

***Ravenelia acaciae-arabicae* and *Ravenelia evansii* are distinct species on *Acacia nilotica* subsp. *indica* in India**

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Abstract

The two rust fungi, *Ravenelia acaciae-arabicae* and *R. evansii*, were both found on *Acacia nilotica* subsp. *indica* in southern (Tamil Nadu) and northern (Gujarat) India. *R. acaciae-arabicae* has been often incorrectly synonymised with *R. evansii*, although each has distinctive urediniospores, viz. echinulate in *R. acaciae-arabicae* and verruculose in *R. evansii*. Both species are re-described and illustrated from fresh specimens collected in India. Herbarium specimens of *R. evansii* from South Africa, including the holotype, were also examined. The difficulty in connecting different anamorphic spore stages to either of these teleomorphic rusts is highlighted by the presence of similar aecidia on plants of *A. robusta* infected with *R. evansii* in South Africa and on *A. nilotica* subsp. *indica* infected with *R. acaciae-arabicae* in India. It is not known whether these aecidial rusts represent the same species, nor is it known if they represent an aecidial stage of either *R. acaciae-arabicae*, *R. evansii* or other rusts.

Key words: biological control, morphology, taxonomy, rust fungi.

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Introduction

During Jan 2010, a survey was carried out in southern (Tamil Nadu) and northern (Gujarat) India to find pathogens of *Acacia nilotica* subsp. *indica* (Benth.) Brenan that might have potential as biological control agents in Australia where it has become a major weed in tropical grasslands and savannahs (Dhileepan *et al.* 2010). The natural distribution of *A. nilotica* subsp. *indica* is restricted to Asia, which include India, Pakistan, Yemen, Oman and Myanmar (Dhileepan, 2009). Two distinctive rust fungi, *Ravenelia acaciae-arabicae* Mundk. & Thirum. and *R. evansii* Syd. & P. Syd., were found on *A. nilotica* subsp. *indica* in both Tamil Nadu and Gujarat. There has been some confusion in the literature over the identity of these two rusts, which resulted in *Ravenelia acaciae-arabicae* Mundk. & Thirum. being considered a synonym of *R. evansii* Syd. & P. Syd. by several authors (Kapoor & Agarwal, 1974; Cannon, 2008). Cannon (2008) hinted at the taxonomic problems when he noted the variation in morphological measurements and morphological characteristics of *R. evansii* as given by various authors and observed that it was possible that not all of the connections made between the different stages of the life cycle were correct.

Materials and methods

Dried herbarium specimens were examined by light microscopy (LM) and scanning electron microscopy (SEM). For LM, microscope slide mounts were prepared

from fresh material, herbarium specimens and cultures by placing small portions of excised material into a drop of clear lactic acid on microscope slides, which were covered with a cover slip and gently heated. The microscope slides were examined immediately and images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope equipped with a Nomarski interference condenser. For SEM, dried spores were dusted on double-sided adhesive tape, mounted on a specimen stub, sputter-coated with gold-palladium, ca. 20 nm, and examined in a SEM at 10 kV.

Taxonomy

***Ravenelia acaciae-arabicae* Mundk. & Thirum.**

Fig. 1 Mycological papers 16: 17. 1946.

Holotype on leaves of *Acacia arabica* Willd. [= *A. nilotica* (L.) Willd. ex Delile], India, Karnataka, Hessarghatta, 26 Mar. 1943, M.J. Thirumalachar, [II, III]; paratype India, Tamil Nadu, Coimbatore, 23 Jan. 1917, C.K. Nayar ex Herb. Coimbatore, no. 993.

Uredinia on leaflets, mostly on lower leaf surface, not associated with spots although leaflet tissue on corresponding surface lightly chlorotic, solitary or in groups, subepidermal, erumpent, pale brown, 150–500 µm diam.; enclosed by a compact band of peripheral paraphyses, cylindrical or gradually tapered to a rounded apex, sinuate to incurved, pale reddish brown 20–50 × 6–17 µm with thin walls 0.5–1.0 µm wide; with

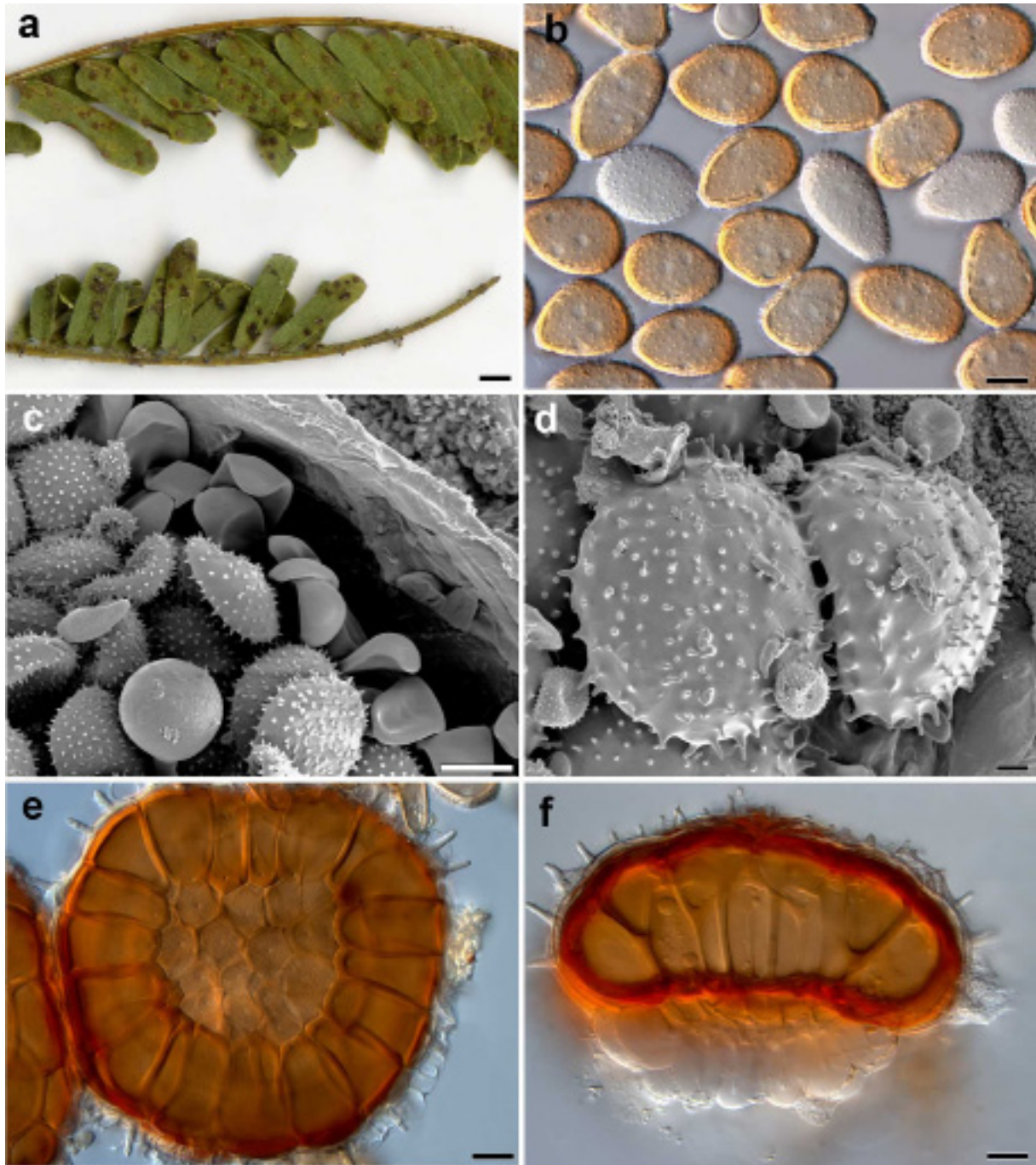


Fig. 1 *Ravenelia acaciae-arabicae* on *Acacia nilotica* subsp. *indica*. **a** Uredinia (upper) and telia (lower) on leaflets ex BRIP 53144; **b** urediniospores ex BRIP 53154; **c** SEM of uredinium with urediniospores, peripheral paraphyses and an intrasoral capitate paraphysis ex BRIP 53144; **d** SEM of teliospores ex BRIP 53144; **e** dorsal view of teliospore ex BRIP 53144; **f** side view of teliospore ex BRIP 53144. Bar: (a) = 1 mm. (b, c, d, e, f) 1 = 10 μ m.

abundant scattered intrasoral capitate paraphyses, sub-hyaline to pale brown, up to 60 μ m long with pedicels 4–6 μ m wide and apex 10–16 μ m diam. with walls 1.5–3.5 μ m. *Urediniospores* obovoid, cylindrical to broadly ellipsoidal, 20–30 \times 15–21 [av. (\pm SD) 26 (\pm 2) \times 18 (\pm 1), n = 30] μ m, yellowish brown; wall even, 2–3 μ m wide, echinulate with spines about 1 μ m high and 1–2 μ m apart, with 6–10 scattered, conspicuous germ pores.

Telia on leaflets, mostly on lower leaf surface, not associated with spots although leaflet tissue on

corresponding surface lightly chlorotic, solitary or in groups, subepidermal, erumpent, black, 150–500 μ m diam., enclosed by a compact band of peripheral paraphyses identical to those in uredinia. *Teliospores* often form within the uredinia, strongly adherent in hemispherical-convex heads, the heads reddish brown, 65–110 [av. (\pm SD) 83 (\pm 7) – 92 (\pm 9), n = 20] μ m diam., 4–6 probasidial cells across; probasidial cells 1-layered, 22–34 μ m long, in surface view unequally hexagonal, 17–29 μ m diam.; wall 2–6 μ m thick at apex, smooth with 2–6 blunt conical spines, 2–10 μ m high; cysts broadly ellipsoidal, sessile, hyaline, ca 15–22 \times 6–10



Fig. 2 *Ravenelia evansii* on *Acacia nilotica* subsp. *indica*. **a** Uredinia on leaflets ex BRIP 53149; **b** urediniospores and paraphyses ex BRIP 53149; **c** SEM of urediniospores and paraphyses ex BRIP 53149; **d** SEM of teliospores ex BRIP 53149; **e** SEM of hub and spoke ornamentation of teliospore ex BRIP 53149; **f** uredinia and telia on leaflets ex PREM 2181 (holotype); **g** teliospore ex PREM 2181 (holotype). Bar: (**a**) = 1 cm. (**b, c, d**) = 10 μ m. (**e**) = 1 μ m. (**f**) = 1 cm. (**g**) = 10 μ m.

μ m, smooth and without processes or appendages; pedicel hyaline, multihyphal, 10–15 μ m wide, up to 35 μ m long, deciduous.

Specimens examined: India: On *Acacia nilotica* subsp. *indica*: Tamil Nadu, Coimbatore District, Sevalur Pond, 24 Dec. 2008, M. Senthilkumar & S. Murugesan (BRIP 52568) [II]; Tamil Nadu, Coimbatore District, Vedappatti, 30 June 2009, M. Senthilkumar & A. Balu (BRIP 52800) [II]; Tamil Nadu, Mettupalayam near Annur, 4 Jan. 2010, R.G. Shivas, K. Dhileepan & A. Balu (BRIP 53152) [I, II]; Tamil Nadu, Veerakeralam, 4 Jan. 2010, R.G. Shivas,

K. Dhileepan & A. Balu (BRIP 53144) [I, II, III]; Gujarat, Tarapur, 23 Jan 2010, R.G. Shivas, K. Dhileepan & S. Singh (BRIP 53154) [II]; Gujarat, Nadiad, 23 Jan 2010, S. Singh (BRIP 54013), [II]; Tamil Nadu, Coimbatore District, Nagarajapuram, 6 Sept. 2010, M. Senthilkumar W2581 (BRIP54015 – glasshouse inoculation ex BRIP 54016 – field material, IMI 398973) [II].

The specimens of *Ravenelia* that we collected on *Acacia nilotica* subsp. *indica* in southern and northern India agree closely with Mundkur & Thirumalachar's (1946) original description of *R. acaciae-arabicae*. Whereas

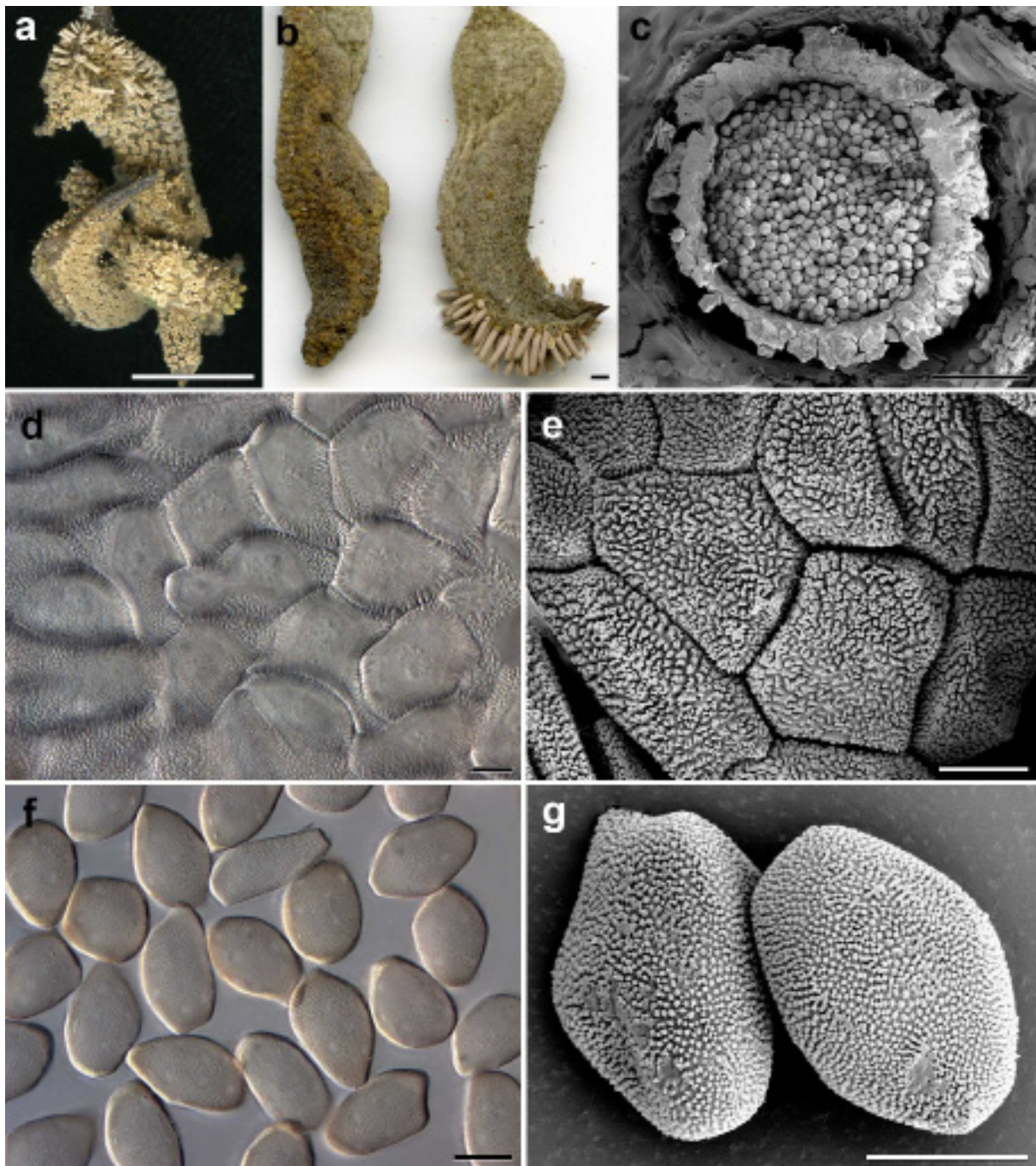


Fig. 3 *Aecidium* sp. on *Acacia nilotica* subsp. *indica*. **a** Aecia on pods ex BRIP 53144; **b** Aecia on pods ex BRIP 53150; **c** SEM of aecium ex BRIP 53144; **d** peridial cells ex BRIP 53144; **e** SEM of peridial cells ex BRIP 53144; **f** aeciospores ex BRIP 53144; **g** SEM of aeciospores ex BRIP 53144. Bar: (**a**) = 1 cm. (**b**) 1 = mm. (**c**) = 100 μ m. (**d**, **e**, **f**, **g**) = 10 μ m.

Mundkur & Thirumalachar (1946) gave the diam. of telial heads as 64–92 μ m with a mean of 78 μ m, we found similar corresponding measurements of 65–105 μ m with a mean of 83 – 92 μ m ($n = 20$). Mundkur & Thirumalachar's (1946) description of *R. acaciae-arabicae* as having urediniospores minutely echinulate, 20–33 x 17–20 μ in size (mean = 27 x 19 μ), with 8 to 10 scattered germ pores was also similar to those on specimens we collected in India.

***Ravenelia evansii* Syd. & P. Syd.**

Fig. 2 *Annales Mycologici* 10: 440. 1912.

Holotype on *Acacia robusta*, South Africa, Transvaal, near Pretoria, The Willows, 23 Mar. 1912, J.B. Pole Evans 2181 (PREM 2181!), [III].

Uredinia amphigenous, not associated with spots although leaflet tissue on corresponding surface lightly chlorotic, solitary or in groups, subepidermal,

erumpent, reddish brown, up to 1 mm diam.; paraphyses abundant, intrasoral, clavate to spathulate, apex layered, yellowish brown at apex becoming lighter downwards, up to 12 µm thick at apex and 18 µm wide, with hyaline pedicels up to 55 µm long. *Urediniospores* subglobose to broadly ellipsoidal, 18–26 × 14–19 µm, yellowish brown; wall uneven, 1–2 µm wide, densely verruculose, apparent under SEM as conical warts 1 µm high on narrow cylindrical bases interconnected by straight ridges, with 4–6 scattered, inconspicuous germ pores.

Telia on leaflets, mostly on upper leaf surface, not associated with spots solitary or in groups, subepidermal, erumpent, black, 150–400 µm diam., lacking paraphyses. *Teliospores* strongly adherent in hemispherical-convex heads, the heads reddish brown, 63–90 µm [av. (± SD) 73 (± 7) – 78 (± 8), n = 16] diam., 3–4 probasidial cells across; probasidial cells 1-layered, 20–40 µm long, in surface view unequally hexagonal, 20–32 µm diam.; wall 2–6 µm thick at apex, smooth with 4–8 blunt conical spines, 2–6 µm high; cysts broadly ellipsoidal, sessile, hyaline, smooth and without processes or appendages; pedicel hyaline, multihyphal, short.

Specimens examined: **South Africa:** On *Acacia robusta*: Transvaal, near Pretoria, The Willows, 18 May 1906, Anon. (PREM 7123), [III]; Natal, Umzinto, 13 July 1938, A.P.O. McClean (Prem 30124), [II, III]; Tshokware tea room, Kruger National Park, 16 May 1991, M. van Reenen (PREM 50693) [III]. On *Acacia gerrardii*: Natal, Verulum, 25 July 1938, R.N. Halse (PREM 30127) [II, III]. **India:** On *Acacia nilotica* subsp. *indica*: Gujarat, Talala, opp. sugar mill, 20 Jan. 2010, R.G. Shivas, K. Dhileepan and S. Singh (BRIP 53149) [II]; loc. id., 26 Oct. 2010, S. Singh (BRIP 54061) [II]; Gujarat, Tarapur, 22 Jan. 2010, R.G. Shivas, K. Dhileepan and S. Singh (BRIP 53151, 53152), [II]; loc. id., 26 Oct. 2010, S. Singh (BRIP 54062) [II]; Gujarat, Varaval, 26 Oct. 2010, S. Singh (BRIP 54063) [II]; Tamil Nadu, Coimbatore District, Nagarajapuram, Sept. 2010, M. Senthilkumar W2590 (BRIP 54017 – glasshouse inoculation ex IMI 399218), [II].

The type specimen of *R. evansii* from South Africa and the Indian specimens that we collected were morphologically similar and agreed with the descriptions given by other authors. Sydow (1912) and Doidge (1926) gave the diameter of the teliospores heads of *R. evansii* as 50–80 µm, compared to our measurements of 63–90 µm. All of the specimens had verruculose urediniospores. Doidge (1939) reported the width of the urediniospores of *R. evansii* as 12.5–15 µm, compared to 14–19 µm, which we found.

Aecidium sp.

Fig. 3

Spermogonia on fruits, inflorescences and shoot tips, subcuticular, indistinct, conical, ca 75–100 µm wide; spermatia globose to ellipsoidal, hyaline, ca 2.5 µm long.

Aecia cupulate, subepidermal, erumpent, in dense clusters causing hypertrophy on fruits, inflorescences and shoot tips; peridium white to grey, cylindrical, slightly flared at apex, up to 300 µm wide; peridial cells irregular with curved and straight sides, 23–40 × 12–27 µm, wall 3–5 µm, thick, densely verruculose. *Aeciospores* subglobose, broadly ellipsoidal or polygonally irregular, hyaline, 22–35 × 15–20 µm, catenulate in columns up to 3 mm long and 300 µm wide, wall 2.5–3.0 µm thick, finely verruculose, with 6–10 scattered, inconspicuous germ pores.

Specimens examined: On *Acacia robusta*: **South Africa:** Transvaal, near Pretoria, The Willows, 12 Nov. 1904, Anon. (PREM 7149), [I]. **India:** On *Acacia nilotica* subsp. *indica*: Tamil Nadu: near Annur, 6 May 2009, M. Senthilkumar, BRIP 52799 [I]; Kuniyamuthur, 6 May 2009, M. Senthilkumar, BRIP 53153 [I]; Vedappatti, 30 June 2009, M. Senthilkumar & A. Balu (BRIP 52801) [I]; Chettyalayam near Perur, R.G. Shivas, K. Dhileepan & A. Balu (BRIP 53150) [I]; Mettupalayam near Annur, 4 Jan. 2010, R.G. Shivas, K. Dhileepan & A. Balu (BRIP 53152) [I, II]; Veerakeralam, 4 Jan. 2010, R.G. Shivas, K. Dhileepan & A. Balu (BRIP 53144) [I, II, III].

Discussion

Ravenelia evansii was first described by Sydow & Sydow (1912) based on a specimen (PREM 2181) that only had spermogonia and telia on *Acacia robusta* Burch. from South Africa. The aecidia and aecidiospores of *R. evansii* were subsequently described by Doidge (1926) on *A. robusta* (PREM 7149), *A. woodii* and other unnamed species of *Acacia* from South Africa. Later still, the uredinia and urediniospores of *R. evansii* were first described by Doidge (1939) on *A. robusta* (PREM 30124) and *A. gerrardi*. What is the evidence that links the aecidia and uredinia on these South African specimens with *R. evansii*? In the case of aecidia, the specimen (PREM 7149) described by Doidge (1926) consisted of several distorted shoots and stems covered in aecidia, and without any other spore stages present. The presumed connection of the aecidia to *R. evansii* is based on having the same host species (which cannot be confirmed), and the same specific locality as the type i.e. The Willows near Pretoria. In the case of uredinia, the specimen described by Doidge (1939) also has teliospores forming within the uredinia, which confirms the connection between these uredinial and telial spore stages.

Ravenelia acaciae-arabicae was first described by Mundkur & Thirumalachar (1946) on *Acacia arabica* Willd., which is a synonym of *Acacia nilotica*, from southern India. Their description was based on uredinial and telial spore stages. Although we were not able to obtain the type specimen, we made several collections of this rust from southern and northern India. These specimens closely matched Mundkur & Thirumalachar's (1946) description and showed teliospores forming in uredinia. Subsequently Bagyanarayana & Ravinder (1988) described spermogonial and aecidial stages of this rust (as *R. evansii*) collected from an unspecified location in India over a three year period, having accepted Kapoor & Agarwal's (1974) synonymy, and without mention of Doidge's (1926) earlier description of spermogonia and aecidia. Bagyanarayana & Ravinder (1988) found spermogonia and aecidia in association with uredinia and telial and also noted these were morphologically similar to *R. evansii*.

An examination of the original descriptions of the uredinial stages of the *R. acaciae-arabicae* with *R. evansii* reveals clear and significant differences between the two species. Mundkur & Thirumalachar's (1946) description of the type of *R. acaciae-arabicae* states that the urediniospores are echinulate with 8-10 scattered germ pores. Doidge's (1939) original description of *R. evansii* gives the urediniospores as verruculose-echinulate with 4 equatorial germ spores.

Kapoor & Agarwal (1974) offered no justification for their proposed synonymy of *R. acaciae-arabicae* with *R. evansii*, other than to indicate that they had examined the type specimens of each, noting that the type of *R. evansii* contained only telia. Cannon (2008) accepted Kapoor & Agarwal's (1974) synonymy despite noting some variation in measurements and morphological characteristics and also observing that not all the connections made between the different stages of the life cycle were correct. In our view the significant morphological differences between *R. acaciae-arabicae* and *R. evansii*, together with different hosts and continental distributions, warrant retaining them as separate species. Considering the wide range of species of *Acacia* in Africa that have been reported as hosts for *R. evansii*, it is possible that *R. evansii* represents more than one species.

Hernández *et al.* (2011) showed that 10 species of *Ravenelia* had urediniospores with a peculiar type of surface ornamentation described by Gardner & Hodges (1985) as knob-like processes interconnected by straight ridges, and by Hernández & Hennen (2002) as hub and spoke. All of these species were distributed in parts of North, Central and South America. Two of these extended further than the New World, namely *R. spegazziniana* Lindq., which occurs in Hawaii on the introduced *Acacia farnesiana* (Gardner & Hodges, 1985), and *R. fragrans* var. *everinia* (Syd.) J. Baxter,

which occurs on some *Mimosa* spp. in India, Nepal and Pakistan (Hernández *et al.* 2011).

The pattern of uredinial ornamentation on *R. evansii* differs from that of *R. spegazziniana*, which has flat topped warts with a central dimple. *R. evansii* has urediniopores with rounded conical warts subtended by a ring-like extension, which is similar to at least nine other species of *Ravenelia* that were examined by SEM (Hernández *et al.* 2011). *R. evansii* differs from other *Ravenelia* spp. with a hub and spoke uredinial ornamentation, by having abundant intrasoral paraphyses and 4-6 scattered germ pores.

During our survey in India we were able to collect several specimens of rust on *A. nilotica* subsp. *indica*. Examination of these specimens together with herbarium specimens of some South African rusts borrowed from PREM allowed us to resolve the confusion of the identity of the rusts referred to *R. acaciae-arabicae* and *R. evansii*. These two rusts are different and easily differentiated by the ornamentation of the urediniospores, with less discernable differences in the teliospores. It is anticipated that DNA sequence analysis will be necessary to demonstrate whether the morphologically similar aecidial stages from South Africa and India are the same, and if so, to which rust they belong.

The confusion surrounding the identity of *R. acaciae-arabicae* with *R. evansii* was fuelled by inadequate descriptive illustrations, lack of specimens, and the risks associated with connecting different spore stages in the rust life cycle to a particular species. One further specimen that we did not examine was *Aecidium schweinfurthii* Henn., which was described on fruits of *Acacia fistula* from Gallabat [Sudan] in north-eastern Africa and listed by Cannon (2008) as possibly another synonym of *R. evansii*.

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