Mycologia, 85(1), 1993, pp. 108–118.

HARKNESSIA SPECIES OCCURRING IN SOUTH AFRICA

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ABSTRACT

Three new species of *Harknessia* are described from leaves of woody hosts in South Africa. *Harknessia eucalyptorum* and its teleomorph, *Wuestneia eucalyptorum*, are described from *Eucalyptus* leaves. In this case, the teleomorph, anamorph connection was proven in culture. *Harknessia fusiformis* is described from *Eucalyptus* leaf litter, while *H. syzygii* is described from *Syzygium cordatum*. Additional collections of *H. uromycoides* and *H. hawaiiensis* are also discussed, and a microconidial state described for the latter species.

Key Words: *Eucalyptus*, foliicolous fungi, *Harknessia*, systematics, *Syzygium cordatum*, *Wuestneia*

Three comprehensive reviews on this genus have been published (Sutton, 1971, 1980; Nag Raj and Di Cosmo, 1981) since *Harknessia* Cooke was first described (Cooke and Harkness, 1881). Recent studies (Galán et al., 1986; Sutton and Pascoe, 1989) have led to the description of three additional species, including the *Cryptosporella* Saccardo teleomorph of *Harknessia karwarrae* Sutton & Pascoe. In a recent study of the genus *Cryptosporella*, Reid and Booth (1989) concluded that *Wuestneia* Auerswald is the correct name for fungi previously assigned to *Cryptosporella*.

Several *Harknessia* spp. are known to produce microconidial states. The first record of a microconidial state for *Harknessia* is that described by Sutton (1971) for *H. antarctica* Spegazzini. Subsequently, Nag Raj and Di Cosmo (1981) described microconidial states for seven species, and Sutton and Pascoe (1989) described an additional two.

Harknessia uromycoides (Speg.) Speg. was the first species of the genus reported to occur on *Eucalyptus* leaves in South Africa (Doidge, 1950). In this paper we describe *Wuestneia eucalyptorum* and its *Harknessia* anamorph from *Eucalyptus* leaves, *H. syzygii* from *Syzygium cordatum* Hochst. and *H. fusiformis* from *Eucalyptus* leaf litter. Additional collections of *H. uromycoides* and *H. hawaiiensis* Stevens & Young are also discussed. A microconidial state is described for the latter species.

MATERIALS AND METHODS

Symptomatic leaves and leaf litter were collected at regular intervals since 1987 at a *Eucalyptus* provenance trial planted on Stellenbosch Mountain in the Western Cape, as well as at different locations in Transvaal, Natal and Orange Free State Provinces. In addition to *Eucalyptus* leaves, leaf litter of *S. cordatum* was collected in Natal and Transvaal Provinces.

Leaves were incubated in moist chambers at 25 C under near-ultraviolet light for 3 days, after which time furfuraceous margins and exuding black spore masses indicated the presence of *Harknessia* conidiomata. To detect the presence of a teleomorph, leaves were incubated in the dark at 4–7 C for 3 days before incubating as explained above. Material was mounted in water, lactophenol cotton blue, erythrosin, 3% KOH as well as Melzer's reagent. Wherever possible, 50 examples of each structure were measured and averages given.

Single conidial and ascospore isolates were obtained using the dilution plating technique on malt extract

agar (15 g Difco agar, 20 g Oxoid malt extract, 1 L water) (MEA). To induce sporulation, cultures were placed on MEA, carnation-leaf agar (CLA) (Fisher et al., **1982**; Crous et al., **1992**) or *Eucalyptus* leaf agar (leaf discs sterilized using 1,2-propylene oxide), and subsequently incubated at 20 and 25 C under near-ultraviolet/white light.

The optimum growth temperature was determined for each of the fungi on MEA. One single-conidial isolate was taken as representative of each species, and used in the growth studies. Optimum growth temperature (expressed as colony diameter) was determined after isolates were incubated for 3 days in the dark at eight temperature settings ranging from 5–40 C at 5 C intervals. Each treatment had three replications and the experiment was repeated.

RESULTS AND DISCUSSION

During a study of fungi occurring on *Eucalyptus* leaves in 1988, a *Harknessia* sp. was found on leaves of *E. globulus* Labill., *E. nitens* (Deane & Maid.) Maid. and *E. maidenii* F. Muell. at Stellenbosch in the Western Cape Province. Examination of the conidiomata showed conidia to be 16–22 × 8–14 μm ($\bar{x}$ = 19 × 12 μm), broadly ventricose with apiculate to obtuse apices. The appendages were 2–18 ($\bar{x}$ = 8.5 μm), suggesting that this fungus was *Harknessia eucalypti* Cooke *apud* Cooke & Harkn. (Crous et al., **1989**). Since these initial collections, additional material has been obtained from the same area on leaves of *E. andrewsii* Maid., *E. grandis* Hill: Maid., *E. tereticornis* Sm. and *E. viminalis* Labill. An examination of these collections together with cultural studies has shown that the South African material differs morphologically from *H. eucalypti*. Conidia were found to vary in shape from ventricose to broadly ventricose with apiculate or rounded apices. Conidia were 16–29 × 9–15 μm ($\bar{x}$ = 22 × 12 μm) in size, thus similar to those of *H. eucalypti* (FIG. 1), 19–28 × 11–15 μm, and *H. podocarpi* Lindquist & Sutton *apud* Sutton, 17.5–26 × 11–15 μm (Nag Raj and Di Cosmo, **1981**).

The conidia from these new collections from South Africa could be distinguished from those of *H. eucalypti* by their more obtuse conidial apices and longer appendages. Although conidial dimensions of these collections fit those of *H. podocarpi*, the conidia differ from this species by not being striate and not having persistent mucous sheaths. The fungus previously recorded as *H. eucalypti* in South Africa (Crous et al., 1989) and that noted in the more recent collections are therefore described below as a new species of *Harknessia*.

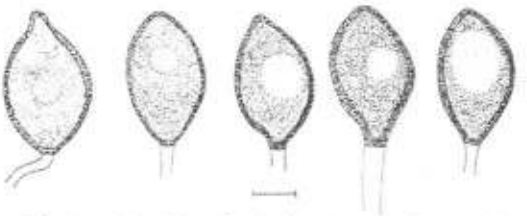


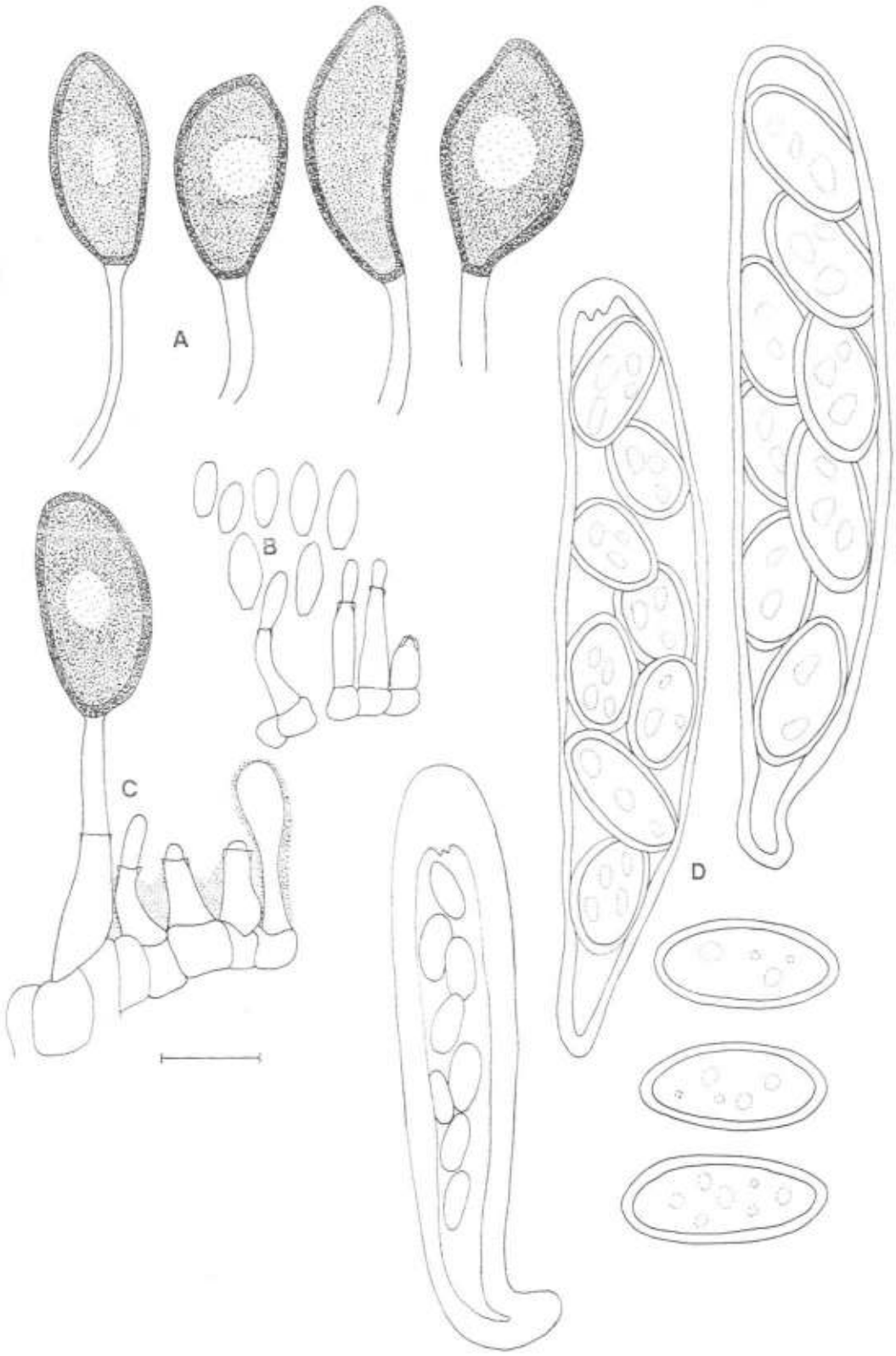
FIG. 1. Conidia of *Harknessia eucalypti* (IMI 146779). Bar = 10 μm.

Harknessia eucalyptorum Crous, Wingfield *et* Nag Raj, *sp. nov.* FIGS. 2, 3, 11

Conidiomata separata, immersa, globosa ad subglobosa, unilocularia, erumpentia et punctiformia, usque ad 350 μm diam, ostiolum margine furfuraceo, pallide brunneo; parietes basales et laterales, 5–7 cellulis crasses, ex textura angulari compositi. Conidiophora ad cellulas conidiogenas deminuta. Cellulae macroconidiogenae discretae, hyalinae, laeves, lageniformes, doliiformes ad cylindricae, 6–20 × 3.5–6.2 μm basi, ex cellulis interioribus parietis conidiomati oriundae, conidium unum efferentes vel proliferatione una enteroblastice. Macroconidia holoblastica, late ventricosa, cum guttula centrali, aseptata, atrobrunnea, apice obtuso ad apiculato, basi truncata, 16–29 × 9–24 μm ($\bar{x}$ = 22 × 11 μm) in foliis, 14.5–24 × 10.5–14 μm ($\bar{x}$ = 19.5 × 12.5 μm) in cultura; appendix hyalina, non ramosa, basalis, 3–16 μm ($\bar{x}$ = 10.5 μm) longa in foliis, usque ad 12 μm longa in cultura. Cellulae microconidiogenae subcylindricae ad lageniformes, hyalinae, laeves, usque ad 15 μm longae, 2.5–4 μm crassae basi. Microconidia holoblastica, apicalia vel lateralia, hyalina, aseptata, laevia, ellipsoidea ad fusiformia, 4.5–9 × 2–3.5 μm.

SPECIMEN TYPICUM in foliis vivis *Eucalypti andrewsii* Maid., Stellenbosch Mountain, Stellenbosch, Western Cape, R.S.A., 20 Dec. 1989, *P.W. Crous*, HOLOTYPUS, PREM 50813 (DAOM 211793, IMI 338270a, ISOTYPI).

Foliicolous and caulicolous. Conidiomata separate, immersed, globose to subglobose, unilocular, erumpent and punctiform, up to 350 μm diam, ostiole with a light brown furfuraceous margin; basal and lateral walls five to seven cells thick, composed of textura angularis. Conidiophores reduced to conidiogenous cells. Macroconidiogenous cells discrete, hyaline, smooth, lageniform, doliiform to cylindrical, 6–20 μm long, 3.5–6.2 μm wide at the base, formed from inner cells of conidiomatal wall, producing a single conidium or proliferating enteroblastically once, periclinal thickening minute, collarette absent. Macroconidia holoblastic, broadly ventricose with central guttule, aseptate, dark brown, astriate, apex obtuse to bluntly apiculate, base truncate, 16–29 × 9–24 μm ($\bar{x}$ = 22 × 11 μm) on


A
B
C
D

leaves, 14.5–24 × 10.5–14 μm ($\bar{x}$ = 19.5 × 12.5 μm) in culture; basal appendage hyaline, unbranched, 3–16 μm ($\bar{x}$ = 10.5 μm) on leaves, up to 12 μm in culture. Conidiogenous cells and appendages sometimes enclosed in a nonpersistent mucilaginous sheath. Microconidiogenous cells in the same or in separate conidiomata, subcylindrical to lageniform, hyaline, smooth walled, with cytoplasmic channel and periclinal thickening but no collarette, up to 15 μm long, and 2.5–4 μm wide at base. Microconidia holoblastic, apical or lateral, hyaline, aseptate, smooth, ellipsoidal to fusiform, 4.5–9 × 2–3.5 μm.

HOSTS: *Eucalyptus andrewsii, E. maidenii, E. globulus, E. grandis, E. nitens, E. tereticornis, E. viminalis.*

SPECIMENS EXAMINED. SOUTH AFRICA. WESTERN CAPE: Stellenbosch, *E. andrewsii*, 20 Dec. 1989, *P.W. Crous* (HOLOTYPE, PREM 50813; ISOTYPES, DAOM 211793, IMI 338270a) *E. andrewsii*, Oct. 1989, *P.W. Crous* (PREM 50814); *E. maidenii*, 20 Dec. 1989, *P.W. Crous* (PREM 50815); *E. maidenii*, Feb. 1988, *P.W. Crous* (PREM 49105); *E. maidenii*, 30 Sept. 1988, *P.W. Crous* (PREM 50816; culture, PPRI 4295); *E. maidenii*, 8 Dec. 1988, *P.W. Crous* (PREM 50817); *E. grandis*, Oct. 1989, *P.W. Crous* (PREM 50818); *E. grandis*, Oct. 1989, *P.W. Crous* (PREM 50819); *E. nitens*, Feb. 1988, *P.W. Crous* (PREM 49104); *E. tereticornis*, 15 Nov. 1988, *P.W. Crous* (PREM 50821); *E. viminalis*, 30 Sept. 1988, *P.W. Crous* (PREM 50822); *E. viminalis*, 15 Nov. 1988, *P.W. Crous* (PREM 50823); *E. viminalis*, 8 Dec. 1988, *P.W. Crous* (PREM 50824); *Eucalyptus* sp., 7 July 1988, *P.W. Crous* (PREM 50826); *Eucalyptus* sp., 29 Sept. 1988, *P.W. Crous* (PREM 50827); *Eucalyptus* sp., 17 Nov. 1988, *P.W. Crous* (PREM 50828); *Eucalyptus* sp., 8 July 1989, *P.W. Crous* (PREM 50829). EASTERN TRANSVAAL: Jessievale, *E. nitens*, 24 Nov. 1988, *P.W. Crous* (PREM 50820).

In this study we observed variation in the symptoms associated with *H. eucalyptorum*. It was usually found associated with a leaf and peduncle necrosis of various *Eucalyptus* spp., and although lesions were always distinct and light brown in color, they were surrounded by a large chlorotic band on *E. tereticornis* but not on other host species. On *E. viminalis*, however, lesions occurred mainly along the leaf margins.

Isolates of *H. eucalyptorum* grew optimally on MEA at 25 C, and sporulated after 2 wk. Colonies were white to pale yellow colored, eventually turning olivaceous green at the center when sporulating. Conidia from cultures derived from different host species were similar in size and appendage length to those occurring on leaves (TABLE I). Conidia from cultures were generally broadly ventricose with obtuse apices, and a central, globose guttule. Although a microconidial state was present on collections made from *E. nitens* and *E. maidenii* leaves, no microconidia were formed in culture.

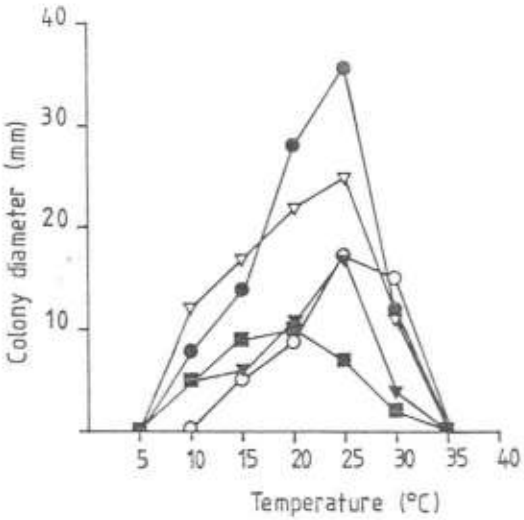


FIG. 3. Colony diameters (mm) of *Harknessia* spp. on MEA after 3 da at various temperatures in the dark. *Harknessia eucalyptorum* (PPRI 4295), ▼; *H. hawaiiensis* (PPRI 4298), ●; *H. uromycoides* (PPRI 4296), ■; *H. fusiformis* (PPRI 4297), ▽; *H. syzygii* (PPRI 4299), ○.

In recent collections of *H. eucalyptorum* [conidia 18–29 × 9–14 μm ($\bar{x}$ = 22 × 11 μm), appendages 3–16 μm ($\bar{x}$ = 10.5 μm)] from leaves of *E. andrewsii* and *E. maidenii*, the conidiomata were associated with the ascocarps of another fungus, and hyphal connections were also observed between the two fructification types. Colonies obtained from single ascospores on MEA were white, flocculent, turning the medium caramel brown in color. Conidiomata with conidia of *H. eucalyptorum* were observed after 3 months on MEA to which sterilized pieces of *Eucalyptus* leaf had been added. We therefore believe that the fungus producing ascomata found on *Eucalyptus* leaves is the teleomorph of *H. eucalyp-*

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FIG. 2. A–D. *Wuestneia eucalyptorum* and its anamorph *H. eucalyptorum*. Bar = 10 μm. A. Macroconidia (PREM 50813). B. Microconidia (PREM 49104). C. Macroconidium and conidiogenous cells. D. Asci and ascospores (PREM 50830).

TABLE I

CONIDIAL AND APPENDAGE DIMENSIONS OF *HARKNESSIA EUCALYPTORUM*

Conidial length × width (μm)[a]	Appendage length (μm)[a]	Host	Conidium (length/ width ratio)	Specimen
16–22 × 8–14 (19 × 12)	2–18 (8.5)	*E. maidenii*	1.6/1	PREM 49105
20–32 × 9–15.5 (27 × 13)	2–18 (10)	*E. maidenii*	2.0/1	PREM 50815
20–27.5 × 11–14.5 (22.5 × 12)	7–20 (14)	*E. nitens*	1.9/1	PREM 50820
21–29 × 11–15.5 (26.5 × 13.5)	5.5–21 (13.5)	*E. tereticornis*	2.0/1	PREM 50821
20–28 × 10–13 (25 × 11)	5–18 (12)	*E. tereticornis*	2.3/1	in vitro
18–22 × 9–12.5 (19.5 × 12)	2–18.5 (10.5)	*E. viminalis*	1.6/1	PREM 50822
20–27.5 × 10–16.5 (24 × 13)	4–23 (13.5)	*Eucalyptus* sp.	1.8/1	PREM 50825
17.5–20 × 9–13.5 (19.5 × 12)	5–14.5 (9)	*Eucalyptus* sp.	1.6/1	PREM 50827
16.5–23 × 11–14 (20 × 12)	7–14 (10)	*Eucalyptus* sp.	1.7/1	PREM 50828
18–29 × 9–14 (22 × 11)	3–16 (10.5)	*E. andrewsii*	2.0/1	PREM 50813
14.5–24 × 10.5–14 (19.5 × 12.5)	3–12 (10)	*E. andrewsii*	1.6/1	in vitro

[a] Averages in brackets are representative of 50 measurements.

torum. Moreover, the structure of the perithecia and morphology of the asci and ascospores are consistent with those of species of *Wuestneia*, which are known to have teleomorphs of *Harknessia*. The yellow brown ectostromata that did not produce a purple reaction in KOH, as well as the brown conidia, place this fungus in the section *Sordida* Reid & Booth (Reid and Booth, **1989**). We describe the teleomorph state of *H. eucalyptorum* as follows:

Wuestneia eucalyptorum Crous, Wingfield et Nag Raj, *sp. nov.* FIGS. 2, 12, 13

Ascomata perithecialia, singula vel usque ad 5 aggregata, immersa, disco furfuraceo brunneo, collo peritheciali erumpenti ad depresso, peritheciis usque ad 250 μm diam, parietibus 7–15 μm crassis, quinque cellullis crassis, ex textura angulari atrobrunnea compositis, versus centrum pallidioribus. Asci unitunicati, cylindrici ad clavati, hyalini, laeves, octospori, 70–110 × 13–20 μm, poro iodo tincto haud caerulescente. Ascosporae asepiatae, uni- vel biseriatae, ellipsoideae, ad apicem et basim obtusae, hyalinae, parietibus crassis, guttulatae, laeves, 13–28 × 8–13 μm.

SPECIMEN TYPICUM in phyllodiis *Eucalypti andrewsii* Maid., Stellenbosch Mountain, Stellenbosch, Western Cape, R.S.A., 20 Dec. 1989, *P.W. Crous*, HOLOTYPUS, PREM 50830 (DAOM 211794, IMI 338270b, ISOTYPI).

Perithecial ascomata single or aggregated in groups of up to five, immersed, disc furfuraceous brown, perithecial neck emergent to depressed, perithecia up to 250 μm in diam, walls 7–15 μm thick, and comprised of up to five cell layers, dark brown, *textura angularis*, with cells becoming paler towards the center. Asci unitunicate, cylindrical to clavate, hyaline, smooth, eight-spored, 7–110 × 13–20 μm, apical apparatus not blueing in iodine after rehydration in 2% aqueous KOH or distilled water. Ascospores aseptate, uni- or biseriate, ellipsoidal, obtuse at ends, hyaline, thick-walled, guttulate, smooth, 13–28 × 8–13 μm.

ANAMORPH: **Harknessia eucalyptorum** Crous, Wingfield *et* Nag Raj, *sp. nov.*

SPECIMENS EXAMINED. SOUTH AFRICA. WESTERN CAPE: Stell., *E. andrewsii*, 20 Dec. 1989, *P.W. Crous* (HOLOTYPE, PREM 50830; ISOTYPES, DAOM 211794, IMI 33827b); *E. maidenii*, 20 Dec. 1989, *P.W. Crous* (PREM 50831); *Eucalyptus* sp., Feb. 1988, *P.W. Crous* (PREM 50825), PARATYPES.

Although asci and ascospores of the teleomorph *Cryptosporella karwarrae* Sutton & Pascoe were similar in size to those of *W. eucalyptorum*, *H. karwarrae* Sutton & Pascoe had smaller conidia with shorter appendages (Sutton and Pascoe, **1989**). No other *Wuestneia* sp. with a *Harknessia* anamorph (Nag Raj and Di Cosmo, **1981**; Reid and Booth, **1989**) is similar to *W. eucalyptorum*.

Harknessia uromycoides (Speg.) Speg., An. Soc. Cient. Argent. **13**: 21 (1882). FIGS. 3, 4, 14

Synonymy in Sutton (**1971**).

This species occurs not only on leaves but also on twigs, petioles and seed capsules of *Eucalyp-*

tus spp. It has been reported from Argentina, Australia, California, U.S.A. and Portugal (Sutton, **1971**, **1980**). *H. uromycoides* is not host specific, and hosts include members of the Leguminosae (Swart, **1972**) and Proteaceae (Sutton and Pascoe, **1989**). Doidge (**1950**) referred to a record of *H. uromycoides* (PREM 2261) on *E. amygdalina* Labill. from a nursery in the Transvaal Province of South Africa, but this collection lacks any fungal material (Crous et al. **1989**).

New collections of *H. uromycoides* have recently been made from necrotic leaf tips and leaf litter of *Eucalyptus* spp. in the Western Cape and Transvaal, where it probably exists as a saprophyte.

Conidiomata of *H. uromycoides* were found on peduncles and laminae of leaves. They were globose to subglobose, initially subepidermal, protruding with age and exuding black conidial masses. Conidiogenous cells were almost always restricted to the basal wall, and were long lageniform to cylindrical, hyaline and unbranched. Appendages attached to conidiogenous cells were up to 110 μm in length. Conidia were oblong-ellipsoidal with apiculate apices and large globose to irregular guttules. Conidia were 15–29.5 × 9.2–13.8 μm ($\bar{x}$ = 22 × 12.5 μm) with hyaline persistent appendages 30–100 μm ($\bar{x}$ = 56 μm). Some conidia had a longitudinal band of paler pigment, characteristic of this species (Sutton, **1971**).

Cultures grew slower and sporulated less than any other *Harknessia* sp. tested in this study. Adequate sporulation was obtained for all isolates after 2–3 wk on CLA. Cultures had a dense white to pale yellow mycelium on MEA with denser flocculent borders forming a ridge around the colonies. After 5 wk, colonies became olivaceous green and sparse sporulation was observed on MEA. Optimal growth occurred at 20 C (FIG. 3). Conidia of *H. uromycoides* from cultures were slightly smaller than those observed in vivo but were still oblong-ellipsoidal and apiculate with characteristic longitudinal bands of paler pigment, and had long appendages attached.

HOSTS: *E. globulus*, *E. amygdalina*, *Eucalyptus* spp.

SPECIMENS EXAMINED. ARGENTINA. Buenos Aires, *E. globulus*, May 1880, *C. Spegazzini* (HOLOTYPE, IMI 14852, 14853). SOUTH AFRICA. TRANSVAAL: Pretoria, *E. amygdalina*, 24 Apr. 1912, *E.M. Doidge* (PREM 2261). WESTERN CAPE: Stellenbosch, *Eucalyptus* sp., 29 Sept. 1988, *P.W. Crous* (PREM 50832); *Eucalyptus* sp., 30 Sept. 1988, *P.W. Crous* (PREM 50833); *Eucalyptus* sp., 8 Dec. 1988, *P.W. Crous* (PREM 50834; culture PPRI 4296); Grabouw, Sir Lowry's Pass, *Eucalyptus* sp., Feb. 1990, *P.W. Crous* (PREM 50835).

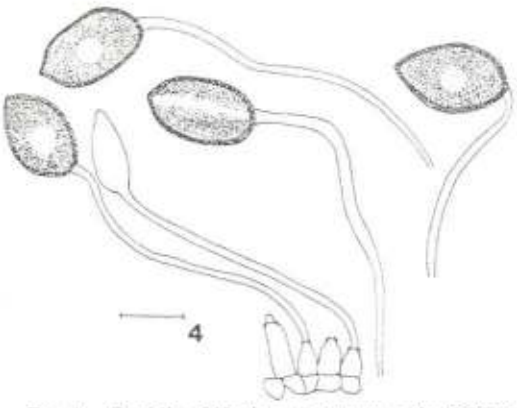


FIG. 4. Conidia of *Harknessia uromycoides* (PREM 50832). Bar = 10 μm.

During Feb. 1990, *Eucalyptus* leaf litter was collected under trees growing at Bloemfontein in the Orange Free State. An examination of these leaves revealed a *Harknessia* sp., chiefly characterized by very long, ventricose conidia and long appendages. The only other species with which it could be confused is *H. spermatoidea* Galán, Moreno & Sutton (FIG. 5). However, an examination of the type collection of the latter species (IMI 295508) showed that it had shorter

FIG. 5. Conidia of *Harknessia spermatoidea* (IMI 295508). Bar = 10 μm.

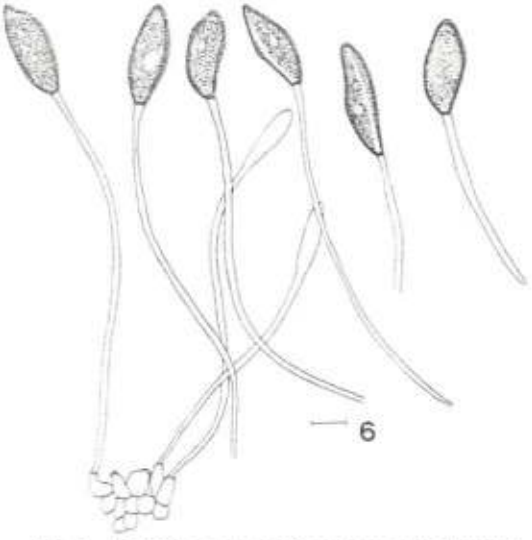


FIG. 6. Conidia and conidiogenous cells of *Harknessia fusiformis* (PREM 50836). Bar = 10 μm.

appendages and smaller conidia. Another important difference between these two fungi was the shape of immature conidia, being spermatoid in *H. spermatoidea* and more fusiform in the South African collection. The collection from *Eucalyptus* leaves at Bloemfontein is described as follows:

Harknessia fusiformis Crous, Wingfield *et* Nag Raj, *sp. nov.* FIGS. 3, 6, 15

Foliicola. Conidiomata immersa, erumpentia, unilocularia, globosa, usque ad 400 μm diam, ostiolo centrali furfuraceo; parietes basales et laterales conidiomatorum 3–6 cellulis crasses, ex textura angulari hyalina ad pallide brunnea compositi. Conidiophora ad cellulas conidiogenas deminuta. Cellulae conidiogenae discretae, hyalinae, laeves, lageniformes ad doliiformes, 7–12 μm longae, 5–7 μm latae basi, usque ad bis proliferatione enteroblastica. Conidia holoblastica, ventricosa vel fusiformi-ellipsoidea cum guttula centrali vel multiguttulata, atrobrunnea, laevia, non striata, saepe longitudinali sectione clariore colore, apices apiculati vel obtusi, 22–45 × 8–12 μm ($\bar{x}$ = 31 × 9 μm), appendix basalis hyalina, non ramosa 45–150 × 2–4 μm ($\bar{x}$ = 80 × 2 μm).

SPECIMEN TYPICUM in foliis emortuis *Eucalypti* sp., Bloemfontein, OFS, South Africa, Feb. 1990, *P.W. Crous*, HOLOTYPUS, PREM 50836.

Foliicolous. Conidiomata immersed, becoming erumpent, unilocular, globose, pycnidioid, up to 400 μm in diam, with furfuraceous central ostiole; basal and lateral walls 3–6 cells thick, composed of hyaline to pale brown *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, hyaline, smooth-walled, lageniform to doliiform, 7–12 μm long and 5–7 μm wide at base, producing a single conidium or proliferating enteroblastically up to two times, periclinal thickening minute, collarette present. Conidia holoblastic, ventricose to fusiform-ellipsoidal, single central guttule, or multiguttulate, dark brown, smooth, nonstriate, frequently with longitudinal band of paler pigment, apices apiculate to obtuse, 22–45 × 8–12 μm ($\bar{x}$ = 31 × 9 μm), basal appendage hyaline, unbranched, 45–150 × 2–4 μm ($\bar{x}$ = 80 × 2 μm). Mean conidial body length : width ratio 3.4:1; microconidia and teleomorph not known.

HOST: *Eucalyptus* sp.

SPECIMENS EXAMINED. SOUTH AFRICA. ORANGE FREE STATE: Bloemfontein, *Eucalyptus* sp., Feb. 1990, *P.W. Crous* (HOLOTYPE, PREM 50836; type culture, PPRI 4297); *Eucalyptus* sp., 28 May 1990, *P.W. Crous* (PARATYPE, PREM 50837).

Cultures of this species sporulated abundantly and grew more rapidly than did those of all the other species tested except for those of *H. hawaiiensis*. Colonies had a dense white mycelium on MEA with denser fluffy borders forming a ridge. Optimum growth occurred at 25 C (FIG. 3). When conidia germinate or field material is incubated in moist chambers for more than 4 wk, conidia tend to become more fusiform and elongated in shape and up to 70 μm in length. Germinating conidia can also develop up to three septa. Under moist conditions, conidiomata on host material also become more erumpent.

Harknessia hawaiiensis Stevens & Young, Bull. Bernice P. Bishop Mus. **19**: 136 (1925). FIGS. 3, 9, 16

Harknessia hawaiiensis occurs on most *Eucalyptus* spp. throughout South Africa (Crous, **1990**). This species also has been found in Brazil, U.S.A. and Zambia (Sutton, **1980**). This fungus is associated with discrete, pale brown, round to irregular lesions extending through the leaf lamina, which are surrounded by a thin red to purple margin. Conidiomata of *H. hawaiiensis* were also found in mixed infections with a globose-spored species. The latter has larger conidia and longer appendages than does *H. globosa* Sutton (FIG. 7), and it also possesses a persistent conidial mucous sheath and a deeply striate conidial wall (FIG. 8) (PREM 50844). This suggests that there is an-

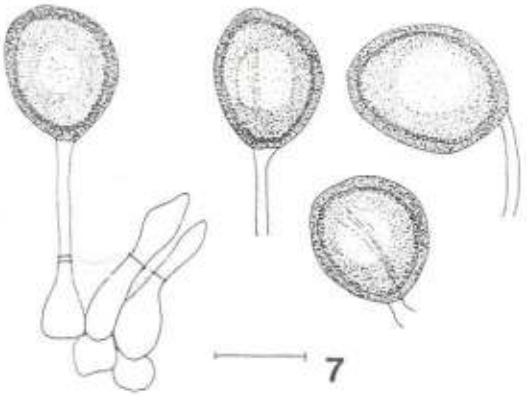


FIG. 7. Conidia and conidiogenous cells of *Harknessia globosa* (IMI 21815). Bar = 10 μm.

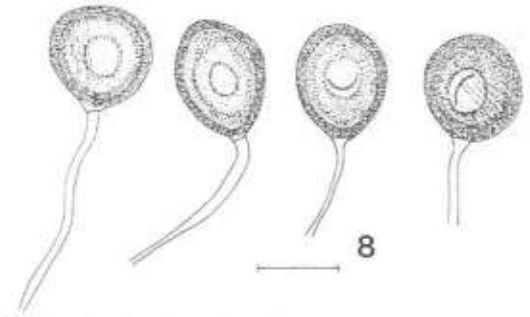


FIG. 8. Conidia of a *Harknessia* sp. (PREM 50844). Bar = 10 μm.

other, as yet undescribed species with globose conidia present in South Africa. More collections are required to describe this taxon adequately.

Cultures of *H. hawaiiensis* grew vigorously on MEA, and optimal growth was attained at 25 C (FIG. 3). Colonies remained white and were less flocculent than those of *H. eucalyptorum*, *H. fusiformis* and *H. uromycoides*. This species sporulated more readily in culture than any other *Harknessia* sp. tested with abundant, distinct conidiomata forming after 1 wk of incubation. The average size of the conidia and conidiophores produced in culture varied little from those formed in vivo.

Examination of the type collection of *H. hawaiiensis* (IMI 148757b) showed its conidia having short appendages (1.2–5 μm) and smooth nonstriate walls. As noted by Sutton (**1971**), the immature conidia lack a mucous sheath. However, examination of fresh South African collections showed this species is more variable than originally believed. For example, striations and a nonpersistent mucous sheath can be present. Furthermore, collections made from *E. nitens* (PREM 50839) and a *Eucalyptus* sp. (K?) contained conidiomata with macro- and microconidia. Sporulation of single-macrospore cultures on CLA induced both conidial types, but only macroconidia were obtained from cultures on MEA. This is the first report of a microconidial stage for *H. hawaiiensis*.

Harknessia globosa and *H. hawaiiensis* are the only two *Harknessia* spp. on *Eucalyptus* spp. described as having globose conidia which apparently lack striations (Sutton, **1971**, **1980**). In this study we found that conidia of both of these species can be finely striate as noted by Nag Raj and Di Cosmo (**1981**), and that nonpersistent mucous sheaths can also be present. The presence of striations and mucous sheaths seem to be highly variable characters that should be recorded with great care. Although the widths of the conidiogenous cells and appendages are described as being different for *H. hawaiiensis* and *H. globosa* (Sutton, **1971**), we found there was considerable overlap when measurements were taken from cultures of *H. hawaiiensis*. We found the two species to have similar appendage sizes

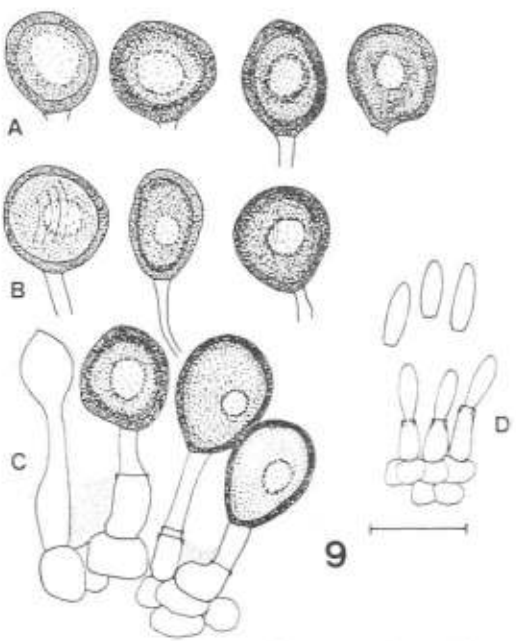


FIG. 9. A–C. *Harknessia hawaiiensis*. Bar = 10 μm. A. Macroconidia (IMI 148757a). B. Macroconidia. C. Macroconidia and conidiogenous cells. D. Microconidia and conidiogenous cells (PREM 50839).

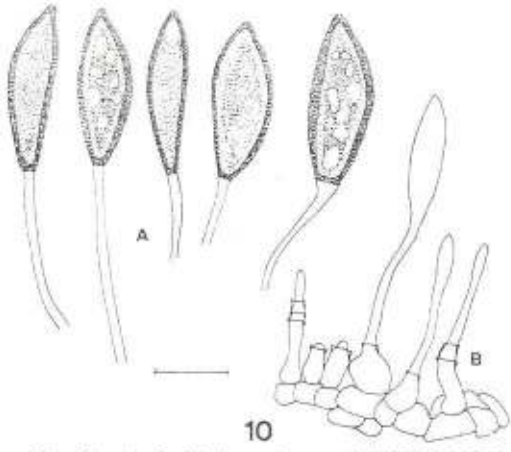


Fig. 10. A, B. *Harknessia syzygii* (PREM 50842). Bar = 10 μm. A. Conidia. B. Conidia and conidiogenous cells.

but could distinguish them by the fact that conidia were larger in *H. globosa* [12.5–17 × 11–13.7 μm ($\bar{x}$ = 14.6 × 12.7 μm)] (IMI 21815) (FIG. 7), and smaller in *H. hawaiiensis* [9–13.5 × 8–11 μm ($\bar{x}$ = 11 × 9 μm)]. Furthermore, the conidiogenous cells of *H. hawaiiensis* were up to 20 μm in length, whereas those of *H. globosa* were up to 31 μm long. On the basis of the collections examined in this study, we provide the following amended description of *H. hawaiiensis*.

Conidiomata sparse, amphigenous on leaves, small, separate to aggregated, globose, almost superficial to subepidermal and protruding, up to 400 μm in diameter in vivo, up to 350 μm in vitro, with black conidial masses exuding from ostioles with furfuraceous margins. Conidiophores reduced to conidiogenous cells. Macroconidiogenous cells, phialides, long lageniform, with the base 2.5–7 μm wide, often becoming septate approximately 4–7 μm from the base, 1.5–3 μm wide towards the apex, hyaline, smooth walled, unbranched, with one to two enteroblastic proliferations, enclosed in a nonpersistent mucilaginous sheath, 7–20 μm in length. Macroconidia holoblastic, aseptate, globose to subglobose, smooth walled, often finely striated in localized areas, with more or less central globose to irregular guttules, 9–13.5 × 8–11 μm, initially enclosed in mucilaginous sheaths, with a persisting hyaline basal appendage, devoid of cytoplasm, 1–8 μm ($\bar{x}$ = 5 μm) long. Microconidiogenous cells formed in the same conidiomata, 4–9 μm long, ampulliform, lageniform or cylindrical, hyaline, smooth, proliferating enteroblastically, with distinct cytoplasmic channels and periclinal thickening without collarettes. Microconidia holoblastic, hyaline, aseptate, smooth, ellipsoidal to fusiform, sometimes with minute marginal frills, 2.5–8.8 × 1.5–3 μm.

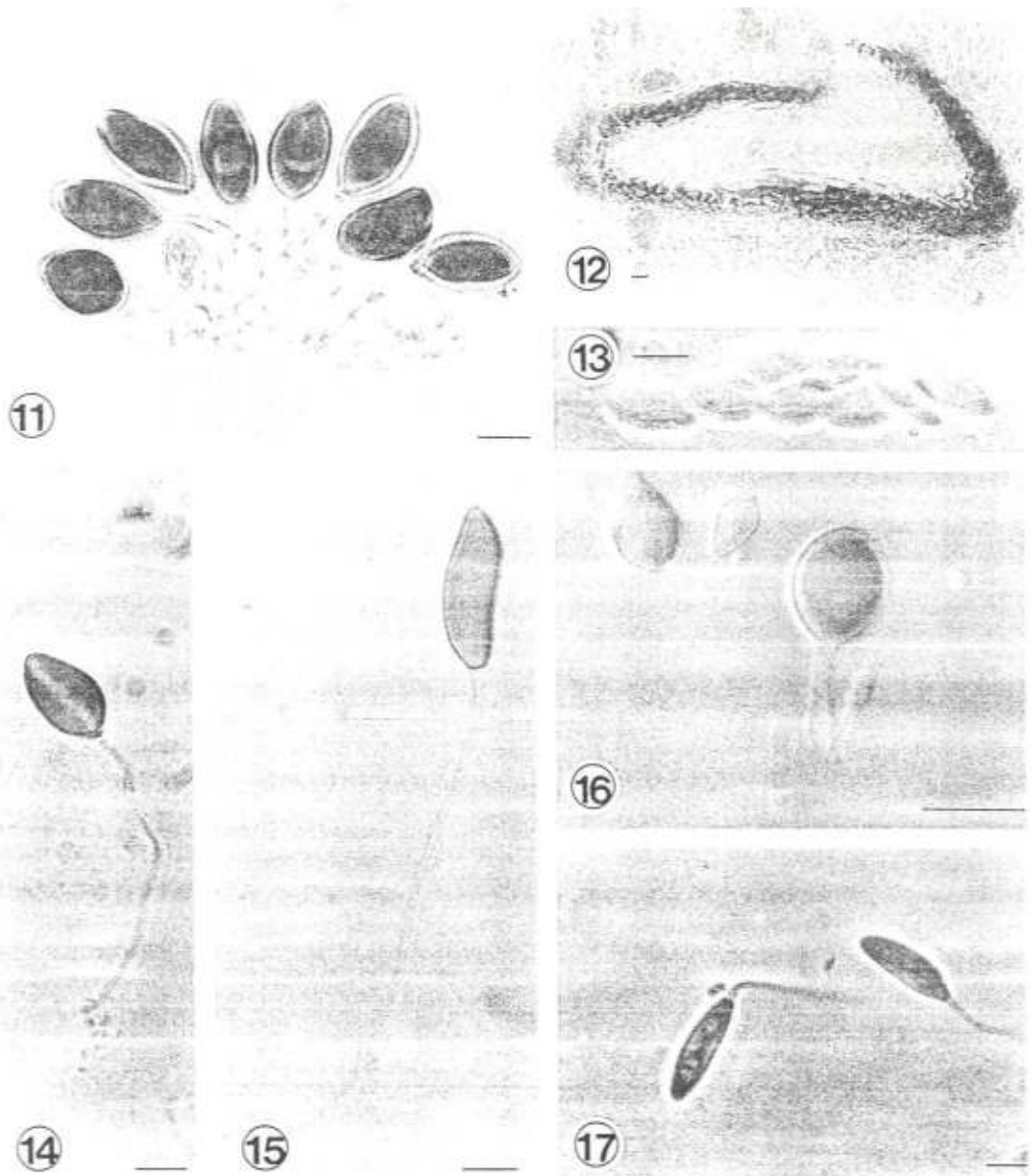
HOSTS: *E. robusta* Sm., *E. grandis*, *E. nitens*, *E. maidenii*, *E. punctata* DC., *Eucalyptus* spp.

SPECIMENS EXAMINED. UNITED STATES OF AMERICA. HAWAII: Oahu, Waipai, *E. robusta*, *Lyon 124* (HOLOTYPE, IMI 147757). SOUTH AFRICA. EASTERN TRANSVAAL: White River, *E. grandis*, 1988, *M.J. Wingfield* (PREM 49165); Sabie, 2 Nov. 1989, *P.W. Crous* (PREM 50838); Jessievale, *E. nitens*, Nov. 1988, *P.W. Crous* (PREM 50839; culture, PPRI 4298); *Eucalyptus* sp., *Mycosphaerella molleriana* (Thüm.) Lindau, Mar. 1960, *H. Schüep* (K); Sabie, *E. maidenii*, Mar. 1990, *M.J. Wingfield* (PREM 50840); *E. punctata*, Mar. 1990, *M.J. Wingfield* (PREM 50841). TRANSVAAL: Pretoria, *Eucalyptus* sp. 30 Oct. 1989, *C. Roux* (IMI 334808, PREM 50627, PPRI 3752).

In an examination of leaf litter collected from *Syzygium cordatum* in the Eastern and Northern Transvaal, another *Harknessia* sp. was found. This species resembled several other species in having ventricose conidia. However, conidia from these leaves were narrower and more ventricose than those of *H. arctostaphyli* Cooke & Harkn., *H. eucrypta* (Cooke & Mass.) Nag Raj & Di Cosmo, *H. fuegiana* Speg., *H. rhoina* Ellis & Everh., *H. ventricosa* Sutton & Hodges and *H. spermatoidea*. Although there are several species with similar appendage lengths, none has the same conidial dimensions. This collection is, therefore, described as a new species of *Harknessia* as follows:

Harknessia syzygii Crous, Wingfield *et* Nag Raj, *sp. nov.* FIGS. 3, 10, 17

Foliicola. Conidiomata stromatica, abundantia, amphigena, subepidermalia, immersa, erumpentia et punctata, globosa ad subglobosa, usque ad 250 μm diam, unilocularia, area dehiscentiae spurie ostiolata, ostioli marginibus furfuraciis; parietes basales et laterales conidiomati 4–7 cellulis crasses, ex textura angulari hyalina ad pallide brunnea compositi. Conidiophora ad cellulas conidiogenas deminuta. Cellulae conidiogenae discretae, hyalinae, laeves, lageniformes ad doliiformes, 4–10 μm longae, 2.5–4.5 μm latae basi, in vagina mucosa non persistentia involutae, semel vel ter enteroblastice proliferantes. Conidia holoblastica, ventricosa ad gibbosa in vivo, fusiformia ad ventricosa in vitro, aseptata, atrobrunnea, laevia vel striis longitudinalibus localibus, cum guttula centrali globosa, apices apiculati ad obtusi, 18.5–23 × 8.5–10.5 μm ($\bar{x}$ = 21 × 9.5 μm), appendix basalis hyalina, non ramosa, 12.5–40 μm in foliis; conidia 13.8–25 × 6–7.5 μm ($\bar{x}$ = 20 × 6.3 μm), appendix 15–50 μm in cultura.



FIGS. 11–17. Conidia and asci of *Harknessia* and *Wuestneia* spp. Bars = 10 μm. 11. Conidia and conidiogenous cells of *H. eucalyptorum* (PREM 50813). 12. Vertical section through a perithecium of *W. eucalyptorum* (PREM 50830). 13. Ascus and ascospores of *W. eucalyptorum* (PREM 50830). 14. Conidium of *H. uromycoides* (PREM 50832). 15. Conidium of *H. fusiformis* (PREM 50836). 16. Macro- and microconidia of *H. hawaiiensis* (PREM 50839). 17 Conidia of *H. syzygii* (PREM 50842).

SPECIMEN TYPICUM in foliis emortuis *Syzygii cordati* Hochst., Barberton, Eastern Transvaal, South Africa, 1 Feb. 1989, *M.J. Wingfield*, HOLOTYPUS, PREM 50824 (IMI 338269, ISOTYPUS).

Foliicolous. Conidiomata stromatic, abundant, amphigenous, subepidermal, immersed, becoming erumpent and punctate, globose to subglobose, up to 250 μm diam, unilocular; area of dehiscence spuriously ostiolate, ostioles with furfuraceous margins; basal and lateral walls four to seven cells thick, composed of hyaline to pale brown *textura angularis*. Conidiophores reduced

to conidiogenous cells. Conidiogenous cells discrete, hyaline, smooth-walled, lageniform to doliiform, 4–10 μm long and 2.5–4.5 μm wide at the base, invested in mucilage, which disappears with maturity, producing a single conidium or proliferating enteroblastically up to three times, channel wide, periclinal thickening minute, collarette present. Conidia holoblastic, ventricose to slightly gibbose, with single central guttule, or irregularly multiguttulate in vivo, fusiform to ventricose and irregularly guttulate in vitro, aseptate, dark brown, smooth, nonstriate, or very finely striate in localized areas, guttulate, occasionally with longitudinal sections of paler pigment at the centers, apices apiculate to obtuse, 18.5–23 × 8.5–10.5 μm ($\bar{x}$ = 21 × 9.5 μm), basal appendage hyaline, unbranched, 12.5–40 μm long on leaves; conidia 13.8–25 × 6–7.5 μm ($\bar{x}$ = 20 × 6.3 μm), appendage 15–50 μm in culture. Mean conidium body length : width ratio 2.3:1 in vivo, 3:1 in vitro; microconidia not seen and no teleomorph known.

HOST: *S. cordatum*.

SPECIMENS EXAMINED. SOUTH AFRICA. EASTERN TRANSVAAL: Barberton, *S. cordatum*, 1 Feb. 1989, *M.J. Wingfield* (HOLOTYPE, PREM 50842; type culture, PPRI 4299; ISOTYPE, IMI 338269), single conidial isolates on sterilized *E. grandis* leaves (IMI 338267). NORTHERN TRANSVAAL: Goudrivier game lodge, *S. cordatum* leaves, May 1991, *P.W. Crous* (PARATYPE, PREM 50843).

Cultures obtained from this collection sporulated on MEA, CLA and *Eucalyptus* leaf agar. Conidia retained their ventricose shape in culture but had less pigment and were slightly narrower than those observed in vivo. Optimum growth occurred at 25 C (FIG. 3).

It is surprising that there are yet more new species of *Harknessia* occurring on *Eucalyptus*. As conidium morphology of many of these species has been found to change on different media and with different conditions of incubation, objective techniques would ultimately prove to be more useful. However, there are clearly many species that remain to be described and mycologists are encouraged to collect these fungi.

ACKNOWLEDGMENTS

Dr. B. C. Sutton (IMI, Bakeham Lane, Egham, Surrey, U.K.) is thanked for his comments on the material collected from *Syzygium cordatum*, and Mr. E. B. H. Oliver (National Botanical Institute, Stellenbosch) is thanked for correcting the Latin diagnosis.

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Accepted for publication October 16, 1992