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 (Hennen et al. 2005). *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...
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. xanthophloeae* on the Vachellia xanthophloeae. 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 albizzicola* (PREM40295), *R. baumiana* (PREM50553, PREM29870, PREM6886), *R. elephantorhizae* (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. pienaarii* (PREM5627, PREM6658), *R.
| Fungus            | Host              | Location                                                                 | Voucher       | GenBank accession |
|-------------------|-------------------|---------------------------------------------------------------------------|---------------|-------------------|
| Ravenelia acaciae-arabicae | Vachellia nilotica | South Africa, KwaZulu-Natal, Weenen Game Reserve                          | KR-M-0006425*| —                 |
| Ravenelia acaciae-arabicae | Vachellia nilotica | South Africa, KwaZulu-Natal, S 29°49′ 29.1″, E 30° 32′ 10.3″             | PREM61853     | MN072675          |
| Ravenelia acaciae-arabicae | Vachellia nilotica | South Africa, KwaZulu-Natal, S 30° 01′ 07.2″, E 30° 13′ 58.7″            | PREM61854     | MN072674          | MN095319 |
| Ravenelia acaciae-nigrescentis | Senegalia nigrescens | South Africa, Mpumalanga, KNP, Orpen Rest Camp               | KR-M-0006132  | MN072686          | MN095329 |
| Ravenelia acaciae-nigrescentis | Senegalia nigrescens | South Africa, Mpumalanga, KNP, Orpen Rest Camp               | KR-M-0006133  | MN072685          | —       |
| Ravenelia acaciae-pennatulae | Vachellia pennatula | Mexico, Veracruz Province, S 19° 26′ 44.1″, W 96° 49′ 51.9″          | BPI841267     | —                 |
| Ravenelia acaciicola | Senegalia senegal var. leiorhachis | South Africa, Zwemkloof, Motse-River, S 24° 44′ 00.6″, E 31° 53′ 25.1″ | PREM61851     | —                 |
| Ravenelia acaciicola | Senegalia senegal var. leiorhachis | South Africa, Mpumalanga, Komatiport                       | PREM609999    | MN072671          | —       |
| Ravenelia acaciicola | Senegalia senegal var. leiorhachis | South Africa, Limpopo, Steelport, S 24° 44′ 00.6″, E 30° 13′ 25.1″      | PREM61861     | MN072683          | MN095326 |
| Ravenelia acaciicola | Senegalia senegal var. rostrata | South Africa, KwaZulu-Natal, S 27° 40′ 49.3″, E 32° 25′ 24.0″         | PREM61847     | MN072677          | MN095320 |
| Ravenelia acaciicola | Senegalia senegal var. rostrata | South Africa, KwaZulu-Natal, S 27° 29′ 27.7″, E 32° 11′ 37.5″          | PREM61849     | MN072676          | —       |
| Ravenelia albizzicola* | Albizia harveyi | —                                                                          | PREM40299**   | —                 |
| Ravenelia argentinica | Vachellia aroma | Argentina, Jujuy Province, Parque Nacional Calilegua                  | BPI841256     | MN072654          | MN095296 |
| Ravenelia argentinica | Vachellia aroma | Argentina, Salta Province, road to Parque del Rey                      | BPI841256     | MN072653          | MN095298 |
| Ravenelia baumiana* | Senna petersoniana | —                                                                          | PREM50553*    | —                 |
| Ravenelia baumiana* | Senna delagoensis   | —                                                                         | PREM29870*    | —                 |
| Ravenelia baumiana* | Senna singueana     | Angola                                                                     | PREM6886**/*  | —                 |
| Ravenelia cebil     | Anadenanthera sp.  | Argentina, Tucuman Province, San Pedro de Colalao                    | BPI841029     | MN072649          | —       |
| Ravenelia cohiana   | Senegalia praecox  | Argentina, Catamarca Province, Rio Las Juntas                           | BPI841185     | MG954487          | —       |
| Ravenelia dichrostachydis | Dichrostachys cinerea | South Africa, Mpumalanga, Nelspruit, SANBI                      | KR-M-0006448  | MN072690          | MN095332 |
| Ravenelia doidgeae  | Senegalia polyacantha subsp. polyacantha | South Africa, Mpumalanga, Nelspruit, Parking area of Botanical Garden | PREM60992**   | MN072672          | MN095316 |
| Ravenelia dumeti    | Senegalia brevispica | South Africa, Mpumalanga, S 25° 54′ 29.8″, E 33° 15′ 48.5″             | ME406**       | MN072680          | —       |
| Ravenelia dumeti    | Senegalia brevispica | South Africa, Mpumalanga, S 25° 54′ 29.8″, E 33° 15′ 48.5″             | PREM61877**   | MN072681          | MN095324 |
| Ravenelia echinata var. ectypa | Calliandra formosa | Argentina, Tucuman Province, San Pedro de Colalao | BPI841034 | DQ323925 | — |
| Ravenelia elephantorhizae | Elephantorrhiza burkei | South Africa, North-West Province, Mountain Sanctuary Park               | KR-M-0006449  | MN072702          | MN095341 |
| Ravenelia elephantorhizae* | Elephantorrhiza elephantina | South Africa, Gauteng, Pretoria, Koedoespoort                   | PREM8955**/*  | —                 |
| Ravenelia escharoides | Senegalia burkei    | South Africa, Mpumalanga, Blyde River Canyon                           | KR-M-00043650 | MG954480          | MN095330 |
| Ravenelia escharoides | Senegalia burkei    | South Africa, Limpopo, John Reservoir                                 | KR-M-00043652 | MG954479          | —       |
| Ravenelia escharoides | Senegalia burkei    | South Africa, Limpopo, Waterberg, Waterberg Hiking Trail               | KR-M-00043651 | MG954481          | —       |
| Fungus               | Host                        | Location                                                                 | Voucher       | GenBank accession |
|---------------------|-----------------------------|--------------------------------------------------------------------------|---------------|-------------------|
| *Ravenelia escharoides* | *Senegalia burkei*          | South Africa, Gauteng, Pretoria, Pienaars River                           | PREM29918*    | —                 |
| *Ravenelia escharoides* | *Senegalia burkei*          | South Africa, Gauteng, Pretoria, Near Hartebeestpoort Dam                 | PREM27586*    | —                 |
| *Ravenelia escharoides* | *Senegalia burkei*          | South Africa, Limpopo, Pruizen (near Mokopane)                           | PREM534*//**  | —                 |
| *Ravenelia evansii*  | *Vachellia robusta* subsp. robusta | South Africa, KwaZulu-Natal, False Bay                                      | PREM61209     | MG945991         |
| *Ravenelia evansii*  | *Vachellia robusta* subsp. robusta | South Africa, North-West Province, Groot Marico, River Still Guest Farm | PREM61208     | MG945992         |
| *Ravenelia evansii*  | *Vachellia davyi*           | South Africa, KwaZulu-Natal, S 27° 19′ 27.2″, E 31° 26′ 39.6″           | PREM61224     | MN275523         |
| *Ravenelia evansii*  | *Vachellia davyi*           | South Africa, Mpumalanga, S 25° 23′ 41.8″, E 31° 05′ 08.0″              | PREM61845     | MG946000         | MN095322 |
| *Ravenelia evansii*  | *Vachellia sieberiana* var. woodii | South Africa, KwaZulu-Natal, S 28° 41′ 30.1″, E 31° 43′ 16.9″           | PREM61028     | MG945996         |
| *Ravenelia evansii*  | *Vachellia hebeclada*       | South Africa, North-West Province, Leeuwvfontein Farm                    | PREM61227     | MG946001         |
| *Ravenelia evansii*  | *Vachellia swazica*         | South Africa, Mpumalanga, Marloth Park, S 25° 20′ 44.4″, E 31° 46′ 26.1″ | PREM61868     | MG945995         |
| *Ravenelia evansii*  | *Vachellia evansii*         | South Africa, KwaZulu-Natal, S 24° 52′ 52.6″, E 31° 23′ 40.3″           | ME384         | MG946003         |
| *Ravenelia evansii*  | *Vachellia luederitzii* var. retinens | South Africa, KwaZulu-Natal, S 27° 19′ 30′ 57.3″, E 32° 00′ 39.1″       | PREM61864     | MG945993         | MN095321 |
| *Ravenelia evansii*  | *Vachellia exuvialis*       | South Africa, Mpumalanga, S 25° 02′ 33.4″, E 31° 06′ 10.3″             | PREM50751     | —                 |
| *Ravenelia evansii*  | *Vachellia borleae*         | South Africa, KwaZulu-Natal, S 28° 39′ 13.8″, E 30° 26′ 59.2″          | KR-M-0006620* | —                 |
| *Ravenelia evansii*  | *Vachellia hieronymi*       | South Africa, Mpumalanga, S 25° 02′ 33.4″, E 31° 06′ 10.3″             | KR-M-0006444* | —                 |
| *Ravenelia evansii*  | *Vachellia sieberiana*      | South Africa, KwaZulu-Natal, S 25° 02′ 33.4″, E 31° 06′ 10.3″          | PREM61876     | MN072682         | MN095325 |
| *Ravenelia evansii*  | *Vachellia evansii*         | South Africa, Mpumalanga, 60 km south of Nelspruit                       | PREM50751     | —                 |
| *Ravenelia evansii*  | *Calpurnea aurea*           | South Africa, KwaZulu-Natal, Camperdown Game Farm                        | KR-M-0006450  | MN072691         | MN095333 |
| *Ravenelia glabra*   | *Calpurnea sylvatica*       | South Africa, KwaZulu-Natal, S 27° 19′ 27.2″, E 31° 26′ 39.6″          | PREM61845     | MG946000         | MN095322 |
| *Ravenelia glabra*   | *Calpurnea sylvatica*       | South Africa, KwaZulu-Natal, S 25° 02′ 33.4″, E 31° 06′ 10.3″          | PREM61845     | MG946000         | MN095322 |
| *Ravenelia halsei*   | *Senegalia ataxacantha*     | South Africa, KwaZulu-Natal, S 28° 39′ 13.8″, E 30° 26′ 59.2″          | CR-M-0006620* | —                 |
| *Ravenelia halsei*   | *Senegalia ataxacantha*     | South Africa, KwaZulu-Natal, close to Oribi Gorge                       | PREM61855     | MG954484         |
| *Ravenelia halsei*   | *Senegalia ataxacantha*     | South Africa, KwaZulu-Natal, S 28° 39′ 13.8″, E 30° 26′ 59.2″          | PREM61867     | MN072682         | MN095325 |
| *Ravenelia halsei*   | *Senegalia ataxacantha*     | South Africa, KwaZulu-Natal, S 28° 39′ 13.8″, E 30° 26′ 59.2″          | PREM50751     | —                 |
| *Ravenelia halsei*   | *Senegalia ataxacantha*     | South Africa, KwaZulu-Natal, Ndwedwe                                       | PREM30117**   | —                 |
| *Ravenelia havanensis* | *Enterolobium cyclocarpum*  | Mexico, Chiapas, N 15° 34′ 39.6″ W 92° 09′ 23.3″                         | BPI871922     | MN275524         | MN095301 |
| *Ravenelia hermosa*  | *Leucaena sp.*              | Colombia, BPI1107966                                                      | BPI872308     | MN072657         | MN095304 |
| *Ravenelia Hernandezii* | *Senegalia tenuifolia*    | Costa Rica, Guanacaste Province, N 10° 50′ 42.1″, W 85° 36′ 27.0″       | BPI872308     | MG954488         | MN095304 |
| *Ravenelia hieronymi* | *Vachellia caven*           | Argentina, Tucuman Province, S 27° 40′ 38.0″, W 65° 46′ 49.4″           | BPI841165     | MN072651         |
| *Ravenelia holwayi*  | *Prospis glandulosa*        | USA, Texas, Del Rio, North Hill/20 Rico                                    | BPI871145     | MN072656         | MN095300 |
| *Ravenelia holwayi*  | *Prospis sp.*               | USA, Texas, San Ygnacio, bird sanctuary along Rio Grande                  | KR-M-0006618  | MN072692         |
| Fungus                  | Host                  | Location                                                                 | Voucher   | GenBank accession |
|------------------------|-----------------------|--------------------------------------------------------------------------|-----------|------------------|
| Ravenelia aff. indigoferae | Indigofera sp.        | South Africa, KwaZulu-Natal, S 27° 28' 32.9", E 32° 35' 44.2"           | PREM61061 | MN072664 MN095308 |
| Ravenelia inornata    | Vachellia natalitia  | South Africa, KwaZulu-Natal, St. Lucia                                 | KR-M-0006613 |                |
| Ravenelia inornata    | Vachellia karroo      | South Africa, Western Cape, Entrance Montagu Nature Reserve             | PREM60135 | MN072684 MN095310 |
| Ravenelia inornata*   | Vachellia karroo      | South Africa, Eastern Cape, Kentani                                     | PREM2368* |                |
| Ravenelia inornata*   | Vachellia karroo      | South Africa, Eastern Cape, Kentani                                     | PREM2541* |                |
| Ravenelia inornata*   | Vachellia karroo      | South Africa, Eastern Cape, Somerset East                               | PREM20734* |                |
| Ravenelia macowaniana | Vachellia natalitia  | South Africa, Limpopo, Steelport, S 24° 08’ 52.4”, E 29° 02’ 21.9”     | PREM61875 | MG946014 MN095327 |
| Ravenelia macowaniana | Vachellia karroo      | South Africa, Limpopo, Sekhukhuneland, Winterveld Mine                 | PREM61222 | MG946007 MN095331 |
| Ravenelia macowaniana | Vachellia karroo      | South Africa,Mpumalanga, Nelspruit                                     | PREM61218 |                |
| Ravenelia macowaniana | Vachellia karroo      | South Africa, Limpopo, Winterveld Mine                                  | PREM61222 | MG946007 MN095331 |
| Ravenelia macrocarpa  | Senna subulata        | Argentina, Catamarca Province, S 28° 03’ 44.1”, W 65° 35’ 19.4”         | BPI841195 | DQ323926         |
| Ravenelia minima*     | Albizia gummifera     | South Africa, KwaZulu-Natal, Durban                                     | PREM30779 |                |
| Ravenelia minima*     | Albizia gummifera     | South Africa, KwaZulu-Natal, SANBI                                      | PREM10697 |                |
| Ravenelia modesta     | Vachellia gerrardii   | South Africa, Mpumalanga, Barberton, S 25° 45’ 37.0’, E 31° 03’ 10.4”  | PREM61878 |                |
| Ravenelia modesta     | Vachellia rehmanniana | South Africa, Limpopo, Louis Trichard                                  | PREM60991* |                |
| Ravenelia modesta     | Vachellia rehmanniana | South Africa, Limpopo, S 23° 57’ 40.3”, E 29° 29’ 17.1”               | PREM60785* |                |
| Ravenelia modesta     | Vachellia rehmanniana | South Africa, Limpopo, S 23° 57’ 40.3”, E 29° 29’ 17.1”               | PREM60791 | MN072660         |
| Ravenelia modesta     | Vachellia luederitzii var. retinens | South Africa, Gauteng, 31 miles North of Pretoria | PREM34572* |                |
| Ravenelia modesta     | Vachellia luederitzii | South Africa, Gauteng, Pienaars River                                   | PREM30110** |                |
| Ravenelia modesta     | Vachellia hebclada    | South Africa, Gauteng, close to Groto Marico                             | KR-M-0006445** | MN072697 |
| Ravenelia moloto      | Senegalia erubescens  | South Africa, North-West Province, Groot Marico                          | PREM61896** | MN072700 MN095339 |
| Ravenelia moloto      | Senegalia erubescens  | South Africa, North-West Province, close to Madikwe                      | PREM61890** | MN072701 MN095340 |
| Fungus                          | Host                        | Location                                                                 | Voucher                  | GenBank accession |
|--------------------------------|-----------------------------|--------------------------------------------------------------------------|--------------------------|-------------------|
| Ravenelia molopa               | Senegalia galpinii          | South Africa, Mpumalanga, Nelspruit, S 28° 29' 50.5", E 30° 59' 27.3" | PREM1879**               | MN072679 MN095323 |
| Ravenelia modjadji             | S. polyacantha subsp.       | South Africa, Limpopo, Louis Trichard                                    | PREM1023**               | MN072667 MN095311 |
| Ravenelia modjadji             | campylacantha               | South Africa, Limpopo, Louis Trichard                                    | KR-M-0006422**           | MN072668 MN095312 |
| Ravenelia modjadji             | campylacantha               | South Africa, Limpopo, S 23° 37' 55.1", E 03° 12' 08.0"                | PREM60788**              | MN072661 MN095305 |
| Ravenelia natalensis           | Vachellia karroo            | South Africa, KwaZulu-Natal, Winkelspruit                                | PREM2514*                | —                 |
| Ravenelia natalensis           | Vachellia karroo            | Australia, Northern Territory, Timber Creek                               | PREM1935**               | —                 |
| Ravenelia neocaledoniensis     | Vachellia farnesiana        | Australia, Western Australia, Kununurra                                  | BRIP56907                | KJ862347 —       |
| Ravenelia neocaledoniensis     | Vachellia farnesiana        | Australia, Western Australia, Kununurra                                  | BRIP56908                | KJ862348 KJ862460 |
| Ravenelia ornata               | Abrus laevigatus            | South Africa, Gauteng, Pretoria, Botanical Garden                        | KR-M-0006447             | MN072687 —       |
| Ravenelia ornata               | Abrus sp.                   | South Africa, Mpumalanga, East of Nelspruit, Pullen Farm                 | KR-M-0006617             | MN072673 MN095317 |
| Ravenelia peglerae*            | Senegalia caffra            | South Africa, Eastern Cape, Kentani                                     | PREM5626*                | —                 |
| Ravenelia peglerae*            | Senegalia caffra            | South Africa, Eastern Cape, Kentani                                     | PREM2331**               | —                 |
| Ravenelia peglerae*            | Senegalia caffra            | South Africa, Eastern Cape, Butteworth                                   | PREM2544**               | —                 |
| Ravenelia pienaarii*           | Senegalia caffra            | South Africa, Gauteng, Garstfontein                                     | PREM6658**               | —                 |
| Ravenelia pienaarii*           | Senegalia caffra            | South Africa, Gauteng, Garstfontein                                     | PREM5627**               | —                 |
| Ravenelia pienaarii            | Senegalia caffra            | South Africa, Mpumalanga, S 25° 34' 24.2", E 31° 10' 51.7"             | KR-M-0045114             | MG954483 —       |
| Ravenelia pienaarii            | Senegalia caffra            | South Africa, Gauteng, Ezemvelo Nature Reserve                          | KR-M-0006442             | MN072699 MN095338 |
| Ravenelia pienaarii            | Senegalia caffra            | South Africa, Gauteng, Ezemvelo Nature Reserve                          | PREM61892                | MG954482 MN095337 |
| Ravenelia piepenbringiae       | Senegalia hayesii           | Panama, Chiriquí Province, Los Algarrobos, N 8° 29' 45.31", W 82° 25' 56.24" | MP5157                   | MG954489 MN095328 |
| Ravenelia platensis            | Erythrina cristagalli       | Argentina, Buenos Aires Province, La Plata                               | BP1841204                | MN072652 MN095297 |
| Ravenelia pretoriensis          | Vachellia natalitia         | South Africa, KwaZulu-Natal, Charters Creek                             | PREM61021                | MN072665 MN095309 |
| Ravenelia pretoriensis*         | Vachellia karroo            | South Africa, Western Cape, Jan Marais Nature Reserve                   | PREM60134*               | —                 |
| Ravenelia pretoriensis*         | Acacia sp.                  | South Africa, Gauteng, Garstfontein                                     | PREM11376**              | —                 |
| Ravenelia pretoriensis          | Vachellia karroo            | South Africa, Gauteng, Roodeplaat, ARC, Experimental Farm               | PREM60809                | MN072659 —       |
| Ravenelia pretoriensis          | Vachellia karroo            | South Africa, Gauteng, Roodeplaat, ARC, Experimental Farm               | KR-M-0006615*            | —                 |
| Ravenelia sp.                  | Vachellia collinsii         | Costa Rica, Guanacaste Area de Conservación Guanacaste                   | BPI872307                | MN0926775 —     |
| Ravenelia stictica             | Mundulea sericea            | South Africa, Gauteng, Pretoria                                        | PREM60784                | MN072663 MN095307 |
| Ravenelia stictica             | Mundulea sericea            | South Africa, Gauteng, Pretoria, hill opposite to Freedom Park          | ME138                    | MN072658 —       |
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 94 °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 CO3J sequences, the primer pair CO3-R1 and CO3-F1 (Viale 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 Sequencer 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 CO3J sequence data using MAFFT v7.154b (Katoh 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 CO3J 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 CO3J 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 CO3J 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.
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 aecia 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 confluenting rings, subepidermal, erumpent; paraphyses 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.
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, echinate, aculei approximately 1 μm in height, germ pores 5–6, equatorially arranged. Telia replacing uredinia, chestnut brown to dark brown; teliospores cinna- mon 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. 2 Cladogram based on a phylogenetic reconstruction using BI showing character states linked to species. Terminal branches were collapsed.
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.
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 &...
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 bilaminate 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 (WM3517), paratype KR-M-0006416; 15 April 2009, W. Maier (WM3516), 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. acaciae-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. Ebingerhaus, W. Maier, & Begerow sp. nov. (Fig. 6a–g)
MycoBank MB831072

Etymology: Name refers to the occurrence of this rust fungus on *Senegalga 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. **e** 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. acaciae-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. Ebingerhaus, W. Maier, & Begerow sp. nov. (Fig. 6a–g)
MycoBank MB831072

Etymology: Name refers to the occurrence of this rust fungus on *Senegalga 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** 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. acaciae-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. Ebingerhaus, W. Maier, & Begerow sp. nov. (Fig. 6a–g)
MycoBank MB831072

Etymology: Name refers to the occurrence of this rust fungus on *Senegalga galpinii*, which is known as Molopa in the local Northern Sotho language.
Type: South Africa, Mpumalanga, Nelspruit, S 28° 29' 50.5", E 30° 59' 27.3" on leaves of Senegalia galpinii (Burtt 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 probasidal cells across, single probasidal cells (27)31–33(36) × (12)14–18(25) μm, cell wall thickened at the top side of the spore and here distinctly bilaminate 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 probasidal 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,
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 × (5)8–10 μm, lateral cell wall 1–1.5 μm thick, at apex 1.5–3 μm; urediniospores ovoidal to ellipsoid, sometimes globose, light brown, (20)22–28(36) × 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 erumpent; paraphyses peripheral, cylindrical to 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) × 6–10(13) μm, cell wall distinctly thickened apically, 3–9 μm and 0.7–1.3 μm laterally; urediniospores light brown, ovoidal to ellipsoid or of irregular shape, (21)24–28(30) × (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, (80)87–95(104) μm in diameter with 5–8 probasidial cells across, single probasidial cells (21)24–28(33) × (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 & Ebinguer subsp. campylacantha (Hochst. ex. A. Rich.) Kyal. & Boattwr., 21 February 2015, M. Ebinghaus (ME418), holotype PREM61860.

Spermatia 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, ellipsoid, sometimes ovoidal, often somewhat curved, (23)25–28(32) × 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 ellipsoid, 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) × 6–10(13) μm, cell wall distinctly thickened apically, 3–9 μm and 0.7–1.3 μm laterally; urediniospores light brown, ovoidal to ellipsoid or of irregular shape, (21)24–28(30) × (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) × (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 Senegalalia polyacantha subsp. campylacantha, 4 April 2013, M. Ebinghaus (ME240), paratype KR-M-0006422; 4 April 2013, M. Ebinghaus (ME239), paratype PREM61023; S 23° 37’ 03.9” E 28° 40’ 47.9”, 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 acia 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
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 accia 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) μm; paraphyses intrasoral, distinctly capitate, pedicel transparent and light brown at the thickened top, total length (22)24–29(35) μm, capitulum 10–15 × (9)11–15 μm, pedicel 3–5 μm in width, cell wall laterally 1.1–1.5–1.8 μm but apically thickened, (2)5–9 μm; urediniospores broadly ovoidal, light ochraceous brown, echinulate, 12–16 × 9–12 μm, spore wall evenly 1–1.5 μm, 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.
Notes: Only the uredinial 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
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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.

Spermogonia andaecia 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, some-
times confluent, 790–1000 μm, subepidermal, erumpent; pa-
raphyses numerous, mostly peripheral but a few intrasoral, cylin-
drical, 46–54 × 6–8 μm, light red-brown; urediniospores
ovoidal to globose, ocreaceous 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
bilaminate 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 arabicae 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. tandoni that was described on Senegalia catechu (Sydow
et al. 1937) but both species can be distinguished by predom-
inantly hypophyllous uredinia in R. acacia-acaciae 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. acacia-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. acacia-arabicae compared with
two rows of four germ pores in R. tandonii. The teliospores of
Ravenelia acacia-arabicae can be distinguished by having
6–9 blunt aculei per probasidial cell in contrast to 3–6 ver-
rucose papillae in R. tandonii.

Phylogenetically, R. acacia-arabicae is most closely
related to R. evansii with which it shares major teliospore
character traits such as size and its spinescent ornamenta-
tion. 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 tis-
sues while all collections of R. acacia-arabicae in South
Africa lack the aecial stage.

Ravenelia acaciicola (as acaciola) 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”,
February 2015, M. Ebinghaus (ME392), PREM61847; brown and clavate paraphyses in a single specimen were observed in our collections. We also observed light in an equatorial position, while 5 to light brown, (57)84 μm, subglobose to ovoidal, often tapered towards the basis, ochraceous brown, erumpent; urediniospores ovoidal, often tapered towards 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 multihyal.

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 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.

Spermatogonia andaecia 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, erumpent; urediniospores ochraceous brown, subglobose to ovoidal, smooth, swelling in water but only slightly in lactophenol solution, number of cysts equal to the number of the probasidial cells; pedicel multihyal.

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. acaciae-nigrescentis. However, in the present study, a small number of peripherals 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
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.

Spermogonia 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) μm, 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 μm in length and 3 μm wide, transparent; paraphyses towards the sorus margin clavate and

![Fig. 12](image1.png)  
*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 μm; e = 50 μm; d = 10 μm; e = 40 μm

![Fig. 13](image2.png)  
*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 μm; d = 3 μm; e–f = 20 μm
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 rachis, 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 bicuspidalis (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 bicuspidalis (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 a paraphysate or having only few paraphyses. This is in contrast to R. mesilliana in which the sori originate subcuticulately and bear numerous intrasoral paraphyses of variable size and shape. Ravenelia mesilliana was originally described on Senna baumiioides (Gray) Irwin & Barneby. Consequently, Sena bicuspidalis is a new host record for this rust fungus. Ravenelia mesilliana was most likely introduced into South Africa on Sena bicuspidalis, which is an invasive species in this country (Foxcroft et al. 2003). Another rust, R. baumiana is known on Sena bicuspidalis 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. gilletiae 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 acia not seen. Uredinia amphigenous but predominantly hypophyllous, subcuticular, surrounded by the tor 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 (24–6(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 uredinal stage of R. modesta based on a specimen (PREM34572) collected from V. luederitzii (Doidge 1948) and which is also...
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 Burtt 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 gerrardi* 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, Ndwwede, 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, apophysate; uredinospores 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, (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 bilaminate 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 multihymphal.

**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 sorus. We further observed 10–12 scattered or bizonate germ pores in the uredinospores, 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* 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)
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 in length, yellow white; aeciospores subhyaline to pale yellow, rectangular to subglobose, 23–27 × 19–22 μ, spore wall densely verrucose, 1–3 μ in height; telia minute, scattered or in small groups, mixed with the aecia, sometimes telia developing within or confluently 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 in diameter with 8–12 single probasial cells across, probasial cells single layered, (32)36–50 (52) × (12)14–17(19) μ, cell wall thickened at the top side of the spore, (3)5.5–8.5(11) μ, each probasial cell bearing a variable number (up to seven) small verrucae, 1–3 μ in height; cysts pendent, hyaline, smooth, number of cysts equal to the number of the probasial 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, Ravenelia 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 Ravenelia inornata in this region might be linked to these environmental factors.

This species shares the hosts V. natalitia and V. karroo with Ravenelia pretoriensis and Ravenelia macowaniana. Mixed infections by these two species were thus occasionally encountered even on a single leaflet. Co-occurrence of Ravenelia inornata with Ravenelia pretoriensis may lead to confusion because the teliospores of both rusts bear verrucose ornamentations. However, the teliospores differ distinctly in size with teliospores of Ravenelia pretoriensis mostly measuring 70–95 μ, while the teliospores of Ravenelia inornata range between 104 and 162 μ (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 Ravenelia 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 tissues ................................. 2
- 1*. No galls/malformations visible, also no old galls ...... 6
- 2. Galls/malformations caused by aecia .............................. 3
- 2*. Galls caused by aecia bearing echinulate aeciospores; reported on S. polyacantha subsp. campylacantha......................................R. modjadji

- 3. Teliospores smooth or with rare verrucae ......................... 4
- 3*. Teliospores spinescent ................................................. R. evansi
- 4. Teliospores < 85 μm ....................................................... 5
- 4*. Teliospores up to 130 μm; reported on Vachellia karroo, V. natalitia, V. permixta......... R. macowaniana

- 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 Caesalpinioideae ................................................. 10

- 7. No urediniospores; peridial 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” pattern ............. 9

- 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. gerhardii.............................................................. R. modesta

- 10. On Senegalia ..................................................................... 18
- 10*. On a different host plant genus .............................. 11

- 11. Teliospores smooth or with only few ornamentes...... 12
- 11*. Teliospores distinctly ornamented.............................. 15

- 12. Teliospores smooth, sometimes with single papillae, up to 3–4 μm; reported on Senna.................. R. mesiliana
- 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. elephantorrhiza

- 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

- 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. indigofera

- 17**. Teliospores verrucose, up to 2 μm long; reported on Tephrosia................................................. R. tephrosia

- 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. pienaarii

- 23. Teliospores spinescent; only few paraphyses present in uredinia; reported on S. nigrescentis...... R. acaciae-nigrescentis

- 23*. Teliospores smooth; uredinia with numerous intrasoral paraphyses; reported on S. senegal (both subspecies)........................................... R. acaciocola

- 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 Paleotropic species while five of these
groups consist of Neotropic and Paleotropic 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. Rugs 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 Mimosoideae. Further rust species with this character have been reported from other host genera within the Mimosoideae but mostly on Albizia including *R. albizzae-zygiae, R. albizicola, 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 intrasporal and distinctly capitata 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 a paraphysate uredinia, while *R. elephantorrhiza* (on Elephantorrhiza, Mimosoideae), *R. macrocarpa*, and *R. mesilliana* (both on Senna, Caesalpinioideae) have intrasporal 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. spicigerae* (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 phylagenetic group. Unfortunately, fresh material of *R. epiphylla* was not available for inclusion in the molecular phylagenetic 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 uredinoid 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. hirnemosa* 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 hieronymyi* and the closely related *R. evansii, R. macowaniana*, and *R. xanthophloeus* by producing telia subsequently on aecial galls in the case of *R. hieronymyi* (Hernández and Hennen 2003, Hennen et al. 2005). Hennen et al. (2005) suggested that *R. hieronymyi* could represent a demicyclic rust as pedicellate urediniospores were never observed. This is in contrast to the macrocyclic *R. evansii, R. macowaniana*, and *R. xanthophloeus* that induce aecial galls but in which the uredial and telial stages are always spatially separated from the aecia (Doige 1939; Ebbinghaus et al. 2018). While the aeciospores of these rusts obviously re-infect the host, the aeciospores of *R. hieronymyi* were suggested to be non-functional (Hernández and Hennen 2003; Hennen et al. 2005). Similar to *R. hieronymyi*, 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 phylagenetic analyses. In the acacia rust *Atelocauda koae* 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 phylagenetic 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 phylagenetic 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 phylagenetic 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* (Shatock 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. cohniana, R. piepenbringiae*, and *R. hernandezii*; compare Ebbinghaus 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.
The Paleotropically distributed rusts in clade I correspond to this host lineage and reasonably explains the Asian-African distribution of *R. acacidicola* (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. polyantha* subsp. *campylacantha*, *S. polyantha* subsp. *polyantha*, and *S. erubesens*, 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. transvaalenensis* 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 unial 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 could 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. evansi* 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 Zvetko 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 spermata and large numbers of aeciospores could also contribute to the wide host range observed in *R. macowaniana* and *R. evansi*, 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                                                                 |
|---------------------------------|----------------------------------------------------------------------|
| *Ravenelia acaciae-arabicae*    | *Vachellia nilotica* subsp. *kraussiana* (Benth.) P.J.H.Hurter & Mabb. |
| *Ravenelia acaciae-nigrescentis* | *Senegalia nigrescens* (Oliv.) P.J.H. Hurter                        |
| *Ravenelia acaciicola*          | *Senegalia senegal* var. *leiorhachis* (L.) Britton                |
| *Ravenelia deformans*           | *Senegalia senegal* var. *rostrata* (L.) Britton                    |
| *Ravenelia dichrostachydis*     | *Dichrostachys cinerea* (L.) Wight & Arn.                          |
| *Ravenelia doidgeae*            | *Senegalia polyacantha* subsp. *polyacantha* (Willd.) Seigler & Ebinger |
| *Ravenelia dumeti*              | *Senegalia brevispica* (Benth.) Kyal. & Boatwr.                    |
| *Ravenelia elephantorhizae*     | *Elephantorrhiza elephantina* (Burch.) Skeels, E. burkei Benth.     |
| *Ravenelia escharoides*         | *Senegalia burkei* (Benth.) Kyal. & Boatwr.                        |
| *Ravenelia evansi*              | *Vachellia borleae* (Burt Davy) Kyal. & Boatwr.                    |
|                                 | *Vachellia davii* (N.E.Br.) Kyal. & Boatwr.                        |
|                                 | *Vachellia exuvialis* (Verdoorn) Kyal. & Boatwr.                   |
|                                 | *Vachellia hebeclada* subsp. *hebeclada* (DC.) Kyal. & Boatwr.     |
|                                 | *Vachellia luederitzii* var. *retinens* (Sim) Kyal. & Boatwr.      |
|                                 | *Vachellia robusta* subsp. *robusta* (Burch.) Kyal. & Boatwr.      |
|                                 | *Vachellia sieberiana* subsp. *woodii* (Burt Davy) Kyal. & Boatwr. |
|                                 | *Vachellia swazica* (Burt Davy) Kyal. & Boatwr.                    |
|                                 | *Vachellia gerrardii* (Benth.) P.J.H.Hurter                       |
| *Ravenelia glabra*              | *Calpurnia sylvatica* E. Mey.                                      |
| *Ravenelia halsei*              | *Senegalia ataxacantha* (DC.) Kyal. & Boatwr.                      |
| *Ravenelia aff. indigoferae*    | *Indigofera* sp. L.                                                |
| *Ravenelia inornata*            | *Vachellia karroo* (Hayne) Banfi & Galasso                        |
| *Ravenelia macowaniana*         | *Vachellia natalitia* (E.Mey.) Kyal. & Boatwr.                    |
|                                 | *Vachellia karroo* (Hayne) Banfi & Galasso                        |
|                                 | *Vachellia natalitia* (E.Mey.) Kyal. & Boatwr.                    |
|                                 | *Vachellia permixa* (Burt Davy) Kyal. & Boatwr.                   |
| *Ravenelia mesilliana*          | *Senna bicapsularis* (L.) Roxb.                                    |
| *Ravenelia minima*              | *Albizia adianthifolia* (Schumach.) W.F.Wight                     |
| *Ravenelia modesta*             | *Vachellia gerrardii* (Benth.) P.J.H.Hurter                       |
|                                 | *Vachellia luederitzii* var. *retinens* (Sim) Kyal. & Boatwr.      |
|                                 | *Vachellia rehmanniana* (Schinz) Kyal. & Boatwr.                   |
| *Ravenelia modjadji*            | *Senegalia polyacantha* subsp. *campylacantha* (Hochst. ex. A.Rich.) Kyal. & Boatwr. |
| *Ravenelia molopa*              | *Senegalia galpinii* (Burt Davy) Seigler & Ebinger                 |
| *Ravenelia moloto*              | *Senegalia erubescens* (Welw. ex Oliv.) Kyal. & Boatwr.            |
| *Ravenelia natalensis*          | *Vachellia karroo* (Hayne) Banfi & Galasso                        |
| *Ravenelia ornata*              | *Abrus precatorius* L.                                             |
| *Ravenelia peglerae*            | *Senegalia caffra* (Thunb.) P.J.H.Hurter & Mabb.                  |
| *Ravenelia pienaarri*           | *Senegalia caffra* (Thunb.) P.J.H.Hurter & Mabb.                  |
| *Ravenelia pretoriensis*        | *Vachellia karroo* (Hayne) Banfi & Galasso                        |
| *Ravenelia spinifera*           | *Senegalia mellifera* subsp. *detinens* (Burch.) Kyal. & Boatwr.   |
| *Ravenelia stictica*            | *Mundulea sericea* (Willd.) A. Chev.                             |
| *Ravenelia tephrosiae*          | *Tephrosia heckmanniana* Harms                                     |
|                                 | *Tephrosia macropoda* var. *macropoda* (E. Mey) Harv.              |
| *Ravenelia transvaalensis*      | *Senegalia mellifera* subsp. *detinens* (Burch.) Kyal. & Boatwr.   |
| *Ravenelia woodii*              | Fabaceae undetermined                                             |
| *Ravenelia xanthophloaeae*      | *Vachellia xanthophloea* (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 (Doigie 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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