**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. enneaagononis* sp. nov., collected on *Enneaagonon* 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*

**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), *Scleropithra* (Thirumalachar et al. 1953), *Sclerospora* (Schröter 1886), and *Viennotia* (Göker et al. 2003), have been described to date. Only three of these genera, *Peronosclerospora*, *Scleropithra*, 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, as well as on species of *Perotis* and *Enneaagonon* 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 *Enneaagonon* 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,
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 GTR+GAMMA 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.
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*Type species*: Baobabopsis donbarrettii

**Description**: Straminipila, Oomycota, Peronosporales, Peronosporaceae. Sporangiophores cover lower leaf surfaces, evanescent, aseptate, hyaline, cylindrical, 75–120 μm long, 20–28 μm wide, unbranched, with 5–20 ampulliform to lageniform ultimate branchlets. Sporangia hyaline, deciduous. Oogonia and oospores in leaves. Oogonia golden yellow, subglobose, 27–45 × 25–39 μm; wall (exosporium includes warts) 3–11 μm thick, uneven, verrucose with rounded warts. Oospores one per oogonium, pale to golden yellow, globose to broadly ellipsoidal, 19–29 × 18–28 μm; wall (endosporium) 1–3 μm 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 × 20–28 (n = 5) μm, with 5–20 terminal ampulliform to lageniform branches with a narrow neck 7–14 × 3–7 (n = 10) μm. Sporangia hyaline, deciduous, broadly ellipsoidal, slightly narrowed towards the base, 16–20 × 11–18 (n = 10) μm. 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) × (25–) 28–31.7–36 (–39) (n = 22) μm diam; wall (exosporium includes warts) 3–9 (n = 20) μm 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) × (18–25)–20–22.5–25(–28) (n = 22) µm diam; wall (endosporium) 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 donbarrettii* 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*...
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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.
**Sporangiophores unbranched (hyphal, club-shaped or cylindrical) ................................................................. 2**
Sporangiophores branched multiple times .......................................................................................... 6

**Sporangiophores hyphal, undifferentiated; oospores 30–80 μm diam .......................................................... 2**
Sporangiophores club-shaped or cylindrical; oospores smaller .................................................................. 3

**Sporangiophores persistent .......................................................... 3**
Sporangiophores evanescent ............................................................................................................. 5

**Sporangiophores stout, immersed in the stomata, ultimate branchlets short, stout, and hardly differentiated**
Sporangiophores slender, with an enlarged apex bearing sporangia on well-differentiated ultimate branchlets
.......................................................................................................................... Benua

**Sporangiophores less than 60 μm in length, club-shaped, sporangia irregular lemon-shaped, tapering towards the apex and pedicel .......................................................... Eraphthora**
Sporangiophores more than 60 μm in length, broadly club-shaped to cylindrical, sporangia obovoid to oval
.......................................................................................................................... Baobabopsis

**Sporangiophores evanescent .......................................................... 7**
Sporangiophores persistent ............................................................................................................. 8

**Sporangia produce zoospores; with an operculum .......................................................... Sclerospora**
Sporangia germinate with a germ-tube; without an operculum .......................................................... Peronosclerospora

**Haustoria small, globose to pyriform .......................................................... 9**
Haustoria large, digitate, globose, lobate or irregular ......................................................................... 15

**Sporangiophores stout and irregularly branched, pedicels wider than 2 μm, broadening widely toward the apex**
Sporangiophores slender, mostly regularly branched, pedicels less than 2 μm wide, not or only slightly broadening toward the apex .................................................................................................................. 10

**Branching monopodial; branches arising at almost rectangular angles .............................................. 10**
Branching sub-monopodial; branches not arising at rectangular angles ............................................. 11

**Sporangia aggregated at slightly to conspicuously widened terminal branches ...................................... 12**
Sporangia not aggregated, usually 2–3 on the pedicels at the terminal branches .................................. 14

**Branching of the sporangiophores often subdichotomous, aggregation regular, end of terminal branches strongly widened, bearing 4–6 sporangia ................................................. 12**
Sporangiophores 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) Sporangiohraphores sympodial, branching at subacute to rectangular angles ....................................... Viennotia
Sporangiophores 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, Breinia, and Paraperonospora on Asteraceae; Plasmovema 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 genera containing all eudicot downy mildews remains unclear (Thines et al. 2009, Thines et al. 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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