

## ***Wuestneia molokaiensis* and its anamorph *Harknessia molokaiensis* sp. nov. from *Eucalyptus***

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*Wuestneia molokaiensis* is newly described from *Eucalyptus robusta* twigs collected in Hawaii. Single ascospore strains produced a *Harknessia* anamorph and microconidial state in culture. *W. molokaiensis* and *H. molokaiensis* are compared with previously described species in these genera and described as new. *W. molokaiensis* is distinguished based on its smaller ascospores with persistent mucous sheaths, and *H. molokaiensis* based on its smaller, ellipsoidal conidia.

Keywords: *Eucalyptus*, *Harknessia*, leaf spot, stem canker.

The genus *Harknessia* Cooke has been the subject of several recent monographs (Sutton, 1971; Nag Raj & DiCosmo, 1981; Nag Raj, 1993), with the latter treatment recognising 26 species. Since then 9 additional species have been described (Crous & al., 1993; Furlanetto & Dianese, 1998; Swart & al., 1998; Yuan & al., 2000). Teleomorphs have been described in *Wuestneia* Auersw. (Reid & Booth, 1989; Sutton & Pascoe, 1989). The first cultural confirmation of this relationship was made by Crous & al. (1993), and later by Yuan & Mohammed (1997), bringing the total number of accepted taxa in *Wuestneia* up to 12 species and one variety.

Although not many data are available relating to their pathogenicity, species of *Harknessia* are commonly associated with leaf spots, as well as stem and branch cankers (Crous & al., 1989, 1993; Yuan & Mohammed, 1997; Yuan & al., 2000). Preliminary results from stem inoculations on eucalypts with *H. eucalypti* Cooke apud Cooke & Harkn. by Yuan & al. (2000) failed to induce lesions, whereas leaf inoculations with *H. hawaiiensis* F. Stevens & E. Young on 12 different eucalypt species produced prominent lesions on some species, and none on others (Crous, unpubl. data). These observations, as well as the fact that *Harknessia* spp. are most commonly encountered on lesions with other primary pathogens,

suggest that the majority of these species are possibly secondary or weak pathogens.

The present paper results from a collection of an undescribed *Wuestneia* species and its *Harknessia* anamorph from *Eucalyptus*. The aim of this study was thus to describe these taxa, and to compare them with others known in these genera.

### Materials and methods

Perithecia of a *Wuestneia* sp. obtained from *Eucalyptus* twigs were dissected in sterile water on plates containing 2% malt extract agar (MEA) (Biolab, Midrand, Johannesburg, South Africa). Plates were inverted, and incubated at 25 C in the dark. Single germinating ascospores were transferred to fresh MEA plates, and later to divided plates containing MEA, and carnation leaf agar (CLA) (Fisher & al., 1982; Crous & al., 1992). Plates were incubated at 25 C under continuous near-ultraviolet light to enhance sporulation. Wherever possible, thirty measurements were made of structures mounted in clear lactophenol, and the averages determined. Colony colours (top and bottom) were rated after 14 d on MEA at 25 C in the dark, using the colour charts of Rayner (1970). The holotype specimen was lodged at the Bishop Museum, Honolulu (BISH), and isotypes at the Dept. of Plant Pathology, Washington State University, Pullman (WSP), and the National Collection of Fungi in Pretoria, South Africa (PREM), and ex-type cultures maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch, South Africa (STE-U), and at the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

### Results and discussion

***Wuestneia molokaiensis*** Crous & J. D. Rogers, **sp. nov.** – Figs. 1, 4–8.

Anamorph: *Harknessia molokaiensis* Crous & J. D. Rogers, sp. nov.

Perithecia solitaria, in ramis inclusa, collis emergentibus, ectostromate cellularum furfuracearum brunnearum vel fulvarum circumcincta, brunnea vel fusca, globosa vel subglobosa, 250–350 µm diam.; peridio 10–15 µm lato, 4–6 stratis cellularum pseudoparenchymatarum sectione texturae angularis praedito, superficie texturae epidermoideae composita; ostiola usque ad 60 µm lata. Asci clavati vel subcylindrici, 65–110(–130) µm longitudine tota × 11–17(–20) µm crassi, unitunicati, brevistipitati, annulo apicali distincto destituti, octo ascosporis uniseriatis vel biseriatis praediti. Ascosporae hyalinae, vagina irregulari 1–3 µm crassa circumcinctae, ellipsoideae, pariete crasso praeditae, intus granulosaе, 14–22(–24) × 5–8 µm.



Figs. 1–3. – *Wuestneia molokaiensis* and its *Harknessia molokaiensis* anamorph (holotype). – 1. Asci and ascospores. – 2. Conidia and conidiophores. – 3. Microconidia and conidiogenous cells. – Bar = 10  $\mu$ m.

***Harknessia molokaiensis*** Crous & J. D. Rogers, **sp. nov.** – Figs. 2, 3, 9–14.

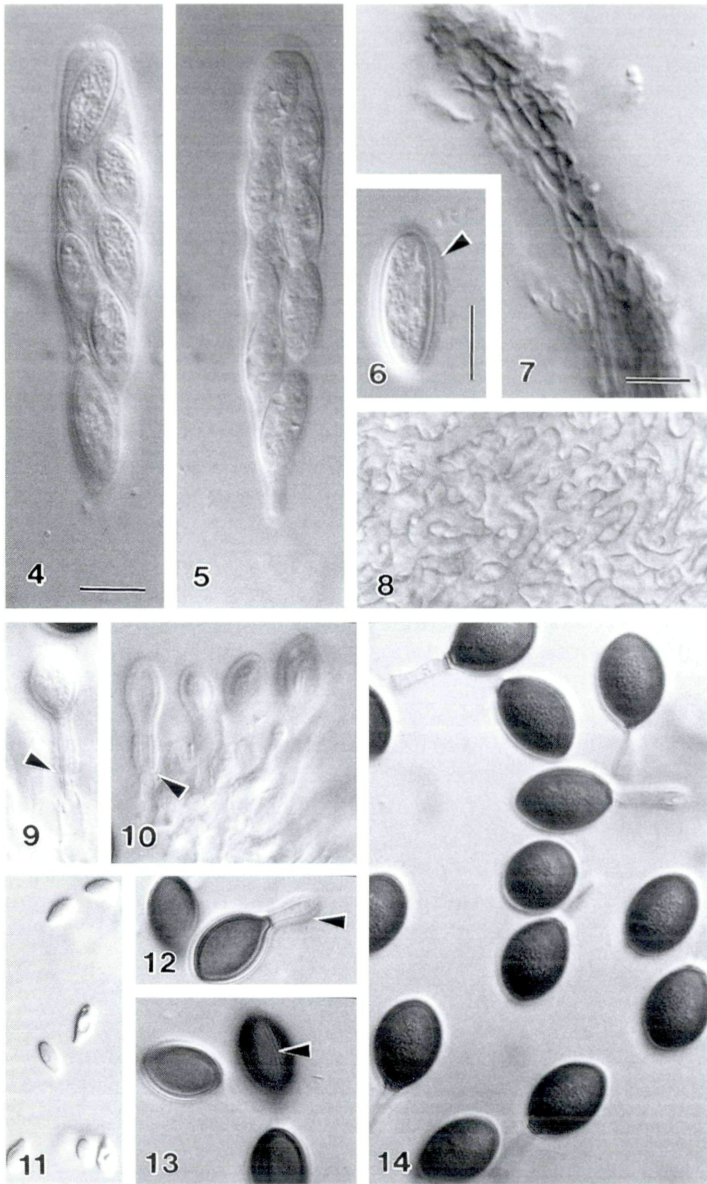
Ab *Harknessia eucalyptorum* differt in macroconidiis  $14\text{--}17 \times 8\text{--}11 \mu\text{m}$  et in microconidiis  $4\text{--}5 \times 2 \mu\text{m}$ .

**Etymology.** – named after the collection locality, Molokai Island, Hawaii.

**Ascomata** caulicolous, perithecial, solitary, subepidermal with emergent necks, surrounded by an ectostromatic layer of furfuraceous brown to yellow-brown cells. – **Perithecia** medium to dark brown, globose to subglobose,  $250\text{--}350 \mu\text{m}$  diam.; peridium  $10\text{--}15 \mu\text{m}$  wide, consisting of 4–6 layers of medium brown pseudo-parenchymatous cells of *textura angularis* (in section), of *textura epidermoidea* in surface view; ostiole up to  $60 \mu\text{m}$  wide. – **Asci** clavate to subcylindrical,  $65\text{--}110\text{--}(130) \times 11\text{--}17\text{--}(20) \mu\text{m}$ , unitunicate, short-stipitate, lacking a well-defined apical apparatus, containing 8, uni- to biseriate ascospores. – **Ascospores** hyaline, surrounded by an irregular sheath,  $1\text{--}3 \mu\text{m}$  wide, ellipsoid, thick-walled, granular,  $14\text{--}22\text{--}(24) \times 5\text{--}8 \mu\text{m}$  ( $\bar{x} = 18 \times 6 \mu\text{m}$ ). – **Conidiomata** produced on MEA in culture, separate, scattered, pycnidial, unilocular, globose to subglobose,  $300\text{--}450 \mu\text{m}$  diam.; walls of 5–7 layers of medium brown *textura angularis*,  $20\text{--}30 \mu\text{m}$  diam. – **Conidiophores** lining conidiomatal cavity, reduced to conidiogenous cells. – **Conidiogenous cells** ampulliform to lageniform, hyaline, smooth, formed from inner layer of the conidiomatal wall, proliferating once or twice percurrently,  $7\text{--}10 \times 4\text{--}6 \mu\text{m}$ . – **Macroconidia** ellipsoid to ovoid with a truncate base, medium brown, aseptate, apex obtuse to bluntly apiculate, smooth, except for longitudinal striations in restricted areas on some conidia, with a lighter central area consisting of small, aggregated droplets,  $14\text{--}17 \times 8\text{--}11$  ( $\bar{x} = 15 \times 10 \mu\text{m}$ ); basal appendage hyaline, unbranched, frequently surrounded by a mucous sheath,  $5\text{--}17 \times 2\text{--}3 \mu\text{m}$ . – **Microconidiogenous cells** in same or separate conidioma, subcylindrical, hyaline, smooth, with minute apical periclinal thickening,  $5\text{--}6 \times 1.5\text{--}2 \mu\text{m}$ . – **Microconidia** hyaline, smooth, aseptate, limoniform to ellipsoid or fusoid with obtuse apex and truncate base,  $4\text{--}5 \times 2 \mu\text{m}$ .

**Cultural characteristics.** – Colonies white to pale luteous (19f) (surface), and pale luteous to luteous (19f–17b) (bottom), sienna in the centre (13i), with feathery margins and moderate, fluffy, off-white aerial mycelium after 14 d at  $25^\circ\text{C}$  in the dark.

**Host.** – *Eucalyptus robusta* Sm. (Myrtaceae).



Figs. 4–14. – *Wuestneia molokaiensis* and its *Harknessia molokaiensis* anamorph (holotype). – 4, 5. Asci. – 6. Ascospore with mucous sheath (arrow). – 7. Vertical section through pseudothecial wall. – 8. Pseudothecial wall in surface view. – 9, 10. Conidiogenous cells with persistent sheath covering conidium and appendage up to conidiogenous locus (arrows). – 11. Microconidia. – 12. Macroconidium with mucous sheath enclosing basal appendage (arrow). – 13. Macroconidium with striations (arrow). – 14. Macroconidia with basal appendage. – Bars = 10  $\mu\text{m}$ .

Distribution. – U.S.A. (Molokai Island, Hawaii).

Specimen examined. – U.S.A., Hawaii, Molokai Island, Kamacou Forest Reserve, dead branches of *E. robusta*, J. D. Rogers, 9 Jan. 2000, holotypes in BISH, isotypes in WSP and PREM, culture ex-type STE-U 3797.

*Wuestneia molokaiensis* can be compared with *W. karvarrae* (B. Sutton & I. Pascoe) Z.Q. Yuan, *W. eucalyptorum* Crous, M. J. Wingf. & Nag Raj and *W. epispora* Z.Q. Yuan. Ascospores of *W. molokaiensis* are surrounded by a mucilaginous sheath, as are those of *W. epispora*, which was also described from eucalypt branches collected in Tasmania (Yuan & Mohammed, 1997). In *W. epispora*, however, the ascospores are larger ( $20\text{--}35 \times 9\text{--}15 \mu\text{m}$ ), and the sheath more prominent than in *W. molokaiensis*. Ascospores of both *W. eucalyptorum* ( $13\text{--}28 \times 13\text{--}20 \mu\text{m}$ ; Crous & al., 1993) and *W. karvarrae* ( $23\text{--}27 \times 9\text{--}11 \mu\text{m}$ ; Sutton & Pascoe, 1989) are without any sheath, and are also larger than those of *W. molokaiensis* ( $14\text{--}22 \times 5\text{--}8 \mu\text{m}$ ).

Several species of *Harknessia* are comparable with *H. molokaiensis* based on macroconidium shape and appendage length. Of these species, however, *H. molokaiensis* has smaller conidia ( $14\text{--}17 \times 8\text{--}11 \mu\text{m}$ ) than *H. eucalypti* Cooke ( $19\text{--}28 \times 11\text{--}15 \mu\text{m}$ ), *H. eucalyptorum* Crous, M. J. Wingf. & Nag Raj ( $16\text{--}29 \times 9\text{--}14 \mu\text{m}$ ), *H. karvarrae* B. Sutton & I. Pascoe ( $17\text{--}18 \times 12\text{--}12.5 \mu\text{m}$ ) and the *Harknessia* anamorph of *W. epispora* ( $18\text{--}35 \times 12\text{--}16 \mu\text{m}$ ) (Sutton & Pascoe, 1989; Crous & al., 1993; Nag Raj, 1993; Yuan & Mohammed, 1997). Based on macroconidium dimensions, therefore, *H. molokaiensis* is easily distinguishable from other species of *Harknessia*.

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