasso), P. MacOwan, holotype (PREM20734).

Spermogonia not seen. Aecia hypophyllous on leaflets, scattered or in small groups, subepidermal, peridium cylindrical, up to 500 μ m in length, yellow white; aeciospores subhyaline to pale yellow, rectangular to subglobose, 23–27 \times 19–22 μ m, spore wall densely verrucose, 1–3 μ m, but distinctly thickened in the angles up to 6 μ m; telia minute, scattered or in small groups, mixed with the aecia, sometimes telia developing within or confluent with the aecia, dark brown to almost black; teliospores subcircular or irregular circular shaped from above, upper side of teliospores convex, often irregularly shaped, chestnut brown to dark brown, variable in size, 104–162 μ m in diameter with 8–12 single probasidial cells across, probasidial cells single layered, (32)36–50 (52) \times (12)14–17(19) μ m, cell wall thickened at the top side of the spore, (3)5.5–8.5(11) μ m, each probasidial cell bearing a variable number (up to seven) small verrucae, 1–3 μ m in height; cysts pendent, hyaline, smooth, number of cysts equal to the number of the probasidial cells, swelling in water but only slightly in lactophenol solution; pedicel multihyphal.

Additional specimens examined: South Africa, Mpumalanga, South of Barberton, on leaves of *V. natalitia*, 2 June 2012, M. Ebinghaus (ME158), PREM61214; KwaZulu-Natal, close to St. Lucia, 9 March 2013, M. Ebinghaus (ME215), KR-M-0006613; Eastern Cape,

Kentani, on leaves of *A. karroo* Hayne, 3 June 1912, A. Pegter (PREM2368); 23 July 1912, A. Pegter (PREM2541).

Notes: Based on collections deposited at the National Mycology Collections (Roodeplaat, South Africa) and our observations, this species appears to have a distribution range in South Africa that is restricted to the southern and southeastern coastal regions and up to the low lying regions of southern Mpumalanga Province. Interestingly, *R. inornata* appeared to be absent in the central plateau region of South Africa despite the common occurrence of its host trees (Coates Palgrave 2005). This region is climatically distinct in having lower levels of precipitation and a pronounced dry season. The absence of *R. inornata* in this region might be linked to these environmental factors.

This species shares the hosts *V. natalitia* and *V. karroo* with *R. pretoriensis* and *R. macowaniana*. Mixed infections by these two species were thus occasionally encountered even on a single leaflet. Co-occurrence of *R. inornata* with *R. pretoriensis* may lead to confusion because the teliospores of both rusts bear verrucose ornamentations. However, the teliospores differ distinctly in size with teliospores of *R. pretoriensis* mostly measuring 70–95 μ m, while the teliospores of *R. inornata* range between 104 and 162 μ m (Table S2). They can furthermore be distinguished by their life cycles: *Ravenelia pretoriensis* might be hemicyclic as only the uredinial and the telial stages are known while *R. inornata* appears demicyclic. All three species are only distantly related in molecular phylogenetic analyses (Figs. 1 and 3).

Key to South African *Ravenelia* species

- 1. Galls/malformations of host tissues2
- 1*. No galls/malformations visible, also no old galls6
- 2. Galls/malformations caused by aecia3
- 2*. Galls caused by aecia bearing echinulate aeciospores; reported on *S. polyacantha* subsp. *campylacantha*.....*R. modjadji*
- 3. Teliospores smooth or with rare verrucae4
- 3*. Teliospores spinescent*R. evansii*
- 4. Teliospores < 85 μm 5
- 4*. Teliospores up to 130 μm ; reported on *Vachellia karroo*, *V. natalitia*, *V. permixta*.....*R. macowaniana*
- Telia 0.1–0.2 mm, on leaflets; reported on *V. xanthophloea*.....*R. xanthophloea*
- 5*. Telia crustaceous, pedicels of teliospores up to 180 μm long; teliospores < 60 μm*R. natalensis*
- 6. On *Vachellia*7
- 6*. On Mimosoideae other than *Vachellia*, or on Faboideae or Caesalpinioideae10
- 7. No urediniospores; peridiate aecia on leaves; teliospores verrucose..... *R. inornata*
- 7*. Urediniospores often present; no aecia, if present teliospores spinescent.....8
- 8. Urediniospores echinulate; teliospores spinescent; reported on *V. nilotica*.....*R. acaciae-arabicae*
- 8*. Urediniospores with “hub & spoke” pattern9
- 9. Teliospores verrucose to spinescent; reported on *V. karroo* and *V. natalitia*.....*R. pretoriensis*
- 9*. Teliospores distinctly spinescent; reported on *V. rehmanniana*, *V. hebeclada*, *V. luederitzii*, *V. gerrardii*..... *R. modesta*
- 10. On *Senegalia*..... 18
- 10*. On a different host plant genus..... 11
- 11. Teliospores smooth or with only few ornaments..... 12
- 11*. Teliospores distinctly ornamented..... 15
- 12. Teliospores smooth, sometimes with single papillae, up to 3–4 μm ; reported on *Senna*.....*R. mesilliana*
- 12*. On a different host plant genus..... 13
- 13. Teliospores > 115 μm , smooth or with solitary verrucae..... 14
- 13*. Teliospores up to 105 μm , smooth; reported on *Elephantorrhiza*.....*R. elephantorrhizae*
- 14. Uredinia aparaphysate; probasidial cells 2-layered; reported on *Calpurnea*.....*R. glabra*
- 14*. Uredinia with numerous clavate paraphyses; teliospores with few solitary verrucae, 115–140 μm *R. woodii*
- 15. Teliospores with pronounced clavate papillae, up to 8 μm long; reported on *Abrus*.....*R. ornata*
- 15*. Teliospores < 70 μm , with distinct spines; reported on *Albizia*.....*R. minima*
- 15**. On a different host plant genus..... 16
- 16. Teliospores spinescent; probasidial cells 1 layered; reported on *Dichrostachys cinerea*.....*R. dichrostachydis*
- 16*. On a different host plant genus..... 17
- 17. Teliospores with papillae; uredinia aparaphysate; reported on *Mundulea sericea*.....*R. stictica*
- 17*. Teliospores with papillae, 4–7 μm long; reported on *Indigofera*.....*R. indigoferae*
- 17**. Teliospores verrucose, up to 2 μm long; reported on *Tephrosia*.....*R. tephrosiae*
- 18. On *S. mellifera*..... 19
- 18*. On a different host plant species..... 20
- 19. Teliospores smooth.....*R. transvaalensis*
- 19*. Teliospores spinescent.....*R. spinifera*
- 20. Teliospores smooth or rarely with solitary papillae; urediniospores with 13–15 scattered germ pores; reported on *S. ataxacantha*.....*R. halsei*
- 20*. Teliospores smooth, verrucose or spinescent; urediniospores with 4–6 equatorially arranged germ pores; on a different host plant species..... 21
- 21. On *S. caffra*..... 22
- 21*. On a different host plant species 23
- 22. Teliospores smooth.....*R. peglerae*
- 22*. Teliospores verrucose.....*R. pienaarrii*
- 23. Teliospores spinescent; only few paraphyses present in uredinia; reported on *S. nigrescens*.....*R. acaciae-nigrescentis*
- 23*. Teliospores smooth; uredinia with numerous intrasoral paraphyses; reported on *S. senegal* (both subspecies).....*R. acaciicola*
- 23**. Teliospores verrucose; uredinia aparaphysate; on *S. burkei*..... *R. escharoides*
- 23***. Teliospores verrucose; only few paraphyses present in uredinia; reported on *S. polyacantha* subsp. *polyacantha*.....*R. doidgeae*
- 23****. Teliospores with small verrucae; uredinia with peripheral paraphyses; reported on *S. galpinii*.....*R. molopa*
- 23*****. Teliospores verrucose; uredinia with peripheral paraphyses; reported on *S. erubescens*..... *R. moloto*

Discussion

In the present study, we analyzed numerous *Ravenelia* specimens with a strong focus on South African species, but included also species from the Neotropics as well as published gene sequences. The South African representatives clustered in seven phylogenetic lineages, one of which was represented by a single species only (clade III). Two of these phylogenetic groups consist only of Paleotropical species while five of these

groups consist of Neotropical and Paleotropical species. Two lineages were found that are of exclusively Neotropical origin and were therefore not highlighted in the phylogenetic trees. In the following sections, the findings of the molecular phylogenetic analyses will be discussed with emphasis on morphological and life cycle traits as well as a consideration of the evolutionary ecological implications relating to host specialization. Finally, the diversity and taxonomy of *Ravenelia* in South Africa is re-evaluated.

Morphology and life cycle traits linked to phylogenetic lineages

Clade I The species in this clade comprised only South African representatives and are exclusively parasitizing species of the genus *Senegalia* (Mimosoideae). They were often difficult to discriminate from each other based on urediniospore or teliospore morphology. Nevertheless, the species could be distinguished by molecular phylogenetic analyses and, in addition, showed distinct host preferences. Rusts that shared teliospore traits like a specific ornamentation type tended to represent distinct lineages that mirror their close phylogenetic relationships, e.g., *R. acacia-nigrescentis* and *R. spinifera* (spinescent teliospores), *R. moloto*, *R. doidgeae* and *R. modjadji* (verrucose teliospores) or *R. transvaalensis* and *R. acaciicola* (smooth teliospores). For species in this clade, only uredinia and telia are known and therefore, they may be hemicyclic. The only exception is here *R. modjadji*. This species produces gall-inducing primary uredinia as well as secondary uredinia.

Clade II This clade was comprised of both Paleotropical and Neotropical species. The South African *R. modesta* and *R. pretoriensis* share a unique urediniospore surface structure with the Neotropical species in this clade that was described as “hub and spoke pattern” (Gardner and Hodges 1985, Hernández and Hennen 2002, see Fig. 14b and e) and appears to be synapomorphic for this lineage. This urediniospore ornamentation had previously only been reported for a few *Ravenelia* species from the Neotropics. The African representatives in this clade were only found on *Vachellia* species, while the South American species had been collected from *Vachellia* and *Mimosa*. Further rust species with this character have been reported from other host genera within the Mimosoideae but mostly on *Albizia* including *R. albiziae-zygiae*, *R. albiziicola*, *R. clemensae* among others. Field observations suggested that the uredinial stage of *R. pretoriensis* and *R. modesta* is consistently the predominant spore stage, with the teliospores observed more rarely. This finding was irrespective of the time point of species sampling and may represent a lineage-specific life cycle characteristic.

Clade III This clade consisted of a single species, *R. dumeti*. Only the uredinial stage is known of this species and specific

traits are thus limited to characterize this rust. The numerous intrasoral and distinctly capitate paraphyses distinguish it from all other *Ravenelia* species investigated in the present study. The extended branch length in the phylogenetic trees that separates this lineage from its sister group of clade II mirrors the distinctiveness of these uredinial traits. *Ravenelia dumeti* is the only *Ravenelia* species of southern African origin that parasitizes a species of a lineage of *Senegalia* with African-American distribution, while all *Senegalia* rusts of clade I are parasitic on a hosts lineage of an African-Asian distribution (Bouchenak-Khelladi et al. 2010; Kyalangalilwa et al. 2013). In this respect, *R. dumeti* is more similar to the *Ravenelia* species studied from South America (Fig. 2), which also parasitize on hosts belonging to this African-American lineage of *Senegalia*.

Clade IV The members of this clade are heterogeneous in terms of morphology and associated hosts as they parasitize representatives of the two traditionally recognized subfamilies Caesalpinioideae and Mimosoideae. Here, *R. halsei* (on *Senegalia*, Mimosoideae) is the only species with aparaphysate uredinia, while *R. elephantorrhizae* (on *Elephantorrhiza*, Mimosoideae), *R. macrocarpa*, and *R. mesilliana* (both on *Senna*, Caesalpinioideae) have intrasoral paraphyses. In the latter two species, the paraphyses are variable in size and shape (Baxter 1965, Hernández and Hennen 2002; Fig. 11). *Senna* spp. are hosts to approximately 14 species of *Ravenelia* and are thus an important host group for these rusts (www.indexfungorum.org). Future studies should clarify whether size variation of the paraphyses is a common morphological character that is synapomorphic for *Ravenelia* species parasitizing *Senna*.

Clade V *Ravenelia holwayi* and *R. dichrostachydis* share the host preference for Mimosoideae but show several striking differences and a significant genetic distance reflecting their distant geographic origin from North America and South Africa, respectively. Most prominently, the species differ in the ability of *R. holwayi* to induce uredinial galls while *R. dichrostachydis* does not share this trait. To date, only *R. dichrostachydis* is known from *Dichrostachys* while six *Ravenelia* species have been described on *Prosopis*, e.g., *R. arizonica*, *R. chacoensis*, *R. prosopidis*, or *R. spicigeriae* (www.indexfungorum.org). The incorporation of these species in future analyses could help to characterize members of this clade in more detail.

Clade VI This is the only lineage comprising *Ravenelia* species infecting members of the Faboideae suggesting that this host association evolved only once. This is in contrast to the *Ravenelia* spp. parasitizing on Mimosoideae, which are present in several lineages of the phylogenetic tree and thus of polyphyletic origin. Within this clade, only uredinial and telial

stages are known for *R. stictica*, *R. glabra*, *R. aff. indigoferae*, and *R. tephrosiae* suggesting a hemicyclic life cycle. They are furthermore characterized by two-layered probasidial cells. Since the type species of *Ravenelia*, *R. epiphylla*, was collected on a related host of the Faboideae and also shares these characters, we assume that it would reside within this phylogenetic group. Unfortunately, fresh material of *R. epiphylla* was not available for inclusion in the molecular phylogenetic studies and its relationship to other species could thus not be resolved.

In our analyses, *Ravenelia ornata* was the sister species to *R. platensis*. Both species develop uredinioid aecia (Hernández and Hennen 2002; Wood 2006), a trait that is shared with several distinct lineages of *Ravenelia* rusts, i.e., the neotropical *R. echinata* var. *ectypa*, *R. havanensis* and *R. hermosa* but also *R. holwayi* in clade V (Fig. 2). However, *R. ornata* apparently lacks the ability to induce galls. The two accessions of this rust collected in South Africa showed considerable sequence variation of 3.8% (6.6% when gaps are included) within a fragment of the LSU gene region, even though they are morphologically indistinct based on teliospore morphology (data not shown). Interestingly, collections indicate a wide distribution within the tropics with this rust being reported from the Caribbean, Asia, and Africa (Farr and Rossman 2017). These findings suggest that a broader sampling including specimens from its full geographical range could reveal a species complex including cryptic species.

Clade VII The rusts in this phylogenetic group are confined to Mimosoideae, mainly *Vachellia* species with the exception of two neotropically distributed rusts, i.e., *R. mainsiana* and *R. cebil* infecting *Mimosa* and *Anadenanthera*, respectively. Several species in this clade possess two-layered probasidial cells, a feature shared with *Ravenelia* spp. on Faboideae (clade VI), and most species are able to induce galls (Fig. 2). No spermogonia were reported for those species within the group (*R. acacia-arabicae*, *R. mainsiana*, *R. cebil*, *R. inornata*) lacking the ability of gall induction. This is in line with observations of Larous and Lösel (1993) that invasion of the vascular system and resulting hypertrophies are only induced by the monokaryotic hyphae of the rust fungi. Remarkable differences in life cycle traits were seen between *Ravenelia hieronymi* and the closely related *R. evansii*, *R. macowaniana*, and *R. xanthophloeae* by producing telia subsequently on aecial galls in the case of *R. hieronymi* (Hernández and Hennen 2003, Hennen et al. 2005). Hennen et al. (2005) suggested that *R. hieronymi* could represent a demicyclic rust as pedicellate urediniospores were never observed. This is in contrast to the macrocyclic *R. evansii*, *R. macowaniana*, and *R. xanthophloeae* that induce aecial galls but in which the uredial and telial stages are always spatially separated from the aecia (Doidge 1939; Ebinghaus et al. 2018). While the aeciospores of these rusts obviously re-infect the host, the aeciospores of

R. hieronymi were suggested to be non-functional (Hernández and Hennen 2003; Hennen et al. 2005). Similar to *R. hieronymi*, only aecia and telia are known for *R. inornata* and they develop intermingled with the aecia, but galls are unknown in this species. However, the two species did not appear to be closely related in our phylogenetic analyses. In the acacia rust *Atelocauda koeae* simultaneously occurring aecio- and teliospores are known to be non-functional and an ongoing transition from demicyclic to microcyclic life cycle has been suggested (Hodges Jr and Gardner 1984, Chen et al. 1996). It remains an open question as to whether this also holds true for *R. inornata*. Dietel (1906) did not observe germ pores in this rust and this could support the view of non-functional aeciospores.

Linking morphological traits to phylogenetic lineages within *Ravenelia* showed that taxonomic systems as proposed by several authors including Long (1903), Dietel (1906), Sydow and Sydow (1915), and Sydow (1921) were often not congruent with the phylogenetic reconstructions based on LSU and CO3 gene regions (Fig. 2, Table S1). Rust fungal structures including the morphology of uredinio- and teliospores have been suggested to be highly adaptive and are consequently prone to convergent evolution (Savile 1971, 1976, 1978). Additionally, our finding of close phylogenetic relationship between *Ravenelia* species that show different life cycle traits highlights the variability of those character states in the rust fungi as has been shown many times before (compare Maier et al. 2007). Nevertheless, several traits such as the host association but also the ability to induce galls in combination with the development of two-layered probasidial cells were found to represent useful criteria to draw conclusions regarding monophyly. In this respect, we found evidence that the macrocyclic and often gall-inducing rusts comprising those on *Vachellia* but also other members of the Mimosoideae most likely represent a more ancestral lineage in *Ravenelia* (Shattock and Preece 2000). Likewise, the “hub and spoke” urediniospore ornamentation pattern appeared synapomorphic for a distinct lineage of *Ravenelia* species.

Evolutionary ecological implications

Species of *Senegalia* and *Vachellia* represent the majority of *Ravenelia* hosts in South Africa.

Two major lineages of *Senegalia* rusts had either an exclusively Paleotropic (clade I) or Neotropic (i.e., *R. cohniiana*, *R. piepenbringiae*, and *R. hernandezii*; compare Ebinghaus and Begerow 2018) distribution. Molecular dating analyses suggest a split of *Senegalia* species into an Asian-African lineage from American ancestors in the late Miocene, some 18–9 Mya (Bouchenak-Khelladi et al. 2010). The Paleotropic lineage is believed to have subsequently diversified in response to oscillating climatic changes during the Pliocene and species likely spread from Africa into Asia (Bouchenak-Khelladi et al.

2010). The Paleotropically distributed rusts in clade I correspond to this host lineage and reasonably explains the Asian-African distribution of *R. acaciicola* (Fig. 2). The ancestor of this rust lineage could have jumped from a non-*Senegalia* host onto an early representative of the diverging African host lineage and diversified simultaneously with the diverging hosts by cospeciation or subsequently via host tracking co-evolution (Ehrlich and Raven 1964; Janz and Nylin 1998). In the latter case, the parasite would have overcome host defense of an ancestral host and, following its radiation, colonized and diversified on these related species of the same clade. These two possible scenarios could be assumed for *R. modjadji*, *R. moloto* and *R. doidgeae* that infect the sister species *S. polyacantha* subsp. *campylacantha*, *S. polyacantha* subsp. *polyacantha*, and *S. erubescens*, respectively (Bouchenak-Khelladi et al. 2010; Kyalangalilwa et al. 2013). Other members of this rust lineage might rather have evolved by host shifts (Roy 2001), e.g., *R. transvaalensis* or *R. spinifera* that both infect *S. mellifera* subsp. *detinens*.

The results of the present study further suggest that the *Ravenelia* species occurring on *Senegalia* represented in clade I are highly host specific, with most species restricted to a single host. The hosts of the rusts in this clade predominantly occur in the semi-arid savannas of South Africa where they occupy specific niches and form dominant floral elements (Coates Palgrave 2005, Smit 2008). This native semi-arid habitat could also have contributed to a predominance of the more heat- and drought-resistant telial stage compared with the uredinial stage (Savile 1976).

The limited numbers of wind-dispersed urediniospores as well as the obvious lack of spermogonia and the aecial stage in these *Ravenelia* spp. may imply that rust fungal propagules are restricted from easy movement to a broader range of potential hosts due to dispersal limits. This may have hampered them from acquiring multiple hosts.

Several rust fungi that lack spermogonia were found to reproduce sexually through self-fertility (Anikster et al. 1980; Anikster and Wahl 1985; Ono 2002). If this is also true for the *Senegalia* rusts, the dispersal limits and the resulting restrictions in gene flow between populations may reasonably explain the observed host ranges but could also have led to an increased speciation rate. On the other hand, apomictic or asexual reproduction was proven to occur in various microcyclic species (Ono 2002) and which potentially can lead to similar speciation patterns. However, examinations of nuclear behavior as well as population genetic approaches are needed to prove either mechanism.

Species in the genus *Vachellia* (formerly *Acacia* subsp. *Acacia*) represent the other major host group for the South African *Ravenelia* species. Our study clearly showed that the rusts infecting this host genus evolved at least twice independently. The gall-inducing macrocyclic rusts *R. macowaniana*

and *R. evansii* are among the most abundant rust fungi in southern Africa. This is also consistent with their wide host range and common occurrence of their hosts (Smit 2008). In contrast to the observations in the south African gall rusts, populations of macrocyclic rust fungi were reported to often lose their aecial stage in more arid environments. Savile interpreted this as an adaptation to low water availability and high UV radiation, because aeciospores often possess little pigmentation and thin spore walls (Savile 1971, but see Zwetko and Pfeifhofer 1991). However, we can only speculate under which conditions these rusts have evolved the capacities to induce aecial galls and whether or not ontogenic constraints may restrict life cycle reductions. But considering the scattered distributions of their hosts in open savannas and in the Karoo semi-desert, the massive production and release of wind-borne aeciospores may be still advantageous by effectively bridging the distance between potential host individuals. The production of spermatia and large numbers of aeciospores could also contribute to the wide host range observed in *R. macowaniana* and *R. evansii*, with greater numbers of potential hosts being exposed to these spores. The South African and gall-inducing *Vachellia* rusts could thus have evolved a different strategy compared with *Ravenelia* species on *Senegalia* to persist and disperse in a similar environment.

The *Vachellia* rusts exhibiting urediniospores with “hub and spoke” ornamentation (clade II) most likely originated in the Neotropics. This emerges from the fact that several *Ravenelia* species displaying this morphological characteristic infect a suite of different mimosoid genera with a Neotropical distribution. We thus assume that an ancestor of *R. modesta* and *R. pretoriensis* was introduced to Africa by long-distance dispersal. Spore dispersal over long distances may readily occur and was shown for other rust fungi like *Melampsora* species (e.g., Barrès et al. 2008). The origin of the African lineage could have coincided with the trans-Atlantic dispersal events that were argued to have occurred in the *Vachellia* species in the Miocene (16–11 mya; Bouchenak-Khelladi et al. 2010). *Ravenelia modesta* and *R. pretoriensis* are difficult to distinguish from each other based on spore morphology or the genetic markers utilized in this study and speciation most likely occurred more recently.

These findings and the fact that *Ravenelia* species parasitizing the major host genera *Vachellia* and *Senegalia* appear in several phylogenetic lineages suggest that diversification of *Ravenelia* in the current circumscription might have been driven by a combination of host shifts and co-evolutionary host tracking or cospeciation in addition to more rare jumps to co-occurring but more distantly related hosts. This situation might be comparable with patterns observed within *Puccinia* and *Uromyces* where a similar situation was observed for the species parasitizing Cyperaceae and Poaceae in relation to all other parasitized groups (Maier et al. 2007; van der Merwe et al. 2007, 2008).

Table 2 List of *Ravenelia* species and their hosts reported in South Africa

Fungus	Host
<i>Ravenelia acaciae-arabicae</i>	<i>Vachellia nilotica</i> subsp. <i>kraussiana</i> (Benth.) P.J.H.Hurter & Mabb.
<i>Ravenelia acaciae-nigrescentis</i>	<i>Senegalia nigrescens</i> (Oliv.) P.J.H. Hurter
<i>Ravenelia acaciicola</i>	<i>Senegalia senegal</i> var. <i>leiorhachis</i> (L.) Britton <i>Senegalia senegal</i> var. <i>rostrata</i> (L.) Britton
<i>Ravenelia deformans</i>	Undetermined acacia (aff. <i>V. nilotica</i>)
<i>Ravenelia dichrostachydis</i>	<i>Dichrostachys cinerea</i> (L.) Wight & Arn.
<i>Ravenelia doidgeae</i>	<i>Senegalia polyacantha</i> subsp. <i>polyacantha</i> (Willd.) Seigler & Ebinger
<i>Ravenelia dumeti</i>	<i>Senegalia brevispica</i> (Benth.) Kyal. & Boatwr.
<i>Ravenelia elephantorrhizae</i>	<i>Elephantorrhiza elephantina</i> (Burch.) Skeels, E. burkei Benth.
<i>Ravenelia escharoides</i>	<i>Senegalia burkei</i> (Benth.) Kyal. & Boatwr.
<i>Ravenelia evansii</i>	<i>Vachellia borleae</i> (Burt Davy) Kyal. & Boatwr. <i>Vachellia davyi</i> (N.E.Br.) Kyal. & Boatwr. <i>Vachellia exuvialis</i> (Verdoorn) Kyal. & Boatwr. <i>Vachellia hebeclada</i> subsp. <i>hebeclada</i> (DC.) Kyal. & Boatwr. <i>Vachellia luederitzii</i> var. <i>retinens</i> (Sim) Kyal. & Boatwr. <i>Vachellia robusta</i> subsp. <i>robusta</i> (Burch.) Kyal. & Boatwr. <i>Vachellia sieberiana</i> subsp. <i>woodii</i> (Burt Davy) Kyal. & Boatwr. <i>Vachellia swazica</i> (Burt Davy) Kyal. & Boatwr. <i>Vachellia gerrardii</i> (Benth.) P.J.H.Hurter <i>Vachellia rehmanniana</i> (Schinz) Kyal. & Boatwr.
<i>Ravenelia glabra</i>	<i>Calpurnia sylvatica</i> E. Mey.
<i>Ravenelia halsei</i>	<i>Senegalia ataxacantha</i> (DC.) Kyal. & Boatwr.
<i>Ravenelia</i> aff. <i>indigoferae</i>	<i>Indigofera</i> sp. L.
<i>Ravenelia inornata</i>	<i>Vachellia karroo</i> (Hayne) Banfi & Galasso <i>Vachellia natalitia</i> (E.Mey.) Kyal. & Boatwr.
<i>Ravenelia macowaniana</i>	<i>Vachellia karroo</i> (Hayne) Banfi & Galasso <i>Vachellia natalitia</i> (E.Mey.) Kyal. & Boatwr. <i>Vachellia permixta</i> (Burt Davy) Kyal. & Boatwr.
<i>Ravenelia mesilliana</i>	<i>Senna bicapsularis</i> (L.) Roxb.
<i>Ravenelia minima</i>	<i>Albizia adianthifolia</i> (Schumach.) W.F.Wight
<i>Ravenelia modesta</i>	<i>Vachellia gerrardii</i> (Benth.) P.J.H.Hurter <i>Vachellia luederitzii</i> var. <i>retinens</i> (Sim) Kyal. & Boatwr. <i>Vachellia rehmanniana</i> (Schinz) Kyal. & Boatwr.
<i>Ravenelia modjadji</i>	<i>Senegalia polyacantha</i> subsp. <i>campylacantha</i> (Hochst. ex. A.Rich.) Kyal. & Boatwr.
<i>Ravenelia molopa</i>	<i>Senegalia galpinii</i> (Burt Davy) Seigler & Ebinger
<i>Ravenelia moloto</i>	<i>Senegalia erubescens</i> (Welw. ex Oliv.) Kyal. & Boatwr.
<i>Ravenelia natalensis</i>	<i>Vachellia karroo</i> (Hayne) Banfi & Galasso
<i>Ravenelia ornata</i>	<i>Abrus precatorius</i> L.
<i>Ravenelia peglerae</i>	<i>Senegalia caffra</i> (Thunb.) P.J.H.Hurter & Mabb.
<i>Ravenelia pienaarii</i>	<i>Senegalia caffra</i> (Thunb.) P.J.H.Hurter & Mabb.
<i>Ravenelia pretoriensis</i>	<i>Vachellia karroo</i> (Hayne) Banfi & Galasso
<i>Ravenelia spinifera</i>	<i>Senegalia mellifera</i> subsp. <i>d. etinens</i> (Burch.) Kyal. & Boatwr.
<i>Ravenelia stictica</i>	<i>Mundulea sericea</i> (Willd.) A. Chev.
<i>Ravenelia tephrosiae</i>	<i>Tephrosia heckmanniana</i> Harms <i>Tephrosia macropoda</i> var. <i>macropoda</i> (E. Mey) Harv.
<i>Ravenelia transvaalensis</i>	<i>Senegalia mellifera</i> subsp. <i>detinens</i> (Burch.) Kyal. & Boatwr.
<i>Ravenelia woodii</i>	Fabaceae undetermined
<i>Ravenelia xanthophloeae</i>	<i>Vachellia xanthophloea</i> (Benth.) P.J.H.Hurter

Diversity of *Ravenelia* in South Africa

A targeted sampling effort in combination with morphological investigations and molecular phylogenetic analyses revealed that the diversity of *Ravenelia* species in South Africa greatly exceeds the current knowledge regarding the diversity of this genus in the region. Six *Ravenelia* species were revealed as new to science and five *Ravenelia* species were new reports for South Africa. In another recent study, one new *Ravenelia* species and seven new acacia hosts were reported from the same region (Ebinghaus et al. 2018). Thus, to date, 32 species of *Ravenelia* are known for South Africa. With a total of 25 species, the majority of *Ravenelia* spp. are confined to the traditionally recognized host subfamily Mimosoideae (in the classic sense). Of these, 13 species occur on 13 *Senegalia* hosts, 9 on 15 *Vachellia* hosts, and 3 on host trees of various other mimosoid genera. Five species occur on Faboideae hosts and a single rust species (*R. mesilliana*) is known on *Senna* (Caesalpinioideae) (Table 2). A single species (*R. woodii*) was collected from an undetermined leguminous host plant (Doidge 1939). Unfortunately, this monotypic rust is not represented in PREM and could not be investigated.

A special effort was made to investigate all of the approximately 42 species of acacias (18 taxa of *Senegalia* and 24 taxa of *Vachellia*) in South Africa (Coates Palgrave 2005; Smit 2008) and scrutinize them for infections. With 22 described *Ravenelia* spp. on at least 28 acacias the genera *Senegalia* (13 host taxa; 72% of total species) and *Vachellia* (15 host taxa; 62.5% of total species) represent the major hosts of this genus in South Africa (Table 2). Based on the number of herbarium specimens present at PREM and our own observations, some of these rusts, e.g., *R. macowaniana* and *R. evansii* likely represent the most abundant and ecologically significant rust fungal species on acacias in this region.

A total of 161 *Vachellia* species and 203 species of *Senegalia* have been described globally (Maslin et al. 2003). Of these, only approximately 26 and 37 species, respectively, were reported as hosts of *Ravenelia* spp. (www.indexfungorum.org). Considering the high percentage of host taxa we found within the genera *Senegalia* and *Vachellia* in South Africa, it is likely that future studies on *Ravenelia* will significantly exceed species numbers currently known for this genus.

Conclusion

In this study, we have revised and illustrated the diversity of *Ravenelia* spp. in South Africa. The first molecular phylogenetic analysis is also presented for this genus. Based on phylogenetic reconstructions, it was possible to consider aspects of the presumed evolutionary strategies in *Ravenelia* spp. that reflect lineage-specific host association patterns and the

biogeography of their hosts. It appears worthwhile for future research in these rust fungi to focus on a global species sampling including rusts especially collected from the Caesalpinioideae and Faboideae as well as to incorporate additional members of the family Raveneliaceae. These are likely to shed light on evolutionary pathways aiding to a more comprehensive understanding of the evolution of character states in the rust fungi.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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