Ravenelia piepenbringiae and Ravenelia hernandezii, two new rust species on Senegalia (Fabaceae, Mimosoideae) from Panama and Costa Rica

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Abstract

Two new rust species, Ravenelia piepenbringiae and R. hernandezii (Pucciniales) on Senegalia spp. (Fabaceae) are described from the Neotropics (Panama, Costa Rica). A key to the species on neotropical Senegalia spp. is provided. Molecular phylogenetic analyses based on 28S rDNA sequence data suggest that the representatives of Senegalia rusts distributed in the neotropics evolved independently from species known from South Africa. This is further supported by the teliospore morphology, which is characterised by uniseriate cysts in the neotropical Senegalia rusts and contrasting multiseriate cysts in the paleotropical Ravenelia species that infect this host genus.

Keywords

Senegalia rust, rust fungi, Phylogeny, Taxonomy

Introduction

With more than 200 described species, the genus Ravenelia is amongst the most speciose genera within the rust fungi (Pucciniales) (Cummins and Hiratsuka 2003). In the tropics and subtropics, members of this genus parasitise a diverse range of hosts of the legume family (Fabaceae), including Caesalpinioideae, Faboideae and Mimosoideae. Numerous species of Ravenelia are known from the neotropics, mostly from Mexico.
(Cummins 1978), Brazil (Dianese et al. 1993, Rezende and Dianese 2001; Hennen et al. 2005) and Argentina (Hernández and Hennen 2002).

However, in the neotropics, occurrence of *Ravenelia* species is poorly known in other countries such as Panama and Costa Rica. Preliminary checklists of abundant fungi in Central America report only a single species of *Ravenelia* in Panama (*R. entadae*) (Piepenbring 2006) and 18 species of *Ravenelia* in Costa Rica, respectively (Berndt 2004).

Specimens of a rust fungus on *Senegalia hayesii* (Benth.) Britton and Rose were collected in Panama in 2013. Another species of *Ravenelia* was discovered through the analysis of herbarium specimens of the U.S. National Fungal Collections (BPI) on *Senegalia tenuifolia* (L.) Britton and Rose. On the basis of morphological and molecular data, these two specimens were herein analysed and described respectively as *Ravenelia piepenbringiae* and *R. hernandezii*.

**Material and methods**

**Light- and electron microscopic investigations**

Spores representing different spore stages were scraped from the leaf surfaces of dried herbarium specimens and stained in lactophenol solution on microscope slides. For the analysis of soral structures, hand sections were prepared under a stereomicroscope. Samples were microscopically studied with a Zeiss Axioskop Light Microscope and Zeiss AxioCam. Cellular structures were measured using ZEN 2 (Blue Edition) Software. Infected leaflets of the herbarium specimens were mounted on double-sided sticky carbon tape on metal stubs and coated with gold in a Sputtercoater BAL-TEC SCD OSO (Capovani Brothers Inc, USA). Superficial ornamentation of spores was investigated using a ZEISS Sigma VP scanning electron microscope at the Ruhr-University Bochum, Germany.

**DNA extraction and PCR**

Genomic DNA extractions were carried out using the INNUPrep Plant DNA Kit (Analytic Jena, Germany) according to the manufacturer’s protocol. Spores were milled in a Retsch Schwingmühle MM2000 (F. Kurt Retsch GmbH &Co KG, Haan, Germany), using two steel beads and liquid nitrogen in three consecutive cycles. An amount of 40 ml of lysis buffer was added to loosen spore remnants by vortexing from the Eppendorf tube lid, followed by centrifuging in a final cycle. Polymerase chain reaction (PCR) of 28S rDNA was conducted using the Taq-DNA-Polymerase Mix (PeqLab, Erlangen, Germany). To compensate for small amounts of spores applied for DNA extractions up to 5ml of genomic DNA extraction were used as the template in 25 ml reactions. Primer pair LR0R (Moncalvo et al. 1995) and LR6 (Vilgalys and Heester 1990) were used to obtain sequences of the 28S rDNA, with thermal cycling conditions set at 96 °C (3 min) followed by 40 cycles of 30 sec at 95 °C, 40 sec at 49 °C and 1 min at 72 °C, with a final extension for 7 min at 72 °C. PCR products, which
Table 1. Specimens analysed in this study, including GenBank Accession Numbers. Published references are given for sequences obtained from GenBank. †: BPI (U.S. National Fungus Collections, USA); ‡: KR (Staatliches Museum für Naturkunde Karlsruhe, Germany); $: PREM (Plant Protection Research Institute, South Africa); |: Z+ZT (Universität Zürich, Switzerland and Eidgenössische Technische Hochschule Zürich, Switzerland); ¶: BRIP (Department of Agriculture and Fisheries, Australia); #: PMA (Universidad de Panamá, Panama).

| Voucher     | Species                        | Substrate                  | Reference        | Origin            | LSU GenBank   |
|-------------|--------------------------------|----------------------------|------------------|-------------------|---------------|
| BPI841185†  | Ravenelia cohniana Hennek.     | Senegalia ptaeoce (Grieseb.) Siegler & Ebinger | This work        | Catamarca Province, Argentina | MG954487     |
| BPI841034†  | Ravenelia echinata var. eczpt (Arthur & Holw.) Cummins | Calliandra formosa (Kunth) Benth. | Scholler and Aime, 2006 | Tucuman Province, Argentina | DQ323925*    |
| KR-M-0043650‡ | Ravenelia escharoides Syd.     | Senegalia burkei (Benth.) Kyal. & Boarwright | This work        | Mpumalanga, South Africa | MG954480     |
| KR-M-0043651‡ | Ravenelia escharoides Syd.     | Senegalia burkei (Benth.) Kyal. & Boarwright | This work        | Limpopo, South Africa | MG954481     |
| KR-M-0043652‡ | Ravenelia escharoides Syd.     | Senegalia burkei (Benth.) Kyal. & Boarwright | This work        | Limpopo, South Africa | MG954482     |
| PREM61223$  | Ravenelia evansii Syd.         | Vachellia sieberiana (Burtt Davy) Kyal. & Boarwtr. | This work        | KwaZulu-Natal, South Africa | MG954998     |
| PREM61228$  | Ravenelia evansii Syd.         | Vachellia sieberiana (Burtt Davy) Kyal. & Boarwtr. | This work        | KwaZulu-Natal, South Africa | MG954998     |
| PREM61855$  | Ravenelia habezi Doidge        | Senegalia ataxacantha (D.C) Kyal. & Boarwright | This work        | Mpumalanga, South Africa | MG954484     |
| Z+ZT RB5788| Ravenelia basivanensis Arthur  | Enterolobium centorvisilicuuum (Vell.) Morong | Aime, 2006       | Tucuman Province, Argentina | DQ354557*    |
| BPI872308†  | Ravenelia hernandezii          | Senegalia tenuifolia (L) Briton & Rose | This work        | Guanacaste, Costa Rica | MG954488     |
| PREM61222$  | Ravenelia macowaniana Pa&zschke | Vachellia karroo (Hayne) Bani & Galasso | This work        | Limpopo Province, South Africa | MG960007     |
| PREM61210$  | Ravenelia macowaniana Pa&zschke | Vachellia karroo (Hayne) Bani & Galasso | This work        | Eastern Cape Province, South Africa | MG960004     |
| PREM61221$  | Ravenelia macowaniana Pa&zschke | Vachellia karroo (Hayne) Bani & Galasso | This work        | North-West Province, South Africa | MG960005     |
| BPI841195†  | Ravenelia macrocarpa Syd. & Syd. | Senna subulata (Grieseb.) H.S Irwin & Barnebyy | Scholler and Aime 2006 | Argentina | DQ323926*    |
| BRIP56908§  | Ravenelia necaledoniensis Huguenin | Vachellia farnesiana (L) Wight & Ann. | McTaggart et al. 2015 | Kununurra, Australia | KJ862348*     |
| BRIP56907§  | Ravenelia necaledoniensis Huguenin | Vachellia farnesiana (L) Wight & Ann. | McTaggart et al. 2015 | Northern Territory, Australia | KJ862347*     |
| KR-M-0045114‡ | Ravenelia pienaaei Doidge     | Senegalia caffeine (Thunb.) P.J.H Hurter & Malbb. | This work        | Gauteng, South Africa | MG954483     |
| PREM61892$  | Ravenelia pienaaei Doidge      | Senegalia caffeine (Thunb.) P.J.H Hurter & Malbb. | This work        | KwaZulu-Natal, South Africa | MG954482     |
| MP5157 (PMA)# | Ravenelia piepenbriniae       | Senegalia haynii (Benth.) Britton & Rose | This work        | Chiriqui Province, Panama | MG954489     |
| BRIP56904§  | Ravenelia sp.                  | Cassia sp. Mill.            | McTaggart et al. 2015 | Northern Territory, Australia | KJ862349*     |
| PREM61858$  | Ravenelia transvaalensis Doidge | Senegalia mellifera (Vahl) Seibler & Ebinger | This work        | North-West Province, South Africa | MG954485     |
| PREM61893$  | Ravenelia transvaalensis Doidge | Senegalia mellifera (Vahl) Seibler & Ebinger | This work        | North-West Province, South Africa | MG954486     |
| BRIP56539§  | Endoreticulum auriculiforme McTaggart & Shivas | Acacia difficilis Maiden | McTaggart et al. 2015 | Northern Territory, Australia | KJ862398*     |
| BRIP27071§  | Endoreticulum ternetzyi (Walker & Shivas) Scholler & Aime | Acacia harpophylla E.Muell. ex Benth. | McTaggart et al. 2015 | Queensland, Australia | KJ862335*     |
| BRIP56557§  | Endoreticulum tropicmum McTaggart & Shivas | Acacia tropica (Maiden & Blakely) Tindale | McTaggart et al. 2015 | Northern Territory, Australia | KJ862337*     |
| BRIP56545§  | Endoreticulum violae-funaeiae Berndt | Acacia difficilis Maiden | McTaggart et al. 2015 | Northern Territory, Australia | KJ862344*     |
showed only weak bands on agarose gels, were purified with Zymo Research DNA Clean & Concentrator-5 Kit (ZymoResearch Corp., Irvine, USA), according to the manufacturer’s protocol. The remaining PCR products were purified using Sephadex G-50 columns (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Sequencing was carried out in both directions using the same primers as in PCR at the sequencing service of the Faculty of Chemistry and Biochemistry of the Ruhr-University Bochum, Germany and by GATC (GATC Biotech, Konstanz, Germany).

**Phylogenetic analyses**

Sequences were screened against the NCBI Genbank using the BLAST algorithm to check for erroneously amplified contaminations and were afterwards edited manually using Sequencher 5.0 software (Gene Codes Corp., Michigan, USA). In total, 26 sequences were included (Table 1) to construct an alignment of the 28S rDNA-sequence data using MAFFT v6.832b (Katoh and Standley 2013). Maximum likelihood (ML) analyses were performed with RxML 8.0.26 (Stamatakis 2014) using RAxML GUI v. 1.31 (Silvestro and Michalak 2012) based on the General Time Reversible model of nucleotide substitution plus gamma distribution (GTR+G; Rodriguez et al. 1990) and 1000 generations. Four representative species of *Endoraecium* (*KJ862335*, *KJ862298*, *KJ862337*, *KJ862344*) were set as multiple outgroups. Maximum Parsimony (MP) analyses were carried out using MEGA6 (Tamura et al. 2013) using the heuristic search option with tree bisection-reconnection (TBR) branch swapping algorithm with 10 initial trees using random step-wise addition. The reliability of topology was tested using the bootstrap method with 1000 replicates.

**Results**

**Phylogenetic analyses**

The alignment of the 28S rDNA sequence data consisted of 26 sequences representing 18 taxa and had a total length of 1015 nucleotides with 305 variable characters, 250 parsimony-informative sites and 55 singletons. The tree topologies of MP and ML analyses were identical and thus only the ML tree is shown. A clade, comprising rusts on neotropical *Senegalia* species, i.e. *R. cohniana*, *R. hernandezii* sp. nov. and *R. piepenbringiae* sp. nov., displays a robustly supported sister-group (MLBS/MPBS = 99/100) to two neotropically distributed rusts which infect non-*Senegalia* hosts (i.e. *R. echinata* var. *ectypa* on *Calliandra formosa*, DQ323925 and *R. havanensis* on *Enterolobium contortisiliquum* DQ354557) (Scholler and Aime 2006, Aime 2006). A second clade, based on sequences obtained from *Ravenelia* species on *Senegalia* spp. with paleotropical origin, appeared only distantly related to the former species cluster (MLBS/MPBS = 100/99) (Figure 1).
**Taxonomy**

*Ravenelia piepenbringiae* Ebinghaus & Begerow, sp. nov. on *Senegalia hayesii* (Benth.) Britton & Rose (Mimosoideae, Leguminosae)
Mycobank: MB 824297
Fig. 2

**Type.** Panama, Chiriquí Province, Dolega District, Los Algarrobos, Casa de la Alemana, Bosquecito, approx. 150 m a.s.l., 8°29'45.31"N, 82°25'56.24"W on *Senegalia hayesii* (Benth.) Britton and Rose, 17 February 2013, coll. M. Piepenbring MP 5157 [holotype: s.n. (PMA), isotypes: KR-M-0043654 (KR). M-0141345 (M)]

**Etymology.** Named after M. Piepenbring, who discovered the rust fungus in her garden and provided the specimens.
Spermogonia and aecia not seen. Uredinia hypophyllous, single or in irregular groups, light brown, often associated with necrotic spots that are also evident on the adaxial surface, 0.1–0.8 mm in diameter, a paraphysate, subepidermal, covered by the epidermis when young, later erumpent. Urediniospores obovoidal, ellipsoidal or slightly curved, often limoniform with an acuminate apex, ochraceous brown, (18)21–25(29) × 12–15(20) mm; spore wall laterally 1–1.5 mm thick, apically and basally often slightly thickened, distinctly verrucose to echinulate; aculei 1.0–1.5 mm high, distances between aculei about 2 mm, germ pores 4–7, in equatorial position. Telia replacing uredinia or developing independently from uredinia, chestnut to dark brown, sometimes confluent. Teliospores roundish to broadly ellipsoidal to oblong in planar view, hemispherical in lateral view, with 4–6 probasidial cells across, single-layered, each teliospore formed by 9–13 probasidial cells, (44)58–73(78) mm in diameter, single probasidial cells (19)22–26(31) × (11)17–22(28) mm; cell wall thickened at the surface of the teliospore (epispore), 2–4(5) mm thick, often with a thin and hyaline outer layer, each probasidial cell with 7–11 rod-shaped, straight spines that are (1)2–3(4.5) mm long; cysts at the
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basis of the teliospores, uniseriate and in the same position and number as the peripheral probasidial cells, globose, hyaline, swelling in water, slightly swelling in lactophenol.

Further specimens. Type locality, 22 January 2014, M. Piepenbring 5203 [M-0141344 (M), s.n. (UCH)]. Type locality, 12 January 2017, M. Piepenbring & I. D. Quiroz González 5333 (UCH, s.n.).

Ravenelia hernandezii Ebinghaus & Begerow, sp. nov. on Senegalia tenuifolia (L.) Britton and Rose (Mimosoideae, Leguminosae)
Mycobank: MB 824298
Fig. 3

Type. Costa Rica, Guanacaste, Area de Conservación Guanacaste, Sendero Bosque húmedo (10°50.702’N, 85°36.450’W) on Senegalia tenuifolia (L.) Britton and Rose, coll. J.R. Hernandez, 1. December 2003. Holotype: BPI 872308 (BPI).

Figure 3. Ravenelia hernandezii. A Infected leaflets of S. tenuifolia B Mixed sori containing urediniospores and teliospores C Teliospore seen in LM D telium seen by SEM E Adaxial view of a teliospore by LM, with arrows indicating the uniseriate cysts F SEM view of spinescent teliospores G LM view of the upper surface H drawing of a urediniospore. Scale Bars: 0.5 mm (A); 0.1 mm (B); 20 mm (C–G); 10 mm (H).
Table 2. Summary of morphological characteristics of *Ravenelia* species infecting *Senegalia* trees in the neotropics. All measurements are given in mm. Absent characters are indicated with dashes.

| Species          | Teliospore size | Probasidial cell size | Epispore | Ornamentsation | Cells in Diameter | Arrangement of Cysts | Source                        |
|------------------|-----------------|-----------------------|----------|----------------|-------------------|----------------------|-------------------------------|
| *R. cohniana*    | 39–45–73 (74)   | 16–22 × 13–15         | not stated | (2)–3–5 (8)     | 3–5              | spinescent           | (3)–4–5 (6) uniseriate        | Hernández and Hennen (2002)  |
| *R. escharoides* | 55–90           | 30–35 × 16–20         | up to 6   | 4–9            | 1–2              | verrucose            | 6–8 uniseriate               | Doidge (1939)                |
| *R. halsei*      | 80–112          | 25–30 × 10–15         | 5–6       | –              | –                 | smooth               | 9–11 uniseriate              | Doidge (1939)                |
| *R. hernandezii* | (59)67–75 (96)  | (19)22–25 (39) × (11)17–22 (28) | (2.5)3–4.5(6) | 3–5            | (1)–3–4 (6)      | spinescent           | 5–6 uniseriate               | This study                    |
| *R. lata*        | 53–64           | (18)22–26 (width)     | not stated | 6–20           | not stated        | spinescent           | 4 multiseriate              | Hennen et al. (2005)          |
| *R. monosticha*  | (50)53–55 × 65–70 | 16–19 × 13–15        | not stated | 4–8            | not stated        | verrucose            | 4–6 uniseriate              | Spegazzini (1923)             |
| *R. piresorum*   | 80–120          | 25–30 × 10–15         | up to 7   | 4–7            | 1–1.5 (2)        | verrucose            | (6)7–10 multiseriate        | Doidge (1939)                |
| *R. piepenbringiae* | (44)58–73 (78) | (19)22–26 (31) × (11)17–22 (28) | 2–4 (5) | 7–11           | (1)–2–3 (4.5)    | spinescent           | 4–6 uniseriate              | This study                    |
| *R. pringlei*    | (55)70–95 (105) | (12)14–18 (20) (width) | not stated | not stated     | not stated        | verrucose            | (5)6–8 uniseriate           | Cummins (1975)               |
| *R. rata*        | (30)33–40 (44)  | 14–20 × 12–17         | 1.5       | not stated     | 2–3              | verrucose            | 2–4 uniseriate              | Hennen et al. (2005)          |
| *R. roemeringiae*| 63–100          | Not stated             | not stated | 3–10           | 2                 | verrucose            | 5–7 uniseriate              | Long (1917)                  |
| *R. scopulata*   | (55)65–100 (110) | (13)16–19 (21) (width) | not stated | not stated     | not stated        | smooth               | 5–8 multiseriate            | Cummins and Baxter (1976)     |
| *R. stevensii*   | 40–63           | Not stated             | not stated | 1–3            | 6–19              | verrucose            | 3–6 multiseriate            | Arthur (1915)                |
| *R. transvaalensis* | 75–100         | 30–35 × 15–17.5       | up to 6   | –              | –                 | smooth               | 5–6 multiseriate            | Doidge (1939)                |
| *R. versatilis*  | 85–105          | 10–16 (width)         | not stated | –              | –                 | smooth               | 7–9 not stated              | Dietel (1894)                |
| Source       | Paraphyses   | Urediniospore characters | Position | Shape          | Size                  | Cell wall | Germ pores | Number | Shape      |
|--------------|--------------|--------------------------|----------|----------------|-----------------------|-----------|------------|--------|------------|
| Hernández and Hennen (2002) |             |                          |          |                |                       |           |            |        |            |
| Doidge (1939) |             |                          |          |                |                       |           |            |        |            |
| This study   |             |                          |          |                |                       |           |            |        |            |
| Cummins (1975) |             |                          |          |                |                       |           |            |        |            |
| Hennen et al. (2005) |             |                          |          |                |                       |           |            |        |            |
| Doidge (1939) |             |                          |          |                |                       |           |            |        |            |
| This study   |             |                          |          |                |                       |           |            |        |            |
| Hennen et al. (2005) |             |                          |          |                |                       |           |            |        |            |
| Long (1917)  |             |                          |          |                |                       |           |            |        |            |
| Cummins and Baxter (1976) |             |                          |          |                |                       |           |            |        |            |
| Arthur (1915) |             |                          |          |                |                       |           |            |        |            |
| Doidge (1939) |             |                          |          |                |                       |           |            |        |            |
| Dietel (1894) |             |                          |          |                |                       |           |            |        |            |

Ravenelia piepenbringiae and Ravenelia hernandezii, two new rust species...
**Etymology.** Named after J.R. Hernández who collected the type specimen.

Spermogonia and aecia not seen. Uredinia hypophyllous, minute, single or in small and often loose groups, ochraceous to light brown, 0.1–0.3 mm in diameter, aperiphysate, subepidermal, erumpent and surrounded by torn epidermis; urediniospores obovoidal, ellipsoidal, often reniform or slightly curved, ochraceous brown, often with an attached fragment of the pedicel, (17)18–21(24) × (8)9–10(12) mm; spore wall thin, laterally (0.5)1–1.5 mm thick, apically and basally slightly thickened, distinctly echinate; aculei approximately 1.0–1.5 mm high, germ pores 5–6, in equatorial position. Telia replacing uredinia, chestnut- to dark brown. Teliospores (59)67–75(96) mm, roundish or broadly ellipsoidal to oblong in planar view, hemispherical in lateral view, 5–6 probasidial cells across, single-layered, central cells often arranged in two rows of 3 or 4 cells, each cell (19)22–25(39) × (11)17–22(28) mm, cell wall thickened at the apex, (2.5)3.0–4.5(6.0) mm thick, often with a thin and hyaline outer layer, probasidial cells each with 3–5 rod-shaped straight spines (1)3–4(6) mm long; cysts on the abaxial side of the teliospores, uniseriate and in same position and number as the peripheral probasidial cells, globose, hyaline, swelling in water, slight swelling in lactophenol.

**Discussion**

A total of 10 species of *Ravenelia* have been described to date from the neotropics parasitising *Senegalia* trees: *R. cohniana* Hennings on *S. praecox* (Griseb.) Seigler & Ebinger, *R. idonea* Jackson & Holway, *R. lata* Hennen & Cummins on *S. glomerosa* (Benth.) Britton & Rose, *R. monosticha* Speg. on *S. bonariensis* (Gillies ex Hook. & Arn.) Seigler & Ebinger, *R. pringlei* Cummins on *S. greggii* (A. Gray) Britton & Rose, *R. rata* Jackson & Holway on *S. pedicellata* (Benth.) Seigler & Ebinger, *R. roemeriana* Long on *S. greggii* (A. Gray) Britton & Rose, *R. stevensii* Arthur on *S. riparia* (Kunth) Britton & Rose ex Britton & Killip and *R. versatilis* (Peck) Dietel on *S. anisophylla* (Watson) Britton & Rose. No species of *Ravenelia* has been reported to affect *Senegalia hayesii* or *S. tenuifolia*. Most of these species known to parasitise *Senegalia* spp. are distinguished from species identified in this study by abundant paraphyses in the uredinia, except for *Ravenelia rata* which also lacks paraphyses in the uredinia. However, this species differs from *R. piepenbringiae* and *R. hernandezii* by abundant tuberculate teliospore ornamentations 2–3µm in length and by formation of only 2–4 cysts per teliospore. Both newly described species exhibit longer tuberculate spines and bear 6–8 cysts per teliospore. *Ravenelia cohniana* is the only species that resembles various teliospore and urediniospore characteristics of *R. piepenbringiae* and *R. hernandezii* (see Table 2). The teliospores of *R. hernandezii*, however, are larger in size than those of the latter two species (Table 2). In contrast to the teliospores, urediniospores of *R. hernandezii* tend to be smaller and more slender, while they mostly lack the characteristic acuminate apex present in urediniospores of *R. piepenbringiae* (Table 2; compare Figures 1H and 2H). Hernández and Hennen (2002) considered *R. concinna* Syd. on *S. riparia* (Kunth) Britton & Rose ex Britton & Killip
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and S. glomerosa, R. distans Arthur & Holway on an unidentified mimosoid host and R. lindquistii Hennen & Cummins on Senegalia praecox as synonyms of R. cohniana due to a nearly identical morphology. However, given the likewise close morphological resemblance in R. piepenbringiae, R. hernandezii and R. cohniana, despite being phylogenetic entities, this assumption needs revision by molecular phylogenetic means.

The resemblance of teliospore characters in R. cohniana and the species identified in the present study suggests a close relationship which is supported by the phylogenetic reconstructions. These neotropical rusts on Senegalia further appear to have evolved independently from those Senegalia rusts that have a paleotropical origin (Fig. 1, Table 1). The phylogenetic distinction of both lineages is also mirrored by a morphological feature: the arrangement of teliosporic cysts is uniseriate in the analysed neotropical species but multiseriate in all investigated paleotropical Senegalia rusts (Table 2).

Key to species of Ravenelia infecting neotropical Senegalia trees

1 Teliospores ≤64 mm; urediniospores with equatorially arranged germ pores .... 2
  – Teliospores >64 mm; urediniospores with bizonate or equatorially arranged germ pores .......................................................... 4
2 Paraphyses present in uredinia .............................................................. R. rata
  – Paraphyses absent in uredinia .......................................................... 3
3 Teliospores with <6 verrucae per cell; on S. riparia ..................... R. stevensii
  – Teliospores with 6–20 spines per cell; on S. glomerosa ............. R. lata
4 Urediniospores with 6–8 bizonate germ pores; teliospores verrucose or smooth ................................................................. 5
  – Urediniospores if present with equatorially arranged germ pores; teliospor espinescent or verrucose; teliospore cysts uniseriate .......... 8
5 Teliospores smooth ....................................................................... R. versatilis
  – Teliospores verrucose ................................................................... 7
6 On S. anisophylla; urediniospores 12–14 × 19–24 mm ................ R. scopulata
  – On S. greggii; urediniospores 13–18 × 26–32 mm ...................... R. roemerianae
7 With intrasoral paraphyses; on S. roemeriana ...................... R. roemerianae
  – On S. greggii ........................................................................... R. pringlei
8 Paraphyses present; teliospores verrucose; on S. bonariensis .... R. monosticha
  – Paraphyses absent; teliospores spinescent .................................. 9
9 Teliospores with 7–11 spines per cell; urediniospores often limoniform; on S. hayesii ................................................................. R. piepenbringiae
  – Teliospores with 3–5 spines per cell; urediniospores obovoidal to ellipsoidal, sometimes limoniform ............................... 10
10 Teliospores 59–96 mm in diameter; urediniospores <13mm in width; urediniospore wall laterally 1–1.5 mm; on S. tenuifolia .... R. hernandezii
  – Teliospores 39–75 mm in diameter; urediniospores 11–19 mm in width; urediniospore wall laterally 1.5–2.5 mm; on S. praecox ........ R. cohniana
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