

Baobabopsis, a new genus of graminicolous downy mildews from tropical Australia, with an updated key to the genera of downy mildews

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Abstract: So far 19 genera of downy mildews have been described, of which seven are parasitic to grasses. Here, we introduce a new genus, *Baobabopsis*, to accommodate two distinctive downy mildews, *B. donbarrettii* sp. nov., collected on *Perotis rara* in northern Australia, and *B. enneapogonis* sp. nov., collected on *Enneapogon* spp. in western and central Australia. *Baobabopsis donbarrettii* produced both oospores and sporangiospores that are morphologically distinct from other downy mildews on grasses. Molecular phylogenetic analyses showed that the two species of *Baobabopsis* occupied an isolated position among the known genera of graminicolous downy mildews. The importance of the *Poaceae* for the evolution of downy mildews is highlighted by the observation that more than a third of the known genera of downy mildews occur on grasses, while more than 90 % of the known species of downy mildews infect eudicots.

Key words:

cox2
genus key
nrLSU
phylogeny
Peronosporaceae
Poaceae

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INTRODUCTION

Graminicolous downy mildews (GDM) occur on diverse wild and cultivated grasses, particularly in the semi-arid tropics and subtropics. Seven genera of GDM, namely, *Eraphthora* (Telle & Thines 2012), *Graminivora* (Thines *et al.* 2006), *Peronosclerospora* (Shirai & Hara 1927), *Poakatesthia* (Thines *et al.* 2007), *Sclerophthora* (Thirumalachar *et al.* 1953), *Sclerospora* (Schröter 1886), and *Viennotia* (Göker *et al.* 2003), have been described to date. Only three of these genera, *Peronosclerospora*, *Sclerophthora*, and *Sclerospora*, cause significant and widespread economic losses (Kenneth 1981, Jeger *et al.* 1998, Spencer & Dick 2002). The other GDM genera are known only from a single host plant genus, and, with the exception of *Eraphthora*, seemingly have limited geographical distributions. The genera *Poakatesthia* and *Viennotia* are only known from their type specimens. Several unusual and unidentified downy mildews have been collected on native Australian tropical grasses, in particular on species of *Perotis* and *Enneapogon* that also extend into New Guinea and Malesia (Simon & Alfonso 2011). The aims of this study were to identify the downy mildews on specimens of *Perotis* and *Enneapogon* in BRIP (Plant Pathology Herbarium, Brisbane), and further, to investigate their phylogenetic relationships with other GDM.

MATERIALS AND METHODS

Microscopy

For morphological characterization, downy mildews were examined directly from infected plant tissue preserved in the fungarium at BRIP. Collection details are provided in the taxonomy section of this manuscript. Specimens were mounted on glass slides in 100 % lactic acid, stained with aniline blue (sporangia and sporangiophores only), and gently heated prior to microscopic examination. Ranges were expressed as either min. – max. or as (min.–) mean minus SD – mean – mean plus SD (–max.), depending on the amount of measurements done, with values rounded to 0.5 µm. Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast.

DNA extraction, PCR-amplification, sequencing and phylogenetic analysis

Specimens for which new sequence data were obtained in this study are listed in Table 1. DNA was extracted and PCR for *cox2* mtDNA was performed as described in Telle *et al.* (2011). PCR for the D1-D3 region of the LSU nrDNA was done as described in Choi *et al.* (2011). Amplicons were sequenced by the sequencing laboratory at the Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main,

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Table 1. Oomycete specimens sequenced in this study.

Species	Host	Voucher	GenBank <i>cox2</i>	GenBank nrLSU	Locality	Date
<i>Sclerospora graminicola</i>	<i>Setaria viridis</i>	FR-0247007	n.a.	KT248942	Germany, Saxony-Anhalt, Dübener Heide	08.08.2007
	<i>Setaria viridis</i>	FR-0247008	n.a.	KT248943	Germany, Thuringia, Jena	18.08.2007
<i>Sclerophthora macrospora</i>	<i>Lolium perenne</i>	FR-0247009	n.a.	KT248941	Poland, Legnica (road to Ules)	28.06.2007
	<i>Lolium perenne</i>	FR-0247010	n.a.	KT248940	Poland, Legnica (road to Ules)	28.06.2007
	<i>Festuca arundinaceum</i>	FR-0247011	n.a.	KT248939	Italy, Spilimbergo (northwards)	26.06.2007
<i>Peronosclerospora</i> sp.	<i>Sorghum</i> sp.	BRIP 39880	n.a.	KT248947	Australia, NT: Alyangula Groote Eylandt	09.03.2003
<i>Baobabopsis enneapogonis</i>	<i>Enneapogon cylindricus</i>	BRIP 49822	n.a.	KT248946	Australia, NT: East MacDonnell Ranges	21.04.2007
<i>Baobabopsis donbarrettii</i>	<i>Perotis rara</i>	BRIP 54675	KT248948	KT248945	Australia, Western Australia, Kununurra	19.04.2011

Germany, with the primers used for PCR. Sequences were edited for bad quality bases and trimmed and assembled in contigs using Geneious v. 5.0 (Biomatters, Newark, NJ). Sequences were aligned using MAFFT (Katoh & Standley 2013), employing the Q-INS-i algorithm (Katoh & Toh 2008). Leading and trailing gaps were removed, and no manual adjustments of the alignment were made in order to prevent bias. Phylogenetic analyses were performed with MEGA v. 6.0 (Tamura *et al.* 2013) for Minimum Evolution inference, with all parameters set to default, except for using the Tamura-Nei substitution model and performing 1000 bootstrap replicates. For Bayesian Inference MrBayes (Huelsenbeck *et al.* 2001) as implemented in the graphical user interface siMBa (Mishra & Thines 2014) was used with default settings, except for running 10 M generations, sampling every 10 000th tree of which the first 25 % were discarded before calculating posterior probabilities. Maximum Likelihood analyses were done using RAxML (Stamatakis 2006, Stamatakis *et al.* 2008) v. 7, using the GTRGAMMA algorithm and performing 1000 bootstrap replicates. Alignments are deposited in TreeBASE, study accession number S11476.

RESULTS

Morphology

The members of the new genus *Baobabopsis* exhibit several characteristics typical for GDM with evanescent sporangia (Fig. 1), including thin-walled sporangiophores and sporangia and thick-walled oospores. Unlike any known downy mildew genus, the ultimate branchlets are ampulliform in *Baobabopsis*. *Baobabopsis* further differs from other GDM in having stout unbranched sporangiophores.

Molecular phylogeny

Alignments contained 495 sites in case of *cox2* (no gaps) and 879 sites in case of nrLSU (4 sites with gaps). Molecular phylogenetic analyses based on LSU nrDNA (Fig. 2) and *cox2* mtDNA (Fig. 3) sequences place the new genus, *Baobabopsis*, among the GDM with evanescent sporangiophores. In the *cox2*-based tree *Baobabopsis* is sister to the genus *Sclerospora*, and in the nrLSU-based tree *Baobabopsis* is sister to a clade containing *Sclerospora* and *Peronosclerospora*, although with weak support in both cases. Further, the monophyly of all genera of GDM is inferred with high to maximum support. Apart from the high support for the grouping of *Eraphthora* and *Sclerophthora*, there was only weak support for other GDM relationships. As the trees from Minimum Evolution, Maximum Likelihood, and Bayesian phylogenetic reconstructions revealed no conflicting topologies with high support, only the Minimum Evolution trees are given (Figs 2 and 3), with support values from the other analyses.

TAXONOMY

Baobabopsis R.G. Shivas, Y.P. Tan, Telle & Thines, **gen. nov.**
MycoBank MB813445
(Fig. 1E)

Etymology – Named after the Australian baobab (also termed boab) tree (*Adansonia gregorii*), which has a trunk and branches that magnify the gross morphology of the sporangiophores. Coincidentally, this fungus was found on its host plant, *Perotis rara*, in the shade of a baobab tree.

Diagnosis: Differs from all other genera of *Peronosporaceae* in having broad club-shaped to cylindrical sporangiophores with a cluster of terminal ampulliform projections bearing sporangia.

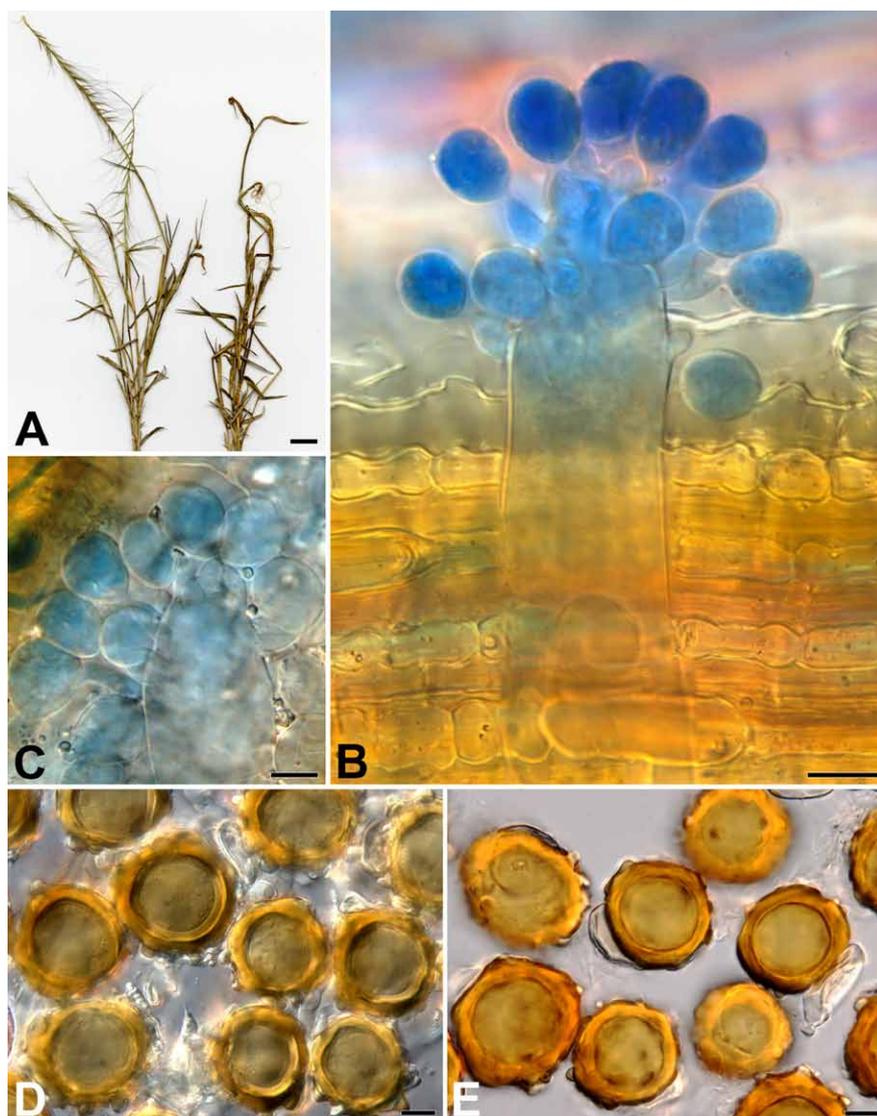


Fig. 1. Morphological features of *Baobabopsis*. **A.** Healthy *Perotis rara* (left), infected with *B. donbarrettii* (right). **B–C.** Sporangiophore and sporangia of *B. donbarrettii* (BRIP 54675). **D.** Oospores of *B. donbarrettii* (BRIP 54675). **E.** Oospores of *B. enneapogonis* (BRIP 49822). Bars: A = 1 cm, B–E = 10 μ m.

Type species: Baobabopsis donbarrettii

Description: *Straminipila*, *Oomycota*, *Peronosporales*, *Peronosporaceae*. *Sporangiophores* cover lower leaf surfaces, evanescent, aseptate, hyaline, cylindrical, 75–120 μ m long, 20–28 μ m wide, unbranched, with 5–20 ampulliform to lageniform ultimate branchlets. *Sporangia* hyaline, deciduous. Oogonia and oospores in leaves. *Oogonia* golden yellow, subglobose, 27–45 \times 25–39 μ m; wall (exosporium includes warts) 3–11 μ m thick, uneven, verrucose with rounded warts. *Oospores* one per oogonium, pale to golden yellow, globose to broadly ellipsoidal, 19–29 \times 18–28 μ m; wall (endosporium) 1–3 μ m thick, even, smooth.

Baobabopsis donbarrettii R.G. Shivas, Y.P. Tan & Thines, **sp. nov.**
Mycobank MB813446
(Fig. 1A–D)

Etymology: Named after Donald Barrett, former Dean of the Faculty of Arts, University of Queensland and scholar in Classics and Ancient History, in appreciation for generously providing many Latin translations for new Australian fungal species.

Diagnosis: Differs from *Baobabopsis enneapogonis* in parasitizing *Perotis rara*, and has densely verrucose oogonial walls.

Type: **Australia:** *Western Australia:* Kununurra, truck stop near Lake Kununurra, alt. about 45 m, on *Perotis rara*, 19 Apr. 2011, R.G. Shivas & T.Y. Chi (BRIP 54675 – holotype; sequences ex-type GenBank KT248945, *cox2* mtDNA, KT248948, nrLSU).

Description: *Sporangiophores* cover lower leaf surfaces, evanescent, aseptate, hyaline, cylindrical, 75–120 \times 20–28 ($n = 5$) μ m, with 5–20 terminal ampulliform to lageniform branches with a narrow neck 7–14 \times 3–7 ($n = 10$) μ m. *Sporangia* hyaline, deciduous, broadly ellipsoidal, slightly narrowed towards the base, 16–20 \times 11–18 ($n = 10$) μ m. Oogonia and oospores in leaves that split into tangled vascular strands up to 5 cm long. *Oogonia* golden yellow, subglobose, (27–)32.5–36.0–39.5(–45) \times (25–) 28–31.7–36 (–39) ($n = 22$) μ m diam; wall (exosporium includes warts) 3–9 ($n = 20$) μ m thick, uneven, densely verrucose with rounded warts. *Oospores* one per oogonium, subhyaline to golden yellow, globose to broadly ellipsoidal, (19–)22–24.1–

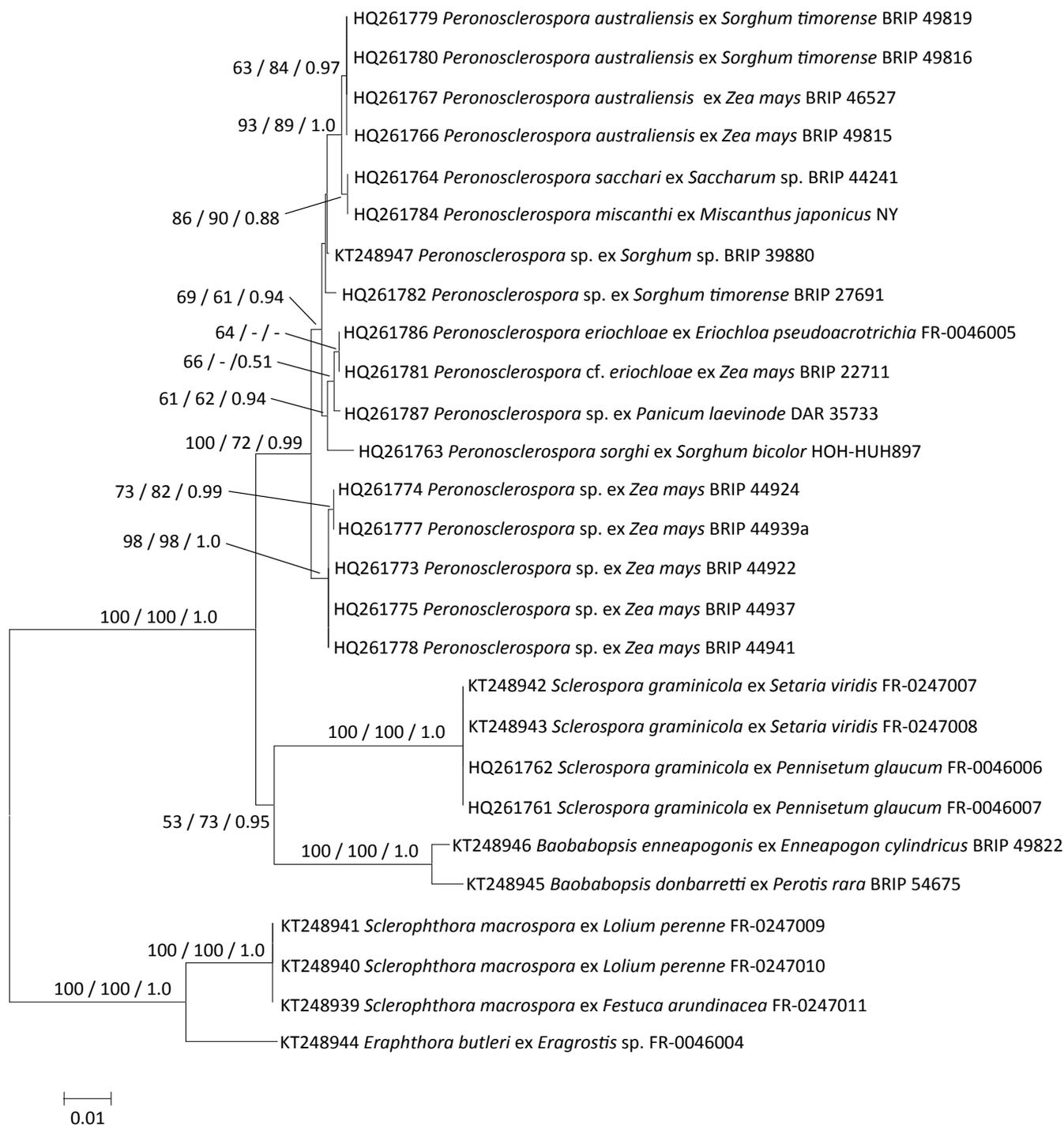


Fig. 2. Phylogenetic tree (Minimum Evolution), based on nrLSU sequences. Numbers on branches denote support from Minimum Evolution, Maximum Likelihood and Bayesian analyses, in the respective order. - = no support for the displayed or an alternate topology.

27(–29) × (18–) 20–22.5–25(–28) ($n = 22$) μm diam; wall (endospore) 1–3 μm thick, even, smooth.

Habitat: In living plants of *Perotis rara*, Australia.

Baobabopsis enneapogonis R.G. Shivas, Y.P. Tan, Telle & Thines, **sp. nov.**

Mycobank MB813447
(Fig. 1E)

Etymology: Named after the host genus *Enneapogon* (*Poaceae*).

Diagnosis: Differs from *Baobabopsis donbarretti* in parasitizing *Enneapogon* spp., and in having slightly less prominent warts and moderately verrucose oogonial walls.

Type: **Australia:** Northern Territory: East MacDonnell Ranges, near turnoff to Corroboree Rock, on *Enneapogon*

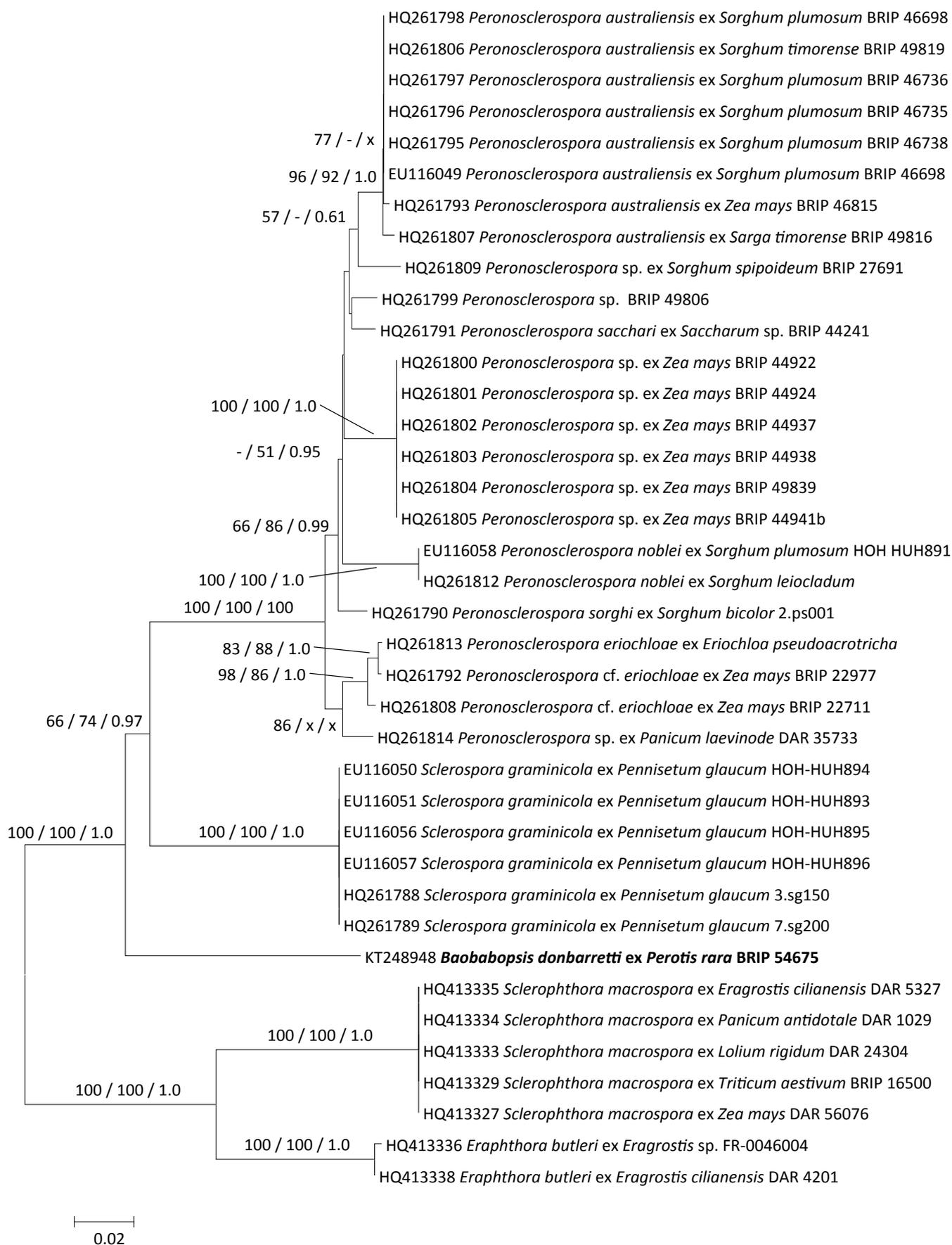


Fig. 3. Phylogenetic tree (Minimum Evolution), based on *cox2* sequences. Numbers on branches denote support from Minimum Evolution, Maximum Likelihood and Bayesian analyses, in the respective order. - = no support for the displayed or an alternate topology, x = support for an alternate topology.

cylindricus, 21 Apr. 2007, A.R. McTaggart, J. Liberato, M.D.E. & R.G. Shivas (BRIP 49822 – holotype; sequences ex-type GenBank KT248946, *cox2* mtDNA).

Description: Asexual morph not seen. Oogonia and oospores in leaves that split into tangled vascular strands up to 10 cm long. Oogonia golden yellow, subglobose, (30–)32.5–36.3–40(–42) × (29–)30–33.1–36(–39) (*n* = 20) µm diam; wall (exosporium includes warts) 3–11 (*n* = 20) µm thick, uneven, moderately verrucose with rounded warts, often with remnants of antheridium attached. Oospores one per oogonium, pale to golden yellow, globose to broadly ellipsoidal, (20–)21.3–23.0–24.7(–26) × (19–)20.5–21.9–

23.5(–24) (*n* = 20) µm diam; wall (endosporium) 1–1.5 (–2) µm thick, even, smooth.

Habitat: In living plants of *Enneapogon avenaceus* and *E. cylindricus*, Australia.

Additional specimens examined: **Australia:** Northern Territory: West MacDonnell Ranges, on *E. avenaceus*, 22 Apr. 2007, A.R. McTaggart, J. Liberato, M.D.E. & R.G. Shivas (BRIP 49630). Western Australia: 2 km south of Wingellina, on *E. cylindricus*, 11 Oct. 2010, A.A. Mitchell AAM9628A (BRIP 53764); Mueller Ranges, on *E. cylindricus*, 14 Apr. 2014, A.R. McTaggart, L.S. Shuey, J. Brands, M.D.E. & R.G. Shivas (BRIP 60962).

Key to the genera of downy mildews

- 1 Sporangiohores unbranched (hyphal, club-shaped or cylindrical) 2
Sporangiohores branched multiple times 6
- 2 (1) Sporangiohores hyphal, undifferentiated; oospores 30–80 µm diam **Sclerophthora**
Sporangiohores club-shaped or cylindrical; oospores smaller 3
- 3(2) Sporangiohores persistent 4
Sporangiohores evanescent 5
- 4 (3) Sporangiohores stout, immersed in the stomata, ultimate branchlets short, stout, and hardly differentiated **Benua**
Sporangiohores slender, with an enlarged apex bearing sporangia on well-differentiated ultimate branchlets **Basidiophora**
- 5 (3) Sporangiohores less than 60 µm in length, club-shaped, sporangia irregular lemon-shaped, tapering towards apex and pedicel **Eraphthora**
Sporangiohores more than 60 µm in length, broadly club-shaped to cylindrical, sporangia obovoid to oval **Baobabopsis**
- 6 (1) Sporangiohores evanescent 7
Sporangiohores persistent 8
- 7 (6) Sporangia produce zoospores; with an operculum **Sclerospora**
Sporangia germinate with a germ-tube; without an operculum **Peronosclerospora**
- 8 (6) Haustoria small, globose to pyriform 9
Haustoria large, digitate, globose, lobate or irregular 15
- 9 (8) Sporangiohores stout and irregularly branched, pedicels wider than 2 µm, broadening widely toward the apex **Plasmoverna**
Sporangiohores slender, mostly regularly branched, pedicels less than 2 µm wide, not or only slightly broadening toward the apex 10
- 10 (9) Branching monopodial; branches arising at almost rectangular angles **Plasmopara**
Branching sub-monopodial; branches not arising at rectangular angles 11
- 11 (10) Sporangia aggregated at slightly to conspicuously widened terminal branches 12
Sporangia not aggregated, usually 2–3 on the pedicels at the terminal branches 14
- 12 (11) Branching of the sporangiohores often subdichotomous, aggregation regular, end of terminal branches strongly widened, bearing 4–6 sporangia **Bremia**
Branching of the sporangiohores usually sub-monopodial to monopodial, end of branches not strongly widened, aggregation of the sporangia irregular 13

13 (12) Aggregation of the sporangia pronounced, sporangia subglobose	Protobremia
Aggregation of the sporangia not always pronounced, sporangia ovoid to oval	Novotelnova
14 (11) Branches of the sporangiophores widening towards the ramifications, sporangia lacking distinct germination papilla	Paraperonospora
Branches of the sporangiophores not widening towards the ramifications, sporangia with germination papilla	Plasmopara
Not matching any of the above characteristics	15
15 (14) Sporangia coloured (grey, purple, olive, brown, black)	16
Sporangia hyaline, white to slightly yellow	17
16 (15) Sporangia produce zoospores; with a germination papilla	Pseudoperonospora
Sporangia germinating with a germ-tube; without a germination papilla	Peronospora
17 (15) Haustoria (globose / pyriform), large globose to lobate; ultimate branchlets sigmoid	Hyaloperonospora
Haustoria digitate or irregular; ultimate branchlets not sigmoid	18
18 (17) Haustoria irregular; intracellular mycelium present	Poakatesthia
Haustoria digitate; without intracellular mycelium	19
19 (18) Sporangia aggregated on dichotomous ultimate branches	Graminivora
Sporangia not aggregated	20
20 (19) Sporangioophores sympodial, branching at subacute to rectangular angles	Viennotia
Sporangioophores determinate, branching at acute angles, often intertwined	Perofascia

DISCUSSION

The majority of downy mildews are pathogenic to eudicots, encompassing more than 700 described species in 19 genera (Dick 2002, Thines 2014). Six of these genera are host-specific to a single eudicot plant family, namely, *Basidiophora*, *Benua*, *Bremia*, and *Paraperonospora* on *Asteraceae*; *Plasmoverna* on *Ranunculaceae*; and *Perofascia* on *Brassicaceae*. Other eudicot-infecting genera have hosts in more than one plant family. During the past decade it has been established that none of the eudicot-infecting genera contain species that infect monocots, the exceptions being two *Peronospora* species (Constantinescu 1991, Dick 2002, Göker *et al.* 2003, Voglmayr 2003). *Alliaceae* and *Poaceae* are the only monocot families infected by downy mildews. The GDM contain less than 30 species, but exhibit a high genetic and morphological diversity (Göker *et al.* 2003, Thines *et al.* 2006, 2007, 2008, Telle & Thines 2012). *Baobabopsis* is the eighth known genus of GDM.

In multigene phylogenetic reconstructions, GDM generally occupy basal positions (Göker *et al.* 2003, 2007, Thines *et al.* 2009). However the phylogenetic relationships amongst the GDM or with the three major monophyletic groups that contain all eudicot downy mildews remains unclear (Thines *et al.* 2009, Thines 2014). Phylogenetic studies have shown that the oomycete genus *Phytophthora* is paraphyletic with respect to the downy mildews (Cooke *et al.* 2000, Göker *et al.* 2007, Thines *et al.* 2009, Runge *et al.* 2011). However, recent phylogenomic studies inferred a monophyly for the four species of *Phytophthora* that were studied (Matari & Blair 2014, or a polyphyly of the downy mildews (Sharma *et al.* 2015). Further studies with more taxa of downy mildew and *Phytophthora* are warranted to ascertain the degree of paraphyly for *Phytophthora*. Morphological studies

also support a close link between the downy mildews and *Phytophthora*, as the basal GDM genera exhibit traits that are reminiscent of *Phytophthora* species (Thines 2009), for example: repeated outgrowth after sporangium dehiscence in *Viennotia* (Göker *et al.* 2003, Thines *et al.* 2009); the presence of intracellular mycelium in *Poakatesthia* (Thines *et al.* 2007); and the hyphal sporangiophores with limoniform sporangia in *Sclerophthora* (Thirumalachar *et al.* 1953, Telle & Thines 2012). This led Thines *et al.* (2009) to speculate that the root of downy mildew evolution might be in hosts of *Poaceae*. Interestingly, some monocot-infecting *Phytophthora* species are not easily cultivated and the *Phytophthora* species on *Cyperaceae*, which have not yet been included in detailed phylogenetic analyses, seem to be obligate biotrophs (Erwin & Ribeiro 1996). For the latter, the genus *Kawakamia* was introduced (Miyabe & Kawakami 1903), but not widely accepted, especially as the genus became heterogeneous after some additional species of *Phytophthora* were transferred to it (Sawada 1942, Sawada 1943). It is unclear if *Kawakamia* should be considered a member of the downy mildews and thereby represents a link between *Phytophthora* and the GDM. The eight genera of GDM exhibit a morphological diversity greater than that of the eudicot-infecting genera. *Baobabopsis* is morphologically unlike any other downy mildew, with broad cylindrical sporangiophores and ampulliform ultimate branchlets. Although the highest diversity of GDM seems to be in eastern Asia and Australia, two of the three genera of GDM with persistent sporangiophores are only known from Africa. More detailed investigations including multigene phylogenies of *Phytophthora* and downy mildews on native grasses, particularly from Australia and Africa, are needed to clarify if the evolutionary origins of the downy mildews are in *Poaceae* and to provide insights into how and from where these pathogens diversified.

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Author contributions: conceived the study RGS and MT; performed microscopy RGS; performed sequencing ST and YPT; analysed the data MT and YPT; wrote the manuscript MT and RGS, with contributions from all other authors.

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